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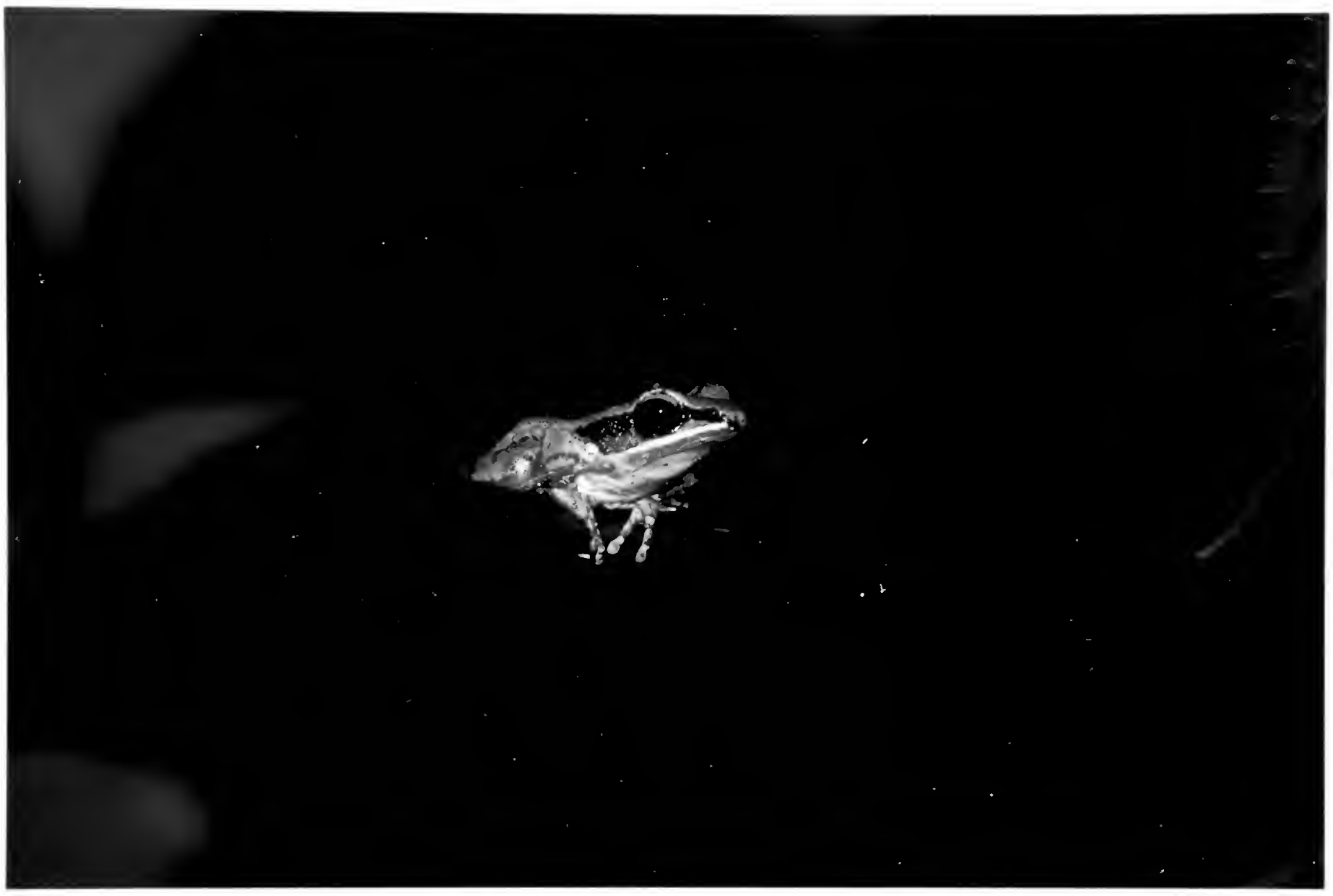

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A Preliminary Study of the Lizard Fauna and Their Habitats in Northwestern Iran

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Abstract.- Northwestern Iran has unique geographical and climatic conditions that support a rich flora and fauna. In view of the lack of in-depth studies on the lizards of the region, an investigation was started in the northern part of Ardabil Province for an inventory of this component of the fauna and their habitats. Collections were made from October 2003 to June 2005 and 165 specimens were collected and identified. Five families, 12 genera and 15 species are represented, including Agamidae: *Laudakia caucasia, Phrynocephalus persicus, Trapelus ruderatus*; Lacertidae: *Lacerta media media, Lacerta strigata, Lacerta brandtii, Darevskia raddei raddei, Eremias strauchi strauchi, Eremias arguta, Ophisops elegans*; Scincidae: *Mabuya aurata transcaucasica, Eumeces schneiderii princeps, Abelepharus bivittatus*; Anguidae: *Pseudopus apodus* and Gekkonidae: *Cyrtopodion caspium caspium*. Comparing this list to the data provided by Anderson (1999), it seems that most of the lizards are being reported for the Province for the first time. The families Gekkonidae and Anguidae are newly recorded, and the gecko *Cyrtopodion caspium* is first recorded from the west and northwest of Iran. With seven species represented in the area, lacertids have the highest species diversity among the lizard families and need further study. Habitat features also have been given for all species.

Keywords.- Iran, Ardabil, fauna, lizard, *Lacerta*.

Introduction

General information about the herpetofauna of Iran has been provided by Mertens (1957), Anderson (1966), Tuck (1971, 1974), Latifi (1984, 1991), Balouch and Kami (1995) and Kami and Vakilipoure (1996a, 1996b). Furthermore, a handbook of amphibians and reptiles of the Middle East has been published by Leviton et al. (1992), a book on the Lizards of Iran was recently published by Anderson (1999) and an updated checklist to the lizards of Iran was provided by Firouz (2000). Despite these publications, the lizards of Iran are still poorly-known and infrequently collected, with many new species still being discovered (Rastegar-Pouyani, 1996; Rastegar-Pouyani and Nilson, 1998). Studies on the lizards of Ardabil Province are also very limited (Ahmadzadeh, 2004).

The aim of this study is to determine in detail the lizard fauna and their habitat features in the northern part of Ardabil Province, which is of particular significance considering the unique geography and vegetation of the region. Moreover, this study will collect baseline population data for future management.

Materials and Methods

The area of study is in the Northwest part of Iran, specifically, the northern part of Ardabil Province (38° 15' E, to 39° 40' E, 47° 30' N to 48° 00' N). The region is surrounded by the Alborz Mountains and the Caspian Sea to the east, Aras village is to the north, Arasbaran protected area and Gare-Dagh Mountain to the west and the Sabalan Mountain chains to the south (Fig. 1). Altitude ranges between 20 m in the Moghan steppe to 4,888 m on the Sabalan Mountain. The study was carried out between October 2003 and June 2005. All of the samples were caught by hand and some lizards which are active and difficult to catch, such as green lizards (e.g., *Lacerta m. media* and *L. strigata*), were captured by dust shot. Locality data and their habitat features were recorded for all species encountered during the study. However, all have been preserved in accordance to standard methods (Formalin 10%) and voucher specimens are stored in the Biodiversity and Ecosystem Management Department Collection (BEMD) at Shahid Beheshti University of Iran. Specimens were identified with Leviton et al. (1992) and Anderson (1999) using morphometric meas-

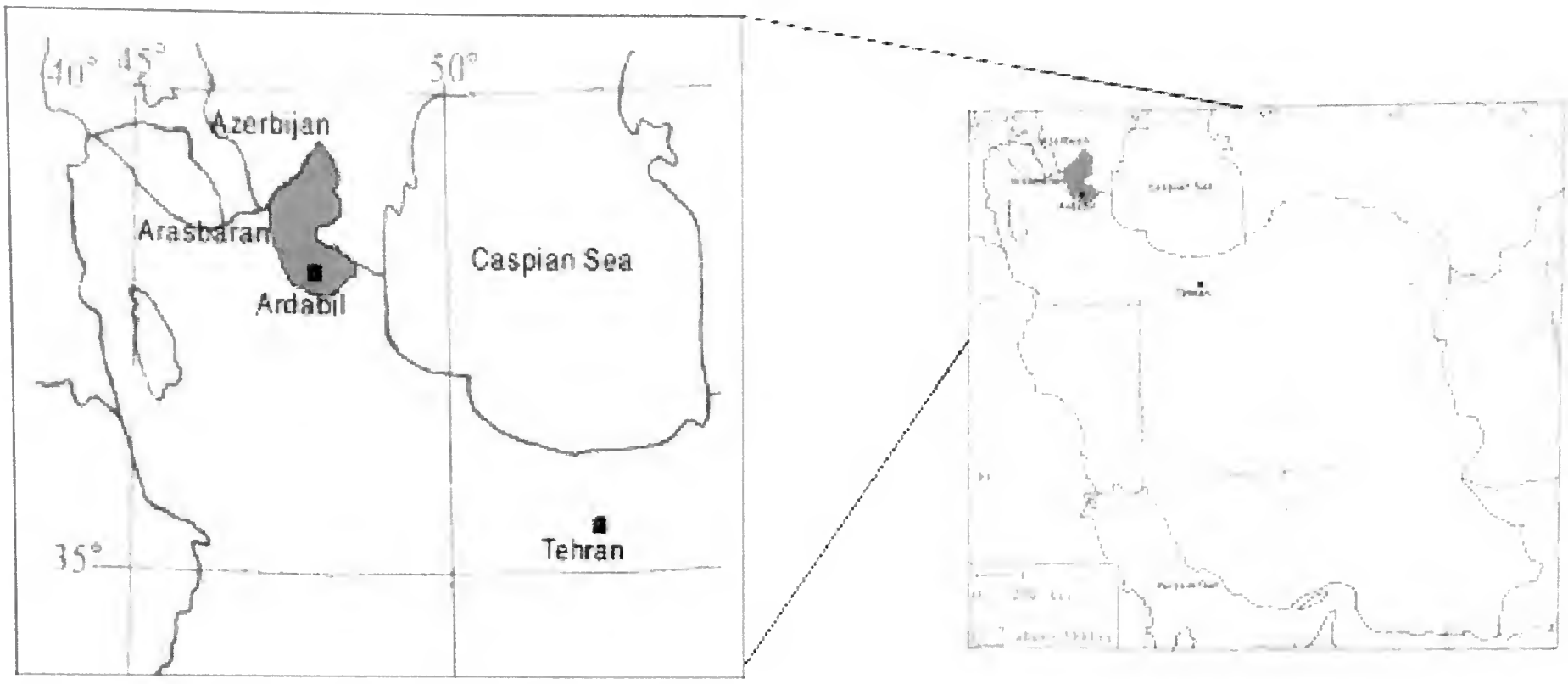

Figure 1. The study area, the northern part of Ardabil Province of Iran.

urements, coloration and pholidosis features (including the number, structure and range of plates).

Results

A total of 165 samples were collected in the study area, comprising 15 species in 12 genera and 5 families. The species composition is given in Table 1. Distribution of species are presented in Figure 2.

Family: Agamidae

Laudakia caucasia caucasia (Eichwald, 1831)

Laudakia c. caucasia is widely distributed in the study area, preferring montainous habitats and eroded sand canyons in flooded plains adjacent to mountains, rock cliffs, old houses and stony walls near roads. At 6 km^2, 210 specimens were recorded on 10 June 2004 in Meshkinshar. This species was also collected on 24 November and 3 March in the Meshkinshahr and Arshagh areas at elevations between 500–2,800 m. Specimens were light olive to dark gray in ground color with adult snout-vent length of 152 mm in males and 155 mm in females.

Phrynocephalus persicus persicus De Filippi, 1863

This species was rarely encountered in the study area. Four specimens were captured in Arshagh, Alma village – a semi-arid area with ephemeral plants in spring and loamy soil. Xerophyte vegetation, both plants and bushes, grow in these areas. Dorsal coloration was light brown with three dark transverse marks, within which on the hind limbs were enlarged tubercular scales. The largest female was 40 and 48 mm snout-vent and tail length, respectively. During the study period, no males were found.

Trapelus ruderatus ruderatus (Olivier, 1804)

The small agamid lizard *Trapelus r. ruderatus* was found on open stony ground and in cultivated fields with sparse weed vegetation in autumn. On sunny summer days, it hides under weeds such as *Euphorbia* spp., *Chenopodium* spp. and *Chrozophora tinctoria*. Its activity appears to begin in early June and extends to late September. In total, 10 specimens were collected on the harvested wheat and barley fields in Gooshe area at approximately at 10:30 AM. Ground color was typically grayish-brown with five dark transverse bars on the trunk which were interrupted by a series of light ovoid vertebral spots (Fig. 3). The largest male examined with distinct callous preanal scales, had a 65 mm snout-vent and 74 mm tail length. The largest female had measurements of 63 mm and 75 mm, respectively.

Family: Anguidae

Pseudopus apodus (Pallas, 1775)

Pseudopus apodus occurs throughout the Hyrcanian forest of northern Iran. It has recently been collected from the Arasbaran protected area, but there are no records for Ardabil Province. This species was found in grassland and shrubby vegetation near streams. On a sunny day, four *P. apodus* were observed in a pond. The ground color of the dorsum was gray with zig-zagging blackish-brown stripes in the juvenile. The head was light yellowish-brown in adults with the remainder of the body dark brown. The longest adult had a 520 mm snout-vent length and a 670 mm tail.

Family: Gekkonidae

Cyrtopodion caspium caspium (Eichwald, 1831)

An isolated population of *Cyrtopodion caspium* was found in the Moghan Steppe for the first time, representing a new family record for northwestern Iran. One spec-

Table 1. Lizard species collected from the study area.

Family	Species	Common Name
Agamidae	*Laudakia caucasia*	Caucasian agama
	Phrynocephalus persicus	Persian toad agama
	Trapelus ruderatus	Olivier's agama
Anguidae	*Pseudopus apodus*	Glass-snake, sheltopusik
Gekkonidae	*Cyrtopodion caspium*	Caspian bent-toed gecko
Lacertidae	*Darevskia r. raddei*	Azarbaijan lizard
	Eremias arguta	Steppe-runner
	Eremias strauchi	Strauch's racerunner
	Lacerta brandtii	Persian lacerta
	Lacerta media	Three-lined lizard
	Lacerta strigata	Caspian green lizard
	Ophisops elegans	Snake-eyed lizard
Scincidae	*Ablepharus bivittatus*	Two-streaked snake-eyed skink
	Eumeces schneiderii	Schneider's skink
	Mabuya aurata	Transcaucasian grass skink

imen was collected at night on walls of an old house at 20 m elevation. After sunset they fed on various nocturnal insects around lights. Dorsal scales are strongly keeled. Dorsal body coloration was light gray with five dark transverse bars on the body and 11–12 on the tail (Fig. 4). In Pars-Abad, one male specimen with a snout-vent length of 75 mm and a tail length of 70 mm was measured.

Family: Lacertidae

Darevskia raddei raddei (Boettger, 1892)

Darevskia r. raddei is common in rocky areas where *Laudakia caucasia* is also frequently found. In March, this species was seen on vertical surfaces of rocks in the Kapas Mountains near Meshkin-Shahr. *Darevskia r. raddei* is various shades of light brown dorsally and more common in the rocky habitats than other lacertids. Specimens were found below altitudes of 900 m in Meshkinshar, but in the Salavat and Arshag Mountains, it was collected at altitude up to 2,400 m. The relationship of this subspecies to *D. r. vanensis* in northwestern Iran requires further study. The largest male had a snout-vent of 71 mm and tail length of 131 mm. One adult female had a 70 mm snout-vent length and 130 mm tail length (Fig. 5).

Lacerta brandtii De Filippi, 1863

Lacerta brandtii was collected under stones, on foothills and in the burrows of other animals in open arid bushy and stony habitats in the Razeye area. Large numbers of this lizard were also found in Samian District, 100 km from the study area on a foothill surrounded by cultivated land. The relationship between the two Iranian populations of this species in Esfahan Province and east Azarbijan Province remains problematic. This species is less active in comparison to other lacertid lizards such as *Darevskia raddei*. The dorsal surface was olive-gray with small black spots (Fig. 6) and the ventral surface had 8 longitudinal rows of plate. The longest male specimen had total length of 192 mm. The tail of this species displays autotomy (approximately 60% of total specimens).

Eremias arguta (Pallas, 1773)

Eremias arguta has a limited distribution in the study area: one adult specimen was collected in a harvested barley field on a sunny day near to the Ardabil-Meshkin road and two juvenile specimens were captured in the Ardabil Airport area in August 2004. This lizard had a white belly and a dorsum with white spots edged with black on a grayish background (Fig. 7) that sometimes formed transverse bands in the adults. Our adult specimens had a 95 mm snout-vent length and a 110 mm tail.

Eremias strauchi strauchi Kessler, 1878

There are two subspecies of this lizard in Iran – *Eremias s. strauchi* and *E. s. kopetdaghica*, of which only the first was found in the study area. The specimen was collected in the eastern part of the study area in the Arshag plain under wheat straw in a dry, stony harvested field. *Eremias s. strauchi* is active and hides in shrubby vegetation. Five eggs of this lizard were found under an *Artemisia* sp. shrub on 14 June 2004 in Amir-Abad village. In the study area one male specimen was measured

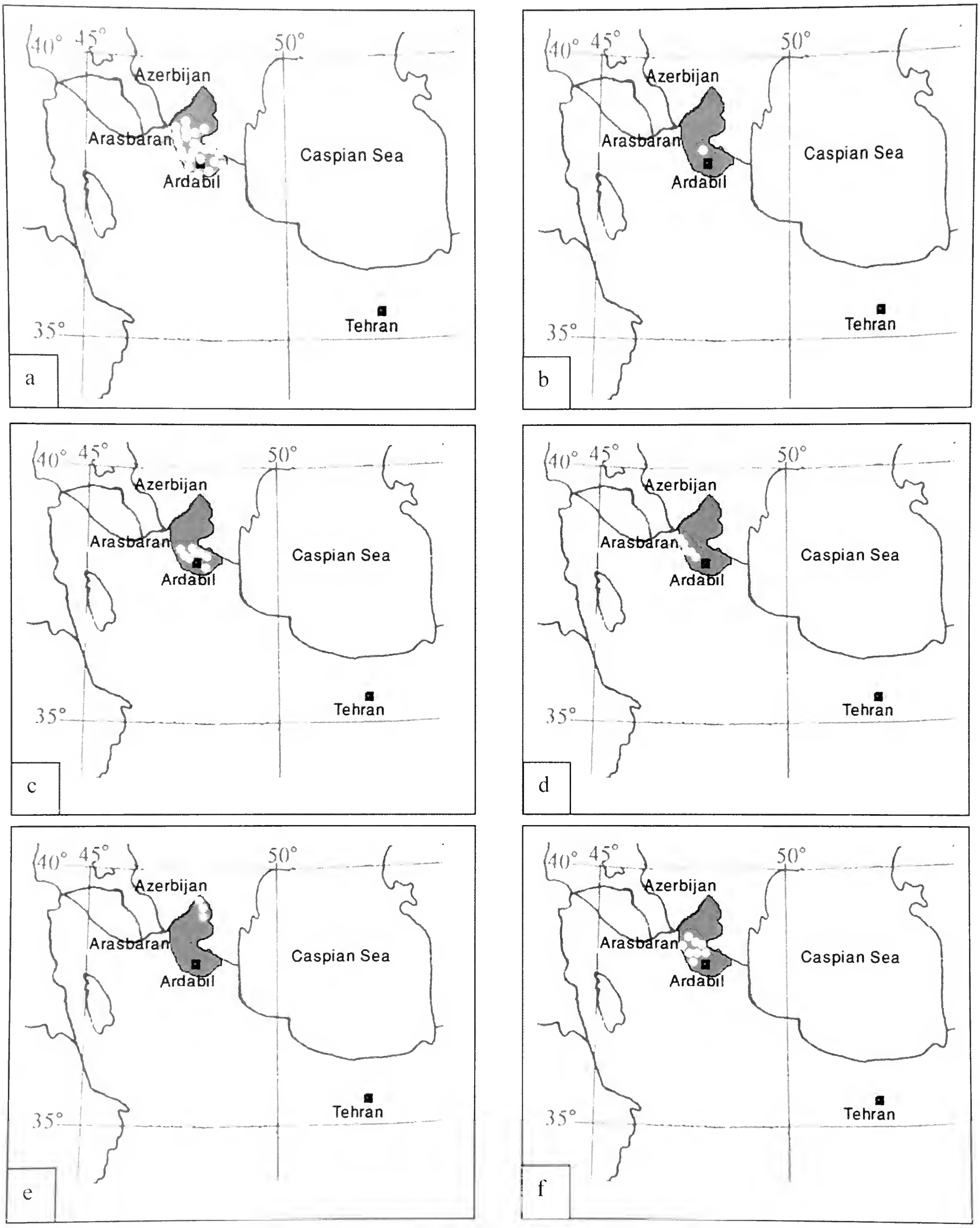

Figure 2. Distribution of species in the study area: (a) *L. caucasia*, (b) *Ph. persicus*, (c) *T. ruderatus*, (d) *P. apodus*, (e) *C. caspium*, (f) *D. raddei*.

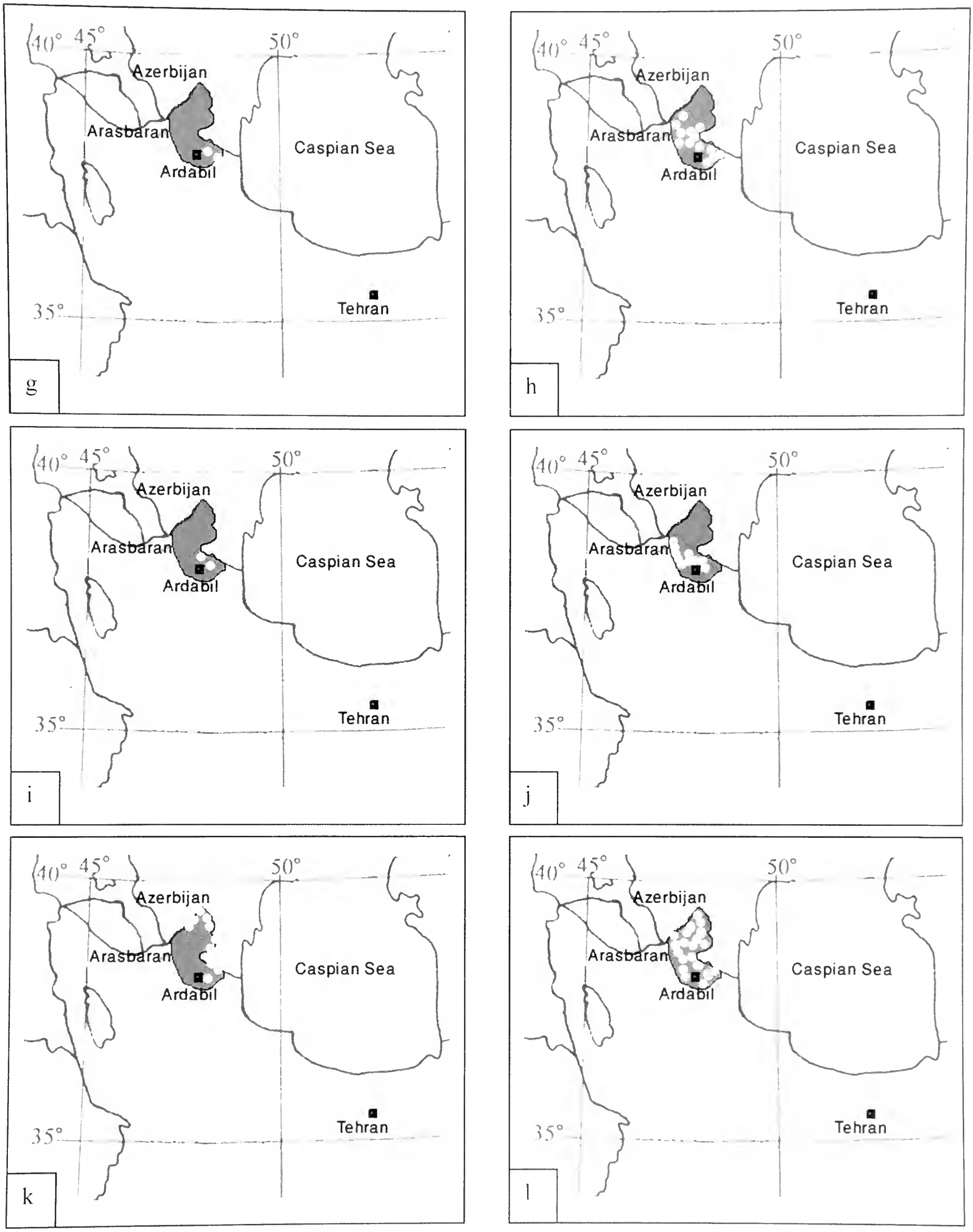

Figure 2. (continued) (g) *E. arguta*, (h) *E. strauchi*, (i) *L. brandtii*, (j) *L. media*, (k) *L. strigata*, (l) *O. elegans*.

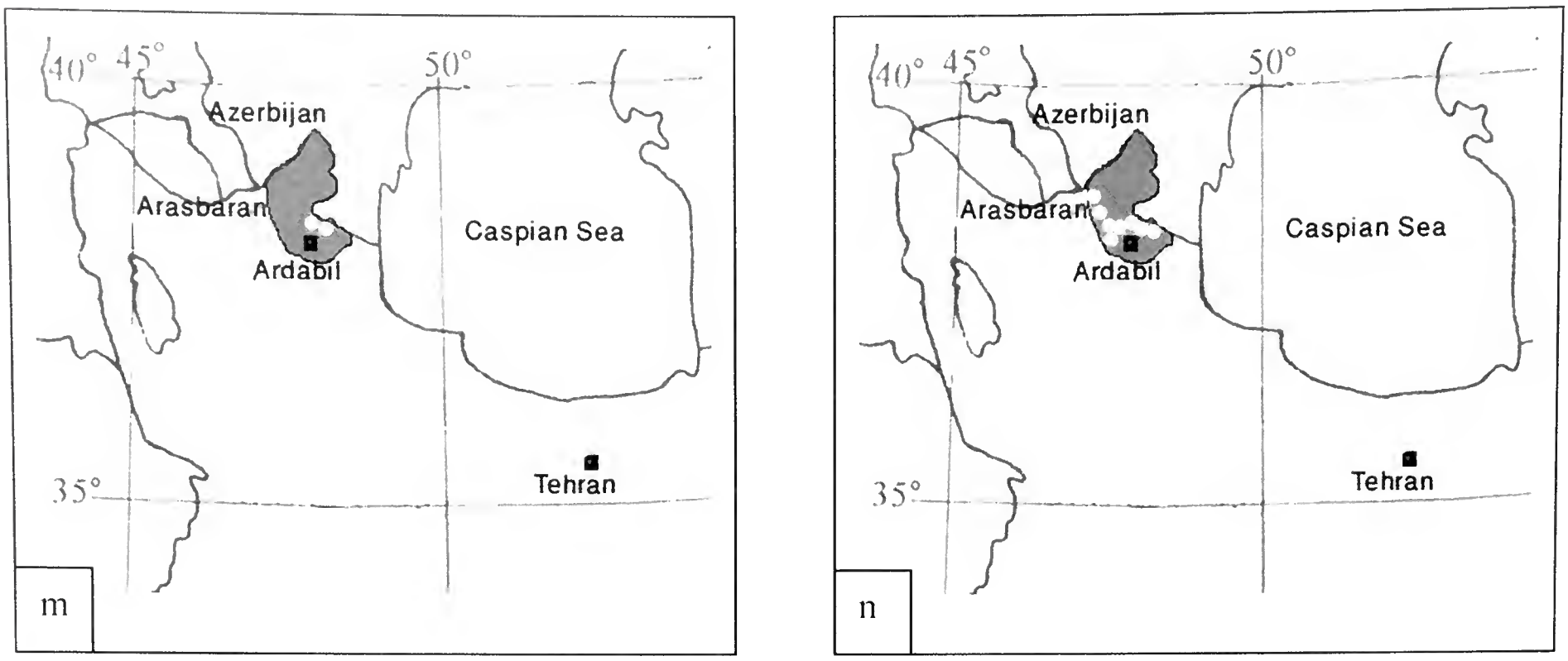

Figure 2. (continued) (m) *A. bivitattus*, (n) *E. schneiderii*, (o) *M. aurata*.

with a 71 mm snout-vent length and had a 128 mm tail. It's olive-gray color pattern does not vary greatly among populations (Fig. 8).

Lacerta media media (Lantz and Cyren, 1902)
Lacerta m. media was very common in the grassy and shrubby areas along the Khyave Chaye and Garesoo river banks. One male specimen was captured under a stone near a bean field. Specimens were observed at an altitude of 2,100 m and males were seen on stony walls near the roads at the end of the winter. The dorsal surface of the adult male, unlike juveniles, was green without any light lines or spots. Females were dark olive-brown with large lateral spots that disappeared with age. The largest lacertid collected during the study was one male specimen with a snout-vent of 117 mm and a tail length of 272 mm. This species exists in two differently-spotted morphs, with specimens from cultivated fields being larger than those from other habitats.

Lacerta strigata Eichwald, 1831
Lacerta strigata was most frequently found in the Hyrcanian Forest in northern Iran and in some bushy and wooded streams banks associated with this forest, such as the Arax River in the northern part of the study area. Large numbers of this species were seen in Pars-Abad, Bilasovar and Germi near streams with dense *Tamarix* and *Rubus* vegetation. One specimen was captured far from the Hyrcanian Forest in an open harvested wheat field on 25 August 2004. Most collection sites represent new locality records. The general color of the dorsum was light green in males and dark-olive to brown in females; it was more strongly spotted than *Lacerta m. media*. Females were also smaller with more numerous dark spots. The largest male had a snout-vent length of 160 mm and a tail length of 100 mm, while the

Figure 3. *Trapelus ruderatus ruderatus*.

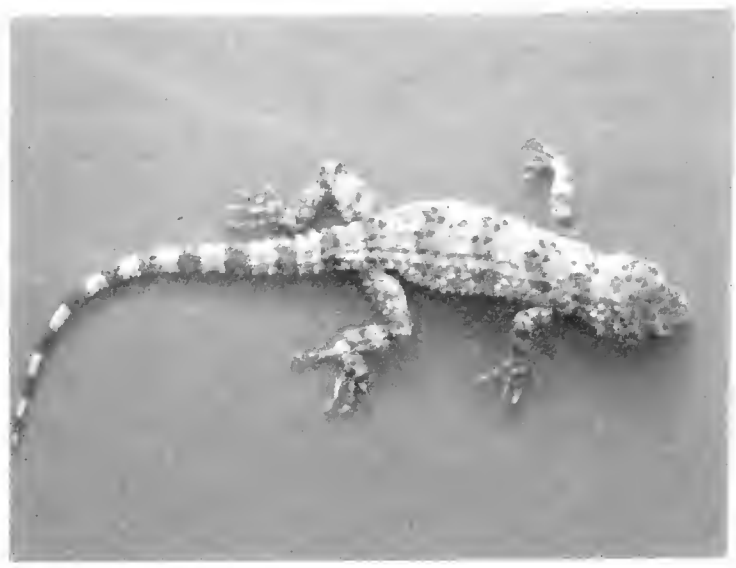

Figure 4. *Cyrtopodion caspium caspium*.

Figure 5. *Darevskia raddei raddei*.

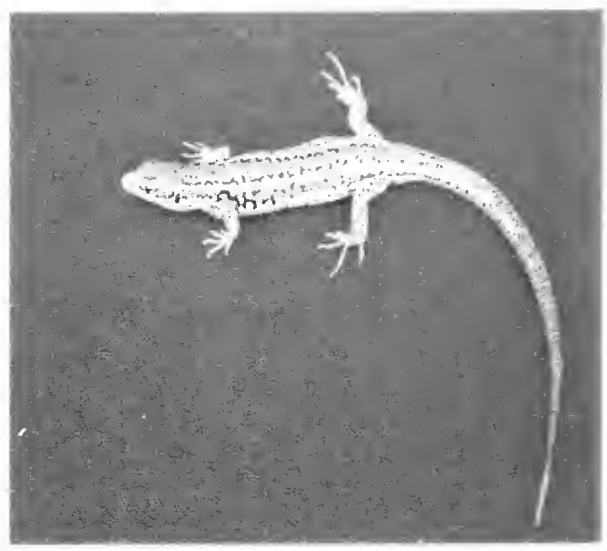

Figure 6. *Lacerta brandtii*.

Figure 7. *Eremias arguta*.

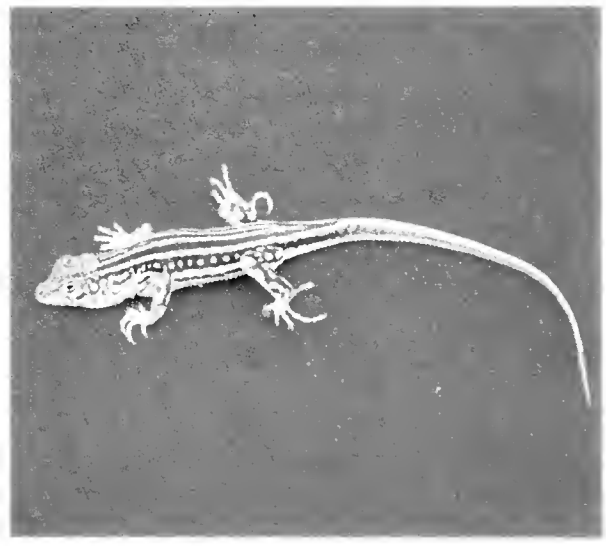

Figure 8. *Eremias strauchi*.

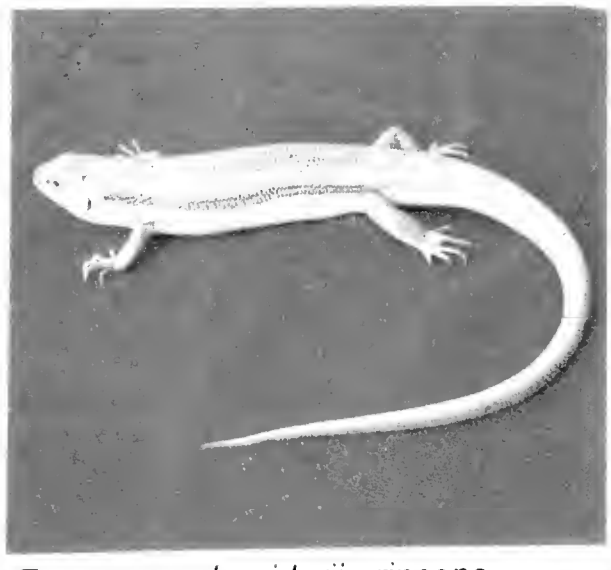

Figure 9. *Eumeces schneiderii princeps*.

Figure 10. *Mabuya aruata transcaucasica*.

largest female had measurements of 97 mm and 187 mm.

Ophisops elegans Menetries, 1832
Ophisops elegans is widely distributed, but is most common on the Moghan Steppe. A large isolated population was found in Amir-Abad village. Specimens were active and encountered almost everywhere, particularly in dry stony habitats. Males and females both showed different color patterns during the reproductive period. Dorsal coloration was generally olive-green to brownish with two light dorsolateral stripes that disappear in the adult. An adult female from the Moghan Steppe had a snout-vent length of 64 mm and a tail length of 110 mm, and a male from Amir-Abad village had a snout-vent of 70 mm and a tail length of 120 mm.

Family: Scincidae

Ablepharus bivittatus (Menetries, 1832)
The Two-streaked Snake-eyed Skink, *Ablepharus bivittatus,* has only been found in Amir-Abad village on Ardabil-Germi Road on a slope with large spiny cushion vegetation where it was sympatric with *Ophisops elegans* and *Eremias strauchi.* This active lizard has a high population density in the Neur Lake area in southern part of Ardabil Province reaches. Body coloration on the dorsum and tail is bronze-brown. The largest adult female specimen reached 60 mm in snout-vent length.

Eumeces schneiderii princeps (Eichwald, 1839)
This species lives on sand dunes, stony hills and dry river beds. We captured one male on a foothill in Meshkinshahr on 16 June 2004 at 08:00 AM, where *Mabuya aruata transcaucasica* was also found. This lizard is very active, hides in burrows and can jump approximately 2 m. In comparison to other scincid lizards, *Eumeces schneiderii* occurs in relatively few localities - overgrazing and destruction of habitat is threatening extirpation of this species in the study area. The dorsum was brownish with a narrow creamy-white lateral line from the posterior labial through the ear along the sides to the groin (Fig. 9). Total length (snout-vent + tail length) of the captured male was 240 mm.

Mabuya aurata transcaucasica (Chernov, 1926)
Mabuya aurata transcaucasica lives in sandy areas and small hills that are covered with *Astragalus* and *Acantolimon* vegetation. This lizard often jumps from stone to stone for hunting insects especially grasshoppers. The sympatric occurrence of *Mabuya aurata transcaucasica*, *Darevskia raddei raddei* and *Laudakia caucasia caucasia* has been documented on Salvat Mountain in a rocky habitat. On the Arshagh Mountains, juveniles with blue tails were found in cliffs, but we could not find adult specimens at this locality. Dorsal coloration is olive-brown with dark spots in longitudinal rows. These spots disappear on the tail and head (Fig. 10). In the study area a specimen with a 115 mm snout-vent length and a 125 mm tail was collected.

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A Second Record of *Ptyctolaemus gularis* (Peters, 1864) from Bangladesh

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Abstract.- *Ptyctolaemus gularis* (Peters, 1864), the blue-throated lizard, was collected from a hilly stream in Rangamat District in Bangladesh in July 2003 and November 2004, representing the second recorded occurrence of this species in Bangladesh.

Keywords.- *Ptyctolaemus gularis*, blue-throated lizard, occurrence, habitat, ecology, morphology, Bangladesh.

Introduction

The blue-throated lizard, *Ptyctolaemus gularis* (Peter, 1864), has been previously described from Meghalaya, Assam, the Chittagong Hill Tracts, Tibet and China (Boulenger, 1890; Hora, 1926; Smith, 1935; Zhao and Adler, 1993). Boulenger (1890), who developed the lizard taxonomy of the Indian Subcontinent, examined two specimens of *P. gularis*, the type specimen from Calcutta, preserved in the Berlin Museum, and a specimen from Sadiya, Assam, in the British Museum. Following Boulenger (op. cit.), Hora (1926) reported ten specimens from Assam and Nainimukh (correctly spelled Mainimukh), Chittagong Hill Tracts, which are presently deposited in the Zoological Survey of India. The single specimen from Nainimukh represented the first record of this species in Bangladesh. This record has been subsequently overlooked by other authors, including Ahsan, 1998; Khan, 1982; Sarker and Sarker, 1988.

Observations and Discussion

During a herpetological survey of Bangladesh, one specimen of *Ptyctolaemus gularis* was collected from Rangamati District (part of the Chittagong Hill Tracts) on 18 July 2003 (Fig. 1). Two other specimens were later collected from Rampahar about 50 km east of Chittagong City in Kaptai National Park (22.30.425′ N, 092.10.446′ E), Rangamati District, on 25 November 2004 (Fig. 1). The first specimen was collected from Rupkari Chara (23.12.126′ N, 092.10.628′ E), a hilly stream of the Rupkari Union Parishad under Baghaichari Upazila. The collection site is approximately 7 km northwest from the Baghaichari Upazila headquarter. At the time of collection, approximately 1300 h, the specimen was observed on a large stone hunting insects. This specimen has been deposited in the departmental museum of Zoology, University of Chittagong, Chittagong, Bangladesh (Fig. 2A, B). The other two specimens, currently in the collection of S. Chakma, were collected approximately 150 m apart between 1300 and 1400 h. These animals were also collected while they were hunting for insects. One of us (MFA) also observed this species in Chittagong at the Chunati Wildlife Sanctuary in 1990.

With these new records, it is likely that *Ptyctolaemus gularis* also occurs in the hills of Sherpur, Jamalpur, Hobiganj, Moulvibazar, Sylhet (i.e., British-Indian Assam), Khagrachari, Bandarbans (part of Chittagong Hill Tracts) and Cox's Bazar Districts in Bangladesh, which share similar habitats.

Habitat and Ecology

Ptyctolaemus gularis is a terrestrial, diurnal species that is frequently found south of the Brahmaputra River in India (Smith, 1935; Daniel, 2002) and uncommonly encountered in the southeastern hilly forests of Bangladesh. It is most often observed in search of food on land, stones and logs near streams and water-logs. The first collection locality visited in 2003 was a narrow stony stream, with the hills on both sides covered with bamboo brakes (muli [*Melocanna bambusoides*]), gameri (*Gmelina arborea*) and teak (*Tectona grandis*) trees. Ferns and some natural herbs grew between the stones. The second and third specimens collected in 2004 were found on the slopes of a hilly, stony stream close to a waterfall. The upper canopy was dominated by garjan (*Dipterocarpus* spp.) and gutgutia (P*rotium serratum*) trees, and the lower canopy and forest floor were densely covered by shrubs and herbs.

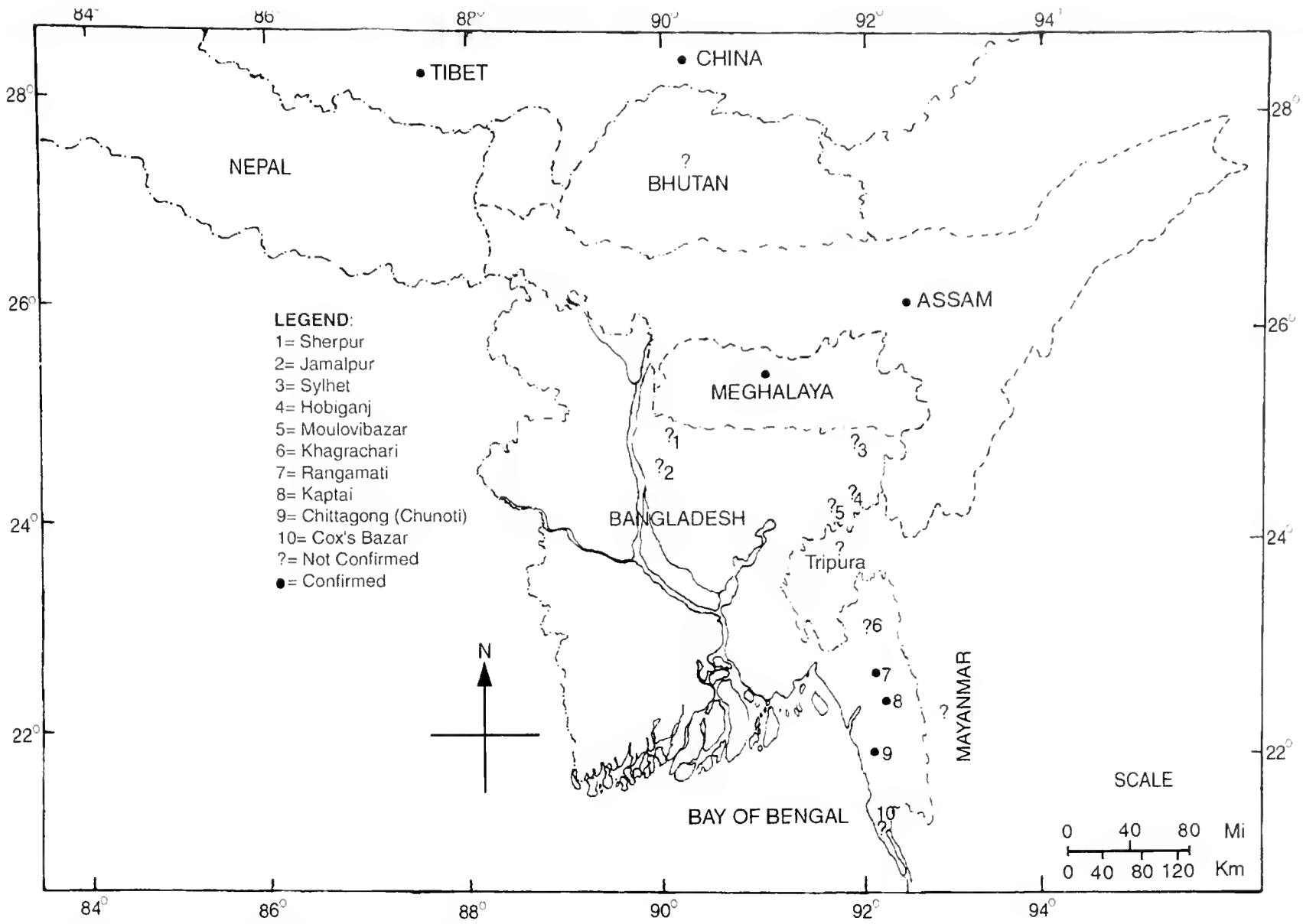

Figure 1. Map showing collection localities.

Identification

The collected specimens can be most readily separated from congeners by having three parallel longitudinal folds on each side of the throat that converge posteriorly (cf. Boulenger, 1890; Smith, 1935) (Fig. 2C). Other useful characters include an olive-brown dorsum with dark transverse bars and/or spots, two curved dark brown cross-bars between the eyes separated by a central light bar, a dark stripe below the eye to the angle of the mouth, dark blue throat folds, and limbs and a tail with dark cross-bars above and yellowish-white cross-bars below (cf. Boulenger, 1890; Smith, 1935).

The head is also rather long and narrow with unequally-sized upper scales that are strongly keeled. The dorsal body scales are also unequally-sized, with large, strongly-keeled scales and smaller feeble ones. Several mid-dorsal rows also point backwards and upwards and the ventral scales are strongly keeled and mucronate. The limbs are moderate in size; the third and fourth fingers are equal while the fourth toe is much longer than the third. The tail is rounded, slender and covered with sub-equal keeled scales (cf. Boulenger, 1890; Smith, 1935). Table 1 compares the lengths of the present specimens with those collected previously.

Acknowledgments

We wish to thank Dr. M. A. G. Khan and an anonymous reviewer(s), who kindly reviewed an earlier version of this manuscript. Mr. M. S. Islam and Mr. M. A. W. Chowdhury kindly helped in drawing the map.

Table 1. Comparison of recently collected specimens with those from earlier collections.

Snout-vent length (mm)	Tail length (mm)	Source
76.3* (77,69, 83)	167.7* (157, 166, 180)	Present report
80	170	Smith 1935
45.7	162.5	Hora 1926
69.85 (2.75")	158.75 (6.25")	Boulenger 1890

* Mean and raw data within brackets

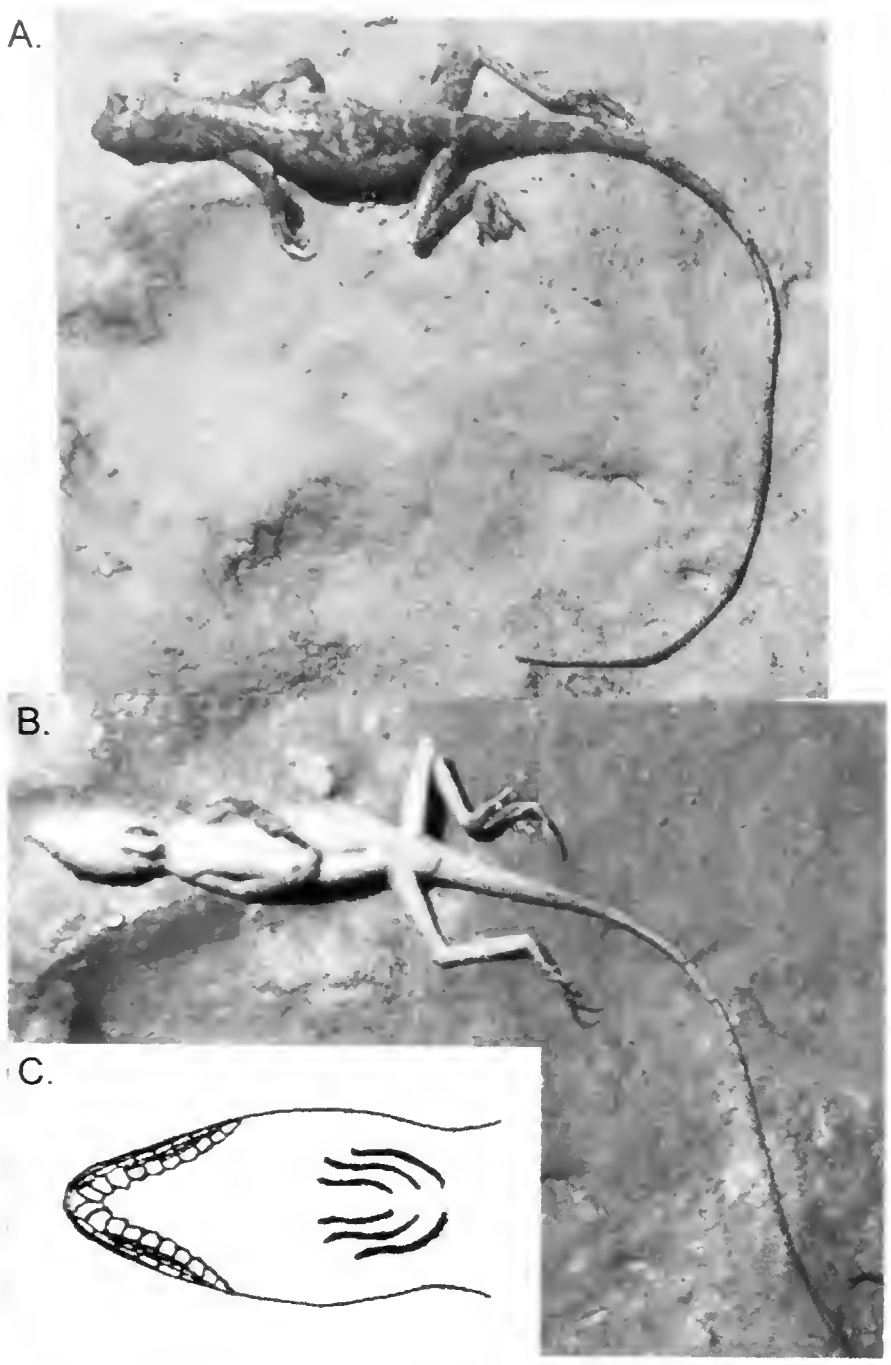

Figure 2. Dorsal (A) and ventral (B) aspects of *Ptyctolaemus gularis* collected. Inset (C) shows the gular region folds converging posteriorly, diagnostic of this species.

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Observations on the Ovipositional Behavior of the Crest-less Lizard *Calotes liocephalus* (Reptilia: Agamidae) in the Knuckles Forest Region of Sri Lanka

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Abstract.- A mature female *Calotes liocephalus* lying on the ground in Pitawala in the Knuckles Forest Region of Sri Lanka. This is the first described observation of the ovipositing of *Calotes liocephalus*. The ovipositional behavior consisted of digging a hole to lay eggs, laying the eggs, scraping soil to bury the eggs, filling of the spaces between the eggs, the tight compression of the soil and camouflaging the nest.

Keywords.- Agamidae, *Calotes*, egg-laying behavior, Knuckles, Sri Lanka, conservation.

Introduction

There are eighteen species of agamid lizards in Sri Lanka, fifteen of them are endemic to the island (Bahir and Surasinghe, 2005; Manamendra-Arachchi et al., 2006; Samarawickrama et al., 2006). Seven species belong to the genus *Calotes*. Five of them (*C. ceylonensis* Muller, 1887; *C. liocephalus* Gunther, 1872; *C. liolepis* Boulenger, 1885; *C. nigrilabris* Peters, 1860; *C. desilvai* Bahir and Maduwage, 2005) are endemic. The remaining two *Calotes* (*C. calotes* [Linnaeus, 1758); *C. versicolor* (Daudin, 1802]) are probably widespread species throughout South East Asia. According to the published literature, *Calotes liocephalus* is a largely arboreal species found only in parts of the Knuckles Forest Region in Sri Lanka (Manamendra-Arachchi and Liyanage, 1994). Its conservation status is Rare and Endangered (Bahir and Surasinghe, 2005). It can be distinguished from its congeners by the presence of an oblique fold in front of the shoulder, a lower jaw that is rather short, a head without spines (or rarely a rudimentary spine above the ear), enlarged supraocular scales and poorly-developed dorsinuchal crests on the head and lower neck (Manamendra-Arachchi, 1990). Adults have a snout to vent length of 91 mm, a head length of 37 mm, a tail length of 261 mm and an axilla to groin length of 43 mm (Deraniyagala, 1953).

Location of observation.- Observations were made approximately 1 km from Matale-Pallegama Road in Pitawala in the Knuckles Forest Region (altitude: 783 m) in Matale District, Central Province. The habitat consisted mainly of disturbed home gardens (Ekanayake and Bambaradeniya, 2001). The ground was covered with small amounts of wet leaf litter and the soil was soft. There was approximately 10% canopy cover and the undergrowth consisted primarily of grasses. Observations of the lizard was made by the unaided eye from 2 m away between the hours of 1420 and 1600 hrs. The animal was not disturbed during observation. All measurements were taken to the nearest 0.1 mm using dial calipers.

Observations

A mature female *Calotes liocephalus* (snout to vent length: 54.0 mm, head length: 19.4 mm, head width: 11.9 mm, tail length: 156.0 mm, axilla to groin length: 26.5 mm) lying on the ground, approximately 50 cm from the road, was observed on 21 June 2006 at about 1420 hr. The temperature was 23.6°C and the humidity 93%. The weather was gloomy and the cloud cover was 8/8.

Digging the nest hole.- First, the lizard lifted the anterior part of its body using its forelimbs. It then looked around for ~10 min. During this period it repeatedly turned its head 180° five times, without moving its body (Fig. 1). The female then began digging into the ground while scraping the soil with its forelimbs, which was thrown backward under its body through its raised hind limbs. This continued for approximately 5 minutes (Fig. 2). After that it stopped digging and looked around for approximately 5 min. while repeatedly turning its head 180° three times, without moving its body (Fig. 3). Again, it continued digging and this time the female dug the hole continuously for approximately 10 min. It stopped and looked around for about 5 min. while turning its head 180° around three times, without moving its body as in Figure 3. After that, it continued to dig the

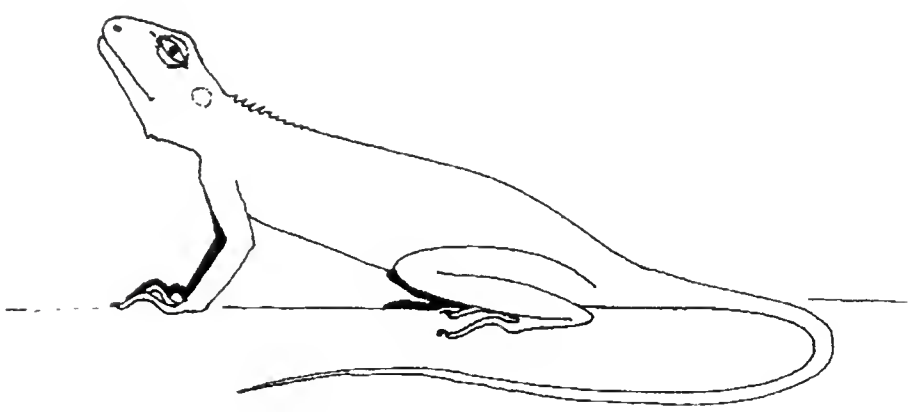
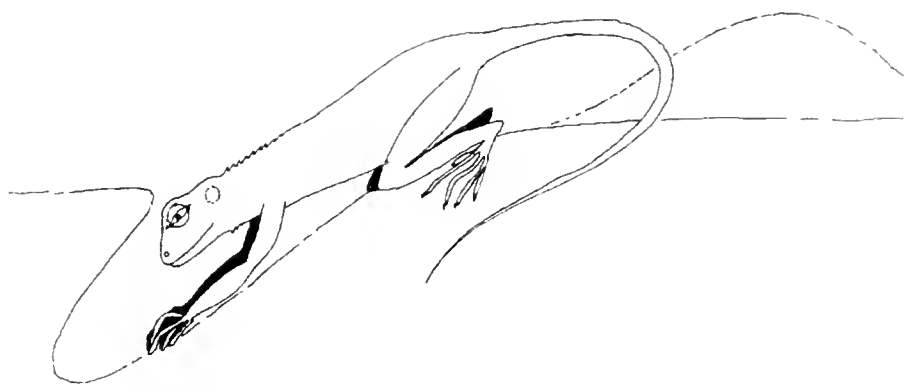


Figure 1.

Figure 2.

Figure 3.



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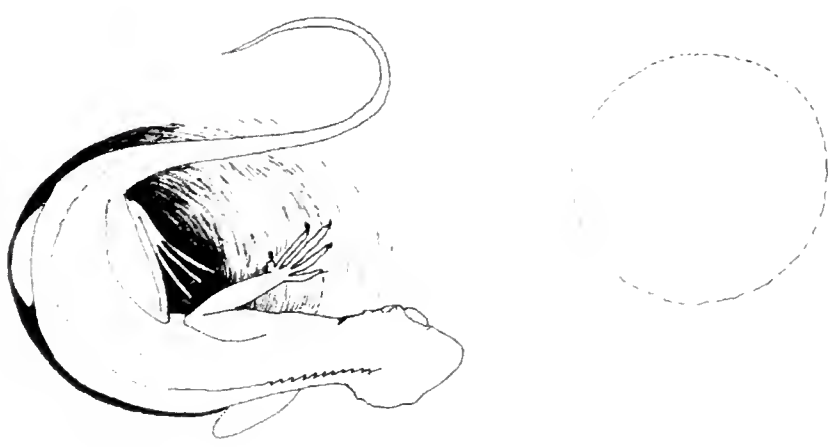

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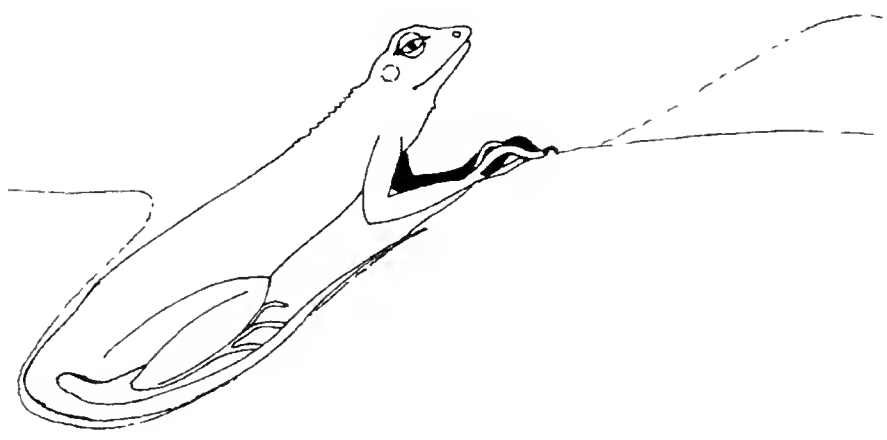

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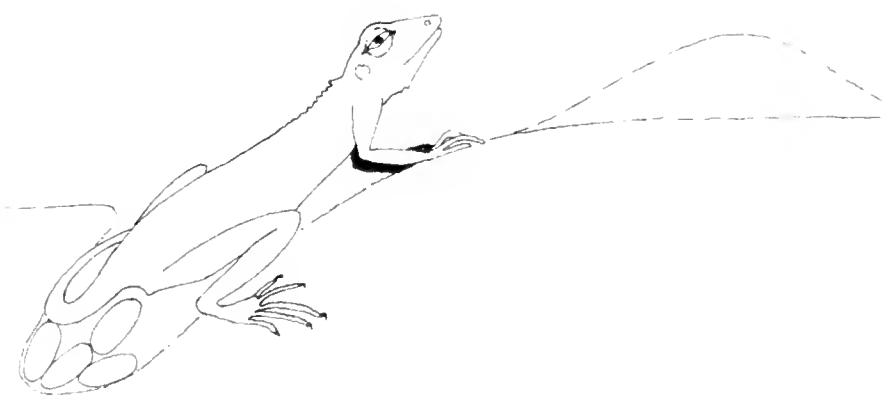

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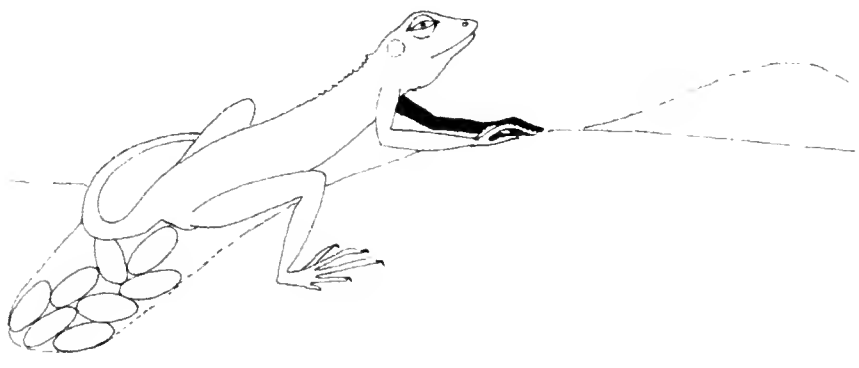

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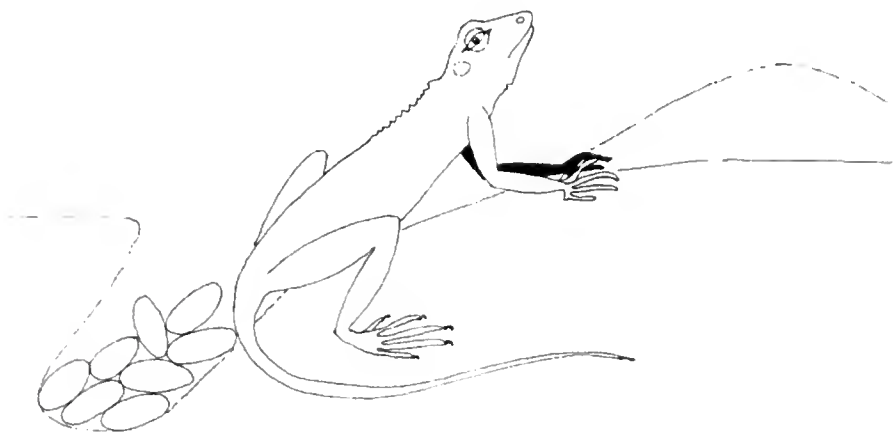
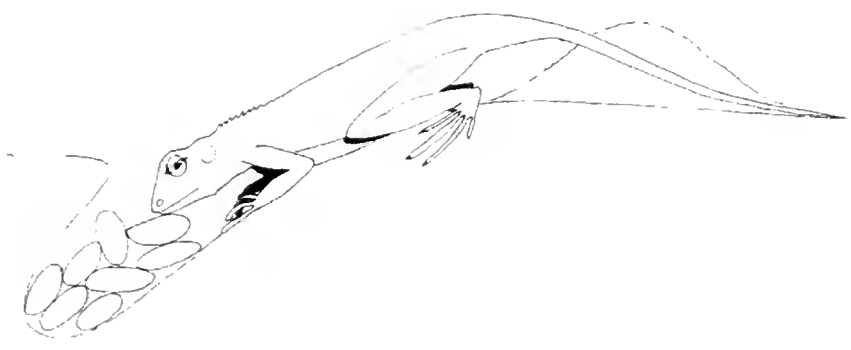


Figure 9a.

Figure 9b.



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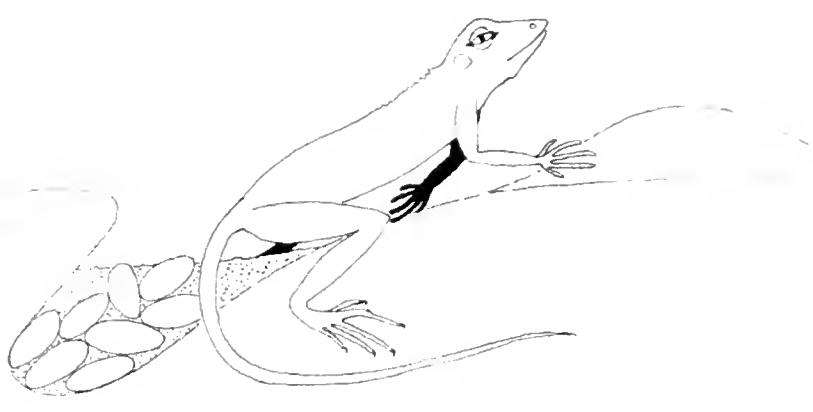

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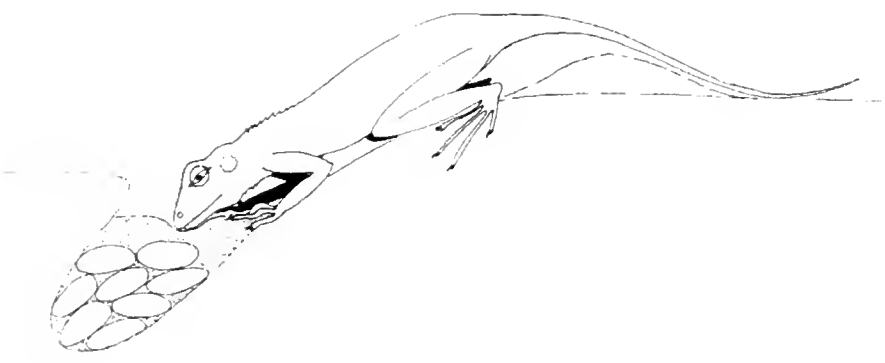

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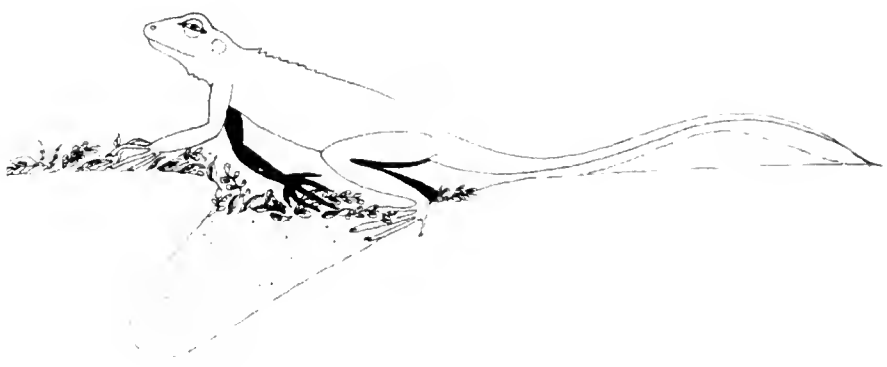

Figure 13.

hole for another half an hour, stopping three more times for 5 min. each, to rest. The hole was dug into the ground at a 45° angle. The final hole was 92.6 mm deep and 79.1 mm in diameter (Fig. 4). During the rest intervals the body was coiled inside the hole with the anterior half bent at an angle of 90° to looking around (Fig. 5). There was a drizzle for ~15 minutes, but the female continued digging.

Laying the eggs. - After half an hour of digging, the female turned its body 180° clockwise, placing the posterior part of its body inside the hole. It then looked around again (Fig. 6). The significance of this egg laying behavior was that the female removed herself slowly from the hole without lifting her limbs while it was laying its eggs (Figs. 7–8). Eight eggs were laid at a rate of one per minute. The eggs were pure white and elliptical, with a mean length of 14.8 mm and a mean width 8.6 mm. After the eggs were laid, the female came out of the hole completely and started looking around (Fig. 9a, b). Then the female crept back into the hole for 15 min. to pack and place the eggs below ground level using the anterior part of its lower jaw (Fig. 10).

Burying the eggs and camouflaging the nest.- After coming out of the hole, the female turned 180° clock-

wise and began to drag the soil towards the hole using its forelimbs. The dragged soil was thrown backwards under its body while it lifted its hind limbs (Fig. 11). After dragging the soil for about 5 min., it turned 180° counter-clockwise and began pressing the soil with the anterior half of its lower jaw for half an hour. The hole was filled up to 18.4 mm below ground level (Fig. 12). After looking around, it dragged the surrounding *Albizia saman* (Family: Fabaceae) leaves over the nest site for camouflage (Fig. 13). It remained motionless for 2 min. and then ran towards the forest, during which time it was caught for measurement and then released.

Discussion

The oviposition behavior of this species varies from the oviposition behavior of *Calotes versicolor*. According to Amarasinghe and Karunarathna (2007), *C. versicolor* places its cloacal aperture over the opening of the hole while laying its eggs, but *C. liocephalus* places the posterior part of the body inside the hole while laying eggs. *C. versicolor* also lifts the anterior part of the body with its forelimbs while turning its head to look around, but *C. liocephalus* coils its entire body inside the hole while bending the anterior part of its body to look around. *C. versicolor* makes a knocking noise while packing and placing the eggs in the hole using its lower jaw while the *C. liocephalus* places them softly without making any noise. After the observation the eggs were removed from the hole and the hole was subsequently examined. The bottom was conical and the soil was soft, dark and wet. Finally the eggs were buried in a home garden to hatch. After approximately two and half months we observed five small holes where the hatchlings had come out. Unfortunately we could not observe the hatchlings.

A Few diagrams, brief descriptions and notes of *Calotes liocephalus* are available in popular journals, books and magazines but almost nothing exists on the pre and post mating behavior, egg laying behavior, captive breeding and their hatchlings. In addition *Calotes liocephalus* is an endemic, rare and threatened species and therefore it may become extinct if their population does not increase. For such a situation to be achieved, captive breeding methods may be needed for ex-situ conservation of this species. In addition further observations are also needed for the conservation of *Calotes liocephalus*.

Acknowledgments

We wish to thank Mr. Kelum Manamendra-Arachchi (WHT – Wildlife Heritage Trust) for reviewing the manuscript and Mrs. Zeenia Nissam of the Department of Zoology, Faculty of Natural Sciences of the Open University, Sri Lanka, for her generous support for the field visit. We also thank Mr. Niranjan Karunarathna and Mr. Gayan Wijeytunga (YZA – The Young Zoologists' Association) for assisting the fieldwork. Finally, Ms. Debbie McCormick and Mr. F. S. Abeywickrama are acknowledged for their help in preparing this paper.

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On the Status of the Chinese Pitviper *Ceratrimeresurus shenlii* Liang and Liu *in* Liang, 2003 (Serpentes, Viperidae), with the Addition of *Protobothrops cornutus* (Smith, 1930) to the Chinese Snake Fauna

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Abstract.- *Ceratrimeresurus shenlii* Liang and Liu *in* Liang, 2003 was described as a new genus and a new species on the basis of the first "horned" specimen of pitviper recorded from the People's Republic of China. This taxon has been overlooked in the literature. The original description is here translated verbatim into English. The holotype is compared with other horned pitvipers known from Asia. On the basis of its scalation and pattern, *Ceratrimeresurus shenlii* is synonymized with *Protobothrops cornutus* (Smith, 1930). The range of this latter species, previously endemic to Vietnam, is expanded northeastwards by approximately 780 airline km. A brief comment on the zoogeography of South China is given.

Keywords.- Serpentes, *Ceratrimeresurus*, *Ceratrimeresurus shenlii, Protobothrops cornutus*, China, Vietnam, taxonomy, zoogeography.

Introduction

A new Chinese genus and new species of Asian pitviper, *Ceratrimeresurus shenlii* Liang and Liu *in* Liang, 2003 has remained overlooked in the literature. *Ceratrimeresurus shenlii* was not included in the latest checklist of the *Trimeresurus* complex (Gumprecht et al., 2004), nor announced in Wolfgang Wüster's invaluable website "Venomous Snakes Systematic Alert" (http://biology.bangor.ac.uk/~bss166/update.htm). Lastly, this taxon was not considered by Malhotra and Thorpe (2004, 2005) in their revision on the pitvipers of the *Trimeresurus* complex.

The description of *Ceratrimeresurus shenlii* Liang and Liu *in* Liang (2003: 411; Plate 8: Fig. 13. Type locality: "Working site 02 at Wuzhishan forest, Ruyuan Xian", Guangdong Province) appeared in a chapter of a book on the natural history of Nanling Nature Reserve, located in Nanling Mountains, in the north of Guangdong Province (Pang, 2003), a fact that may explain that the new taxon remained overlooked by the herpetological community. However, this new species was merely mentioned in September 2004 in the forum of a website dedicated to venomous snakes (http://www.venomdoc.com/forums - last viewed on May 30th, 2005), where this species was regarded, without explanations, as a synonym of *Protobothrops cornutus*. It was also tentatively regarded as a synonym of *Protobothrops cornutus* by Vogel (2006).

Only three other taxa of Asian pitvipers with more or less raised supraoculars were previously known from the mainland: *Trimeresurus wiroti* (see David et al., 2006), *Protobothrops cornutus* and *Triceratolepidophis sieversorum*. In this paper, we provide a translation of the original description. The new taxon is, according to the re-examination of the holotype, compared with the currently known horned species of mainland Asia and its status is discussed.

Materials and Methods

Body and tail lengths were measured to the nearest mm. The number of ventral scales is counted according to Dowling (1951). The terminal scute is not included in the number of subcaudals. The numbers of dorsal scale rows are given at one head length behind head, at mid-body (i.e. at the level of the ventral plate corresponding to a half of the total number of ventrals), and at one head length before vent, respectively. Values for symmetric head characters are given in left/right order.

Abbreviations of measurements and other meristic characters.-

MEASUREMENTS AND RATIOS: HL: head length; SVL: snout-vent length; TaL: tail length; TL: total length; TaL/TL: ratio tail length /total length.

MERISTIC CHARACTERS: DSR: formula of dorsal scale rows; MSR: number of dorsal scale rows at midbody; IL: infralabials; SC: subcaudals; SL: supralabials; VEN: ventrals.

Museum abbreviations.- BMNH: Natural History Museum, London, UK. - FMNH: Field Museum of Natural History, Chicago, USA. - MNHN: Muséum National d'Histoire Naturelle, Paris, France. - ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

Results

***Translation of the original description of* Ceratrimeresurus shenlii**.- The original description appeared in Liang (2003: 411), but is credited to Liang and Liu. It was published in Chinese with a short English summary. A poor quality, small color photograph of the holotype appears on Plate 8, Figure 13 of the book. A verbatim translation of the original description should read as follows (numbers placed in square brackets refer to our comments placed after this translation):

Jiao laotietou[1] *Ceratrimeresurus shenlii* Liang and Liu, gen. and sp. nov. (See colour plate 13[2])

The upper eyelid forms on each side a raised triangle covered with small scales, the base of the triangle being slightly like a three-sided pyramid; the length of the triangular horn is 1.5 times the diameter of the eye, and the height from the tip of the triangle to the edge of the upper eyelid is equal to the eye diameter. The tip of the snout is blunt, the upper snout surface is broad and flattened, with the lateral edge slightly upturned. The length of the snout is about a 1/4 of head length and 1/3 of head width. The nostril is placed near the tip of the snout, slightly directed sideward and almost rounded in shape, the opening being in the middle of a divided nasal scale; there is one upper nasal scale. There are 5 small scales between the two nostrils[3]. The upper head surface is covered with granular scales, which become progressively larger and more keeled from the front to the back of the head. The cephalic surface is slightly convex and becomes progressively flat and broader backwards. There are 7 small interorbital scales[4]. There are 11 small scales surrounding the base of the triangular horn. The tip of the triangular horn is one rather large scale, to which is adjacent a smaller scale which makes the horn looking as bifurcated. The eye is rather large and the third of its volume is out of the head. The iris is a vertically elongated oval. There is a row of small scales around the orbit and many scales between the snout side and temporal region. The forward sunken part of the occiput is roughly equal to the length of the snout, with the end of the neck plunged in it. The neck is slender, its diameter being less than half of the head width. There are 14 supralabial scales[5], among which the first and second ones are separated from the nasal scales by a row of 3 small scales, and the third to fifth ones do not enter the loreal pit[6], but are separated from it by a row of small scales; the edge of the upper jaw is convex downwards; 14 infralabials, the fourth being the largest, the first to the fourth ones in contact with the anterior chin shields, and the posterior chin shields being rather large. One large fang is located on the anterior part of the upper jaw, followed by 2 smaller teeth. No teeth on the palatine and pterygoid[7]. Five small teeth are present on the lower jaw. The loreal pit is placed antero-inferiorly to the eye.

The head is peach-shaped and slightly flattened, with a narrow and elongated neck; the body is rather wide but slender and long, with a long, pointed tail. The dorsal scales are strongly keeled, 23-23-21-17 rows, the rows of cervical scales are more oblique than those of the body scales. There are 187 ventral scales +77 pairs of subcaudals. The anal scale is single. The vertebral scales are normal.

The back of the body is of a grey color, which becomes light grey on the sides. On the head upper surface, there is a 'X' shaped pattern, made of a blotch extending from the nasal scale to the front of the opposite triangular, and of a blackish brown elongated blotch extending from the posterior of the horn through the rearward supraorbital scale (behind the horn) backwards up to the posterior of the head. A white streak extends from the posterior margin of the eye through the temporal up to the posterior of the head, followed by a blackish brown stripe from the corner of the mouth to the sides of the throat. The upper and lower lips are marked with blackish brown square shaped spots. Diffuse and irregular spots cover the rest of the body throughout. The dorsal side of the head and the body is brown in color. Dorsal blotches extend on each side from the middle of the body to the vent, the right and the left rows being offset and these blotches being linked together by their inner corner; they are merged together in the middle of the body and linked each other again by the inner corner on the posterior part of the body, then merged together again from the vent to the end of the tail. Light grey-brown spots on the lower part on the sides of the body, roughly square in shape or almost rounded, oval or

reduced to short crossbars. The venter is pale grayish-brown, without other spot in the middle, so that the ventral side of the body is light grey-brown color throughout. There is a pointed scale on the tip of the tail. The total length of the specimen: 362 + 78 = 440 mm, length of the tail/total length = 0.564, weight = 24 g.

The specimen was collected in the thatch on the top of a house near a forest, coiled with the head in the middle of its body. Not very active. It was knocked down with a little stick on the ground and captured. The locality is the "Working site 02" at Wuzhishan forest, Ruyuan Xian[8], July 1996. The specimen is deposited in the Laboratory of Zoology of the Faculty of Biotechnology, Jinan University[9].

Comments on the original description. - In this original description, no mention of the sex of the specimen is given, although it is a female according to the shape of the base of the tail. Other comments are:

[1]: Translation: "Horned iron-head [snake]". The vernacular name "*Laotietou*" is given in China to *Protobothrops mucrosquamatus*, due to the shape of its head, looking like an ancient iron.

[2]: It should rather appear as Plate 8: fig. 13.

[3]: This value includes the two supranasals plus the scales separating these latter ones.

[4]: If one counts the cephalic scales on a line connecting the middle of the supraoculars, the value is 14 (see below).

[5]: This value is obviously erroneous; we counted 8 SL.

[6]: This is obviously a lapsus for the orbit.

[7]: this lack of teeth on the palatine bones is surprising and may be an artifact. Teeth are very commonly miscounted, as they are masked by the tissues and one usually needs to peel back the gum tissues to count the sockets. All the tooth counts in this description may be unreliable (A. Malhotra, pers. comm, January 2006).

[8]: Ruyuan County, in northern Guangdong Province, close to the Guangdong-Hunan border. The Nanling National Nature Reserve is located in the centre of Nanling Mountains, which extend from northeast Guangxi to southwest Fujian.

[9]: Guangzhou, Guangdong Province.

Redescription of the holotype of **Ceratrimeresurus shenlii** ***(Figs. 1-3).***- The original description includes some imprecision or mistakes. In combining data of the original description and our own data, the morphology is as follows:

Body moderately stout; head subtriangular, wide at its base, clearly distinct from the neck, thick and swollen when seen from the side, depressed between the uplifted areas of the supraoculars. Snout average in relative length, about one quarter of HL, bluntly rounded when seen from above, depressed in its center, obliquely truncated when seen from the side, with a distinct *canthus rostralis*. Eye relatively large. Tail long and tapering. The holotype is now in average condition and somewhat dessicated:

SVL: 362 mm; TaL: 78 mm; TL: 440 mm; ratio TaL/TL: 0.177.

VEN: 187; SC: 77, paired, plus one terminal scale; anal shield entire.

DSR: 23-21-17 scales, rhomboid, distinctly keeled.

Rostral visible from above, broader than high, triangular; nasals subrectangular, divided, with a round nostril opening near the tip of the snout, directed slightly sideward and almost rounded in shape; 2 internasals on each side, separated by 2 small scales; 4/4 canthal scales, slightly larger than adjacent snout scales, bordering the *canthus rostralis*; 2 elongate upper preoculars above the loreal pit; lower preocular forming the lower margin of loreal pit; 3/3 small postoculars; 5/5 supraoculars on an uplifted triangular area, of which the two central supraocular scales are strongly enlarged, triangular and strongly obliquely erected ("horn like") and extending out of the head margin, 1.5 times the diameter of the eye, convergent and originating from the same base covered with small scales; 8 or 9 slightly enlarged scales on upper snout surface on a line between the scales separating the internasals and a line connecting the anterior margins of eyes, smooth, juxtaposed, irregular in shape; 14 cephalic scales on a line between the base of the supraoculars (including the scales covering the uplifted basal area), smooth, flat and juxtaposed; occipital scales larger than cephalic scales, distinctly keeled; temporal scales small, obtusely keeled; 9 SL, 1st SL separated from nasal; 2nd SL bordering the anterior margin of the loreal pit, 3rd SL largest, separated from the subocular by 1 scale; 4th SL much smaller than 3rd SL, separated from the subocular by 1 scale; 5th and posterior SL much smaller; 14 IL, first four pairs in contact with anterior chin shields.

The coloration and pattern agree with that of the original description. The light postocular streak is quite conspicuous, but the overall pattern of the body is rather faded.

Discussion

Nomenclatural considerations.- The combined description of the genus and species was brief and may raise some questions about its validity. Two points need to be discussed. The first one relates to the combined description of the genus *Ceratrimeresurus* and of its sole

Figure 1. *Ceratrimeresurus shenlii*, holotype. General view of body. Photograph by Tian Mingyi.

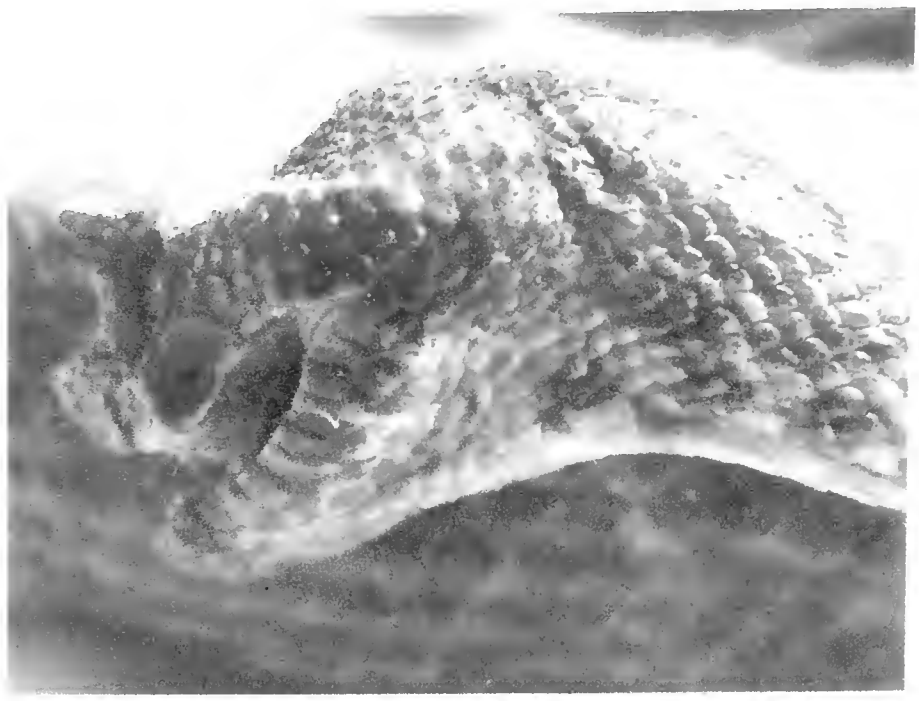

Figure 2. *Ceratrimeresurus shenlii*, holotype. General view of body. Photograph by Tian Mingyi.

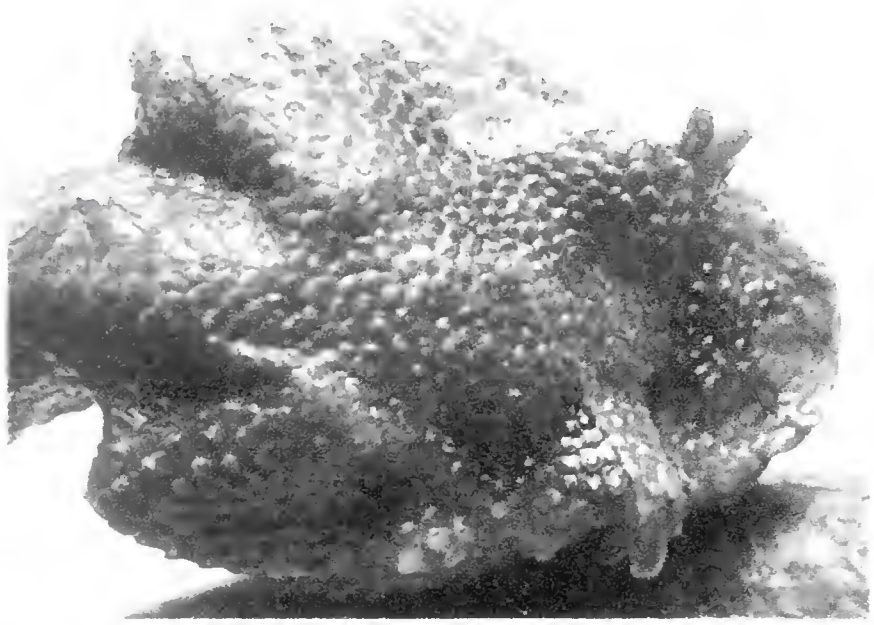

Figure 3. *Ceratrimeresurus shenlii*, holotype. Lateral view of the head. Photograph by Tian Mingyi.

Figure 4. Head of the second Chinese specimen of *Protobothrops cornutus*. Photograph by Fu Jie.

included species, *shenlii*. According to Art. 13.4 of the *International Code of Zoological Nomenclature* (1999), in the present case, such a "description of a new nominal genus and a single included new nominal species is deemed to confer availability on each name under Article 13.1.1." Art. 13.1.1 requires that every new name published be accompanied by a description purported to differentiate the taxon. This combined description is hence considered to be complying with the requirements of the *Code*.

The second point relates to the identification of the holotype. No number was cited in the description, and this specimen was still deposited in Prof. Liang Qishen's private collection in December 2005. Nevertheless, as the name of the collection into which the specimen will be eventually deposited was clearly indicated in the original description, and as it was confirmed to us that the specimen will eventually be deposited in this collection, we consider that this point complies with Art. 16.4.2 of the *Code* and makes valid the description of the specific nomen.

A comparison of* Ceratrimeresurus shenlii *with other horned species.- In the Asian mainland, only *Protobothrops cornutus* and *Triceratolepidophis sieversorum* have erected supraoculars. *Trimeresurus wiroti* has only slightly raised supraoculars (see David et al., 2006). Another species occurring in western Indonesia, *Trimeresurus brongersmai*, has erected horn-like supraoculars. However, this latter species, related to *Trimeresurus wiroti*, differs from the other horned species by several characters, including the shape of its snout (David et al., 2006). On the basis of Ziegler et al. (2001), Herrmann et al. (2002), Herrmann and Ziegler (2002), Herrmann et al. (2004) and of specimens examined by ourselves, a comparison between horned species and *Ceratrimeresurus shenlii* is given in Table 1.

Triceratolepidophis sieversorum differs by its greater number of ventral scales and a different structure of horns, free and strongly divergent. This species also

Figure 5. Distribution map of of *Protobothrops cornutus*.

shows a peculiar structure of keels of dorsal scales, which is not found in other horned pitvipers (Ziegler et al., 2001). The dorsal keels of *Ceratrimeresurus shenlii* are narrow and made of a single ridge.

In contrast, *Ceratrimeresurus shenlii* cannot be distinguished from *Protobothrops cornutus* otherwise than by minor characters. In both species, the horns stem from the same base and are free only in their outermost part. All scalation characters are similar, including the keeling of the dorsal scales (see Table 1). Other characters include the keeled occipital scales, the large 3rd SL separated from the subocular by 1 scale row, 4th and 5th separated from the subocular by 1 or 2 scales, the number of internasals and of supraoculars. The sole difference bears on the numbers of pairs of infralabials in contact with the anterior chin shield, first 3 pairs in *P. cornutus* vs. first 4 pairs in *Ceratrimeresurus shenlii*. The pattern is also similar, especially the dorsal blotches, the upper head dark pattern and the postocular streak.

On the basis of the similarities in morphological characters, we synonymize *Ceratrimeresurus shenlii* Liang and Liu *in* Liang (2003) with *Trimeresurus cornutus* Smith, 1930, now *Protobothrops cornutus* (see Herrmann et al. [2004] for the generic position of this species).

At the generic level, the point to be now discussed is if the horned species *Trimeresurus cornutus* Smith, 1930 should be referred to a genus distinct from *Protobothrops*, in which all other included species are hornless. In this case, the generic *nomen Ceratrimeresurus* is available. Pending molecular analyses that should clarify the relationships between the Chinese and the Vietnamese populations, we have to rely only on morphology. Only two currently recognized genera of mainland Asia include species with truly erected supraoculars ("horns"), namely *Protobothrops* (only for *P. cornutus*) and *Triceratolepidophis* (*T. sieversorum*). On the basis of the similarities between *P. cornutus* and *C. shenlii*, we adopt a conservative approach in considering that erected supraoculars have been convergently evolved in several lineages. Consequently, we synonymize the genus *Ceratrimeresurus* Liang and Liu *in* Liang (2003) with *Protobothrops* Hoge and Romano Hoge, 1983.

A second Chinese specimen of *Protobothrops cornutus* has appeared on Internet in summer 2005 (Fig. 4), from Shimentai Nature Reserve (24° 22′-24° 31′ N, 113° 05′-113° 31′ E), Nanling Mountains, Yingde County, Guangdong Province (see Jim and Xu [2002] for a description of the area). The characters visible on this picture of a freshly killed specimen are identical with

Table 1. A comparison between known specimens of Asian horned pitvipers.

Taxon	Sex	Tal/TL	MSR	Ven	Sc	Cep	SL	IL	Sup/Oc	SupOc
"*C. shenlii*"	F	0.177	21	187	77	14	9/?	14/14	5/5	convergent
P. cornutus (1)	M	0.203	21	189	78	16	9/9	14/14	6/6	convergent
P. cornutus (2)	F	0.184	21	192	71	13	9/9	13/14	6/6	convergent
P. cornutus (3)	F	0.182	21	193	72	13	9/9	12/13	7/7	convergent
T. sieversorum (4)	M	0.167	23	228	82	15	8/9	13/14	2/2	divergent
T. sieversorum (5)	F	0.157	22	235	79	15	8/9	13/14	2/2	divergent
T. sieversorum (6)	?	—	21	—	—	16	10/10	13/14	—	divergent

List of cited specimens. *Protobothrops cornutus*. (1) ZFMK 75067, Phong Nha-Ke Bang National Park, Quang Binh Province, Vietnam (not seen; after Herrmann et al., 2004); (2) BMNH 1946.1.19.25, "Fan-si-pan Mts., Tonking", now Mt. Phang Si Pang, Lai Châu Province, Vietnam; (3) MNHN 1937.35, "Tonkin", northern Vietnam. - *Triceratolepidophis sieversorum*. (4) ZFMK 71262 (holotype), Phong Nha village, Phong Nha-Ke Bang Nature Reserve, Quang Binh Province, Vietnam; (5) ZFMK 75066, Phong Nha-Ke Bang Nature Reserve, Quang Binh Province, Vietnam; (6) FMNH 255258, Hin Nammo NBCA, Boualapha District, Khammouan Province, Laos (specimens (5) and (6) not seen; after Herrmann et al., 2002).

those of *Ceratrimeresurus shenlii*. The pattern is much similar to that of *Protobothrops cornutus* depicted in Herrmann et al. (2004). According to the data kindly communicated to us by Mr. Fu Jie, the author of the photograph, this second specimen was seen in May 2005. It was held in alcohol in the home of a member of the Yao minority, to be most likely used as a medicinal beverage. The locality of this second specimen lies in a hilly area at approximately 75 airline km southeastwards from the type locality of *Ceratrimeresurus shenlii* in Nanling Nature Reserve. According to the Yao owner of this specimen, this snake was caught by himself while he was acting as a guide to a scientific survey of the local herpetological fauna. Several specimens were collected, but all died in captivity within some days. This species is there considered very rare.

Conclusions

The occurrence of *Protobothrops cornutus* in China makes a considerable northeastward range extension for this latter species, previously considered endemic to Vietnam (Nguyên et al., 2005) (see map on Fig. 5). Ziegler et al. (2006) recorded a specimen of *Protobothrops cornutus* from Ha Giang Province, in extreme northern Vietnam, close to the border with Yunnan and Guangxi Zhuang Autonomous Provinces. The previously northernmost known locality, Mt. Phang Si Pang (formerly Mt. Fan Si Pan) and Nanling Mountains are separated by about 980 airline kilometers. Chinese specimens are separated from the new locality cited in Ziegler et al. (2006) by about 760 airline km.

We also consider that the wide gap between the Chinese population and its Vietnamese relatives may most likely reflect a lack of appropriate collecting effort in elevated areas of Guangxi Zhuang Autonomous and Guangdong Provinces than a real geographical gap. *Protobothrops cornutus* should be searched for in forests of mountain or hill ranges such as Daming Shan and Dayao Shan (Guangxi Zhuang Autonomous Province) and in various hills of northern Guangdong located between the Nanling Mountains and the mountain ranges of Guangxi Autonomous Region. The herpetofauna of this latter province is still quite poorly known. Besides the provinces of Guangxi and Guangdong, this species should be searched for in forested areas of southern Yunnan (see Herrmann et al., 2004). Herrmann et al. (2004) also showed that, in Central Vietnam, *P. cornutus* also occurs in the lowlands. However, lowland areas of southern China are quite dry (Anonymous, 1998). These lowlands separate the hill or mountain ranges of southern China which share a subtropical humid climate with high annual amounts of rainfall (above 2000 mm), a situation which leads to the isolation of the populations of *P. cornutus* in this region.

A discussion on the zoogeographical affinities between North Vietnam and various regions of South China are outside the scope of this paper, but a mere comparison between the snake faunas of northern Vietnam and the hills of Guangxi and Guangdong suggests strong similarities. The occurrence of *Protobothrops cornutus* in northern Guangdong, as well as of *Shinisaurus crocodilurus* and *Amphiesma bitaeniatum* in northern Vietnam (David et al., 2005; respectively Le and Ziegler, 2003) reinforces the zoogeographical relationships of these areas connected by more or less contiguous hill or mountain ranges receiving high annual amounts of rainfall.

Acknowledgments

We are indebted to Q. Liang (Guangzhou, China), who was instrumental in letting TM re-examine the holotype of *Ceratrimeresurus shenlii*. We are grateful to A. Malhotra (Bangor, United Kingdom), M. W. Lau (Hong Kong, China) and T. Ziegler (Köln, Germany) for their comments on the draft of this paper; we especially thank T. Ziegler who made available and permitted us to cite his paper in press. Lastly, we thank J. Fu (Guangzhou, China) and N. Wu (Beijing, China) for their kind permission to use in our paper the picture of the second Chinese specimen of *Protobothrops cornutus*.

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The Effects of Incubation Temperature On Hatching Success, Embryonic Use of Energy and Hatchling Morphology in the Stripe-tailed Ratsnake *Elaphe taeniura*

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Abstract.- We incubated eggs of *Elaphe taeniura* at 22, 24, 27, 30 and 32°C to examine the effects of incubation temperature on hatching success, embryonic use of energy and hatchling morphology. Incubation temperature affected incubation length and most hatchling traits examined in this study. Incubation length increased nonlinearly as temperature decreased, with the mean incubation length being 101.7 d at 22°C, 86.0 d at 24°C, 66.3 d at 27°C, 53.9 d at 30°C, and 50.5 d at 32°C. Hatching successes were lower at the two extreme temperatures (41.2% at 32°C, and 50.0% at 22°C) than at the other three moderate temperatures (78.1–79.3%). Hatchlings from the extreme high incubation temperature (32°C) were smaller in body size and wet body mass. High incubation temperatures resulted in production of less developed hatchlings that characteristically had less developed carcasses but contained more unutilized yolks. The proportion of energy transferred from the egg contents to the hatchling was 71.1% at 22°C, 80.2% at 24°C, 81.5% at 27°C, 82.6% at 30°C and 83.9% at 32°C. Taking the lowest hatching success at 32°C and the substantially prolonged incubation lengths at 22°C into account, we conclude that these temperatures are not suitable for embryonic development in *E. taeniura*. Our data confirm the prediction that there are some thresholds over which incubation temperatures can affect hatching success, embryonic use of energy and hatchling morphology.

Keywords.- Reptilia, Colubridae, *Elaphe taeniura*, egg, incubation, temperature, hatchling phenotype.

Introduction

As in other vertebrate and invertebrate taxa, temperature may profoundly influence embryonic development in reptiles. Compared to avian embryos, reptilian embryos can develop under a relatively wide range of temperatures (Birchard, 2004, Booth, 2004). Low temperatures slow embryogenesis but usually have little lethal effect on embryos; high temperatures result in faster embryonic development (and thus, shortened incubation or gestation length) but often increase embryonic abnormality or mortality (e.g. Andrews and Rose, 1994; Andrews et al., 1997; Deeming and Ferguson, 1991; Sexton and Marion, 1974; Shine and Harlow, 1996). Apart from the effects on the rate of embryonic development and embryonic abnormality or mortality, thermal environments experienced by developing embryos also affect a number of phenotypic attributes of the hatchling, including morphology (Du and Ji, 2002; Ji and Du, 2001a, b; Overall, 1994), energy reserves (Du and Ji, 2001), behavior (Burger, 1991; 1998), post-hatching growth (Braña and Ji, 2000; Du and Ji, 2003; Rhen and Lang, 1995), and gender in species with temperature-dependent sex determination (Janzen and Paukstis, 1991). It has been repeatedly reported for oviparous reptiles that eggs incubated at optimal temperatures not only exhibit high hatching success but also produce good-quality hatchlings.

The range of optimal temperatures for reptilian embryos is often narrow and varies not only among but also within species. For example, the optimal incubation temperatures fall within the range from 24°C to 26°C in *Xenochrophis piscator* (checkered keelback; Ji et al., 2001) and *Deinagkistrodon acutus* (five-paced pit-viper; Lin et al., 2005) but, in *Elaphe carinata* (king ratsnake; Ji and Du, 2001b), *Naja atra* (Chinese cobra; Ji and Du, 2001a), *Rhabdophis tigrinus lateralis* (red-necked keelback; Chen and Ji, 2002), *Dinodon rufozonatum* (red-banded wolf snake; Ji et al., 1999b; Zhang and Ji, 2002), *Ptyas korros* (gray ratsnake; Du and Ji, 2002) and *P. mucosus* (mucous ratsnake; Lin and Ji, 2004), generally within the range from 26°C to 30°C. *Pelodiscus sinensis* (Chinese soft-shelled turtle), however, has a wider range of optimal incubation temperatures, because temperatures exert no important effects on hatching success and hatchling phenotypes within the range from 24°C to 34°C (Du and Ji, 2003; Ji et al., 2003). In *Eumeces chinensis* (Chinese skink), eggs from a lower latitudinal population have a narrower range of optimal incubation

temperatures than do those from a higher latitudinal population, primarily because of more stable thermal environments in the former population (Ji et al., 2002). Overall, previous studies suggest that optimal temperatures for developing embryos differ among reptiles differing in habitat use and/or distributional range.

The stripe-tailed ratsnake *Elaphe taeniura* is a large sized (to 1800 mm SVL [snout-vent length]) oviparous colubrid snake that ranges from the central and southern provinces of China to Korea, Burma, Laos, Vietnam and India (Zhao, 1993). Wild population of this snake have declined dramatically due to habitat loss and over-harvesting over the past two decades (Zhao, 1998). Fecundity, reproductive output and embryonic mobilization of energy and material during incubation have been reported for snakes from Zhoushan Islands (Ji et al., 1999a; 2000). Because eggs were never incubated at multiple temperatures, the range of incubation temperatures optimal for developing embryos of *E. taeniura* remains unknown. To fill this gap, we incubated eggs produced by females from a southern population (Guangxi, China) at five constant temperatures ranging from 22°C to 32°C. Specifically, our aims are to (1) examine the effects of incubation temperatures on hatching success, embryonic use of energy and hatchling morphology, and (2) determine the range of optimal temperatures for embryos of *E. taeniura.*

Materials and Methods

We obtained 13 gravid *E. taeniura* (SVL: 1220–1690 mm; postoviposition body mass: 277.9–755.0 g) in June 1998 from a private hatchery in Guilin (Guangxi, southern China), and brought them to our laboratory in Hangzhou, where they were maintained in a 2000 x 800 x 800 (length x width x height) mm wire cage placed in a room inside which air temperatures were never higher than 30°C. Food (eggs of *Coturnix coturnix*) and water were provided *ad libitum.* The snakes laid eggs between 23 June and 3 July (clutch size: 8.8±0.4, range: 7–11). We collected the eggs within a few hours after being laid. Each egg was measured (to the nearest 0.01 mm) for length and width with a Mitutoyo digital caliper and weighed (to the nearest 1 mg) on a Mettler balance. One freshly laid egg from each clutch was dissected to determine the composition of eggs. Egg contents (yolk plus embryo) were placed in pre-weighed glass dishes, and weighed. Shells were briefly rinsed, dried by blotting with paper towels and weighed. Egg contents and shells were weighed again after oven drying to constant mass at 65°C. The remaining eggs were incubated systematically at five constant temperatures (22, 24, 27, 30, and 32 [± 0.3]°C); such that eggs from single clutches were distributed almost equally among the five temperature treatments.

Eggs were individually incubated in covered plastic jars containing known amounts of vermiculite and distilled water at approximately -12 kPa water potential (vermiculite: water = 1:2). One-third of the egg was buried lengthwise in the incubating substrate, with the surface near the embryo exposed to air inside the jar. Jars were equally assigned to five incubators (Guanzhou medical instrument, China), with incubation temperatures set at 22, 24, 27, 30, and 32 (± 0.3)°C, respectively. We moved jars among the shelves in the incubator daily according to a predetermined schedule to minimize any effects of thermal gradients inside the incubator. Jars were weighed on alternate days, and distilled water was added evenly into substrates when necessary to compensate for evaporative losses and water absorbed by eggs, thereby maintaining the substrate water potential constant.

The duration of incubation, measured as the number of days to pipping, was recorded for each egg. Hatchlings were collected, measured (for SVL and tail length), and weighed a few hours after hatching, and then euthanized by freezing to -15°C for determination of composition and sex. The killed hatchlings were separated into carcass, residual yolk and fat bodies. The

Table 1. The effect of temperature on incubation duration, hatching success, and sex ratio in *Elaphe taeniura*. Data on incubation duration are expressed as mean±1 SE.

Temperature (°C)	Duration of incubation (d)	Hatching success (%)	Sex ratio (♀♀/♂♂)
22	101.7±1.3	50.0 (6/12)	2/4
24	86.0±0.6	78.1 (25/32)	12/13
27	66.3±0.7	79.2 (19/24)	13/6
30	53.9±0.4	79.3 (23/29)	11/12
32	50.5±0.5	41.2 (7/17)	2/5

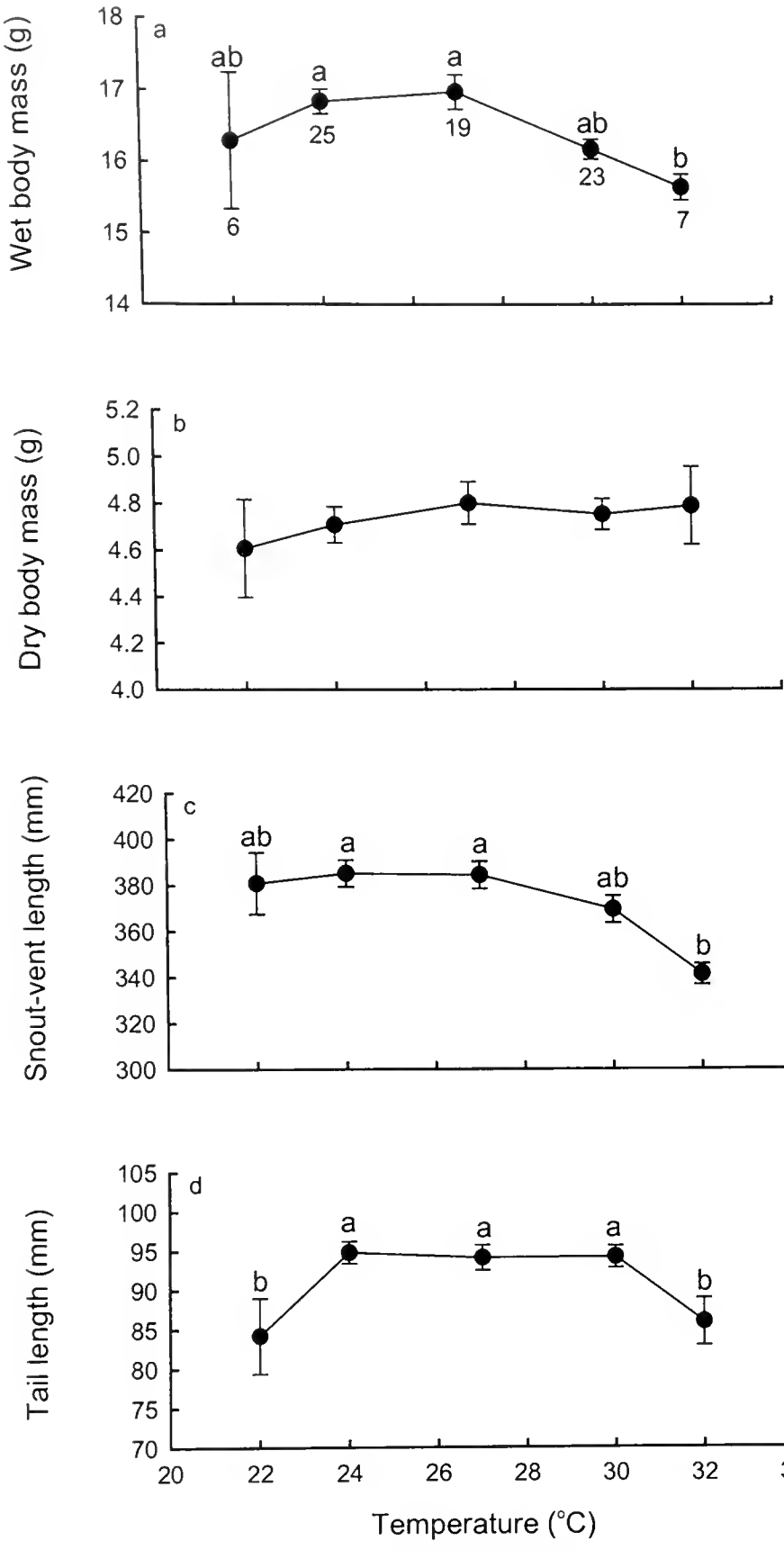

Figure 1. Adjusted means (±1 standard error) for body mass, SVL and tail length of hatchlings from different incubation temperatures, with initial egg mass being set at 23.5 g. Adjusted means with different letters differ significantly. Numbers under the error bars in the upper plot are sample sizes, and are applicable to the other two plots.

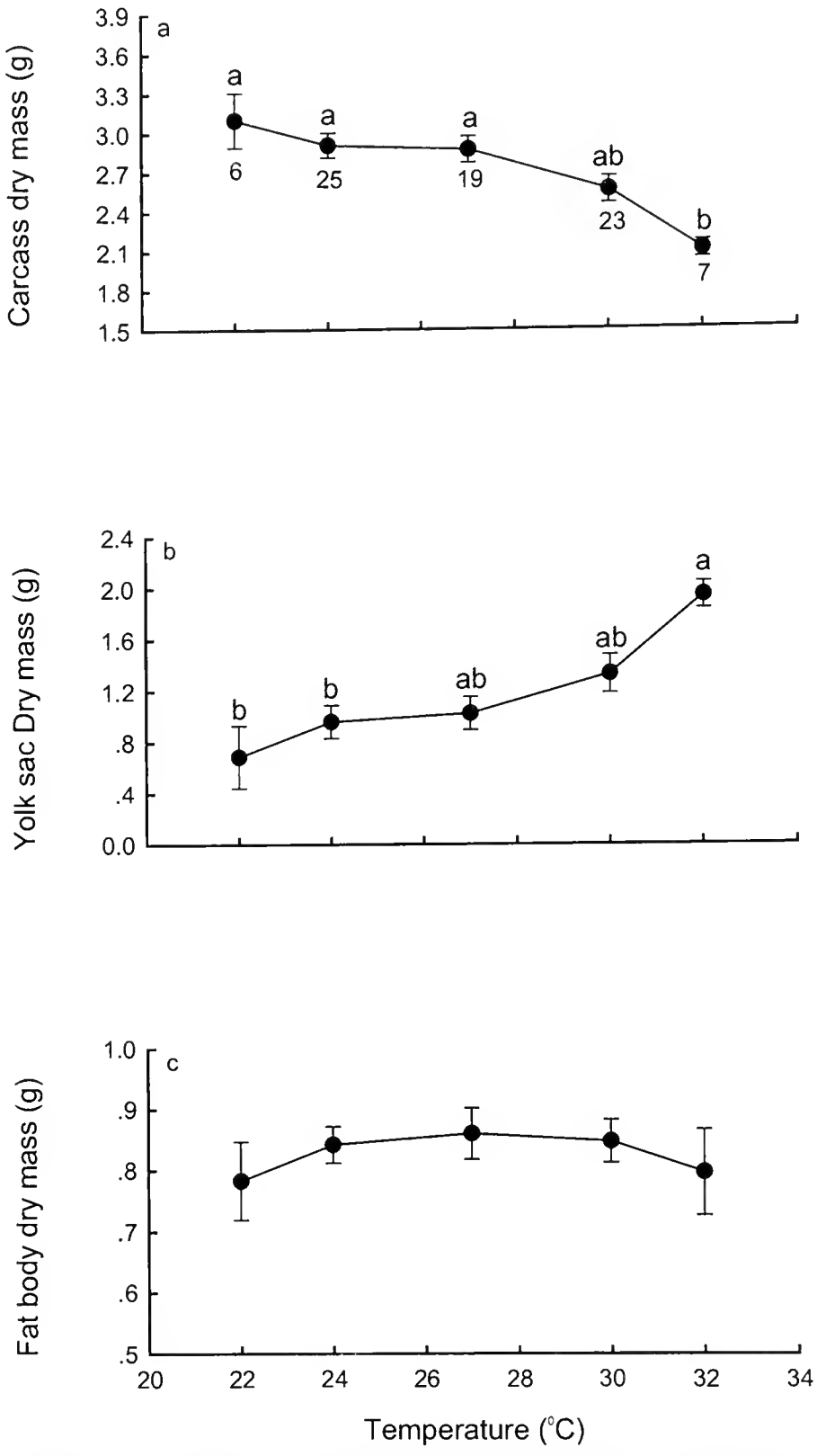

Figure 2. Carcass, yolk sac and fat bodies of hatchling *Elaphe taeniura* incubated at different temperatures. Graphs show adjusted mean (± standard error), with initial egg mass as the covariate. Adjusted means with different letters above the error bars differ significantly. Numbers under the error bars in the upper graph are sample sizes, and apply to all graphs within this figure.

three components of the hatchling were dried in an oven (65°C) to constant mass, weighed and preserved frozen for later analyses. We determined the sex of hatchlings by pressing on both sides of the ventral tail base with forceps to record the presence or absence of hemipenes; hatchlings with everted hemipenes were recorded as males.

We extracted non-polar lipids from dried samples of egg contents and hatchlings in a Soxhlet apparatus for a minimum of 5.5 h using absolute ether as solvent. The amount of lipids in each sample was determined by subtracting the lipid-free dry mass from the total sample dry mass. We determined energy density of each dried sample using a GR-3500 adiabatic bomb calorimeter (Changsha Instruments, China).

All data were tested for normality using Kolmogorov-Smirnov test and for homogeneity of variance using Bartlett's test. Parametric analyses were used to analyze data when the assumptions for these analyses were met; otherwise, nonparametric analyses were used. Values are presented as mean ± 1 standard error, and the significance level is set at $\alpha = 0.05$.

Table 2. Lipids and energy in egg contents and shell dry mass of hatched eggs and freshly laid eggs in *Elaphe taeniura*. Data are expressed as adjusted mean ±1 SE, with initial egg mass as the covariate.

	Egg contents (n = 11)	Hatchlings				
		22°C (n = 6)	24°C (n = 25)	27°C (n = 19)	30°C (n = 23)	32°C (n = 7)
Lipid mass (g)	1.956±0.079	1.082±0.047	1.269±0.039	1.316±0.048	1.362±0.046	1.387±0.101
Energy (kJ)	143.9±4.3	102.3±3.9	115.4±2.5	117.3±3.1	118.9±2.6	120.8±4.9
Shell dry mass (g)	1.555±0.031	1.401±0.075	1.411±0.011	1.408±0.029	1.380±0.029	1.397±0.030

Results

The mean values for incubation length varied considerably among the five temperature treatments (ANOVA; $F_{4,75} = 687.2$, $p < 0.0001$). Incubation length decreased as incubation temperature increased, but not in a linear pattern. The mean incubation length was shortened by 3.5 d from 30°C to 32°C, 12.4 d from 27°C to 30°C, 19.7 d from 24°C to 27°C, and 15.7 d from 22°C to 24°C (Table 1). Incubation temperature affected hatching success (G-test, $G = 11.51$, $df = 4$, $p < 0.05$), but not the sexual phenotype of hatchlings ($G = 4.73$, $df = 4$, $p > 0.25$). Hatching successes were apparently lower at the two extreme temperatures (22°C and 32°C) than at the other three moderate temperatures (24, 27, and 30°C) (Table 1).

There were no between-sex differences in all examined hatchling variables (all $p > 0.05$), so we pooled data for both sexes. All examined hatchling variables, except yolk sac, were positively correlated with initial egg mass). ANCOVAs with initial egg mass as the covariate showed that incubation temperatures significantly affected wet body mass ($F_{4,74} = 3.67$, $p < 0.01$; Fig. 1a), SVL ($F_{4,74} = 4.21$, $p < 0.01$; Fig. 1c) and tail length ($F_{4,74} = 4.31$, $p < 0.01$; Fig. 1d) of hatchlings, but not hatchling dry body mass ($F_{4,74} = 0.38$, $p > 0.05$; Fig. 1b). Mean values for wet body mass, SVL and tail length were all smaller in hatchlings incubated at 32°C than in hatchlings incubated at the other four temperatures (Fig. 1a, c, d). Hatchlings from different incubation temperatures differed in carcass mass (ANCOVA with the initial egg mass as the covariate: $F_{4,74} = 6.36$, $p < 0.001$; Fig. 2a) and yolk sac dry mass (ANOVA: $F_{4,75} = 4.79$, $p < 0.01$; Fig. 2b), but not fatbody dry mass ($F_{4,74} = 0.37$, $p > 0.05$; Fig. 2c). Hatchlings from 32°C had lighter carcass dry mass but larger residual yolk sac, whereas hatchlings from 22°C had heavier carcass but smaller residual yolk sac (Fig. 2a, b).

Energy contents ($F_{4,74} = 2.78$, $p < 0.05$) and non-polar lipids ($F_{4,74} = 2.80$, $p < 0.05$) differed among hatchlings from different incubation temperatures, with hatchlings incubated at 22°C containing lower quantities of energy and non-polar lipid than did those incubated at other four temperatures (Table 2). Conversion efficiency of energy during incubation at 22°C (71.1%) was thus lower than those at 32, 30, 27, and 24°C (83.9%, 82.6%, 81.5%, and 80.2%). Similarly, conversion efficiency of lipid at 22°C (55.3%) was lower than those at 32, 30, 27, and 24°C (70.9%, 69. %, 67.3% and 64.9 %). Additionally, shells of hatched eggs were significantly lighter than shells of freshly laid eggs ($F_{5,84} = 3.19$, $p < 0.05$; Table 2).

Discussion

As in numerous other reptiles, thermal environments experienced by developing embryos affect hatching success, incubation length, embryonic expenditure of energy, and linear dimensions (SVL and tail length) and body composition of hatchlings in *Elaphe taeniura*. Our results provide support for the previous conclusion that reptilian embryos developing at relatively low or moderate temperatures produce well developed and thus, larger hatchlings (e.g. Deeming and Ferguson, 1991; Du and Ji, 2002; Ji and Du, 2001a, b). The larger hatchling size has an association with the greater carcass dry mass (Chen and Ji, 2002; Du and Ji, 2002; Ji et al., 1997; Ji and Sun, 2000). Data from the current study proved that this conclusion is also true in *E. taeniura* (Fig. 1; Fig. 2). The finding that more yolks remain unutilized at hatching when eggs are incubated at high temperatures has been reported for nearly all reptiles studied to-date (e.g., Beuchat, 1988; Du and Ji, 2002; Ji and Du, 2001a, b; Lin et al., 2005; Phillips et al., 1990; Phillips and Packard, 1994). In *E. carinata* (Ji et al., 1997), *E. taeniura* (Ji et al., 1999a), *D. rufozonatum* (Ji et al., 1999b) and *P. korros* (Ji and Sun, 2000), hatchlings exhibit a substantial increase in size (SVL) during the first post-hatching days due to the transfer of resources in the residual yolk into carcass.

The proportion of lipids transferred from the egg contents into the hatchling was noticeably lower than those of energy. Given that mass-specific energy density is much higher in lipids than in proteins and carbohydrates, this result provides evidence that lipids are the main source of energy for embryonic development.

Hatchling size and mass were primarily determined by the embryonic expenditure of energy during incubation after removing the influence of variation in initial egg mass (Du and Ji, 2001, 2003; Ji and Du, 2001a, b). Given that the total energy invested in an egg is fixed, any increase in embryonic expenditure of energy during incubation may inevitably result in production of small sized or lighter hatchlings. Incubating eggs at low temperatures or high temperatures may increase energy expenditure for embryonic development due to the increased incubation length and/or embryonic metabolism (Booth, 1998; Booth and Astill, 2001; Packard and Packard, 1988). Consequently, eggs incubated at moderate temperatures usually produce larger and heavier hatchlings than did those at low or high temperatures.

A prolonged exposure of eggs of *E. taeniura* to temperatures lower than 24°C or higher than 30°C may have a detrimental effect on embryonic development, as indicated by the fact that hatching success decreases dramatically at temperatures outside this temperature range (Table 1). The mean incubation length 30°C is 53.9 days, approximately 3.4 days longer than that at 32°C, so the ecological disadvantage of the increased incubation length (and thus, decreased growth period prior to the onset of the first winter) due to a decrease in incubation temperature from 32°C to 30°C is less pronounced. Considering that less developed hatchlings are produced at 32°C and that hatching success is low at this temperature, we conclude that the temperature of 32°C is outside the range of optimal temperatures for incubating eggs of *E. taeniura*. Eggs incubated at 24°C exhibit high hatching success and produce well developed hatchlings. However, the majority of hatchlings from eggs incubated at 24°C appear between late September and mid-October, so the growth prior to the onset (late November) of the first winter for these hatchlings is about 1.5–2 months. As incubation length increasingly increases as temperature decreases in *E. taeniura* (Table 1), we expect that the disadvantage of incubating eggs of *E. taeniura* at temperatures lower than 24°C can be very pronounced due to the increasingly prolonged incubation length. For eggs of reptiles incubated under natural conditions, the prolonged incubation length at low temperatures also increases exposure of eggs to the effects of adverse biotic (increased microbial contamination) and abiotic factors(extreme thermal and hydric conditions) in the incubation environment of the eggs, which potentially reduces hatching success. Thus, the temperature of 24°C is suitable but not optimal for incubating eggs of *E. taeniura*. Taking the energy expenditure during incubation, the rate of embryonic development and hatchling phenotypes into account, we consider that temperatures around 27°C are optimal for incubating eggs of *E. taeniura*.

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Behavioral Observations and Descriptions of the Endangered Knobby Newt *Tylototriton wenxianensis* and Their Application in Conservation

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Abstract.- *Tylototriton wenxianensis* is an endangered species at the status of VU. This paper introduces observations and studies including breeding, territorial, communication and antipredatory behaviors. The rules and mechanisms of the behaviors are recorded separately and described in the paper. Relationships between the behaviors and environment are also explained for the purpose of describing how the environment acted on the behaviors and how the behaviors adapted to the environment. The paper ends with conservation plans in connection with behavioral ecology: A) protect the particular habitat and avoid anthropogenic threats, B) artificial construction for natural migration and gene communication and C) artificial breeding and re-introduction into nature.

Keywords.- Observations, *Tylototriton wenxianensis*, behaviors, habitats, conservation.

Introduction

Tylototriton wenxianensis (Caudata: Salamandridae) is peculiar in China and poorly known. It is only found along the boundary between Gansu and Sichuan Province, narrowly distributed in Wenxian, Qingchuan and Pingwu (details see Table 1 and Fig. 1). It was defined as a threatened species with the status of VU (IUCN, 2006).

A representative of the Family Salamandridae, *T. wenxianensis* has rough and toxic skin with seasonal colors. The bilateral warts stay longitudinal and clustered in the same size and the boundaries between them are not clear enough. It is dark all over apart from the red-orange fingers, toes and the venter of the tail (Fei, 1993).

The newt lives in the heavily forested mountains at approximately 940 m. The adult is terricolous and usually wanders about the pool. It stays hidden under wet gravel or small muddy caves covered by fallen leaves in the daytime and appears at night for preying (Gong and Mou, 2006).

Behavioral studies on tailed amphibians have recently been reported. The studies were mainly focused in the following fields: breeding behaviors, including sexual recognition and sexual selection (Dawley, 1984), modes of courtship and mating (Arnold et al., 1972, 1977; Salthe, 1967, 1974), sperm competition (Arnold, 1977; Halliday, 1998; Massey, 1988; Sparreboom, 1995; Verrel et al., 1998), reproductive behavior (Fang, 1984; Harris et al., 2002; Park et al., 2000), parental care (Cramp, 1995; Nussbaum, 1985; Peterson, 2000), evolution of reproduction (Arnold, 1977; Veith, 1998; Verrell and Krenz, 1998,); migratory behavior (Arntzen, 2002; Douglas, 1979; Griffiths, 1996; Serbiolova, 1995; Twitty, 1966); territorial behavior (Mathis, 1991; Mathis et al.,1998; Mathis et al.,2000; Simons et al., 1997); communications (Holliday, 1997; Houck, 1988; Houck and Sever, 1994; Rollmann, 1999; Verrell, 1989,1989a;); antipredatory behavior (Brodie, 1990; Graves and Quinn, 2000; Maerz et al.,2001; Sih et al., 2000, 2003; Storfer and Sih, 1998; Sullivan, 2002; Woody and Mathis, 1997). All the above provide a source of reference for the behavioral study on *T. wenxianensis*.

Methods

Migratory behavior.- We chose the habitat in Qingchuan as the site to observe, where a large population was found (Gong and Mou, 2006). The newts are not active year round except for the breeding season, so their migratory behaviours seem quite obvious and clear. We

Figure 1. Location of the habitats and observation sites.

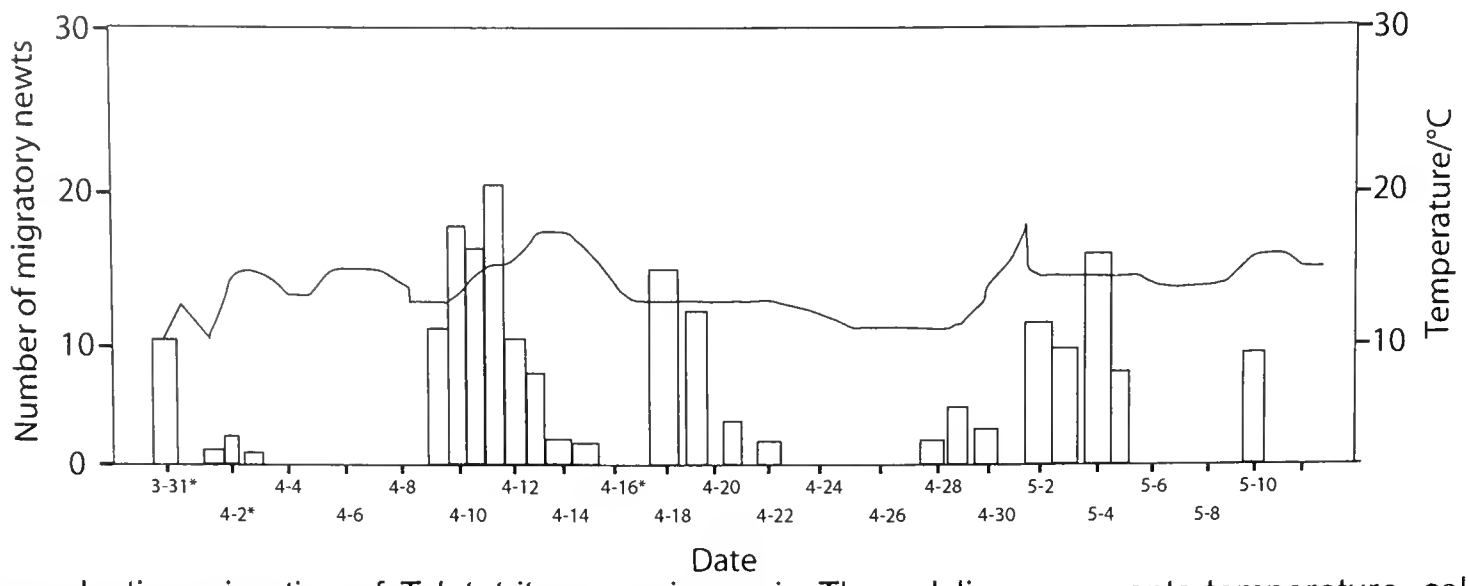

Figure 2. Reproductive migration of *Tylototriton wenxianensis*. The polyline represents temperature, columns represent number of the migratory newts and " * " rainy days.

made continuous observations when they migrated for breeding towards the pools nearby. We marked each migratory group with varisized loops and observed by radio tracking and GPS. Finally we made records of the time, route length, size of groups and sex ratio in the migration.

Reproductive behavior.- Reproductive behaviors included mating and spawning. We observed the course and took down the data of sex ratio, time, envioronment and quantity of the spawns etc.

Commmunication.- Communication happens along with courtship. We tried to understand how the newts communicate with each other and why. The communications included chemical signals and body-contacts (Jiang, 2004). Mechanisms of chemical signals from glands are expected to be understood through dissection. Types of the body-contacts and their effects on courtship were studied.

Territorial behaviors.- We measured the size of the territory occupied by 6 female newts and size of the tails and bodies in contrast. Aggressive behaviors related to the available prey in the environment were also observed. Relationships between the data and phenomenon were discussed and concluded.

Antipredatory behavior.- The newts were less aggressive without structures for aggressivity (Jiang, 2004). They moved too slowly to escape attacks from enemies and to defend themselves. We made some model enemies and demonstrated the predation and antipredatory behaviors artificially.

Results and Analysis

Behaviors.- We made continuous observations on the migratory behaviors and found 186 adults including 170 females and only 16 males, the ratio was 170:16 (♀:♂) = 10.625:1. Breeding lasted from early April to late May with a peak from April 8th to May 6th. The average temperature was 15.5±4°C (Fig. 2).

Like many other newts, *T .wenxianensis* does not seem to have significant secondary sexual characters. It distinguishes and selects the opposite sex primarily by sight and sense of smell. The male seemed more active. It courted the female by wagging its tail and dancing round. Meanwhile, the female was secreting chemicals and emitting a strong smell to attract the male. Soon the male bit the female on the tail (or hind limb), then they circled around both with their tails wagging (Fig. 3). Experiments show that the male prefers to choose a bigger one with more ovums and a stronger smell.

Mating did not accompany spawning in the meantime. It was the female that selected where to spawn. The male moved to the pool and settled down earlier than the female. However, the female left soon after spawning, while the male stayed untill the end of the

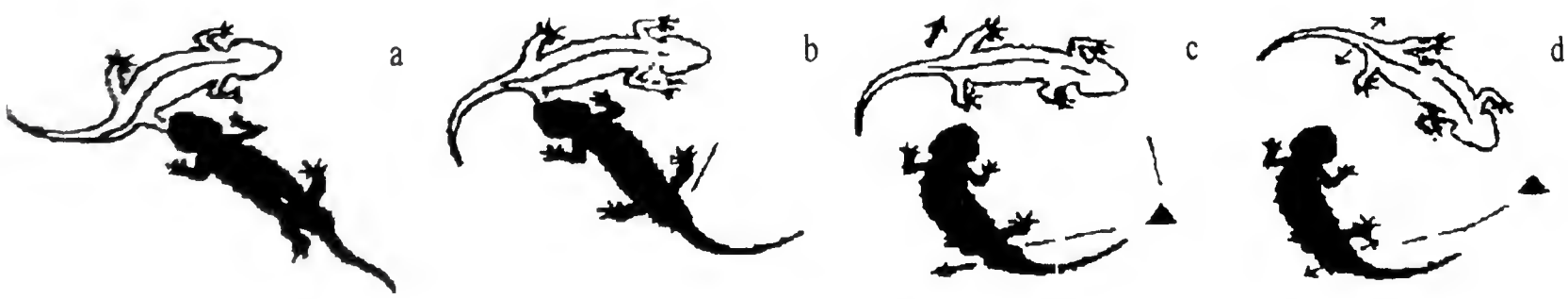

Figure 3. Model of mating behavior. Shows the male (black) wagging its tail and dancing round.

Table 1. Distribution and flora of *T. wenxianensis* along the boundary between Gansu and Sichuan Province.

Province	City	Country (town), county	Village	Latitude and longitude	Altitude (m)	Flora
Gansu	Longnan	Bikou, Wenxian	Bifenggou	103.7°E 33.95°N	927	semitropics
Gansu	Longnan	Liziba, Wenxian	Moziping	104.6°E 33.2°N	940	semitropics-intersemitropics
Gansu	Longnan	Liziba, Wenxian	Hanjialiang	104.4°E 33.1°N	1100	semitropics-intersemitropics
Sichuan	Guangyuan	Daba, Qingchuan	Wuxing	105.1°E 32.5°N	1160	semitropics-intersemitropics
Sichuan	Guangyuan	Daba, Qingchuan	Dawuji	105.1°E 32.46°N	998	intersemitropics
Sichuan	Guangyuan	Daba, Qingchuan	Laowuji	105°E 32.68°N	1092	semitropics-intersemitropics
Sichuan	Guangyuan	Daba, Qingchuan	Baishuling	105.14°E 32.32°N	1210	intersemitropics
Sichuan	Mianyang	Shuitian, Pingwu	Dafenling	104.23°E 32.58°N	1134	semitropics-intersemitropics
Sichuan	Mianyang	Bazi, Pingwu	Yinshanli	104.3°E 32.44°N	962	intersemitropics
Sichuan	Mianyang	Bazi, Pingwu	Miaoshanli	104.34°E 32.45°N	970	intersemitropics

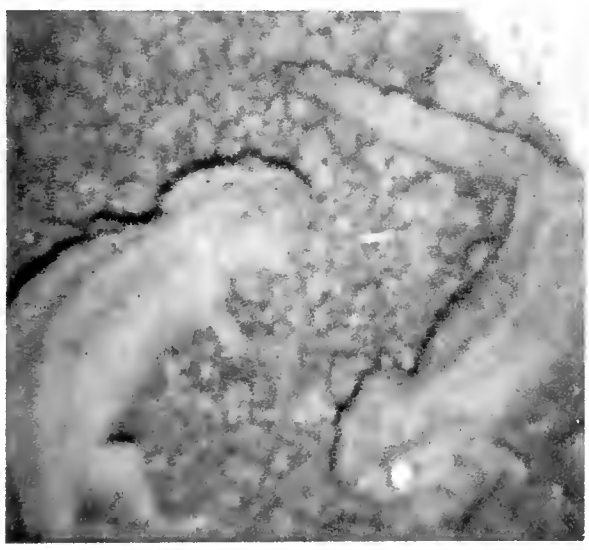

Figure 4. The sperm transmission. Swells show the colloidal secretion.

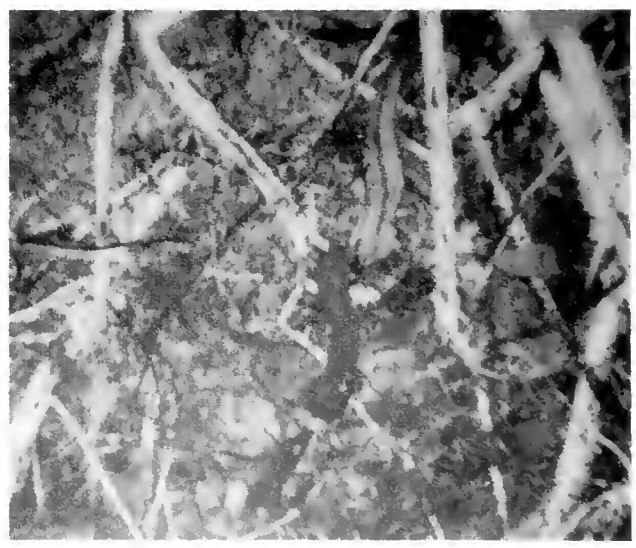

Figure 5. The courtship behavior. Shows the transmission of pheromones in the fantail.

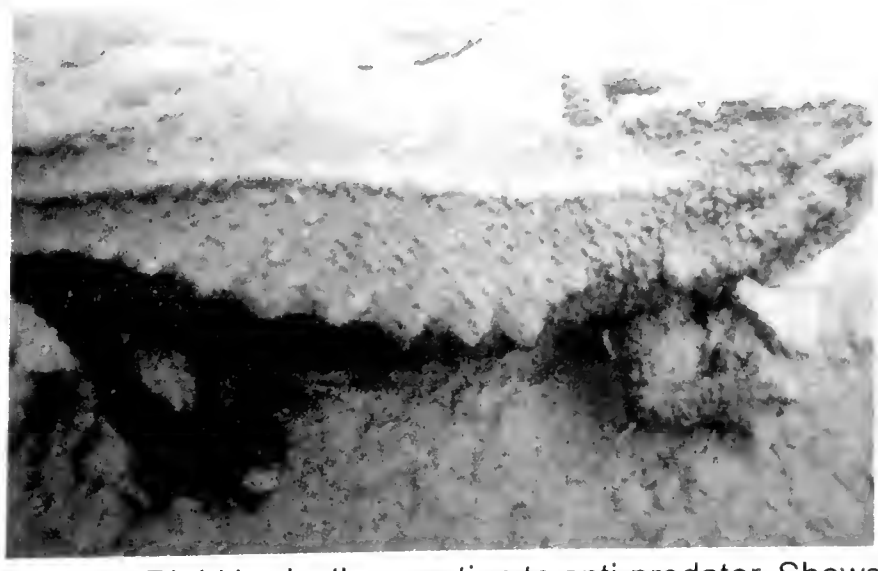

Figure 6. Rigid body: the reaction to anti-predator. Shows ribs jutting through the side warts.

Figure 7. The raised tail and warning color on the ventral surface of the tail.

Figure 8. The protuberant glands and warning color on the back. Shows harmful and toxic skin secretion, glandiform warts, spine glands, caudal glands, glandiform warts, spine glands, caudal glands, tergal glands, etc.

Figure 9. Playing dead. Shows the newts played dead with upward bottom and rigid body.

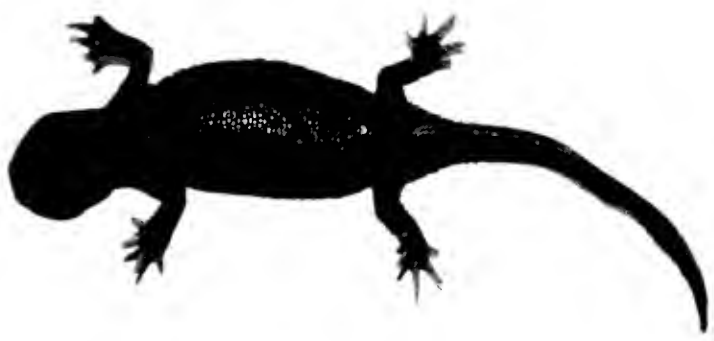

Figure 10. Mimicry: newt playing dead, with venter upward and body rigid.

Figure 11. The breeding pool (after spawning). Vicinity of Wuxing Village, Sichuan Province, southwest China.

Table 2. Relation between spawning territory, food and size of six female newts.

No.	Areas of spawning territory (m^2)	Percent of total (%)	TL (mm)	SVL (mm)	Food environment
1♀	0.64	10.39	58	71	poor
2♀	0.81	13.15	59.2	72.2	poor
3♀	0.55	8.93	55.7	70.1	poor
4♀	1.26	20.45	65.9	73.2	medium
5♀	2.3	37.34	67.4	74.6	high
6♀	0.6	9.74	56.1	71.4	poor

Table 3. The antipredatory behaviors of *T. wenxianensis*, grouped by different types. The observations were made in the field and in artificial conditions. a. harmful skin secretion; b. toxic skin secretion; c. parotids; d. glandiform warts; e. spine glands; f. caudal glands; g. tergal glands; h. tergal colors; i. ventral colors; j. rigid body; k. roll of body; l. exposed abdomen; m. raised tail; n. raised jaw; o. arched body; p. larger head; q. swagging tail; r. curved forehead; s. extended nerve.

Types	Imitative toxicant body						Warning colors		Warning postures									Particular skeleton	
No.	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s

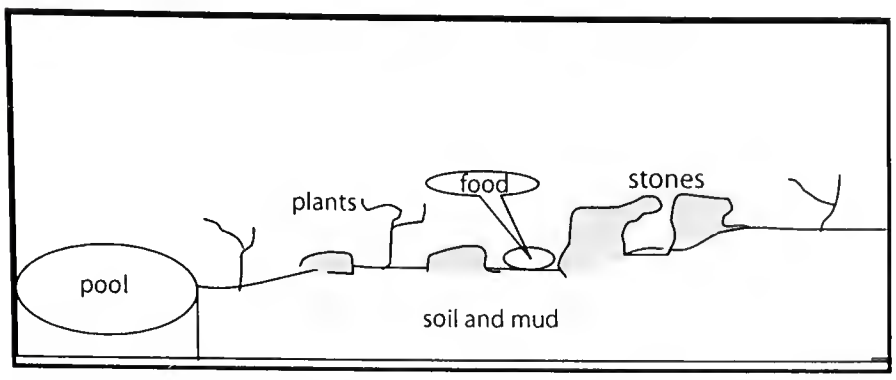

Figure 12. The artificial breeding box made of glass, 110 × 40 × 60 cm (lateral view). Shows the constitution and features of the breeding ecotope under artificial control.

breeding season. The analysis from Douglas (1979): First, to choose the optimum condition for spawning and to reduce risks in breeding migration; Second, to control the male genotype effectively through sexual selection.

Mating behavior happened on land, while spawning underwater. We analyzed the reason: the adult newt mainly lives a terricolous life. Sometimes it approaches the pools or streams for food. The observations on the behaviors in captivity indicate that the adults needed almost no water unless they felt too dry. They had adapted to the climate overland. In addition, in reproductive seasons, inorganic conditions overland near the pools may help them to secrete pheromones and sex hormones which could be significantly reduced underwater.

The newt took no attack on its kin, but became quite different towards non-related individuals. The newt recognized its kin through chemical signals. In the hot summer, the adult admitted the posterity into its territory sometimes and food became insufficient. Occupying territory usually accompanied attacks and fights. That depended on food quantity, size of body and environment (Table 2). The newt with longer tail and body occupied larger territory, closely related to the food quantity.

Communications were drived mainly by chemical signals and body contact. Chemical signals are necessary for the accurate and regular courtship behavior. The newts distinguished the sex and attracted each other by transmission of pheromones (Fig. 4) and secretion of hormones (Fig. 5), which later brought about the mating behavior.

Body contact was also important, particularly in breeding behavior. Tactile signals were transmitted through touch on the mouth, collisions and friction between bodies.

In the long course of evolution, series of antipredatory behaviors had formed against captures, such as escapes, cryptic coloration, rigid body (Fig. 6), aposematic coloration (Fig. 7, Fig. 8), camouflage (Fig. 9), warning postures (Fig. 7), imitative toxicants, particular skeletal adaptations (Fig. 6), chemical defenses and playing dead (Fig. 10) etc. (Table 3).

Relationship between the behaviors and environment.- The female finished spawning in a short time without male involved. Spawning lasted 2–3 days smoothly in rainy seasons without interference. They got to the spawning pools no sooner than they selected the proper sites. Spawning began one day later. The female spawned one a time lasting half a hour, and the whole course 5–6 hours. The course maintained a even pace slowly without a peak.

In Qingchuan, three pools were found with female newts and a mass of spawn. The three conditions for spawning sites were: A) ground covered with plants (the plants may be divided into three layers: the upper consisting of tall sparse laurisilvae, bushes in the middle and wet weeds at the bottom), B) spawning sites with semipermanent pools, and the water soaked out after rain, C) it chose a mesa with loose soil as the spawning site on the hillside, covered by fallen leaves (Fig. 11).

The newt preyed mainly on insects, earthworms and snails. Like other amphibians, it stayed hidden in the day and preyed in the night. The behaviors changed sensitively with the temperature and sunlight.

In summers, plants flourished and numerous insects appeared, when the newt acts frequently. After thunderstorms, earthworms come out from the soil and supply food to the newts. In winter, the newts have to hibernate in response to the lack of food and low temperature.

Conservation plans.-

(1) *Protect the particular habitat and avoid anthropogenic threats.*

•Figure 10 shows the particular breeding habitat. The newts enter the pools and ground nearby only in

Table 4. Relationship between the conditions and hatching rate under artificial control.

No.	Temperature (°C)	Relative humidity (%)	Water pH*	Sunlight period (hour/day)	Incubation period (day)	No. of larvae	No. that hatched out	Hatching rate (%)
1	5~15	30~40	5.2	0.5	32	40	5	12.5
2	10~20	40~50	5.4	2	28	50	11	22
3	15~25	50~60	5.6	3	23	45	28	62.2
4	20~30	60~70	6	1.5	20	52	40	76.9

*Huang (2007) reported that pH 5.2~6.0 was most suitable for the development of larvae.

reproductive seasons, so it is of great significance to protect the similar pools. The pools were more or less 50 cm deep, 10 m^2 in size and at an elevation of 1000 m. Pieces of rocks piled around. Soil and mud around the pool was covered densely by rotted leaves. The vegetation nearby were mainly made up of shrubs and arbors (see Table 1).

Human activities, especially farming and poaching severely threatens breeding. Recommendations about plans to avoid these threats: A) build up fences around the mating sites and breeding pools to prevent the natural enemies and poachers from breaking in, B) reduce farming and grazing by livestock, especially around irrigation water from the pools, C) make (and enforce) laws to punish poachers.

(2) *Artificial construction for natural migration and gene communication.*

•Protect the whole habitat and ensure the natural migratory behavior. The migratory routes are uaually blocked by farmland etc., so it may help keep the proper migration to build up artificial passages across the farmland.

Connect the adjacent pools by building canals as much as possible in order to ensure the communication of gene from different populations, especially from breeding groups. The measure may help avoid inbreeding depression and loss of genetic diversity in a small population and also help with evolution of the species.

(3) *Artificial breeding and re-introduction into nature*

•In view of the high sex ratio (♀:♂ = 10.625:1 on average, n = 186) in the breeding season and the low rate of hatchability (46.54%; n = 1272) in nature, it is quite feasible to increase the hatching rate and reduce the sperm competition between the males in artificial conditions. We directly gathered the females and eugenic males with longer bodies (longer tails and bodies seem dominant in the sex competition apt to survive under natural selection (Jiang, 2004) and higher sperm density, and also keep the sex ratio at 1:1 in a artificial glass box (Fig. 12) to avoid competition and injury as well as to ensure high-quality inheritance.

Studies showed that factors influencing the hatching rate were natural enemies (such as snakes), climate changes and pollution (Tian et al., 1997). It is an effective approach to raising the survival rate and enlarging the population by hatching out the larvae in artificial conditions and re-introducing them into nature. The conditions include temperature, humidity, sunlight period and pH of water etc. (Table 4). Re-introduce the juveniles into nature when they complete metamorphosis and get ready to land.

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Observations on the Influence of Seasonality, Lunar Cycles, and Weather Condition on Freshwater Turtle Activity in Sarawak, East Malaysia (Borneo)

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Abstract.- Freshwater turtles were surveyed at two sites in Sarawak: Loagan Bunut National Park and Balai Ringin. Capture results were tested against environmental factors such as lunar phase, weather and seasonality to examine differences in activity level. Proportionally, soft-shelled turtles were most active during the full moon (29.0%) and the last quarter lunar phase (29.4%). Hard-shelled turtles were active during the full moon 50.0% of the time. Both soft-shelled and hard-shelled turtles were more active during overcast periods (53.0% and 66.0%, respectively). Seasonality did not seem to affect soft-shelled turtle activity, while hard-shelled turtles were active 50.0% of the time during the dry South-west Monsoon from June to September.

Keywords.- Testudines, Malaysia, Borneo, capture success, environmental factors.

Introduction

Southeast Asia has a highly diverse freshwater turtle fauna due to a combination of factors, including the presence of major mountain massifs, some of the largest archipelago systems in the world, large tracts of lowland forests, streams and rivers, high precipitation and tropical climate (Iverson, 1992; Lovich, 1994). Due to their cryptic nature and the presence of intense hunting in the recent past, however, these enigmatic species have been difficult to study. Consequently, there is a paucity of published information regarding the current status and basic ecology of the Southeast Asian fauna, including those populations endemic to Borneo. As part of a larger study on the ecology of *Amyda cartilaginea* (described in Jensen, 2006), the activity of freshwater turtles in Borneo in relation to environmental factors, such as lunar phase, precipitation, and seasonality, were assessed.

Materials and Methods

The primary study area was Loagan Bunut National Park (03° 44'–03° 00' N, 114° 09'–114° 17' E) in northern Sarawak, which is within a three hour drive to the town of Miri. Field work was concentrated at the Park, but two visits were also made to Balai Ringin (01° 03' 00" N, 110° 45' 00" E), a fishing village about two hours driving distance from Kuching. Both sites are located within peat swamp forests (Fig. 1).

Loagan Bunut National Park contains the only freshwater floodplain lake in Sarawak (Sayer, 1991), encompassing 650 ha[2] at its maximum diameter. The lake is completely dry during prolonged droughts. Annually, the lake completely dries between three and six times, typically in February, May, and June.

A variety of techniques were attempted to assess the most effective method for capturing *Amyda cartilaginea*. One method employed was the use of hoop traps according to techniques described by Frazer et al. (1990), Legler (1960), and Vogt (1980). Native hoop traps called 'bubu' were ineffective in trapping turtles. Another local fishing device called a 'selambau' caught a single turtle in Balai Ringin.

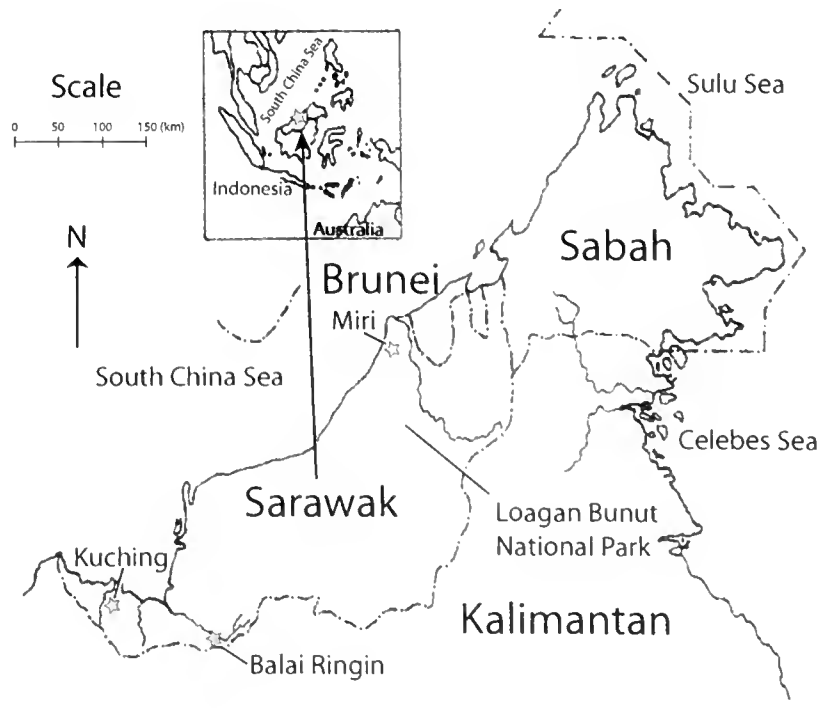

Figure 1. Locations of Balai Ringin and Loagan Bunut National Park in Sarawak, East Malaysia.

Table 1. Total number of individuals of freshwater turtle species caught during the present study. The asterisk refers to an unsexed carcass. Sites include: 1 = Loagan Bunut National Park; 2 = Balai Ringin; 3 = Matang Wildlife Centre; and 4 = Vicinity of Mulu National Park.

	Site 1	Site 2	Site 3	Site 4	Total
Amyda cartilaginea	14	5	0	1	20
Adult males	6	0	0	1	7
Adult females	6	4	0	0	10
Juveniles	2	1	0	0	3
Cuora amboinensis	3	0	0	0	3
Adult males	0	0	0	0	0
Adult females	0	0	0	0	0
Juveniles	3	0	0	0	3
Cyclemys dentata	6 (1)*	1	0	0	7
Adult males	0	0	0	0	0
Adult females	1	1	0	0	2
Juveniles	4	0	0	0	4
Heosemys spinosa	0	1	3	0	4
Adult males	0	0	1	0	1
Adult females	0	1	1	0	2
Juveniles	0	0	1	0	1

Manual capture, otherwise known as 'muddling' (Cagle, 1943), was an effective, albeit labor-intensive, method of collecting turtles in the surrounding forests and streams, although it was only effective during low water periods. The technique consists of wading through streams and probing areas of sand or mud and among roots with a stick, hands, or feet. Comparisons with other studies were not possible as there are no comparative studies of *Amyda cartilaginea* available.

Hard-shelled turtles were searched for in forested areas by walking 100 m transects (2 m wide) through the forest, looking under leaves, tree roots, and debris. The turtles were most frequently located as they crossed trails or other open areas.

At Loagan Bunut National Park, 51 field days were spent during five sampling trips. Over the course of 33 evenings, two hoop nets and 60 baited hand lines were set, totaling 2,046 trap nights. At Balai Ringin, 16 field days were spent during two sampling trips; in total, 45 baited hand lines were set over 16 evenings, totaling 720 trap nights. There were 2,766 trap nights in total.

With the exception of the Balai Ringin trips, traps were set for a minimum of seven days. The baits used, chosen on the basis of availability, consisted of parts of chicken, pork, or local fishes such as Ikan Kali (*Clarius nieuhofii*), Ikan Toman (*Channa micropeltes*) or Ikan Haruan (*Channa striatus*). If habitat conditions changed noticeably, the traps were re-located to a nearby site with at least one meter of water depth.

Lunar phase was recorded at the time of capture to test for possible differences in turtle activity level during the different phases of the lunar cycle. Turtles that were captured while physically active, as opposed to resting, were used in the present study. "Active" animals were qualified as those specimens caught with traps or lines because they must have swam or walked to the area of capture. Three *Amyda cartilaginea* were found by muddling and one *Cyclemys dentata* was found buried near the trunk of a tree. These animals were inactive at the time of capture and were not included in subsequent analyses. One *A. cartilaginea* was caught by baited line on a trip near Gunung Mulu National Park, and was included in subsequent analyses, as were any active hard-shelled turtles found in localities other than the study sites.

Lunar phase was divided into four categories; new moon, first quarter, full moon, and last quarter. New moon was defined as when the non-illuminated side is facing the Earth; at this time, the moon is not visible, except during a solar eclipse. First quarter moon was defined as the phase when one half appears to be illuminated by direct sunlight; during this phase, the illuminated fraction of the moon's disk increases. Full moon was the phase when the moon was completely illuminated by direct sunlight. Last quarter moon was defined as the phase when one half of the moon appears to be illuminated by direct sunlight; during this phase, the illuminated fraction of the moon's disk decreases. Moon phases were obtained from the U.S. Navy Astronomical Applications Department website (U.S. Navy, 2003). In the analyses, hard-shelled and soft-shelled species were pooled separately due to their presumed different behavior, capture method, and habitat use.

To determine if weather affected capture success, weather conditions at the time of collection was recorded, being divided into three categories: clear, overcast, and raining. Overcast was defined as times when the sky was completely cloudy and grey, and clear weather was defined as entirely clear to having some white cumulus clouds.

Seasonality was divided into the North-east Monsoon (wet season), the South-west Monsoon (dry season) and non-monsoonal times, which occurred during April, May and October. The North-east Monsoon prevails from November to March and the South-west Monsoon occurs from June to September. The North-east Monsoon brings the majority of precipitation to Sarawak, while the South-west Monsoon season is typically characterized by dry weather.

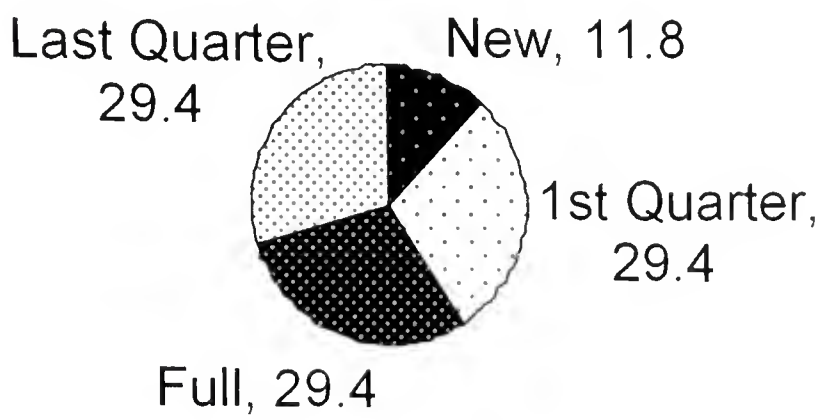

Figure 2. Percentage of *Amyda cartilaginea* collected while physically active during various lunar phases.

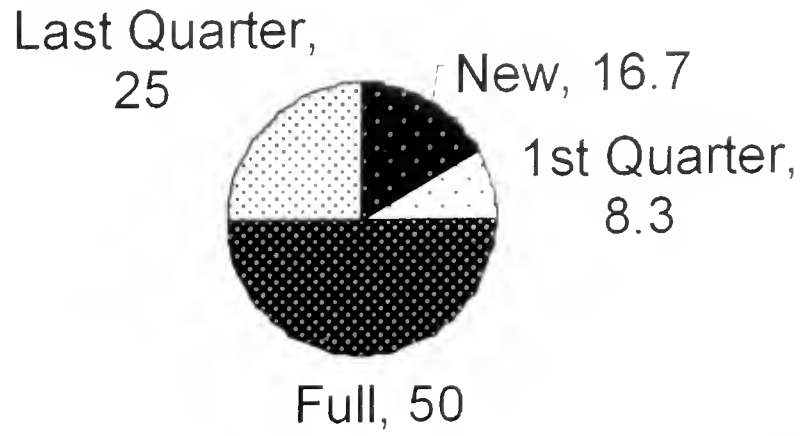

Figure 3. Percentage of hard-shelled turtles collected while physically active during various lunar phases.

Twenty-two days were spent searching for turtles during the North-east Monsoon, 15 days were spent searching during the South-west Monsoon and 30 days were spent searching during non-monsoonal times.

Results

Species richness.- A total of 34 individual turtles from four species were found at the sites examined (Table 1).

Loagan Bunut National Park.- A total of five freshwater turtle species were recorded over a period of 51 field days and 2,046 trap nights. In all, 14 *Amyda cartilaginea* were captured: six males, six females and two juveniles. Six *Cyclemys dentata* were collected: one female, four juveniles and one unsexed carcass. Three juvenile *Cuora amboinensis* were collected.

Of the 14 *Amyda cartilaginea* captured at Loagan Bunut National Park, three individuals were found by muddling, and 11 were caught during the 2,046 trap nights. Of the 11 trapped animals, ten were caught on handlines with Ikan Kali and one was caught on a handline baited with Ikan Haruan. No more than a single turtle was collected per night, representing a 0.54% trap success, with only 11 out of 2,046 trap nights being successful.

Balai Ringin.- Three freshwater turtle species were collected over a period of 16 days and 720 trap nights: one female *Cyclemys dentata*, one female *Heosemys spinosa* and five *Amyda cartilaginea* (four females and one juvenile).

Of the turtles captured at Balai Ringin, three were caught using handlines baited with Ikan Kali, one was found in a selambau and one was caught in a bubu. The trapping success rate was 0.69%.

Additional species.- An adult *Amyda cartilaginea* was caught in the vicinity of Mulu National Park (4° 1' 15" N, 114° 54' 2" E). Three *Heosemys spinosa* (one male, one female, and one juvenile) were also found by various Universiti Malaysia Sarawak personnel while performing wildlife surveys at Matang Wildlife Centre (01° 36' 398" N, 110° 11' 33" E).

Effects of lunar phase on capture success.- During the new moon phase, traps and lines were set for 8 days; two adult *Amyda cartilaginea* (one male, one female) were captured. During the first quarter moon phase, traps and lines were set for 18 days; five adult *A. cartilaginea* (one male, four females) were captured.

During the full moon phase, traps and lines were set for 14 days; five *A. cartilaginea* were captured (four females, one juvenile). During the last quarter phase, traps and lines were set for nine days; five *A. cartilaginea* were captured (two males, one female, two juveniles). These data are shown in Figure 2. The active hard-shelled turtle species captured were *Heosemys spinosa* (four individuals), *Cuora amboinensis* (three individuals) and *Cyclemys dentata* (five individuals, one of which was inactive). During the new moon lunar phase, one juvenile *Cuora amboinensis* and one juvenile *Cyclemys dentata* were collected over a period of three days. During the first quarter lunar phase, 10 days were spent searching for turtles with only one female *Heosemys spinosa* (8.3% of the total) captured. During the full moon lunar phase, collections over 11 days produced one female and one juvenile *Heosemys spinosa*, one juvenile *Cuora amboinensis*, and one female and two juvenile *Cyclemys dentata*. During the last quarter lunar phase, collections over five days yielded one male *Heosemys spinosa*, one juvenile *Cuora amboinensis* and one juvenile *Cyclemys dentata* (Fig. 3).

Results for both soft-shelled and hard-shelled turtles indicate that lunar phase may not have an influence on their activity patterns. A larger sample size with at least one radio-tagged species would provide more significant results.

Effects of weather on capture success.- During clear weather, three *Amyda cartilaginea* (one male, two juve-

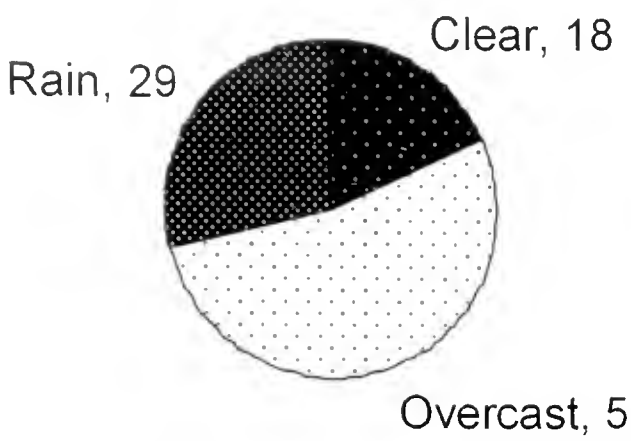

Figure 4. Percentages of *Amyda cartilaginea* collected during different weather conditions.

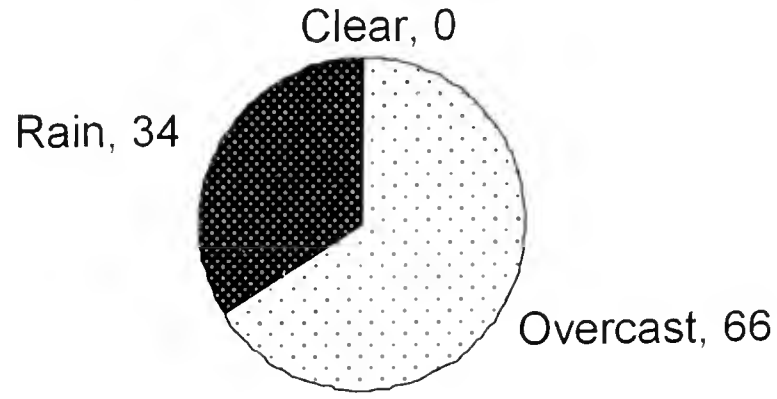

Figure 5. Percentages of hard-shelled turtles collected during different weather conditions.

Figure 6. Percentages of all *Amyda cartilaginea* collected based on seasonality.

Figure 7. Percentages of all hard-shelled turtles collected based on seasonality.

niles) were collected, nine individuals were captured during overcast weather (three males, six females), and six individuals were captured when it was raining (four females and one juvenile) (Fig. 4).

For hard-shelled turtles, 12 individuals were found on the forest floor or on forest trails when it was clear, while only four were found when it was raining (one female, one male and one juvenile *Heosemys spinosa* and one female *Cyclemys dentata*). When it was overcast, eight turtles were found (one female *Heosemys spinosa*, three juvenile *Cuora amboinensis*, and four juvenile *Cyclemys dentata*) (Fig. 5).

Effects of seasonality on capture success.- *Amyda cartilaginea* capture success was examined between seasons. As might be expected, the water levels of both the lake and its tributaries at Loagan Bunut National Park and the riparian habitats in Balai Ringin were lower in the dry season. Consequently, three of the 20 individuals captured were found buried in the mud. During the wet season, six female *Amyda cartilaginea* were captured, while in the dry season, only seven soft-shelled turtles (three males, two females, and two juveniles) were captured. During the non-monsoon seasons, seven soft-shelled turtles (four males, two females, one juvenile) were captured, representing 41% of the total (Fig. 6).

Thirteen hard-shelled turtles were collected. One female *Cyclemys dentata* was found buried under the hollowed trunk of a tree, and although inactive at the time of capture, was included in this component of the analysis since we were looking at the overall effects of capture and seasonality. Six of these turtles were collected during the wet season (one female *Heosemys spinosa*, two juvenile *Cuora amboinensis*, and three juvenile *Cyclemys dentata*). Three of the turtles were found during the dry season (one juvenile *Heosemys spinosa*, one juvenile *Cuora amboinensis*, and one juvenile *Cyclemys dentata*). Four turtles were captured during non-monsoon times (one male and one female *Heosemys spinosa*, and two female *Cyclemys dentata*) (Fig. 7).

All four turtles found buried in mud or hidden in a tree hollow were collected in the dry season. During the North-east Monsoon, these localities would have been covered by at least two meters of water.

Discussion

Capture rates for both *Amyda cartilaginea* and hard-shelled turtles were low, (0.54% and 0.69%, respectively), indicating that the populations of these species may be at critically low levels, although this is difficult to substantiate considering the paucity of historical data for southeast Asia.

Turtle capture rates were tested against three environmental factors: lunar phase, weather, and season. In the lunar phase analysis, it appeared that a new moon may have some influence on the movements of *Amyda cartilaginea*. At a capture rate of 11.8% (compared to

29.4% for all other phases), the darkness of the sky may have an effect on the foraging capabilities of this species. Other predatory species have also been noted as having increased foraging activity with increased moonlight (Brigham and Barclay, 1992). A capture rate of 50.0% during the full moon phase indicated that hard-shelled turtles may need lunar illumination for foraging activity.

The effects of lunar phase on changes in animal behavior are well known. Tigar and Osborne (1999) hypothesized that fewer predaceous arthropods were active during full moons than new moons, possibly because of the increased risk of vertebrate predation. Álvarez-Castañeda et al. (2004) concluded that fewer rodent remains were present in barn owl (*Tyto alba*) pellets during full moons, indicating that rodent activity may be decreased during this phase, which is supported by other studies that have found rodent activity to be linked to lunar phase. O'Farrell (1974) found that the most important factors affecting rodent activity was the amount of time between sunset and sunrise, as well as lunar phase. Price et al. (1984) reported that bright moonlight reduces the overall activity of nocturnal rodents. Church (1960a), concluded that ovulation of the common Asian toad (*Duttaphrynus melanostictus*) was correlated with the lunar cycle in Java. Church (1960b) also found this to be the case with the crab-eating or mangrove frog (*Fejervarya cancrivora*) in Java.

In the present study, 53.0% *Amyda cartilaginea* collections occurred during overcast weather, 29.0% ocurred during rain events, and 18% occurred when the skies were clear. A total of 66.0% of hard-shelled turtles captured during overcast conditions, 34.0% were captured during rainy conditions and none were captured during clear weather, indicating that turtles may favor overcast weather for moving and foraging. Seasons did not have a dramatic affect on the capture rate of turtles, however, more information is necessary to make this determination with any confidence. Clearly, a large amount of effort is required to examine the behavior of turtles, as well as other animals, especially when conducted across multiple seasons, lunar phases, weather conditions, or even years. This paper thus presents preliminary information on the influences of environmental factors on turtle behavior in Borneo.

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Effect of Stocking Density on the Energy Budget of Juvenile Soft-Shelled Turtles (*Pelodiscus sinensis*)

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Abstract.- The present work investigates the effect of stocking density on the energy budget of juvenile soft-shelled turtles (*Pelodiscus sinensis*). Turtles (body weight: 16.22±0.28 g) were stocked at densities of SD1 (8 animals/m^2, 0.14 kg/m^2), SD2 (48 animals/m^2, 0.81 kg/m^2) and SD3 (96 animals/m^2, 1.62 kg/m^2) in aquaria in triplicate for each treatment. The experiment lasted for 35 days. Survival rate, coefficient of size variation, productivity, and apparent digestibility coefficient were not significantly different at the three stocking densities. While there were no significant differences between treatments SD2 and SD3, turtles in group SD1 showed a lower excretion rate and significantly higher food intake and growth rate. Turtles in group SD1 also showed higher crude lipid content and lower crude ash content. No significant differences were found among the treatments in body moisture and crude protein.

Keywords.- Survival, growth, food consumption, stress, body composition.

Introduction

Stocking density is one of the most important biotic factors in aquaculture because it directly influences survival, growth, behavior, health, feeding, and production. High densities may interfere with intra-population interactions and eventually affect biomass gain. The relationship between stocking density and growth for fish has been shown to be positive (Papst et al., 1992), negative (Hengsawat et al., 1997; Irwin et al., 1999) or density-independent (Fairchild and Howell, 2001; Rowland et al., 2004; Rowland et al., 2006), depending on different experimental density ranges. In the fish farming industry, it is very important for the farmer to know the optimum stocking density of the animals being reared to maximize production and profitability.

The soft-shelled turtle (*Pelodiscus sinensis*) is a commonly cultured aquatic reptile species in China with a yield of more than 140,000 tons in 2004 (Shen et al., 2006; Zhang, 2005). Despite the fact that the aquaculture of this species is widespread, scientific studies concerning the effects of stocking density on biological characters are limited (Mayeaux et al., 1996). The objective of the present study is to evaluate the effect of stocking density on the energy budget of juvenile *P. sinensis*.

Materials and Methods

Turtles and rearing conditions.- Juvenile *Pelodiscus sinensis* (body weight: 16.22±0.28 g) were obtained from a commercial turtle farm in Beijing. Turtles were reared in rectangular aquaria (80 length [L] × 35 width [W] × 30 cm height [H]), with 11 individuals per aquarium, at a water depth of 15 cm. Water temperature was maintained at 29.5±0.5°C by a thermo-controlled heater. Aquaria were supplied with dechlorinated water. The dissolved oxygen level was over 5 mg/L and the pH was 7.95. Natural photoperiod was followed. Turtles were fed to satiation once daily at 1500 h. Commercial turtle food was used with 0.5% Cr_2O_3 added for the apparent digestibility coefficient assay. Proximate dry matter composition of the diet was as follows: moisture 3.97%; crude protein 40.27%; crude lipid 7.04%; and crude ash 15.73%. Energy content was 16.06 kJ/g. Turtles were allowed to acclimate to the laboratory conditions for three weeks before the experiments began.

Experimental process.- Healthy turtles were randomly stocked at initial densities of SD1 (8 animals/m^2, 0.14 kg/m^2), SD2 (48 animals/m^2, 0.81kg/m^2) and SD3 (96 animals/m^2, 1.62 kg/m^2) in aquaria (40 L ×30 W ×30 cm H) in triplicate for each treatment. There were no significant differences in initial average body weight or coefficient of size variation within each aquarium among the treatments. The experiment lasted for 35 days. The final densities were 0.34 kg/m^2, 1.20 kg/m^2 and 2.26 kg/m^2, respectively. To maintain a constant numbers of animals, an alternative turtle with approximately the same body weight was added when an initial turtle died. All the water in the tanks was replaced by an equal amount of fresh water daily after surplus food was removed. The aquaria were inspected once daily for mortalities and dead turtles were removed immediately after detection.

Table 1. Survival, specific growth rate, food consumption, apparent digestibility coefficient, and excretion of juvenile soft-shelled turtles (*Pelodiscus sinensis*) held at different stocking densities (Mean ± S. E.)[1].

Treatment	SD1	SD2	SD3	*P*-Value
Initial average weight (g)	16.90±0.45	16.27±0.23	16.15±0.16	0.294
Final average weight[3] (g)	40.30±1.96[a]	24.09±0.38[b]	22.49±0.70[b]	0
Survival rate (%)	100±0.00	91.67±8.33	77.78±10.02	0.1
Coefficient of size variation (%)	—	30.82±5.24	26.84±2.72	0.504
Specific growth rate[2] (%/day)	2.48±0.22[a]	1.12±0.09[b]	0.94±0.11[b]	0.002
Productivity (g/day/m^2)	5.57±0.57	7.08±4.96	7.60±7.98	0.963
Food consumption[3] (mg/day/g)	40.54±0.46[a]	25.76±0.43[b]	24.34±0.62[b]	0
Apparent digestibility coefficient (%)	74.29±2.12	75.60±0.10	78.14±1.44	0.319
Excretion (mg N/day/kg)	60.44±10.64[b]	104.36±5.75[a]	108.59±3.54[a]	0.012

[1]Values in each row with different superscript letters are significantly different ($p < 0.05$).

[2]Means are significantly different among treatments ($p < 0.01$).

[3]Means are extremely different among treatments ($p < 0.001$).

Turtles were weighed to an accuracy of 0.1 g before and after the experiment following three days starvation. Six turtles at the beginning of the experiment and all turtles remaining at the end of the experiment were sacrificed and dried at 65°C to constant weight for analysis of body biochemical composition. Crude protein was determined by the Kjeldahl method, crude lipid was extracted by ether, and crude ash was determined after 12 h of burning at 550° in a muffle furnace. Energy contents were measured using a calorimeter (CA-4P, Shimadzu, Japan). All samples were analyzed in triplicate.

Measurements of various components of the energy budget.- A weighed excess of feed pellets was fed to the turtles once daily (at 1500 h) with a fraction of feed retained for determination of dry matter content. Uneaten food was collected an hour later and dried. Food intake was determined as the difference between the food supplied and the food left uneaten.

Fresh complete feces were collected once daily. Cr_2O_3 content in the diet and feces were determined by the method described in detail by Bolin et al. (1952). The apparent digestibility coefficient (ADC) and the energy lost via feces (F) were calculated by the following expressions:

$$ADC\ (\%) = 100 \times (1 - Cr_2O_3 \text{ content in diet}/Cr_2O_3 \text{ content in feces})$$

$$F = I \times (100\text{-}ADC) \times E_f/100$$

where I and E_f are food consumption in dry weight and feces energy content, respectively.

The coefficient of variation in body weight (CV) within each aquarium, specific growth rate (SGR), and productivity were calculated by the following formulae:

$$CV\ (\%) = 100 \times \text{Standard deviation/Average body weight}$$

$$SGR\ (\%/day) = 100 \times (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

$$\text{Productivity } (g/day/m^2) = (W_{t2} - W_{t1})/S/(t_2 - t_1)$$

where W_2 and W_1 express final average weight at time t_2 and initial average weight at time t_1 in days, respectively. W_{t2}, W_{t1} and S express biomass at day t_2, biomass at day t_1, and aquarium surface area, respectively. Energy allocated to growth was calculated from weight gain (g) and energy content (kJ/g) of the whole body.

Energy lost via excretion was calculated from the ammonia and urea excreted using energy equivalents for ammonia (24.83 J/mg N) and urea (23.03 J/mg N) (Elliott, 1976). Ammonia and urea concentrations inside the water were measured by firstly catalyzing urea to ammonia using urease, and then assaying the total ammonia via standard Nessler's colorimetric technique. The turtles were kept in a given amount of renewed experimental water for 48 h and fasted during the measurement. Water samples were taken before and after this period.

The energy budget for juvenile animals can be described as:

$$C = F + U + R + G$$

where C is the energy in the food consumed, F is the energy lost in fecal production, U is the energy lost in nitrogenous excretory products, R is the energy spent in metabolism, and G is growth energy. In this study, C, F,

Table 2. Chemical composition of juvenile soft-shelled turtles (*Pelodiscus sinensis*) held at different stocking densities in 35-day experimental period (Mean ± S. E.)[1].

Treatment	Moisture (%)	Crude protein*(%)	Crude lipid* (%)	Crude ash*,[2] (%)
SD1	75.86±0.28	63.58±0.65	13.46±0.62[a]	19.16±0.15[b]
SD2	76.27±1.45	65.05±0.13	8.31±1.01[b]	22.39±0.54[a]
SD3	76.46±0.83	61.89±1.04	10.13±0.73[b]	23.11±0.22[a]

*Crude protein, crude lipid, and crude ash based on the contents in the dry matter.
[1]Values in each column with different superscript letters are significantly different $p < 0.05$).
[2]Means were extremely different among treatments ($p < 0.001$).

U, and G were determined directly, and R was calculated by the equation:

$$R = C - F - U - G.$$

Statistical analysis.- All data were analyzed with SPSS for Windows, Version11.0. A one-way ANOVA was used to test the differences among treatment means when assumptions of normality and homogeneity were met. When a significant treatment effect was found, the Least-Significant-Difference (LSD) test was applied to determine which specific pairs differed. The nonparametric Kruskal-Wallis test was applied when the required homogeneity of variance and normality were not satisfied. A regression analysis was carried out to estimate the relationship between stocking density and growth rate. The significant level was set at $p < 0.05$.

Results

All turtles in one aquarium of treatment SD2 died due to a malfunction of the thermo-controlled heater. This replicate was not taken into account for any statistical comparisons.

The effects of stocking density on survival, specific growth rate, food consumption, apparent digestibility coefficient, and excretion.- Survival rates were not significantly different among the three treatments (Table 1), but all showed a negative relationship with increased stocking density ($r = -0.708$, $p = 0.050$). Stocking density showed a clear influence on final body weight, specific growth rate, food consumption, and excretion, as identified by the statistical significance (Table 1). Turtles in group SD1 showed significantly higher food intake and growth rate than those held at the other two densities, which did not differ significantly from each other. Lower excretion rate was observed in group SD1 compared to groups SD2 and SD3. The apparent digestibility coefficients of juvenile turtles ranged from 74.29% to 78.14%. The relationship of SGR and stocking density (SD, animals/m^2) can be described as the linear model or the quadratic model:

$$SGR = -0.0171 \times SD + 2.4360$$
$$(p < 0.01, R^2 = 0.769)$$

$$SGR = 0.0003 \times SD^2 - 0.0531 \times SD + 2.8805$$
$$(p < 0.01, R^2 = 0.915).$$

The effect of stocking density on body composition.- Body composition for each treatment group is shown in Table 2. There were significant differences in lipid and ash contents between treatments ($F_{2,5} = 11.520$, $p = 0.013$; $F_{2,5} = 64.577$, $p = 0.000$). Crude lipid contents of group SD1 were much higher than those of groups SD2 and SD3 while crude ash contents were lower. No significant differences were found among treatments in body moisture and crude protein ($F_{2,5} = 0.155$, $p = 0.860$; $F_{2,5} = 3.412$, $p = 0.116$).

The effect of stocking density on energy budget.- No marked differences were found among treatments in F/C and R/C (Table 3; $F_{2,5} = 0.058$, $p = 0.945$; $F_{2,5} = 2.561$, $p = 0.171$). Stocking density significantly influenced U/C and G/C ($F_{2,5} = 27.151$, $p = 0.002$; $F_{2,5} = 6.243$, $p = 0.044$). Energy budgets for the different treatments can be described as:

$$100C = 10.0F + 0.3U + 73.3R + 16.5G; \text{ SD1}$$
$$100C = 10.3F + 0.7U + 78.3R + 10.8G; \text{ SD2}$$
$$100C = 10.0F + 0.8U + 79.1R + 10.2G; \text{ SD3}$$

Discussion

Stocking density has been considered to be chronically stressful to reared animals (Vijayan and Leatherland, 1988). Several studies have also demonstrated that increased stocking density has a negative effect on survival and growth (Penha-Lopes et al., 2006; Schram et al., 2006), except in some fish species that exhibit schooling behavior (Jørgensen et al., 1993; Papoutsoglou et al., 1998). This impaired growth by stocking density may be attributed to reduced food con-

Table 3. Energy budget of juvenile soft-shelled turtles (*Pelodiscus sinensis*) held at different stocking densities in 35-day experimental period (Mean ± S. E.)[1].

Treatment	C[3] (J/day/g)	F/C (%)	U/C[2] (%)	R/C (%)	G/C (%)
SD1	651.21±7.33[a]	10.04±0.83	0.31±0.06[b]	73.29±2.61	16.45±1.77[a]
SD2	413.83±6.89[b]	10.31±0.04	0.74±0.06[a]	78.32±0.63	10.75±0.63[b]
SD3	390.96±9.94[b]	9.96±0.65	0.79±0.03[a]	79.12±1.56	10.23±1.13[b]

C: food intake in energy; F: energy lost in fecal production; U: energy lost in nitrogenous excretion; R: energy lost in metabolism; G: energy allocated to growth.
[1]Values in each column with different superscript letters are significantly different ($p < 0.05$).
[2]Means are significantly different among treatments ($p < 0.01$).
[3]Means are extremely different among treatments ($p < 0.001$).

sumption, lowed food conversion rate or increased metabolic cost (Jørgensen et al., 1993; Li and Brocksen, 1977; Vijayan and Leatherland, 1988).

In the present study, an obvious trend of decreased survivorship of juvenile soft-shelled turtle with elevated stocking density was observed. This agrees with the results of Mayeaux et al. (1996), who reported that common snapping turtles (*Chelydra serpentine*) stocked at 58 animals/m^2 exhibited greater mortality, lower weight gain, and higher food consumption compared to those stocked at 29 animals/m^2. Food consumption also reduced with increasing stocking density in the present study, however, conflicting with the above snapping turtle results. This discrepancy may be caused by differences in life habit or varying experimental conditions. Knights (1985) observed that more aggressive (and usually larger) eels of *Anguilla anguilla* ate more, while the feeding of smaller subordinates was inhibited, even when food was offered to excess. In the present experiment, despite food being divided between at several spots in each tank, a similar phenomenon was observed during the feeding process. It is likely that the appetite of the subordinate turtles was suppressed and their growth inhibited when the turtles were grouped at high density.

The lower feed intake in treatments SD2 and SD3 appear to explain the lower growth in the same treatments, because reduced food ingestion reduces the amount of energy available for growth (Table 1). Moreover, the proportions of food energy spent in growth (indicated by the gross energy efficiency, Table 3) in treatments SD2 and SD3 were obviously lower in relation to that in treatment SD1 (10.8% and 10.2%, compared to 16.5%). These results may suggest that the ingested energy was not efficiently converted to body reserves, especially at high stocking density.

Juvenile soft-shelled turtles tend to grab each other with their sharp claws, and grabbing activities often result in injuries of the toes and neck, and may even result in death. Agonistic interactions among individuals and elevated swimming activity also lead to increased metabolic expenditure. In the present experiment, turtles of groups SD2 and SD3 exhibited hyperactivity compared to group SD1, and had reduced lipid and higher ash contents. The difference in chemical composition in these turtles suggest that elevated stocking density may induce extra energy expenditure, subsequently allocating less energy to storage.

In conclusion, the pattern of energy allocation of the turtles in the present experiment was significantly influenced by different stocking densities. Turtles cultured at lower density had a relatively higher survival rate, distinctly higher growth rate and transfer more consumed energy to growth. The lower energy input and lower gross energy efficiency in treatments SD2 and SD3 may have contributed to their reduced growth rate. Furthermore, the excretion of nitrogenous wastes to the environment was relatively lower with reduced stocking density. Conversely, higher stocking density could result in higher productivity to some degree, since there were no significant differences in productivity among the treatments. We suggest that the turtle farmer pursue an optimal stocking density based on profitability, considering that lower stock densities are shown to be related to increased survivorship, growth rate and feed utilization, while also being associated with a reduction in nitrogenous wastes.

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Genetic Variation and Trans-species Polymorphism of MHC Class II B Genes in Reptiles

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Abstract.- Trans-species polymorphism has been extensively documented for the major histocompatibility complex (MHC) in mammals, fishes and birds, but not for non-avian reptiles. Our study has addressed this by focusing on three species of the reptiles: *Chinemys reevesii*, *Plestiodon chinensis* and *Alligator sinensis*. Using polymerase chain reaction (PCR) and nucleotide sequence analyses, we examined a total of twenty-five sequences of exon 2 of the MHC class II B genes in these species. High allelic variability was observed among sequences within each of these species, indicating extensive MHC polymorphism. Nonsynonymous substitution rates (d_N) exceeded synonymous substitution rates (d_S) greatly within the antigen-binding sites (ABS), suggesting the effect of balancing selection. Phylogenetic analysis of these reptile sequences clearly supports the hypothesis of trans-species polymorphism. We therefore confidently conclude that trans-species polymorphism in the MHC is now known for reptiles, as well as mammals, fishes and birds. This suggests that the main function of the MHC (presentation of peptides to T lymphocytes) has remained largely unchanged despite of long periods of evolution.

Keywords.- Major Histocompatibility Complex (MHC), reptiles, trans-species polymorphism.

Introduction

The genes of the major histocompatibility complex (MHC) code for polymorphic membrane glycoproteins that play a key role in the T-cell mediated immune response (Klein, 1986). There are two distinct classes of MHC molecules, class I and class II, which are encoded by separate but tightly linked loci. The diverse, but always specific, antigen-binding properties of the MHC class I and II molecules determine which foreign peptides can be identified to trigger an immune response. Such MHC-dependent recognition of certain antigens has been considered as an important contributing factor in susceptibility to disease (Klein, 1986). In many vertebrate species, the MHC class I and class II loci exhibit an extraordinarily high degree of polymorphism, particularly in exon 2 of the beta genes. This variation is probably maintained through some kind of balancing selection related to interactions between the immune system and pathogens (Parham and Ohta, 1996), although it has not been resolved as to whether the selection is overdominant (heterozygote advantage hypothesis), frequency dependent (rare-allele advantage hypothesis) or a combination of these factors (Hughes and Hughes, 1995; Hughes and Yeager, 1998; Hill et al., 1992; Hughes, 2000; Thurz et al., 1997).

A characteristic feature of the MHC genes is trans-species polymorphism, i.e. the existence of allelic lineages shared by related species, supporting the theory that the divergence of MHC allelic lineages predate speciation (Graser et al., 1996; Klein, 1987; Ottova' et al., 2005). For MHC genes, this kind of polymorphism has been well-documented in mammals, but for other vertebrate classes, the data on trans-species polymorphism are either fragmentary or unavailable. Only in fish (Klein et al., 1998; Ottová et al., 2005) and birds (Hess and Edwards, 2002; Richardson and Westerdahl, 2003) is there clear evidence for the interspecific sharing of MHC alleles, but these species are of recent origin and do not provide information about long-term persistence of allelic lineages. In an attempt to obtain such information, we decided to compare the polymorphism in three reptiles (*Alligator sinensis*, *Chinemys reevesii* and *Plestiodon chinensis*) with that in two other closely-related reptiles (*Alligator mississippiensis* and *Caiman crocodilus*).

In this study, we investigate genetic variation at exon 2 of the MHC class II B genes, including part of the putative antigen-binding sites, in three reptiles. We choose this particular exon because it is known to be highly polymorphic in primates and a variety of other terrestrial species. Our purposes in this study are: first, to analyze the variability of MHC class II B genes among the species listed above; second, to test for the influence of selection on amino-acid polymorphism, i.e. a positive (balancing) selection in exon 2; and finally, to document whether the trans-species polymorphism in MHC class II B genes also exits in reptiles.

Table 1. The genetic parameters of the sequences within each analysed species.

Species	N	L	S	π	p
C. reevesii	8	166	84	0.22203	35.80%
P. chinensis	7	166	12	0.0218	5.20%
A. sinensis	10	166	38	0.09236	18.40%

Note: N: number of sequences; L: sequence length (pb); S: variable sites; π: nucleotide diversity; p: amino acid diversity.

Materials and Methods

Isolation of genomic DNA.- Total genomic DNA was isolated from 20–50 µL of blood using standard phenol-chloroform extraction methods (Sambrook and Russell, 2001). The sampled individuals (without existing sequence data on GenBank) included a total of four *Chinemys reevesii* (terrapin) and two *Plestiodon chinensis* (saurian) from natural populations.

Polymerase chain reaction (PCR).- A 166 bp fragment of exon 2, from the class II B genes coding for part of the peptide-binding region, was amplified by PCR using the following degenerate primers reported by Shi et al. (2004): the forward (sense) primer MHC-UP 5'-AAGG(T/G/C)C(C/G)AGTG(T/C)TACT(T/A)(C/T)A(T/G/C)(T/G/C)AACGG-3'; the reverse (anti-sense) primer MHC-DP 5'-TAGTTGTG(C/G)C(G/T)GCAG(A/T)A(C/G)GTGTC-3'. PCR reaction were performed in 30 µL of reaction mixture containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 150 µM dNTP, 1 µM of each primer, 20–100 ng of isolated genomic DNA and 1 unit of *Taq* DNA Polymerase (Promega). Thermocycler conditions were as follows: an initial denaturation for 5 min at 94°C, followed by 35 cycles, each consisting of 30 s at 94°C, 40 s at 52°C, and 40 s at 72°C. The final extension at 72°C was for 10 min. PCR products were separated in a 2% agarose gel containing ethidium bromide (0.5 µg mL^{-1}). Separated PCR products were visualized under UV light and photographed to examine the banding patterns.

Cloning and sequencing.- Following agarose gel electrophoresis, PCR products of appropriate size were recovered, purified and concentrated using the DNA Gel Extraction Kit (V-gene Biotechnology Limited). The purified PCR products was ligated into the pGEM®-T Vector using the TA cloning kit (Promega); Competent *Escherichia coli* DH5α cells were transformed in a ligation reaction, and positive clones were identified by blue/white selection, as described in the manufacturer's protocol. Twenty to thirty positive clones were selected for each individual. Insert size was verified by PCR using M13 universal forward and reverse primer. Different inserts were screened by single-strand conformation polymorphism (SSCP) analysis and sequenced using the dideoxy nucleotide chain termination method (Sanger et al., 1977) on an Applied Biosystems 377 automated sequencer.

Data analysis.- Nucleotide and inferred protein sequences were aligned using the CLUSTAL X software (Jeanmougin et al., 1998). MHC sequences from closely-related species were acquired using the GenBank BLAST program (Altschul et al., 1990). Genetic distances were measured using the two-parameter method (Kimura, 1980). The computer package MEGA 2.1 (Kumar et al., 2001) was used to estimate the rate of nonsynonymous (d_N) and synonymous (d_S) substitutions according to Nei and Gojobori (1986), applying the Jukes and Cantor (1969) correction for multiple hits. The differences between these rates was evaluated with a *t*-test with infinite degrees of freedom according to the test statistic $t = d/s(d)$; $s(d)$ is the standard error of d and

Table 2. Numbers (mean±standard error) and relative rate (d_N/d_S) of nonsynonymous (d_N) and synonymous (d_S) substitutions per nucleotide in exon 2 sequences given for all sites and for pABS and non-pABS for comparison of three species.

Species	Sites	No. of codons	d_N (S.E.)	d_S (S.E.)	d_N /ds
C. reevesii	pABS	14	0.669±0.171	0.429±0.149	1.60*
	non-pABS	41	0.230±0.046	0.149±0.047	1.54*
	Total	55	0.311±0.049	0.204±0.047	1.52**
P. chinensis	pABS	14	0.043±0.027	0.032±0.036	1.34
	non-pABS	41	0.016±0.007	0.018±0.013	0.89
	Total	55	0.023±0.008	0.020±0.012	1.15
A. sinensis	pABS	14	0.245±0.087	0.045±0.034	5.44**
	non-pABS	41	0.083±0.025	0.058±0.032	1.43**
	Total	55	0.120±0.028	0.055±0.024	2.18**

Asterisks indicated the significance of two-tailed t-test in the order: * $p < 0.01$, ** $p < 0.001$.

Figure 1. Amino acid sequences translated from nucleotide sequences of exon 2 from MHC class II B genes of *C. reevesii, P. chinensis* and *A. sinensis*. The *Alsi* sequences are from our laboratory work (Shi et al. 2004). Asterisks (*) indicate the putative antigen-binding sites correspond with those for human class II sequences; dots (.) indicate identity with the consensus sequence at the top; dashes (–) gaps introduces to achieve optimal sequence alignment.

	10	20	30	40	50	55
Chre-1	T E R V R Y L Y R D	I Y N G R Q D L H F	D S D V G V H V A D	T E L G Q P D A E Y	W N S Q P E I L A D	R R A A V
Chre-2	. . . . .	. . . . .	. . . . R	. . . . .	. . . . .	. . . . .
Chre-3	. . . A	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
Chre-5	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	M . . . E . . . K .	. . . . K . V M E L	. . G E .
Chre-4	. . . . . H . Y	. . D R . . I A . .	. . . L . . Y . . .	. . . . R L	. . . . . T . Q Y	Y . . V .
Chre-6	. . . I . L . V . W	. . . . Q . Y . . .	. . E L . R Y . . .	. . P . R . S . . .	. . K D K A E . D H	. . G E A
Chre-7	. . . . . L . V S W	. . . . Q . Y A . .	. . E L . R Y . . .	. . P . R . S . . F	. . K D K A V . D H	. . G E A
Chre-8	. . . . . F . F . Y	T . . R Q P L A . .	. . . . . F E . .	. . . . R A Q . . I	. . K D K A L . . Q	A . G E .
Plch-1	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	M . . . G . . . K .	. . . . K . V M E L	. . G E .
Plch-2	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	M . . . E . . . K .	. . . . K . V M E L	. . G E .
Plch-3	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	M . . . E . . . K .	. . . . K . V M E L	. . G E .
Plch-4	. Q . . . . . Y	V W . Q E . . A . .	. . . . . Y . . .	M . . . E . . . K .	. . . . K . V M E L	. . G E .
Plch-5	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	M D . . E . . . K .	. . . . K . V M E H	. . G E .
Plch-6	. Q . . . . . H	V W . Q E . . A . .	. . . M . . Y . . .	M . . . E . . . K .	. . . . K . V M E H	. . G E .
Plch-7	. Q . . . . P P . H	V W . Q E . . A . .	. . . . . Y . . .	M . . E E . . . K .	. . . . K . V M E L	. . G E .
Alsi-1	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	M . . . E . . . K .	. . . . K . V M E L	. . G E .
Alsi-2	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	. . . . E . . . K .	. . . . K . V M E L	. . G E .
Alsi-3	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	. D . . E . . . K .	. . . . K . D M E Y	. . I G P
Alsi-4	. Q . . . . V S . .	. W D R . . . V . .	. . . . . Y . . .	. . . . E . . . K .	. . . . K . E . E .	D W . . .
Alsi-5	. Q . . . . . H	V W . Q E . . A . .	. . . . . Y . . .	. . . . E . . . K .	. . . . K . V . E Y	. . G S .
Alsi-6	. Q . . . . P . . H	V W . Q E . . A . .	. . . . . Y . . .	M . . . E . . . K .	. . . . K . V . E Y	. . G S .
Alsi-7	. Q . . . . V . . .	V W D R . . . V . .	. . . . . Y . . .	. . . . E . . . K .	. . . . R . E . E .	D W . . .
Alsi-8	. Q . . . . . V S	. W D R . . . V . .	. . . . . W . . .	. . . . E . . . K .	. . . – – – V E . E L	. . G V .
Alsi-9	. Q . . . . V . . .	V W D R . . . V . .	. . . . . Y . . .	. . . . E . . . K .	. . . . K . V . D Y	. . G . .
Alsi-10	. Q . . . . V . . .	V W D R . . N V . .	. . . . . Y . . .	. . . . E . . . K .	. . . . K . V M E Y	. . G S .
	* * *	* * * *	*	* * * * *	* * * *	* *

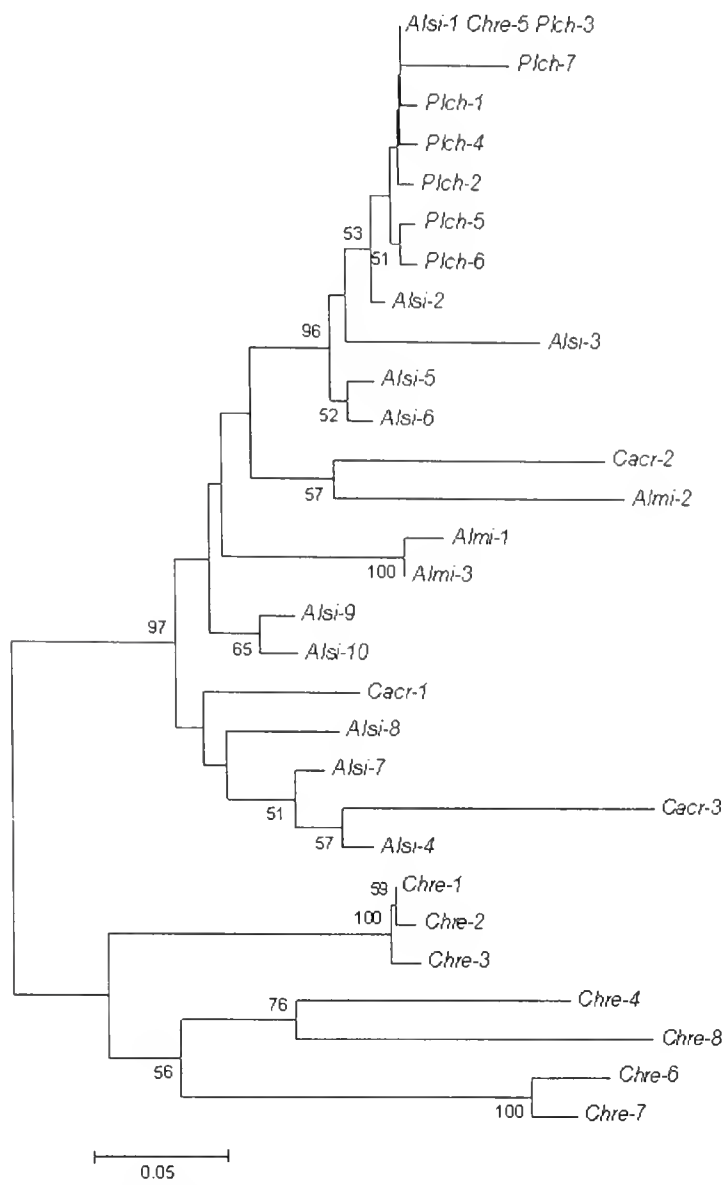

Figure 2. Phylogenetic tree of MHC class II B genes nucleotide sequences in different reptiles constructed by neighbor-joining method in MEGA. Bootstrap values from 1000 replications were indicated above the branches, values less than 50% are not shown.

is given by $s(d) = [\mathrm{Var}(d_S) + \mathrm{Var}(d_N)]^{1/2}$ (Kumar et al., 1993). These analyses were performed on the 24 codons comprising the ABS sites (as defined in the crystal structure of a human class II molecule, DRB1 (Brown et al., 1993)) and on all remaining non-ABS codons in the amplified segment. The phylogenetic tree was constructed using MEGA 2.1 program for distance-based methods, applying the neighbour-joining (NJ) algorithm. Bootstrap analysis (1000 replications) was performed to determine the reliability of the branching pattern in the phylogenetic tree. To further quantify polymorphism, nucleotide diversity (π) was also calculated using the computer application DnaSP (Rozas et al., 1999).

Results

Amount and extent of variation.- In total, fifteen sequences were obtained from *Chinemys reevesii* and *Plestiodon chinensis* (see Table 1); sequence identity was confirmed by sequencing multiple clones in both directions. These sequences are designated *Chre* for *Chinemys reevesii* and *Plch* for *Plestiodon chinensis*, in accordance with the proposed nomenclature (Klein et al., 1990), and have been deposited in GenBank under the accession numbers AY937200~AY937207 (for *Chre*-1~*Chre*-8), AY772946~AY772951 and AY764032 (for *Plch*-1~*Plch*-7). Ten sequences (*Alsi*-1~*Alsi*-10; GenBank accession numbers AY491421~AY491430) of exon 2 of the class II B genes from three *Alligator sinensis*, used for the subsequent data analysis, were also submitted. All sequences except for one from *A. sinensis* (*Alsi*-8), which had six nucleotides deletions, were 166bp in length. Published sequences were aligned with those derived here, illustrating numerous variable sites in *Chinemys reevesii* (84 [=50.6%]), *P. chinensis* (12 [=7.2%]) and *A. sinensis* (38 [=22.9%]); these numbers are consistent with those seen for this fragment in other species. Nucleotide diversity was also calculated within species, with all three species exhibiting a mean pairwise nucleotide diversity of 0.22203, 0.02180 and 0.09236, respectively. Furthermore, *Chre*-5 was also found to be identical to *Plch*-3 in *P. chinensis*, and *Alsi*-1 in *A. sinensis*. Sharing of these same alleles in different species was also reported in Hedrick et al. (2002).

Amino acid variation within *Chinemys reevesii*, *Plestiodon chinensis* and *Alligator sinensis* was 35.8%, 5.2% and 18.4%, respectively (Table 1). Aligned amino acid sequences are presented in Figure 1. The putative antigen-binding sites (pABS), corresponding to those in the human class II sequences (Brown et al., 1993), are indicated by an asterisk. The term "putative" has used here because the actual antigen-binding sites for reptiles have not yet been verified. Among the *Chinemys reevesii* sequences, 92.9% (13 out of 14 codons) of pABS are variable and 63.4% (26 out of 41 codons) of the nonbinding sites (non-pABS) are variable. Within *A. sinensis*, 71.4% of pABS and 31.7% of the non-pABS are variable, and in *P. chinensis*, 21.4% of pABS (3 of 14) are variable, while 12.2% (5 of 41 positions) of non-pABS are variable. The numbers of synonymous substitutions (d_S) and nonsynonymous substitutions (d_N) per nucleotide in exon 2 sequences are given in Table 2. The ratio of d_N to d_S tended to be greater than 1.0, particularly for pABS, which has a ratio consistent with that seen in other MHCs, suggesting that there is selection for amino acid replacements in the antigen-binding region. Meanwhile, d_N/d_S for pABS and non-pABS in *Chinemys reevesii* and *A. sinensis* were all larger than 1.0.

Phylogenetic analysis.- BLAST searches in genome sequence databases have revealed that a number of alleles from other reptiles exhibit a high degree of similarity to the sequences derived here. Of these, three *Caiman crocodilus* alleles (*Cacr*-1~*Cacr*-3; Accession Numbers AF256651, AF256652 and AF277661) and three *Alligator mississippiensis* alleles (*Almi*-1~*Almi*-3; Accession Numbers U24402~U24404) were chosen for

phylogenetic analysis. A neighbor-joining tree showing the relationships among these nucleotide sequences is presented in Figure 2. The sequences of *Chinemys reevesii* and *Plestiodon chinensis* tend to cluster together. Interestingly, *Caiman crocodilus* sequences were widely dispersed in the tree (supported by high bootstrap values), being more similar to the crocodile sequences. A cluster of four *Alsi* sequences (*Alsi*-2, *Alsi*-3, *Alsi*-5, *Alsi*-6) showed a higher degree of similarity to *Plch* sequences than to other lineages. The phylogenetic tree supports the hypothesis of trans-species polymorphism, as indicated by the clustering of lineages from different species and the presence of sequences from different species in the same allelic lineage. This trans-species allelic similarity is not unusual for MHC genes, as it has been proposed that MHC allelic lineages are maintained by selection and are often older than the species themselves.

Discussion

In the present study, we investigated exon 2 (of MHC class II B genes) sequences from three reptiles and examined within species polymorphism. The results revealed relatively high amounts of variability in both nucleotide and amino acid sequences (Table 1), as well as a pattern of evolution consistent with those seen in a variety of mammalian species, including humans. However, the level of genetic variation within each of these species differed, possibly reflecting different patterns of evolution and population genetic structure. Levels of polymorphism are higher in *Chinemys reevesii* and *Alligator sinensis* compared to *Plestiodon chinensis*, which may prove to be of value in future studies on population genetics and conservation biology.

As an important genetic component of the vertebrate immune system, variation in the MHC is significant to consider selective pressure due to parasitic or pathogen resistance. It has been suggested that species or populations with low MHC diversity might be particularly susceptible to infectious disease and parasites (Hedrick and Kim, 2000; O'Brien and Evermann, 1988). Furthermore, in this and other studies (Hedrick et al., 2002; Ottová et al., 2005), identical MHC alleles have been found amongst different species; the sharing of these likely homologous sequences may be due to exposure to similar (or the same) antigens present throughout the evolution of each species.

Balancing selection appears to play a determinant role in MHC evolution (Bernatchez and Landry, 2003), evidence of which is the presence of more nonsynonymous (d_N) than synonymous (d_S) substitutions in antigen-binding sites (Binz et al., 2001; David and Helena, 2003; Hedrick et al., 2002). In the present study, the observed excess of nonsynonymous substitutions, particularly at putative antigen-binding sites, indicates that nonsynonymous sites evolve faster than synonymous sites. This implies the presence of balancing selection (or positive Darwinian selection), which favors new variants and increases MHC diversity, which has been observed in a number of species (Hughes and Nei, 1989). In the case of *Chinemys reevesii* and *Alligator sinensis*, however, a significant excess of nonsynonymous substitutions was also found in non-pABS sites, possibly suggesting that reptile ABS sites may not exactly correspond to those in humans as originally defined by Brown et al. (1993). Similar findings were reported for the Pacific salmon (Miller and Withler, 1996) and Sonoran topminnow (Hedrick et al., 2001).

The phylogenetic tree suggests that some *Alsi* sequences are more similar to *Plch* sequences than to other *Alsi* sequences, with species sequences intermingling to form several significantly-supported clusters (Fig. 2). This intermixing suggests a trans-species persistence of MHC class II exon 2 sequences, with some allelic lineages predating species cladogenesis. The prolonged maintenance of MHC alleles is contrary to what would be expected from neutral loci, supporting the idea that long-term balancing selection on the MHC alleles has occurred (Figueroa et al., 1988). Our results are consistent with the theory of trans-species evolution in MHC alleles (Klein, 1987), which has been previously supported by studies on mammals and fish (reviewed in Hedrick, 2001), suggesting that MHC polymorphism is widespread in the Vertebrata.

In conclusion, we are presenting strong evidence for trans-species polymorphism at exon 2 of class II gene in reptiles. The polymorphism is putatively maintained by balancing selection and is restricted to what are apparently functional loci on (primarily) the ABS sites. These observations suggest that the MHC carries out the same function in reptiles as it does in mammals, but additional research needs to be conducted, particularly with regards to trans-species polymorphism and specific binding loci across taxa.

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The Biology and Taxonomic Status of the Sunken Ear Frog (*Rana tormotus* Wu, 1977)

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Abstract.- The biology and taxonomic status of the sunken ear frog, *Rana tormotus* Wu, 1977, are reviewed and briefly discussed. This is a rare species restricted the mountain streams and rivers of Anhui and Zhejiang provinces in East China, and is characterized by a sunken tympanic membrane that forms an external ear canal, similar to that seen in birds. The male, which is known to produce ultrasonic sounds when calling, has a more deeply sunken membrane. The karyotype of this frog is 2n = 26, having five pairs of large, eight pairs of small and seven pairs of submetacentric chromosomes. The frog is active during the night and females are uncommonly encountered. Specimens are often found in the same habitat as *Megophrys boettgeri, Bufo gargarizans, Rana limnocharis, R. schmackeri, Paa spinosa, Amolops wuyiensis* and *A. ricketti*. This frog was first described within *Rana*, but it was recombined in *Amolops* because its tadpole was of the "*Amolops* type", even though the tadpole was unknown at the time. The recently-discovered tadpole has no abdominal sucker and the poison glands, smaller than and similar to those of *R. schmackeri* with LTRF (I:4-4/III:1-1), making it distinct from the "*Amolops*-type" tadpole. Adult and larval morphology, as well as developmental characters, support the placement of this species in *Wurana*, a new genus setup recently.

Keywords.- Amphibia, Ranidae, *Rana*, *tormotus*, *Wurana, Amolops*.

Introduction

Rana tormotus Wu, 1977 is a characteristic frog with a sunken ear membrane that is particularly distinct in the male, giving this species the common name of "sunken eared frog" or "concave-eared torrent Frog," the former of which translates to "Ao Er Wa" or "Wa Er Wa " in Chinese. In 1972 and 1974, Ermi Zhao, Guanfu Wu and two assistants collected one female and 18 male frogs with sunken tympana at Taohua Creek on Mt. Huanshan. These frogs were subsequently described as members of the new species *Rana tormotus* (Sichuan Institute of Biology [Wu, G. F.]). During the next 23 years, only a few reports on this frog's taxonomy and karyotype were published (Chen, 1991; Fei et al., 1991; Guo and Dong, 1986; Huang et al., 1990). The species was subsequently moved to *Amolops* by Fei et al. (1991), because its tadpole might be of the "*Amolops* type", even though the tadpole was unknown at the time. The correct taxonomic placement of this species is currently ambiguous (Global Amphibian Assessment, 2005; Zhao and Adler, 1993; Zhao and Zhao, 1994; Zhao et al., 2000;).

Recently, the ecology, bioacoustics and evolutionary history of this species have been explored by Liu and Hua (2001), Wu and Wu (2002) and Feng et al. (2002, 2006). We have also completed surveys on its distribution and habitat (at Jiande, Zhejiang and its type locality at Huanshan, Anhui), which are presented below. We further placed this species in the new genus *Wurana* on the basis of developmental characters and adult and larval morphology (Li et al., 2006).

Distribution and habitat.- This sunken ear frog is a rare endemic species restricted to the mountain streams and rivers of East China at elevations between 150 and 750 m. It is currently known only from the type locality (Taohua Creek, Hotspring Creek, Fu Creek and Xiang Creek of Huanshan, Anhui Province) and two locations in Zhejiang province (Huang, et al, 1990, personal communication with Prof. Qinghui Gu): two small creeks in the Jiangde Forestry Centre and the creeks of Anji County. The latter creeks are filled with large rocks and are surrounded by trees, brushes and grass (Figs. 1–3).

All frogs were collected at night since they could not be found during the day. Adult males were found on rocks in the river and in the surrounding trees and shrubs, and were located by their calls. No females were observed; in Liu and Hua (2001), it was reported that females were collected only after midnight following the appearance of males. It was suggested that the females were not commonly found because they occupied higher tree branches (Wu and Wu, 2002). Neither sex was observed following the breeding season.

Wu and Wu (2002) reported that the frogs were only found in shrubs along the flat parts of the river. In comparison, at the bases of mountains we found that trees

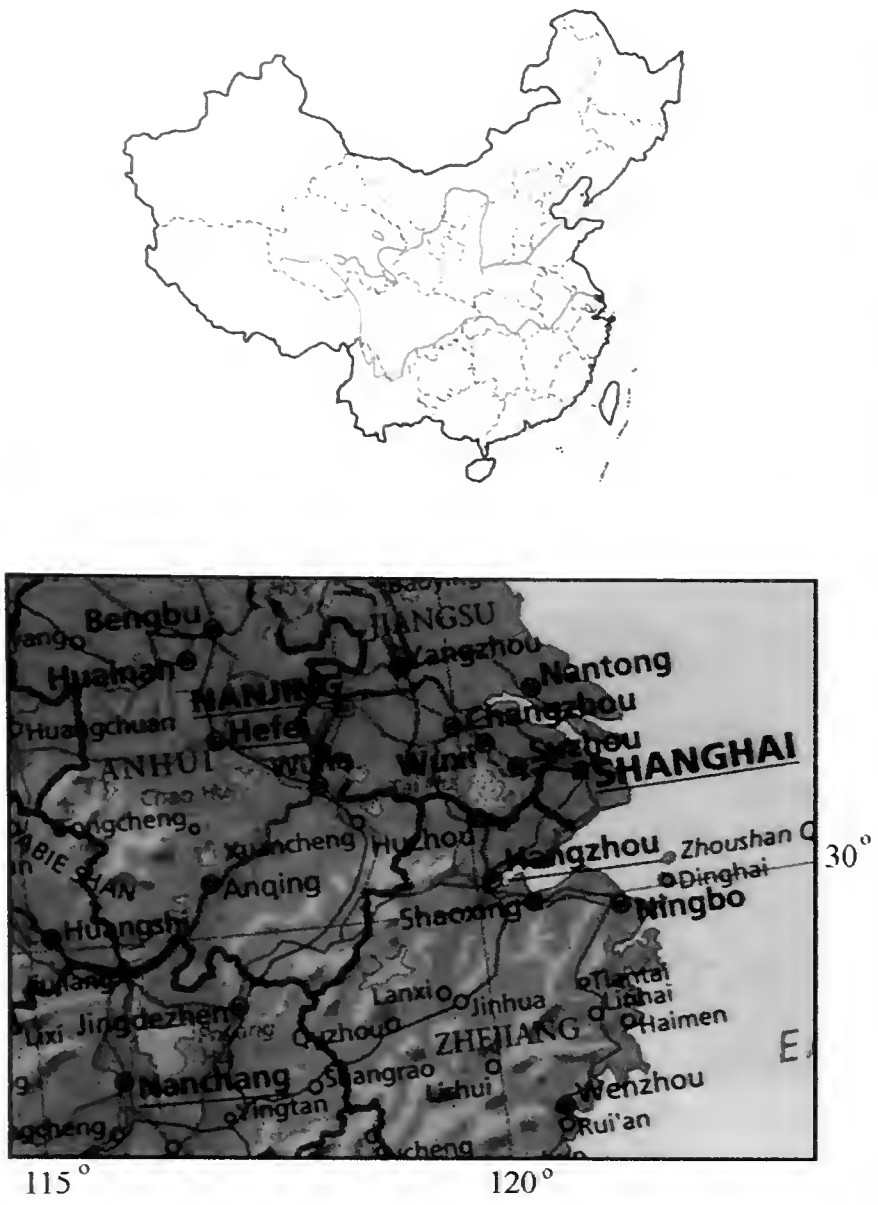

Figure 1. Distribution of *Rana tormotus* (red square) in Anhui and Zhejiang province.

Figure 2. Type locality of *Rana tormotus*, Taohua Creek of Mt. Huanshan in Anhui province, China.

were preferentially chosen (57.32% at Taohua Creek and 51.9% at Fu Creek), followed by shrubs (31.71% and 40.51%) and then rocks in the water (10.98% and 7.95%). Liu and Hua (2001) observed 11 of 53 frogs on branches, four on sand near vegetation along the river bank and the remainder on exposed sand and rock in the river far from any vegetation. In the present study at Taohua Creek, 20% of frogs were found on tree branches, 40% on shrubs, 20% on grass leaves and 20% on rocks; specimens were never found along sandy river banks, where they may have been excluded by *Rana* species. The differences in observations between surveys may be due to climatic variation.

Males were kept in an aquatic box simulating the natural environment. During the day, the frogs hid in gaps between stones and vegetation, but at night they emerged on the surrounding leaves and rocks despite variation in weather (including rain).

The other frogs found in the same habitat as *Rana tormotus* at the type locality were *Megophrys boettgeri*, *Bufo gargarizans*, *Rana limnocharis*, *Rana schmackeri* (Boettger, 1892), *Paa spinosa* (David, 1875) and *Amolops wuyiensis* (Liu and Hu, 1975) (Liu and Hua, 2001; Li, Lu and Lü, 2006). *Amolops ricketti* was found instead of *A. wuyiensis* at Jiande. Frogs observed in the surrounding areas included *Rana nigromaculata*, *Microhyla heymonsi, R. livida* and *R. japonica* (Wu and Wu, 2002). Tadpoles of *Rana schmackeri, Paa spinosa* and *Amolops wuyiensi* were also collected from Taohua Creek.

Karyotype and Ag-banding pattern.- Guo and Dong (1986) reported the karyotype and Ag-banding pattern of *Rana tormotus*. The karyotype is 2n = 26, consisting of five pairs of large and eight pairs of small chromosomes. A secondary constriction is present near the centromere on the long arms of chromosome 6 and 10. No heteromorphic chromosomes were present. One homologous pair of NORs were found in the secondary constriction of chromosome 10 using Ag-AS staining techniques. There were also seven pairs of submetacentric chromosomes, the largest among frogs with a 2n = 26 karyotype in the Raninae (Guo and Dong, 1986; Pan et al., 2002).

Calling and related morphological characters.- Recently, Feng et al. (2002, 2006) detailed the extraordinarily rich vocal repertoire of the sunken ear frog. These frogs produce countless vocalizations, some of which share features of bird songs or primate calls – e.g., ultrasonic frequency, multiple upward and downward FM sweeps and sudden the onset and offset of selective harmonic components within a call note. Most frog calls go either up or down, and no others are known to extend into the ultrasonic range. Frame-by-frame video analysis of the frog's calling behavior suggests the presence of two pairs of vocal sacs that may contribute to its remarkable call-note complexity. Electrophysiological studies of the frog's auditory midbrain confirmed that its audible range extends into the ultrasonic (Xu et al., 2005). This characteristic can likely be explained by the fact

Figure 3. Another locality of *Rana tormotus* at Jiande county of Zhejiang province, China.

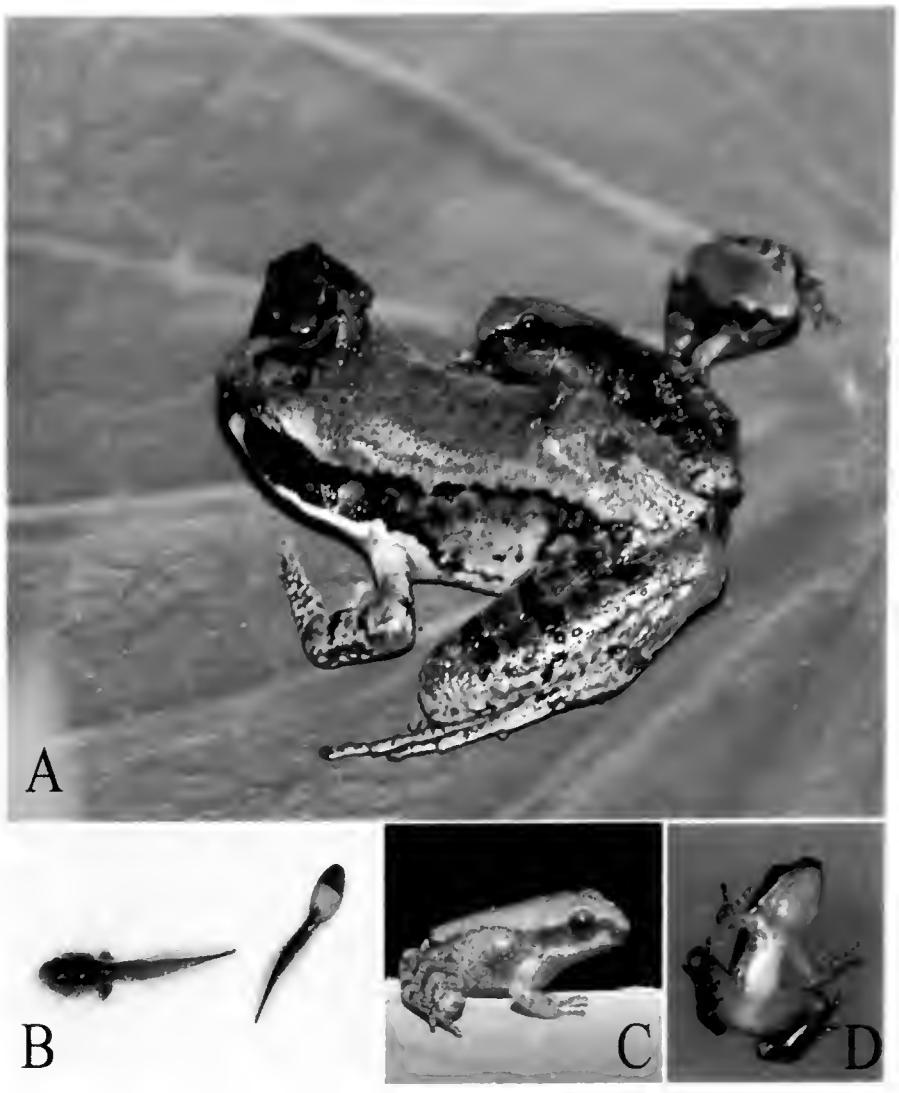

Figure 4. Tadpole, froglets and adult male of *Rana tormotus* (Wu, 1977). (A) Adult male and froglets, (B) tadpoles, (C) froglet just after metamorphosis, (D) froglet in ventral view.

that the frog lives by noisy streams that produce acoustic signals with significant ultrasonic harmonics that would mask normal calls; selective pressure on this species could eventually produce a call that would not be masked by the wideband noise produced by the river (Peter et al., 2003).

With regard to sound reception, the sunken ear frog has a unique structural autapomorphy not seen in other Anura – a sunken tympanic membrane that forms an external ear canal like that seen in birds. The tympanic membrane is more deeply sunken in the male, suggesting that it receives the airborne sound in a manner somewhat different from that in the female (Feng et al., 2006; Xu et al., 2005).

Tadpoles and taxonomic status.- Several different species of tadpoles from the frog's type locality were reared to metamorphosis in the lab. The first morphotype was identified as *Amolops wuyiensis*, which had an abdominal sucker and poison glands; the second to fourth morphotypes were without either of these structures. Among latter, one identified as *Paa spinosa* had a relatively larger body and body-tail length, and froglets that very closely resembled the adult in general morphology. The remaining two morphotypes were significantly smaller and had the same LTRF (I:4-4/III:1-1); of these, one was externally similar to the adult of *Rana schmackeri* and one was similar to the adult of *R. tormotus* (Fig. 4); the characters used to aid in the separation of these two species were those listed in Li et al. (2006) and another paper discussing this species in the present journal issue (Li et al., 2007).

These characters are enough to verify that *Rana tormotus* does not have an *Amolops*-type larva, and should be placed elsewhere. This conclusion was verified by examining and comparing the skeletons of *Rana tormotus* to the ranine genera *Amolops*, *Pseudoamolops*, *Rana* and *Staurior* (Li et al., 2006).

Li et al. (2006) designated *Rana tormotus* as the type species of the new monotypic genus *Wurana* (etymologically, the specific epithet honors Wu Guanfu for the research on this and other frogs), which is known from Anhui and Zhejiang Provinces, China. *Wurana* is diagnosed as follows:

> ADULT.- Dorsolateral folds relatively thick and wide; tympanum deep, forming an external auditory canal that is pronounced in the male; no temporal folds; male without humeral glands; tarsal folds absent; tips of fingers and toes expanded into small disks with circummarginal grooves on outer three digits; width of crossbar on terminal phalanx much less than 0.3 times phalanx length.
>
> LARVA.- Small type tadpole with weak horny beak; two rows of lower labial papillae with bases originating in same line; oral disc emarginate laterally, with single row of truncate marginal papillae in posterolateral margin of upper lip and with wide rostral gap; labial tooth row formula (LTRF) usual-

ly I:4-4/III:1-1 (sometimes I:3-3/III:1-1); without external gland groups; spiraculum on the left with free tube.

From the general external morphological and skeletal characters, *Wurana* is closer to *Rana*, especially to some of the odorous frogs, than *Amolops*.

The surveys done by Qinghui Gu, Pipeng Li and others have shown that *Wurana tormota* is likely either a threatened or "rapidly declining" species (Global Amphibian Assessment, 2005; Liu and Hua, 2001). It has a restricted distribution in a region that is heavily-impacted by human activities such as sight-seeing, therefore continued biological research and regional conservation are strongly recommended.

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A New Species of Brown Frog from Bohai, China

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Abstract.- A new species of brown frog is described from Mt. Culai in Shandong province, China. The new species differs from other Chinese members of the *R. longicrus* group (*R. zhenhaiensis, R. chaochiaoensis* and *R. omeimontis*) in that the head is wider than long and female leg is longer than that of the male. Furthermore, the body is larger the web on the inner side of the male fifth toe nearly extends to the toe tip, the dorsal color is reddish-brown, there are no gray or dark bars across the eyes or spots on the back, the labial tooth row formula is frequently 3(2-3)/3, the male tibia is slightly longer than the foot, the dorsal masculine line is absent and the ventral masculine line is weakly developed.

Keywords.- Ranidae, *Rana*, brown frog, new species, China.

Introduction

Brown frogs, also known as wood frogs (Liu, 1946), are a widespread, complex and diverse group in the genus *Rana*. Thirteen brown frogs are known from China, five of which were previously recognized as *R. japonica* (Pope and Boring, 1940). These five species, together called the southern Chinese Brown Frog (2n = 26), occur south of the Yangtse River and in Taiwan. The brown frog in Taiwan was revived as *R. longicrus* Stejneger, 1898 and the species on the mainland were subsequently considered to be *R. chaochiaoensis* Liu, 1945, *R. chevronta* Hu and Ye, 1981, *R. omeimontis* Ye and Fei, 1993, *R. zhenhaiensis* Ye, Fei and Matsui, 1995 and *R. japonica* (Fei et al., 1993; Fei et al., 2005; Liu and Hu, 1961; Ye et al., 1995).

The species of southern Chinese brown frog do not overlap in their distributions. *Rana chaochiaoensis* and *R. omeimontis* are more western in distribution, with the former found in Yunnan, Guizhou and Sichuan Provinces, and the latter found in Sichuan and Gansu Provinces, as well as some counties in Guizhou, Hunan and Hubei Provinces (Fei et al., 2005, Li et al., 2005). *R. zhenhaiensis* occurs in eastern and southeastern China, in Anhui, Jiangsu, Zhejiang, Jiangxi, Hunan, Fujian, Guandong and Guangxi Provinces (Fei et al., 2005; Li et al., 2005). *R. chevronta* is also western in distribution, found only on Mt. Omei (Fei, 1999; Fei et al., 2005; Li et al., 2005). In northern China, *R. japonica,* now replaced by *R. zhenhaiensis* and no *R. japonica* in China (Ye et al., 1995), was recorded formerly at Mt. Culai in Shandong Province and Jixian county in Tianjin (Wang et al., 1997; Wang et al., 1995). The two other species of brown frog found in northern China are *R. chensinensis* and *R. kunyuensis,* found in Shandong peninsula (Li et al., 2006; Lu and Li, 2002), 500 km away from Mt. Culai.

The present study, part of a project funded by the National Natural Science Foundation of China, was conducted in a region near the Bohai Sea, where the authors collected three species of brown frogs (Li et al., 2005; Li et al., 2006; Lu and Li, 2002). Several specimens resembling *Rana zhenhaiensis* and *R. omeimontis* were collected on Mt. Culai, and were subsequently described as a "species belong to *Rana longicrus* species group in Shandong Province" (Lu et al., 2005). However, after comparing these specimens with representatives of *R. zhenhaiensis* and *R. omeimontis* collected from their type localities and the original descriptions of these species, the frog from Mt. Culai appears to be a distinct and separate species. This new species is here placed within *Rana* as part of the *R. longicrus* group and its relationship to other Chinese members of the group is discussed.

Materials and Methods

From May 2005 to March 2006, surveys were conducted at Mt. Culai, Shandong Province (Fig. 1), and the type localities of *Rana zhenhaiensis* (Caiqiao of Beilun (formerly Zhenhai County), Zhejiang Province) and *R. omeimontis* (Longdong of Mt. Omei, Sichuan Province), where adults, juveniles and tadpoles were collected. Some tadpoles were reared through metamorphosis to confirm their identification, as well as to describe and compare their coloration with respect to the adults, or at least until stages 36–38 for proper description. Specimens were raised in captivity in plastic boxes (260 x 175 x 160 mm) filled with 1.5 L of water. Egg yolk and

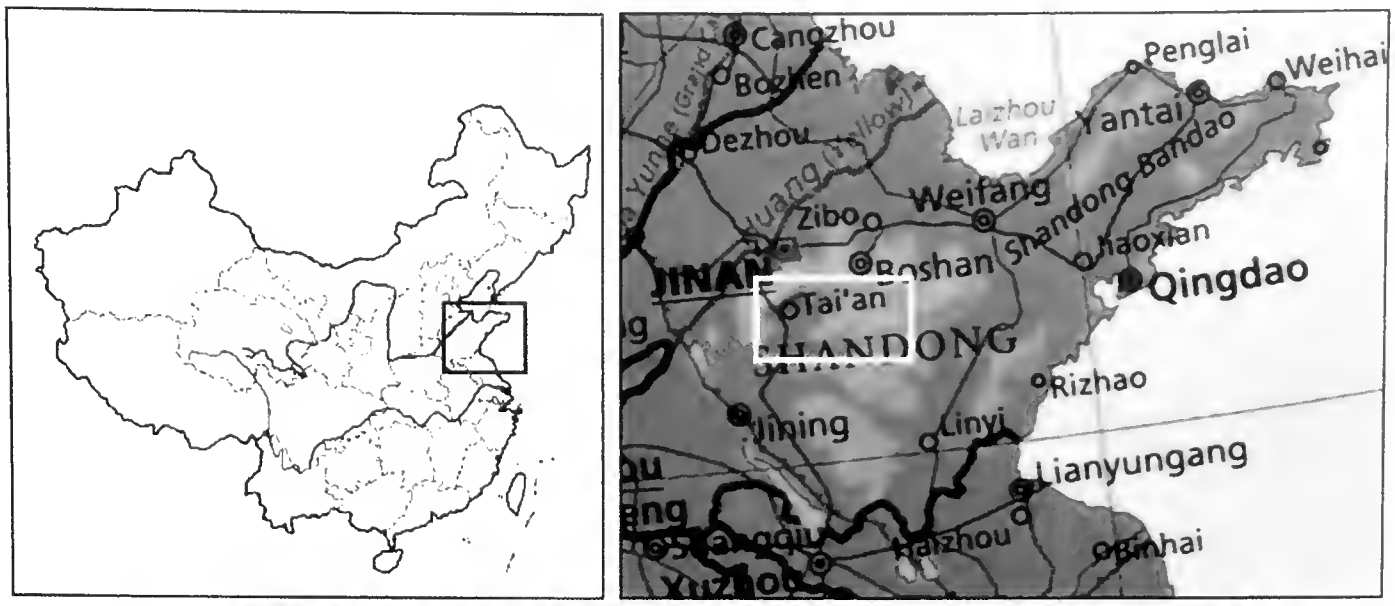

Figure 1. Collection Area in Shandong province, China. △: Culai Mountain.

vegetable leaves were regularly provided. Frogs and tadpoles were preserved in 10% formalin and deposited in the collections of Shenyang Normal University.

Measurements were made with digital calipers to the nearest 0.01 mm. Abbreviations are as follows: SVL = snout-vent length; HDL = head length, from tip of snout to rear of jaws; HDW = maximum head width; SNT = snout length, from tip of snout to anterior corner of eye; EYE = diameter of exposed portion of eyeball; IND = internasal distance; IOD = interorbital distance at narrowest point; TMP = horizontal diameter of tympanum; TEY = tympanum-eye distance, from anterior edge of tympanum to posterior corner of eye; FAHL = forearm and hand length; FAW = forearm width; TLL = total length of leg; TIB = tibia length; TFL = tarsus and foot length; FL = foot length, from proximal edge of inner metatarsal tubercle to tip of fourth toe.

All tadpoles were staged according to Gosner (1960). Tadpoles in stage 33, including both reared specimens and those preserved immediately after capture, were measured and used in descriptions. Measurements and terminology follow McDiarmid and Altig (1999). The labial tooth row formula follows those outlined by McDiarmid and Altig (1999) and Dubois (Li, 2006).

All measurements were taken with a digital caliper (to the nearest 0.01 mm) under a stereomicroscope, except for total length, which was measured with the caliper directly. Photographs were taken with Nikon D100 and Sony 717digital cameras.

Data from *Rana zhenhaiensis* and *R. omeimontis* were taken from their original descriptions and from additional specimens collected from their respective type localities and other localities stored in Chengdu Institute of Biology (CIB) and Museum of Natural History of Shenyang Normal University (SYNU).

Taxonomy

***Rana culaiensis*, new species**

(Figs. 2–3)

Holotype and type locality.- An adult male, Field number YT050526007, collected by Lu Yuyan on 27 May 2005 from Mt. Culai (117° 18′ E, 36° 02′ N), Taian City, Shandong province, China (Fig. 1), at 900 m elevation.

Paratypes.- An adult female, Field number YT050526005, other information as forthe holotype.

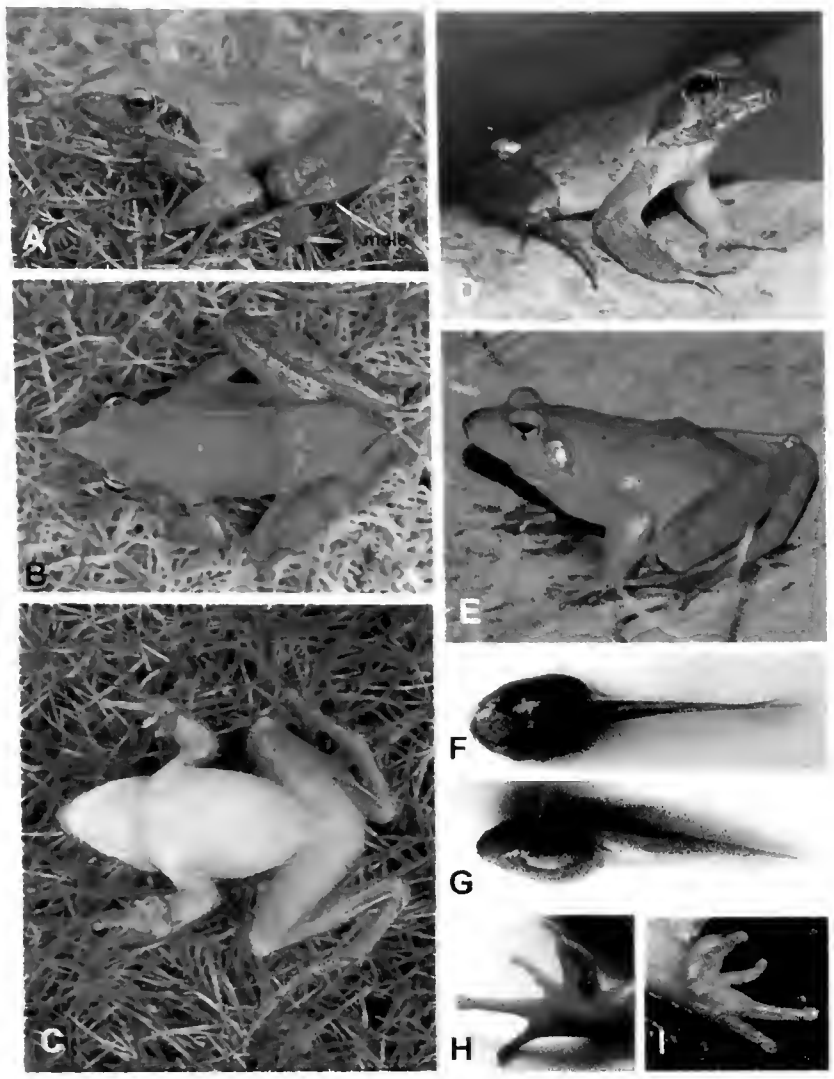

Figure 2. Holotype of *Rana culaiensis*, sp. nov. and its allied species from type localities. (A) Dorsal, (B) Lateral and (C) Ventral view of *R. culaiensis*, (D) Lateral view of *R. zhenhaiensis*, (E) Lateral view of *R. omeimontis*, (F) Dorsal and (G) Lateral view of tadpole of *R. culaiensis* at stage 33, (H) Palmar view of hand in *R. culaiensis*, (I) Palmar view of hand in *R. zhenhaiensis*.

Five males, Field number YT050526001-004, YT050526006, collected by Lu Yuyan on 27 May 2005 from Mt. Culai (36° 02~03′ N, 117° 17~18′ E, Taian City, Shandong province, China, at 690–900 m elevation.

Tadpoles.- Other information as for the holotype.

Diagnosis and comparisons.- This new species is superficially similar to *Rana zhenhaiensis*, but it can be distinguished by the following characters: 1) average snout-vent length larger in adult males (53.6 mm) and females (62.0 mm); 2) head length slightly less than head width; 3) toes 3/4 webbed, with web on inner side of male fifth toe nearly extending to tip; 4) dorsal color reddish brown, without gray or dark bar across eyes or spots on back; 5) dorsal masculine line absent and ventral masculine line weakly developed; 6) male tibia slightly longer than foot; 7) labial tooth row formula frequently 3(2-3)/3. Furthermore, with respect to similar brown frogs in China, the female leg of *R. culaiensis* is longer than the male leg.

On the other hand, this new species can be separated from all other southern Chinese brown frogs on the basis

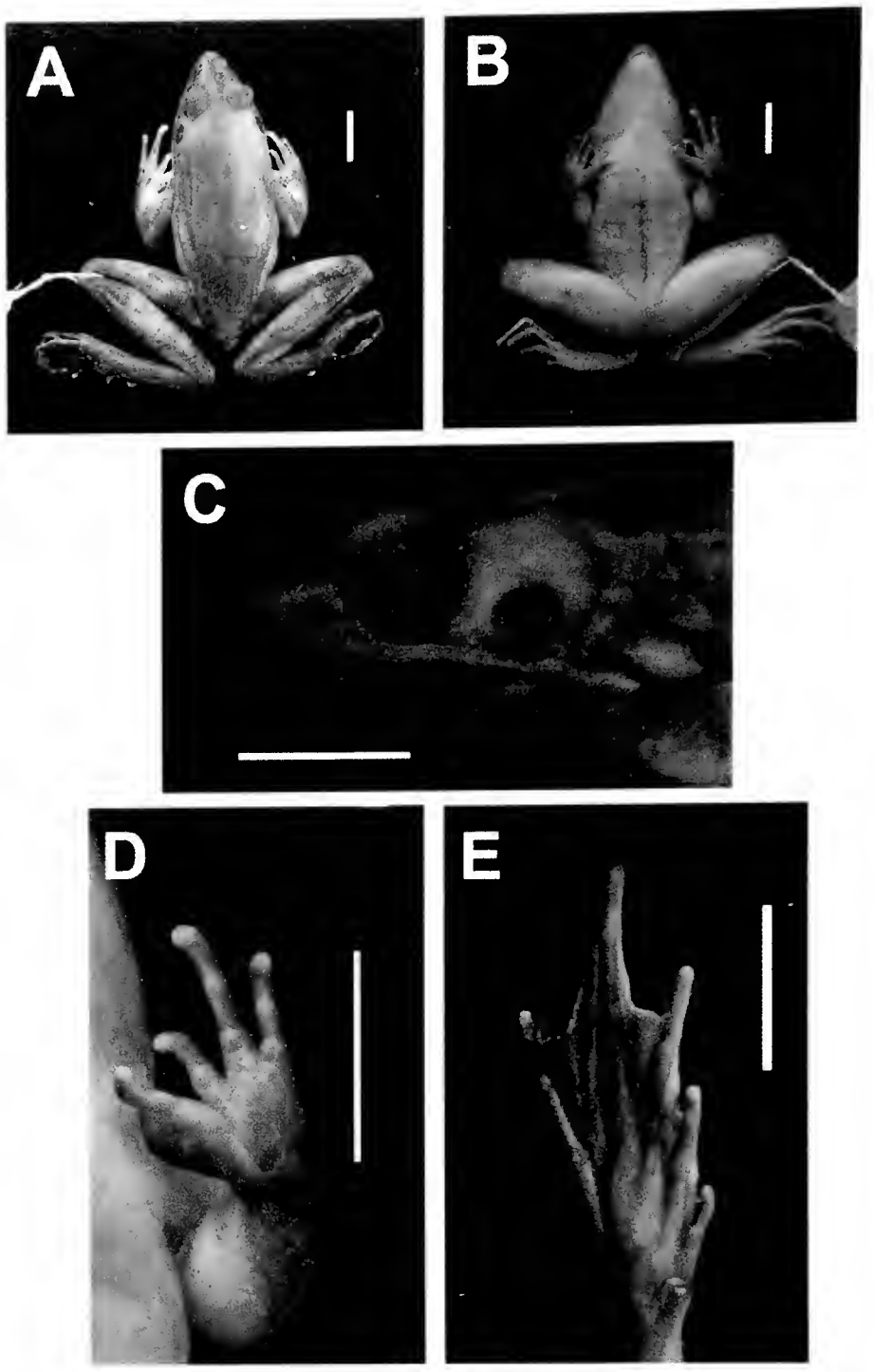

Figure 3. Holotype of *Rana culaiensis*, sp. nov. (A) Dorsal view, (B) Ventral view, (C) Lateral view of head, (D) Palmar view of hand, (E) Tarsal view of Foot. Scale bar = 10 mm.

of a head that is wider than long and a female leg that is longer than that of the male. When comparing *Rana culaiensis* to other southern Chinese brown frogs in the *R. longicrus* group, it appears to be a very well-defined species with conspicuous diagnostic features (Fig. 4; Table 2) that easily separate it from the superficially similar *R. zhenhaiensis*, *R. omeimontis* and *R. chaochiaoensis*.

From *R. zhenhaiensis* in having larger males and females, longer female legs, a more well-developed web on inner side of male toe 5 (ill developed in *R. zhenhaiensis*), an indistinct ventral masculine line (on both sides in *R. zhenhaiensis*) and a different breeding season (from March to April in *R. culaiensis* and January to March in *R. zhenhaiensis*).

From *R. omeimontis* in having slightly curved dorsolateral fold (straight in *R. omeimontis*), relatively longer female legs, an indistinct ventral masculine line (on both sides in *R. omeimontis*), a larger TMP:EYE ratio (0.66) and different breeding seasons (July to

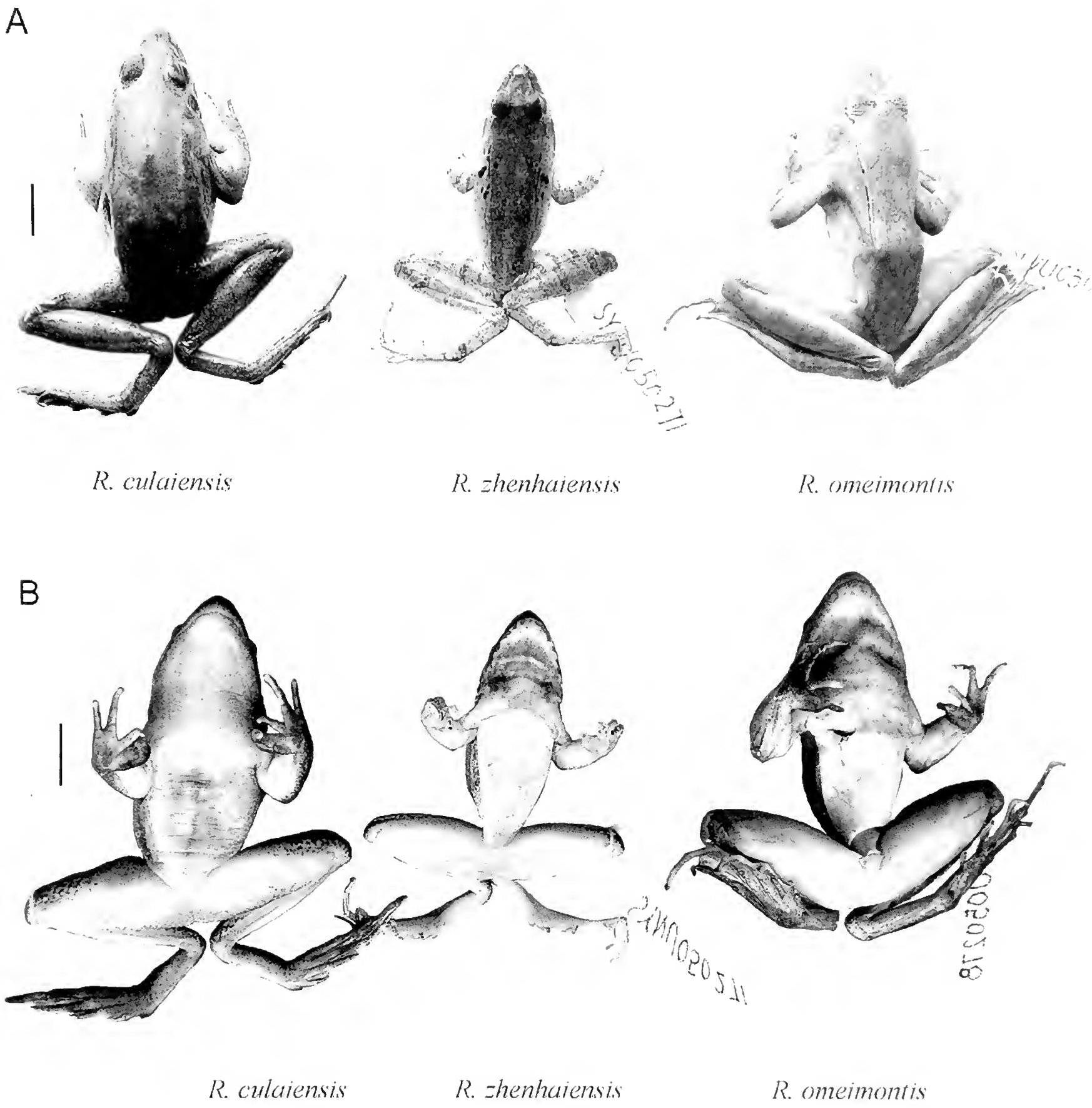

Figure 4. *Rana culaiensis*, sp. nov. and its allied species from their type localities. (A) dorsal view of male and (B) ventral view of male (scale bar = 10 mm).

October in *R. omeimontis*).

From *R. chaochiaoensis* in having slightly curved dorsolateral fold, relatively longer female legs (legs of subequal length in both sexes of *R. chaochiaoensis)*, an indistinct ventral masculine line, a yellowish-white female venter (reddish-orange in *R. chaochiaoensis*), three rows of teeth on the tadpole lower lip (four rows in *R. chaochiaoensis*) and a different breeding season (April to May in *R. chaochiaoensis*, with some breeding seen as late as August).

In distribution, *Rana culaiensis* is allopatric to its related species. It is a common case in the brown frogs in China, such as *Rana chevronta* which only found located at a narrow area in Mt. Omei (Fei et al., 2005) and *R. kunyuensis,* which is closed to *R. amurensis* and located in Mt. Kunyu only (Che et al., 2007; Li et al., 2005).

Description of holotype.- An adult female with SVL 62.0 mm; HDW slightly wider than HDL and head strongly depressed; snout rounded (more so on projection beyond lower jaw) and SNT slightly longer than EYE; *canthus rostralis* distinct, loreal region slightly oblique; nostril slightly closer to tip of snout; IND wider

Table 1. Measurements (mm) of allotype and paratypes of *Rana culaiensis*, sp. nov.

Characters	Allotype (♀) (n=1)	Paratypes (♂) (n=5)	Characters	Allotype (♀) (n=1)	Paratypes (♂) (n=5)
SVL	62.04	48.47~59.06*	TMP	4.78	3.41~4.32
		53.57±4.29**		7.70%	3.69±0.37
					6.89%
HDL	18.45	14.63~17.37	FAHL	28.25	21.52~26.62
	29.74%***	16.01±1.22		45.54%	23.71±1.96
		29.89%			44.26%
HDW	19.99	15.43~18.24	FAW	4.96	5.53~7.18
	32.22%	16.72±1.25		7.99%	6.31±1.00
		31.22%			11.78%
SNT	9.41	7.05~8.24	TLL	121.29	82.78-108.12
	15.17%	7.64±0.65		195.50%	95.92±10.84
		14.27%			179.05%
IND	5.48	4.39~5.44	TIB	39.35	26.29~34.99
	8.83%	5.07±0.45		63.43%	30.80±3.68
		9.46%			57.49%
IOD	4.51	3.45~3.91	TFL	53.12	38.99~48.89
	7.27%	3.64±0.19		85.62%	44.25±4.10
		6.80%			82.59%
UEW	4.07	3.12~3.97	FL	36.95	28.62~33.65
	6.56%	3.51±0.36		59.56%	30.29±3.18
		6.55%			56.54%
EYE	7.62	5.13~6.73			
	12.28%	5.91±0.62			
		11.03%			

Note: * size range, ** mean ± SD (n = 5), *** % SVL.

than IOD and IOD wider than upper eyelid width; tympanum large, round, TMP two-thirds diameter of EYE and separated from eye (TEY) by one-third of TMP; vomerine teeth developed in slightly oblique groups between and behind choanae, with groups narrowly separated in "\ /" shape; tongue deeply notched and with papillae.

FAHL less than half of SVL; fingers obtuse with relative length of fingers II < IV < I < III; subarticular tubercles prominent; three metacarpal tubercles distinct (inner one large and outer two separated at bases). TLL relatively long; tibio-tarsal joint reaching nostril, making TIB about 57.8% of SVL; heels overlapping when limbs folded at right angles to body; TIB slightly longer than FL; toes also obtuse with tips similar to those of fingers, toes 3/4 webbed with subarticular tubercles well developed; web of inner side of male fifth toe nearly extending to tip of toe; inner metatarsal tubercle oval, outer metatarsal tubercle weakly developed.

Skin rather smooth above, with few warts; glandular dorsolateral fold running along each side of body behind eye to insertion of hind leg and slightly curved to temporal fold above tympanum; temporal fold distinctly curving posteriorly from above tympanum to large triangular gray patch behind eye; ventral surface of body smooth except for posterior and median surfaces of femora, which are covered with coarse granular glands (small granules).

Measurement of holotype.- SVL 56.8 mm; HDL 17.15 mm; HDW 17.9 mm; SNT 7.8 mm; IND 5.32 mm; IOD 3.51 mm; UED 3.97 mm; EYE 6.26 mm; TMP 3.69 mm; FAHL 23.63 mm; FAW 7.6 mm; TLL 106.17 mm; TIB 34.2 mm; TFL 47.91 mm; FL 33.7 mm.

Coloration of holotype in life (in preservative).- Dorsum evenly reddish or yellowish-brown (gray), without gray or dark interorbital bars; stripe on upper lip dark brown with white blotches, extending from tip of snout to venter of eye, joining with dark reddish-brown

(yellow) stripe running to behind arm insertion; lower lip with brown speckles; rictal glands brown (yellow); limbs gray dorsally with four and five dark cross-bars on thigh and tibia, respectively; sides of body light yellow. Dorsolateral fold pale brown (yellow); throat and belly creamy-yellow (white with pale gray nebulous marks); triangular patch gray to somewhat black (gray with tympanum dark brown); metacarpal tubercles and nuptial pad dark brown (black).

Description of paratypes.- The paratypes, six adult specimens, one female (YT050526005) and five males (YT050526001-004, YT050526006) approximate the holotype in almost all pertinent details. The measurements of paratypes summarized in Table 1. However, there are some minor differences between paratypes and the holotype as follows:

MALE PARATYPES.- One male specimen with small tubercles on sides of body covered with black. And the following characters of male paratypes as secondary sexual characters: smaller than female with FAW much thickened; male with strong nuptial pads on the inner-dorsal side of first fingers, extended to figure tip and separated at metacarpus; no vocal sacs; lineae musculinae indistinct ventrally and absent dorsally; TLL is shorter than the female TLL.

FEMALE PARATYPE.- The female specimen with light jacinth spots ventrally in life (pale gay flecks in preservative).

Tadpoles.- The body ovoid in dorsal profile, pear-like in lateral profile; darkly colored in life (tail more grey in preservative). In stage 33, body length 16 mm, tail length 34 mm, length of hind limb 9 mm; snout rounded, eye dorsolateral, nostril slightly closer to snout tip; spiraculum small, on left side of body and with no free tube; vent dextral, tube of vent continuous with ventral caudal fin; dorsal fin rising from base of tail. Tail height about half of body length with apex obtuse; musculation weakly developed on pointed tip; mouth anteroventral, with row of labial papillae on lower lip and mouth corner (papillae of lower lip regularly arranged); corner of mouth with several additional papillae; labial tooth row formula frequently 3(2-3)/3, length of tooth row long, horny beak weak and narrow.

Habitat.- During field work at Mt. Culai of Shandong province in 2005 and 2006, we surveyed the Mt. Culai and the nearby mountains and collected the frogs described here as a new species in a forest brook (alt. 630–900m) covered with gramineous grass following the breeding season. The tadpoles in stage 28–34 were collected on 27 May 2005. No eggs were found at that time, suggesting that the breeding season for this species was in March and April.

Etymology.- *Rana culaiensis* is so named as it is apparently restricted to Mt. Culai of Shandong procince, East China.

Taxonomic account.- As Liu (1946) indicated, "among the Chinese amphibian the woodfrog group presents a problem most difficult to solve. Great confusion exists in the literature, as there has been no satisfactory comparative study of preserved museum specimens of different species, and no careful investigation in the fields." In some species groups, the frogs are quite conservative in their morphology and very difficult to separate (Liu and Hu, 1961; Lu and Li, 2002; Tanaka et al., 1996; Xie et al., 2000), as is also the case for the many species of Eurasian brown frogs (Kim et al., 2002). Although much progress has been made in the systematics of these species, many brown frogs are still difficult to identify and some species may yet remain undescribed (Che, 2007; Lu and Li, 2005).

The brown frogs of the *Rana longicrus* group (formerly treated as the *Rana japonica* group) included four species (*Rana zhenhaiensis*, *R. chaochiaoensis*, *R. omeimontis*, and *R. chevronta*) in the mainland of the southern China and one species (*Rana longicrus*) in Taiwan (Fei et al., 2005; Xie et al., 2000). These frogs once classified as *R. japonica* by Pope and Boring (1940), The species-level status of *R. chaochiaoensis*, *R. chevronta* and *R. longicrus* has never been questioned, but some researchers still treat *R. omeimontis* and *R. zhenhaiensis* as *R. japonica* (Zhao et al., 2000), even though Xie et al. (2000) provided significant morphological, ecological, cytological and morpholometrical support to verify the status of all five species. Well-supported phylogenetic analyses have also been provided (Che, 2007; Jiang and Zhou, 2001; Yang et al., 2001). The cluster tree provided by Xie et al. (2000) illustrates a close relationship between *R. omeimontis* and *R. chaochiaoensis*, as well as a close relationship between *R. zhenhaiensis* and *R. chevronta*.

The Culai frogs once reported as new province record under *Rana japonica* (Wang et al. 1997), but *R. japonica* was replaced by *R. zhenhaiensis* by Ye, Fei and Matsui (1995). Here we treated it as a new species with some difference from other related species in the group and it maybe close to *R. zhenhaiensis* and *omeimontis* (Fig. 4; Table 2). As allopatric to its congeners, it shows the same case of as *Rana chevronta* and *R. kunyuensis*.

Material examined.-

Rana chaochoensis from CIB (n = 70). (males)

Table 2. Comparisons between *Rana culaiensis* and its allies.*

Species	*R. chiaochiaoensis*	*R. omeimontis*	*R. zhenhaiensis*	*R. culaiensis*
Chromosome	2n = 26	2n = 26	2n = 26	2n = 26*
SVL (mm)	♂52.7, ♀52.9	♂58.5, ♀61.0	♂46.1, ♀48.9	♂53.6, ♀62.0
Head shape	HL > BW	BL > BW	BL > BW	BL < BW
Dorsolateral fold	Straight	Straight	Slightly curved	Slightly curved
IOD:UED	smaller	smaller	smaller	larger
TMP: EYE	3-Feb	5-Mar	3-Feb	3-Feb
IOD:TMP	0.68	0.75	0.73	0.98
TIB:FL	1.05	1.03	0.96	1.03
HL/SVL (%)	♂181.1	♂196.7	♂178.8	♂178.8
	♀183.5	♀182.4	♀171.5	♀195.5
Male's web of inner side of toe 5	Ill developed	Well developed	Ill developed	Well developed
Color of female ventral side	Reddish Orange	Yellowish white	Yellowish white	Yellowish white
Nuptial pad	Extend to the figure tip, no separated at metacarpus	Extend to the figure tip, separated at metacarpus	Extend to the figure tip, slightly separated at metacarpus	Extend to the figure tip, separated at metacarpus
Masculine line	Both	Both	Both	Ventral only
Denticle formula on the lower lip of tadpole	4 lines	3 lines	3 lines	3 lines
Tadpoles live through winter or no	No or yes	Yes	No	No
Breeding season	Apr.–May, some to Aug.	Sep.–Oct.	Jan.–Mar.	Mar.–Apr.
Elevation range	2000–2000m	1500 m	10–1400 m	630–900 m

* data of *Rana chaochiaoensis*, *R. omeimontis* and *R. zhenhaiensis* cited from Xie et al. (2000).

CIB37841, CIB37842, CIB37847, CIB37850, CIB37851, CIB37852, CIB37855, CIB37857, CIB37859, CIB37866, CIB37867, CIB37870, CIB37873, CIB37877; (females) CIB378853, CIB37860, CIB37862, CIB37869, CIB37874, CIB37876 and CIB84495 from China, Sichuan Province, Zhaojue County (as Chaocho formerly, type locality). (males) CIB37643, CIB37683, CIB37703; (females) CIB37641, CIB37642, CIB37644, CIB 37681, CIB37699, CIB37700 and CIB37707 from China, Sichuan Province, Yuexi, Mianning, and Yanyuan Counties. (males) CIB37712, CIB37713, CIB37715, CIB37723, CIB37731, CIB37734, CIB37735, CIB377747, CIB37748, CIB37754, CIB37755, CIB37756, CIB37758, CIB37760; (females) CIB37720, CIB37721, CIB37724, CIB37725, CIB37773 and CIB37777 from China, Guizhou Province, Weining County. (males) CIB37796, CIB37799, CIB37798, CIB37801, CIB37802, CIB37803, CIB37804, CIB37805, CIB37806, (females) CIB37786, CIB37787, CIB37788, CIB37790, CIB37792, CIB37793 and CIB37830 from China, Yunnan Province, Kunming City. (males): CIB37840, CIB37839 and (female) CIB37838 from China, Yunnan Province, Lijiang County.

Rana chevronta from CIB (n = 1): (male) CIB65I0028 from China, Sichuan Province, Mt. Omei (type locality).

Rana omeimontis from SYNU (n = 8): (males) SYNU050274, SYNU050275, SYNU050278, SYNU06080522, SYNU06080523; (females) SYNU050276, SYNU050277 and SYNU050279 from China, Sichuan Province, Omei Mt (type locality).

Rana zhenhaiensis from SYNU (n = 18): (males) SYNU050267, SYNU050268, SYNU050271, SYNU06020126, SYNU06040129, SYNU06040130, SYNU06040131, SYNU06040132, SYNU06040133, SYNU06040134, SYNU06040135; (females) SYNU050269, SYNU050270, SYNU050272,

SYNU050273, SYNU06020127, SYNU06020128 and SYNU06040136 . China, Zhejiang Province, Beilun region (formaerly Zhenhai county, type locality).

Acknowledgments

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The Tadpole of A Little-known Frog, *Rana tormotus* Wu, 1977

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Abstract.- The tadpoles of a Chinese endemic and rare frog, *Rana tormotus* were collected from the type locality and reared with other coexisting tadpoles of *Paa spinosa, Rana schmackeri* and *Amolops wuyiensis* in comparison. The tadpole of *Rana tormotus* is small (about 27 mm in total length) and brown to slightly olive in color. Its body is ovoid in dorsal view, widest at about midpoint, depressed and elliptical in lateral view. Body length is nearly one-third of total length. Lateral line pores are visible on the body and tail. No glands (such as ventral and dorsal glands in tadpoles of genus *Amolops*) are visible. The snout is round, slightly flatted in dorsal profile, and rounded in lateral and ventral profiles. Eyes directed dorsolaterally with diameter 31% of body height, closer to tip of snout than eye. Spiracle is short and sinistral posteriorly. Tail approximately 2.1 times body length. Tail fins convex and approximately fusiform. Tail tip is V-shaped or narrowly rounded. Vent tube is dextral and attached to ventral fin.

Oral disc is large and anteroventral in position. Labial tooth row formula 5(2-5)/4(1). Upper jaw sheath is finely serrate and narrow, lower jaw sheath is finely serrate and shallowly V-shaped. No abdominal sucker was observed behind the oral disc. It is similar to those of *Rana andersonii* and *R. schmackeri* in shape and oral disc characters.

From the characters of the tadpole of *Rana tormotus*, it does not belong to the *Amolops* type. It should be placed in the genus *Rana* (*sensu lato*) as *Rana tormotus* firstly or placed in a new genus (as *Wurana* by Li et al. [2006]) by further analysis.

Keywords.- *Amolops tormotus, Rana tormotus*, *Wurana tormota,* tadpole, sunken ear frog, concave-eared torrent frog.

Introduction

Rana tormotus Wu, 1977 is an arboreal frog in the family Ranidae found in the mountain streams of the Anhui and Zhejing Provinces of Eastern China (Sichuan Institute of Biology (Wu, G. F.), 1977; Zhao and Adler, 1993). This frog has an interesting characteristic: the males warble melodies like a bird in order to attract females (Feng et al., 2002), calling nightly from the low vegetation along the banks of rivers and streams. Their vocal repertoire is extraordinarily rich; individual calls exhibit multiple upward and downward frequency sweeps, rapid frequency "steps," and sudden onset and offset of selective harmonic components within a note (Feng et al., 2002; Peter et al., 2004). This is the first species of frog known to use diverse rising and falling modulations – most frog calls only go either up or down. These calls are also the first terrestrial frog noises known to extend into the ultrasonic range (Feng et al., 2002; Peter et al, 2004). Both of these phenomena are related to the frog's unique ear.

This frog, which is called the "sunken ear frog" or "concave-eared torrent frog" in Chinese, has a conspicuous character that makes it different from most other frogs: the males have visible ear canals leading to eardrums within the skull, similar to *Amolops cavitympanum* Boulenger (Sichuan Institute of Biology [Wu G. F.], 1977; Feng et al., 2002; Fei et al., 1991, 2005). Fei et al. (1991, 2005) and Dubois (1992) placed this species in *Amolops* because its tadpole might "belong to [the] *Amolops* type" (Fei et al., 1990 (1991); Zhao and Zhao, 1994). Because the tadpoles have never been recorded, however, it has been argued that this species should instead be assigned to "*Rana*" (Zhao and Zhao, 1994; Zhao et al., 2000; Global Amphibian Assessment, 2005).

Here, for the first time, we describe the tadpole of this little-known species of frog and provide information on its natural history. The importance of these findings lies in the necessity for larval characters in anuran classification (Chou and Lin 1997), and may allow for clarification of the uncertain position of *Rana tormotus* in relation to *Rana* and *Amolops*.

Materials and Methods

Field work was conducted in a small stream of Longjiang Forest, Zhejiang Province (China), and the type locality of Taohuaxi stream in the Huangshan Mts, Anhui province. Field studies were done from June to August 2005.

The tadpoles of *Rana tormotus* Wu, 1977, *Paa spinosa* (Daivd, 1875), *Rana schmackeri* Boettger, 1892 and *A. wuyiensis* (Liu and Hu, 1975) were observed and sampled in Taohuaxi, a permanent stream. Some tad-

poles of *R. tormotus* and *R. schmackeri* were reared until stages 36–38 (Gosner, 1960) for description, or through metamorphosis to confirm their identification and to describe and compare adult coloration. Tadpoles were raised in captivity in a plastic box (260 x 175 x 160 mm) with 1.5 L of water. Egg yolk and vegetable leaves were provided regularly. The tadpoles were preserved in 5% formalin. This material, together with adult voucher specimens, is deposited in the collections of Shenyang Normal University.

Tadpoles were staged according to Gosner (1960). Tadpoles in stage 38, included both reared and freshly captured specimens, were used in the descriptions and measured. No changes were observed in the oral morphology or general shape of reared tadpoles. Measurements, terminology, and labial tooth row formula follow Altig and McDiarmid (1999); labial tooth row formula also follows Dubois (1995).

All measurements were taken with a digital caliper (0.01 mm) and a stereomicroscope, except for total length, which was measured directly with a hand caliper. Drawings were made with the aid of a camera lucida attached to a stereomicroscope. The photographs were taken with a Nikon D100 camera.

Measurements are abbreviated as follows: BL (body length), TL (total length), TaL (tail length), BW (maximum body width), BH (maximum body height), TH (height of tail), DFH (dorsal fin height), VFH (ventral fin height), SO (snout-ocular axis distance), SN (snout-nasal axis distance), E (eye diameter), IN (internarial distance), IO (interorbital distance), SS (snout-spiracle distance), ODW (oral disc width). Standard measurements are shown in Figure 1.

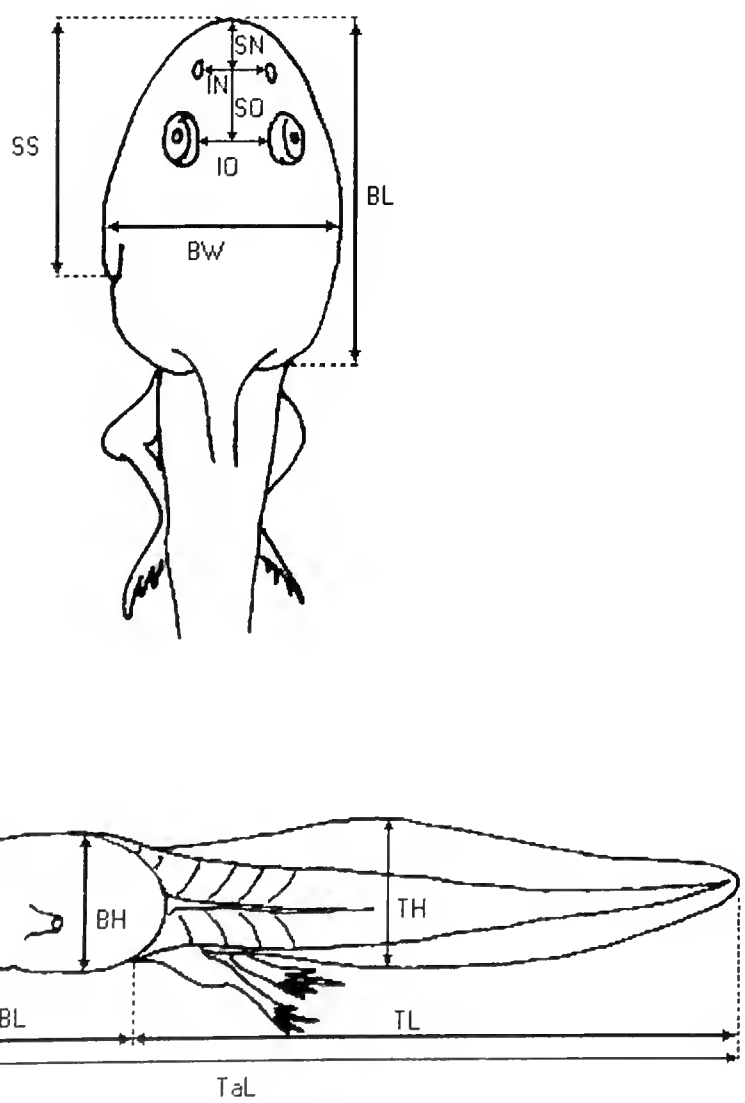

Figure 1. Standard morphometric measurements for tadpoles used in this study.

Decription of external morphology at stage 38.- The mean measurements and standard deviations of eight tadpoles in stage 38 are shown in Table 1. Mean total length at stage 38: 27±1.07 mm (n = 8; Table 1). Body ovoid in dorsal view, widest at midpoint, depressed and elliptical in lateral view; lateral part of marginal papillae of oral disc slightly visible; Body length nearly one-third (32%) of total length, body 1.5 times longer than wide, 2.1 times longer than high, 1.4 times wider than high. Lateral line pores (neuromasts of caudal, dorsal, supranaso-orbital, infranaso-orbital, lateral, mental, postgular, postspiracle and pregular lines) visible on body and tail. No glands visible. Snout rounded, slightly flatted in dorsal profile, rounded in lateral and ventral profiles; eyes moderate, not part of dorsal profile, directed dorsolaterally, diameter 31% of body height, separated by distance about 2.8 times eye diameter; interorbital distance 88% of body width; nostrils directed dorsolaterally, closer to tip of snout than eye, internarial distance 60% of interorbital distance.

Spiracle sinistral, short, posterior, opening slightly above midline at about 5/7 of body length, directed posterodorsally at about 15°, lateral wall longer than medial wall, inner wall confluent with body, wall forming around aperture.

Tail approximately 2.1 times body length and 3.0 times body width, maximum height 28% of tail length, maximum tail height at end of first third of tail length. Tail musculature highest at base, slightly higher than dorsal and ventral fins, gradually tapering to pointed tip, weakly developed. Tail fins convex and approximately fusiform. Dorsal fin originates from tail muscle (the posterior edge of the first section) near tail-body junction, tallest just past to midpoint; ventral fin of nearly equal height throughout its length; dorsal fin height 1.3 times ventral fin height at highest point. Tail tip V-shaped or narrowly rounded; vent tube dextral, short, attached to ventral fin.

Oral disc large, anteroventral in position, width about 0.73 times distance between eyes and approximately 45% of body width, emarginate laterally, with single row of truncate marginal papillae in lateral posterior margin of upper lip and wide rostral gap, two rows of completely marginal papillae on lower lip but bases of papillae originate in same line; rostral gap equal in length of A-1. No lateral submarginal papillae. Labial tooth row formula 5(2-5)/4(1) and 1:4+4/1+1:3 (following Dubois [1995]); A-1 and A-2 longest, slightly longer

Table 1. Mean measurements and standard deviation (mm) of eight tadpoles in stage 38 of Gosner (1960).

	Mean±SD	Range
TL	27.13±1.07	25.91–27.86
BL	8.69±0.13	8.52–8.92
BW	5.83±0.24	5.47–6.30
BH	4.12±0.20	3.81–4.46
TaL	18.45±1.06	17.10–20.43
TH	5.14±0.36	4.82–5.71
DFH	1.58±0.14	1.40–1.72
VFH	1.21±0.14	1.00–1.41
E	1.28±0.09	2.07–2.20
SO	3.07±0.14	2.68–3.19
SN	1.26±0.13	1.07–1.35
IO	3.62±0.09	3.48–3.75
IN	2.16±0.07	2.07–2.30
SS	6.6±0.23	6.24–6.96
ODW	2.63±0.09	2.51–2.74

than all other rows and similar in length, A-2 gap narrow, length of row becoming progressively shorter from A-3 to A-5 (A-5 22% length of A-2); P-2 and P-3 equal in length and longer than P-1 and P-4 (80% P-2); A-2 gap narrower than P-1. Labial teeth small, blunt and devoid of cusps; longest at middle of row, teeth becoming progressively smaller from P1 to P4, but equal in P2 and P3, teeth of A1 smaller than A2 and those of A2 equal. Jaw weakly developed. Upper jaw sheath finely serrate, narrow, width slightly less than width of lower jaw; lower jaw sheath finely serrate, shallowly V-shaped.

From stage 26 onward, labial tooth row formula stabilizes with very small variation in some specimens. Larval denticles disappear after disappearance of vent tube. Tadpole matching Orton's Type-IV category (Orton, 1953): oral disc elaborate and spiracle sinistral.

In preserved specimens, dorsal surface of body dark brown; gut and heart visible ventrally, not visible laterally; anterior half of ventral part of body pale brown with dark brown spots on sides, and abdominal region whitish without dark brown spots. Coloration of muscular part of tail similar to that of body in reticulated pattern. Tail fins with small brown spots.

In life, tadpole body coloration dark-brown with head pale brown. Pupil of eye round, black, and enclosed by narrow sliver ring.

Natural history notes.- Relative to the size of the adult (male 35 mm, female 48 mm), the tadpole is small. If the tadpole had not been reared to adulthood, it would have been difficult to believe that the tadpole and frog were of the same species.

The tadpoles gathered in groups in the small stream, and were found to swim freely among small stones where there was no swift water current or side pools that were poorly connected with the main permanent stream at night. During the daytime, the tadpoles hid under stones and were rarely seen. The tadpoles were nocturnal and used their sites for grazing, avoiding areas with silty sediment and fast water flow. The large oral disc and numerous blunt rows of teeth suggested a greater capacity for grazing than for suspension feeding.

The color of the tadpoles camouflaged them against the small stones and sand on the bottom of the stream where they spent the day. Although collected from a mountain stream, the tadpoles were able to live in their jars for a long time, sometimes in good condition after three day's travel. After 40 days of captivity, the larvae completed metamorphosis and became froglets similar to the adult in body shape and coloration. The biggest of the tadpoles was less than 30 mm in total length. Froglets were 11.4 mm (10–11.6 mm, n = 5, SD = 0.73) from snout to vent just after metamorphosis.

The frogs and larvae that were found coexisting with *Rana tormotus* in the stream were *Paa spinosa*

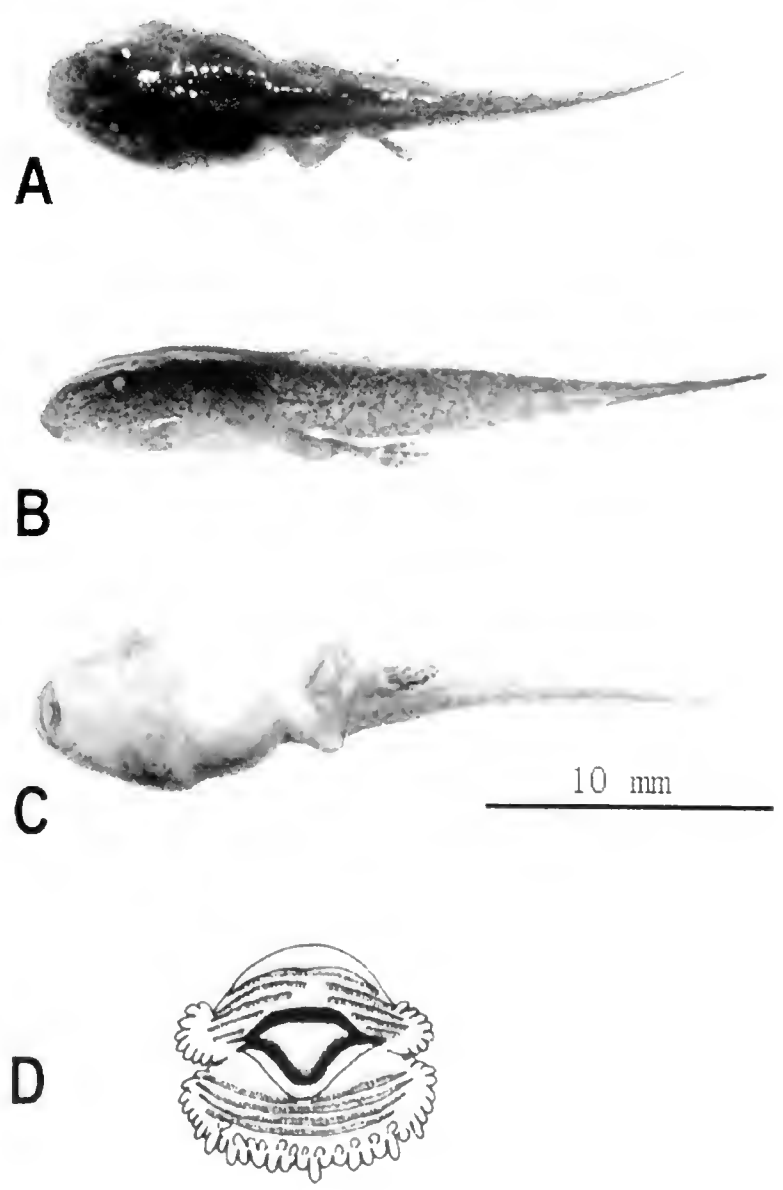

Figure 2. *Rana tormotus* tadpole at stage 38 (Gosner, 1960), (A) Dorsal view, (B) Lateral view, (C) Ventral View, (D) Oral disc.

(Daivd, 1875), *Rana schmackeri* and *A. wuyiensis* (Liu and Hu, 1975). While the tadpole of *R. schmackeri* was similar to *R. tormotus* in shape, coloration and general oral disc characters, the other two appeared conspicuously different.

Although *Rana schmackeri* also had a small tadpole belonging to Orton's Type-IV category (Orton, 1953), it was larger than the tadpole of *Rana tormotus*. The total length of *R. schmackeri* tadpole was more than 30 mm from stage 35, while the maximum total length of the *R. tormotus* tadpole was less than 30 mm at any later stage. At stage 36, the body and tail length was 11.1 mm and 20.25 mm for *R. schmackeri*, and 8.36 mm and 18.83mm for *R. tormotus*. At stage 44, the back of *R. schmackeri* became greenish, and after metamorphosis, yellow patches appeared on the back.

The tadpole of *Amolops wuyiensis* was also of the small type, but the ventral suctorial disc easily identified it. This species was found at the edge of side water-bodies of the stream with little current. The tadpole used the suctorial ventral disc to adhere to the rocky substrata to overcome the stream's water current.

The tadpole of *Paa spinosa* is of the big type, and is found alone or in groups of a few individuals at the bottom of pools beside or below cascades and gently flowing parts of the Taohuaxi stream. The oral disc of this species is emarginate, with two rows of marginal papillae, lateral submarginal papillae and a strong beak. The most conspicuous character of the tadpole is a gray or black band at the base of tail. The labial tooth row formula is 5(2-5)/3(1).

Discussion of taxonomic status.- Examination of the tadpole of *Rana tormotus* reveals that it is not of the "*Amolops* type", because there is no abdominal sucker and no ventral or dorsal glands (Yang, 1991). The tadpole of this species is more similar to that of *Rana andersonii* (Liu, 1940) and *O. schmackeri* in shape and oral disc morphology, but the tadpole and froglet just after metamorphosis is smaller, differently shaped, and without green or yellow back patches.

The body and tail length of the *Rana andersonii* tadpole averaged 12.27 mm (12–12.5 mm, n = 3) and 26.27 mm, and the froglet 12.7 mm (Liu, 1940). Four days after metamorphosis, uneven green patches appeared on the back (Liu, 1940).

While the larvae are similar in appearance, the adults of odorous frogs and *Rana tormotus* are remarkably different in morphology, with the sunken ear and absence of odor gland cells in the skin of *R. tormotus* being most the notable characters.

Based on the above evidence, this species should either be left to *Rana* as *Rana tormotus*, or placed in the new genus as *Wurana tormota*, although further study is needed.

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A Brief Report on the Life History of *Batrachuperus taibaiensis* at Ping He Liang of Tsinling Mts.

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Abstract.- *Batrachuperus taibaiensis* is a high-mountain, stream salamander endemic to China, and compared to congeners, is more northeastern in distribution and found at lower elevations. The distribution, life history and measurements of the larva and adult of this species were recorded from 2005 to 2006 at Ping He Liang of Tsinling Mts. The life history of the genus is discussed in the context of refining species definitions for those *Batrachuperus* found in the Tsinling Mts. Past surveys carried out in Tsinling Mts found several different *Batrachuperus* species and there is a need to clarify which species really exist. The distribution of *Batrachuperus taibaiensis* is discussed in the paper.

Keywords.- Life history, stream salamander, *Batrachuperus taibaiensis.*

Introduction

Much of the taxonomy and phylogeny of the Chinese Amphibia remain unresolved, as is much of the knowledge on their basic natural history, generally because observations on natural history are not considered to be worthy of publication and the gathering observations is often time intensive (Greene, 1993). Nevertheless, these observations may contribute to phylogenetically informative characters (de Queiroz and Wimberger, 1993) and data critical for developing conservation and management strategies (Mendelson et al., 1999).

The high-mountain stream salamanders, genus *Batrachuperus* Boulenger, 1878, contains seven species that occur in Western China and adjacent Myanmar (Frost, 2007). The natural history of the species in this genus remains largely unknown or unpublished because individuals generally hide under stones in small mountain streams at high altitude, although reports on the common species *B. pinchonii* (David) have been made by Liu (1945).

Recently, one new species (*Batrachuperus taibaiensis*) was found in the upper Heihe River in the Tsinling Mts. of Shaanxi Province, China. Compared to congeners, this salamander is more northeastern distribution and found at lower elevations (Song et al., 2001). During collecting trips (1987–2005) along the rivers and streams of Ping He Liang, the senior author collected several *B. taibaiensis* and collected data on their natural history. The following is a brief excerpt from the results of a series of herpetological surveys made from 2005 to 2006.

Materials and Methods

Amphibian surveys were made on the south side of the Tsinling Mts. (33° 36′ N, 108° 28′ E, 1800–2000 m) between Huo Di Tang and Xun Yang Ba of Ningshan County, Shaanxi province, China, from April 2005 to June 2006. Ping He Liang reaches an altitude of 2160 m at the National Way. *Batrachuperus taibaiensis* was also surveyed at its type locality on the north side of the Tsinling Mts in April 2005.

Adult specimens were stored in 10% formalin. Larvae and egg cases were observed live in the lab.

Results

Identification.- *Batrachuperus taibaiensis* (Fig. 1) can be separated from congeners by its relatively large body size and lack of horny covers on the palms and tarsa.

Distribution.- The distribution of *Batrachuperus taibaiensis* is concentrated in the upper part of two rivers and their associated streams on the northern and southern sides of Ping He Liang (Fig. 1). The pH of the water was somewhat acidic (pH = 5.5–6.0). Specimens were found under rocks in river headwaters and streams in the study area 2–3 km from the top of Ping He Liang (above 1800 m).

In the streams and rivers where *Batrachuperus taibaiensis* was found, adults of a number of other amphibians occurred: *Ranodon tsinpaensis* Liu and Hu, *Bufo gargarizans* Cantor, *Rana chensinensis* David, *Paa quadrana* (Liu, Hu and Yang), *Bufo andrewsi* Schmidt. Tadpoles of *Bufo andrewsi* and *S. ningshanensis* Fang

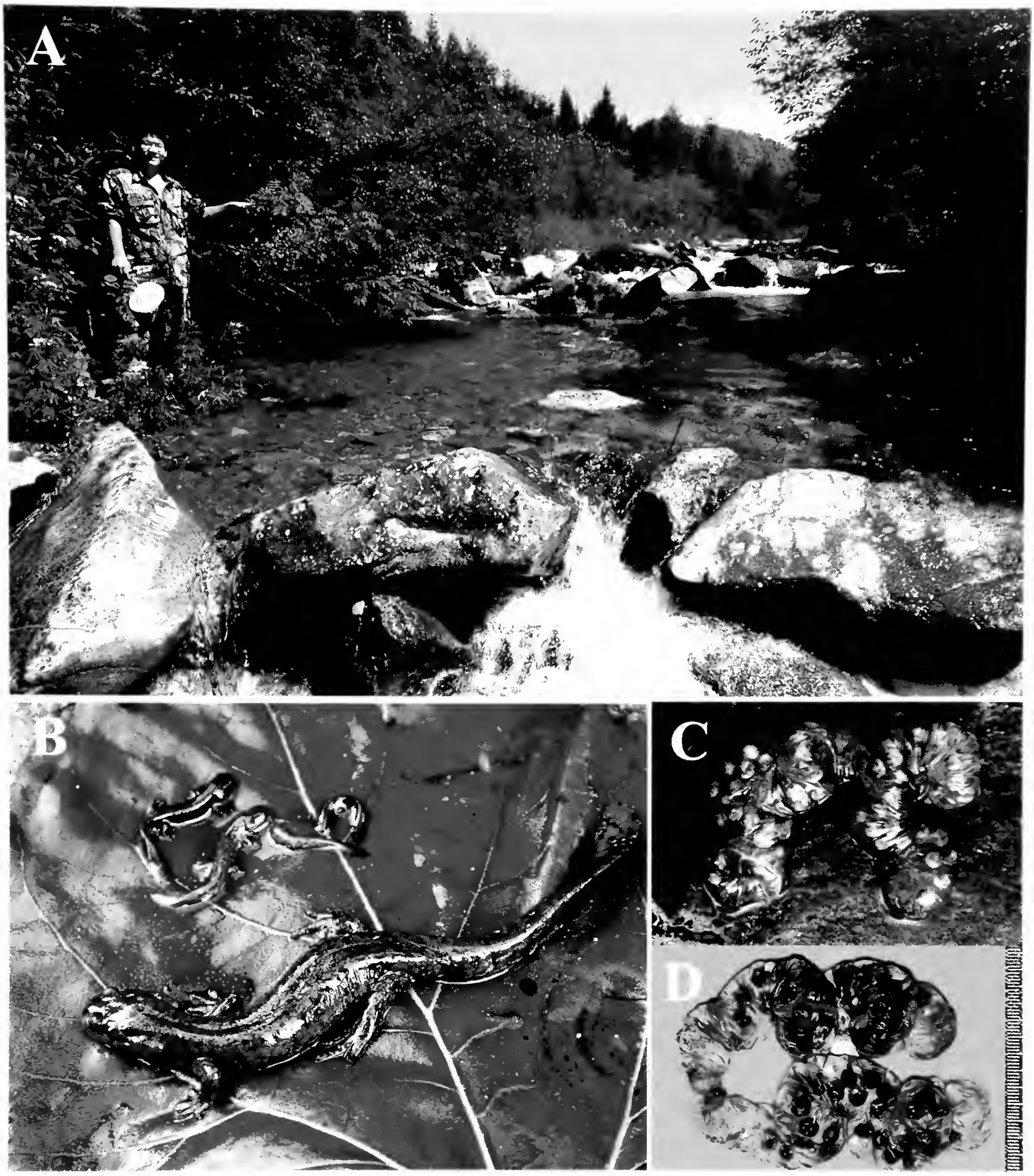

Figure 1. (A) Habitat of *Batrachuperus taibaiensis.* (B) Adult and larvae of *B. taibaiensis* from Ping He Liang. (C) and (D) are egg cases of *B. taibaiensis.*

were also observed, the latter of which has its type locality in the same region. *Hyla tsinlingensis* Liu and Hu, another endemic Chinese amphibian, was found in nearby ponds. *Bufo gargarizans* Cantor, *Bufo andrewsi* Schmidt, *H. tsinlingensis* Liu and Hu, *Rana chensinensis* David *P. quadrana* (Liu, Hu and Yang) and *Batrachuperus pinchonii* were more widespread at lower elevations near the study area.

The study area encompassed approximately 15 km^2 of Subalpine conifer habitat covered with well-developed vegetation. The canopy often arched over the rivers and streams.

Reproduction and growth.- The breeding season most likely occurs from April to July, as the youngest larvae with external gills and the most number of young salamanders in different stages of development were collected on August 3rd, 2005; young salamanders were not collected after April 16th, 2005.

Egg-cases, previously unknown for this species, were found adhered to the under-side of rocks in the river in April 16th, 2005. The body of the egg-case was a curled columnar tube with tapered ends; the case was smooth and almost entirely transparent with thin longitudinal striations; fresh cases were the color of milk; for-

Table 1. Measurements of *Batrachuperus taibaiensis* (n=3)

	larvae			adults	
	A	B	C	♂	♀
Body-Length (mm)	19.47	30.27	40.94	93.77	96.08
Head-Length (%)	26.3	32.58	34.96	23.91	24.69
Head-Width (%)	21.73	24.29	24.54	20.56	19.85
Length-Eye (%)	6.27	6.26	6.03	5.18	4.73
Trunk-Length (%)	44.94	43.99	41.28	54.85	53.44
For-Leg (%)	15	20.45	26.21	27.17	23.09
Hind-Leg (%)	11.61	20.34	31.45	29.43	31.51
Tail Length (%)	58.04	69.82	78.47	99.62	93.17
Tail Width (%)	9.14	12.59	12.41	12.16	11.82
Tail Height (%)	20.54	22.52	18.38	13.85	12.92

A: larva just after hatching; B: developed larva; C: larva with fill shrivel-ing

malin-fixed cases became brittle. The cup-like cap at the free end of the case was more soft and delicate than the remainder of the case (Fig. 1). Cases were 15.0–17.0 mm in length with a diameter of 18.0-20.0 mm. There were 27–29 eggs or embryos in each egg case. Egg diameter was 5.0–5.5 mm.

Free larvae appeared at stream edges under small stones at the beginning of August. The early larvae, compared to developed larvae, juveniles and adults, in that the color was lighter gray with the dorsum yellowish-green and the venter yellowish; the dorsal pigment faded after fixation. The forelimb of the early larva was fully developed with four formed fingers; the fourth toe bud appeared after formation of the first three toes. The fore limbs developed earlier than the hind limbs. The labial folds were well-developed and the pores of the lateral line organ were visible on the head. Eyes were small, black and covered with a transparent membrane; eyelids were absent. Balancers were absent from the side of head. There were four pairs of external gills (decreasing in size posteriorly); the filaments of the last gill were very short, white and hidden beneath the third gill; the first to third gills resemble those of *Batrachuperus pinchonii* (Liu, 1945). The vertebral groove was distinct along the length of the trunk. The tail was much higher and shorter than that of the adult.

The developed larva was blacker than the younger larva, and larvae with gill regeneration were nearly black. Pores of the lateral line organs were more conspicuous in the head and shoulder regions. Eyelids were well developed (as in *Batrachuperus pinchonii* [Liu, 1945]). Gill filaments were blacker and longer than those seen in earlier larva, but shorter than larva with gill regeneration. Fingers and toes were well-developed.

Fifteen specimens from various developmental stages were measured (Table 1). Larval head length was 26.3%, 32.58% and 34.96% of total body length, while adults head length was 23.91% in the male and 24.69% in the female. Larval eye length was slightly larger than that of adults.

Discussion

Need for clarification of* Batrachuperus *species in Tsinling Mts.- Song (1983) and Yuan (1984) reported *Batrachuperus pinchonii* from the Tsinling Mts. in Mao Tai Zi, Liuba County, and Huo Di Tang Forest, Ningshan County. Later, from 1985 to 1989, the senior author and a colleague from Shaanxi Normal University collected two species of *Batrachuperus* from Huo Di Tang and Xun Yang Ba Forests, Ningshan County, which are on the northern and southern slopes of the same Mountain: *B. pinchonii* was collected at lower elevations while *B. tibetanus* was collected at higher elevations (Li and Fang, 1993 and unpublished data). At the same time, Song collected a new species of *Batrachuperus* from Zhouzi County - *B. taibaiensis* – which was supported by sequence data from cytochrome b (Song et al., 2001). Unfortunately, these species were either excluded (Fei et al., 2005) or treated under incorrect names (Han and Lu, 2003; Zhang and Jia, 2002) in subsequent publications.

Batrachuperus occurs throughout the Tsinling Mts., extending to Foping, Zhouzi, Zashui and Ningshan Counties. *Batrachuperus taibaiensis* has also been found in Kangxian, Wenxian and Fenxian in Gansu Province, as well as Liuba and Nanjiang in northern Sichuan Province (Zeng, 2004); these identifications were made using molecular techniques because it is often difficult to identify species of *Batrachuperus* using traditional methods (Zeng, 2004).

Based on what is now known about the biology, morphology and distribution of *Batrachuperus taibaiensis*, we consider that all specimens of *B. tibetanus* collected from the Tsinling Mts south to Shaanxi, Southeastern Gansu and the North Sichuan Provinces (such as Micanshan and Dabashan) are actually representatives of *B. taibaiensis*. Considering this, the full range of *B. taibaiensis* may actually extend as far as southeastern Shaanxi Province and the Shenlongjia Mts in northwestern Hubei Province. To corroborate these assumptions, it will be necessary to re-examine previously collected specimens from these localities to verify their identity. Further fieldwork and molecular analyses will likely be necessary.

The need to focus on* Batrachuperus *life history.- The high-mountain stream salamander *Batrachuperus* currently was considered to contain seven species that occur in Western China and adjacent Myanmar (Frost,

2007). Most of these species are restricted to China, and *B. tibetanus* and *B. pinchonii* are significant in their use as experimental animals in embryological, morphological and ecological investigations (Li and Fang, 1993; Xu, 1992, 1993; Zhang and Jia, 2002; Han and Lu, 2003; Fei et al., 2005).

Excluding one report by Liu (1945) on *Batrachuperus pinchonii*, little data on life history has previously been available for species in this genus. Limited notes on *B. longdongensis* Liu and Tian, 1978, *B. cochranae* Liu, 1950 and *B. yenyuanensis* Liu, 1950 have also been published for populations in White-drag-on-pool at Chin-ting (Jinding) of Mt. Omei, Pao-hsing-hsien (Baoxing County), Yen-yuan-hsien (Yanyuan County) and Tien-shui (Tianshui) and Sia-ho (Xiahe) Counties of Gansu Province. Data from Fei et al. (2005) are also useful for understanding the biology of these native animals.

Here we have reported additional life history data for a new species in the genus, *Batrachuperus taibaiensis*, one of the largest stream salamanders in China. The tube-like egg case is longer and thicker than those in other species, it is strongly coiled and contains more (27–29) eggs. In comparison, the egg case of *B. pinchonii* is cylindrical and cayenne-shaped with 7–12 eggs (Liu, 1945); the case of *B. yenyuanensis* is linear and has 6–13 eggs (Zhao and Yang, 1997). The larvae of this species are also unique.

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An Investigation of the Morphometric Characteristics of Eggs of the Chinese Alligator (*Alligator sinensis*)

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Abstract.- In this study we analyzed morphometric intraspacific differences in the eggs of the Chinese alligator, *Alligator sinensis*, (egg mass, egg width and egg length). Data were collected for 1460 eggs from 40 clutches at the Anhui Research Center for Chinese Alligator Reproduction (ARCCAR). Our results found that the mean clutch size was 36.5 eggs, mean egg mass per clutch was 41.9 g, and clutch mass was 1519.7 g. We generated three regression equations relating to relationships among egg width, length and mass. The average of clutch size in 2004 was much higher than it was in 1985 as indicated by a two-sample t-test.

Keywords.- Egg shape index, egg length, egg width, egg mass, clutch size.

Introduction

Studies of the reproductive ecology of *Alligator sinensis* are important for our understanding of its conservation biology and status. Several studies about the reproductive ecology of *Alligator sinensis* are available and include information on egg incubation in captivity (Gu and Zheng, 1983; Liang and Pan, 1990), nest excavation (Huang and Watanabe, 1986), the relationships between egg hatching and environmental factors (Wang et al., 2000) and captive breeding (Cheng and Wang, 1984; Wang and Zhang, 2000; Xu et al., 1989). Egg characteristics are important when examining reproductive ecology, as has been illustrated in previous studies (Cariello et al., 2004; Du, 2003; Huang et al., 2003; Reese, 2000), but little of this research has been devoted to *Alligator sinensis*. We herein record data on egg characteristics of the Chinese alligator in the ARCCAR, and analyze these characteristics to investigate intraspecific differences, providing fundamental information for further study of the influence of egg shape on hatching rate and quality of young alligators.

Materials and methods

Measurements of eggs.- A total 1460 eggs from 40 clutches were collected between 5 and 16 July 2004 at the artificial breeding area of the ARCCAR. Eggs were collected within 12 hours of laying and taken to a hatching room where their length and width were measured with digital calipers (precision 0.01 mm). Egg mass was taken using a scale (precision 0.1 g).

Statistical analysis.- We analyzed the morphological characteristics of the eggs using the statistical software SPSS (Version 10.0). Data on the distribution of egg characteristics (including egg length, width, mass, egg shape index [length/width], clutch size, clutch mass and clutch mean egg mass) were analyzed using descriptive statistics. The coefficient of variation (SD/Mean) was used to study variation in egg characteristics. Regression analyses were used to examine the correlation among egg width, length and mass. Hoyt (1979) put forward an empirical formula:

$$W = K_W X Y^2$$

where K_W is the coefficient of mass, W is egg mass, X is egg length, and Y is egg width.

Results

Descriptive statistics of the morphological characteristics of the eggs of the Chinese alligator.- Table 1 provides descriptive statistics for the morphological characteristics of the eggs examined. From the frequency distributions of these morphological characteristics (Fig. 1), it is apparent that in most cases, egg length ranges from 51.42 mm to 60.00 mm, egg width from 32.49 mm to 37.51 mm, egg mass from 33.34 mm to 48.33 mm, clutch mass from 1200 g to 1800 g, clutch size from 30 to 45, and clutch mean egg mass from 35.0 g to 47.5 g.

The egg of the Chinese alligator usually has the form of an elongate ellipse, although some eggs deviated from this shape. From the frequency distribution of egg

Table 1. Descriptive statistics of the morphological characteristics of the eggs of *Alligator sinensis*.

	Maximum	Minimum	Mean	SD	N
Egg length (mm)	87.15	32.86	56.58	3.73	1460
Egg width (mm)	57.3	29.01	35.24	1.9	1460
Egg shape index	2.42	0.59	1.61	0.11	1460
Egg mass (g)	83.7	27	41.9	5.1	1460
Clutch size	46	15	36.5	6.2	40
Clutch mass (g)	1978.4	630.9	1519.7	266.7	40
Clutch mean egg mass (g)	51.1	32.7	41.8	3.9	40

shape index, it was evident that in most cases, egg shape falls between 1.50 and 1.72.

Intraspecific difference of egg morphological characteristics.- Since there were a high proportion of malformed eggs in four of the 40 clutches, only the 36 remaining clutches were analyzed. Figure 2 shows the CV (coefficient of variation) of egg morphological characteristics, indicating morphological variation among eggs of the same clutch.

There were differences in variation among the morphological characteristics of the eggs laid by different females. The CV of egg morphological characteristics were calculated from Table 1, revealing that variation in egg mass (CV = 0.18) and clutch size (CV = 0.17) were the greatest.

The correlations among the CV of egg morphological characteristics were analyzed using a Pearson correlation analysis (Table 2). Table 2 indicates that the CV of the egg shape index had a significant positive correlation with the CV of egg width. The CV of egg mass had a significant positive correlation with the CV of egg length.

Correlations of egg length, egg width and egg mass.- Data (including egg length, width and mass) collected from 1445 eggs (malformed and broken eggs not included) were used to generate scatter plots. These plots indicated that both egg width and length had a positive correlation with mass, while length was negatively correlated with width. In order to obtain the exact correlation between the two parameters, the influence of other parameters was eliminated by using a partial correlation analysis. The results showed that egg length had a significant positive linear relationship with egg mass ($r = 0.875$, $p < 0.001$); egg width had a significant positive linear relationship with egg mass ($r = 0.856$, $p < 0.001$); and egg width had a significant negative linear relationship with egg length ($r = -0.638$, $p < 0.001$).

Table 2. Pearson Correlations of CV of four egg morphological characteristics.

	Egg shape index	Egg length	Egg width	Egg mass
Egg shape index	1.000	0.424**	0.900**	0.287
Egg length	0.424**	1.000	0.242	0.774**
Egg width	0.900**	0.242	1.000	0.314
Egg mass	0.287	0.774**	0.314	1.000

** Correlation is significant at $p < 0.01$ (2-tailed).

Linear regression analysis was used to analyze the relationships between egg width (Fig. 4), length, and mass (Fig. 3). Two regression equations were generated

(1) $W = -29.855 + 1.265\ X_L$
$R^2 = 0.681,\ p < 0.001;\ df = 1443$

(2) $W = -53.078 + 2.699\ X_W$
$R^2 = 0.637,\ p < 0.001;\ df = 1443.$

Where W is egg mass (g), X_L is egg length (mm), X_W is egg width (mm).

We were able to approximate the original egg mass from data about egg width and length using the two equations.

In order to estimate egg mass (W) more exactly, we used the empirical formula given by Hoyt (1979) to derive another regression equation. From the scatter plots of egg mass and the volume (V) of the cube approximately the volume of the egg (egg width x egg width x egg length), it is evident that egg mass had a significant positively correlation with the volume of this cube. Linear regression analysis (Fig. 5) yielded the following equation expressing the relationship between egg mass and volume:

(3) $W = 1.656 + 0.00057V$
$R^2 = 0.928,\ p < 0.001;\ df = 1443,$

where $V = X_L X_W^2$, so the final equation is:

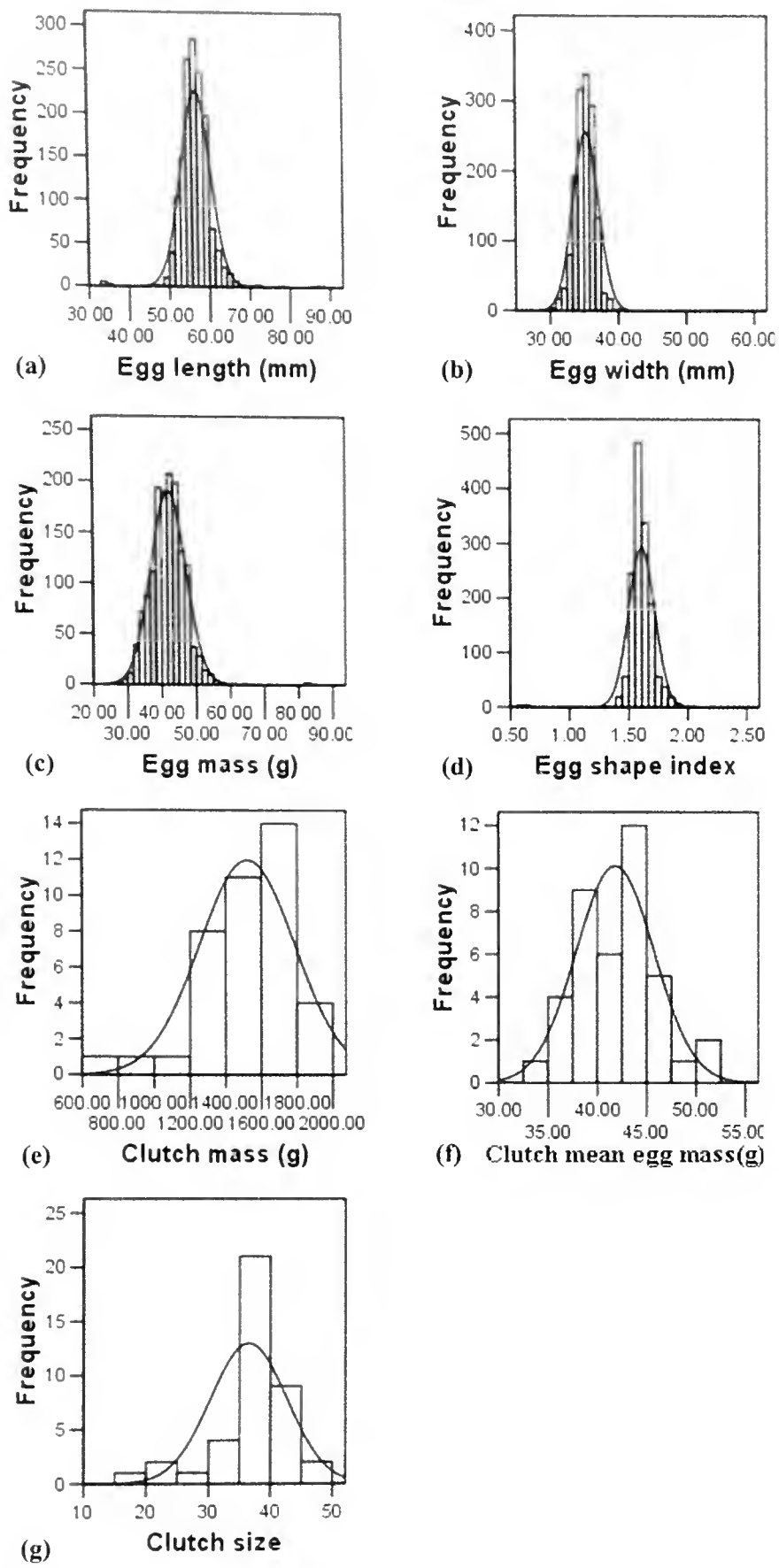

Figure 1. Frequency distributions of egg morphological characters of *Alligator sinensis*.

$$(4) \quad W = 1.656 + 0.00057X_L X_W^2,$$

where W is egg mass (g), X_L is egg length (mm) and X_W is egg width (mm).

Discussion

In 1985 it was recorded that the clutch size of Chinese Alligators at the ARCCAR, based on 29 clutches, was 26.2 (SD = 3.9; n = 29) (Xu et al., 1989). Means of clutch sizes in 1985 and 2004 were compared by a two-sample t-test, revealing that the average clutch size in 2004 was much higher than it was in 1985 ($t = 7.77$, $p < 0.05$). The data collected in 1985 were from the original parent generation captured from the wild, which is no longer breeding, leaving the F1 generation to constitute the dominant portion of the breeding population (Wu et al., 1999, 2005). Many reproductive characteristics of squamate reptiles are fictile, clutch size being one of them. In the wild, adult alligators must face pressures relating to natural selection potentially reducing their full reproductive potential, but in the ARCCAR, the nutrition of the alligators is regulated by artificial diets, maximizing their reproductive potential. One way to quantify this potential is to examine egg morphological characteristics, which are directly influenced by nutrition.

Egg width and length data can be collected easily, but data on egg mass are more difficult to obtain because some eggs might break while the female alligator protects her clutch, or if it is usurped by other females. Furthermore, there is variation in egg mass during incubation (Wang and Zhang, 2000). In bird species examined in the wild, investigators have correlated egg weight, width and length (Hoyt, 1979; Zhao and Ma, 1997; Zhou, 1994) in order to calculate egg mass. The regression equations (1), (2), and (4) established in this study have been found to accurately estimate the original egg mass from data on egg width and length, providing an efficient means of measurement that can also be applied to the mass of recently-hatched young alligators if only the eggshell is available. This information subsequently be used to estimate the constitution of the young alligators, gain basic information on wild populations, and develop a sound basis for investigation of wild populations. We conducted an analysis of variance test (ANOVA) to determine whether the egg masses predicted by the three equations differed from actual observations. The results showed no significant differences ($F = 0.891$, $p > p_{0.05}$), illustrating that all three equations can be used to calculate egg mass, although the accuracy of equation (3) ($R^2 = 0.928$) is higher than that of the other two [(1) ($R^2 = 0.681$); (2) ($R^2 = 0.637$)].

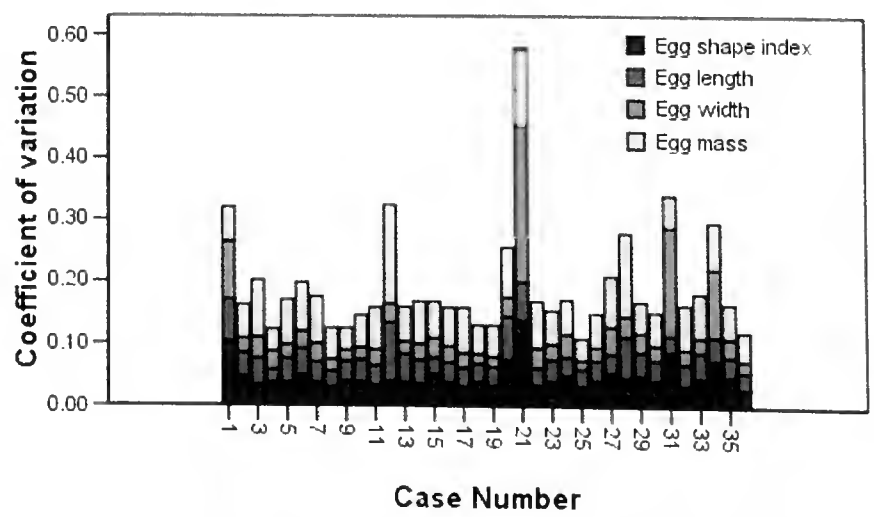

Figure 2. Coefficient of variation of egg morphological characteristics.

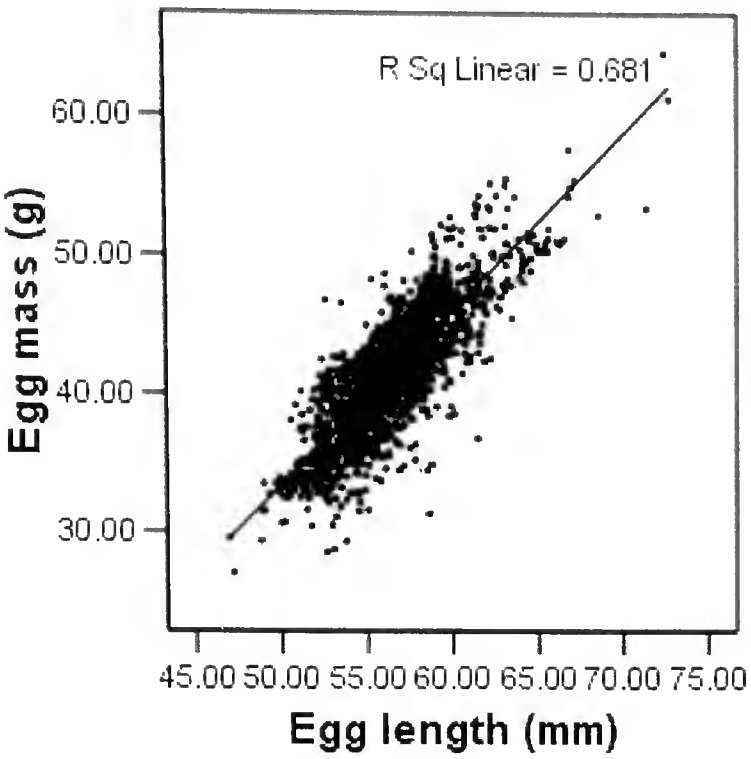

Figure 3. The relationship between egg mass and length.

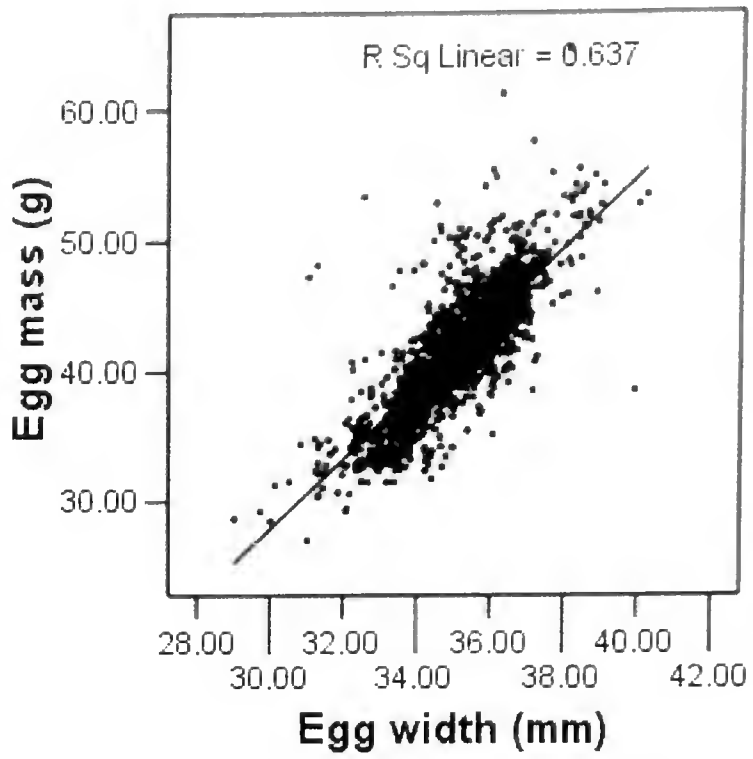

Figure 4. The relationship between egg mass and width.

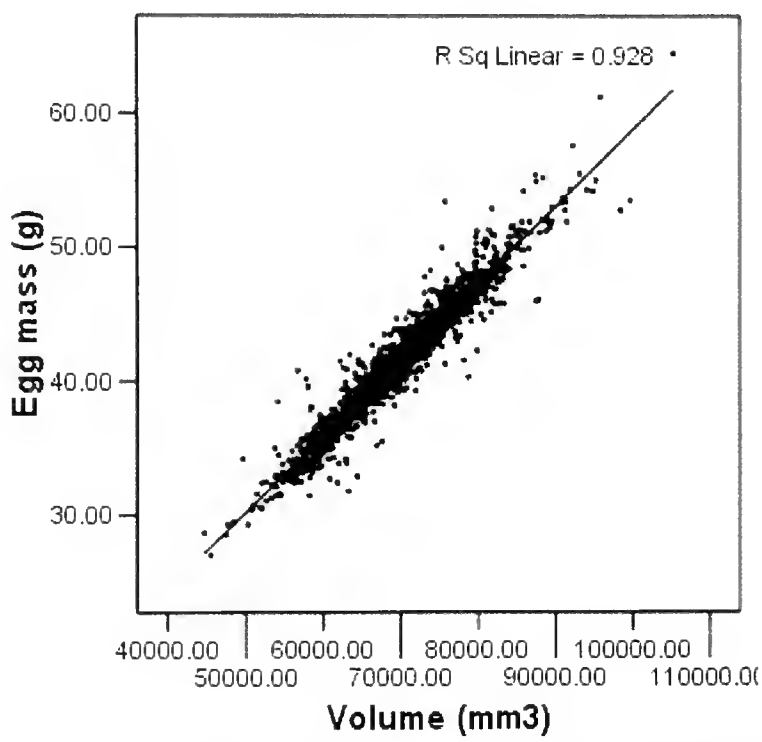

Figure 3. The relationship between egg mass and length.

Though selection females produce progeny of a certain size that are able to escape natural enemies and obtain food efficiently. Compared to progeny size, the number of progeny should show larger variation within a single brood (Lin and Ji, 2004). The results of this study suggest that the CV of egg mass (0.12) is smaller than the CV of clutch size (0.17), meaning that clutch size in the Chinese alligator exhibits greater variation than does egg mass.

Egg shape index can be used to describe the shape of eggs. From Table 2 we conclude that the CV of egg shape index has a significant positive correlation with the CV of egg width. This suggests that, compared to egg length, egg width has a greater influence on egg shape. According to studies on the hatching rate of some birds and reptiles (Fang et al., 2004; Fang et al., 2001; Zhu, 2002), egg shape had been found to be an important variable in hatching rate, however, little research on the relationship between egg shape and hatching rate has been made, and is in need of further investigation.

Acknowledgments

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A New Species of *Rhacophorus* (Anura: Ranidae) from China

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Abstract.- A new brown tree frog species, genus *Rhacophorus*, is described on the basis of seven adult male specimens collected from Cenwanglaoshan Nature Reserve, Guangxi, in southern China. This frog can be distinguished from all other Asian *Rhacophorus* Kuhl and van Hasselt, 1822 by the combination of: skin brown and smooth; Y-shaped cartilage visible dorsally on tips of fingers and toes; outer fingers one-third webbed; distinct dermal ridges present on forearms, above vent, and calcars present on heels; anterior and posterior surface of thighs tangerine in color without distinct dark or light spots; tympanum distinct and large, about 6.6% of SVL; dorsum brown with wide dark cross-shaped mark.

Keywords.- Amphibia, Rhacophoridae, *Rhacophorus*, new species.

Introduction

The genus *Rhacophorus* Kuhl and van Hasselt, 1822, a member of the family Rhacophoridae, contains approximately 60 species (*sensu stricto*) (Frost, 2007) that are distributed in the tropical and temperate zones of East, South, and Southeast Asia. Liem (1970) outlined a conservative definition of the genus (*Rhacophorus sensu stricto*), and while some authors recognize the genus in this sense (Jiang et al., 1987; Fei et al., 1990; Zhao and Adler, 1993; Inger et al., 1999; Malkmus et al., 2002; Frost, 2007; Frost et al., 2006), others have adopted a broader definition (*Rhacophorus sensu lato*) that includes the genus *Polypedates* (Tian and Jiang, 1986; Dubois, 1987, 1992; Fei, 1999; Fei et al., 2005). In a recent review of the Rhacophrinae, a new generic classification was proposed for the Rhacophorinae (Delorme et al., 2005), where some members of *Rhacophorus* were transferred to the new genus *Aquixalus*. This classification was adopted by Frost (2007) and Frost et al. (2006) with some modification. In view of the various interpretations of the Rhacophoridae, the classification of the treefrogs should still be considered unstable (Wilkinson et al., 2002; Matsui and Orlov, 2004), and as stressed by Frost et al. (2006), the boundaries of *Rhacophorus* should be considered tentative.

All Chinese *Rhacophorus* (*sensu lato*) have been found in southern region north to Qinling, with most species distributed in the tropical and subtropical regions. Some new species were recently added to the genus from the regions in and around southern China: *R. hainanus* Zhao et al., 2005 from Hainan, *R. minimus* Rao et al., 2006 from Guangxi, *R. yinggelingensis* Chou et al., 2007 from Hainan, and *R. jarujini* Matsui and Panha, 2006 from eastern Thailand.

During the survey of the herpetofauna of Cenwanglaoshan Nature Reserve, Guangxi, China, in May 2004 and 2005, seven specimens of a small brown *Rhacophorus* were collected (Fig. 1), which appeared to be distinct from other congeners hitherto known from China (Fei et al., 2005) and nearby countries (Bourret, 1942), including Vietnam (Inger et al., 1999; Orlov et al., 2001), Thailand (Taylor, 1962) , Laos (Stuart, 1999), India (Inger and Dutta, 1987), and Burma (Zug et al., 2003). These specimens, which resemble some southeastern Asia members of the genus, are described below as a new species.

Materials and Methods

Morphological data.- The seven specimens included in the new species were collected by hand in Cenwanglaoshan Nature Reserve, Guangxi, China, in May 2004 and 2005. Five were preserved in 10% buffered formalin, and two were preserved in 70% ethanol. Morphological information of related species used for comparison was obtained from the literature listed below.

Measurements.- Sixteen body measurements were made using dial callipers to the nearest 0.1 mm: SVL = snout-vent length; HL = head length from tips of snout to the commissure of the jaws; HW = head width at the commissure of the jaws; SL = snout length from tip of snout to the anterior corner of the eye; INS = internarial space; IOS = interorbital space, i.e., the smallest space between the inner edge of upper eyelid; UEW = width of

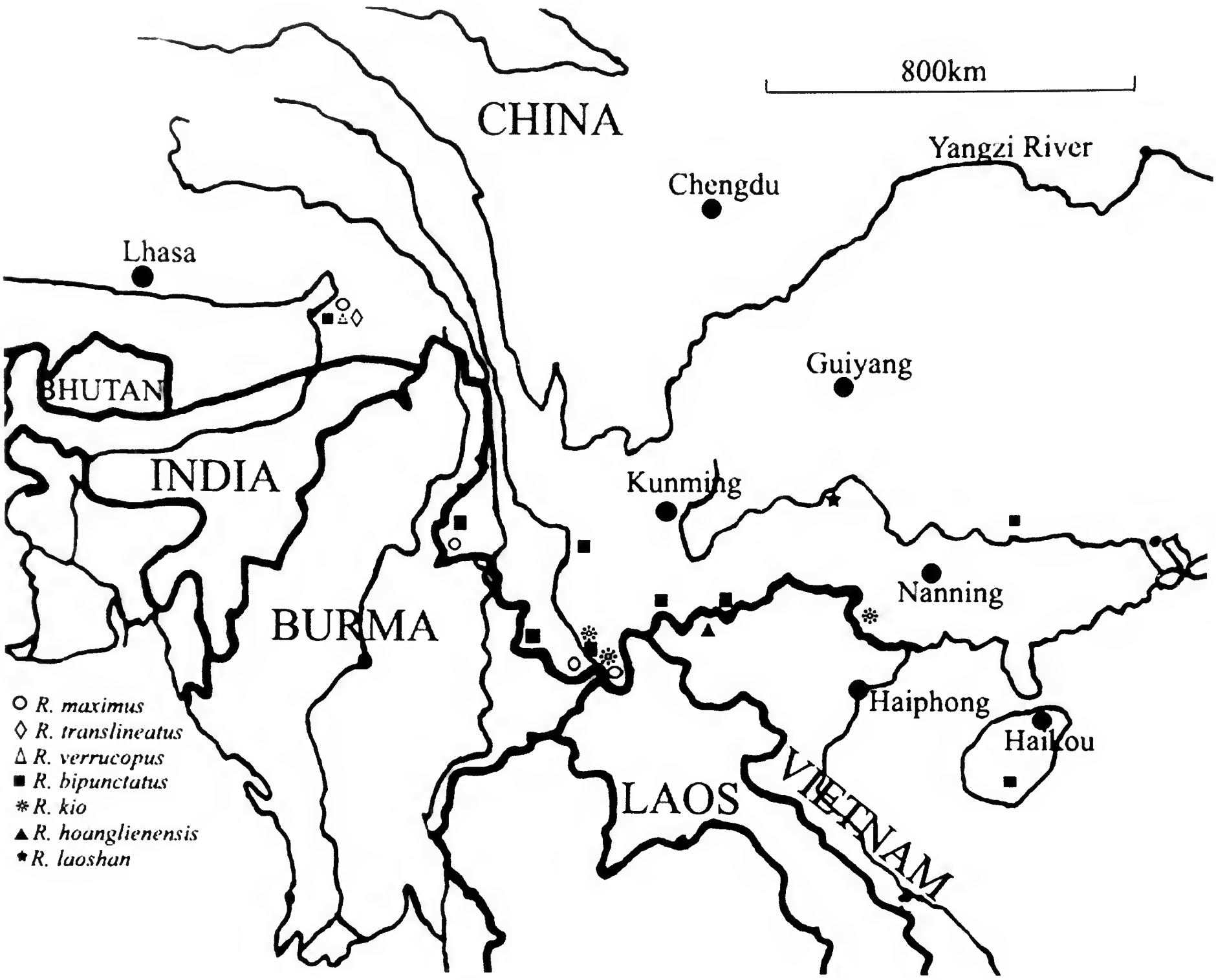

Figure 1. Localities recorded for the six species belonging to the *Rhacophorus reinwardtii* group in China, with the locality recorded for *R. hoanglinensis* in Vietnam.

upper eyelid; ED = diameter of eye; TD = horizontal diameter of tympanum; LAHL = length of lower arm and hand; HAL = hand length; HLL = hindlimb length; TL = tibia length; FTL = length of foot and tarsus; FL = foot length; and TFDD = third-finger disc transverse diameter.

Analyses of advertisement calls.- We recorded the advertisement calls of this new species using a Panasonic SV-MP21V recorder (parameter set as 22050Hz, 16 bit, monophone, wav file). Calls were analyzed using Cool Edit Pro V2.1 and BatSound V3.0. Environmental parameters recorded during collection (2010–2340 h) were as follows: air temperature 22°C; moisture 92%.

Museum acronyms.- CIB, Chengdu Institute of Biology, Chinese Academy of Sciences; GXNM, Natural History Museum of Guangxi, Nanning, China.

Taxonomy

Rhacophorus laoshan, new species
Fig. 2

Holotype.- Catalog number GXNM 2005081. Adult male collected in Cenwanglaoshan Nature Reserve (Guangxi, China) on 19 May 2005 (106° 24′ 8.22″ E, 24° 29′ 1.98″ N) at 1389 m altitude by Yunming Mo.

Paratypes.- Six adult males, all from the same site as the holotype: CIB 2831k collected on 19 May 2004 by Jianping Jiang, Annemarie Ohler, Yunming Mo, and Feng Xie; GXNM2005079, GXNM2005082, GXNM2005095, CIB2005080, and CIB2005094 collected on 19–20 May 2005 by Yunming Mo.

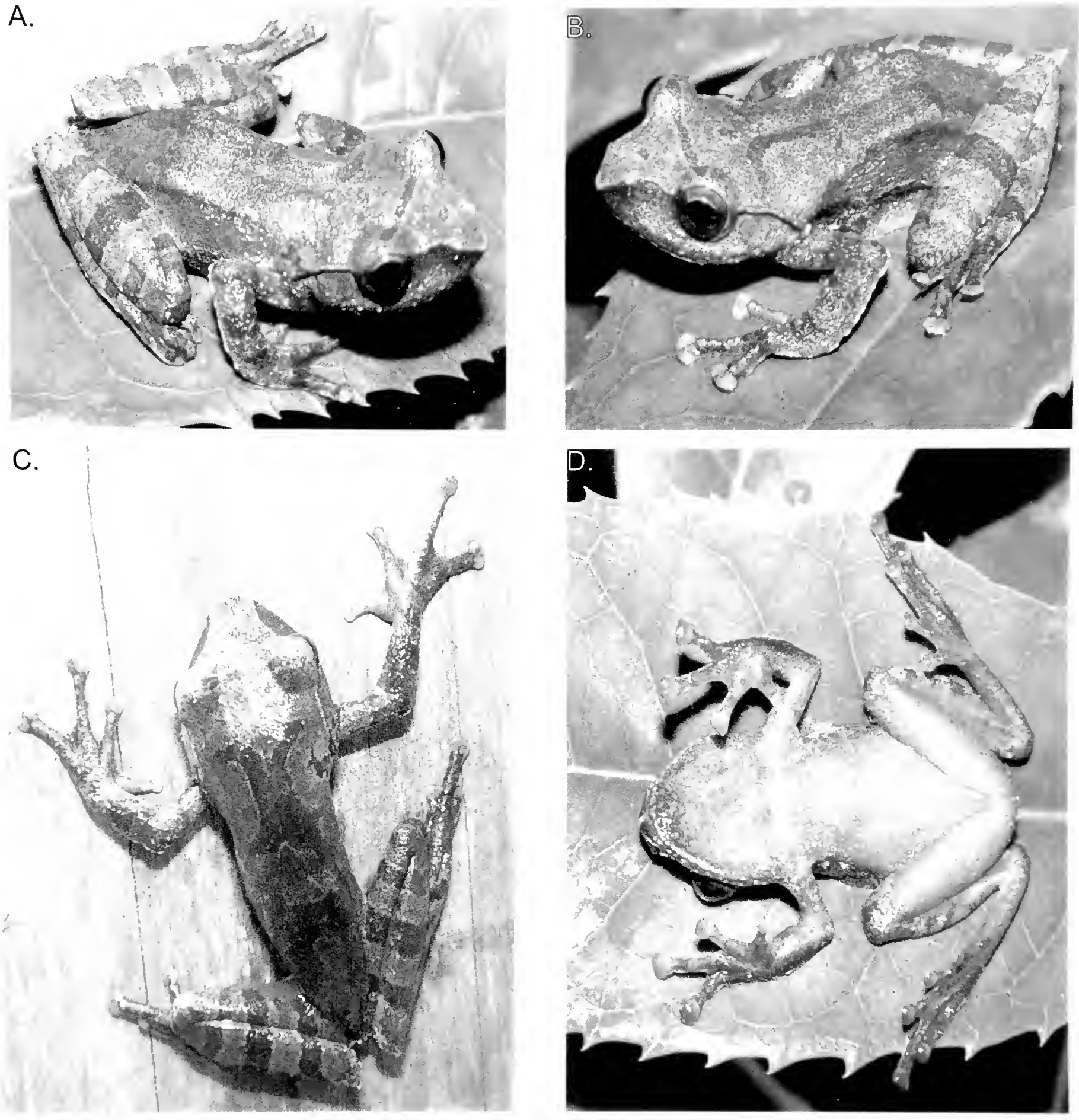

Figure 2. (A-D) Holotype of *Rhacophorus laoshan* sp. nov.; adult male GXNM2005081.

Diagnosis.- This new species *Rhacophorus laoshan* is a small brown treefrog (SVL 35.1±1.3 mm; n = 7) that can be distinguished from all other Asian Rhacophoridae by the combination of the following characters: skin brown and smooth; Y-shaped cartilage visible dorsally on tips of fingers and toes; outer two fingers one-third webbed; dermal ridges present on forearm, above vent, and on heel calcars; anterior and posterior surfaces of thigh tangerine in color and without distinct dark or light spots; tympanum large, about 6.6% of SVL; dorsum brown with wide dark cross.

Description of holotype.- Body size small (SVL = 35.4 mm) and moderately elongate. Head moderately compressed, dorsally flat, and wider (HW = 13.9 mm) than long (HL = 13.8 mm). Snout bluntly pointed, projecting, with length (SL = 6.4 mm) longer than horizontal diameter of eye (ED = 4.6 mm). *Canthus rostralis* distinct with loreal region slightly concave. Nostril oval and closer to tip of snout than eye; interorbital space almost flat, and larger (IOS = 4.5 mm) than upper eyelid (UEW = 3.9 mm) and internarial space (INS = 4.0 mm). Tympanum rounded with diameter (TD = 2.3 mm) half that of eye; tympanum-eye distance about half tympanum diameter. Supratympanic fold distinct, present from posterior corner of eye to above and behind insertion of

arm. Vomerine ridges oblique, at an angle of 45° to body axis, and closer to choanae than to each other. Vomerine teeth on tongue pear-shaped, with deep notch on posterior end.

Forearm plus hand about half snout-vent length, with forearm (6.7 mm) shorter than hand (10.9 mm); forearm with distinct dermal ridge from elbow to wrist (Fig. 2); relative length of fingers: 3 > 4 > 2 > 1; fingers with dermal fringes (Fig. 2); third and fourth fingers with web on basal one-third; tips of all fingers with well-developed disks with distinct circummarginal grooves; discs relatively wide compared to fingers, except for first finger; Y-shaped cartilage easily observable on backs of fingers; subarticular tubercle developed; supernumerary tubercles below base of fingers distinct; inner metacarpal distinct, large, flat, and oval.

Hind limbs rather long, with tibiotarsal articulation reaching middle of eye when leg stretched forward; heels strongly overlapping when limbs folded at right angles to body; tibia (TL 18.0 mm) about half SVL and longer than thigh (12.8 mm) and foot (FL 14.9 mm); relative length of toes: 4 > 3 = 5 > 2 > 1; tips of all toes with moderately-sized disks (slightly smaller than those of fingers except that of first finger); discs with distinct circummarginal grooves that are relatively wide compared to toe width; Y-shaped cartilage easily observable on backs of toes; web present, half developed, I 1 - 2 $^{1/2}$ II 1 - 2 $^{1/2}$ III 1 - 2 $^{1/2}$ IV 2 - 1 V dermal fringe along toe V distinct; subarticular tubercles prominent on all toes; inner metatarsal tubercle distinct and oval; outer metatarsal tubercle absent.

Dorsum smooth with granules scattered along sides of body and head, along lower mandible, and back of forearm; venter, head, and limbs covered with flat granules; outer edge of forearm and tarsus-metatarsus with granulose ridge; dermal calcars present on heels; granules above vent forming transverse skin fold.

Color in life.- Dorsum chocolate brown; broad transverse strip present medially on upper lids and interorbital space; back with broad cross-shaped marking (Fig. 2); limbs with broad transverse stripes: 2 on forearms, 2–3 on carpals and fingers, 3 on thighs and tibiae, and 4–5 on feet and toes; anterior and posterior surfaces of thighs tangerine in colour and usually without distinct dark or light spots; inner surface of tarsus and foot tangerine; belly light gray-brown and without dark spots.

Male secondary sexual characters.- Adult males with nuptial pad on the base of first finger; internal subgular vocal sacs present with two elongate openings; linea masculina absent.

Variation.- Most variation was found in the appearance of the wide cross-shaped mark on the dorsum. Usually, this mark was visible except for when the dorsum was more darkly-colored. The snout of some specimens was green, and some specimens had a green spot on the shoulder; ovate yellow spots on the sides of the body sometimes formed a line.

Etymology.- The species is named after the locality, i.e., Cenwanglaoshan Natural Preserve in northwestern Guangxi, China, where it was found.

Measurements.- Sixteen body measurements are provided in Table 1.

Habitat and ecological notes.- This new species was found in a secondary broad-leaf forest with bamboo undergrowth. Dense grass and deciduous leaves covered the ground (Fig. 3). There were almost no perennial streams in the region. During April and May, especially at night after rainfall, males were heard calling loudly in the forest, with six to nine calls making up a chorus. The holotype and paratypes were found on branches and leaves of trees one to three meters from the ground.

Advertisement calls.- The analyzed results of the calls by Cool Edit Pro 2.1 and BatSound indicated that calls were emitted every 17–25 seconds and lasted about 3.6–4.6 seconds. The calls had 19-26 notes (6 individuals, 12 calls) (Fig. 4a), with a note interval of 0.171–0.267 seconds (mean ± SD: 0.189±0.0184, 6 individuals, 12 calls, 44 notes). The dominant frequency was 2000 Hz and the second dominant frequency was 4000 Hz (Fig. 4b).

Comparisons.- The new species *Rhacophorus laoshan* is most similar in appearance to *R. hoanglienensis* (Orlov et al., 2001) and *R. verrucopus* Huang, 1983. It species can be distinguished from *R. hoanglienensis* by a combination of the following characters: (1) anterior and posterior surfaces of the thighs are orange-red and without distinct dark or light spots (black and white vermiculation is present in *R. hoanglienensis*); (2) body size smaller (SVL 35.1±1.3 mm; n = 7) than in *R. hoanglienensis* (SVL 43.2 mm; n = 1); (3) tympanum more distinct and larger (TD = 2.3 mm, about 6.6% of SVL) than that of *R. hoanglienensis* (TD = 2.1 mm, about 4.86% of SVL); (4) wide dark cross-shaped mark present on dorsum; (5) venter light gray-brown and without spots (not creamy-white with small dark spots that merge in the distal parts of the fore and hind limbs). Furthermore, the new species emits a call every 17–25 sec. (see above), while *R. hoanglienensis* calls every 3–5 min (Orlov et al., 2001), and white lines running from the supratympanic fold to tip of snout through eyelid and

Table 1. Measurements (in mm) of *Rhacopnorus laoshan* sp. nov. Variations (mean ± SD) are shown for paratypes and ratios to SVL (%). Abbreviations as used in text.

Character	Holotype	Paratypes (n = 6)	Character	Holotype	Paratypes (n=6)
SVL	35.4	33.2–36.7 (35.1±1.4)	TD	2.3	2.1–2.7 (2.3±0.2)
				-6.50%	6.6% (5.9–6.8)
HL	13.8	12.2–13.4 (12.8±0.4)	LAHL	17.6	16.1–17.7 (17.1±0.5)
	-39.00%	36.5% (35.2–39.0)		-49.70%	48.7% (47.1–51.2)
HW	13.9	12.8–13.5 (13.1±0.3)	HAL	10.9	10.4–10.9 (10.6±0.2)
	-39.30%	37.3% (36.1-39.3)		-30.80%	30.3% (29.4–31.9)
SL	6.4	5.5–6.2 (5.9±0.3)	HLL	54.7	53.5–56.1 (54.7±1.1)
	-17.10%	16.7% (15.7–17.1)		-154.50%	156.1% (154.0–169.0)
INS	4	3.6–4 (3.8±0.1)	TL	18	17.6–18.1 (17.9±0.2)
	-11.30%	10.9% (10.4–11.7)		-50.80%	51.1% (49.3–53.9)
IOS	4.5	4.2–4.4 (4.3±0.1)	FTL	23.9	22.4–24.6 (23.7±0.7)
	-12.70%	12.2% (11.7–13.3)		-67.50%	67.6% (63.1–72.3)
UEW	3.9	3.5–3.9 (3.7±0.2)	FL	14.9	14.4–15.5 (15.1±0.4)
	-11.00%	10.6% (10.5–11.0)		-42.10%	43.1% (41.4–45.5)
ED	4.6	4.1–4.7 (4.5±0.2)	TFDD	2.3	2.2–2.4 (2.3±0.1)
	-13.00%	12.7% (12.3–13.0)		-6.50%	6.6% (6.3–6.8)

canthal ridge of female of *R. hoanglienensis* (Bain and Truong, 2004). This new species can be distinguished from *R. verrucopus* by the latter lacking vocal sac, head longer than width, outer fingers 1/2 webbed, the dermal ridge on forearm and above vent more weak, and the dorsum without the wide dark cross mark present (Huang, 1983). *Rhacophorus laoshan* can be separated from related species that also have dermal flaps on the forearms, tarsus, vent, or heel as follows. *Rhacophorus laoshan* shows reduced toe webbing, which is more extensive in *R. annamensis*, *R. bipunctatus*, *R. exechopygus*, and *R. reinwardtii* (Inger et al., 1999); complete webbing on the feet is seen in *R. kio*, *R. nigropalmatus, R. reinwardtii* (Ohler and Delorme, 2006), *R. maximus* (Liu and Hu, 1961; Fei, 1999), *R. pardalis* (Brown and Alcala, 1998; Inger, 1954, 1966; Inger and Stuebing, 1997; Malkmus et al., 2002), *R. prominanus* (Taylor, 1962; Inger, 1966), and *R. robinsoni* (Taylor, 1962; Inger, 1954) have. The absence of a dark spot in temporal region distinguishes the new species from *R. cyanopunctatus* (Malkmus et al., 2002); and *R. bipunctatus* (Inger et al., 1999).

Rhacophorus baluensis can be separated from the new species by being larger (male SVL = 50–55 mm) (Malkmus et al., 2002) and by having dark transverse bars and irregular light or dark blotches on the dorsum (Inger and Stuebing, 1997; Malkmus et al., 2002). *Rhacophorus bimaculatus* (Peters, 1867) is also larger in size (SVL = 65 mm) and has fingers that are almost fully webbed (Fei, 1999). Almost fully-webbed fingers are also seen in *R. dulitensis* (Taylor, 1962; Inger and Stuebing, 1997). *Rhacophorus gauni* can be separated from the new species by having a conspicuous white spot below the eye (Inger and Stuebing, 1997). The skin of *R. kajau* is leafy green dorsally and usually has minute white spots scattered on the back, head, and exposed surface of the limbs (Inger and Stuebing, 1997; Das, 2007). *Rhacophorus rhodopus* has more developed finger and toe webbing, which is also red in color (Liu and Hu, 1959; Fei, 1999), not grey-brown. *Rhacophorus translineatus* has dark transverse bars on dorsum (Sichuan Institute of Biology [Hu et al., 1977; Fei, 1999]) while *R. laoshan* has a dark wide cross-shaped mark.

The new species can be distinguished from *Rhacophorus appendiculatus* by having irregular low ridges on the back, as well as a narrow flap on the heel (Inger and Stuebing, 1997; Malkmus et al., 2002). *Rhacophorus calcaneus* differs by having oval or round dark brown spots on the dorsum, small enameled white spots, or pinkish dorsolateral bands and a thin black network enclosing large white spots ventrolaterally (Inger et al., 1999). *Rhacophorus verrucosus* has the vomerine teeth absent (not present) and a low conical tubercle on the heel (Inger et al., 1999). *Rhacophorus bisacculus* has no prominent projections in the infra-anal area, but it may have several short, pointed tubercles (Inger et al., 1999). *Rhacophorus naso* has a dorsolateral fold and a skin projection on the tip of the snout (Fei, 1999).

Figure 3. Habitat of *Rhacophorus laoshan* sp. nov.

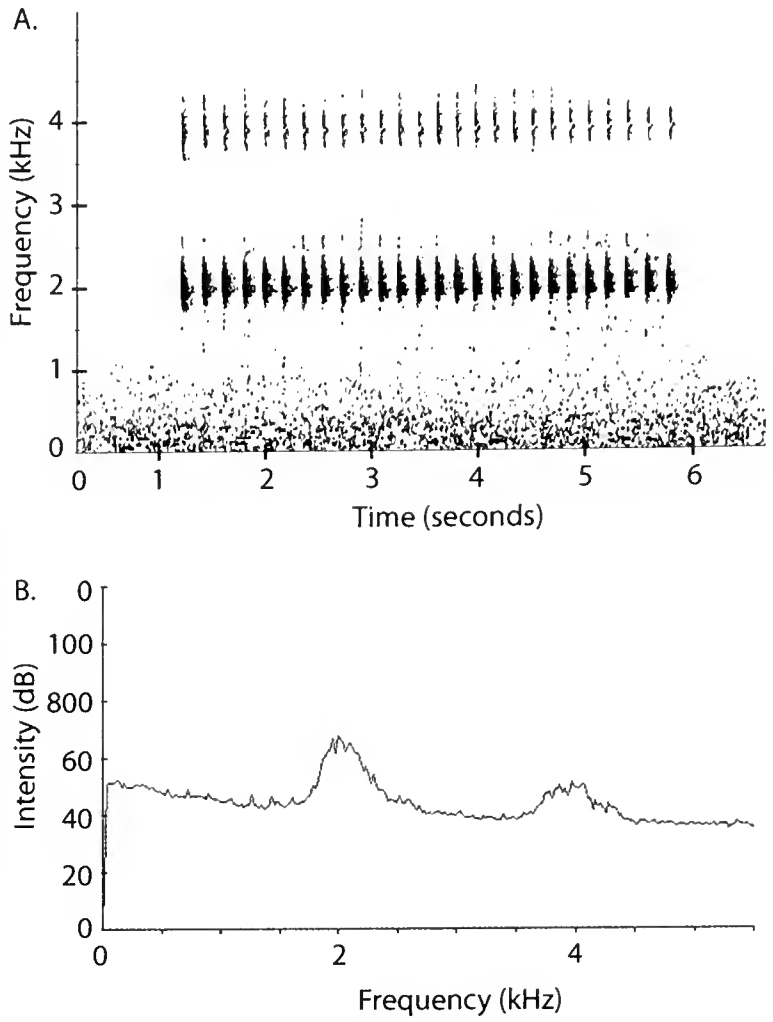

Figure 4. Audio-spectrogram with FFT size 512 (A), and power spectrum (B) of advertisement calls of the new species *Rhacophorus laoshan* (Holotype)

Remarks.- Those *Rhacophorus* with dermal flaps are distributed in the tropical region of southeastern Asia, extending north to the southern slopes of the eastern Himalayas, the Hengduan Mountains, the Yunnan-Guizhou Plateau, and the Nanling Mountains. Within this area, most species are distributed north of 20° N. Of these taxa, *R. bipunctatus*, *R. maximus*, *R. kio*, *R. hoanglienensis*, *R. laoshan*, *R, translineatus*, and *R. verrucopus*, are distributed along the southern border of China (Fig. 1).

Rhacophorus bipunctatus (recorded as *R. bimaculatus* by Fei (1999) and Fei et al. (2005) in their list of Chinese amphibians) has the widest Chinese distribution of these seven species (Fig. 1), and is also known from Northeastern India, Myanmar, Thailand, Laos and Vietnam. In China this species occupies the tropical region of the southern slopes of the Yunnan-Guizhou Plateau, the Nanling Mountains, and southern Medog. *Rhacophorus maximus* has and *R. bipunctatus* are known from Yunnan and Tibet. The northern extent of *R. kio* in China is the southern border regions of Yunnan and Guangxi (Ohler and Delorme, 2006; Fei, 1999). *Rhacophorus translineatus* and *R. verrucopus* are found along the southern slope of the eastern Himalayas in Medog, while *R. hoanglienensis* and *R. laoshan* are known from the southern slope of the Yunnan-Guizhou Plateau.

In summary, there are only five *Rhacophorus* species with dermal flaps along the southern slopes of the Yunnan-Guizhou Plateau and the Nanling Mountains: *R. maximus*, *R. kio*, *R. hoanglienensis*, *R. laoshan*, and *R. bipunctatus*. Of these, the new species *R. laoshan* is the most northern, *R. bipunctatus* the most eastern and *R. kio* and *R. hoanglienensis* the most southern.

Lastly, only four rhacophorids were recorded in Cenwanglaoshan Nature Reserve (Mo and Xie, 2005), including the new species, suggesting that further surveys of the herpetofauna along the southern slopes of Yunnan-Guizhou Plateau and the Nanling Mountains should be carried out to further explore the little-known biodiversity of this region. Such efforts may elucidate more species or populations of Asian treefrogs or other new species.

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The Life History of *Triturus vittatus vittatus* (Urodela) in Various Habitats

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Abstract.- The life cycle of *Triturus v. vittatus* at localities of various altitudes in Israel, ranging from 212 to 740 m above sea level (ASL), were studied. Mature newts were observed only around winter rain pools, where they arrived before the pools filled with water. The males left the ponds after spawning, while females left after eggs were oviposited on plants and other substrata, according to the conditions of the ponds. Males (9–11 cm long, weighing 4.3–5.3 g) were slightly bigger than females (8.5–10 cm long, weighing 3.1–4.3 g) Females laid 18–68 eggs each. Fifteen to 30 days after oviposition, larvae hatched and from April to July, remained in the ponds to develop. Various anuran larvae were found in the same breeding sites, including *Hyla savignyi, Bufo viridis, Rana bedriagae* and *Pelobates syriacus*. Larval and adult *Salamandra infraimmaculata* were found to inhabit several of the rain pools simultaneously, although the period during which both stages existed together was brief. Although temperature and oxygen levels in the pools were not significantly different between breeding sites in the various habitats, development took longer to complete at the more elevated sites.

Keywords.- Newt, larvae, *Triturus v. vittatus*, winter pool, Israel, life cycle.

Introduction

The life cycle and ecological conditions necessary for the banded newt (*Triturus vittatus*) have not been well studied, although some aspects of the biology and life cycle of the subspecies *T. v. vittatus* have been documented in Israel, Europe and the Mediterranean region (Raxworthy, 1989; Olgun et al., 1997). Three subspecies of the banded newt are currently recognized: *T. v. vittatus*, found along the eastern edge of the Mediterranean, ranging from Turkey to Israel; *T. v. cilicensis*, found in areas bordering the east and northeast of the Mediterranean; and *T. v. ophryticus*, located in the Caucasus to the east and south of the Black Sea.

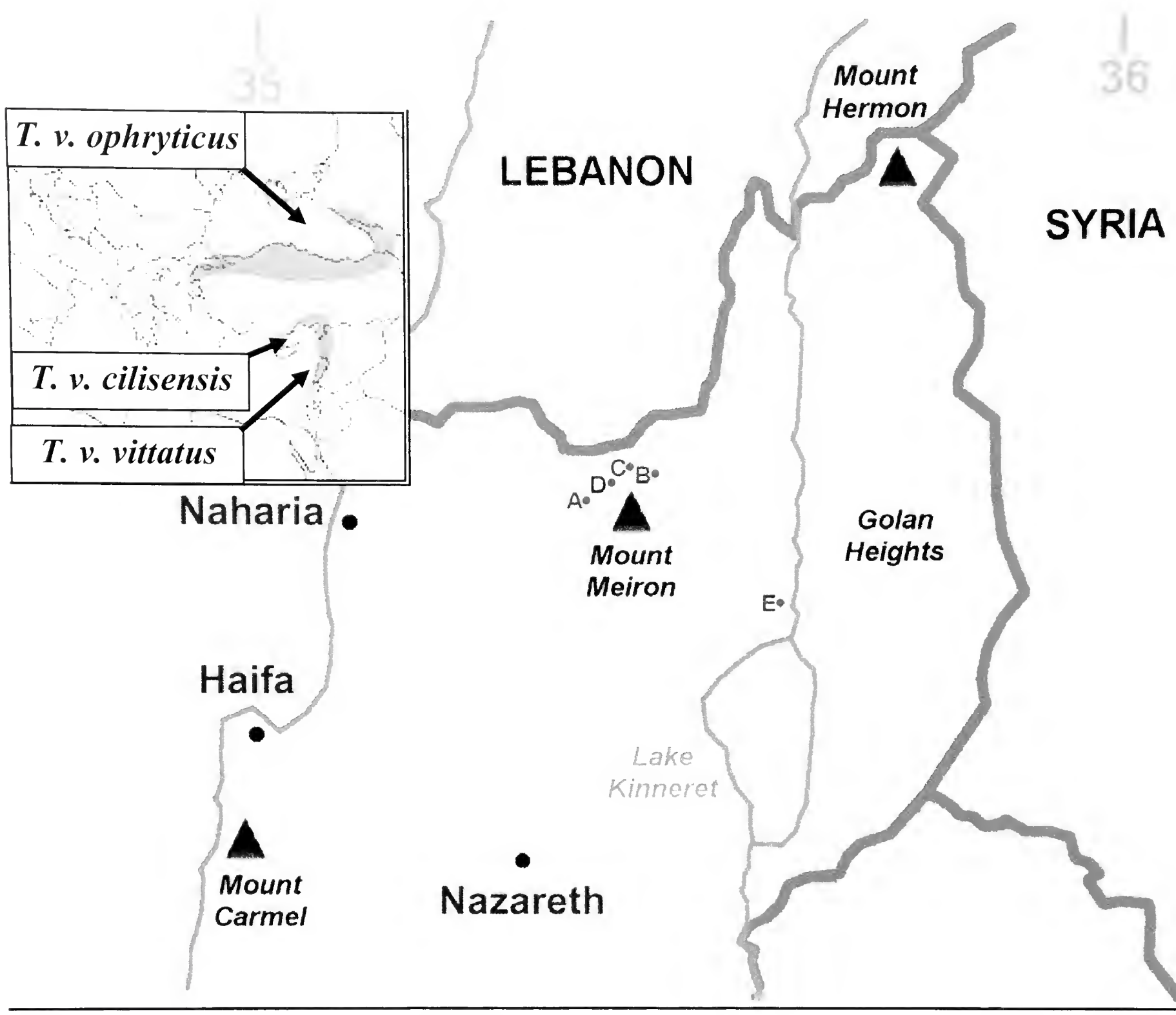
In Israel, *Triturus v. vittatus* is found from the north to the central coastal plains, where conditions are most extreme. The biology and life cycle of the populations in northern Israel and Upper Galilee have been previously described (Degani, 1986; Degani and Mendelssohn, 1983). Throughout their adult aquatic phase and larval periods, *T. v. vittatus* mainly inhabits winter pools that sometimes disappear at the beginning of summer (Degani and Kaplan, 1999). The terrestrial adults reach the pond at the beginning of the rainy season before the ponds fill with water, and when the ponds are filled, enter them for their aquatic phase.

Materials and Methods

In order to locate *Triturus v. vittatus,* all of the main types of aquatic habitats inhabited by amphibian larvae in northern Israel were investigated. The procedure followed was that described by Degani and Kaplan (1999).

The various *Triturus v. vittatus* populations around winter rain ponds and rock pools in northern Israel were studied during four consecutive years (2001–2005) (Fig. 1). The elevations of these habitats ranged from 212 to 740 m ASL and represented a number of extreme ecological and physical conditions. Water parameters were measured every two weeks during the time the pools were filled. *In situ* temperature and dissolved oxygen data were obtained by a hand-held oxygen meter (WTW, Oxi330 set, Germany). Water parameters were analyzed by one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls (SNK) test, for which Graph-Pad Prism software (Graph Pad, San Diego, CA) was used. The level of significance between groups was set at $p < 0.05$.

Larvae were collected with a hand net (Degani and Mendelssohn, 1983), identified to species and grouped by specific water body.



Name of pond	Longitude	Latitude	Altitude m (ASL)
Dovev pond (A)	239158	772801	740
Nahalit pond (B)	243657	776401	665
Matityahu Q. pond (C)	242783	774855	670
Pharaa pond (D)	242784	774580	682
Amiad water holes (E)	251721	757994	212

Figure 1. Various ponds in Israel colonized with newts examined in the study.

Results

The life cycle of *Triturus v. vittatus* is presented in Figure 2. Males and females arrived at the dried ponds before the beginning of the heavy rains in October and November and entered them when they were filled. Male newts (9–11 cm long and weighing 4.3–5.3 g) were found to be slightly bigger than females (8.5–10 cm long and weighing 3.1–4.3 g), with no significant difference in size detected for either sex between ponds (Fig. 3).

After mating, the males left the ponds while the females remained in the water to deposit between 18–68 eggs on plants or rock surfaces. The larvae hatched 19–29 days later and remained in the rain pool for 30 to 75 days. Hatching time and duration spent in the pool was dependent on water temperature (Fig. 4), with development being slower at higher altitudes.

Various anuran larvae were found in the same breeding sites, including *Hyla savignyi*, *Bufo viridis*, *Rana bedriagae*, and *Pelobates syriacus*. Larval and adult *Salamandra infraimmaculata* were found to inhab-

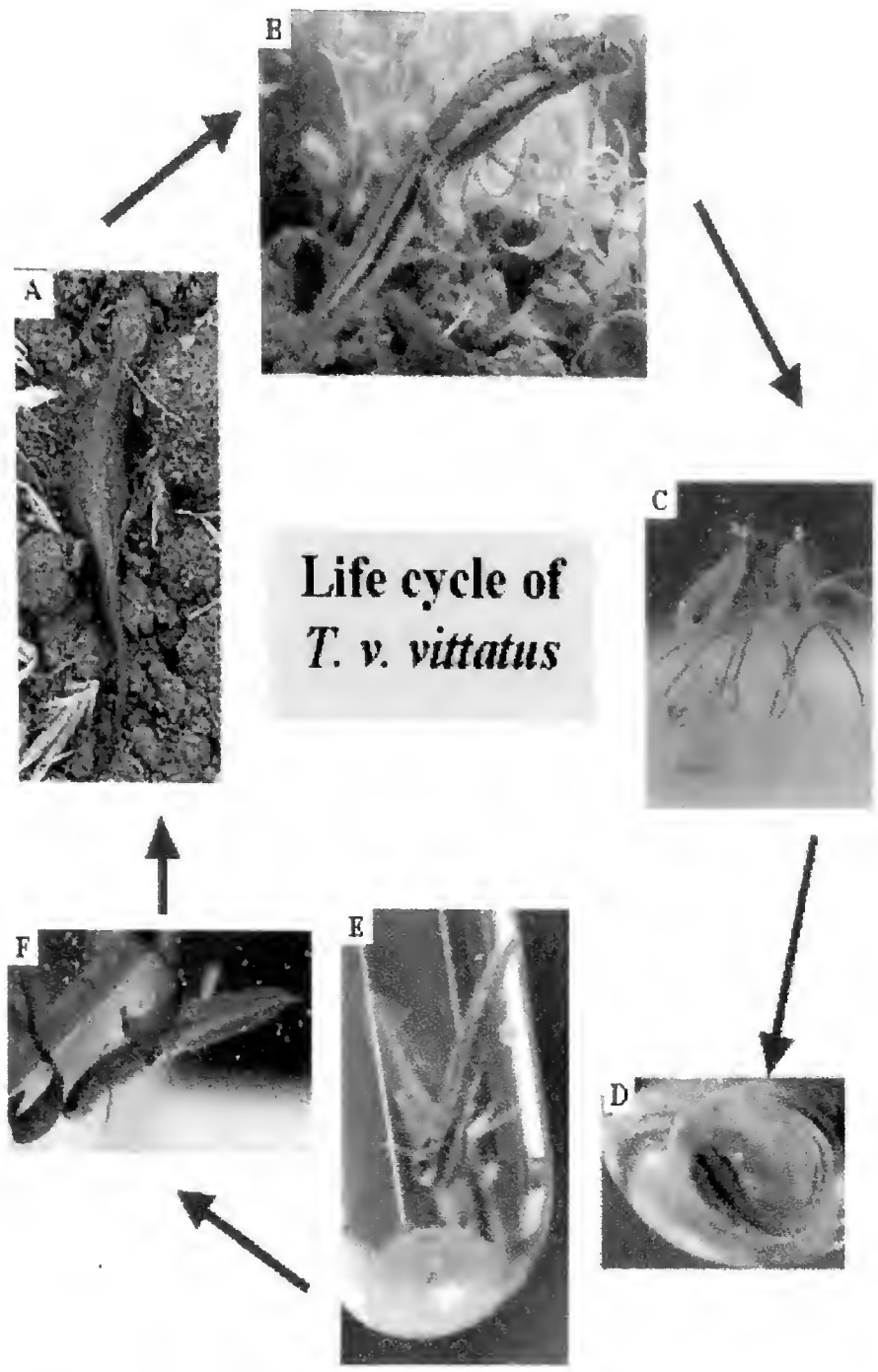


Figure 2. Life cycle of *T. v. vittatus*: Terrestrial (A) and aquatic females (B), eggs on plants in the pond few days after spawning (C), larvae one day before hatching (D) and 2 days after hatching (E), and developing larvae (F).

it several of the rain pools simultaneously, although the period during which both stages existed together was brief. The eggs and larvae developed in the ponds only when temperatures rose above 18°C, the threshold temperature also necessary for metamorphosis in *S. infraimmaculata*.

From winter to spring temperatures rose from 5°C to 30°C (Fig. 4). No significant differences in temperature were observed between the various ponds ($p > 0.05$, F-value = 0.1766–1.186) during the periods when the newt larvae were present, although temperatures in Dovev pond were lower at the beginning of the growth period during the years 2001–2002, 2003–2004 and most of the growth period in 2004–2005. Dissolved oxygen concentrations did not vary between sites ($p > 0.05$, F-value = 0.3489–2.326), ranging between 2–27 mg/L; concentrations stayed between 5–10 mg/L for most of the growth period. High oxygen concentrations were detected during the larval growth period and during completion of metamorphosis (Fig. 4).

Discussion

Since newt larval development is dependent on an aquatic habitat, the locations of breeding sites changed from year to year depending on water availability. This site flexibility is an important environmental adaptation in a semi-arid country such as Israel. The data obtained from the five different newt populations examined in this study were consistent with those data obtained in previous studies on the same subspecies in Upper Galilee (Degani and Kaplan, 1999; Degani and Mendelssohn, 1983) and the coastal plains of Israel (Geffen et al., 1987), supporting observations that *T. v. vittatus* is present in water in Israel between December and April. Adults of *T. v. ophryticus* in northern Turkey differ in that they are usually found in the water from

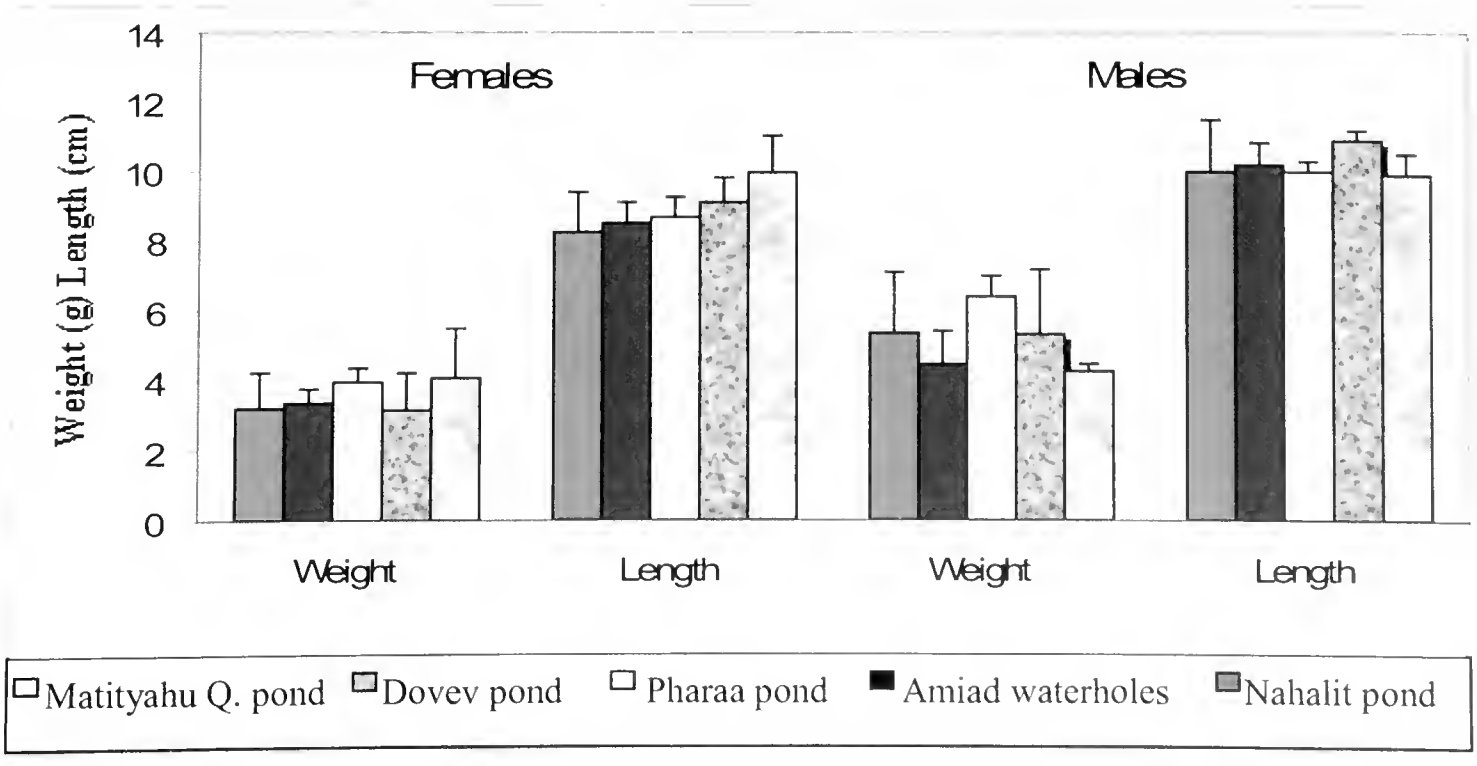


Figure 3. The measurements of mature females (N = 27) and males (N =15) from various populations.

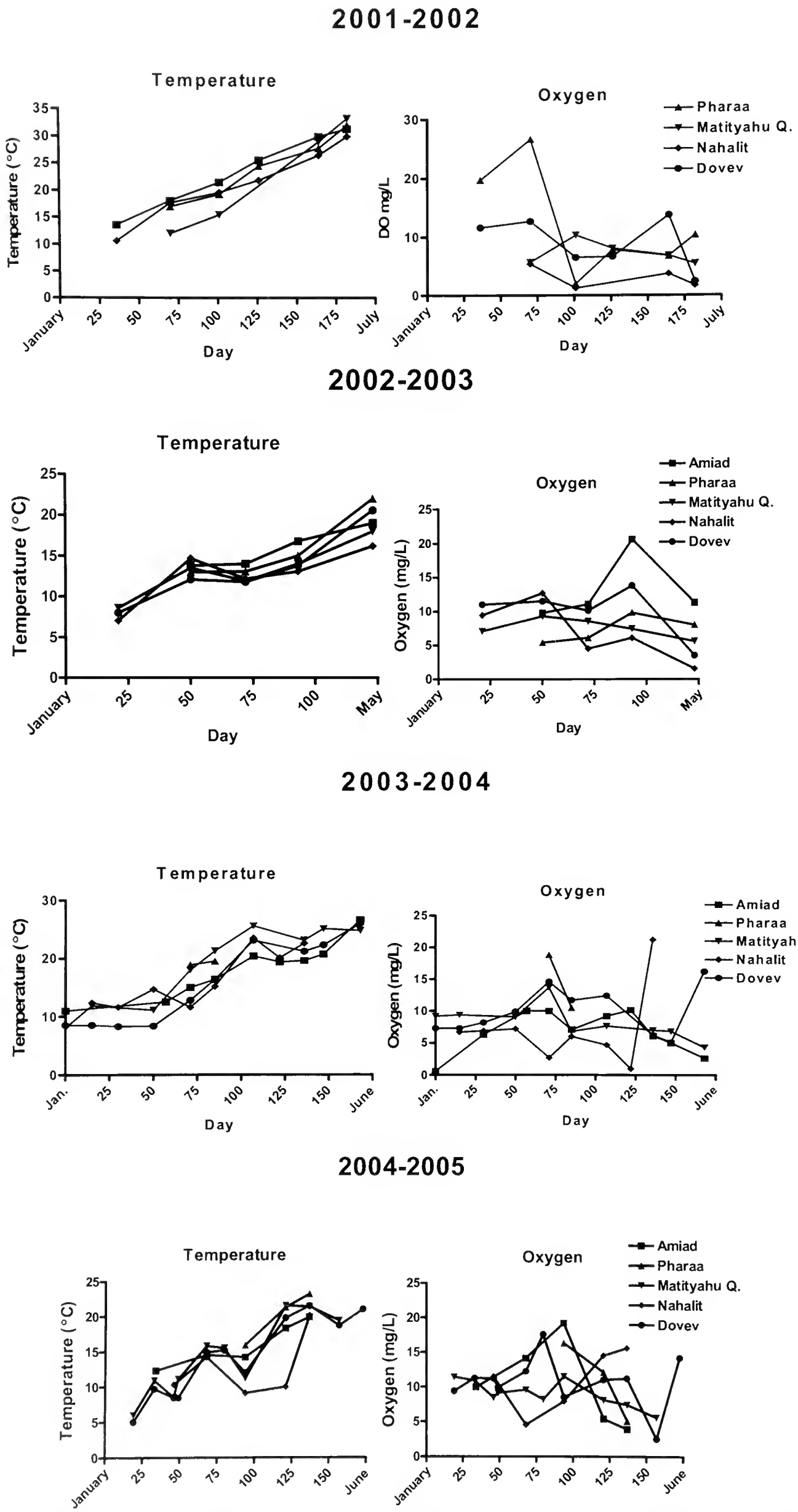


Figure 4. Water temperatures and oxygen concentration of various breeding sites where *T. v. vittatus* newts were present during winter and spring 2001 to 2005.

early March to late Octoberor November, depending on the climate and altitude (Kutrup, 2005b). We suggest that the differences between the two subspecies are due to regional climate differences.

Kutrup et al. (2005a) studied the food of the banded newt, *Triturus v. ophryticus*, at different sites in Trabzon in northern Turkey and discovered that the newts consume a wide variety of invertebrates during their aquatic phase. In Israel, the *Salamandra infraimmaculata* and *T. v. vittatus* have a very similar diet, composed of various invertebrates (Degani and Mendelssohn, 1978; Geffen et al., 1987).

In summary, the present study examined the life cycle of *Triturus v. vittatus* in northern Israel, which was found to vary depending on the unpredictable presence of rain pools necessary for juvenile development. Among the different ponds, large variations were found in the length of the larval growth period, as well as in the time required for completion of metamorphosis. In contrast, no differences were observed in the ecological parameters and water quality of the ponds during the larval growth period.

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Sexual Dimorphism and Female Reproduction in *Lacerta vivipara* in Northeast China

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Abstract.- Lacerta vivipara is a small lacertid lizard that inhabits much of Europe and northern Asia. From the end of May to the beginning of October in 2003, these common lizards were collected from a population in Heilongjiang Province (northeast China) in order to study sexual dimorphism and female reproductive traits. Through the examination of external morphological traits, such as snout-vent length, head length, head width, head height, tail length, body weight, rows of ventral and mid-dorsals scales, ventral color, tail base and femoral pores, analyses revealed the presence of a distinct sexual dimorphism. Males possessed a bulging tail base, a salmon-pink venter and a thorn in the femoral pore. Females had significantly more rows of ventral scales and fewer mid-dorsal scales than males. Adult males were larger in head size and had a longer tail, whereas adult females were larger in body size and weight. Male juveniles and neonates were larger in head size than females of the same age and female neonates were larger in body size than male neonates. The rates at which head length, head width and head height increased with increasing SVL (snout-vent length) was allometric in females.

Females produced a single clutch every breeding season, with 3–12 young per clutch. While clutch size and neonate mass were not positively correlated with maternal SVL, clutch mass was, suggesting that sexual dimorphism in this species is due (in part) to differences in reproductive investment between the sexes. The larger head of males is likely an adaptation for male-male combat while the larger relative body length of females is a result of selection for higher fecundity.

Keywords.- Sexual dimorphism, female reproductive adaptations, allometry, Lacertidae, *Lacerta vivipara.*

Introduction

Sexual dimorphism in body size, body shape, and coloration is widespread in many Chinese lizards, including *Takydromus septentrionalis*, *Sphenomorphus indicus*, *Eremias argus*, *Gekko japonicus*, *Plestiodon elegans*, *Plestiodon chinensis*, *Erendas brenchleyi*, *Phrynocephalus vlangalii* and *Eremias multiocellata* (Du and Ji, 2001; Ji and Du, 2000; Lin and Ji, 2000; Li et al., 2006; Xu and Ji, 2003; Zhang et al., 2005). Previous studies strongly suggest that sexual dimorphism results from a balance between numerous selective pressures differing in influence between the sexes (Shine, 1989; Schoener et al., 1982; Vitt and Cooper, 1985). Consequently, various hypotheses have been proposed to explain sexual dimorphism, including, female choice in mate selection, male aggressive behavior (Andersson, 1994; Cooper and Vitt, 1993), fecundity selection (a selection leading to larger body-cavity size in females) (Griffith, 1990), differential mortality due to differences in longevity (Shine et al., 2002), and food-niche divergence (Lin and Ji, 2000). Because reproductive output is associated with numerous morphological traits in lizards, data on female physiology and reproduction are crucial to understanding the origin of sexual dimorphism in the group (Du and Ji, 2001; Ji and Du, 2000; Lin and Ji, 2000; Li et al., 2006; Zhang et al., 2005).

The common lizard, *Lacerta vivipara* Jacquin, 1787, has the largest geographic range of any terrestrial squamate reptile, extending across Eurasia from western Europe to Japan. In China, it is found in Heilongjiang Province, Xijiang Province and Inner Mongolia. It is a small (approximately 4–5 g), diurnal, non-territorial lizard typically found in open spaces surrounded by pine-broadleaf mixed forest (Zhao et al., 2006).

Due to both its abundance in nature and unique dual oviparous and ovoviviparous reproductive modes, *Lacerta vivipara* has been the focus of numerous morphological studies (Guillaume, 2006; Šmajda and Majláth, 1999; Lecomte et al., 1992; Wermuth, 1955). Despite this abundance of detailed quantitative examination (Dong, et al. 2004; Fang and Tang, 1983; Zhao et al., 2006), however, details on Chinese populations of the species and the relationship between its reproductive ecology and morphometry remains poorly understood . To examine the relationships between sexually dimorphic, morphometric traits in males and females (from adults to neonates), and their relationships to offspring

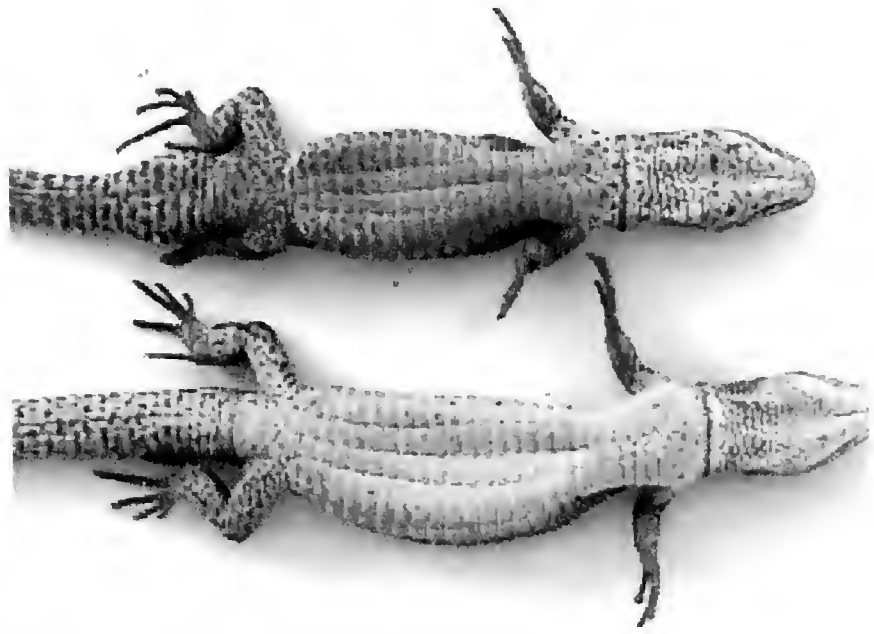


Figure 1. The tail base and ventral color of a male (top) and female (bottom) *Lacerta vivipara*.

number and mass, we studied a population of *L. vivipara* in Sunwu County, Heilongjiang Province, in northeast China (49° 39′ 19.2″ N, 127° 34′ 10.1″ E; elevation 304 m). Morphological measurements were taken from lizards collected in the field. Females gave birth to young under simulated field conditions. Particular attention was paid to examining (1) sexual dimorphism in ecologically important morphological traits and (2) the relationship between female size and offspring size and number. The results demonstrate that increased male head size is an adaptation for combat and increased female body length is an adaptation for higher fecundity.

Materials and Methods

Specimen collection and housing.- From the end of May to the beginning of October in 2003, 183 lizards (121 females, 62 males) were collected and analyzed. It was assumed that the lizards were collected randomly, thereby making the sample representative of the population as a whole. Most of the males sampled were used only for the collection of morphological data and were released immediately following measurement; all females were retained for subsequent analysis. The retained lizards were transported to a nearby field station and housed in a 7.5 x 1.8 x 1.0 (length x width x height) m^3 enclosure on the ground. The bottom of the enclosure was covered with grass, branches and stones to simulate the lizards' natural habitat. Food (insects and spiders) and water in small dishes were provided ad libitum. A humid environment was maintained by spraying the substrate with water daily. The lizards were marked by toe clipping and back-painting.

Morphometry.- For each lizard collected, the following five variables were measured with digital calipers to the nearest 0.01 mm: snout-vent length (SVL; from the tip

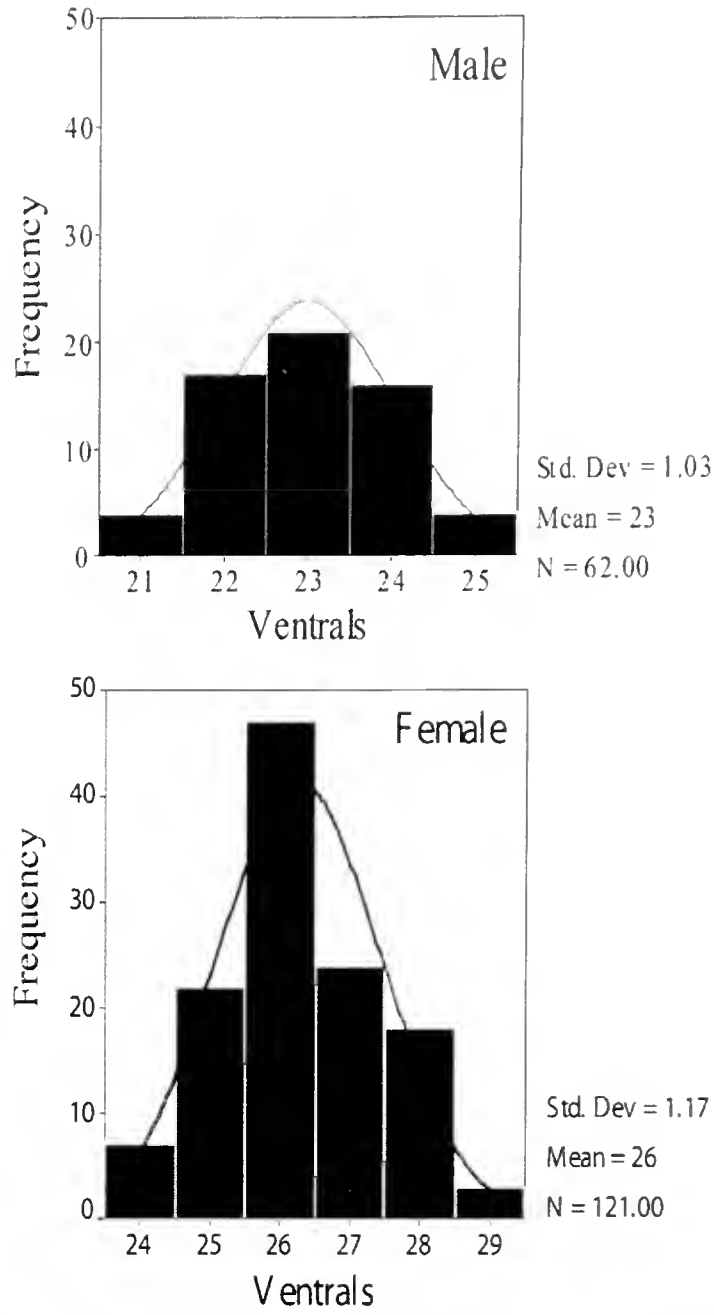


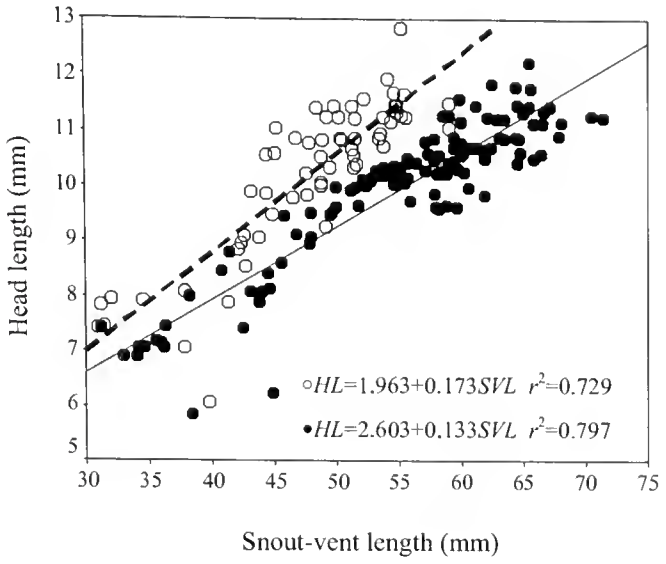
Figure 2. Frequency distribution of rows of ventral scales in males and females.

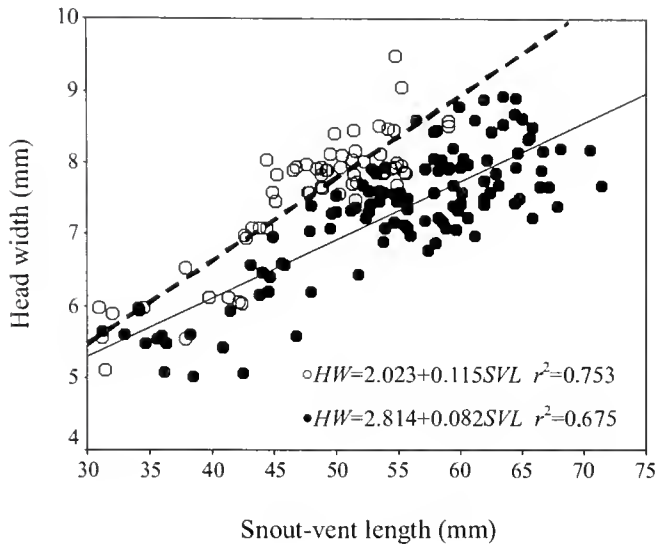
of the snout to the anterior margin of the cloacal lips); head length (HL; from the tip of the snout to the posterior margin of the skull); head width (HW; the largest width of the head); head height (HH; the largest height of the head); tail length (TL; from the anterior margin of the cloacal lips to the tip of the tail; specimens with regenerated tails were excluded); body weight (BW), number of ventral and mid-dorsals scale rows. Femoral pores, venter color and tail base width were used to sex individuals. Specimens with a SVL of 47 mm or more were considered to be sexually mature adults (139 specimens total); specimens with a SVL of 36-47 mm were considered juveniles (30 specimens); specimens with a SVL of less than 36 mm were considered neonates (14 specimens). .

Female reproduction.- Gravid females were separated from each other in 30 x 25 x 25 (length x width x depth) cm^3 cages in order to accurately associate newborns with their mothers. Enclosures were checked at least once a day for neonates, which were immediately measured and weighed after birth. Postpartum females were individually weighed and measured for SVL. Clutches

Table 1. Descriptive statistics of morphological traits of *Lacerta vivipara* in China. Data are expressed as mean±SE and range (minimum-maximum) and compared using *t*-test (*t*) and ANCOVA (F) with SVL as the covariate. Length units are in mm, and mass in grams.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

	Neonates				Juveniles				Adults			
Trait	Male (n = 5)	Female (n = 9)	*t*	*F*	Male (n = 15)	Female (n = 15)	*t*	*F*	Male (n = 42)	Female (n = 97)	*t*	*F*
	31.80±1.30	34.56±1.74			42.39±2.27	43.11±2.55			51.86±3.13	58.69±5.44		
SVL	31.00–34.12	31.06–36.00	-3.073**		38.01–45.14	38.21–46.08	-0.821		47.05–59.07	50.03–71.40	-9.304***	
	47.0±3.74	47.5±2.21			65.74±9.34	56.77±8.09			84.71±1.71	79.37±1.25		
TL	43.00–52.19	44.0–51.03	-0.244		43.25–80.14	41.84–66.25	1.59		57.07–96.21	43.76–93.86	2.519**	
	0.78±0.14	0.82±0.09			2.33±0.79	1.87±0.43			3.79±0.57	4.35±0.71		
BW	0.54–0.87	0.66–0.93	-0.601		1.17–3.97	1.00–2.52	1.954		2.65–5.00	2.26–7.36	-3.710***	
	7.80±0.27	7.11±0.22			9.07±1.43	7.90±1.10			10.94±0.65	10.47±0.69		
HL	7.53–8.03	7.11–7.52	5.155***	18.515**	6.03–11.00	6.03–9.50	2.490*	11.380**	10.10–11.69	9.00–11.03	3.791***	140.145***
	5.70±0.44	5.5±0.30			6.81±0.77	6.07±0.64			8.02±0.41	7.67±0.57		
HW	5.53–6.03	5.00–6.00	0.727	0.357	5.50–8.03	5.03–7.02	2.831**	12.134**	7.50–9.50	6.45–9.01	4.037***	48.800***
	4.40±0.42	4.61±0.42			4.81±0.51	4.52±0.39			5.90±0.64	5.66±0.65		
HH	4.14–5.04	4.13–5.03	-0.907	0.13	4.20–6.55	4.12–5.22	1.756	2.932	5.50–7.50	4.50–7.01	1.937	50.035***





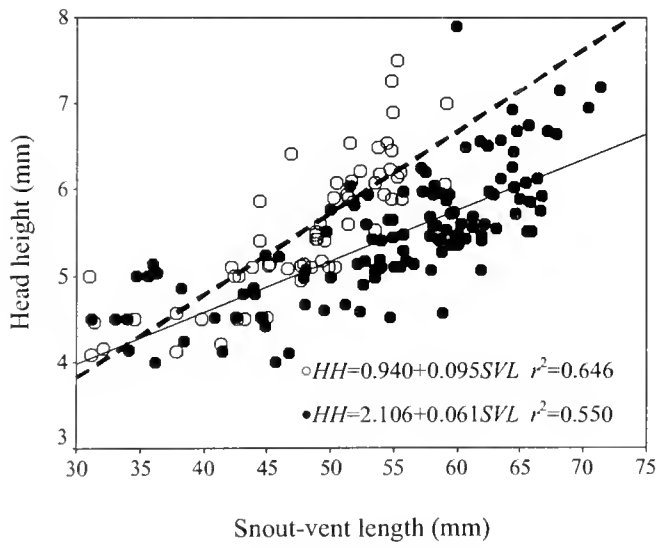


Figure 3. Linear regressions of head length, head width and head height with SVL in *Lacerta vivipara*. The regression equation is indicated in the figure. See text for statistical analyses. Solid dots and lines: females; open dots and dashed lines: males.

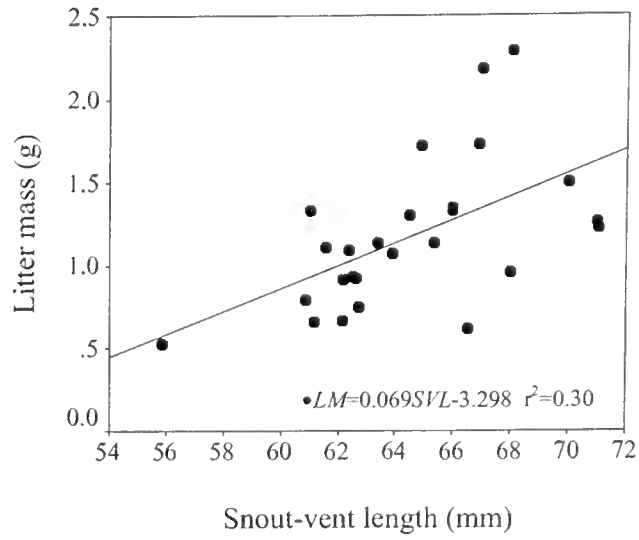


Figure 4. Linear regression of litter mass on female SVL in *Lacerta vivipara*. The regression equation is indicated in the figure. See text for statistical analyses.

including dead young, stillborns, or unfertilized eggs were excluded from statistical analyses. Clutch mass (RLM) was calculated by dividing litter mass by post-partum female mass (Shine, 1992). Relative fecundity was calculated by using the residuals derived from the regression of litter size on maternal SVL (Olsson and Shine, 1997).

Statistical analysis.- Whenever parametric statistics were applied, a normal distribution was verified using the Kolmogorov-Smirnov test. Homoscedasticity was verified using Levene's Test for Equality of Variances. For significant departures from normality or homoscedasticity, data were $\log_e$-transformed before analysis. To test for sexual dimorphism in the data, absolute values of morphometric measurements were compared between sexes using a linear regression analysis, one-way analysis of variance (ANOVA) and one-way analysis of covariance (ANCOVA) with SVL as the covariate.

All statistical analyses were performed using SPSS (Statistical Package for the Social Science) v11.5 for Windows. Homogeneity of slopes was checked prior to testing for differences between adjusted means. Values are presented as mean ± standard error and the significance level in set at $p \leq 0.05$ for all statistical tests.

Table 2. Slope (b), intercept (a) and adjusted R square (r^2) estimated from reduced major axis regressions for each trait against SVL in female neonates, juveniles, and adults.

	Neonates			Juveniles			Adults		
	n = 9			*n* = 15			*n* = 97		
Y	a	b	r^2	a	b	r^2	a	b	r^2
Head length	8.617	-0.044	0.118	4.316	0.083	0.037	4.939	0.094	0.555
Head width	7.378	-0.053	0.093	-0.968	0.163	0.42	4.653	0.051	0.241
Head height	1.837	0.08	0.112	3.939	0.014	0.008	1.117	0.077	0.407

Table 3. Descriptive statistics of female reproductive traits and snout-vent length of *Lacerta vivipara* (n = 26).

	Mean	Standard error	Range
Female snout-vent length (mm)	64.52	3.52	55.85–71.04
Postpartum body mass (g)	4.82	0.71	3.63–6.03
Litter size	7.11	2.47	3–12
Litter mass (g)	1.18	0.44	0.53–2.29
Neonate mass (g)	0.17	0.03	0.11–0.24
Relative litter mass	0.25	0.1	0.09–0.32

Results

Sexual dimorphism.- In males, the base of the tail bulged because of the presence of the hemipenes and the venter of the tail was salmon pink. In females, the base of the tail was slender and the venter had a saffron-yellow to off-white tint (Fig. 1). Femoral pores (8–12) were small and black in females and neonates while it was accompanied by a thorn in adult male. There were more vertical scale rows in females (24–29) than in males (21–25) (Mann-Whitney Test, $Z = -10.377$, $p < 0.01$; Fig. 2), and males had more mid-dorsal scales (31.63±1.46) than females (30.61±1.48) (Mann-Whitney Test, $Z = -3.514$, $p < 0.01$).

The largest male and female were 59.07 and 71.40 mm SVL, respectively. The mean SVL was larger in adult females (58.69±5.44 mm) than in adult males (51.86±3.13 mm) ($t = -9.304$, $p < 0.001$). Body weight was greater in adult females ($t = -3.710$, $p < 0.001$) and tail length was larger in adult males ($t = 2.519$, $p < 0.01$). An ANCOVA test controlling for SVL found that adult males had a larger head size (head length, head width and head height) compared to adult females of the same SVL (ANCOVA; HL, $F = 140.145$, $p < 0.001$; HW, $F = 48.800$, $p < 0.001$; HH, $F = 50.035$, $P < 0.001$). Head length and head width were larger in juvenile males than in juvenile females (ANCOVA; HL, $F = 11.380$, $p < 0.01$; HW, $F = 12.134$, $p < 0.01$) and head length was larger in neonate males than in neonate females (ANCOVA; HL, $F = 18.515$, $p < 0.01$). Body length was larger in neonate females than in neonate males ($t = -3.073$, $p < 0.01$) (Table 1).

The rates at which head length, head width, and head height increased with increasing SVL were all greater in males than in females (Fig. 3). Although the rates of increase were the same in adult males as they were for juvenile and neonate males (ANCOVA; HL, $F = 2.972$, $p = 0.059 > 0.05$; HW, $F = 0.476$, $p = 0.624 > 0.05$; HH, $F = 5.091$, $p = 0.09 > 0.05$), this was not the case for females (ANCOVA; HL, $F = 8.175$, $p < 0.001$; HW, $F = 5.586$, $p = 0.005 < 0.01$; HH, $F = 6.143$, $p = 0.03 < 0.05$). In female neonates, head length and width did not increase proportionally to SVL ($b < 0$) and rate of head width was greater in female juveniles than in female adults ($b = 0.163$ vs. $b = 0.051$) (Table 2).

Female reproductive traits.- Female *Lacerta vivipara* produced a single clutch of 3–12 young every breeding season (Table 3). Clutch mass was positively correlated with maternal SVL ($r = 0.55$, $F = 5.43$, $p < 0.01$; Fig. 4), whereas clutch size ($r = 0.38$, $F = 3.75$, $p = 0.06$) and neonate mass ($r = 0.37$, $F = 3.38$, $p = 0.06$) were not. Neonate mass was independent of relative fecundity ($r = 0.15$, $F = 0.56$, $p = 0.46$).

Discussion

Consistent with previous studies of European populations of *Lacerta vivipara* (Gvoždík and Damme, 2003; Kratochvil et al., 2003; Šmajda and Majláth, 1999; Wermuth, 1955), the present study found that sexual dimorphism in head size, abdomen length, and tail length was widespread in Chinese populations, suggesting that these sexually dimorphic traits evolved a very long time ago and has remained in the species as it dispersed across Asia. *Lacerta vivipara* is similar to other lizards (e.g., *Plestiodon laticeps*, *Plestiodon elegans*, *Phrynocephalus vlangalii*, *Takydromus septentrionalis*, *Tropidurus torquatus*) (Du and Ji, 2001; Vitt and Cooper, 1985; Zhang and Ji, 2000; Zhang et al., 2005) in that the males have a larger head and longer tail while females have a longer snout-vent length, increased body weight a longer abdomen, and more rows of ventral scales.

Sexual differences in head size are common within the Lacertidae (Huang, 1998; Molina-Borja et al., 1998). Since long periods of evolutionary time are often required to manifest these differences (Kratochvil et al., 2003), proximate environmental factors can be less important determinants of sexual dimorphism in head size than ultimate ones, such as phylogenetic history. Sexual dimorphism may simply be the result of phylogenetic history and is maintained through competition over mates (intra- and inter-sexual selection) (Kratochvil et al., 2003; Shine, 1989).

According to most speculation, variations in allometry in *Lacerta vivipara* are adaptive responses related to differences in both the ecology and reproductive behavior of the two sexes (Kratochvil et al., 2003). Although it has been reported that a larger head is a male adaptation to feeding on larger prey (Schoener et al., 1982), there is little intersexual dietary divergence in *L. vivipa-*

ra (Zhao et al., 2006). In contrast, the present study supports the conclusion that larger male heads are an adaptation for intersexual combat (Gvoždík and Damme, 2003). There is also evidence to support the possibility that a longer male tail provides armament in combat and improves the male's ability to escape (Barbadilloo and Bauwens, 1997; Barbadilloo et al., 1995; Braña, 1996; Herrel et al., 2001). Color dimorphism is hormonal in origin, becoming noticeable at the onset of sexual maturity; this dimorphism apparently aids in sexual identification and maintaining social hierarchy (Adriana, 2005).

Females have a considerably larger number of transverse rows of scales covering the venter of the abdomen and have a relatively large abdomen compared to males of the same size. The present data showed that maternal size is the main determinant of reproductive output in *Lacerta vivipara*, with larger females producing heavier clutches. This offers strong evidence to support the hypothesis that selection for higher fecundity results in the evolution of a longer trunk.

In other species of lizards such as *Takydromus septentrionalis*, *Podarcis muralis*, *Gekko japonicus*, *Plestiodon chinensis* and *Sphenomorphus indicus*, sexual dimorphism in head size occurs at earlier ontogenetic stages (Zhang et al., 2005). Our results reveal a similar pattern in *Lacerta vivipara*, in that changes in allometry vary at different ontogenetic stages between the sexes, resulting in a distinct dimorphism. The neonates have larger heads to obtain more foods to increase the trunk, so that the sex individuals have no significant difference in the size. With the growth of the body, the rate growth of the head slows in female and head length and head width is decreased, and quickly increased in juveniles till the adults. Adult females of *L. vivipara* sacrifice head and tail growth for increased abdomen (and body cavity) length in order to achieve a greater reproductive output. In conclusion, *Lacerta vivipara* exhibits a sexual dimorphism in size, color, and shape that can be linked to sexual selection. In females, characteristics allowing for higher reproductive output are selected for in females, resulting in larger bodies and energy allocation directed to early reproduction instead of growth. In males, characteristics selecting for increased numbers of copulations are selected for.

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Sperm Morphology of Five *Rhacophorus* (Amphibia: Anura: Rhacophoridae) Species from China

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Abstract.- Sperm shape and size of five species of the genus *Rhacophorus* from China were investigated in the present study. Our results reveal the presence of two possible monophyletic lineages: the first is composed of *R. chenfui, R. dugritei* and *R. omeimontis*, has relatively small spermatozoa with a with a coiled head and a thin tail, while the second, composed of *R. mutus* and *R. megacephalus*, have spermatozoa that are longer with a straight head and filiform shape.

Keywords.- Amphibian, Rhacophoridae, *Rhacophorus*, *Polypedates*, spermatozoa, morphology.

Introduction

The genus *Rhacophorus* (*sensu lato*) is a member of the family Rhacophoridae and contains approximately 80 species worldwide, 24 of which are found in China (Fei et al., 2005). Rhacophorids are predominantly arboreal, sharing with basal ranids expanded digital pads and mantellids the intercalary phalangeal elements (Frost et al., 2006). These frogs are distributed in the tropical and sub-tropical regions of eastern and southern Asia; in China, they inhabit southern areas north to Qinling. Phylogenetic and taxonomic relationships within this genus are still conjectural and controversial. For instance, the genus *Polypedates* is valid according to Frost et al. (2007), but it is absent in Fei et al. (2005). Furthermore, Frost et al. (2007) placed *R. chenfui*, *R. dugritei*, *R. omeimontis* in *Rhacohporus*, while *R. mutus* and *R. megacephalus* were placed in the genus *Polypedates* by Fei et al. (2005).

Previous studies have revealed that some morphological characters of the spermatozoa were unique to given taxa in the Anura (Kuramoto and Joshy, 2000; Kuramoto, 1996; Zheng et al., 2000a, 2000b, 2002). For example, the spermatozoa of most *Rana* species are characterized by a cylindrical head and a thin tail, while spiral and corkscrew-shaped sperm head and waved tail are typical of spermatozoa in the family Megophryidae (Zheng et al., 2002), and fusiform-shaped spermatozoa are unique to the family Bombinatoridae (Zheng et al., 2000a). These studies make it evident that sperm morphology is not only variable between taxa, but can be useful for elucidating taxonomic relationships (Kuramoto and Joshy, 2000). In this study, the shape and size of spermatozoa in *Rhacophorus chenfui*, *R. dugritei*, *R. omeimontis*, *R. megacephalus* and *R. mutus* were examined for the purpose of resolving their cryptic phylogenetic relationships.

Materials and Methods

Collecting localities are listed in Table 1. All specimens were collected during their breeding seasons, (i.e., May to July) from 1998 to 2005.

Frogs were euthanized by inserting a medical needle through the occipital ostium to destroy the spinal cord. Next, the testes were removed and immediately fixed with 10% formaldehyde, squashed and macerated with a clean toothpick; sperm were suspended on slides, air-dried and stained with acid carmine for 40 seconds. Slides were examined on a ZEISS Axioplan2 light microscope (LM). Other testes were fixed with 3% glutaraldehyde for about two hours and centrifuged at 3000 rpm for 30 s; the supernatant was discarded and the pellet rinsed with double distilled water. A drop of the resulting sperm suspension was placed on a cover slip, air-dried, coated with gold, and observed on a JEOL JSM-5900LV scanning electron microscope (SEM).

Spermatozoan pictures were shot by ZEISS AxioVision 4.0. Sperm length was measured by software ArcView GIS 3.2. Sperm length data were analyzed with SPSS 11.5. The length of sperm head of *Rhacophorus chenfui*, *R. dugritei*, *R. omeimontis* were calculated by the formulate $L=Nl\pi$ (L: the length of sperm head, $\pi = 3.14$, l = the diameter of helix, N = the number of turns in the helix).

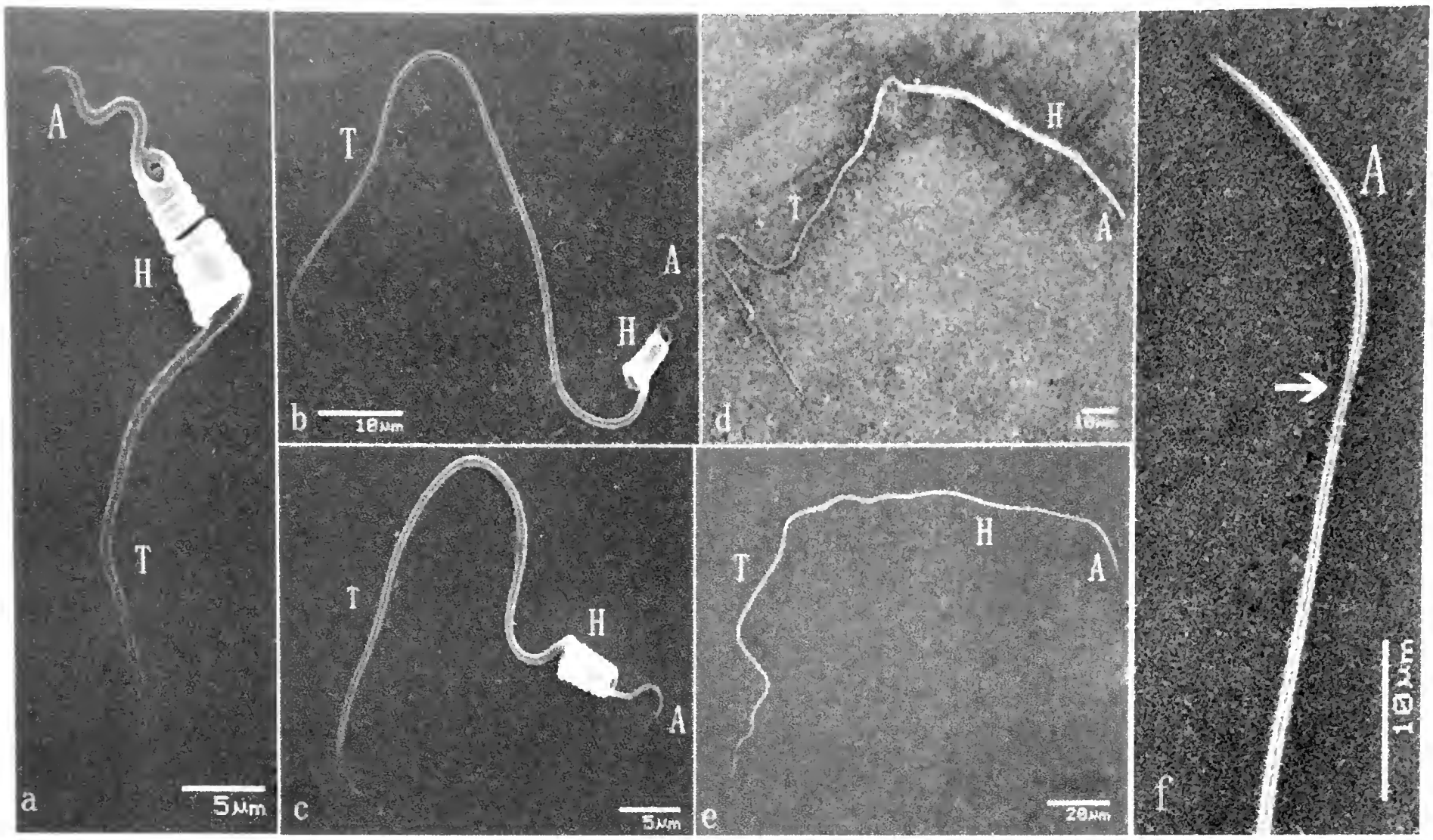

Figure 1. Spermatozoa of five tree frogs. a: *Rhacophorus chenfui*; b: *R. dugritei*; c: *R. omeimontis*; d: *R. mutus*; e: *R. megacephalus*; f: detail of the sperm head of *R. mutus*. A: acrosome; H: head; T: tail.

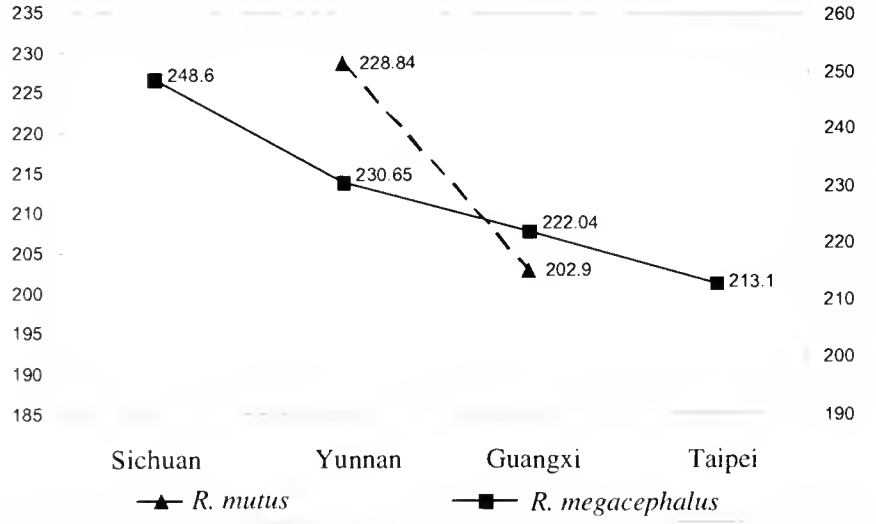

Figure 2. The total length of spermatozoa in different populations of *Rhacophorus mutus* and *R. megacephalus*. Units: μm.

Results

The spermatozoa of all five species consisted of two portions, the head and the tail. The sperm head of *Rhacophorus chenfui* (Fig. 1a) was shaped like a coiled spiral, the tail was thin and wavy (shared with characteristic shared with *R. dugritei* (Fig. 1b) and *R. omeimontis* (Fig. 1c)), and the slightly-coiled apical section was likely the acrosome. The shape of the sperm in *R. mutus* (Fig. 1d) was filiform, consisting of a straight, thick head and a thin wavy tail. A thinner section (Fig. 1f, see arrow) at the tip of sperm head was likely the acrosome (Fig. 1f, a). Bends and waves could usually be observed from the midpoint of the sperm.

Spermatozoa measurements are shown in Table 1. Total sperm length in *Rhacophorus chenfui* and *R. dugritei* is much shorter than those measured for *R. megacephalus* and *R. mutus*; the sperm of *R. omeimontis* was of intermediate length.

The sperm head of *Rhacophorus dugritei* was the shortest and thinnest, and the head of *R. omeimontis* was longer than that of *R. dugritei*, and thinner than those of all other four species. Head length in *R. chenfui* was also relatively short, and it was also slightly thicker than that of *R. dugritei*. *R. megacephalus* had the largest sperm head among all five species, with the head of *R. mutus* being only slightly smaller.

The coiled head of *Rhacophorus chenfui*, *R. omeimontis* and *R. dugritei* were largely similar. However, the sperm head of *R. chenfui* was longer than the tail , with the ratio of sperm head to total sperm length being 1:0.86.

Sperm size of *Rhacophorus megacephalus* and *R. mutus* differed remarkably between populations. The spermatozoa of *R. megacephalus* in Sichuan population were longer than those in the Guangxi population, and those of *R. mutus* were longer than those in the Yunnan population compared to the Guangxi population (Table 1; Fig. 2).

Table 1. Sperm measurements of *Rhacophorus* species.

Species	Locality	N	Head length	Tail length	Total length	Head width	Turns of head coil
R. chenfui	E`mei, Sichuan	20	66.35±7.05 (53.75 %)	57.10±4.36 (46.25 %)	123.45±8.60	0.76±0.1	8.15±0.59
R. dugritei	Hongya, Sichuan	20	59.97±10.67 (47.59 %)	63.60±16.73 (50.47 %)	126.02±10.97	0.52±0.1	5.90±0.72
R. omeimontis	Hongya, Sichuan	20	75.43±8.17 (41.12 %)	107.99±14.12 (58.88 %)	183.42±17.00	0.50±0.12	6.80±0.62
	Hejiang, Sichuan	20	104.31±12.36 (43.25 %)	136.85±9.54 (56.75 %)	241.16±11.61	0.89±0.15	—
R. megacephalus	Tengchong, Yunnan	20	94.05±15.30 (40.78 %)	136.60±14.38 (59.22 %)	230.65±20.20	0.85±0.04	—
	Shangsi, Guangxi	20	80.33±12.08 (36.18 %)	141.71±12.00 (63.82 %)	222.04±11.01	0.83±0.09	—
R. mutus	Tengchong, Yunnan	20	95.32±8.96 (41.65 %)	133.52±8.66 (58.35 %)	228.84±14.01	0.79±0.08	—
	Shangsi, Guangxi	20	78.22±10.66 (38.55 %)	124.68±9.81 (61.45 %)	202.90±11.02	0.83±0.09	—

Unit: μm. N: number of spermatozoa;

Discussion

Spermatozoa morphology.- The morphology of the sperm observed here for *Rhacophorus chenfui*, *R. dugritei* and *R. omeimontis* appeared to be very similar to that reported by Mizuhira et al. (1986) for *R. arboreus* and *R. schlegelii,* as well as that reported by Kuramoto (1996) for *R. viridis amamiensis*, *R. owstoni* and *R. moltrechti*. The sperm heads of latter species, however, while coiled, did not appear to have the two twisted subcoils characteristic of the former three species. Conversely, the shape of the spermatozoa in *R. mutus* and *R. megacephalus* were very similar to those seen in *Polypedates leucomystax*, *P. megacephalus* (Kuramoto, 1996) and *P. maculatus* (Kuramoto and Joshy, 2000), where the head is linear (not coiled) and no fibers in the tail. Based on the variation of sperm morphology observed here, the five species examined can be divided into two groups: tthe first group (consisting of *R. chenfui*, *R. dugritei* and *R. omeimontis*) is characterized by a helical sperm head and a thin sperm tail, and the second group (consisting of *R. mutus* and *R. megacephalus*) is characterized by a thread-like sperm head and a thin tail.

Sperm size differ remarkably between species, also between populations. The average length of spermatozoa in *Rhacophorus chenfui* was 123.45±8.60μm, while that of *R. megacephalus* was 230.25±19.81 μm, a difference of statistical significance ($P < 0.01$, $n = 20$, one-way analysis of variance). In the different populations of *R. megacephalus*, total spermatozoa length in the Sichuan, Yunnan, Guangxi and Taipei populations was 241.16 μm, 230.65 μm, 222.04 μm, 213.1 μm (Kuramoto, 1996), respectively, illustrating a reduction in length towards the Pacific Ocean (Fig. 3). This phenomenon was also observed in *R. mutus* (average length 228.84 μm in the Yunnan population and 202.9 μm in the Guangxi population).

The implications for taxonomy from spermatological data.- On the basis of skeletal characters, Liem (1970) placed *Rhacophorus dugritei* and *R. omeimontis* into the genus *Polypedates*. Uncertain as to the limits of these two genera, Fei (1999) and Fei et al. (2005) tentatively treated all *Polypedates* as *Rhacophorus*. Based on molecular evidence, however, Frost et al. (2007) placed *R. chenfui*, *R. dugritei* and *R. omeimontis* in *Rhacohporus,* and placed *R. mutus* and *R. megacephalus* in *Polypedates*. In view of the spermatological data presented here, the latter hypothesis presented by Frost et al. (2007) appears to be best supported.

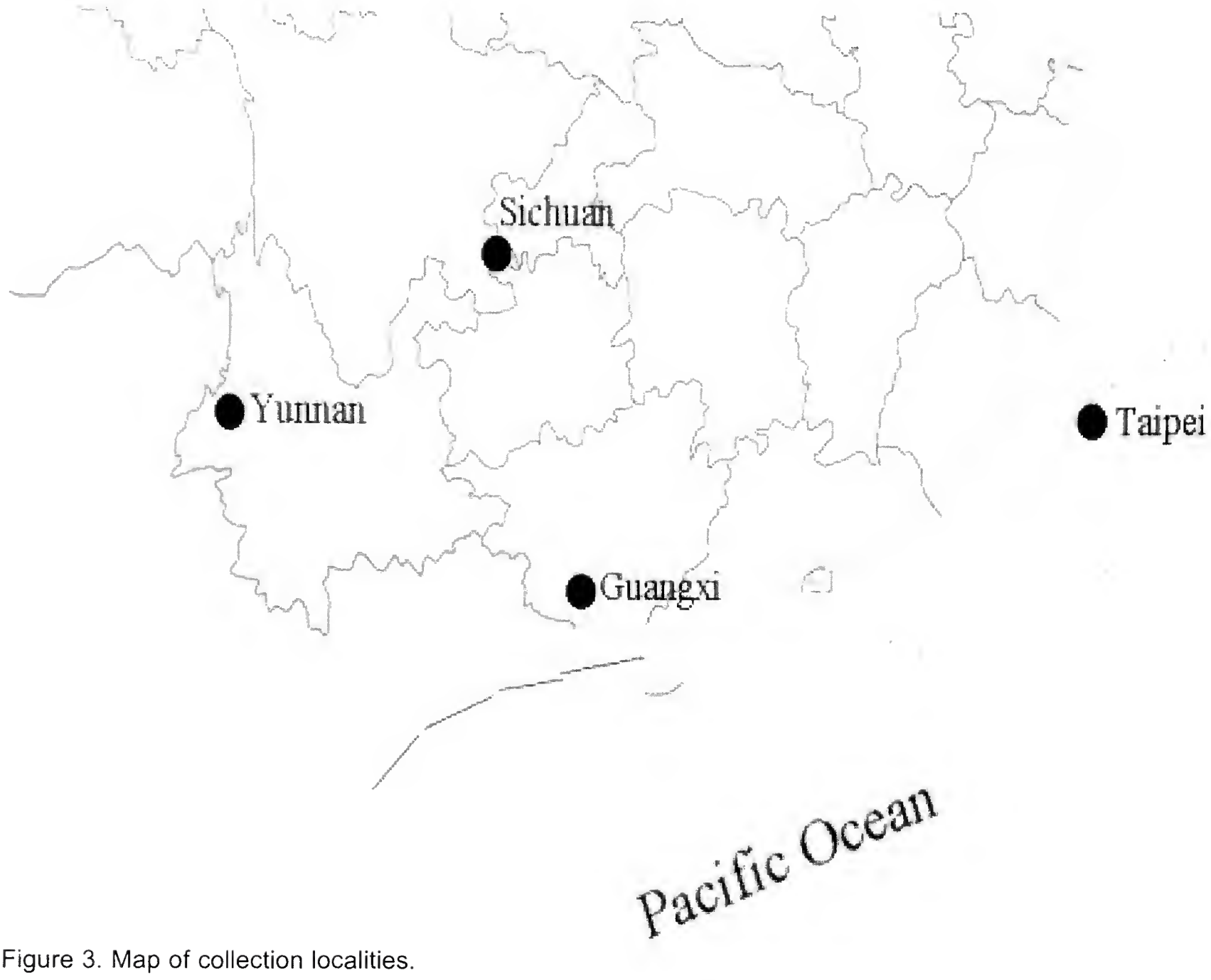

Figure 3. Map of collection localities.

Acknowledgments

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The Herpetofauna of Nallamala Hills, Eastern Ghats, India: An Annotated Checklist, With Remarks on Nomenclature, Taxonomy, Habitat Use, Adaptive Types and Biogeography

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Abstract.- We present an inventory of the herpetofauna of the Nallamala Hills, Eastern Ghats, south-eastern India. The fauna, as currently known, includes 20 species of amphibians belonging to 14 genera in six families and 64 species of reptiles belonging to 42 genera in 15 families. Divided in habitat types, the herpetofauna can be classified into species tolerant of disturbed habitats; exclusively scrub species (and for reptiles, from rocky biotopes); scrub and bordering agricultural fields; and exclusively mesic forest species. For one species, lack of ecological information precludes its allocation to a specific habitat category. Significant diversity of squamates (including gekkonids, scincids, and colubrids) are known from these ranges, several of which endemic or largely restricted to scrub forests of Peninsular India. Mesic forests remain poorly explored, and support hitherto undescribed species among the herpetofauna. Adaptations seen amongst the herpetofauna of the Nallamala Hills include a diversity of dietary and habitat types, including, among amphibians, ant specialists; predators of small vertebrates; folivores; fossorial; terrestrial; aquatic or aquatic-margin; and arboreal forms. Amongst reptiles, adaptive types includes ant- and worm-eaters; predator of crop pests; predator of small or medium-sized vertebrate prey; egg-predators; fish-eaters; frog- and toad-eaters; and one near-exclusive snake-eater. In terms of habitat usage, reptiles exceed amphibians in species richness, on account of their greater capacity of surviving in relatively arid regions.

The Eastern Ghats contributes significantly to both species richness and endemicity of the Indian region, including representatives of endemic genera and species. Nonetheless, these hills continue to receive less attention for conservation compared to the relatively better-known Western Ghats.

Keywords.- Amphibians, reptiles, biodiversity, ecology, Nallamala Hills, Eastern Ghats, India.

Introduction

Nallamala Hills (14° 26' – 16° 31' N and 78° 30' – 80° 10' E) are a group of low hill ranges with an average altitude of ca. 500 m in the central Eastern Ghats complex in the state of Andhra Pradesh, south-eastern India (Fig. 1). From the Palnad Basin in the north to the Tirupati Basin in the south, the Nallamala Hills runs for a distance of ca. 430 km with an average width of 30 km (Anon, 1965; Srinivasulu and Nagulu, 2002). An unbroken chain of rugged hills with precipitous cliffs covering an area of ca. 7,640 km^2, it encompasses six districts (Nalgonda, Mahbubnagar, Kurnool, Cuddapah, Prakasam and Guntur) in Andhra Pradesh State. Running parallel to it in the south-eastern side is the Balapalli and Palakonda Ranges, while on the western side, towards the north, are the Erramala Range. The vegetation is typically of the southern tropical dry deciduous and southern tropical moist deciduous forest types intermingled with scrub (Champion and Seth, 1968), although the Nallamalas show representatives of many vegetation types known from the Eastern Ghats, including dry deciduous, moist deciduous, dry evergreen, riverine and scrub forest (see R. K. Rao, 1998; R. S. Rao, 1998). Dry deciduous forests are dominant. Common species found here include *Antidesma acidum*, *Canthium parviflorum*, *Cerisoides turgida*, *Cissus pallida*, *Cochlospermum religiosum*, *Colebrookea oppositifolia*, *Dalbergia lanceolaris*, *Dalbergia paniculatum*, *Diospyros melanoxylon*, *Ehretai laevis*, *Lagerstroemia parviflora*, *Pterocarpus marsupium*, *Syzygium alternifolium,* and *Tamilnadia uliginosa*. A forest type with *Boswellia serrata* and *Chloroxylon swietenia* as the dominant species occurs near Chalama, a *Terminalia coriacea* and *Anogeisus latifolia* type occur in eastern Nallamala, a *Phoenix* type with *Phoenix loureivie* as the dominant species forming a pure stand on rocky substrata occur between Ramannapenta and Gundla Brahmeshwaram Metta Wildlife Sanctuary (GBM). Moist deciduous forests are restricted to sheltered sites with high rainfall such as GBM, upper Ahobilam and Iskagundam; common species include: *Careya arborea*,

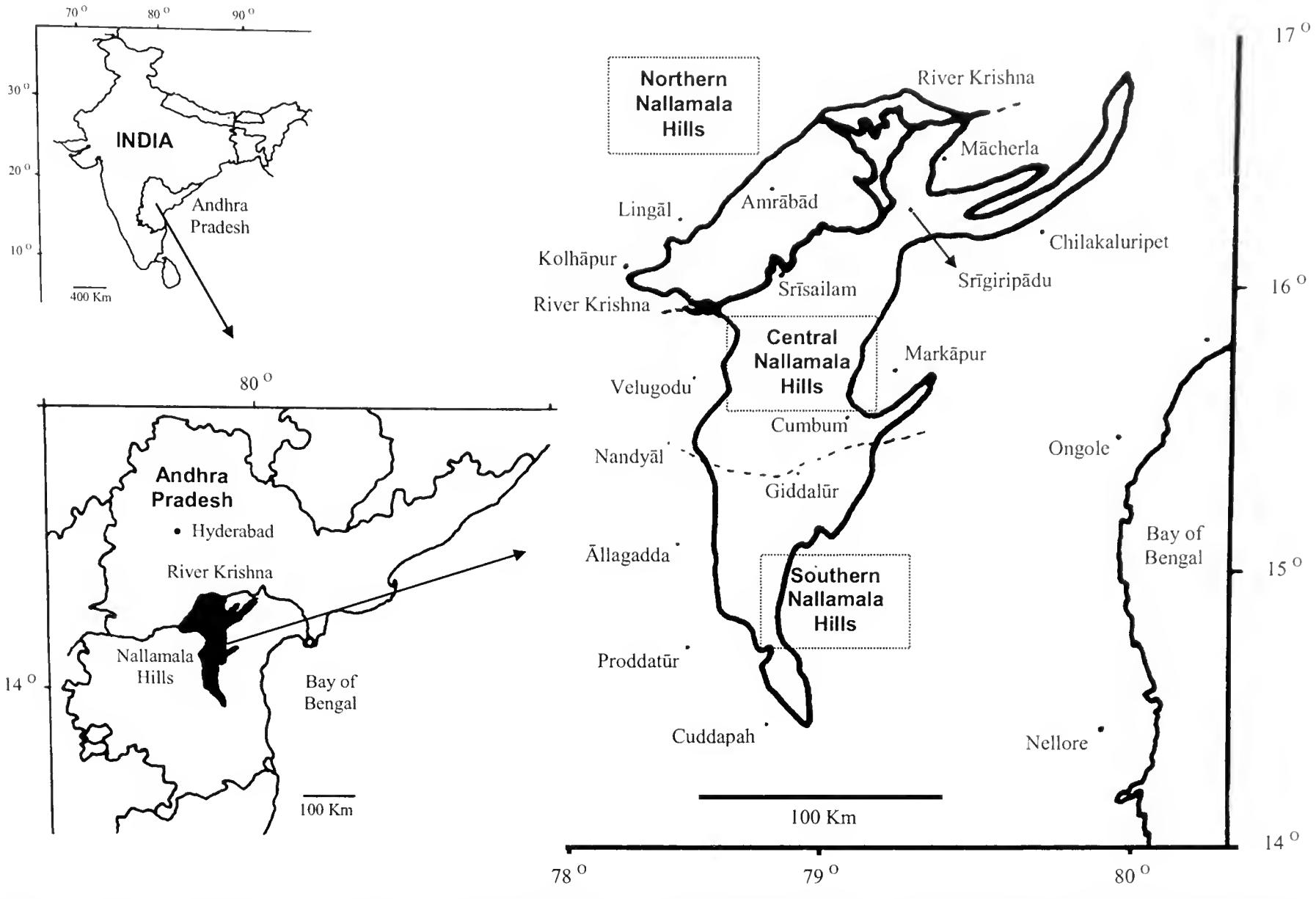

Figure 1. Maps showing the location of the Nallamala Hills, Andhra Pradesh, south-eastern India. On top left, map of India, showing Andhra Pradesh State; on bottom left, map of Andhra Pradesh, showing location of Nallamala Hills; and on right, the Nallamala Hills, with localities mentioned in the text.

Dillenia pentagyna, *Ficus hispida*, *Barleria strigosa*, *Adiantum lunulatum*, *Oroxylona indicum*, *Trema orientalis* and *Pimpinella wallichiana*. The Nallamalas are home to many endemic species of Eastern Ghats including: *Andrographis nallamalayana*, *Ericaulon lushingtonii*, *Dicliptera beddomei*, *Premna hamiltonii*, *Euphorbia linearifolia* var. *nallamalayana*, *Rostellularia vahlii*, *Andrographis beddomei*, *Rostellularia vahlii* var. *rupicola*, *Boswellia ovalifoliata*, *Cycas beddomei*, *Chaemaesyce linearifolia*, *Chaemaesyce senguptae*, *Crotalaria madurensis*, *Crotalaria paniculata nagarjunakondensis*, *Indigofera barberi*, *Pterocarpus santalinus*, *Albizia sikharamensis*, and *Eriolaena lushingtonii*. *Pterocarpus marsupium* and *Cycas beddomii* are well known endemics. Another interesting feature of the flora is the exhibition of gigantism as exemplified by the shrubby climber *Marsdenia tenacissima*, the leaves of which measure up to 32 cm. Other climbers such as *Bauhinia vahlii* and *Enteda pursetha* are dominant over other vegetation. The climate is generally hot and dry with temperatures rising up to 43–45°C during May and dropping to 8–12°C in December. The Nallamala Hills receive on an average 900–1,000 mm rainfall annually.

The Nallamala Hill Range has been conveniently divided into three zones (Fig. 1): i.) the Northern Nallamala Hills (the expanse of hill ranges between the Palnad Basin and the River Krishna that flows approximately 130 km through the hills); ii.) the Central Nallamala Hills (the expanse of hill ranges between the River Krishna and the British railway track between Nandyal and Guntur passing through Chalama, Bogada, and Diguvametta); and iii.) the Southern Nallamala Hills (the expanse of hills between the British railway track and the Tirupati basin near Rajampet (14° 11' N and 79° 10' E). Two contiguous protected areas, the Nagarjunasagar Srisailam Tiger Reserve and the Gundla Brahmeshwaram Metta Wildlife Sanctuary (with a collective area of 4,762 km^2) have been set aside to conserve the rich biodiversity of this tract.

The first of the faunal surveys conducted in the Nallamala Hills dates back to 1930 when the ornithologist, Sálim Ali (1896–1987), of the Bombay Natural History Society, collected bird specimens from Mannanur and Farahabad on the Amrabad Plateau in the Northern Nallamala Hills during the Hyderabad State Ornithological Survey (see Lozupone et al., 2004, for a gazetteer of localities; Srinivasulu and Nagulu, 2002).

Subsequently, the Zoological Survey of India conducted two faunistic surveys to collect vertebrate fauna in the vicinity of the area in the Northern Nallamala Hills that was to be submerged due to the construction of the Nagarjunasagar Dam on River Krishna. Between 1980 to present date many surveys and other studies (Agrawal and Bhattacharyya, 1976; Bhushan 1986, 1994;Murthy, 1968, 1986; Nagulu et al., 1998; Rao et al., 1997; Rao et al. 1999; Rao et al., 2005; Rao et al., 2004a,b,c,d; Reddy et al., 2004; Sharma, 1969, 1971, 1976; Srinivasulu and Nagulu, 2002; Srinivasulu and Rao, 1999; Srinivasulu and Rao, 2000; Srinivasulu, 2001b, 2002, 2003) have been conducted documenting the faunal elements found in the Nallamala Hills.

History of Herpetofaunal Studies

The earliest zoological collections from these hill ranges were made by Thomas Claverhill Jerdon (1811–1872), a member of the Asiatic Society, and also an important contributor to mammalogy, ornithology, and herpetology (see Das, 2004, for a brief biographic account). Jerdon's papers were published in the Journal of the Asiatic Society of Bengal. As Civil Surgeon of Nellore in 1842, Jerdon collected extensively in the then poorly-known region between Madras and Nellore, discovering many novelties amongst the vertebrate fauna, and most famously, the Jerdon's courser, *Rhinoptilus bitorquatus* (see an account in Bhushan, 2003). As a result of his collections during the time, the following now familiar herpetological species were described by Jerdon himself: *Microhyla rubra* (Jerdon, 1854), *Hoplobatrachus crassus* (Jerdon, 1854), *Hemidactylus subtriedrus* Jerdon, 1853, and *Oligodon taeniolatus* (Jerdon, 1853).

The Zoological Survey of India undertook the first herpetological survey of the Nallamala Hills, between 1962 and 1963 (reported by Murthy, 1968; Sharma, 1969; 1971; 1976). As part of the Eastern Ghats Herpetological Survey, Dr. Hem Singh Pruthi (?–1953), Plant Protection Adviser to the Government of India and entomologist with the ZSI (see Lal, 1954, for an obituary), collected herpetofauna from the Nallamala Hills in 1929 which were identified by Dr. Malcolm Arthur Smith. Under the State Faunal Diversity Documentation Project, initiated by the Survey, additional specimens were collected from localities in the Nallamala Hills (Murthy, 1986; Sanyal et al., 1993; Sarkar et al., 1993).

The first author of the present report made observations on the herpetofauna of Northern and Central Nallamala Hills between late 1995 and early 2000. A research team from the Department of Zoology, Osmania University, Hyderabad, also documented the herpetofaunal diversity while executing an Andhra Pradesh Forest Department-sponsored project on the effects of man-made barriers on wildlife in Gundla Brahmeshwaram Metta Wildlife Sanctuary in the Central Nallamala Hills, between 1998 and 2000. Observations on the herpetofaunal diversity made during these two studies between 1995 and 2000 have been listed in an unpublished document (Srinivasulu, 2001a). The Andhra Pradesh Forest Department, in collaboration with Department of Botany, Sri Krishnadevaraya University, Anantapur (for flora) and Department of Zoology, Osmania University, Hyderabad (for fauna) initiated All Taxa Biodiversity Inventorization Project in 2001 (Rao et al., 2004e) in which the first author was involved. Voucher specimens of amphibians and reptiles collected during this project have been deposited in the State Forest Department's Eco-Resource Monitoring Lab, located in Sunnipenta, Kurnool District. Between 3 – 16 June 2003, CS along with a research scholar from Zoological Survey of India, Hyderabad, and other volunteers visited Nagarjunasagar Srisailam Tiger Reserve to study the voucher specimens of the herpetofauna in Eco-Resource Monitoring Lab, Sunnipenta and collect fresh voucher specimens to be deposited in the National Zoological Collection housed at the Freshwater Biological Station of the Zoological Survey of India, Hyderabad, India (Srinivasulu et al., 2006).

Materials and Methods

Literature review and faunistic surveys by the first author for acquiring voucher specimens, both for the Andhra Pradesh Forest Department (January 2001 to June 2003) and the Zoological Survey of India (June 2003), and records of observations made by the first author between December 1995 to December 2004 in the Nallamala Hills form the basis of the diversity of herpetofauna reported here. Voucher specimens collected following techniques detailed in Heyer et al. (1994), including collections along 100–200 m transects and sampling within 50 sq m quadrats, at elevations between 150–570 m ASL. Vegetation in the area of sampling is dry deciduous and scrub forest types. Several moist decidous forest patches were also surveyed, including along seasonal streams, particularly for amphibians. Specimens were preserved and were deposited both at the State Forest Department Collection housed at ERM Labs, Sunnipenta, Kurnool District and the National Zoological Collection at the Freshwater Biological Station, Zoological Survey of India, Hyderabad. Certain large-growing (and threatened/protected) species considered easily-identifiable in the field (e.g., *Crocodylus palustris* and *Python molurus*) were not collected. Photographic vouchers were deposited in the Natural History Museum at the Department of Zoology, Osmania University, Hyderabad. All records from the southern Nallamala Hills are based on sight records.

The annotated lists of amphibians and reptiles provided below include information on their distribution in the Nallamala Hills, their habitat and qualitative impressions of abundance. Details of the vouchers are also provided. If the voucher specimen/s and/or photographic voucher are present, they are indicated by [S] or [P] followed by abbreviation of the place where housed. Abbreviations used include: ZSIK (National Zoological Collection, Zoological Survey of India, Kolkata), ZSIH (National Zoological Collection, Freshwater Biological Station, Zoological Survey of India, Hyderabad), ERM (Eco-Resource Monitoring Lab, Andhra Pradesh Forest Department, Sunnipenta), and NHMOU (Natural History Museum, Department of Zoology, Osmania University, Hyderabad). Nomenclatural remarks concerning species are for those names that are different from that generally prevailing in the literature in Indian herpetology, especially the Fauna of British India volumes by Smith (1931–43).

Results

The herpetofauna of the Nallamala Hills, as currently known, includes 20 species of amphibians belonging to 12 genera in four families and 64 species of reptiles belonging to 42 genera in 15 families. Recently, Rao et al. (2005) published an account of herpetofauna of the Nallamala Hills putting on record about 66 species of herpetofauna (including 18 species of amphibians in 11 genera in 4 families and 48 species of reptiles in 34 genera in 12 families) based on collections made from 16 locations between 15° 35'N (Isukagundam) to 16° 37' N (Vijayapuri) and 78° 39'E (Saileshwaram) to 79° 17' E (Vijayapuri) between November 2001 and September 2004. Rao et al.'s (2005) paper suffers from numerous problems (including misidentifications and erroneous nomenclature, in addition to dubious first record claims), and grossly under-represents the herpetofauna of the region, while ignoring to emphasize the endemic reptiles of the Nagarjunasagar area.

Of the herpetofaunal species listed in this work, voucher specimens of 76 species are either at the National Zoological Collection of the Zoological Survey of India, at Kolkata (48 species) and Hyderabad (20 species) or in the Eco-Resource Monitoring Laboratory, Sunnipenta (62 species). Vouchers of 11 species are at Kolkata, and 14 are at Sunnipenta. Eight taxa listed in this report are either based on literature reports or on sightings.

Annotated List of Amphibians

Order: Anura
Family: Bufonidae

1. *Bufo stomaticus* Lütken, 1862

Bufo stomaticus C. F. Lütken. 1862. Vidensk. Meddr. Danske Naturh. Foren. 1862: 305.

Northern (Sarkar et al., 1993; Srinivasulu et al., 2006), Central (Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (A1844, A1141), ZSIH (ZSI/FBS/N/1148, 1150–1153).

2. *Bufo scaber* Schneider, 1799

Bufo scaber J. G. Schneider. 1799. Hist. Nat. Amph.: 222.

Northern (Srinivasulu et al., 2006), Central (Rao et al., 2005; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ERM (ERMA–5a); [P] NHMOU (NHMOU.Amph.P.1–03).

Remarks: First record claim from the region by Rao et al. (2005) is erroneous as it has been already reported from the Nallamala Hills by Subba Rao et al. (1994). Dubois and Ohler (1999) showed that *Bufo scaber* Schneider, 1799 has priority over *Bufo fergusonii* Boulenger, 1892.

3. *Duttaphrynus melanostictus* (Schneider, 1799):

Bufo melanostictus J. G. Schneider. 1799. Hist. Nat. Amph.: 216.

Northern (Rao et al., 2005; Sarkar et al., 1993; Srinivasulu et al., 2006), Central (Rao et al., 2005; Sarkar et al., 1993; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ZSIK (A1144, A1145, A1845, A1997–98, A8373–75, A8377–81), ERM (ERMA–1a).

Family: Dicroglossidae

4. *Euphlyctis cyanophlyctis* (Schneider, 1799)

Rana cyanophlyctis J. G. Schneider. 1799. Hist. Nat. Amph.: 137.

Northern (Rao et al., 2005; Sarkar et al., 1993; Srinivasulu et al., 2006), Central (Rao et al., 2005; Sarkar et al., 1993; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ZSIK (A6941, A6943, A1948, A8424–26, A1108–13, A1130, A1989, A7810–11, A6947, A1131–32, A1830–34, A1838, A1941–47),

ERM (ERMA–3a); [P] NHMOU (NHMOU.Amph.P.7–03).
Remarks: *Euphlyctis* Fitzinger, 1843 was revived from synonymy of *Rana* Linnaeus, 1758 by Dubois (1992).

5. *Euphlyctis hexadactylus* (Lesson, 1834)
Rana hexadactyla R. P. Lesson. 1834. Voyage Indes-Orient.: 331.
Northern (Rao et al., 2005; Srinivasulu et al., 2006), Central (Rao et al., 2005; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ERM (ERMA–10a); [P] NHMOU (NHMOU.Amph.P.8–03).

6. *Fejervarya* cf. *limnocharis* (Gravenhorst, 1829)
Rana limnocharis J. L. C. Gravenhorst. 1829. Rept. Mus. Zool. Vratis. Delic. Mus. Zool: 42.
Northern (Sarkar et al., 1993; Srinivasulu et al., 2006), Central (Rao et al., 2005; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (A8438–40, A7813–14, A1990, A6946), ERM (ERMA–17a); [P] NHMOU (NHMOU.Amph.P.9–03).
Remarks: *Fejervarya* Bolkay, 1915 was recognized as a subgenus of *Rana* Linnaeus, 1758 by Dubois (1992), and as a genus by Iskandar (1998).

7. *Hoplobatrachus crassus* (Jerdon, 1854) Jerdon's Bull Frog
Rana crassa T. C. Jerdon. 1854. J. Asiatic Soc. Bengal 22(5): 531.
Northern (Sanyal et al., 1993; Srinivasulu et al., 2006), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ZSIK (A1843, A6945, A8409–14), ERM (ERMA–13a); [P] NHMOU (NHMOU.Amph.P.10–03).
Remarks: *Hoplobatrachus* Peters, 1863 was revived from synonymy of *Rana* Linnaeus, 1758 by Dubois (1992). See also Grosjean et al. (2004).

8. *Hoplobatrachus tigerinus* (Daudin, 1803)
Rana tigerina F.-M. Daudin. 1803. Hist. Nat.: 64; Pl. XX.
Northern (Sanyal et al., 1993; Srinivasulu et al., 2006), Central (Rao et al., 2005; Sanyal et al., 1993; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ZSIK (A6944, A8443–45, A1138–39, A1995), ZSIH (ZSI/FBS/N/1145), ERM (ERMA–15a).

9. *Sphaerotheca breviceps* (Schneider, 1799)
Rana breviceps J. G. Schneider. 1799. Hist. Nat. Amph.: 140.
Northern (Srinivasulu et al., 2006), Central (Rao et al., 2005; Sanyal et al., 1993; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ZSIK (A8400, A8448, A1940), ZSIH (ZSI/FBS/N/1143, 1144, 11461, 1147, 1155–58), ERM (ERMA–12a); [P] NHMOU (NHMOU.Amph.P.11–03).
Remarks: Rao et al. (2005) included an erroneously identified photograph (image 13 at www.zoosprint.org/), which is, in fact, that of *Sphaerotheca dobsoni*, a taxon that also is present in the Nallamala Hills (see below). In support of long-separated evolutionary lineages, representing distinct monophyletic radiations of the Africa, Madagascar and southern Asia, Vences et al. (2000) argued for the partition of *Tomopterna* into three lineages. Thus, the earliest available name for the Asian species is *Sphaerotheca*.

10. *Sphaerotheca dobsonii* (Boulenger, 1882)
Rana dobsonii G. A. Boulenger. 1882. Cat. Bat. British Mus.: 32; Pl. 3, Fig. 1.
Northern (Srinivasulu et al., 2006), Central (Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [P] NHMOU (NHMOU.Amph.P.12–03).

11. *Sphaerotheca rolandae* (Dubois, 1983)
Rana (*Tomopterna*) *rolandae* A. Dubois. 1983. Alytes: 2(4): 163.
Northern (Srinivasulu et al., 2006), Nallamala Hills. Open forests. Rare. [P] NHMOU (NHMOU.Amph.P.13–03).
Remarks: Rao et al.'s (2005) claim of this taxon (as *Tomopterna rolandae*) as the first record from Andhra Pradesh is based on erroneous identification. The voucher specimen and the photograph included in the report are that of *Sphaerotheca breviceps* (image 14 at www.zoosprint.org /).

Family: Microhylidae

12. *Kaloula taprobanica* Parker, 1934
Kaloula pulchra taprobanica H. W. Parker. 1934. Monogr. Frogs. Microhylidae: 86.
Northern (Rao et al., 2005) and Central (Rao et al., 2005; Srinivasulu et al., 2006) Nallamala Hills. Open forests and scrub areas. Uncommon. [S] ZSIH (ZSI/FBS/N/1159), ERM (ERMA–4a); [P] NHMOU (NHMOU.Amph.P.2–03).
Remarks: The first record of its occurrence in Andhra Pradesh reported by Rao et al. (2005) is erroneous, as Sivakumar et al. (2003) had reported its occurrence in the State from Sriharikota Island Nellore District. Rao et al. (in review, a) puts on record for its occurrence in the Nallamala Hills.

13. *Microhyla ornata* (Duméril and Bibron, 1841)
Engystoma ornatum A.-M.-C. Duméril & G. Bibron. 1841. Erp. Gen. 8: 745.
Northern (Sarkar et al., 1993; Srinivasulu et al., 2006), Central (Rao et al., 2005; Sarkar et al., 1993; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (A1846–48, A7817, A8451, A1140, A1996), ZSIH (ZSI/FBS/N/1141, 1160), ERM (ERMA–8a); [P] NHMOU (NHMOU.Amph.P.3–03).

14. *Microhyla rubra* (Jerdon, 1854)
Engystoma rubrum T. C. Jerdon. 1854. J. Asiatic Soc. Bengal 22(2): 534.
Northern (Srinivasulu et al., 2006), Central (Rao et al., 2005; Sarkar et al., 1993; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ZSIK (A8463–64), ERM (ERMA–6a); [P] NHMOU (NHMOU.Amph.P.4–03).

15. *Ramanella variegata* (Stoliczka, 1872)
Callula variegata F. Stoliczka. 1872. Proc. Asiatic Soc. Bengal 1872(6): 111.
Central (Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub and open to close forests. Uncommon. No vouchers, based on sightings.

16. *Uperodon globulosus* (Günther, 1864)
Cacopus globulosus A. C. L. G. Günther. 1864. Reptiles British India: 416.
Central (Rao et al., 2005; Srinivasulu et al., 2006; Srinivasulu et al., 2006) Nallamala Hills. Scrub and open forests. Rare. [S] ZSIH (ZSI/FBS/N/1138), ERM (ERMA–7a); [P] NHMOU (NHMOU.Amph.P.5–03).

17. *Uperodon systoma* (Schneider, 1799)
Rana systoma J. G. Schneider. 1799. Hist. Nat. Amph.: 144.
Northern (Rao et al., 2005; Srinivasulu et al., 2006), Central (Rao et al., 2005; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIH (ZSI/FBS/N/1142, 1149, 1154), ERM (ERMA–2a); [P] NHMOU (NHMOU.Amph.P.6–03).

Family: Petropedetidae

18. *Indirana leithii* (Boulenger, 1888)
Rana leithii G. A. Boulenger. 1888. Ann. & Mag. nat. Hist. Ser. 6, 2: 506.
Northern (Srinivasulu et al., 2006) and Central (Srinivasulu et al., 2006) Nallamala Hills. Scrub forests. Rare. [S] ERM (ERM/A24).
Remarks: *Indirana* Laurent, 1986 was revived from synonymy of *Rana* Linnaeus, 1758 by Dubois (1992). This species had been sighted on two occasions near Rollapenta in Central Nallamalla Hills and on one occasion near Ahobilam in Southern Nallamala Hills by the first author (Srinivasulu et al., 2006), who has also studied a single specimen in the collection of ERM Labs (ERM/A24), Sunnipenta that had been identified by Varad Giri of the BNHS.

Family: Ranidae

19. *Hylarana* sp.
Central (Rao et al., 2005; Rao et al., in review, b) Nallamala Hills. Riparian forest. Rare. [S] ERM (ERMA–14a).
Remarks: The systematic status of the population, referred to *Rana temporalis* (Günther, 1864) by previous workers, is under study by the second author, who assigns it to Dubois' (1992) subgenus *Sylvirana*, elevated to generic rank in Frost et al. (2006). Currently this generic name is a synonym of *Hylarana* (See Frost et al., 2007).

Family: Rhacophoridae

20. *Polypedates maculatus* (Gray, 1834)
Hyla maculata J. E. Gray. 1834. Ill. Indian Zool.: Pl. LXXXII; Fig. 1.
Northern (Rao et al., 2005; Sanyal et al., 1993; Srinivasulu et al., 2006), Central (Rao et al., 2005; Sanyal et al., 1993; Srinivasulu et al., 2006) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ZSIK (A8403), ZSIH (ZSI/FBS/N/1140, 1161), ERM (ERMA–11a); [P] NHMOU (NHMOU.Amph.P.14–03).

Annotated List of Reptiles

Order: Crocodilia
Family: Crocodylidae

1. *Crocodylus palustris* Lesson, 1831
Crocodylus palustris R. P. Lesson. 1831. Bull. Sci. Nat. Geol. 25: 121.
Northern Nallamala Hills. Under the Central Government sponsored crocodile rehabilitation programme, some crocodiles were reintroduced both at backwaters of Nagarjunasagar Reservoir in Vijaypuri vicinity, Srisailam Reservoir and Ethipothala (described by Srinivas et al., 1999). Their numbers have dwindled due to poaching, but some crocodiles do survive in both these areas. No vouchers, based on the literature and indirect evidence.

Order: Chelonia
Family: Geoemydidae

2. *Melanochelys trijuga* (Schweigger, 1812)
Emys trijuga A. F. Schweigger. 1812. Prod. Monogr. Chel.: 310.
Northern, Central and Southern Nallamala Hills. Waterbodies, streams and rivers. Uncommon. No vouchers, based on sightings.
Remarks: Assumed, on the basis of locality, to belong to the nominotypical form.

3. *Pangshura tentoria* (Gray, 1834)
Emys tentoria J. E. Gray. 1834. Proc. Zool. Soc. London 1834(2): 54.
Northern, Central and Southern Nallamala Hills. Waterbodies, streams and rivers. Uncommon. No vouchers, based on sightings.
Remarks: Allocated to *Pangshura* Gray, 1869 rather than *Kachuga* Gray, 1856 by Spinks et al. (2004).

Family: Testudinidae: Tortoises

4. *Geochelone elegans* (Schoepff, 1795)
Testudo elegans J. D. Schoepff. 1795. Hist. Test. 3: 111; Pl. XXV.
Northern (Rao et al., 2005), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub forests and near agricultural fields. Common. [S] ERM (ERMR–5a).
Remarks: First reported from the Nallamala Hills by Subba Rao et al. (1994).

Family: Trionychidae

5. *Nilssonia gangetica* (Cuvier, 1825)
Trionyx gangeticus G. L. C. F. D. Cuvier. 1825. Recherch Ossemens Foss. 5: 203.
Northern Nallamala Hills (Sharma, 1971). Waterbodies, streams and rivers. Uncommon. [S] ZSIK (R21238).
Remarks: The generic nomen *Aspideretes* Hay, 1904, was revived from the synonymy of *Trionyx* Geoffroy Saint-Hillaire, 1809 by Meylan (1987). Praschag et al. (2007) placed *Aspideretes* in the synonymy of *Nilssonia*, but provided an incorrect (feminine) termination of the species name.

6. *Nilssonia leithii* (Gray, 1872)
Trionyx Leithii J. E. Gray. 1872. Ann. & Mag. nat. Hist. ser. 4 10: 334.
Northern (Sharma, 1971; Sanyal et al., 1993) and Southern Nallamala Hills. Waterbodies, streams and rivers. Uncommon. [S] ZSIK (R21403).

7. *Lissemys punctata* (Bonnaterre, 1789)
Testudo punctata M. Bonnaterre. 1789. Tableau Encycl. Method. Nat.: 30.
Northern (Sanyal et al., 1993), Central and Southern Nallamala Hills. Waterbodies, streams and rivers. Common. [S] ZSIK (Specimen not traceable).

Order: Squamata
Family: Agamidae

8. *Calotes rouxii* (Duméril & Bibron, 1837)
Calotes rouxii A.-M.-C. Duméril & G. Bibron. 1837. Erp. Gén. 4: 407.
Northern (Rao et al., 2005), Central (Rao et al., 2005) and Southern Nallamala Hills. Rocky outcrops in open and scrub forests, and agricultural fields. Common. [S] ZSIH (ZSI/FBS/N/1172), ERM (ERMR–10a); [P] NHMOU (NHMOU.Rep.P.1–03).

9. *Calotes versicolor* (Daudin, 1802)
Agama versicolor F.-M. Daudin. 1802. Hist. nat. Rept. 3: 395; Pl. XLIV.
Northern (Rao et al., 2005; Sharma, 1971; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Rocky outcrops in open and scrub forests, and agricultural fields. Abundant. [S] ZSIK (R20249–60, R20181–85, R21286–88, R21289, R21431–33, R21367, R21275, R21434–35, R21276, R21422–25, R21277–80, R21368–70, R21281, R21283–85, R24456, R20293, R20214–15), ZSIH (ZSI/FBS/N/1165, 1169), ERM (ERMR–12a).

10. *Psammophilus blanfordanus* (Stoliczka, 1871)
Charasia blanfordana F. Stoliczka. 1871. Proc. Asiatic Soc. Bengal 1871(9): 194.
Northern (Sanyal et al., 1993; Sharma, 1971), Central and Southern Nallamala Hills. Rocky outcrops in open and scrub forests. Common. [S] ZSIK (R21436, R24685, R24659).

11. *Psammophilus dorsalis* (Gray in Griffith & Pidgeon, 1831)
Agama Dorsalis J. E. Gray in: E. Griffith & E. Pidgeon. 1831. Class Reptilia 9: 56.
Northern (Rao et al., 2005; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Rocky outcrops in open and scrub forests. Common. [S] ZSIK (R21291, R20295), ERM (ERMR–11a).

12. *Sitana ponticeriana* Cuvier, 1829
Sit. (= *Sitana*) *ponticeriana* G. J.-L.-N.-F. D. Cuvier. 1829. Reg. Anim. 2: 43.
Northern (Rao et al., 2005; Sharma, 1971; Sanyal et al.,

1993), Central (Rao et al., 2005; Sanyal et al., 1993) and Southern (Sanyal et al., 1993) Nallamala Hills. Rocky outcrops in open and scrub forests and agricultural fields. Abundant. [S] ZSIK (R20284, R21256, R21270, R21413–15, R2127–72, R21348–50, R21267, R21257, R21419–21, R21258–61, R21253–56, R21269, R21357–64, R24436, R24439, R20178, R20216, R21262–68, R21352, R21416, R21357–62, R24668, R24455, R24462, R20223, R20294, R24684), ZSIH (ZSI/FBS/N/1166, 1170, 1171), ERM (ERMR–9a); [P] NHMOU (NHMOU.Rep.P.2–03).

Family: Chamaeleonidae

13. *Chamaeleo zeylanicus* Laurenti, 1768
Chamaeleo zeylanicus J. N. Laurenti. 1768. Syn. Rept.: 46.
Northern (Rao et al., 2005), Central (Rao et al., 2005) and Southern Nallamala Hills. Open and scrub forests, and agricultural fields. Common. [S] ERM (ERMR–13a); [P] NHMOU (NHMOU.Rep.P.1–01).

Family: Gekkonidae

14. *Cnemaspis* sp.
Remarks: An unidentified species of *Cnemaspis* was encountered in Central and Southern Nallamala Hills. Three specimens that were collected by the first author, deposited in the Eco-Resources Monitoring Labs, Sunnipenta in March 2002, were lost due to attack by ants. Specimens were collected from the leaf litter in a dry stream near Chinnarutla. Rare. [S] ERM (Lost). *Cnemaspis otai* Das and Bauer (2000) is known from Vellore region, in extreme northern Tamil Nadu State, adjacent to Andhra Pradesh, and the Nallamala specimens may be either this nominal species, or an undescribed species.

15. *Hemidactylus bowringii* (Gray, 1845)
Doryura bowringii J. E. Gray. 1845. Cat. Lizards British Mus.: 156.
Northern (Sanyal et al., 1993) Nallamala Hills. Inhabits human-altered habitats and other dilapidated man-made structure. Rare. [S] ZSIK (R24465).

16. *Hemidactylus brookii* (Gray, 1845)
Hemidactylus brookii J. E. Gray. 1845. Cat. Lizards British Mus.: 153.
Northern (Rao et al., 2005; Sharma, 1971; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Open forests, old temples, also human commensal, found in houses and other dilapidated man-made structures. Abundant. [S] ZSIK (R21240–44, R24669, R21404–05, R23237, R24435, R20179, R23687, R23699), ZSIH (ZSI/FBS/N/1174), ERM (ERMR–2a).

17. *Hemidactylus flaviviridis* Rüppell, 1835
Hemidactylus flaviviridis E. Rüppell. 1835. Neue Wirbelth.-Fauna Abyss., Amph. 18: Pl. 6; Fig. 2.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Human commensal, found in houses and other man-made structures. Uncommon. [S] ZSIH (ZSI/FBS/N/1173), ERM (ERMR–3a).

18. *Hemidactylus frenatus* Duméril & Bibron, 1836
Hemidactylus frenatus A.-M.-C. Duméril & G. Bibron. 1836. Erp. Gén. 3: 366.
Northern (Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Open forests, old temples, also human commensal, found in houses and other dilapidated man-made structures. Common. [S] ZSIK (R23700), ERM (ERMR–30a).

19. *Hemidactylus giganteus* Stoliczka, 1871
Hemidactylus giganteus F. Stoliczka. 1871. Proc. Asiatic Soc. Bengal 1871(9): 193.
Northern (Rao et al., 2005; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Open forests, old temples, and other dilapidated man-made structures. Uncommon. [S] ZSIK (R21411–12), ZSIH (ZSI/FBS/N/1167, 1168), ERM (ERMR–1a).

20. *Hemidactylus leschenaultii* Duméril & Bibron, 1836
Hemidactylus leschenaultii A.-M.-C. Duméril & G. Bibron. 1836. Erp. Gén. 3: 364.
Northern (Rao et al., 2005; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Open forests, old temples, also human commensal, found in houses and other dilapidated man-made structures. Common. [S] ZSIK (R20180, R23693, R24458, R24466, R24660, R24510, R24513), ERM (ERMR–4a).

21. *Hemidactylus reticulatus* Beddome, 1870
Hemidactylus reticulatus R. H. Beddome. 1870. Madras Monthly J. Med. Sci. 1: 33.
Northern (Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Rocky outcrops in open and scrub forests. Uncommon. [S] ZSIK (R21245–46, R21247–53, RR21346, R21343–45, R21406–10, R23216), ERM (ERMR–17a).

22. *Hemidactylus triedrus* (Daudin, 1802)
Gecko triedrus F.-M. Daudin. 1802. Hist. nat. Rept. 4: 155.
Northern (Rao et al., 2005; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S]

ZSIK (21239, R24509, R24512), ERM (ERMR–7a).

Family: Lacertidae

23. *Ophisops jerdoni* (Blyth, 1853)
Ophisops jerdoni E. Blyth. 1853. J. Asiatic Soc. Bengal 22: 653.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Abundant. [S] ZSIK (R21302–14, R21381–92, R21440, R24661, R24670), ZSIH (ZSI/FBS/N/1176–1178), ERM (ERMR–18a).

24. *Ophisops leschenaultii* (Milne-Edwards, 1829)
Lacerta leschenaultii H. Milne-Edwards. 1829. Ann. Sci. nat. 16: 86; Pl. VI; Fig. 9.
Northern (Sanyal et al., 1993; Sharma, 1971), Central (Murthy, 1986) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ZSIK (R21296–97, R21438–39).

25. *Ophisops minor* (Deraniyagala, 1971)
Cabrita jerdoni minor P. E. P. Deraniyagala. 1971. Ceylon J. Sci. 32(1): 104; Fig. 1.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005; Sanyal, et al., 1993) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21298–301, R21377–80, R24440, R24459, R24464), ERM (ERMR–8a).
Remarks: Reviewed by Böhme and Bischoff (1991). See also nomenclatural remarks in Arnold (1989).

Family: Scincidae

26. *Lygosoma ashwamedhi* Sharma, 1969
Riopa ashwamedhi R. C. Sharma. 1969. Bull. Syst. Zool., Calcutta 1(2): 73; Fig. 2.
Endemic to Andhra Pradesh, known only from type locality. Northern (Sharma, 1969, 1971; Sanyal et al., 1993) Nallamala Hills. Rocky scrub forests. Rare. [S] ZSIK (R21173–77, R21179).

27. *Lygosoma guentheri* (Peters, 1879)
Eumeces guntheri W. C. H. Peters. 1879. S.-Ber. Ges. Naturf. Freunde Berlin 1879(3): 36.
Central (Rao et al., 2005) Nallamala Hills. Scrub forest. Rare. [S] ERM (ERMR–43a).
Remarks: Hitherto known only from the Western Ghats, from Gujarat to Kerala States (Daniel and Shull, "1963";1964; Daniel, 1962; Smith, 1935: 322), this is the first record of the species from the Eastern Ghats,

28. *Lygosoma punctata* (Linnaeus, 1758)
Scincus punctatus C. Linnaeus. 1758. Syst. Nat. 10th ed. 1: 209.
Northern (Sanyal et al., 1993; Sharma, 1971), Central and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK(21294, R21376, R20327, R20318), ZSIH (ZSI/FBS/N/1175); [P] NHMOU (NHMOU.Rep.P.3–03).

29. *Eutropis carinata* (Schneider, 1801)
Scincus carinatus J. G. Schneider. 1801. Hist. Amphib.: 183.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005; Sanyal et al., 1993) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21292–93, R21373–75, R21437, R20313, R21437, R24438, R24457, R24463, R23694, R24511), ZSIH (ZSI/FBS/N/1179, 1180), ERM (ERMR–15a); [P] NHMOU (NHMOU.Rep.P.4–03).
Remarks: Mausfeld et al. (2002) suggested partitioning the genus *Mabuya* Fitzinger, 1826 into several genera, allocating the Asian species to *Eutropis* Fitzinger, 1843.

30. *Eutropis macularia* (Blyth, 1853)
Euprepes macularius E. Blyth. 1853. J. Asiatic Soc. Bengal 22: 652.
Northern (Sanyal et al., 1993; Rao et al., 2005), Central (Sanyal et al., 1993; Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R24437, R23701), ERM (ERMR–16a).

31. *Eutropis nagarjuni* Sharma, 1969
Mabuya nagarjuni R. C. Sharma. 1969. Bull. Syst. Zool., Calcutta 1(2): 71; Fig. 1.
Endemic to Andhra Pradesh. Northern (Sanyal *et al.*, 1993; Sharma, 1969, 1971) Nallamala Hills. Rocky scrub forests. Rare. [S] ZSIK (R21170–72), ZSIH (ZSI/FBS/N/1164); [P] NHMOU (NHMOU.Rep.P.5–03).
Remarks: The photo purported to be of *Mabuya beddomei* (Jerdon, 1870) in Rao et al. (2005; image 30 at www.zoosprint.org/) is that of *Eutropis nagarjuni* (Sharma, 1969), as shown by Srinivasulu et al. (2005).

Family: Varanidae

32. *Varanus bengalensis* (Daudin, 1802)
Tupinambis bengalensis F.-M. Daudin. 1802. Hist. nat. Rept. 3: 67.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultur-

al fields. Common. [S] ZSIK (R21315, R21441–42), ERM (ERMR–26a).
Remarks: For a history of the name *Varanus monitor* Linnaeus, 1758, a junior synonym of *Tupinambis bengalensis* Daudin, 1802, see Mertens (1946; 1956; 1957) and Sprackland (1982).

Family: Boidae

33. *Eryx conicus* (Schneider, 1801)
Boa conica J. G. Schneider. 1801. Hist. Amphib. 2: 268.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21331), ERM (ERMR–14a).

34. *Eryx johnii* (Russell, 1801)
Boa Johnii P. Russell. 1801. Continuation Account Indian Serpents: 18; Pl. xvi–xvii.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21332–33), ERM (ERMR–22a).

Family: Pythonidae

35. *Python molurus* (Linnaeus, 1758)
Coluber molurus C. Linnaeus. 1758. Syst. Nat. 10th ed. 1: 225.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Rocky scrub, open forests and near agricultural fields. Uncommon. No vouchers collected, based on sightings.

Family: Colubridae

36. *Ahaetulla nasuta* (Lacepède, 1789)
Coluber nasuta B.-G.-É. de L. V.-S.-I. Lacepède. 1789. Hist. Nat. Serp. 1: 100.
Northern, Central and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ERM (ERMR–6a).
Remarks: Rao et al., (2005) did not include this taxon in their catalogue, but provided its picture (image 42 at www.zoosprint.org/). In their list they included the subspecies, *Ahaetulla nasutus isabellinus* (Wall). The single specimen based on which the presence is reported by Rao et al. (2005) requires further studies to confirm the validity of the so-called subspecies, whose correct termination of subspecies nomen should be rendered *isabellina*, to match the gender of the genus.

37. *Amphiesma stolatum* (Linnaeus, 1758)
Coluber stolatus C. Linnaeus. 1758. Syst. Zool. 10th ed.: 219.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ERM (ERMR–27a).
Remarks: The gender of *Amphiesma* Duméril, Bibron and Duméril, 1854 has been treated erroneously treated as feminine since it was resurrection by Malnate (1960). Toriba (1994) showed that the genus is neuter, and the termination of the species name should therefore be *stolatum* (see also David et al., 1998).

38. *Argyrogena fasciolata* (Shaw, 1802)
Coluber fasciolatus G. Shaw. 1802. Gen. Zool.: 528.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ERM (ERMR–28a).
Remarks: Revived from the synonymy of *Coluber* for *C. fasciolata* Shaw, 1802, by Wilson (1967).

39. *Atretium schistosum* (Daudin, 1803)
Coluber schistosus F.-M. Daudin. 1803. Hist. Nat. Rept. 6: 132.
Northern, Central and Southern Nallamala Hills. Near waterbodies and paddy fields. Common. No vouchers, based on sightings.

40. *Boiga forsteni* (Duméril, Bibron & Duméril, 1854)
Triglyphodon forsteni A.-M.-C. Duméril, G. Bibron & A.-H.-A. Duméril. 1854. Erp. Gén. 7: 1077.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ERM (ERMR–20a).

41. *Boiga trigonata* (Schneider in Bechstein, 1802)
Coluber trigonatus J. G. Schneider in: J. M. Bechstein. 1802. La Cepede's Nat. Amphib.: 256; Pl. 40; Fig. 1.
Northern (Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21457), ERM (ERMR–37a).

42. *Coelognathus helena* (Daudin, 1803)
Coluber helena F.-M. Daudin. 1803. Hist. nat. Rept. 6: 277; Pl. LXXVI.
Northern (Rao et al., 2005; Sharma, 1971; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21334), ZSIH (ZSI/FBS/N/ 1181), ERM (ERMR–32a); [P] NHMOU (NHMOU.Rep.P.6–03).
Remarks: *Coelognathus* Fitzinger, 1843, was revived from the synonymy of *Elaphe* Fitzinger in: Wagler,

1833, by Helfenberger (2001a; b), based on visceral and vertebrae morphology and allozyme variations.

43. *Coluber bholanathi* Sharma, 1976
Coluber bholanathi R. C. Sharma. 1976. Comp. Physiol. Ecol. 1(3): 106; Fig. 1.
Endemic to Andhra Pradesh, known only from type locality. Northern (Sanyal et al., 1993; Sharma, 1976) Nallamala Hills. Scrub and open forests. Rare. [S] ZSIK (R21335–37).

44. *Dendrelaphis tristis* (Daudin, 1803)
Coluber tristis F.-M. Daudin. 1803. Hist. nat. Rept. 6: 430.
Northern (Rao et al., 2005), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ERM (ERMR–25a); [P] NHMOU (NHMOU.Rep.P.2–01).

45. *Enhydris enhydris* (Schneider, 1799)
Hydrus enhydris J. G. Schneider. 1799. Hist. Amphib. 1: 245.
Northern and Central (Rao et al., 2005) Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. No vouchers collected, based on sightings.

46. *Liopeltis calamaria* (Günther, 1858)
Cyclophis calamaria A. C. L. G. Günther. 1858. Cat. Colubrine Snakes British Mus.: 250.
Central (Rao et al., 2005) Nallamala Hills. Scrub and open forests. Rare. [S] ERM (ERMR–29a).

47. *Lycodon aulicus* (Linnaeus, 1758)
Coluber aulicus C. Linnaeus. 1758. Syst. Nat. 10th ed 1: 220.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ERM (ERMR–35a).

48. *Lycodon striatus* (Shaw, 1802)
Coluber striatus G. Shaw. 1802. Gen. Zool.: 527.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ERM (ERMR–44a).
Remarks: First record claim from the region by Rao et al. (2005) is erroneous as it has been already reported from the Nallamala Hills by Subba Rao et al. (1994).

49. *Lycodon travancoricus* (Beddome, 1870)
Cercaspis travancoricus R. H. Beddome. 1870a. Madras Monthly J. Med. Sci. 1: 169.
Central (Rao et al., 2005) Nallamala Hills. Scrub and open forests. Rare. [S] ERM (ERMR–38a).

50. *Macropisthodon plumbicolor* (Cantor, 1839)
Tropidonotus plumbicolor T. Cantor. 1839. Proc. Zool. Soc. London 1829(7): 54.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ERM (ERMR–36a).

51. *Oligodon arnensis* (Shaw, 1802)
Coluber arnensis G. Shaw. 1802. Gen. Zool.: 526.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ERM (ERMR–41a).

52. *Oligodon taeniolatus* (Jerdon, 1853)
Coronella taeniolata T. C. Jerdon. 1853. J. Asiatic Soc. Bengal 22(6): 528.
Northern (Rao et al., 2005; Sharma, 1971; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ZSIK (R21450–51, R24460), ERM (ERMR–42a).

53. *Oligodon travancoricus* (Beddome, 1877)
Oligodon travancoricum R. H. Beddome. 1877. Proc. Zool. Soc. London 1877(4): 685.
Northern (Sanyal et al., 1993), Central and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Uncommon. [S] ZSIK (R21338).

54. *Ptyas mucosa* (Linnaeus, 1758)
Coluber mucosus C. Linnaeus. 1758. Syst. Nat. 10th ed 1: 216.
Northern (Rao et al., 2005; Sharma, 1971; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21446–47, R21449), ERM (ERMR–39a); [P] NHMOU (NHMOU.Rep.P.3–01).
Remarks: David and Das (2004) showed that the correct termination of the species nomen should be *mucosa*, rather than *mucosus*, to match the gender of the generic nomen.

55. *Sibynophis subpunctatus* (Duméril, Bibron & Duméril, 1854)
Oligodon subpunctatum A.-M.-C. Duméril, G. Bibron & A.-H.-A. Duméril. 1854. Erp. Gén. 7: 58.
Central Nallamala Hills. Scrub and open forests. Rare. [S] ERM (ERMR–40a).
Remarks: The taxon *Sibynophis subpunctatus* (Duméril, Bibron & Duméril, 1854), has been recently been resurrected from the synonymy of *Sibynophis sagittaria* (Cantor, 1839) by Captain et al. (2004).

56. *Xenochropis piscator* (Schneider, 1799)
Hydrus piscator J. G. Schneider. 1799. Hist. Amphib. 1: 247.
Northern (Sharma, 1971; Rao et al., 2005; Sanyal et al., 1993), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21339, R21452, R21454–55).

Family: Elapidae

57. *Bungarus caeruleus* (Schneider, 1801)
Pseudoboa caerulea J. G. Schneider. 1801. Hist. Amphib. 2: 284.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21458–59), ERM (ERMR –24a).

58. *Calliophis melanurus* (Shaw, 1802)
Coluber melanurus G. Shaw. 1802. Gen. Zool.: 552.
Northern (Sanyal et al., 1993; Sharma, 1971) Nallamala Hills. Scrub forests. Rare. [S] ZSIK (R21460).

59. *Naja naja* (Linnaeus, 1758)
Coluber naja C. Linnaeus. 1758. Syst. Nat. 10th ed. 1: 221.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21461), ERM (ERMR–33a); [P] NHMOU.

Family: Typhlopidae

60. *Grypotyphlops acutus* (Duméril, Bibron & Duméril, 1844)
Onychocephalus acutus A.-M.-C. Duméril, G. Bibron & A.-H.-A. Duméril. 1844. Érp. Gen. 6: 333.
Northern (Sanyal et al., 1993; Sharma, 1971), Central and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21330).
Remarks: Wallach (2003) revived *Gyptotyphlops* Peters, 1881 from the synonymy of *Rhinotyphlops* Fitzinger, 1832, to accommodate the present species.

61. *Ramphotyphlops braminus* (Daudin, 1803)
Eryx braminus F.-M. Daudin. 1803. Hist. Nat. Gen. Rept. 7: 279.
Northern (Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21316–20, R21327–29, R21394–99), ERM (ERMR–19a).

Family: Viperidae

62. *Daboia russelii* (Shaw & Nodder, 1797)
Coluber russelii G. Shaw & F. P. Nodder. 1797. Nat. Misc. 8: Pl. 108.
Northern (Rao et al., 2005; Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21341), ERM (ERMR–34a).
Remarks: Although Obst (1983) revived *Daboia* Gray, 1842 from the synonymy of *Vipera* Laurenti, 1768, his concept of the genus included *Daboia* Gray, 1842, *Macrovipera* Reuss, 1927, *Pseudocerastes* Boulenger, 1896 and the *Vipera xanthina* (Gray, 1849) complex. Hermann et al. (1992) separated these genera from each other, and from *Vipera* Laurenti, 1768, restricting *Daboia* to *Vipera russelii* Shaw & Nodder, 1797. For comments on the spelling of the specific name, see Dowling (1993) and Adler et al. (2000).

63. *Echis carinatus* (Schneider, 1801)
Pseudoboa carinatus J. G. Schneider. 1801. Hist. Amphib. 2: 285.
Northern (Sanyal et al., 1993; Sharma, 1971), Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIK (R21342, R21401–02, R24461) ERM (ERMR–23a).

64. *Trimeresurus gramineus* (Shaw, 1802)
Coluber gramineus G. Shaw. 1802. Gen. Zool.: 420.
Northern, Central (Rao et al., 2005) and Southern Nallamala Hills. Scrub, open forests and near agricultural fields. Common. [S] ZSIH (ZSI/FBS/N/1182, 1183), ERM (ERMR–31a).

Erroneous or Doubtful Records

In addition to the records presented in the preceding pages, the following species have been recorded from the Nallamala Hills in the literature. These have been shown to be in error, stemming from the use of incorrect names or from misidentifications.

Bufo hololius Günther, 1876, which had been reportedly collected by Pillai and Murthy (1983) from Nagarjunasagar area (also cited by Sarkar et al., 1993), has not been included in this list as this taxon is known only from type specimen and all other specimens assigned to this nomen need reevaluation, according to Dubois and Ohler (1999).

Polypedates leucomystax (Gravenhorst, 1829), has been recorded from the Nallamala Hills by Rao et al. (2005), and earlier, from the Eastern Ghats, by Pillai and Murthy (1983). The records possibly refers to either *P. maculatus* or another member of this complex, since Gravenhorst's species (type locality: Java) is a mesic area frog, approaching the present study area only in the northeast of the country (see Dutta, 1997).

In a series of papers dealing with the ecology and physiology of squamate reptiles, Subba Rao (1970; 1972) and Subba Rao and Rajabai (1972a; 1972b; 1974) recorded *Calotes nemoricola* from Tirupati, and Subba Rao (1994) recorded this species from the Nallamala Hills. Whitaker and Das (1990) showed this to be erroneous identifications for the widespread *Calotes versicolor* (Daudin, 1802).

Subba Rao et al. (1994) reported *Eutropis beddomei* (Jerdon, 1870) from all districts encompassing the Nallamala Hills range. Recently Rao et al. (2005) also reported it from Vijayapuri and Mallelatheertham in Northern Nallamala Hills. This nominal species is restricted to the Western Ghats of south-western India and south-central Sri Lanka (Smith, 1943). As mentioned earlier, Rao's (2005) image 30 represents *Eutropis nagarjuni* Sharma, 1969.

Rao et al. (2005) listed *Cerberus rynchops* (Schneider, 1799) from Sundipenta/Sikharam, within the Nallamala Range. This is an estuarine/coastal species (see Das, 2002b; Whitaker and Captain, 2004), and its record in the literature from the Eastern Ghats Complex (e.g., Pillai and Murthy, 1983) may be from the plains.

Sibynophis sagittarius (Cantor, 1839) was reported by Rao et al. (2005), based on a specimen collected from Sunnipenta. This taxon was previously reported from the area southeast of the Nallamala Hills, from Sriharikota Island, Nellore District, by Rao and Sekar (1993). *Sibynophis subpunctatus* (Duméril, Bibron & Duméril, 1854) was recently resurrected from the synonymy of *S. sagittarius* (Cantor, 1839) by Captain et al. (2004) for this population. A record of this species from East Godavari District by Sanyal et al. (1993) is erroneous, as the specimen is from Godaveri (27° 34′ N and 85° 22′ E), 10 km southeast of Kathmandu, central Nepal.

Remarks on Habitat Use

Divided in habitat types, the amphibian fauna of Nallamala Hills can be classified into: 1. Human commensals or otherwise tolerant of disturbed habitats (14 species: *Bufo stomaticus, Duttaphrynus melanostictus, Kaloula taprobanica, Microhyla ornata, M. rubra, Uperodon globulosus, U. systoma, Ramanella variegata, Euphylyctis cyanophlyctis, E. hexadactylus, Fejervarya* cf. *limnocharis, Hoplobatrachus crassus, H. tigerinus,* and *Polypedates maculatus*); 2. Exclusively scrub forest species (four species: *Bufo scaber, Sphaerotheca breviceps, S. dobsonii,* and *S. rolandae*); and 3. Exclusively mesic forest species (two species: *Hylarana* sp. and *Indirana leithii*). Corresponding classification for reptiles include: 1. Human commensals or taxa otherwise tolerant of disturbed habitats (seven species: *Calotes versicolor, Hemidactylus bowringii, H. brookii, H. flaviviridis, H. frenatus, H. leschenaultii,* and *Ptyas mucosa*); 2. Exclusively scrub forest species or from rocky biotope (21 species: *Geochelone elegans, Psammophilus blanfordanus, P. dorsalis, Sitana ponticeriana, Hemidactylus reticulatus, H. triedrus, Ophisops jerdoni, O. leschenaultii, O. minor, Lygosoma ashwamedi, L. guentheri, L. punctata, Eutropis macularia, Varanus bengalensis, Eryx conicus, E. johnii, Python molurus, Coluber bholanathi, Liopeltis calamaria, Sibynophis subpunctatus,* and *Calliophis melanurus*); 3. Scrub and bordering agricultural fields (22 species: *Hemidactylus giganteus, Chamaeleo zeylanicus, Eutropis carinata, Ahaetulla nasuta, Amphiesma stolatum, Argyrogena fasciolata, Boiga forsteni, B. trigonata, Coelognathus helena, Dendrelaphis tristis, Lycodon aulicus, L. striatus, Oligodon arnensis, O. taeniolatus, O. travancoricus, Bungarus caeruleus, Naja naja, Grypotyphlops acutus, Ramphotyphlops braminus, Daboia russelii, Echis carinatus,* and *Trimeresurus gramineus*); 4. Exclusively mesic forest species (three species: *Calotes rouxii, Cnemaspis* sp., and *Lycodon travancoricus*); 5. Wetland species (10 species: *Crocodylus palustris, Melanochelys trijuga, Pangshura tentoria, Nilssonia gangetica, N. leithii, Lissemys punctata, Atretium schistosum, Enhydris enhydris, Macropisthodon plumbicolor,* and *Xenochropis piscator*); and 6. Habitat category unknown (one species: *Eutropis nagarjuni*).

In summary, all six microhylids, two bufonids, five ranids, one rhacophorid, in addition to one agamid, five gekkonids and one colubrid are human commensals. Human activities may promote creation or maintenance of certain habitats conducive for these species (e.g., perennial water sources, in the form of wells, drainage areas, etc.). Low amphibian diversity characterize scrub forests, where community members are such as *Bufo scaber, Sphaerotheca breviceps, S. dobsonii* and *S. rolandae* show xeric-region and/or fossorial adaptations (e.g., thickened skins and burrowing adaptations, such as enlarged metatarsal tubercles on pes) and adaptations for retaining moisture.

All turtles and crocodilians reported from the Nallamalla Range are associated with wetlands. The sole non-aquatic species (*Geochelone elegans*) is a scrub forest dweller. Significant diversity of gekkonids (*Hemidactylus reticulatus* and *H. giganteus*), scincids

(*Lygosoma ashwamedhi, L. guentheri, L. punctata,* and *Eutropis macularia*) and colubrids (*Coluber bholanathi, Liopeltis calamaria*, and *Sibynophis sagittaria*) are known from the ranges, several of these endemic (*L. ashwamedhi* and *C. bholanathi*) or largely restricted to scrub forests of Peninsular India. All lacertids (*Ophisops jerdoni; O. leschenaultii,* and *O. minor*) and all boids (*Eryx conicus*, *E. johnii,* and *Python molurus*) reported from the Nallamalla Hills are restricted to this biotope. Nonetheless, mesic forests remain poorly explored, perhaps for which reason both unidentified species from the Nallamala Ranges of amphibian (*Hylarana*) and reptile (*Cnemaspis*) may represent taxa undescribed by science.

Remarks on Adaptive Types

Adaptations seen amongst the amphibians of the Nallamala Hills include a diversity of dietary and habitat types. Representatives of ant specialists include all the microhylid and most bufonid species locally represented. Additional categories include: predators of small vertebrates (*Polypedates maculatus*) and folivores (*Euphlyctis hexadactylus* and some *E. cyanophlyctis*). In terms of gross habitat usage are the fossorial (*Kaloula taprobanica, Microhyla ornata, M. rubra, Uperodon globulosus,* and *U. systoma*), terrestrial (*Duttaphrynus melanostictus, B. scaber,* and *B. stomaticus*), aquatic or aquatic-margin (*Euphlyctis cyanophlyctis, E. hexadactylus, Fejervarya* cf. *limnocharis, Hoplobatrachus crassus,* and *H. tigerinus*) and arboreal (*Polypedates maculatus* and sometimes *Ramanella variegata*) species. At least three species enter bathrooms of human dwellings (*Ramanella variegata, Kaloula taprobanica,* and *Polypedates maculatus*) and one (*Polypedates maculatus*) is known to apply a coat of protein on the surface of its body prior to emerging for foraging, to prevent evaporative water loss. Skittering on the water surface is known for two species (*E. cyanophlyctis* and juvenile *E. hexadactylus*).

Adaptive types among the reptiles, when classified by diet, include eaters of soft-bodied (e.g., ant- and worm) prey (*Grypotyphlops acutus* and *Ramphotyphlops braminus*); predators of crop pests, such as rodents (*Argyrogena fasciolata*, *Ptyas mucosa,* and *Varanus bengalensis*); predator of small or medium-sized vertebrate prey (*Python molurus, Crocodylus palustris, Ptyas mucosa, Daboia russelii, Trimeresurus gramineus,* and *Echis carinata*); egg-predators (*Oligodon arnensis, O. taeniolatus* and *O. travancoricus*); primarily fish-eaters (*Crocodylus palustris*, *Nilssonia gangetica, N. leithii*, *Atretium schistosum, Enhydris enhydris,* and *Xenochrophis piscator*); frog- and toad-eaters (*Macropisthodon plumbicolor* and *Dendrelaphis tristis*) and near-exclusive snake-eaters (*Bungarus caeruleus* and *Calliophis melanurus*).

In terms of habitat usage, reptiles exceed amphibians in species richness, on account of their greater capacity of surviving in relatively arid regions. The regional gekkonid diversity, within the genus *Hemidactylus* includes arboreal (*H. bowringii, H. brookii, H. flaviviridis*, *H. frenatus,* and *H. leschenaultii*), terrestrial (*H. triedrus*) and semi-fossorial (*H. reticulatus*) types. Usage of specific habitat types include walls of houses (*Hemidactylus bowringii, H. brookii, H. flaviviridis*, *H. frenatus,* and *H. leschenaultii*), rupicolous habitats such as rocky boulders (*Psammophilus blanfordanus* and *P. dorsalis*); fossorial habits in terms of usage of soft substratum for burrowing (*Grypotyphlops acutus* and *Ramphotyphlops braminus*); and arboreal species, utilizing trees or some sort of vegetation (*Ahaetulla nasuta, Boiga forsteni, B. trigonata, Dendrelaphis tristis, Lycodon aulicus, Calotes rouxii*, *Chamaeleo zeylanicus,* and *Lycodon travancoricus;* the typhlopid *Ramphotyphlops braminus* is also known to occasionally ascend trees in search of prey). Other adaptive types shown by the fauna include vegetation mimics (*Chamaeleo zeylanicus* and *Ahaetulla nasuta*); Batesian mimicry is shown by *Sibynophis subpunctatus* (for which the model presumably is *Calliophis melanurus*); bipedal locomotion (*Sitana ponticeriana*); and side-winding (*Echis carinatus*, when moving on sand or other loose substrate).

Biogeography of the Eastern Ghats

The Eastern Ghats remain the poor sister of the more well-known Western Ghats, a recognized global hotspot of biological species diversity (e.g., Ward, 2002). Inger (1999) lamented about the low species richness of the amphibian fauna of the Eastern Ghats (21 species), while Das (1996) reported 84 species of reptiles, both significantly different from the known diversity of the Western Ghats, which has seen an explosion of new as well as spectacular species discoveries in recent years (see Biju, 2001; Biju and Bossuyt, 2003). Nonetheless, enough documentation exists to reveal a highly diverse biota of the hill ranges that run approximately parallel to the east coast of India.

The range itself is a weathered relict of the peninsular plateau, characterized by a series of low hills that extend from the Khondmal Hills of Orissa State, south to the Shevaroys of central Tamil Nadu, where they meet the Western Ghats in the Nilgiris region (descriptions in Das, 1996; Mani, 1995). The northern and southern sections of the Eastern Ghats are separated by the delta of the River Godavari, which is approximately 130 km in width. Other important breaks are formed by the drainages of the rivers Mahanadi and Krishna. The Biligirirangan Hills, at 1,750 m, is the highest range in the Eastern Ghats. Moisture regimes show a general gra-

dient, from a relatively mesic northern range, with dry and moist deciduous forests, to a relatively dry southern subzone, with dry deciduous and thorn scrub (vegetational analysis in Legris and Meher-Homji, 1983). Detailed analysis of faunal relationships along these hill ranges, including comparative diversity of lineages as an effect of breaks in the continuity of the ranges, humidity, and elevational effects remain to be conducted.

We adopt Wikramanayake et al.'s (2002) ecoregional approach to interpreting the distribution of the regional herpetofauna. These workers have classified the Indo-Pacific Region (stretching from Afghanistan in the west to New Guinea and the Solomons to the east), recognizing 129 ecoregions on the basis of vegetation, geology and geological history. Within this framework, the Nallamala Range falls within the Deccan Thorn Scrub zone (Ecoregion 23), abutting (and being influenced by) Ecoregions 21 (Central Deccan Plateau Dry Deciduous Forest); Ecoregion 22 (South Deccan Plateau Dry Deciduous Forest); and Ecoregion 6 (East Deccan Dry-Evergreen Forest). Although the Nallamala Hills also are adjacent to Ecoregion 34 (the Godavari-Krishna mangroves), herpetofaunal influences are absent, on account of geological-vegetational differences.

The herpetofauna of the Eastern Ghats has a long history of exploration, commencing with Patrick Russell (1727–1805), the first Western herpetologist in India, and medical doctor and naturalist with the British East India Company, based at Vizagapatam (at present Visakhapatnam). Russell explored the herpetofauna, primarily snakes, of that region and produced a two volume folio of water colors of snakes (also including *Barkudia melanosticta* (Schneider, 1801), which was published in 1796 and 1801–1802.

Collections for faunistic inventories within the Eastern Ghats complex have also been made by McCann (1945), Pillai and Murthy (1983), Daniels and Ishwar (1993), besides the contributions of the Zoological Survey of India in the Nallamala Hills referred to earlier. Rao and Rao (1998) studied the ecology of *Barkudia melanosticta* (as *B. insularis*); Bauer and Das (2000) studied the ecology of *Calodactylodes aureus* in Vellore; Das and Chanda (1998) described a new species of *Philautus* from the Visakhapatnam region; Dutta (2003) described a new *Philautus* from Simlipal Hills; and Das and Bauer (2000) described two new species of gekkonid lizards of the genus *Cnemaspis* from the Eastern Ghats.

Although less species rich than the more mesic adjacent regions, Ecoregion 23 supports a distinctive herpetofauna, including arid region representatives whose relatives are Eurasian and Afro-Ethiopian (e.g., *Chamaeleo*, *Ophisops, Eryx,* and *Echis*) and the region also supports lineages that may be termed distinctly autochthonous (i.e., Indian lineages, such as the genera *Uperodon, Ramanella, Indirana, Sphaerotheca, Melanochelys, Pangshura, Nilssonia, Psammophilus, Sitana, Argyrogena, Atretium,* and *Grypotyphlops*). The presence of representatives of Indo-Malayan elements represented here (e.g., *Kaloula, Hylarana, Calliophis,* and *Trimeresurus*) are explainable using Hora's (1949) Satpura Hypothesis model, of emigration of the biota of the Indo-Malayan region westwards. Alternative models are available to explain the presence of these taxa in the Eastern Ghats, including a more mesic climate in the Indian Subcontinent up to the Eocene (van der Hammen, 1983). The climatic changes were perhaps accelerated by widespread agriculture, specifically through the cultivation of graminaceous crops (Misra, 1983), helping further in the conversion of what was once tropical subhumid and dry deciduous forests into savannas.

Within the Eastern Ghats herpetofauna, endemic genera include the limbless skinks, *Barkudia* (with two species, *B. insularis* and *B. melanostictus;* see Das, 2000) and *Sepsophis* (a monotypic genus, containing *S. punctatus*). A number of species hitherto considered endemic to the Western Ghats have in recent years been found within the Eastern Ghats complex, including *Indirana leithii* (this report), *Hylarana malabarica* (Tschudi, 1838) by Daniel and Selukar (1963), a member of the genus *Hylarana* (this report) and *Lygosoma guentheri* (Peters, 1879) (this report). Balachandran and Pittie (2000) reported the occurrence of *Draco* from these hills, that they allocated to *D. dussumieri* Duméril & Bibron, 1837, a Western Ghats species. Eastern Ghat endemics found in the Nallamala Range include *Hemidactylus reticulatus, Eutropis nagarjuni, Lygosoma ashwamedhi,* and *Coluber bholanathi.* New species have been described from these ranges in recent years, including the geckos *Cnemaspis otai* and *C. yercaudensis* (see Das and Bauer, 2000).

Several species known from the Eastern Ghats have not (yet) been recorded from the Nallamala Ranges. Some may be regional endemics or appropriate habitats may be missing on the site under study, although the absence of some (e.g., *Calodactylodes aureus*), that are known from both north and south of the range here reinforce the argument for more sampling of the fauna. Other Eastern Ghat endemics (e.g., *Barkudia*, with two species, the monotypic *Sepsophis,* and *Hemiphyllodactylus aurantiacus*) among the reptiles, and *Philautus terebrans* and *Ichthyophis peninsularis* occur in adjacent ranges of the Ghats (see Das and Chanda, 1998; Pillai and Murthy, 1983), and with further collection, may prove their presence here, or be represented by hitherto unknown sister species.

Conservation and Management

Parts of the Nallamala Range are within the Protected Areas System, the levels of protection for each component varying from Forest Reserves, that lie within the jurisdiction of the Andhra Pradesh Forest Department, to National Park, that are gazetted and their protection implemented by the Central (= Federal) Government. The most well-known of the protected areas is the Nagarjunasagar Srisailam Tiger Reserve and the recently gazetted Gundla Brahmeshwaram Metta Wildlife Sanctuary.

Conservation of amphibians and reptiles represent special challenges, for which reason, arguments have been made to move away from species-based conservation strategies, to that addresses entire landscapes. Given the large number of known components of the biodiversity of these Protected Areas, especially non-homoeothermous members (or non-mammal and bird species), and the general lack of expertise to identify, let alone understand, conservation requirements, this is apparently a safer approach to the conservation of biodiversity. The situation is not unique to the Eastern Ghats: in the Indo-Pacific region, centinelan extinction (or species loss even before their formal description) is known for both amphibians and reptiles (Das, 2002a; Erdelen, 2002).

A handful of the recorded species from the Nallamala Range are human commensals, or so-called 'weed-species', including, amongst amphibians: *Duttaphrynus melanostictus, B. stomaticus*, *Kaloula taprobanica, Microhyla ornata*, *M. rubra, Uperodon globulosus, U. systoma, Ramanella variegata, Euphylyctis cyanophlyctis, E. hexadactylus, Fejervarya* cf. *limnocharis*, *Hoplobatrachus crassus, H. tigerinus,* and *Polypedates maculatus.* Scrub species of amphibians include: *Bufo scaber, Sphaerotheca breviceps, S. dobsonii*, and *S. rolandae*). Human-commensals among the reptiles include: *Melanochelys trijuga, Lissemys punctata, Calotes versicolor, Hemidactylus bowringii*, *H. brookii*, *H. flaviviridis, H. frenatus*, *H. giganteus, H. leschenaultii*, *Lygosoma punctata, Eutropis carinata, Amphiesma stolatum, Dendrelaphis tristis, Lycodon aulicus, Ptyas mucosa*, *Naja naja,* and *Ramphotyphlops braminus.* Scrub species of reptiles include: *Geochelone elegans*, *Sitana ponticeriana*, *Chamaeleo zeylanicus, Hemidactylus reticulatus*, *H. triedrus, Ophisops jerdoni, O. leschenaultii, O. minor, Lygosoma ashwamedhi, L. guentheri, Eutropis nagarjuni, Coluber bholanathi, Liopeltis calamaria, Calliophis melanurus, Daboia russelii,* and *Echis carinatus.* Two species are exclusively rupicolous (*Psammophilus blanfordanus* and *P. dorsalis*). And only two species are considered mesic region taxa, in that their respective congeners are exclusively distributed in such areas (e.g., *Hylarana* and *Cnemaspis*).

Human-commensals generally refer to species tolerant of environments altered by humans. However, many still have life histories intimately dependent on certain habitat features, such as ponds or other standing bodies of water, substrates that serve as burrowing refugia, etc. Changes from rural to urban environments are known to cause local extinction of amphibian species (including *Sphaerotheca*), through the removal of such habitats, as observed in the Chennai region (Das, unpubl.).

Three herpetofaunal species from the Nallamala Hills are recognised as threatened, under the Red List categories of the IUCN (World Conservation Union; see Hilton-Taylor, 2000). These include the turtles, *Nilssonia gangetica* and *N. leithii*, and the crocodilian, *Crocodylus palustris* (all in the 'Vulnerable' category).

In the end, species protection in countries such as India, where the pressure on land and water are large, can only be assured in areas within protected areas. It is therefore imperative to bring additional areas of these hills with high diversity and/or faunal endemicity into the country's protected areas system.

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Spermatogenesis Timing in a Population *Ophisops elegans* (Sauria: Lacertidae), Western Iran

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Abstract.- During biological activity, specimens of *Ophisops elegans* were collected in western Iran, from March to November. Testis were removed and H&E techniques were used for histological study. The results show three phases in spermatogenesis timing as follows: (a) active phase, spermatogenesis in all specimens is active, (b) transitional phase, spermatogenesis in many specimens are active an in other is inactive, and finally (c) inactive phase, spermatogenesis in all specimens is inactive.

Keywords.- Spermatogenesis timing, testicular cycle, *Ophisops elegans*, Zagros mountains, western Iran.

Introduction

Lizards show two type of spermatogenesis; continuous and alternate (Torki, in press a, b). In the continues type, spermatogenesis is year-round and spermatozoa are foudn in the lumen of the seminiferous all year (e.g., Hernandez-Gallegos et al., 2002; Sherbrooke, 1975; Vieira et al., 2001). In contrast, the alternate type of spermatogenesis occurred during a well defined period in which spermatozoa were not found in the lumen of seminiferous (Castilla and Bauwens, 1990; Fitch, 1970; Torki, 2006, in press a, b, c). Continuous spermatogenesis occurs in tropical regions (Fitch, 1970; Hernandez-Gallegos et al., 2002; Vieira et al., 2001), this region limited by author into ITCZ region (Torki, 2006). Alternate spermatogenesis occurred in non-tropical regions, especially in temperate zones (Castilla and Bauwens, 1990; Torki, 2006). In the temperate-zone, the male testicular cycle is divided into two well-defined phases as follows: (a) the regenerative phase that occurs in the spring and is characterized by sustained sperm production, and (b) the degenerative phase, that begins in late summer, where a break in spermatogenesis is observed (Castilla and Bauwens, 1990; Fitch, 1970; Lofts, 1987; Torki, in press b). Likewise, tropical species in seasonal habitats also display, if less pronounced, a regenerative phase during the wet (reproductive) season

Table 1. shows descriptive statistics of five characters in *Ophisops elegans*. SVL (mm), TV (0.1 mm^3), GS and LS (µm), spermatozoa (1 is present, 0 sabsent, 0.67 and 0.40 is transition phase). Sp: mean of Spermatozoa observed in the lumen.

Month		SVL	TV	GS	LS	Sp
March	N	9	9	9	9	9
	Mean	43.2	211.5	56.55	79.88	1
April	N	10	10	10	10	10
	Mean	42.93	158.1	48.5	63.6	1
May	N	10	10	10	10	10
	Mean	42.05	133.6	40	45.6	1
June	N	9	9	9	9	9
	Mean	41.77	71.41	24.22	39.22	0.67
July	N	10	10	10	10	10
	Mean	43.19	46.42	23.2	32	0.4
August	N	8	8	8	8	8
	Mean	44.2	42.68	12.5	7.5	0
September	N	10	10	10	10	10
	Mean	43.9	40.81	6.2	1.1	0
October	N	9	9	9	9	9
	Mean	44.77	38.13	6.33	0	0

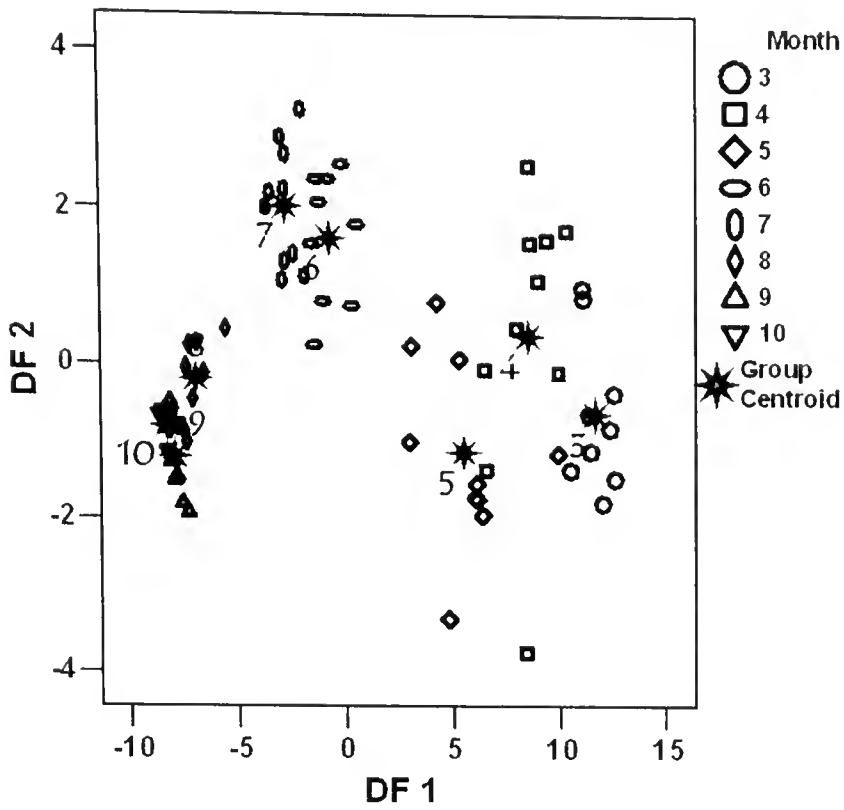

Figure 1. Shows phase significant during degeneration period based on DF analysis.

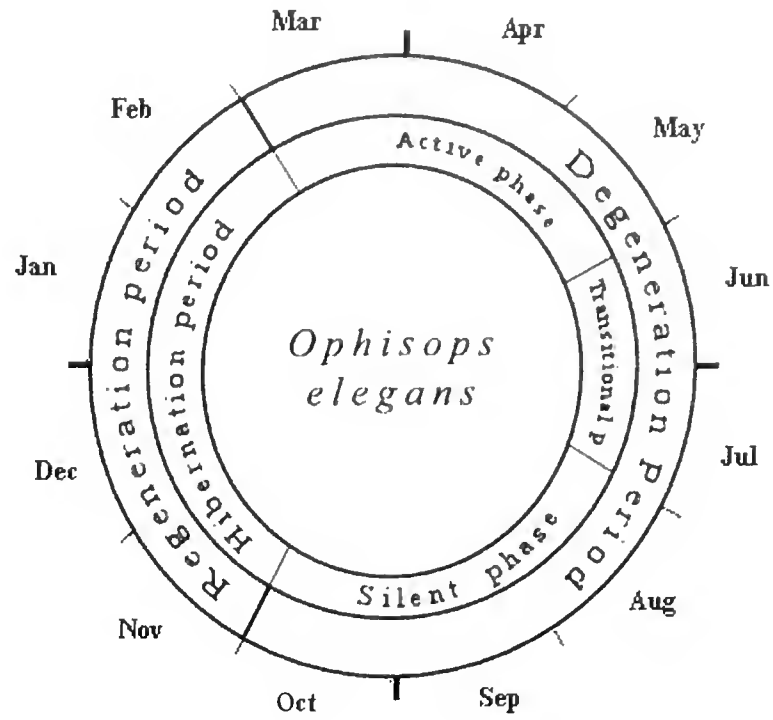

Figure 2. Show annual period and phase in spermatogenesis of *O. elegans* in western Iran, central Zagros.

and a degenerative phase during the dry (non-reproductive) season (Marion and Sexton, 1971; Wilhoft and Reiter, 1965).

Spermatogenesis of some lizards in Iranian plateau especially in Zagros Mountains described by author (Torki, 2006, in press a, b, c), and shows alternation spermatogenesis in Zagros Mountains. In this study, my purpose is determination spermatogenesis timing in *Ophisops elegans* in Zagros Mountains.

Materials and Methods

Seventy-five mature male specimens of *O. elegans* were collected by hand north of Lorestan province. The size of males *O. elegans* is between 38.9 < SVL < 47.2 mm. *O. elegans* go to hibernation period from Oct. to Feb. (Torki, 2005). Testis were removed from each individual by dissection, during each month from after hibernation to before hibernation. Snout-vent lengths (to the nearest 0.5 mm) were measured for each lizard. In each lizard maximum length and width of the left testis was measured (with electronic calipers to the nearest 0.01 mm) and Testis Volume (TV) and estimated TV (0.1 mm^3) using the ellipsoid formula; $v = 4/3\pi\, abc$, where v is volume, a and c are equal to half testicular height, and b is half testicular length (Vieira et al., 2001; Torki, 2006). For histological analysis, testes were fixed and the epididymis was fixed in 3.7% formalin, dehydrated in a graded series of ethanol, cleared in xylem, and embedded them in paraffin. Sections were stained with hematoxylin-eosin (H&E) and were observed with an Zeiss Axiophoto microscope. For each individual, two characters (µm) were measured: the Lumen of Seminiferous (LS) diameter, Germinative Seminiferous (GS) diameter. For data collecting, using the mean of twenty transversally oriented tubules at the same section, next to the core of the testes. Measurements were taken with an ocular micrometer, to the nearest 1 µm. Same as author study (Torki, 2006, in press a, b, c), Tukey HSD test and Canonical Discriminant Functions Analysis (DFA) to show phases significance were used.

Table 2. Shows Tukey HSD test (α = 0.05) for determination significant phase.

Month	N	Subset for alpha = .05		
		1	2	3
August	8	0		
September	10	0		
October	9	0		
July	10		0.4	
June	9		0.67	0.67
March	9			1
April	10			1
May	10			1
Sig.		1	0.38	0.11

Results

Description of GS, LS, and SVL are shown in Table 1; 82% of testis volume from Mar. to Oct. decreased. Based on Tukey test (α = 0.05) (Table 2) spermatogenesis timing of *O. elegans* divided into three phases: phase (1) from Mar. to Jun., phase (2) from Jun. to Jul., and phase (3) from Jul. to Oct. But based on DF analysis distinguishable three phases (Fig 1) as follows: phase (1) from Mar. to May, phase (2) during Jun.-Jul., and phase (3) from Aug. to Oct. occurs. On the other hand, based on histological survey the three phase distinguishable as

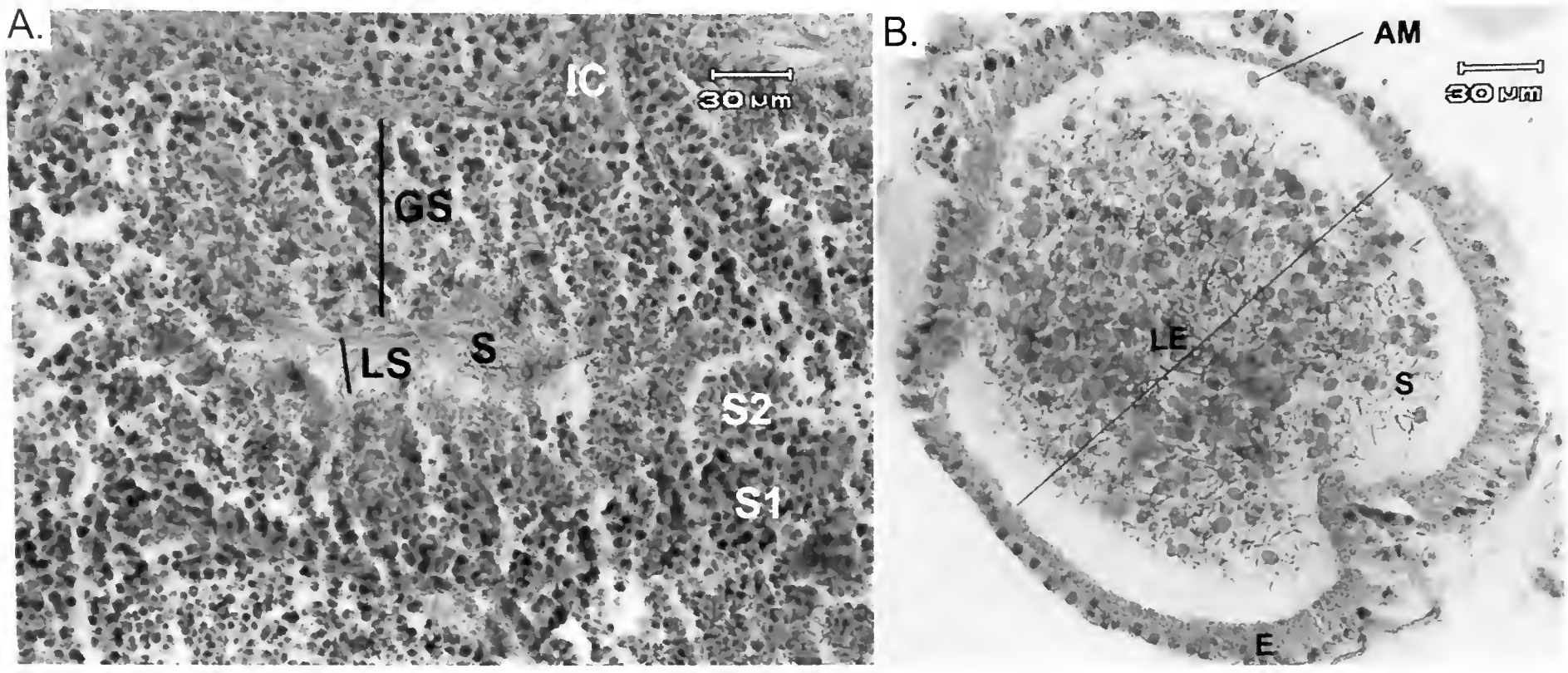

Figure 3. Light microscopy shows active phase, histological section of (a) seminiferous, (b) epididymis. LE, Lumen of Epididymis, E: Epididymis layer, LS: Lumen of Seminiferous, GS: Germinative layer of Seminiferous, S: Spermatozoa, S1: primary Spermatocytes, S2: secondary Spermatocytes, AM: Amorphous Material, IC: Interstitial tissue Cell.

follows: phase (1) from Mar. to Jul., phase (2) during Jun. to Jul., and phase (3) from Aug. to Oct.

Discussion

In this study, there was no significant relationship between SVL*Month ($p > 0.05$), because adult specimens were collected. Based on statistical and histological study, I presented three phases (Fig. 2) during the degeneration period in *O. elegans*; active, transitional and inactive phase. (a) Active phase: because spermatozoa in the lumen of seminiferous and epididymis are found (Fig. 3), this phase occurred from Mar. to May. (b) Transitional phase: because spermatozoa in many specimens found in lumen of seminiferous and epididymis and in other specimens not found, this phase occurred from Jun. to Jul. (c) Inactive phase: because in all specimens lumen of seminiferous and epididymis is without spermatozoa, this phase occurred during pre-hibernation or from Aug to Oct. Same as *O. elegans*, *Trapelus lessonae* show three phases (Torki, 2006, In Press c). Two species (*O. elegans* and *T. lessonae*) show synchronism in three phases during degeneration period. In both species (*T. lessonae* and *O. elegans*) spermatogenesis activity occurred during post-hibernation. In contrast, in the agama, *Laudakia nupta* spermatogenesis occurred after post hibernation in late spring and early summer (Torki, In Press b). Body length of *T. helenae* is lowest than other taxa and body length in *L. nupta* is biggest other taxa and *O. elegans* with *T. lessonae* is between to other. Based on timing of spermatogenesis activity and body length, three types of activities of spermatogenesis timing in lizards inhabitant Zagros Mountains as follows: (a) early active spermatogenesis, that occurred in lowest body length, (b) late spermatogenesis that occurred in highest body length, and finally normal active spermatogenesis that occurred in *T. lessonae* and *O. elegans*. Three taxa (*L. nupta*, *T. lessonae* and *O. elegans*) are sympatric; therefore, divergeny in timing spermatogenesis occurred due to body length. This is pronounced confirmed by *T. helenae* as a lowest body length. Torki and Rastegar-Pouyani briefly report affects of body size to timing of spermatogenesis (2006). However timing of spermatogenesis is many lizards is different, but histological structure in these lizards is similar (Gharzi and Torki, 2006 a). Nevertheless, in many lizards timing of spermatogenesis activity occurred due to climate condition (e.g., Duvall et al., 1982; Fitch, 1970; Whittier et al., 1987). Nevertheless, climate condition and geographic position are two main factors that strongly regulated spermatogenesis timing in lizards (Gharzi et al., 2006; Torki, 2005 b, in press a). On the other hand, geographic variation in timing spermatogenesis activity occurred due to climate gradient or latitude gradient (Gharzi and Torki, 2006 b; Torki, in press a). In many lizards such as genus *Trapelus* divergeny in spermatogenesis activity occurred in *T. lessonae* and *T. agilis*, is related to climate condition. In additional, speciation process due to dispersal or vicariance evidence in two taxon of genus *Trapelus* are important factors for the divergeny in timing of spermatogenesis activity (Torki, 2006).

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An Assessment of Solitary and Arribada Nesting of Olive Ridley Sea Turtles (*Lepidochelys olivacea*) at the Rushikulya Rookery of Orissa, India

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Abstract.- The solitary and arribada population of Olive Ridley Sea Turtles at the Rushikulya rookery of Orissa of India was monitored for two nesting seasons (2003–04 and 2004–05). Mass nesting population census of turtles was carried out using standard IUCN/SSC Marine Turtle Specialist Group recommended statistical technique (number of turtles counted: n = 11024). Curved carapace measurements of egg laying females were recorded (67.16±3.65). There was a reduction in the size class of nesting females as compare to the data available on turtle morphometry from Orissa coast in the last decade. The sporadic nesting was documented at the rookery from December to April with a peak in March and with no major intermediate nesting activities in between. The mass nesting census differs greatly as compare to the nesting figures projected by the state wildlife authority. While the state wildlife authority projects a higher figure of nesting turtles, the actual number of turtles that nests during arribada is quite low. Continuous monitoring of the beach for assessment of solitary nesting activities along with accurate methods of mass nesting census is required for proper assessment of the Olive Ridley Sea Turtle population at the Rushikulya rookery of Orissa.

Keywords.- *Lepidochelys olivacea*, solitary, arribada, estimation, technique, India.

Introduction

Nesting of Olive Ridley Sea Turtles (*Lepidochelys olivacea*) takes place either in solitary or in great simultaneous aggregations (mass nesting) where upto 100,000 females come onto the beach to lay their eggs also popularly known as arribada; a Spanish term meaning mass arrival (Pritchard, 1997). Orissa, a state in India along the easten coast, harbours three major arribada sites *viz.* Gahirmatha, Devi and the Rushikulya rookery (Pandav et al., 1994; see Fig. 1). Besides solitary nesting all along the coast of Orissa, more than a hundred thousand turtles are believed to nest annually at Gahirmatha (Dash and Kar, 1990) and tens of thousands nest at other two locations, i.e. Devi and the Rushikulya rookery (Kar, 1982; Pandav et al., 1994). In spite of its biological importance, solitary nesting has never been evaluated adequately in many important nesting rookeries (Castro, 1986). Although some information is available on solitary nesting of *L. olivacea* at Gahirmatha and Devi rookery (Pandav, 2000), there is little or no information on solitary nesting activities at the Rushikulya rookery. Similarly, the mass nesting events at the Rushikulya rookery have not yet been monitored properly; current data are from anecdotal accounts (Pandav et al., 1994) and the imprecise census by the Orissa State Forest Department due to improper statistical techniques (Pandav, 2000; Shanker et al., 2003; http://www.wildlifeorissa.org). The Orissa State Forest Department have reported mass nesting at this rookery every year since 2001, but accurate estimates of the number of nesting turtles in arribadas are not available in the absence of a standard technique for mass nesting census (Patnaik et al., 2001; Shanker et al., 2003). The IUCN/SSC Marine Turtle Specialist Group (MTSG) has recommended for use of strip transect method for estimating the arribada on mass nesting beaches, on the basis of successful experiment by Valverde and Gates (1999).

The Olive Ridley Sea Turtle is an endangered species according to the protection status of IUCN and as per CITES prohibited for trade of any kind and also is included in the schedule I of Indian Wildlife (Protection) Act (1972) and is legally protected. However, over the past decade, more than 100,000 dead turtles have been reported along the Orissa coast due incidental and accidental fishing related casualties in the sea. Whether this mortality has an impact on the population size of *L. olivacea* is yet to be known (Pandav and Choudhury, 2000). In this paper, in light of its importance, the sporadic nesting and mass nesting census of *L. olivacea* at the Rushikulya rookery was evaluated for two season from November to April (2003–04 and 2004–05) using standard techniques recommended by the MTSG to ascertain the actual arribada nesting population of turtles at this rookery and compare this figure with estimates from the Orissa State Forest Department.

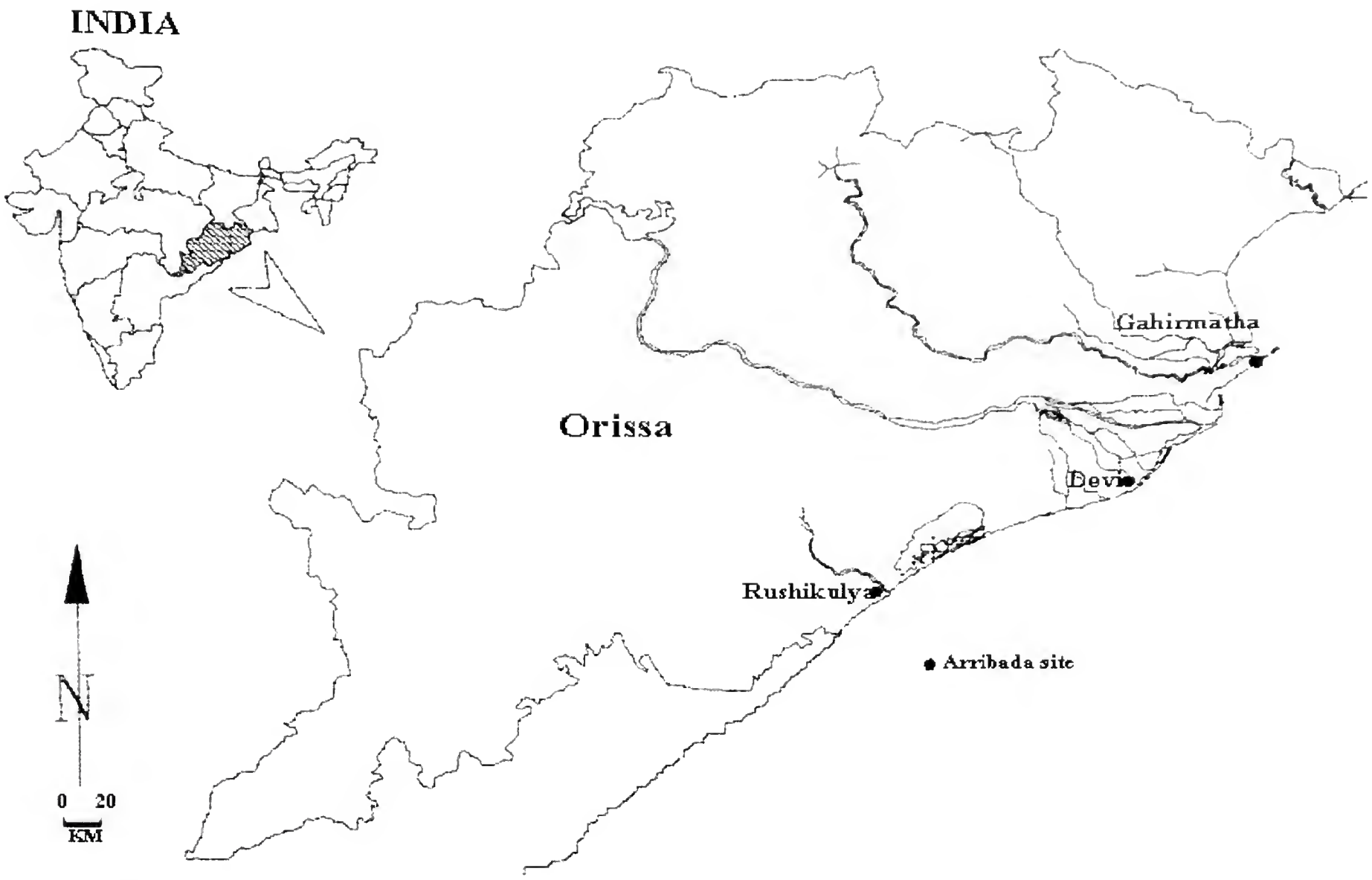

Figure 1. Map of Orissa coast in India with three arribada sites.

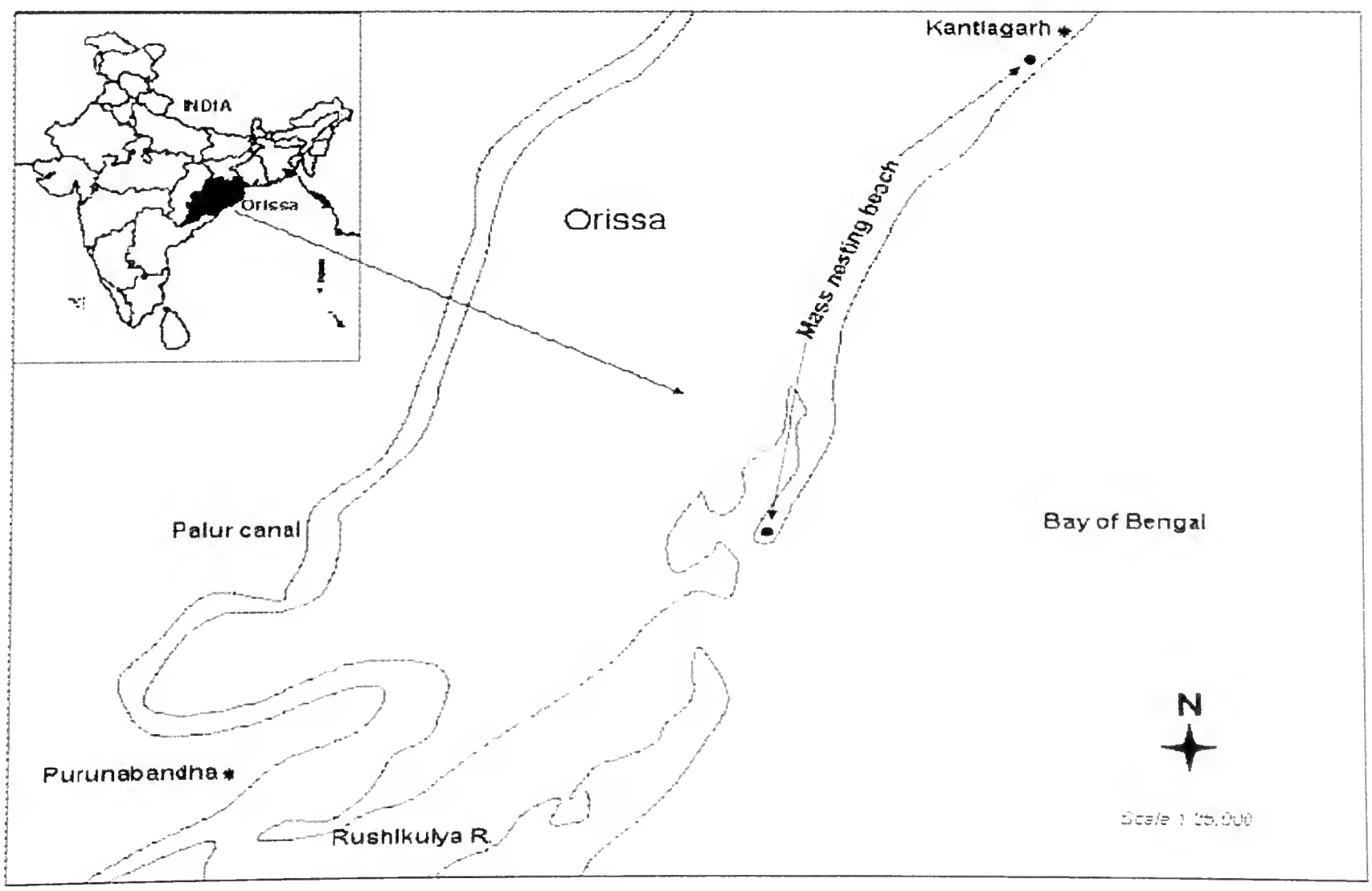

Figure 2. Map of Rushikulya sea turtle rookery, Orissa.

Table 1. Sporadic and intermediate nesting of *L. olivacea* at the Rushikulya rookery of the Orissa coast.

Month	2003–04		2004–05	
	No. of sporadic nesting (No. of nights)	No. of intermediate nesting (No. of nights)	No. of sporadic nesting (No. of nights)	No. of intermediate nesting (No. of nights)
November	5 (5)	0	0	0
December	6 (5)	0	2 (2)	0
January	17 (8)	0	12 (3)	0
February	30 (22)	0	31 (6)	119 (6)
March	166 (21)	258 (9)	170 (29)	0
April	86 (14)	0	23 (10)	0

Being a migratory species *L. olivacea* are known to arrive in the Orissa coast during late October and remain in the coastal waters until May and thereafter migrate back to the southern Bay of Bengal and Indian Ocean area. There are no turtle activities after April/May until October in Orissa. Therefore, the field work was concentrated at Rushikulya between November and May. The mass nesting beach at the Rushikulya rookery is located on the sand spit along the northern end of the Rushikulya River mouth. Rushikulya is situated 320 km south of Gahirmatha mass nesting beach (Lat. 19° 22′ N and Lon. 85° 02′ E). Turtle nesting at Rushikulya takes place along a stretch of ~5 km immediately north of the Rushikulya River mouth from the village Purunabandha (1 km north of the Rushikulya River mouth) to Kantiagada village (Fig. 2).

For systematic coverage, the entire stretch of nesting beach was divided into 100 m segments and was marked with wooden poles. To monitor nesting activities, patrolling was done by foot every night between 1700 and 0700 hr from November to April of 2004 and 2005 (1st November to 30th April for both years). Sea turtles are known to nest along the Rushikulya rookery towards the end of December (Basudev Tripathy, personal observation) and therefore the chance of missing out of some crawls during the nesting season was minimal. Turtle crawls onto the beach were classified into nesting and non-nesting types based on crawl mark pattern and sign of nest (Schroeder and Murphy, 1999). There is no standard classification of solitary nesting or arribada nesting based on the number of nests per night. However, keeping the beach length of the study area (~5 km) in mind, the author classified night with less than 20 nesters (~4 nests/km) as solitary nesting, nesting densities of 20 to 99 turtles (<20 nests/km) were considered intermediate nesting, while those with >100 (>100/km) or above turtles as arribada nesting. A modified strip

Table 2. Sea turtle (*L. olivacea*) mass nesting census at the Rushikulya rookery.

Parameters	Year	
	2004	2005
No. of days sampled	4	2
Total area of nesting (m^2)	150,000	200,000
Duration of arribada (in minutes)	780	780
Total number of egg laying turtles	1,144	3,908
Width of transect (m)	20	20
Number of sampling period (one hour sampling)	13	13
Total length of transect (m^2) (100 x 100 x No. of transect)	1,500	2,000
Average duration of oviposition (in minutes)	14.5	13.5
Estimated number of turtles nested during arribada	23,461	86,688
Estimated variance of the total number of egg laying females	1,138.571	4,599.4

transect method was used to estimate the mass nesting at the Rushikulya rookery (Valverde and Gates, 1999). This method was effective in arriving at an estimate of the number of nesting females, with a mean, variance and confidence intervals that provide rigorous statistical support for the results. A 20 m strip transect was laid at every 100 m segment of the nesting beach. Only egg-laying females (turtles in oviposition) within the strip were counted on hourly intervals starting with the first individual ascending the beach in the evening until morning when there were no nesting activities.

The formula below was used for computation of the mass nesting data (see Valverde and Gates, 1999):

$$\text{Estimate of nesting} = \frac{A \times H \times N}{W \times t \times L \times h}$$

Where:

A	=	Total available nesting area (in m^2)
H	=	Duration of arribada (in minutes)
N	=	Total of number of egg laying turtles
W	=	Width of the transect (in m)
t	=	Number of sampling period (in days)
L	=	Total of length of all transects (in m)
h	=	Average time spent by turtles for egg laying (in minutes)

Size of female *L. olivacea* was determined by the measurement of curved carapace length (CCL) at the time of egg lying. Each turtle was measured down the midline from the nuchal notch to the posterior carapace

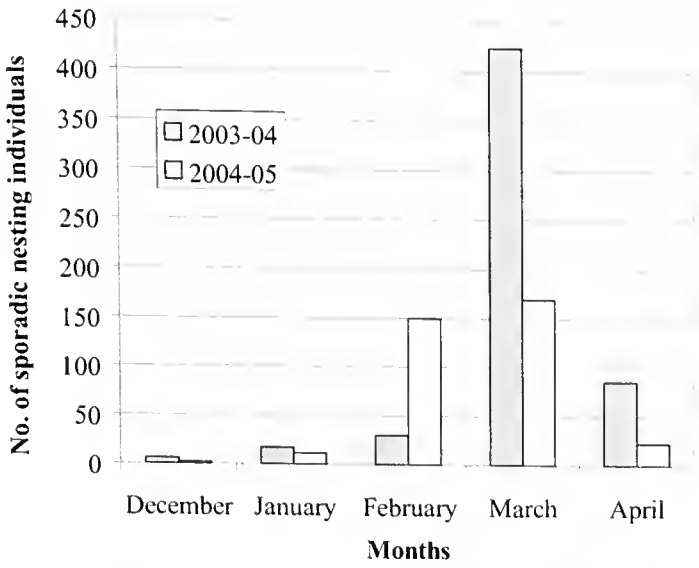

Figure 3. Monthly turtle nesting at Rushikulya for *L. olivacea* (2003–04 and 2004–05).

tip using a flexible measuring tape. Values were rounded to the nearest 0.5 cm.

Of the 568 nests observed during the 2003–04 nesting season between December 2003 and April 2004, 45.2% of the nesters were intermediate nesters, with rest being sporadic nesters (Table 1). Similarly, during the 2004–05 nesting season, intermediate nesting was calculated to be 33.3% (Table 1). There was a distinct pattern of solitary nesting observed at Rushikulya rookery, with a peak in activity during March for both the season (Fig. 3).

A total of 15 and 20 transects with 20 m width and 100 m length were established for counting turtles in arribadas during 2004 and 2005 respectively (Table 2). During 2005, the topography of the beach changed drastically and mass nesting was extended from the estuarine mouth and 2 km northward, and therefore, five more transects were laid. Although arribada took place twice during 2004 (February 9–10 and March 10–13), the February arribada could not monitored due to logistic constraint and hence the census was done only for the March 2004 arribada. During four days of peak nesting in March, a total of 23,461 turtles were estimated to have nested in arribada. However, the 2005 arribada was larger and was estimated to be 86,688 nesting individuals in two nights when censuses were carried out (Table 3).

Nesting females in arribadas at the Rushikulya rookery had an average CCL of 67.163.65 cm (n = 515; min: 60.8, max: 73.61), a value slightly greater than that of the solitary nesters at 66.024.34 cm (n = 335; Mann-Whitney = 505, $p = 0.0004$). No significant differences in CCL existed for arribada of February and March 2004 (Kruskal-Wallis $\chi^2 = 6.9$, $p = 0.2412$) and also between 2004 and 2005 season (Kruskal-Wallis, $\chi^2 = 5.80$, $p = 0.1225$).

Solitary nesting emergence of *L. olivacea* is known to occur almost every month along rest the Orissa coast (Dash and Kar, 1990). However, solitary nesting is found in greater numbers during January to May, indicating that this is the main nesting season for this species (Pandav and Choudhury, 2000). Although year round sporadic nesting is not known from the Rushikulya rookery, this study confirms sporadic nesting of olive ridley turtles at the rookery between December and April, with a peak in March and is identical to other sea turtle rookeries along the Orissa coast. Temperature, weather condition, physiography of nesting beaches and the adjacent sea, conditions of tide, temperature and surface current circulation all play an important role in determining female nest selection (Pandav and Choudhury, 2000). However, this study could not incorporate the above variables at the Rushikulya rookery due to logistic constraints. Unlike Gahirmatha and Devi (Basudev Tripathy, personal observation) where sporadic nesting is almost continuous for the entire season (>10 turtles/night), at Rushikulya rookery, sporadic nesting is irregular, with nesting intensity increasing before the commencement of the arribada. During the other nights, there is either no nesting or low sporadic nesting (<5 turtles/night). However, it is likely that the females emerging on nights with intermediate levels of nesting are responding to arribada cues (cue such as southerly strong wind, cloudy weather and strong wave action in the sea) and are truly arribada nesters. What actually comprises solitary or arribada nesting must also be evaluated in light of the total population for a given beach (Dash and Kar, 1990). During the present study, there was no major intermediate nesting events observed at the Rushikulya rookery except for nine nights in 2004 February and six nights during 2005 March, when nesting per night was over 100. However, it is likely that these turtles were early arribada nesters, since the arribada commenced in the rookery few days later.

At the Rushikulya rookery, although arribadas were reported for many years, precise mass-nesting censuses have not been carried out. The Orissa Forest Department report estimates the number of turtles during the arribada every year, but the methods used are unpublished and unavailable (estimated by Orissa State Forest Department; Table 4). Furthermore, it is not clear that methods are standardized, unbiased and therefore comparable. The State Forest Department staff counts all female turtles that remain on the beach during arribada. However, during arribada emergence, many turtles do not deposit their eggs (~30–40%) and hence are not part of true nesting population. While estimating nesting arribada population, this factor greatly affects the population size estimation and leads to bias. In the past 25 years, a variety of approaches and methods have been used to estimate female populations at arribada beaches

Table 3. Estimates of arribada (nesting number) for the 2004 and 2005 nesting season.

Date	Number of nesting turtle ($\bar{x}$)	Lower confidence interval	Upper confidence interval (95%)	Standard error
2004				
10th March 2004	4,262	3,534.1	4,990.03	364.36
11th March 2004	12,434	11,270.86	13,598.1	507.45
12th March 2004	4,362	4,227.1	4,297.1	273.25
13th March 2004	2,503	2,204.03	2,802.86	149.8
Total (4 nights)	23,561	21,236.09	25,688.09	
2005				
14th March 2005	44,466	42,017.33	46,915.99	7,379.48
15th March 2005	42,222	40,444.45	44,001.99	6,129.67
Total (2 nights)	86,688	82,461.78	90,917.98	

of Orissa (reviewed by Shanker et al., 2003). The present study estimated very low nesting populations during arribadas (using the standard technique as suggested by the IUCN/MTSG (Valvarde and Gates, 1999) compared to the figures projected by the Orissa state forest department (Tables 3 and 4). While projection of a large nesting figure attracts attention, particularly to the national and international media and conservation communities at large, it may results in the downgrading of this species in the Indian Wildlife (Protection) Act and IUCN's Red List.

In recent years (at least between 1996 and 2000), a small but significant decrease in curved carapace length (CCL) of female Olive Ridley Sea Turtles has been documented (Pandav et al., 1994; Kalb, 1999). Similarly, the average CCL of females at Gahirmatha from 1978 to 1985 were larger than those measured during 1996–2000 (Dash and Kar, 1990; Pandav and Choudhury, 2000). The present study found that arribada nesters are significantly larger than the solitary nesters, with a mean CCL being 1.14 cm greater, but was within the range. The decrease in size class (as compare to 1996–2000) was not detected during the current study, but this could be due to a small sample size, the lack of sufficient data, and a less accurate measuring technique (measuring tape for CCL *Vs* metallic calipers for SCL).

In conclusion, it is apparent that the solitary and arribada nesters are not different, but from the same population stock. The genetic study on Olive Ridley Sea Turtles from Orissa also supports this view (Shanker et al., 2004). Also, sporadic nests contribute equally to the population recruitment as that of arribada nesters being hatching success is higher for the later (Castro, 1986, also see Tripathy et al., 2003). Olive Ridleys along the Orissa coast are known to exhibit fidelity to their breeding as well as nesting ground (Dash and Kar 1990, Pandav et al., 2000). Nesting females are known to exhibit movement between rookeries in Orissa both within and between seasons (Tripathy and Pandav, in press; Pandav and Choudhury 2000). Migration and inter-rookery movement by females during the breeding and nesting season along Orissa coast has also been documented (Pandav and Choudhury, 2006). Hence, knowl-

Table 4. Estimates of arribada at the Rushikulya rookery by the Orissa Forest Department and other researchers (1994-2005).

Year	Nesting estimate	Date of arribada	Reference
1994–1995	2,000,000	14–16 March 1995	Pandav et al., (1994)
1995–1996	?	06–08 March 1996	- do -??
1996–1997	?	31 January–3 February 1997	B. Pandav (Personal Observation)
1997–1998	?	20–23 March 1998	—
1998–1999	—	No arribada	—
1999–2000	—	No arribada	—
2000–2001	1,59,000	26 February–4 March 2001	—
2001–2002	—	No arribada	—
2002–2003	2,08,000	09–14 March 2003	Orissa State Forest Department
2003–2004	2,01,000	10–15 March 2004	—
2004–2005	?	15–18 February 2005	—

edge of the location and temporal use of nesting grounds of Olive Ridley Sea Turtles in Orissa is important in view of the habitat loss and large-scale mortality of turtles in the offshore waters. Therefore, along with protection of arribada nesters, it is necessary to monitor the beach and safeguard the sporadic nesters and their habitat as well.

Prior to this study, there were no proper estimates of the number of turtles that nest during arribada at the Rushikulya rookery. Our estimates show that the number of turtles could be much less than what is projected by various governmental agencies. Thus, declaration of a mass nesting population in broad terms (e.g. hundreds of thousands) without a proper assessment may result in the reduction of protection required for *L. olivacea* in their breeding ground, which is already meager. Hence, standard and accurate techniques for mass nesting census are urgently required for additional years for monitoring the status and nesting trends of *L. olivacea* at the in Rushikulya rookery of Orissa. As evidence from the last decade of sea turtle mortality data from Orissa (Pandav and Choudhury, 2000), reduction of the size class of individuals participating in arribadas (Shanker et al., 2004; Tripathy, 2005), and elimination of the older females from the breeding stock over a period of time. However, to confirm this, extensive and accurate measurements of the nesting females and clutch sizes need to be performed to determine if there is a difference/reduction in size of *L. olivacea* over the years at rookeries in Orissa, and thereby a declining of the Olive Ridley Sea Turtle population of the Indian Ocean area and or the rest of the world.

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Effects of Starvation on Urinary Nitrogen Composition of Juvenile Chinese Three-keeled Pond Turtles (*Chinemys reevesii*)

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Abstract.- We investigated the effect of starvation on urinary nitrogen composition in the juvenile Chinese three-keeled pond turtle (*Chinemys reevesii*). Under normal conditions, ammonia, urea, and uric acid constitute 2.3, 95.8 and 1.9% of total urinary nitrogen, respectively. During starvation periods of one to four weeks, the concentration of urea changed little, while that of ammonia rose sharply and that of uric acid fell significantly. After feeding was resumed for four weeks, the levels of ammonia and uric acid returned to control levels. Changes in urinary nitrogen composition during starvation may be related to the anti-oxidative function of uric acid during periods of stress.

Keywords.- Pond turtle, *Chinemys reevesii*, bladder urine content, stress, uric acid.

Introduction

The nature of an animal's nitrogenous waste, including ammonia, urea and uric acid, is dependent on the environment in which it lives (Delaunay, 1931). Furthermore, the spatial distribution of nitrogenous end products in the body provides important data on how that animal responds physiologically to that environment. The highly diverse excretory function of the Reptilia has been well-documented (Campbell, 1995), and although aquatic and semiaquatic turtles are primarily ureotelic, a number of exceptions are known where the predominant form of nitrogenous waste is ammonia (Lee et al., 2007).

The first objective of this study was to determine the composition of excretory nitrogen in the bladder of the Chinese Three-keeled pond turtle *Chinemys reevesii*. This research will add to the body of literature on nitrogen excretion in freshwater turtles, which is currently very limited (Lee et al., 2007; Singer, 2003).

Nitrogenous end-products have diverse physiological functions in different animal groups, including acid-base regulation, osmoregulation, etc. (Wright, 1995). In turtles, two of the major factors affecting nitrogen excretion are availability of water and amount of dietary nitrogen ingested (Singer, 2003), which are of course highly influenced by starvation and dehydration, which turtles have developed a magnificent physiological capability to resist. The aquatic Sonoran mud turtle (*Kinosternon sonoriense*), for example, can aestivate for 11 weeks without food or water; during the initial period of deprivation, urine in the bladder is apparently used for osmoregulation (Peterson and Stone, 2000). While ammonia is quite toxic, more inert forms of excretory nitrogen (e.g. urea and uric acid) are more costly to synthesize (in ATP equivalents; Baze, 1970), making ammonia the most frequently produced form of waste in aquatic reptiles because they can lose this waste rapidly to the environment (Cragg et al., 1961). Uric acid is considered to be an endogenous antioxidant, and as such, might play a role in the clearance of free radicals that are produced during starvation (Zhu et al., 2005). The second objective of the present work is to investigate how *Chinemys reevesii* changes its nitrogen metabolism during starvation.

Materials and methods

Experimental animals and diet.- Juvenile turtles were obtined from a turtle farm in Guangzhou, China and acclimated to a fixed photoperiod of 12L:12D at a temperature of 29±°C for three weeks in December 2004. Each of the three turtles were placed in a glass tank with the dimension of 19 × 23 × 27 cm. Turtles were fed to apparent satiation twice a day on a commercial formulated feed powder (composition as percentage of dry matter: crude protein 41.66%, crude lipid 5.84%, ash 18.24%, and water 8.62% of total weight), which was added to water and extruded to strip pellets before usage. Each turtle was marked by sawing one to three notches at the edge of its carapace.

Experimental procedure.- Before the experiment, 108 healthy turtles with a mean body mass of 13.58±1.84 g (mean±*SD*) were chosen, randomly divided into five groups (C [control], S1, S2, S3 and S4), and placed into a total of 36 tanks. This experiment lasted eight weeks, with a period of starvation interrupting periods when the

Table 1. Percentage of urine in body weight, nitrogen concentration (mmol•L^{-1}) and partition (percent) formed as ammonia, urea and uric acid in the bladder of fresh-water turtles (*Chinemys reevesii*) at the end of fasting and after feeding for four weeks. Data expressed as Mean ± SE. Different letters denote significant differences within the same column, p <0.05.

Group C was the control group. Group S1 was starved during the fourth week; group S2 was starved during the third and fourth weeks; group S3 was starved from the second to the fourth week; group S4 was starved for the first four weeks.

Group	N	Percentage of urine in body weight	Concentration: mmol•L^{-1}				Partition of total Nitrogen		
			Urea	Ammonia	Uric acid	Total N	Urea	Ammonia	Uric acid
					At the end of fasting				
C	5	2.1±0.6	172.5±33.0	9.21±0.44 ^{b}	2.19±0.20 ^{a}	363.0±66.5	94.5±0.8	2.9±0.6 ^{b}	2.6±0.3 ^{a}
S1	2	2.1±0.5	166.3±38.5	17.45±3.64 ^{ab}	0.44±0.42 ^{b}	352.0±82.0	94.6±0.3	5.0±0.1 ^{ab}	0.4±0.4^{ab}
S2	5	4.0±1.2	136.1±45.6	9.37±3.21 ^{b}	0.71±0.14 ^{b}	284.5±94.2	94.7±0.9	3.4±0.3 ^{ab}	1.9±0.8 ^{ab}
S3	6	3.2±0.6	139.4±41.9	13.24±5.44 ^{ab}	0.36±0.13 ^{b}	267.4±104.1	95.6±0.4	3.6±0.5 ^{ab}	0.9±0.3 ^{b}
S4	6	3.1±0.5	235.7±32.3	24.25±2.61^{a}	0.22±0.11 ^{b}	496.6±66.8	94.8±0.3	5.0±0.2 ^{a}	0.2±0.1 ^{b}
F		0.915	1.178	3.103	25.559	1.311	0.417	5.015	4.789
p		0.475	0.352	**0.040**	**0.000**	0.304	0.794	**0.007**	**0.008**
					After feeding for four weeks				
C	6	2.4±0.2	165.7±24.9 ^{ab}	6.10±0.56	0.96±0.17	341.3±49.7 ^{ab}	96.8±0.5	1.9±0.2	1.3±0.3
S1	5	2.1±0.5	242.4±40.5 ^{a}	7.43±1.1	1.08±0.32	496.6±80.9 ^{a}	97.5±0.5	1.6±0.3	1.0±0.4
S2	6	2.1±0.3	150.0±26.7 ^{ab}	7.40±1.58	1.37±0.33	312.8±53.8 ^{ab}	95.1±0.9	2.6±0.5	2.3±0.7
S3	7	2.8±0.3	119.8±10.1 ^{b}	5.59±0.88	1.45±0.19	251.1±21.0 ^{b}	95.4±0.5	2.2±0.3	2.3±0.3
S4	7	2.7±0.2	107.3±21.6 ^{b}	4.81±0.83	1.21±0.17	224.1±43.8 ^{b}	94.7±1.2	2.6±0.6	2.7±0.6
F		1.261	4.077	1.138	0.776	4.064	1.947	1.010	2.010
p		0.313	**0.012**	0.362	0.552	**0.012**	0.135	0.422	0.125

turtles were fully fed. The C group included eight tanks where the turtles were always fed to satiation. The S1, S2, S3 and S4 groups included seven tanks each. The S4 group was starved in the first four weeks of the experiment. The S3 group was fed in the first week and then starved for three weeks. The S2 group was fed for two weeks and then starved for two weeks. The S1 group was fed for three weeks and then starved for one week. All groups were fed to satiation from the fifth to eighth weeks.

Analytical methods.- One turtle was sampled randomly from every tank at the end of the fourth and eighth weeks, starved for 24 hours to empty the gut, toweled off, weighed to within 0.01 g with an electronic balance, placed in a plastic bag and euthanized at a temperature of -80°C.

The turtles were dissected and the urinary bladders, which contained frozen urine, were extracted and weighed. Urine samples were centrifuged and diluted twenty times with 0.9% NaCl. Urea and uric acid concentrations were determined using a Roche Diagnostics Cobas INTEGRA 400. The concentration of ammonia was measured using Roche Modular-P and Integra systems.

Data processing.- All statistical analyses were performed using the SPSS13.0 software package. The Kolmogorov-Smirnov test revealed that the data (including percentages) followed a normal distribution. A one-way ANOVA was employed to assess the effects of starvation. The Tukey HSD or Games-Howell test was used for making multiple comparisons between the means of different groups; $p < 0.05$ was taken as the level of significance.

Results

Table 1 shows the changes in percentage of urine, total excretory nitrogen concentration, and the proportion of ammonia, urea, and uric acid among the different groups at the end of the fasting and refeeding periods.

After fasting, significant differences between the control and deprived groups were found in the concentrations of ammonia ($F_{4,19} = 3.103$, $p = 0.040$)and uric acid ($F_{4,18} = 25.559$, $p = 0.000$), and in the composition of total excretory nitrogen ($F_{4,18} = 5.015$, $p = 0.007$; $F_{4,18} = 4.789$, $p = 0.008$). Ammonia concentration and its relative concentration showed a positive relationship with increading periods of starvation, while those of uric acid showed the reverse trend. Excretory urea did not appear to be significantly affected by starvation ($p > 0.05$).

After feeding for four weeks, urea concentration and total excretory nitrogen of S1 were found to be significantly higher in groups S3 and S4, while there were no clear differences between the other groups for these two parameters. Furthermore, all groups did not differ clearly in other parameters measured ($p > 0.05$). Urinary nitrogen composition was approximately 2.3% ammonia, 95.8% urea, and 1.9% uric acid in the control group.

Discussion

In our study, *Chinemys reevesii* was found to be primarily ureogenic like other freshwater turtles, but it exhibited a relatively higher proportion of urea (about 95.8%) in excretory nitrogen compared to that of other freshwater turtles such as *Trachemys scripta* (about 70%, urine in ureter; Dantzler and Schmidt-Nielson, 1966) and *Pelodiscus sinensis* (54%, water samples; Lee et al., 2007). Schmidt-Nielsen and Skadhauge (1967) reported that ureotelic fresh water turtles excreted 45–95% of their waste nitrogen in the form of urea, comparable to the results found here.

During food deprivation, the concentration of uric acid in the turtle's bladder fell significantly ($p = 0.000$) while that of ammonia clearly rose ($p = 0.040$), even though they constitute only a small proportion of total nitrogenous end products. Rapatz and Musacchia (1957) found that the fasted fresh water turtle *Chrysemys picta* (fasted for 6–8 weeks at 22°C) showed characteristic biochemical properties in decreased liver total fatty acids, decreased blood glucose and increased urine uric acid levels. Meanwhile, specimens in cold torpor (4–8 weeks at 4°C) had increased liver total fatty acids, a significant increase in liver glycogenolysis and increased urine uric acid levels. Zhu et al. (2005) found that *Chinemys reevesii* retained higher uric acid in its bladder during cold torpor, but these contents rapidly decreased when exposed to air because of oxidation. They presumed that retention of uric acid in the bladder during cold torpor (they induced hibernation for about one year) might have a benificial function during long periods of food deprivation, as uric acid or urate is known to have antioxidative properties similar to those of vitamin C and E. This may be the cause of the observed decrease of uric acid in the bladder during starvation in the present study. Conflicting results observed between this and previous studies may be due to differences in rearing conditions, the use of a different species (*Chinemys reevesii* vs. *Chrysemys picta*) or ontogenetic differences (juvenile vs. adult). The increase of ammonia in the bladder of the turtles may be the result of increased activity in innate protein catabolism during starvation. Further research on nitrogen metabolism in stressful

environments should be conducted.

The concentration of total urinary nitrogen and urea in our experiment did not significantly change with starvation, conflicting with prior experiments (Zhu et al., 2005). This conflict may be due to differences in nitrogen metabolism when hibernation is not induced.

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Survival and Metabolic Responses to Freezing Temperature in the Northeast Forest Frog *Rana dybowskii*

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Abstract.- Dynamic changes in water content, crude oil, general proteins, blood sugar and hepatic glycogen during freezing temperatures in the Northeast forest frog (*Rana dybowskii* Günther, 1876) were investigated by establishing frog freeze-tolerant models. Chemical and biochemical analyses showed that a temperature drop from 4°C to -3°C resulted in (1) increase in integrative water content and decrease in in vivo moisture and dissociative water contents; (2) decrease in hepatic glycogen and crude oil and significant increase in blood sugar; (3) significant increase ($p > 0.05$) in general protein content; (4) mortality below temperatures of -1°C; (5) and increase in blood sugar and glucose levels in skeletal muscle following injection of glucose at 4°C and -2°C (hepatic glycogen levels showed similar increases in test groups injected with 650 mmol/L and 1500 mmol/L glucose-PBS, but not in groups injected with 2000 mmol/L glucose-PBS). These physiological and metabolic responses suggest that the Northeast forest frog adopts a positive freeze-tolerant strategy in which glucose serves as the primary mechanism by which damage due to freezing is prevented.

Keywords.- Freezing tolerance, moisture contents, blood sugar, crude oil, general proteins.

Introduction

The Northeast forest frog (Ranidae: Raninae: *Rana dybowskii* Günther, 1876), formerly classified as a northeastern population of *Rana chensinensis* David, 1875 (Xie et al., 1999), is found throughout eastern Asia, with records from Heilongjiang Province, Jilin Province, Liaoning Province, the northeast of the Inner Mongolia Autonomous Region, as well as the Russian Far East, eastern Mongolia, the Republic of Korea, and Tsushima island (Japan) (Fei et al., 2005). The climate in the northern province of Heilongjiang, the region from which research material was collected, is typified by intermediate and frigid-temperature zones with a continental monsoon climate. From November to March, the average temperature is usually less than 0°C, while in January, temperatures reach -15°C to -30°C. Due to climatic factors such as west-wind circumfluence, Siberian air mass, Mongolia high pressure and Baikal cyclone, winters in Heilongjiang Province are often dry and without snow.

As is typical of northern amphibians facing freezing temperatures, the Northeast forest frog hibernates to subtly adjust its physiological functions and metabolism to survive the winter. Although little physiological and biochemical investigations have been made on this species, various freeze-tolerant strategies have been examined in species with similar biochemical metabolisms, including *Rana sylvatica* LeConte, 1825, *Hyla versicolor* LeConte, 1825, *Hyla chrysoscelis* and *Rana ridibunda* Pallas, 1771 (Storey and Storey, 1986; Voituron et al., 2000). These have included studies on ecological behavior, genetic characteristics (Jiang and Zhou, 2001; Yang et al., 2001; Xia et al., 2006), classification (Jiang and Zhou, 2001; Xie et al., 1999; Yang et al., 2001), artificial breeding and reproduction (Wei et al., 2005) and biochemical composition (Xiao et al., 2005). To further explore freeze-tolerant mechanisms and cryobiology in the Amphibia, we here examine the moisture, crude oil, proteins, blood sugar and glycogen contents of the Northeast forest frog when subjected to freezing temperatures.

Materials and Methods

Materials and freeze-tolerant models.- Adult male frogs weighing 20 to 22 g were collected from the Yichun area of Heilongjiang Province in September. Following one week of acclimation to room temperature (25°C), ten randomly-selected frogs were placed into separate glass boxes (40 × 40 × 40 cm) and transferred to digitally-controlled refrigerators. Temperature dropped at a rate of 1°C every 12 h and was held at 4°C for up to 60 d. One third of the water in each box was exchanged with pre-cooled fresh water (4°C) every two days.

Freeze temperature impacting survival ratios.- To investigate survival ratios at different temperatures, six test groups (held at 2°C, 1°C, 0°C, -1°C, -2°C, and -3°C,

respectively) and one control group (25°C) were established with ten frogs per group. For each test group, water temperature was lowered from 4°C to the target temperatures specified above at a rate of 2°C per day. Frogs in those test groups with target temperatures less than -1°C were placed on water-soaked sponges. Survivorship was checked once daily and PT100 thermo-sensors were used to monitor temperatures. Following 10 d of freezing stress, each test group was returned to room temperature (25°C) for 24 hrs and survivorship examined, followed by euthanasia and biochemical analysis. Each test was repeated three times.

Measurement of moisture content, crude oil, general proteins, blood sugar and glycogen.- The drying method outlined by Han et al. (2005) was used to measure dissociative and integrative water content: three 10 g samples from each frog (recorded as W_w) were incubated at 70°C for 6 or 7 hrs until the semi-dry samples reached a constant weight (recorded as W_{70}). ; the semi-dried samples were incubated at 105°C for 5 or 6hrs until the samples again reached a constant weight (recorded as W_{105}). Calculated dissociative and integrative water percentages were calculated as follows:

$$\text{Dissociative water (\%)} = 100 - \frac{W_{70}}{W_w} \times 100$$

$$\text{Integrative water (\%)} = \frac{W_{70} - W_{105}}{W_w} \times 100$$

The Soxhlet extraction method was used for measuring crude oil (Wei et al., 2004): for each group, 10 dry, 1 g samples (recorded as W_{fat}) were placed into the Soxhlet extraction flask and degreased with low-melt-point aether/petroleum; samples were packaged and incubated at 105°C for 1hr until the weight again became constant (recorded as W'_{fat}). Crude oil percentage was calculated using the following equation:

$$\text{Crude oil (\%)} = \frac{W_{fat} - W'_{fat}}{W_{fat}} \times 100$$

General protein content was determined by the Kjeldahl nitrogen determination method: for each group, semi-dried 0.5 g samples were transferred into a digestion tube; 2.5 g Na_2SO_4, 0.13 g $CuSO_4$, and 10 ml H_2SO_4 were added and digested at 400°C for 3 h until the solution color changed to pea green; the tubes were then placed on the Kjeldahl nitrogen determination apparatus for distillation; 15 ml 1% H_3BO_3 were added to the Erlenmeyer flask and connected to the condensator exit; 20 ml saturated NaOH was added to the reaction and methyl red/bromocresol green indicator was added until the solution turned grey; the solution was finally titrated with HCl standard buffer until the solution color changed from grey to blue. General proteins percentages were calculated with following equation:

V_S is the volume of HCl standard buffer added to adjust the distilled sample solution, V_0 is the volume of

$$\text{General proteins (\%)} = \frac{(V_s - V_o)}{W} \times 0.01 \times 0.014 \times 6.25 \times 100$$

HCl standard buffer needed to adjust the control solution, and W is the dry weight of sample.

The ortho-toluidine o-toloidine colorimetry method and the anthrone colorimetry method were used for measuring blood sugar and glycogen, respectively (He et al., 2004): after 24 h of freezing-temperature stress, blood sugar was measured from heart tissue (immediately treated with heparin from phlebotomized specimens within each group); 1 g of liver tissue was used to measure glycogen; tissue was homogenated and centrifuged, the supernatant was collected and added to an equal volume of ethanol; the solution was centrifuged, and 100 ml of distilled water was added to the precipitated glycogen, which was then measured.

Exogenous glucose intervention assay.- The method described by Costanzo et al. (1991) was used to detect the effects of exogenous glucose on glucose metabolism in frog liver and muscle tissue, and to demonstrate whether glucose was involved in any freeze-tolerance mechanisms. For each target temperature (4°C or -2°C), ten frogs in three test groups and one control group (25°C) were examined. For the control group, approximately 2.3–2.5 ml 115 mmol/L PBS was injected into the dorsal lymph bursa, comprising approximately 6.7 % of total volume in the bursa. (For the three test groups, different concentrations (650, 1500, 2000 mmol/L, respectively) of glucose-PBS (pH 7.4) were injected to again comprise approximately 6.7% of total bursa volume. All frogs were euthanized following 72 hrs of freezing temperatures. One gram of blood, liver and muscle were immediately collected to analyze glucose contents by the same methods described above.

Statistical analysis.- SPSS (ver.15.0) software was used for statistic analysis. Confidence intervals were set to 0.05. Data were presented as means±standard error (SE) and analyzed using a one-way ANOVA; these results were subsequently analyzed using the Tukey test.

Results

Freezing temperatures and their impact on survival ratios.- During the temperature decrease from 4°C to -1°C, ice crystals became visible on the skin of the frogs. After ten days, mortality was observed only below -1°C (see Fig. 1).

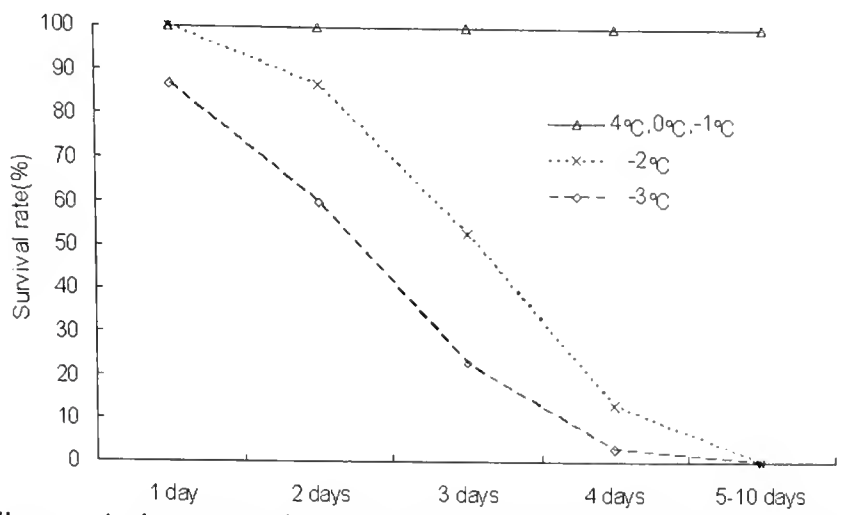

Figure 1. Impact of freeze temperatures and their duration on survival ratios of *Rana dybowskii.* Y-axis is survival ratios in percentage, X-axis is duration. The continuous line with empty triangles represents data from the test groups at 4°C, 0°C and -1°C. The dotted line with crosses is for the test groups at -2°C. The dashed line with empty diamonds is for the test groups at -3°C.

***Changes of moisture contents* in vivo, *crude oils, general proteins and glycogen.*-** Following the temperature decrease from 4°C to -3°C, moisture contents *in vivo* and dissociative water contents decreased gradually, whereas integrative water was found to increase; furthermore, blood sugar was found to increase while hepatic glycogen and crude oil decreased significantly. During freezing-temperature stress, general proteins were found to decrease slightly with decreases in temperature ($p > 0.05$) (see Table 1).

***Changes in blood sugar and glycogen levels with addition of exogenous glucose under freezing-temperatures.*-** At 4°C and -2°C, blood sugar and glucose concentrations in skeletal muscle were always found to increase with the addition of exogenous glucose. Hepatic glycogen also increased with increasing concentrations of exogenous glucose in the test groups injected with 650 mmol/L and 1,500 mmol/L glucose-PBS, however, it was found to decrease in the test groups injected with 2,000 mmol/L, particularly at -2°C ($p < 0.01$) (see Table 2–3).

Discussion

Since the 1980s, investigators have studied the freezing-tolerance mechanisms of amphibians, and have found that species such as *Rana sylvatica* can precisely regulate their metabolic levels in order to tolerate extracellular ice crystallization (Storey and Storey, 1988), which plays as key role in survival and evolution. Investigations on *Rana sylvatica*, *Pseudacris triseriata* and *Rana ridibunda* (Churchill and Storey, 1995; Costanzo et al., 1991; Edwards et al., 2004; Layne and Jones, 2001; Storey and Storey, 1985) illustrate that in at least some amphibians, endogenous glucose is used as a protectant during hibernation.

In the present study, it was found that in *Rana dybowskii,* freezing temperatures are associated with dehydration, an increase in blood sugar and a decrease in hepatic glycogen; temperatures below -1°C are also associated with increased mortality. Some investigations have proposed that endogenous water redistribution aids in the tolerance of freezing temperatures by changing dissociative water into integrative water, condensing extra-cellular solutes and promoting intracellular water trafficking out of cells. This prevents intracellular icing, lowers the freezing point of the body, induces antifreeze

Table 1. Effects of lowering temperature on percentage of water, crude oil, general proteins and sugar in *Rana dybowskii.*

	Dissociative water (%)	Integrative water (%)	Moist contents (%)	Crude oil (%)	General proteins (%)	Blood sugar (mg%)	Hepatic glycogen (%)
Control groups at 25°C	81.25±0.70d	1.44±0.11a	82.68±0.73c	4.64±0.06b	70.49±1.71b	152.38±6.57a	5.60±0.02e
Test groups at 4°C	79.69±0.47cd	1.79±0.06ab	81.49±0.50c	4.63±0.03b	64.90±0.61ab	184.97± 13.18a	4.68±0.08d
Test groups at 0°C	77.67±1.44bcd	1.90±0.13ab	79.58±1.57bc	4.55±0.03ab	64.51±0.66a	196.98± 15.08a	4.12±0.05c
Test groups at -1°C	76.24±2.07bc	2.07±0.14b	78.32±2.21bc	4.49±0.03ab	63.33±1.50a	203.78± 1.84a	3.65±0.03b
Test groups at -2°C	73.33±1.24ab	2.18±0.02b	75.52±1.23ab	4.41±0.05a	63.19±1.18a	266.98± 23.08b	3.21±0.02a
Test groups at -3°C	70.40±0.94a	2.26±0.04b	72.66±0.91a	4.41±0.09a	63.09±1.90a	305.26± 5.88b	3.10±0.01a

*Different letters in the same column represent significant differences ($p < 0.05$).

Table 2. Effect of exogenous glucose on blood sugar and liver glycogen in *Rana dybowskii* at 4°C.

	Blood sugar (mg %)	Hepatic glycogen (%)	Skeletal-muscle glucose (%)
Control groups	124.20±0.69a	5.15±0.45a	0.10±0.01a
Test groups injected with 650 mmol/L	475.50±1.27b	28.68±0.55b	2.03±0.07b
Test groups injected with 1,500 mmol/L	539.07±1.24c	47.65±2.16d	2.57±0.06c
Test groups injected with 2,000 mmol/L	945.07±1.65d	37.94±0.89c	4.59±0.13d

*Different letters in the same column represent significant differences ($p < 0.05$).

Table 3. Effect of exogenous glucose on blood sugar and liver glycogen in *Rana dybowskii* at -2°C.

	Blood sugar (mg %)	Hepatic glycogen (%)	Skeletal-muscle glucose (%)
Control groups	131.18±0.17a	4.19±0.59a	1.63±0.03a
Test groups injected with 650 mmol/L	651.69±0.28b	18.43±1.13b	2.54±0.07b
Test groups injected with 1,500 mmol/L	715.67±2.16c	33.92±1.66c	5.66±0.02c
Test groups injected with 2,000 mmol/L	1,012.58±3.90d	20.99±1.67b	7.33±0.24d

*Different letters in the same column represent significant differences ($p < 0.05$).

synthesis, and prevents damage to critical organs from intracellular ice crystallization (Churchill and Storey, 1995; Hermes-Lima and Storey, 1996; Horton, 1996). Below a certain temperature, however, small ice crystals enlarge to a point that causes damage to cell membranes and cellular substructure, thereby causing death. Stability of the cell membrane would also be compromised, resulting in plasma-membrane fusion, phase transformation from liquid crystal to gels, membrane lipids deficiencies, phospholipid separation, etc., and would destabilize membrane structure (Tong and Nie, 1996). In amphibians glucose is known to function as antifreeze, lowering the freezing-point of the body, maintaining cell membranes and stablizing internal environments (Costanzo and Lee, 1994; Katz, 1989; King *et al.*, 1995). In situations involving dehydration, such as those observed here, hydrogen bond formation between glucose hydroxyls and the heads of membrane phospholipids have been found to stabilize lipid bilayers at the liquid crystalline state, preventing membrane fusion, phase change, side phase separation, cell leakage and membrane protein displacement (Tong and Nie, 1996). Simultaneously, freezing temperatures stimulate the catabolism of hepatic glycogen and crude oils, increasing blood sugar concentrations, initiating the glucose antifreeze system, stabilize cell membranes, and enhancing physiological cold-tolerance. These factors combined provide a physiological and biochemical strategy that the Northeast forest frog uses to survive freezing annual temperatures.

In the present study, it has been illustrated that the injection of exogenous glucose into the lymph bursa results in increased levels of blood sugar and skeletal muscle glucose levels, increasing as higher concentrations of glucose solution are injected, particularly in the test groups kept at -2°C. In comparison, hepatic glycogen levels also showed an increase with higher concentration of the injected glucose solution, although the test groups injected with 2000 mmol/L glucose-PBS showed a significant decrease. A possible explanation for this unexpected result is that extremely high concentrations of glucose induce diabetes-like symptoms, causing glycol-metabolism disorders and decreased glycogen synthesis (Wang and Zhuang, 2001).

Acknowledgments

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Clone and Sequence Analysis of Sox Genes in *Rana tientaiensis*

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Abstract.- The *Sox* genes of *Rana tientaiensis* were amplified and cloned using highly degenerate primers designed from the conservative motif (HMG-box) of the human SRY gene. The SSCP technique was used to detect different clones. Seven distinct *Sox* gene fragments were obtained from both male and female *R. tientaiensis*; no sexual differences were observed. Seven of these fragments (named *RtSox3a, RtSox3b, RtSox3c, RtSox4, RtSox11, RtSox12,* and *RtSox14*) exhibited 95%, 95%, 95%, 97%, 98%, 97%, and 97% similarity (respectively) to the corresponding homologous human SOX genes. The eighth fragment showed 79% and 77% similarity to the human *SOX21* and *SOX14* genes, as well as varying levels of similarity to other group B Sox genes. The eighth gene, provisionally named Rt*SoxB*14, may be a new member of the Sox gene family or a derivative of an existing *Sox* gene. Phylogenetic analysis suggests that the *RtSox* genes are highly conserved members of the *SoxB, SoxC* and *SoxD* gene groups. Sequence analysis further illustrates that the gene *Sox3* found in *R. tientaiensis* are duplicates of those seen in the mammalian *Sox* gene family. Amino acid positions 15–19 are characteristic of each group in the *Sox* family.

Keywords.- *Sox* genes, SSCP, *Rana tientaiensis*, subgroup diagnosis.

Introduction

The Y chromosome-linked gene SRY is a dominant inducer of testis development in mammals (Sinclair et al., 1990) and a founding member of a gene family with sequence homology to the High Mobility Group (HMG) domain (Fawcett and Klymkowsky, 2004). Since discovery of the *SRY* gene, many members of the *SOX/Sox* (SRY-related HMG box) gene family have been found throughout the vertebrates, showing at least 60% protein similarity to the SRY HMG domain. Genes in each subgroup show over 80% similarity. These *SOX/Sox* genes have also been found to be involved in physiological processes such as sex determination and the development of the CNS, neural crest and endoderm (Bowles et al., 2000). *Sox1, Sox2, Sox3* and *Sox11*, for instance, are expressed mainly in the developing nervous system (Collignon et al., 1996; Pevny et al., 1998), and *Sox*4 is essential for heart and lymphocyte development (Schilham et al., 1996). The *SOX/Sox* genes have been divided between ten subgroups, named A to J (Table 1), not all of which occur in the same taxa (Bowles et al., 2000); groups I and J (containing *sox31, sox32* and *sox33*), for instance, are only found in Zebrafish (Girard et al., 2001; Lunde et al., 2004).

Some amphibians have ZZ/ZW or XX/XY modes of sex determination, but most species do not have heteromorphic chromosomes, making the study of evolution and the mechanism of sex determination in these organisms interesting. *Rana tientaiensis* (2n = 26) is one of these species without identifiable sex chromosomes (Guo et al., 1991). In this paper, we describe the cloning and sequencing of the eight Sox genes in *Rana tientaiensis* with the aim of researching the diversity and evolution of this gene family. Sequence analysis indicates that some of these genes in *R. tientaiensis* are duplicated.

Materials and Methods

Two male and two female *Rana tientaiensis* were captured from Taolin and Tingxi Anhui Provinces, China. Genomic DNA was isolated from muscle tissues using routine protocols. A pair of degenerate primers (sn1: ATGAAYGCNTTYATGGTNTGG; sn2: GGNCGRTAYTTRTARTCNGG) were designed using multiple alignments of the HGM-box sequence of *SRY*, corresponding to the MNAFMVW and PDYKYRP motifs found in the HMG boxes of a wide range of *Sox* proteins.

PCR reactions were 30 µl in volume, including 18.75 µl ddH_2O and approximately 100 ng of genomic DNA, 1.5mM Mg^{2+}, 120 µM dNTP, 0.3 µM/primer and 1.25 µl Taq polymerase. PCR cycling conditions were 1 cycle for 5 minutes at 97°C, followed by 35 cycles with 40 sec. at 94°C, 40 sec. at 53°C, 1 min. at 72°C, and finally 72°C for 10 minutes to complete the final reaction.

Clones were genetically sequenced to detect the positive clones with *Sox* DNA insertions. PCR products were detected by 1.5% agarose gels and cloned by pMD 18-T Vector (purchased from TAKARA). Positive clones were screened by SSCP (single-strand conforma-

Table 1. Classification of the *Sox* gene family.

A	B	C	D	E	F	G	H	I	J
SRY	*Sox1*	*Sox4*	*Sox5*	*Sox8*	*Sox7*	*Sox15*	*Sox30*	*Sox31*	*Sox32*
	Sox2	*Sox11*	*Sox6*	*Sox9*	*Sox17*	*Sox16*			*Sox33*
	Sox3	*Sox24*	*Sox12*	*Sox10*	*Sox18*	*Sox20*			
	Sox14	*Sox22*	*Sox13*						
	Sox21		*Sox23*						
	Sox25								

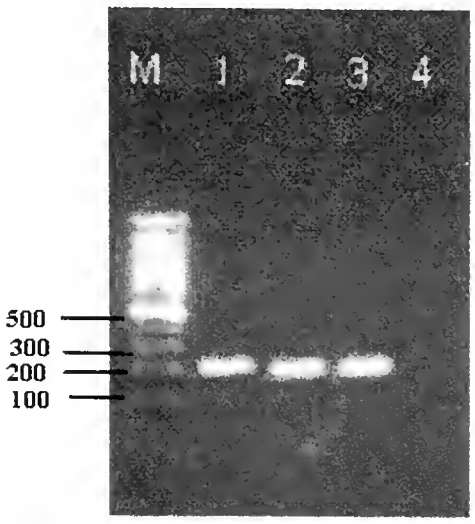

Figure 1. Amplified *Sox* gene fragments from 1: male *Rana tientaiensis*, 2: female *R. tientaiensis*,3: Human; 4: negative control; M: DL2000 marker (TaKaRa).

tion polymorphism) analysis and sequenced with the universal sequencing primers on an ABI377 auto-sequencer. DNA sequences were analyzed using the BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and CLUSTALX programs. Molecular Evolutionary Genetic Analysis (MEGA) software was used to construct the phylogenetic tree.

Results and Discussion

A 203 bp fragment of *Rana tientaiensis* genomic DNA was obtained using the degenerate PCR primers listed above. The fragment was identical to that found in the human genome (Fig. 1), indicating that these fragments belong to homologous genes.

Of the 113 white clones (i.e., those with insertions), 64 were positive for the *Sox* insertion, as confirmed through nucleotide analysis following genomic amplification. Eight distinct positive clones were found in both male and female *Rana tientaiensis*; there were no sexual differences.

Seven of the eight distinct genes were named as follows: *RtSox3a, RtSox3b, RtSox3c, RtSox4, RtSox11, RtSox12, and RtSox14.* The amino acid sequences from these genes had 95%, 95%, 95%, 97%, 98%, 97%, 97% and 97% similarity (respectively) to homologous *SOX* genes in Human. These seven genes belonged to the *SoxB*, *SoxC* and *SoxD* subgroups, all of which lack introns (Bowles et al., 2000). The 9th *Sox* gene was provisionally named *RtSoxB14*; the amino acid sequences from this gene had 79% similarity to the Human *SOX21* gene, 77% similarity to the *Human Sox14* gene, as well as varying levels of similarity to other group B *Sox* genes. Nucleotide and putative amino acid sequences for the eight *Sox* genes are listed (Fig. 2)

The amino acid sequences from the eight clones were compared to 44 published *Sox* gene sequences in GenBank, including sequences from Human (*HomoSRY, SOX1, 2, 3, 4, 7, 9, 11, 12, 14, 15, 21, 30*), Mouse (*MusSox1, 2, 3, 4, 7, 9, 11, 12, 14, 15, 21, 30)*, *Gallus gallus* (*GallSox*2, 3, 9, 11 , 14, 21), *Danio rerio* (*DaniSox1, 2, 4, 11*), *Xenopus laevis* (*XenopusSox2, 4, 11*), *Takifugu rubripes* (*TakifuguSox1, 14b*) and *Eremias breuchleyi* (*EbSox2, 4, 11, 12, 14 ,21*) (Table 2). All sequences were analyzed using neighbor-joining (NJ) methods by MEGA 2.0 (Fig. 3).

Amino acid sequences between the *RtSox* genes were highly conserved. Representatives of the *Sox* gene in other species were also highly conserved with much similarity between sequences. Gene duplication has likely caused most of the diversity seen in the HMG box superfamily, for which the *Sox* genes show the highest mutation rate (Laudet et al., 1993). The high similarity seen between the *Rana* and human genes in this study are certainly indicative of gene duplication.

In the case of *Sox12*, amino acid sequences were nearly identical, although the 10th amino acid was N instead of H in Human and Mouse (Fig. 2). In *Sox 4*, the 48th amino acid was R instead of Q in Mouse, and in *Sox11*, the 48th amino acid was D instead of N in Mouse, Human and several other species. The high degree of similarity amongst these genes suggests that they belong to the same gene family, and may perform similar roles amongst taxa. For example, the three highly conserved genes in group C display overlapping expression patterns, and *Sox4* and *Sox11* display overlapping expression patterns in the mouse embryonic pancreas (Lioubinski et al., 2003).

According to Laudet et al. (1993), *Sox4* is considered to be an early offshoot of the *SRY* gene in the *Sox* family phylogeny. The conservative nature of *Sox4* homologues in non-mammalian amniotes is interesting because in mammals, the *SRY* gene exhibits rapid evolu-

Table 2. Sox genes sequence in different species

Sequence	Accession number	Sequence	Accession number
Human sapiens		***Mouse musculus***	
HomoSOX1	NP_005977	*MusSox1*	BAC75667
HomoSOX2	CAA83435	*MusSox2*	NP_035573
HomoSOX3	CAA50465	*MusSox3*	AAH52024
HomoSOX4	NP_003098	*MusSox4*	NP_ 033264
HomoSOX7	NP_113627	*MusSox7*	NP_035576
HomoSOX9	CAA86598	*MusSox9*	AAH04064
HomoSOX11	BAA88122	*MusSox11*	NP_033260
HomoSOX12	CAB81632	*MusSox12*	NP_035568
HomoSOX14	AAI06731	*MusSox14*	XP 284529
HomoSOX15	NP_008873	*MusSox15*	NP_033261
HomoSOX21	NP_009015	*MusSox21*	NP_808421
HomoSOX30	NP_848511	*MusSox30*	AAF99391
HomoSRY	AAT37462	***Danio rerio***	
Gallus gallus		*DaniSox1*	NP_001032751
GallSox2	NP_990519	*DaniSox2*	NP_001002483
GallSox3	NP_989526	*DaniSox4*	BC065354
GallSox9		*DaniSox11*	CAB87379
GallSox11	NP_990518	***Xenopus laevis***	
GallSox14	NP_990092	*XenopusSox2*	AAB62821
GallSox21	BAA77266	*XenopusSox3*	P55863
Eremias breuchleyi		*XenopusSox11*	*Q91731*
EbSox2	DQ067423	***Takifugu rubripes***	
EbSox4	DQ067426	*TakifuguSox1*	AAQ18494
EbSox11	DQ067427	*TakifuguSox14b*	AAQ18499
EbSox12	DQ067428		
EbSox14	DQ067430		
EbSox21	DQ067433		

tion, possibly caused by Y-linked inheritance (Tucker and Lundrigan, 1993). The limited diversity of this gene in non-mammalian taxa may be due to the retention of an ancient conserved function.

It is likely that *Sox3* is the closest homologue to the *Sry* gene based on nucleotide sequence data. Furthermore, *Sox3* is located on the mammalian X chromosome, and is highly similar to SRY (Sinclair et al., 1990), suggesting that they arose through duplication of their common ancestral during differentiation of the sex chromosomes (Collignon et al., 1996; Foster and Graves, 1994; Stevanovic et al., 1993). This is significant because the X and the Y chromosomes are thought to have arisen from a common "autosome" ancestor in the lineage that gave rise to mammals (Wright et al., 1993). Further examination of *Sox* genes in lower vertebrates, Prototheria (monotremes) and Metatheria (marsupials) will be necessary to establish the evolutionary origins of *Sry*. The three conservative *Sox3* genes (sometimes found within the same species or individual) can be identified by the following variations in amino acid sequence: *RtSox3a* has an F at position 46 and an H at position 58; *RtSox3b* has an F at position 46 and an M at position 58; *RtSox3c* has an I on position 46 and an M on position 58.

The *RtSoxB14* is unique among the Sox genes in having the amino acid sequence VITEH at positions 15–19, a K at position 44 and an S at position 50. In the phylogenetic tree (Fig. 3), although *RtSoxB14* was more closely related to subgroup B than other groups, the origin and classification of this gene is ambiguous.

Previously, genes encoding proteins with more than 60% similarity to the *SRY* HMG domain have been named *Sox* (*SRY* box) genes, and *Sox* genes with at least

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RtSox12     ATGAATGCGTTTATGGTATGGTCCCAGAACGAGCGGCGGAAGATCATGGACCAGTGGCCG
RtSox11     ATGAATGCTTTTATGGTATGGTCCAAGATCGAGCGGAGAAAAATCATGGAGCAGTCGCCC
RtSox4      ATGAACGCGTTTATGGTTTGGTCGCAGATCGAGCGGCGCAAGATCATGGAGCAGTCGCCC
RtSox3a     ATGAATGCTTTTATGGTTTGGTCGCGGGGGCAGCGGCGCAAGATGGCTCAGGAAAACCCC
RtSox3c     ATGAATGCGTTTATGGTTTGGTCGCGGGGGCAGCGGCGCAAGATGGCTCAGGAAAACCCC
RtSox3b     ATGAATGCGTTTATGGTTTGGTCGCGGGGGCAGCGGCGCAAGATGGCTCAGGAAAACCCC
RtSoxB14    ATGAACGCGTTTATGGTCTGGTCCAGAGTACAGAGGAGGAAGGTGATTACAGAACATCCT
RtSOX14     ATGAATGCGTTCATGGTGTGGTCCAGGGGGCAGAGGAGGAAGATGGCCCAGGACAATCCC
            ***** ** ** ***** *****       ** ** * **  *       *    **

RtSox12     GACATGCACAACGCTGAGATCTCCAAGCGCCTCGGCCGTCGCTGGCAGCTCCTGCAGGAC
RtSox11     GACATGCACGACGCCGAGATCTCCAAGCGCCTGGGCAAGCGGTGGAAAATGCTGAAGGAC
RtSox4      GACATGTACAACGCCGAGATCTCCAAGCGGCTAGGCAAACGCTGGAAGCTGCTCAAGGAC
RtSox3a     AAGATGCACAACTCGGAGATCTCCAAGCGCCTGGGCGCGGACTGGAAGCTGCTGAGCGAC
RtSox3c     AAGATGCACAACTCGGAGATCTCCAAGCGCCTGGGCGCGGACTGGAAGCTGCTGAGCGAC
RtSox3b     AAGATGCACAACTCGGAGATCTCCAAGCGCCTGGGCGCGGACTGGAAGCTGCTGAGCGAC
RtSoxB14    AAAATGCACAACTCTGAAATTAGCAAAAAGTTGGGGGCACAGTGGAAGATCCTTGGCGAT
RtSOX14     AAGATGCACAATTCGGAGATCAGTAAAAGACTTGGGGCTGAGTGGAAACTTCTGTCTGAA
             * *** ** *  * ** **    **     * **       *** *  * **    **

RtSox12     TCGGAGAAGATCCCCTTTGTGAAGGAGGCTGAGCGGCTGCGACTCAAGCACATGGCTGAC
RtSox11     AGCGAGAAGATCCCCTTCATCCGCGAGGCCGAGCGGCTGCGACTCAAGCACATGGCTGAC
RtSox4      AGCGACAAGATTCCGTTCATCCAGGAGGCGGAGCGACTGCGCCTCAAGCACATGGCTGAC
RtSox3a     GCGGAGAAGCGCCCTTTCATCGACGAGGCCAAGCGGCTCCGCGCCGTCCACACGAAGGAA
RtSox3c     GCGGAGAAGCGCCCTTTTATCGACGAGGCCAAGCGGCTCCGCGCCGTCCACATGAAGGAA
RtSox3b     GCGGAGAAGCGCCCTATTATCGACGAGGCCAAGCGGCTCCGCGCCGTCCACATGAAGGAA
RtSoxB14    TCAGAGAAGAAGCCTTTTATAGACGAATCAAAAAGGCTGAGAGCTCAGCATATGGTTGAG
RtSOX14     GTCGAGAAAAGACCCTACATTGACGAAGCCAAAAGGTTGAGGGCTCAACACATGAAGGAA
               ** **    **     *    **  *  *  *  *  *       ** * *   **

RtSox12     TACCCTGACTACAAATATCGCCC
RtSox11     TACCCCGATTACAAATACCGCCC
RtSox4      TACCCTGACTACAAATACCGCCC
RtSox3a     TACCCGGATTACAAATACCGCCC
RtSox3c     TACCCCGATTACAAATACCGCCC
RtSox3b     TACCCTGACTATAAATACCGCCC
RtSoxB14    CATCCCGACTACAAATACCGCCC
RtSOX14     CACCCTGACTACAAATATCGCCC
            * ** ** ** ** ** *****
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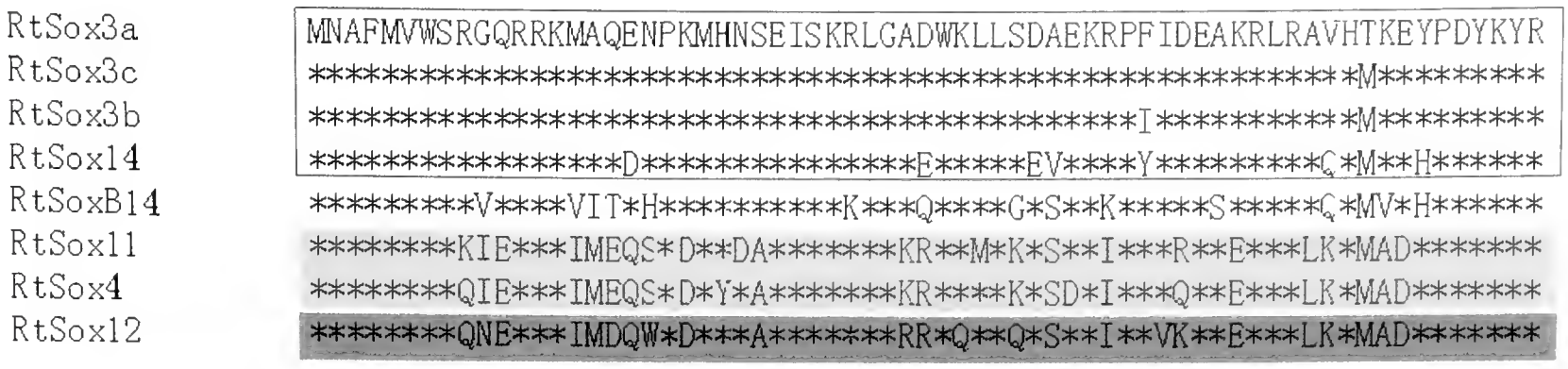
Figure 2. (A) Alignment of nucleotide sequences (above), (B) Alignment of amino acid sequences (Opposite page, top), (C) Percentage amino acid similarity between *Rana tientaiensis Sox* clones as determined by the sequence identity matrix function in Bioedit (Opposite page, bottom).



	HomoSRY	RtSox3a	RtSox3b	RtSox3c	RtSox14	RtSoxB14	RtSox11	RtSox4	RtSox12
HomoSRY	100	67.6	66.1	67.6	63.2	60.2	54.4	54.4	52.9
RtSox3a	---	100	97.0	98.5	88.2	76.4	64.7	64.7	64.7
RtSox3b	---	---	100	98.5	89.7	76.4	64.7	64.7	64.7
RtSox3c	---	---	---	100	89.7	77.9	66.1	66.1	66.1
RtSox14	---	---	---	---	100	76.4	61.7	61.7	61.7
RtSoxB14	---	---	---	---	---	100	63.2	61.7	61.7
RtSox11	---	---	---	---	---	---	100	91.1	83.8
RtSox4	---	---	---	---	---	---	---	100	85.2
RtSox12	---	---	---	---	---	---	---	---	100

Figure 2 (continued).

80% similarity have been placed in the same subgroup. However, as more and more *Sox* genes are identified, the ability to accurately classify these genes decreases. For example, the genes *sox30* and *Ce-soxj* have only 46% and 48% similarity to *Human SRY* HMG. Bowles (2000) attempted to alternatively diagnose the gene family by possession of the amino acid sequence "RPMNAF", which is highly conserved across genes, however, this sequence was also found in the taxonomically ubiquitous gene *cic*, so the sequence "RPMNAFMVW" was provided as a replacement (Bowles et al., 2000).

Now that a sequence identifying the *Sox* gene family has been identified, can sequences be found to characterize the *Sox* subgroups? Following analysis of the available sequences (Figure 4), it appears that positions 15–19 may have be useful for this purpose. The sequence "MAQE(D)N" may work for group B (except for *HomoSOX3* and *MusSox3*), "IMEQS" for group C, "IMEQW" (for *Sox12*) or "ILQAF" (for *Sox5, Sox6* and *Sox13*) for group D, "LADQY" for group E, "LAVQN" (for *Sox7*) or "LAQQN" (for *Sox17* and *Sox18*) for group F, "MAQQN" for group G and "LAKAN" for group H. *RtSoxB14* can be separated from the remaining Sox genes by the unique sequence VITEH, although in mammals (*HomoSOX3* and *MusSox3*) it is changed to "MALEN", like the sequence for *SRY*. This further supports a close relationship between the *SOX3* and *SRY* genes.

Several Sox genes appear to have been duplicated in *Rana tientaiensis*: *RTSox3a, RTSox3b* and *RTSox3c*. Similar duplications in amphibians are uncommon, but they are more frequently encountered in teleosts: Sox1, 4, 9 and 14 has been duplicated in the *sea bass* (Malyka et al., 2003) and Sox21 has been duplicated in the *Zebrafish* (Argenton et al., 2004). The "duplication-degeneration-complementation" model developed by Force et al. (1999) suggests that the partition of ancestral subfunctions is an important mechanism leading to the preservation of multiple gene copies; this model predicts that the probability of gene conservation will be higher in more complex genes with a larger number of subfunctions (Force et al. 1999). Most duplicate genes in *Rana tientaiensis* have silent mutations (except *RtSox3a*, which has an encoded amino acid mutation), but it would appear that the sequences are under selective pressure and may indeed perform separate subfunctions. Future studies investigating *Sox* genes in *Rana tientaiensis* will likely provide much insight into duplicate genes.

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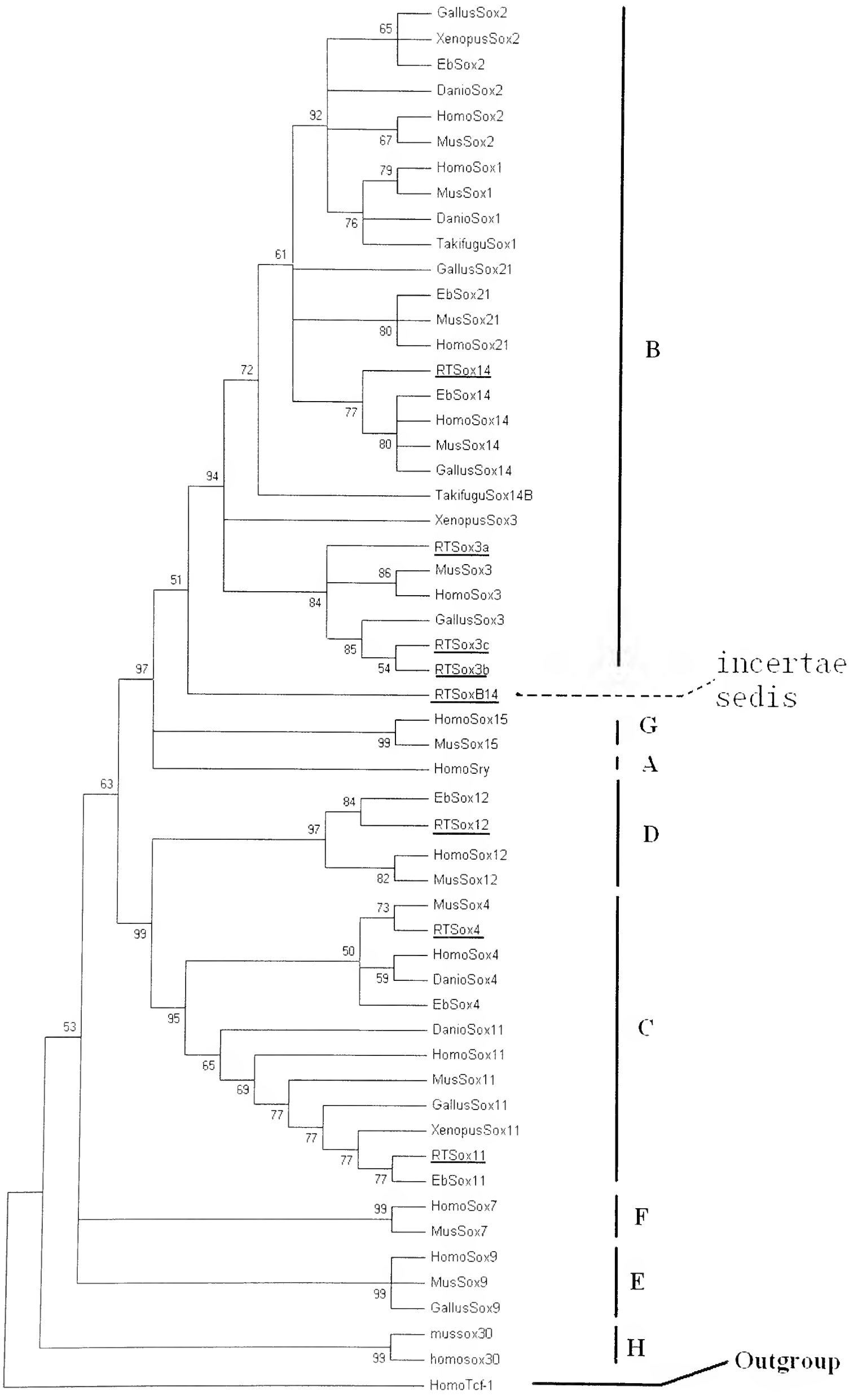

Figure 3. Phylogenetic analysis of Sox/SOX gene family

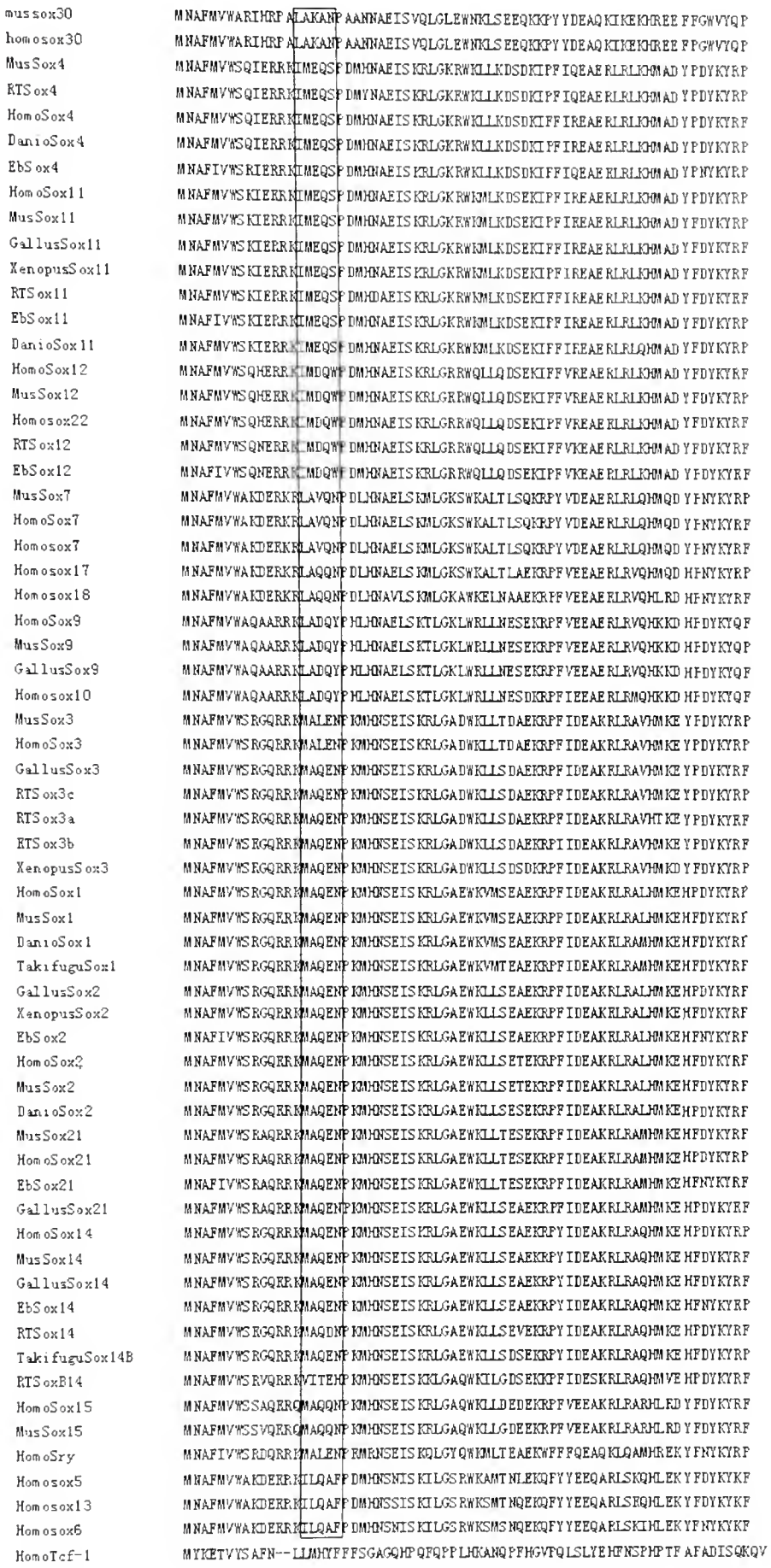

Figure 4. Characteristic Sox amino acid sequences.

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