Journal of
ARACHNOLOGY

PUBLISHED BY THE AMERICAN ARACHNOLOGICAL SOCIETY

The *Journal of Arachnology* (ISSN 0161-8202), a publication devoted to the study of Arachnida, is published three times each year by *The American Arachnological Society*. **Memberships (yearly):** Membership is open to all those interested in Arachnida. Subscriptions to *The Journal of Arachnology* and *American Arachnology* (the newsletter), and annual meeting notices, are included with membership in the Society. Regular, $55; Students, $30; Institutional, $125. Inquiries should be directed to the Membership Secretary (see below). **Back Issues:** James Carrel, 209 Tucker Hall, Missouri University, Columbia, Missouri 65211-7400 USA. Telephone: (573) 882-3037. **Undelivered Issues:** Allen Press, Inc., 810 E. 10th Street, P.O. Box 368, Lawrence, Kansas 66044 USA.

Cover photo: An adult male jumping spider, *Habronattus pyrrithrix* (Salticidae), displaying characteristic green forelegs, white pedipalps and red face during courtship (see page 268). Photo by Colin Hutton.

Troglomorphic pseudoscorpions (Arachnida: Pseudoscorpiones) of northern Arizona, with the description of two new short-range endemic species

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Abstract. This study reports on the pseudoscorpion fauna of the subterranean ecosystems of northern Arizona, U.S.A. Our work resulted in the descriptions of two new species, *Hesperochernes bradybaughi* sp. nov. and *Tuberochernes cohni* sp. nov. (Chernetidae) and the range expansion of one species, *Larca cavicola* (Muchmore 1981) (Larcidae). All of these species were cave-adapted and found within caves on Grand Canyon-Parashant National Monument in northwestern Arizona. Based upon this work, the genus *Archeolarca* Hoff and Clawson is newly synonymized with *Larca* Chamberlin, and the following species are transferred from *Archeolarca* to *Larca*, forming the new combinations *L. aalbui* (Muchmore 1984), *L. cavicola* (Muchmore 1981), *L. guadalupensis* (Muchmore 1981) and *L. welbourni* (Muchmore 1981). Despite intensive sampling on the monument, the two new species were detected in only one cave. This cave supports the greatest diversity of troglomorphic arthropod species on the monument—all of which are short-range endemics occurring in only one cave. Maintaining the management recommendations provided by Peck and Wynne (2013) for this cave should aid in the long-term persistence of these new pseudoscorpion species, as well as the other troglomorphic arthropods.

Keywords: Nearctic, troglomorphy, troglobite, new synonymy, cave

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The pseudoscorpion fauna of North American caves is moderately well known, thanks largely to the efforts of J.C. Chamberlin, C.C. Hoff, E.M. Benedict, D.R. Malcolm and W.B. Muchmore who have characterized and described many different North American troglobites and troglophiles. There are currently 144 named species found in cave habitats across the United States including six species in five families from Arizona: *Pseudogarypus hypogeus* Muchmore 1981 (Pseudogarypidae), *Albiorix anophthalmus* Muchmore 1999 (Ideoroncidae), *Chitrellina chiricahuae* Muchmore, 1996 (Syarinidae), *Archeolarca cavicola* Muchmore 1981, *A. welbourni* Muchmore 1981 (Larcidae) and *Tuberochernes ubicki* Muchmore 1997 (Chernetidae) (Muchmore 1996; Muchmore & Pape 1999; Harvey & Muchmore 2013). Only *A. anophthalmus* and *C. chiricahuae* had troglobitic modifications including the complete lack of eyes and pallid body color (Muchmore 1996; Muchmore & Pape 1999; Harvey & Muchmore 2013), whereas the others are less obviously modified with only the slightly attenuated appendages hinting at an obligate subterranean existence (Muchmore 1981, 1997).

Prior to this work, all of these cavernicolous species from Arizona occurred south of the Colorado River with *P. hypogeus*, *A. cavicola* and *A. welbourni* from northern Arizona (Coconino County) and *A. anophthalmus*, *C. chiricahuae* and *T. ubicki* from south-eastern Arizona (Pima, Cochise and Santa Cruz Counties, respectively). During biological inventories of caves on the Grand Canyon-Parashant National Monument (hereafter referred to as Parashant) in northwestern Arizona, one of us (J.J.W.) and colleagues found

representatives of three different pseudoscorpion species, which are the subject of this study.

Over the past several years, Parashant caves have yielded other significant and interesting arthropod species—many of which are restricted to the cave environment. These include two new genera (comprising two new species)—a book louse (order Psocoptera, family Sphaeropsocidae: *Troglosphaeropsocus voylesi* Mockford 2009 (Mockford 2009), and a cave cricket (family Rhaphidophoridae: cf. *Ceuthophilus* n. gen. n. sp., Cohn and Swanson, unpublished data). This work also resulted in the identification of several cave-adapted and cave-limited species including a leiodid beetle, *Ptomaphagus parashant* Peck and Wynne 2013 (Peck & Wynne 2013), an undescribed species of centipede (family Anopsobiidae; Wynne, unpublished data), an undescribed Isopod species, *Brackenridgia* n. sp. (S. Taiti, in litt.), and a recently described cave limited millipede, *Pratherodesmus voylesi* Shear 2009 (Shear et al. 2009). Additionally, three new species of trogloxenic beetles were reported from Parashant caves including *Eleodes wynnei* Aalbu, Smith, and Triplehorn 2012 (Tenebrionidae; Aalbu et al. 2012), an undescribed species of the carabid beetle genus *Rhadine* LeConte (Carabidae: the *perlevis* species-group; T.C. Barr, in litt.), and an undescribed carabid beetle species *Pterostichus* Stephens (Carabidae, K. Will, in litt.).

METHODS

The junior author and colleagues sampled caves on Grand Canyon-Parashant National Monument during 4–14 August

2005, 1–6 May 2007, 16–25 August 2007, 12–21 May 2008, and 5–12 March 2009. They sampled all caves identified as having deep zone like conditions ($n = 10$). Given the short duration of study (between two to four site visits), and potential seasonal effects, confidently identifying this zonal environment was not possible. The cave deep zone is required habitat for cave-adapted arthropods and is characterized by complete darkness, stable temperature, a near-water saturated atmosphere and limited to no airflow (as in Howarth 1980, 1982). Parashant is located in northwestern Arizona, encompasses approximately 4,451 km², and is characterized by rugged terrain containing deeply incised canyons, mesas, and mountains. Vegetation zones include Mojave Desert containing creosote bush (*Larrea tridentata*) and Joshua trees (*Yucca brevifolia*) at lower elevations, gradating through Great Basin pinyon (*Pinus edulis*) and juniper (*Juniperus* spp.) woodlands to Colorado Plateau grasslands and Ponderosa pine (*Pinus ponderosa*) forest with aspen (*Populus tremuloides*) groves on Mt. Trumbull (elevation 2,447 m). All of the caves referred to in this paper were located within the Supai, Kaibab, or Redwall limestone formations. Elevation for the caves that were studied ranges from 736 to 1,590 m.

Although we inventoried 10 Parashant caves, we provide descriptions for only the three caves (PARA-1001, PARA-2204 and PARA-3503) where pseudoscorpions were detected. PARA-1001 Cave was the second most biologically diverse cave on Parashant (Wynne, unpublished data), and supports the largest known cricket roost in northern Arizona (Wynne & Voyles 2014). A small solution cave within the Kaibab limestone, it had a total surveyed length and depth of 76.2 m and 10.4 m, respectively. This cave had a small south-facing vertical entrance (135° aspect) at bottom center of a large sinkhole. Vegetation was characterized as juniper scrublands at 1,585 m elevation, and was located on the north side of the lower Colorado River along the western extent of the Grand Canyon. PARA-2204 Cave was the most biologically diverse cave on the monument (Wynne, unpublished data). The deepest extent of this cave contained active speleothem formations and supported a near-saturated water atmosphere year-round. Located within the Supai formation, this large solution cave (total surveyed length 175 m) was comprised of several sinuous phreatic passages. This cave has one horizontal entrance (330° aspect) and was situated within a canyon near the base of the canyon's north-face. Located at 1,272 m elevation, this cave occurred within the vegetation transition zone of Mojave Desert scrub and juniper woodlands. PARA-3503 Cave was a dry cave with no evidence of recent speleothem activity, and supported a summer roost of bats, *Myotis* sp. (Wynne, unpublished data). The cave had a large horizontal entrance (135° aspect) situated upon a high bench (1,102 m elevation on an exposed cliff face). This cave was situated along the south-face of one of the largest canyons draining into the Colorado River from the north. Occurring within the Redwall formation, this large solution cave contained 540 m of surveyed length with an estimated survey depth of 14.2 m. Vegetation was characterized as Mojave Desert scrub.

The work conducted in 2005 was part of a biological baseline study [refer to Wynne & Voyles (2014) for a description of sampling methods]. Later (between 2007 and 2009), these caves were systematically sampled to characterize the cave-dwelling arthropod communities. Interval sampling using baited pitfall traps, timed searches, and opportunistic sampling techniques were used. To apply these techniques, detailed maps for each cave were required. For interval sampling, we established up to 10 sampling intervals (which included a sampling station at either wall and one at cave centerline for a total of ≤ 3 sampling stations per interval). We used 10% of the total cave length to establish the sampling interval (e.g., for a 1,000 m long cave, the sampling interval was every 100 m).

At each sampling station, we deployed live capture baited pitfall traps and conducted timed searches. For pitfall traps, we used two 907 g stacked plastic containers (13.5 cm high, 10.8 cm diameter rim and 8.9 cm base). A teaspoon of peanut butter was used as bait and placed in the bottom of the exterior container. At the bottom of the interior container, we made several dozen holes so the bait could "breathe" to attract arthropods (e.g., Barber 1931). Attempts were made to counter-sink each pitfall trap within the cave sediment or rockfall. When this was not possible, we built ramps around each trap using local materials (e.g., rocks, wooden debris, etc.) so arthropods could access the trap and fall in (e.g., Ashmole et al. 1992). To discourage small mammals, we placed small rocks around the edges of the trap and then covered the mouth of the trap with a cap rock. Pitfall traps were deployed for three to four days (a three day deployment occurred once due to scheduling constraints). For timed searches, we established a 1 m radius around each sampling station (where the pitfall trap would be deployed) and searched for arthropods within that ~3 m circle. A one to three minute timed search (one minute if no arthropods were observed, three minutes if arthropods were detected) was conducted before pitfall trap deployment and prior to trap removal.

Opportunistic collecting was executed by two to three trained observers as they traversed the length of each cave. This technique was applied as the observers were in transit between sampling intervals while deploying and removing pitfall traps and conducting timed searches. Opportunistic collecting was not conducted while at sampling stations and was resumed only when the observers were in transit once again. This technique was used at least twice per cave (both during pitfall trap deployment and retrieval trips). For example, a cave containing 10 sample station arrays, there were 27 individual "random walks" per site visit (i.e., nine random walk samples times three observers collecting along their between stations). Because we conducted two site visits per cave, there would be a total of 54 samples. For one cave, PARA 1001 Cave, we had two observers conduct the opportunistic collecting.

An alpha-numeric coding system developed by the National Park Service (NPS) was used to safeguard the location of both caves and their resources. We only provide generalized latitude and longitude coordinates of the area to keep the precise location of the cave confidential. Parashant National Monument headquarters in Saint George, Utah has the cipher table with cave codes. A copy of this paper with actual cave names is on file at both monument headquarters, National Park Service and the National Cave and Karst Research Institute, Carlsbad, New Mexico.

Specimens representing three species collected by one of us (J.J.W.) and colleagues form the basis of this study. All specimens were collected and stored in 70% ethanol. The holotypes of both new species and specimens of the known species are deposited in the Museum of Northern Arizona, Flagstaff, Arizona (MNA). Temporary slide mounts were prepared by mounting them on microscope slides with 10 or 12 mm coverslips supported by small sections of 0.25, 0.35 or 0.50 mm diameter nylon fishing line in a drop of lactic acid at room temperature for two or more days. After study the specimens were rinsed in water and returned to 75% ethanol with the dissected portions placed in 12 × 3 mm glass genitalia microvials (BioQuip Products, Inc.). All specimens were studied using a Leica DM2500 compound microscope and illustrated with the aid of a drawing tube. Measurements were taken at the highest possible magnification using an ocular graticule. Terminology and mensuration mostly follow Chamberlin (1931), with the exception of the nomenclature of the pedipalps, legs and with some minor modifications to the terminology of the trichobothria (Harvey 1992), cheliceral setation (Harvey & Edward 2007), cheliceral rallum (Judson 2007) and faces of the appendages (Harvey et al. 2012).

TAXONOMY

Family Larcidae Harvey 1992
Larca Chamberlin 1930

Larca Chamberlin 1930:616.
Archeolarca Hoff and Clawson 1952:2–3. **Syn. nov.**

Type species.—*Larca: Garypus latus* Hansen 1884, by original designation.

Archeolarca: Archeolarca rotunda Hoff and Clawson 1952, by original designation.

Remarks.—The genus *Larca* was created by Chamberlin (1930) for the type species *L. lata* (Hansen) from Europe and *L. granulata* (Banks 1891) from eastern U.S.A. Since then, other species have been added from Europe (Beier 1939a; Gardini 1983; Henderickx & Vets 2002; Zaragoza 2005) and North America (Hoff 1961; Benedict & Malcolm 1978; Muchmore 1981). *Archeolarca* was described for the type species *A. rotunda* which was collected from pack rat middens and porcupine nests in Utah (Hoff & Clawson 1952). Since then, four additional species have been described from other parts of western North America, all from cave ecosystems (Muchmore 1981, 1984), and *A. rotunda* has been found in New Mexico and Oregon (Hoff 1956a; Benedict & Malcolm 1978). *Archeolarca* only differs from *Larca* in the possession of four trichobothria on the movable chelal finger of adults, whereas species of *Larca* have only two or three trichobothria (e.g. Hoff 1961; Benedict & Malcolm 1978; Muchmore 1981; Gardini 1983; Muchmore 1984, 1990; Henderickx & Vets 2002; Zaragoza 2005). Most adult specimens from the Parashant have four trichobothria on the movable chelal finger (Fig. 12), consistent with being a species of *Archeolarca*, but one male has four on the right chela and three on the left (Fig. 11) raising the issue of whether the genera should be retained.

The maintenance of garypoid genera based solely on trichobothrial number has been abandoned for several other groups including the garypid genera *Anagarypus* Chamberlin 1930 with seven trichobothria on the fixed finger and one or two on the movable finger forming a pattern of 7/1–2 (Muchmore 1982), *Eremogarypus* Beier 1955, with a pattern of 5–8/1–3 (e.g., Beier 1962; Beier 1973), *Synsphyronus* Chamberlin 1930, with a pattern of 5–8/1–3 (e.g., Chamberlin 1943; Harvey 1987b, 2011) and *Thaumastogarypus* Beier 1947, with a pattern of 7–8/3–4 (e.g. Beier 1947; Mahnert 1982), and the geogarypid genus *Geogarypus* Chamberlin 1930 in which adults normally have an 8/4 pattern, but *G. bucculentus* Beier 1955 and *G. connatus* Harvey 1987 have a 7/4 pattern (Harvey 1986, 1987a). Intra-specific variation in the number of trichobothria of the movable chelal finger has been reported in the genus *Serianus* Chamberlin 1930 (Garypinidae). Hoff (1950) found that a small series of specimens of *S. minutus* Hoff 1950 (now known as *S. argentinae* Muchmore 1981 due to secondary homonymy of the original name) included adults with the normal four trichobothria on the movable chelal finger, as well as some with only two or three trichobothria. Similarly, Mahnert (1988) found that the type series of *Paraserianus bolivianus* Beier 1939 possessed three or four trichobothria on the movable chelal finger. Given that the main feature used to substantiate the genus *Paraserianus* by Beier (1939b) was the presence of only three such trichobothria (as opposed to four in *Serianus*), Mahnert (1988) placed *Paraserianus* as a synonym of *Serianus*.
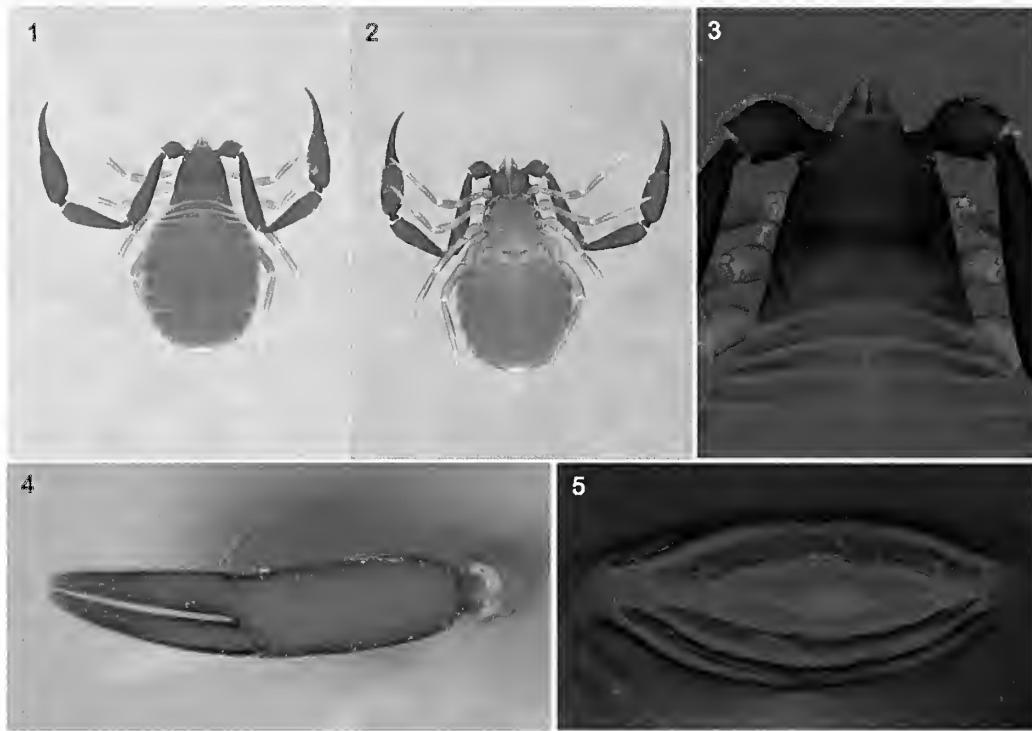
Comparison of specimens of many species of *Larca* and *Archeolarca* by one of us (M.S.H.), including the type species of both genera, has revealed no other significant differences that could be considered to maintain distinct genera, and *Archeolarca* is here regarded as a synonym of *Larca*, resulting in the following new combinations: *L. aalbui* (Muchmore 1984), **comb. nov.**, *L. cavicola* (Muchmore 1981), **comb. nov.**, *L. guadalupensis* (Muchmore 1981) **comb. nov.** and *L. welbourni* (Muchmore 1981) **comb. nov.**

Larca cavicola (Muchmore) comb. nov.
(Figs. 1–14)

Archeolarca cavicola Muchmore 1981: 55–56, Figs. 11, 12.

Material examined.—U.S.A.: *Arizona*: Mohave County: 1 male, PARA-3503 Cave, Grand Canyon-Parashant National Monument, ca. UTM 0247400 N, 4020000 E, Zone 12S, baited pitfall trap 1A, 20 May 2008, J.J. Wynne (MNA); 1 female, same data except baited pitfall trap 1C, 6 March 2009, J.J Wynne (MNA); 1 tritonymph, 1 deutonymph, same data except trap 2B, 10 March 2009, J.J. Wynne (MNA); 1 tritonymph, same data except trap 7A (MNA); 1 tritonymph, same data except opportunistic collecting in a possible deep zone (MNA); 1 male, PARA-2204 Cave, Grand Canyon-Parashant National Monument, ca. UTM 025100 N, 4041000 E, Zone 12S, M, baited pitfall trap 2B, 17 May 2008, J.J. Wynne (MNA); 1 female, same data except 20 May 2008 (MNA); 1 tritonymph, same data except trap 1A (MNA); 1 male, same data except trap 1B (MNA).

Diagnosis.—*Larca cavicola* resembles the other species previously included in the genus *Archeolarca* in possessing four trichobothria on the movable chelal finger, but occasionally this is reduced to three trichobothria. It differs from these species by having reduced eyes, especially the posterior pair, which are noticeably smaller than the anterior pair.

Figures 1–5.—*Larca cavicola* (Muchmore), male from PARA-2004 Cave: 1. Body, dorsal; 2. Body, ventral; 3. Carapace, dorsal; 4. Left chela, lateral; 5. Anal region, posterior.

Description.—*Adults:* Color: carapace, pedipalps and coxae deep red-brown, abdomen pale red-brown and legs pale yellow-brown.

Chelicera: with 4 setae on hand, with *sbs* absent, and 1 subdistal seta on movable finger (Fig. 7); all setae acuminate; seta *bs* slightly shorter than others; with 2 dorsal lyrifissures and 1 ventral lyrifissure; galea of ♂ and ♀ very long with 3 terminal rami, rami of male smaller than on female; rallum of 4 blades, the most distal blade with several serrations on leading edge, other blades smooth; serrula exterior with 14 (♂), 16 (♀) blades; lamina exterior present.

Pedipalp (Fig. 9): most surfaces of trochanter, femur, patella and chelal hand lightly and sparsely granulate, chelal fingers smooth; trochanter, femur, patella and chelal hand with prominent, curved, slightly denticulate setae arranged sparsely; patella with 3 small sub-basal lyrifissures; trochanter 1.83–1.99 (♂), 1.90–1.93 (♀), femur 4.74–5.94 (♂), 4.57–4.95 (♀), patella 3.63–4.47 (♂), 3.69–3.94 (♀), chela (with pedicel) 4.47–5.28 (♂), 4.08–4.54 (♀), chela (without pedicel) 4.22–5.02 (♂), 3.85–4.26 (♀), hand (with pedicel) 2.17–2.49 (♂), 1.94–2.08 (♀) × longer than broad, movable finger (with pedicel) 0.96–1.01 (♂), 0.99–1.00 (♀) × longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 12), although *sb* absent from left chela of one male (Fig. 11): *eb, esb, ib* and *ist* situated subbasally, *est, isb* and *it* submedially, *et* subdistally, and *est* opposite *it*; *b* and *sb* situated subbasally, and *st* and *t* situated submedially, with *st* situated very close to *t*; patch of microsetae not present on external margin of fixed chelal finger near *et*. Venom apparatus present in both chelal fingers, venom ducts fairly short, terminating in nodus ramosus slightly distal to *et* in fixed finger (Figs. 11, 12). Chelal teeth pointed, slightly
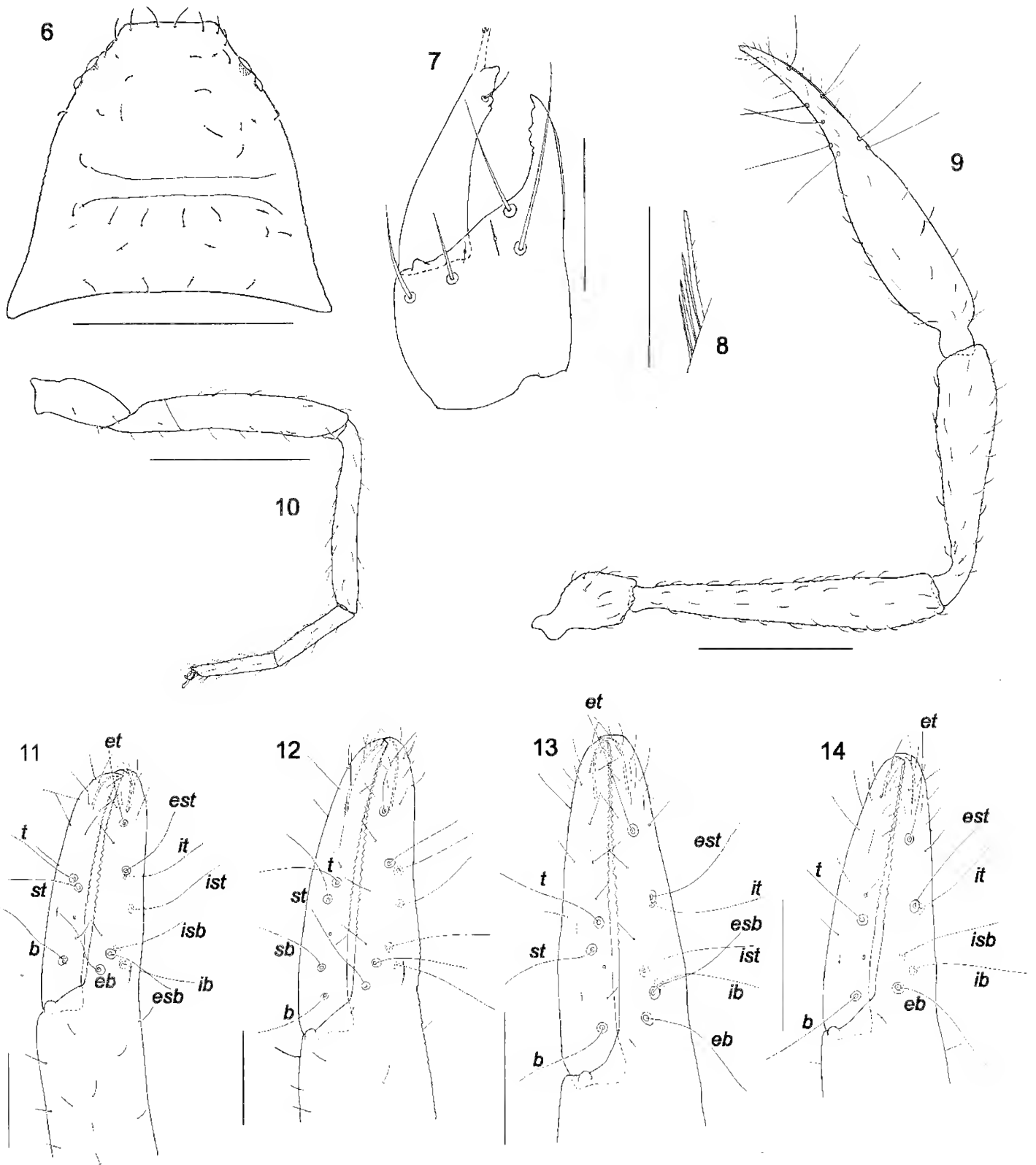
retrorse, becoming rounded basally; fixed finger with 32 (♂, ♀) teeth; movable finger with 32 (♂), 33 (♀) teeth; accessory teeth absent.

Carapace (Figs. 3, 6): 0.77–0.86 (♂), 0.74 (♀) × longer than broad; anterior margin straight; with 2 pairs of rounded corneate eyes, tapetum present; with 31 (♂), 32 (♀) setae, arranged with 4 (♂, ♀) near anterior margin and 4 (♂, ♀) near posterior margin; with 1 deep, broad median furrow.

Coxal region: manducatory process rounded with 1 small sub-oral seta, and 9 (♂), 12 (♀) additional setae; median maxillary lyrifissure large, rounded and situated submedially; posterior maxillary lyrifissure rounded. Coxae I to IV becoming progressively wider. Chaetotaxy of coxae I–IV: ♂, 6: 6: 6: 14; ♀, 6: 7: 9: 16.

Legs: femora I and II longer than patellae; junction between femora and patellae III and IV very angulate; femora III and IV much smaller than patellae III and IV; femur + patella of leg IV 5.92 (♂), 5.27 (♀) × longer than broad (Fig. 10); metatarsi and tarsi not fused; tarsus IV without tactile seta; subterminal tarsal setae arcuate and acuminate; claws simple; arolium much longer than claws, not divided.

Abdomen: tergites II–X and sternites IV–VIII of male and female with medial suture line fully dividing each sclerite, sternite IX partially divided. Tergal chaetotaxy: ♂, 4: 6: 10: 10: 11: 12: 11: 10: 10: 6 (arranged T4T): 7: 2; ♀, 6: 5: 7: 9: 10: 11: 11: 13: 9: 6 (arranged T4T): 8: 2; tergites I–X uniseriate. Sternal chaetotaxy: ♂, 19: (0) 19 [3 + 3] (0): (0) 6 (0): 7: 9: 7: 8: 8: 6: 3: 2; ♀, 14: (0) 8 (0): (0) 4 (0): 6: 7: 6: 8: 9: 6: 4: 2; sternites IV–X uniseriate; ♂ and ♀ sternite II and III with all setae situated near posterior margin. Spiracles with helix. Anal plates (tergite XII and sternite XII) situated between tergite XI and sternite XI, and surrounded by desclerotized region of

Figures 6–14.—*Larca cavicola* (Muchmore), specimens from PARA-3503 Cave: 6. Carapace, dorsal, male; 7. Chelicera, dorsal, male; 8. Rallum, lateral, male; 9. Right pedipalp, dorsal, male; 10. Left leg IV, male; 11. Left chela, lateral, male; 12. Left chela, lateral, female; 13. Left chela, tritonymph; 14. Left chela, deutonymph. Scale lines = 0.1 mm (Figs. 7, 8), 0.2 mm (Figs. 11–14), 0.5 mm (Figs. 6, 9, 10).

tergite XI and sternite XI; sternite XI with ca. 18 (♂), 24 (♀) small lyrifissures. Pleural membrane finely wrinkled-plicate; without any setae.

Genitalia: male: very similar to that described for *L. laceyi* Muchmore, 1981 by Muchmore (1981). Female with 1 pair of lateral cribriform plates and 2 pairs of median cribriform plates; spermathecae absent.

Dimensions: male (PARA-3503 Cave) followed by other males (where applicable): Body length 2.40 (2.14–2.42). Pedipalps: trochanter 0.371/0.186 (0.351–0.387/0.192–0.207), femur 1.021/0.172 (0.923–0.976/0.187–0.206), patella 0.859/0.192 (0.768–0.832/0.200–0.229), chela (with pedicel) 1.220/0.231 (1.173–1.286/0.262–0.272), chela (without pedicel) 1.160 (1.106–1.216), hand length 0.576 (0.569–0.622), movable finger length 0.582 (0.547–0.595). Chelicera 0.200/0.115, movable finger length 0.130. Carapace 0.605/0.784 (0.621–0.656/0.763–0.772); anterior eye diameter 0.059, posterior eye diameter 0.043. Leg I: femur 0.382/0.090, patella 0.249/0.092, tibia 0.350/0.067, metatarsus 0.252/0.042, tarsus 0.218/0.042. Leg IV: femur + patella 0.740/0.125, tibia 0.605/0.079, metatarsus 0.285/0.055, tarsus 0.270/0.048.

Female (PARA-3503 Cave) followed by other female (where applicable): Body length 2.85 (2.72). Pedipalps: trochanter 0.422/0.219 (0.408/0.215), femur 1.108/0.224 (0.978/0.214), patella 0.992/0.252 (0.822/0.223), chela (with pedicel) 1.394/0.307 (1.304/0.320), chela (without pedicel) 1.309 (1.232), hand length 0.640 (0.621), movable finger length 0.643 (0.616). Chelicera 0.240/0.131, movable finger length 0.150. Carapace 0.708/0.960); anterior eye diameter 0.049, posterior eye diameter 0.048. Leg I: femur 0.410/0.103, patella 0.289/0.117, tibia 0.382/0.075, metatarsus 0.261/0.059, tarsus 0.237/0.048. Leg IV: femur + patella 0.828/0.157, tibia 0.660/0.095, metatarsus 0.300/0.067, tarsus 0.282/0.058.

Tritonymph: Color: carapace, pedipalps and coxae red-brown, abdomen pale red-brown and legs pale yellow-brown.

Chelicera: with 4 setae on hand and 1 on movable finger; galea long and slender with 3 terminal rami.

Pedipalp: trochanter 1.97, femur 5.05, patella 3.90, chela (with pedicel) 4.58, chela (without pedicel) 4.32, hand (without pedicel) 2.17 × longer than broad, and movable finger 1.02 × longer than hand (without pedicel). Fixed chelal finger with 7 trichobothria, movable chelal finger with 3 trichobothria (Fig. 13): *eb, esb, ist* and *ib* situated basally; *est* and *it* medially; *et* distally, *isb* absent; *b* subbasally, *st* and *t* submedially, *sb* absent. Fixed chelal finger with 26 teeth; movable finger with 22 teeth.

Carapace: 0.85 × longer than broad; with 2 pairs of small rounded corneate eyes; with 4 setae near anterior margin and 3 near posterior margin; with deep median furrow.

Legs: much as in adults.

Abdomen: tergal chaetotaxy: 4: 4: 6: 7: 8: 7: 8: 6: 6: 6 (arranged T4T): 7: 2. Sternal chaetotaxy: 2: (0) 7 (0): (0) 3 (0): 4: 4: 4: 5: 6: 4: 2: 2.

Dimensions (mm) (PARA-3503 Cave): Body length 1.75. Pedipalps: trochanter 0.314/0.159, femur 0.768/0.152, patella 0.643/0.165, chela (with pedicel) 1.040/0.227, chela (without pedicel) 0.981, hand length 0.493, movable finger length 0.501. Carapace 0.544/0.640.

Deutonymph: Color: carapace, pedipalps and coxae pale red-brown, abdomen and legs pale yellow-brown.

Chelicera: with 4 setae on hand and 1 on movable finger; galea long and slender with 3 terminal rami.

Pedipalp: trochanter 2.11, femur 5.16, patella 3.50, chela (with pedicel) 4.19, chela (without pedicel) 3.94, hand (without pedicel) 2.02 × longer than broad, and movable finger 0.97 × longer than hand (without pedicel). Fixed chelal finger with 6 trichobothria, movable chelal finger with 2 trichobothria (Fig. 14): *eb, ist* and *ib* situated basally; *est* and *it* medially; *et* distally; *it* subdistally, *esb* and *isb* absent; *b* subbasally, *t* submedially, *sb* and *st* absent. Fixed chelal finger with 24 teeth; movable finger with 21 teeth.

Carapace: 0.82 × longer than broad; with 2 pairs of small rounded corneate eyes; with 4 setae near anterior margin and 4 near posterior margin; with deep median furrow.

Legs: much as in adults.

Abdomen: tergal chaetotaxy: 4: 4: 4: 6: 6: 6: 6: 6: 6: 6 (arranged T4T): 4: 2. Sternal chaetotaxy: 0: (0) 2 (0): (0) 2 (0): 3: 2: 4: 4: 4: 4: 4: 2.

Dimensions (mm) (PARA-3503 Cave): Body length 1.49. Pedipalps: trochanter 0.278/0.132, femur 0.629/0.122, patella 0.514/0.147, chela (with pedicel) 0.850/0.203, chela (without pedicel) 0.800, hand length 0.410, movable finger length 0.397. Carapace 0.490/0.600.

Remarks.—*Larca cavicola* was described from a single female collected in Cave of the Domes, Grand Canyon National Park, Coconino County, Arizona (Muchmore 1981). The new specimens were taken from two different caves within the Parashant, PARA-3503 Cave and PARA-2204 Cave, expanding the known range of this species some 160 km west of the type locality. Specimens from both cave localities have shorter and slightly thinner pedipalpal segments than the female holotype. In addition, the PARA-3503 Cave specimens have slightly longer and thinner pedipalps than those from PARA-2204 Cave. There do not appear to be any other morphological features that would warrant the recognition of more than one species amongst these specimens which are all here attributed to *L. cavicola*. As noted by Muchmore (1981), this species shows some obvious troglomorphic features consistent with an obligate subterranean existence including long, slender pedipalps and legs, reduced posterior eyes, and fewer setae on the carapace. Given the findings of both Muchmore (1981) and the present study, we consider this species to be troglobitic. A useful measure of troglomorphic adaptation in larcid pseudoscorpions was proposed by Gardini (1983), who found that the ratio pedipalpal femur length/carapace length was lower in epigean species of *Larca* than in cavernicolous species. This pattern was also observed in two new Spanish species of *Larca* (Zaragoza 2005). A similar condition is found in the species formerly described in *Archeolarca*. The epigean *L. rotunda* has a low ratio of 1.20 (male), 1.36 (female) (Hoff & Clawson 1952), whereas the cavernicolous species generally have higher ratios: *L. aalbui* 1.57 (male), *L. cavicola* 1.44 (female), *L. guadalupensis* 1.34 (female) and *L. welbourni* 1.47 (female) (Muchmore 1981, 1984). The ratios of the new specimens of *L. cavicola* recorded here [1.69 (male), 1.56 (female)] are higher than the female holotype, but we ascribe this to individual variation.

Two of the three post-embryonic nymphal stages (deutonymph and tritonymph) are present in the samples, and they

exhibit the same trichobothrial pattern as illustrated for *L. aalbui* (under the name *Archeolarca aalbui*) by Harvey (1992).

Family Chernetidae Menge 1855
Subfamily Chernetinae Menge 1855
Hesperochernes Chamberlin 1924

Hesperochernes Chamberlin 1924:89–90.

Type species.—*Hesperochernes laurae* Chamberlin 1924, by original designation.

Remarks.—The genus *Hesperochernes* currently comprises 19 North American species, ranging as far south as the Dominican Republic and Mexico (e.g., Ellingsen 1910; Chamberlin 1924; Beier 1933, 1976) and as far north as Canada (Hoff 1945), and a single Japanese species (Sato 1983). Muchmore (1974) provided details on how to separate *Hesperochernes* from the morphologically similar genera *Chernes* Menge 1855 and *Dinocheirus* Chamberlin 1929, but admitted that the composition of the genus was not fully resolved due to uncertainties in the morphology of several species. *Hesperochernes* is currently diagnosed by the following combination of characters: rallum composed of 4 blades; tarsus III and IV without conspicuous tactile seta; setae of pedipalps and tergites not large and leaf-like; female spermathecae with long paired ducts terminating in rounded sacs; and cheliceral setae *bs* and *sbs* usually dentate or denticulate. Of these characters, Muchmore (1974) was only able to nominate the spermathecal morphology and the denticulate *bs* and *sbs* as features that distinguish it from *Chernes*. It appears, however, that some species currently assigned to *Hesperochernes* have an acuminate *bs*, including *H. canadensis*, *H. holsingeri*, *H. molestus*, *H. montanus*, *H. occidentalis* and *H. riograndensis* (Chamberlin 1935; Hoff 1945; Hoff & Clawson 1952; Hoff 1956b; Hoff & Bolsterli 1956; Muchmore 1994). Moreover, the new species described below clearly demonstrates the labile nature of this feature, with the male having a strongly denticulate *bs* on both chelicerae, but the two females having an acuminate *bs*. It would seem that this feature should be used with considerable caution, and that the nature of the spermathecae is the only feature that can be reliably used to separate *Hesperochernes* from *Chernes*.

Although Muchmore (1974) was able to confirm the generic placement of several species from the U.S.A. and Canada [*H. laurae*, *H. minuhus* Chamberlin 1952, *H. mirabilis* (Banks 1895), *H. molestus* Hoff 1956, *H. occidentalis* (Hoff and Bolsterli 1956). *H. riograndensis* Hoff and Clawson 1952, *H. tamiae* Beier 1930, and *H. utahensis* Hoff and Clawson 1952], he was not able to ascertain whether others were correctly placed [*H. canadensis* Hoff 1945, *H. montanus* Chamberlin 1935, *H. pallipes* (Banks 1893), *H. paludis* (Moles 1914), *H. thomomysi* Hoff 1948, and *H. unicolor* (Banks 1908)]. The same can be said of the Central American and Asian species currently included in *Hesperochernes*, *H. globosus* (Ellingsen 1910), *H. tumidus* Beier 1933 and *H. inusitatus* Hoff 1946 from Mexico, *H. vespertilionis* Beier 1976 from Dominican Republic, and *H. shinjoensis* Sato 1983 from Japan, as the morphology of the spermathecae has not yet been ascertained (Ellingsen 1910; Beier 1933; Hoff 1946a; Beier 1976; Sato 1983).

Species of *Hesperochernes* are frequently collected in caves or are associated with other animals. The cave-dwelling species include three eyeless species that have long slender pedipalps consistent with strong troglomorphisms, *H. holsingeri*, *H. mirabilis* and *H. occidentalis*, as well as the new eyeless species described below that has long legs but has robust pedipalps. The species associated with rodents include *H. minuhus*, *H. molestus*, *H. riograndensis*, *H. tamiae*, *H. thomomysi* and *H. utahensis* (Beier 1930; Hoff 1945, 1946b; Chamberlin 1952; Hoff & Clawson 1952; Hoff 1956b), while *H. vespertilionis* was collected within a bat roost (Beier 1976). *Hesperochernes laurae* and *H. unicolor* were found within both wasp's and ant's nests (Banks 1908; Chamberlin 1924; Hoff 1947), respectively, *H. montanus* was found in a bird's nest (Chamberlin 1935), and *H. tumidus* was collected "lying on the ground in pods of *Inga* sp." (translated from the original German) (Beier 1933). The poorly described and most likely misplaced *H. paludis* was taken from both rotten poplar tree logs on the ground and live standing poplar trees (Moles 1914), and the only species recorded from outside of North America, *H. shinjoensis* from northern Japan, was collected from under tree bark (Sato 1983). The other species lack any habitat data.

Hesperochernes bradybaughi sp. nov.

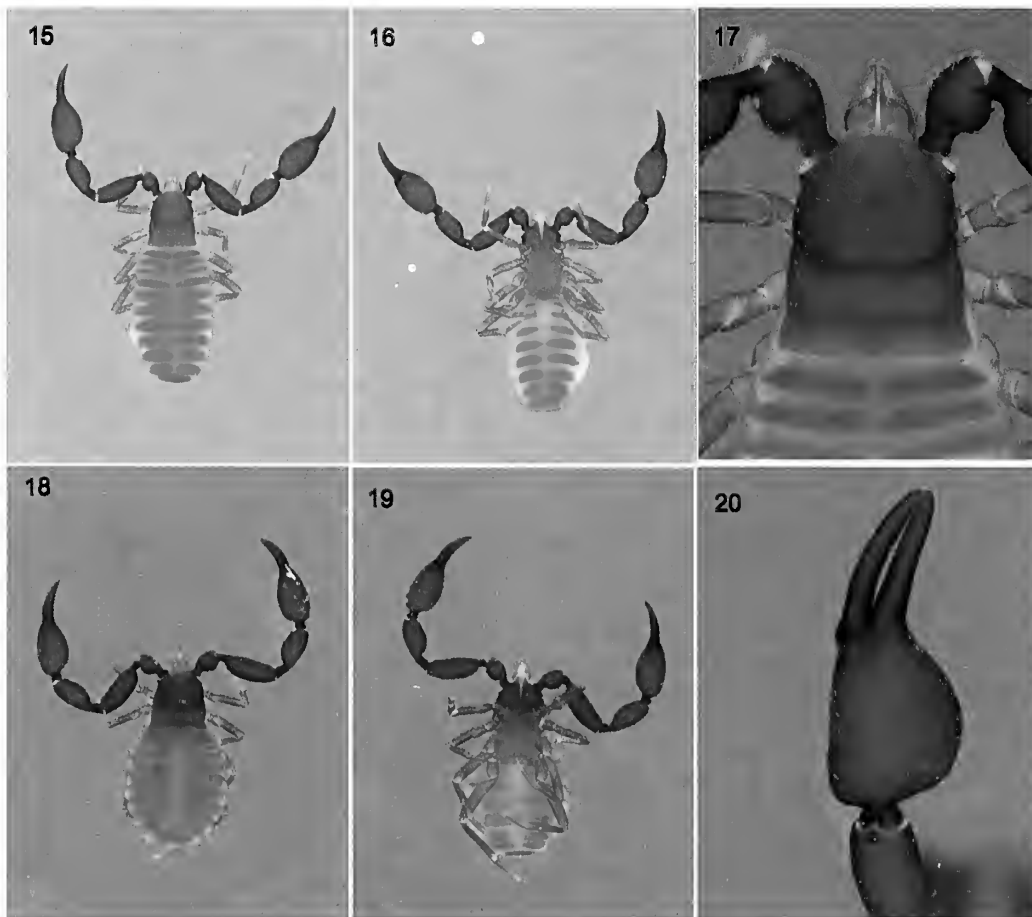urn:lsid:zoobank.org:act:5419D319-EF22-4722-926F-1F8EC 080400B

Figs. 15–26

Material examined.—*Types*. U.S.A.: *Arizona*: Mohave County: holotype male, PARA-1001 Cave, Grand Canyon-Parashant National Monument, ca. UTM 0264500 N, 4060700 E, Zone 12S, baited pitfall trap 3B, 20 August 2007, J.J. Wynne (MNA); 1 female, same data as holotype except baited pitfall trap 5A (MNA); 1 female, same data as holotype except opportunistic, mid cave, 13 August 2005 (NMA).

Etymology.—This species is named for Jeff Bradybaugh, former superintendent of Grand Canyon-Parashant National Monument and an advocate for cave research, conservation and management both on Parashant and within the National Park Service.

Diagnosis.—*Hesperochernes bradybaughi* most closely resembles three other species of the genus that are also completely eyeless and have long slender legs [e.g. femur + patella IV 5.19 (male), 5.37–5.56 (female) × longer than broad], *H. mirabilis*, *H. holsingeri* and *H. riograndensis*. *Hesperochernes bradybaughi* lacks the slender pedipalps characteristic of *H. mirabilis* and *H. holsingeri*, and the male chela of *H. bradybaughi* is markedly swollen, especially on the dorsal face (Fig. 21), unlike the male of *H. riograndensis* which is not swollen. It is also substantially larger than *H. riograndensis*, e.g., chela (without pedicel) of *H. riograndensis* is 0.956 (male), 0.970 (female) mm, whereas *H. bradybaughi* is 1.434 (male), 1.502–1.510 (female) mm.

Description.—*Adults:* Color: pedipalps and carapace dark red-brown, legs light red-brown, tergites yellow-brown, sternites pale yellow-brown.

Chelicera: with 5 setae on hand and 1 subdistal seta on movable finger (Fig. 23); setae *ls* and *is* acuminate, *es* and *bs* dentate, *sbs* denticulate in female, acuminate in male; with 2 dorsal lyrifissures and 1 ventral lyrifissure; galea of ♂ and ♀ with 6 rami; rallum of 4 blades, the 2 distal blades with several

Figures 15–20.—*Hesperochernes bradybaughi*, sp. nov.: 15. Body, dorsal, male holotype; 16. Body, ventral, male holotype; 17. Carapace, dorsal, male holotype; 18. Body, dorsal, female paratype; 19. Body, ventral, female paratype; 20. Left chela, lateral, male holotype.

serrations on leading edge, other blades smooth; serrula exterior with 18 (♂), 17 (♀) blades; lamina exterior present.
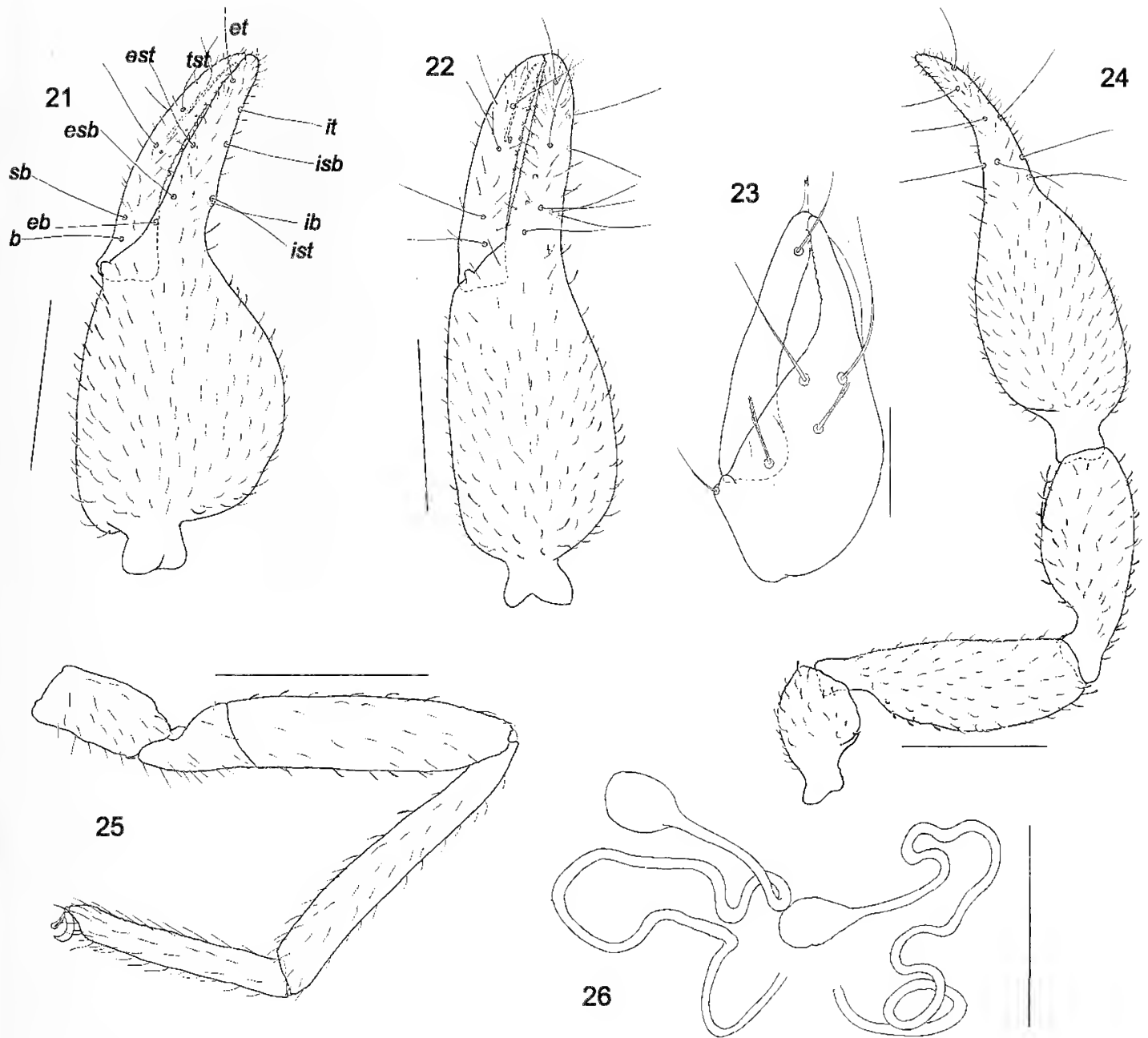
Pedipalp (Fig. 24): surfaces of trochanter, femur, patella and chelal hand coarsely granulate, chela fingers mostly smooth; patella with 5 small sub-basal lyrifissures; trochanter 1.84 (♂), 1.86–1.88 (♀), femur 3.17 (♂), 2.95–3.09 (♀), patella 2.62 (♂), 2.54–2.66 (♀), chela (with pedicel) 3.07 (♂), 3.23–3.34 (♀), chela (without pedicel) 2.83 (♂), 2.98–3.09 (♀), hand 1.49 (♂), 1.34–1.64 (♀) × longer than broad, movable finger 0.93 (♂), 0.86–0.96 (♀) × longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Figs. 21, 22): *eb* and *esb* situated basally, *ib* and *ist* subbasally, *est* and *isb* submedially, *et* and *it* subdistally, *isb* situated midway between *ist* and *it*, and *et* slightly distal to *it*; *t* situated subdistally, *st* situated closer to *t* than to *sb*. Venom apparatus only present in movable chelal finger, venom ducts long, terminating in nodus ramosus distal to *st* (Figs. 21, 22). Fixed finger with 2 large sensillae on retrolateral face, and 2 on prolateral face; movable chelal finger with sensilla slightly proximal to *sb* in male and slightly distal to *sb* in female, with 2 receptors. Chela of male without mound. Chelal teeth pointed and slightly retrorse, basal teeth more rounded; fixed finger with 44 (♂), 48 (♀) teeth, plus 11 (♂), 9 (♀) retrolateral and 10 (♂), 7 (♀) prolateral accessory teeth; movable finger with 46 (♂), 50 (♀) teeth, plus 9 (♂, ♀) retrolateral and 6 (♂), 4 (♀) prolateral accessory teeth.

Carapace (Fig. 17): coarsely granulate, 1.15 (♂), 0.98–1.10 (♀) × longer than broad; without eyes or eyespots; with 100 (♂), 83 (♀) setae, arranged with 61 (♂), 42 (♀) (including 6 near anterior margin) in anterior zone, 25 (♂), 34 (♀) in median zone, and 14 (♂), 17 (♀) in posterior zone; with 2 deep furrows, posterior furrow situated slightly closer to posterior carapace margin than to anterior furrow.

Coxal region: maxillae granulate; manducatory process somewhat acute, with 2 apical acuminate setae, 1 small sub-oral seta and 37 (♂), 32 (♀) additional setae; median maxillary lyrifissure rounded and situated submedially; posterior maxillary lyrifissure rounded. Leg coxae smooth; chaetotaxy of coxae I–IV: ♂, 18: 19: 23: ca. 60; ♀, 18: 21: 25: ca. 65.

Legs: very slender; junction between femora and patellae I and II strongly oblique to long axis; junction between femora and patellae III and IV very angulate; femora III and IV much smaller than patellae III and IV; femur + patella of leg IV 5.19 (♂), 5.37–5.56 (♀) × longer than broad; all tarsi with slit sensillum on raised mound; male leg I not modified; tarsi III and IV without tactile seta, but with paired subdistal setae; subterminal tarsal setae arcuate and acute; claws simple; arolium about same length as claws, not divided.

Abdomen: tergites I–X and sternites IV–X of male and female with median suture line fully dividing each segment. Tergal chaetotaxy: ♂, 11: 12: 11: 18: 19: 18: 20: 18: 17: 18: 13: 2; ♀, 12: 13: 13: 17: 17: 18: 19: 19: 21: 16: 14: 2; uniseriate, except

Figures 21–26.—*Hesperochernes bradybaughi*, sp. nov.: 21. Left chela, lateral, male holotype; 22. Left chela, lateral, female paratype; 23. Chelicera, dorsal, female paratype; 24. Right pedipalp, dorsal, male holotype; 25. Left leg IV, male holotype; 26. Spermathecae, female paratype. Scale lines = 0.1 mm (Fig. 23), 0.2 mm (Fig. 26), 0.5 mm (Figs. 21, 22, 24, 25).

for medial and lateral discal seta on tergites IV–IX; setae thickened and strongly dentate. Sternal chaetotaxy: ♂, 30: (3) 22 [2 + 2] (3): (1) 8 (1): 19: 21: 21: 20: 20: 16: 8 (arranged T6T): 2; ♀, ca. 40: (3) 10 (3): (1) 5 (1): 14: 21: 20: 20: 18: 16: 11 (arranged T9T): 2; uniseriate, except for lateral discal seta on sternites VII–X; setae of anterior sternites acicular, becoming progressively more denticulate on posterior sternites. Spiracles with helix. Anal plates (tergite XII and sternite XII) situated between tergite XI and sternite XI, anal setae not denticulate. Pleural membrane wrinkled and somewhat stellate; without any setae.

Genitalia: male of the chernetid type. Female (Fig. 26): with a pair of long thin-walled spermathecae terminating in rounded sacs.

Dimensions: Male holotype: Body length 3.11. Pedipalps: trochanter 0.518/0.282, femur 974/0.307, patella 0.824/0.314, chela (with pedicel) 1.552/0.506, chela (without pedicel) 1.434, hand length 0.756, movable finger length 0.704. Chelicera 0.322/0.165, movable finger length 0.252. Carapace 0.956/0.830. Leg I: femur 0.268/0.161, patella 0.495/0.136, tibia 0.503/0.102, tarsus 0.495/0.079. Leg IV: femur + patella 0.883/0.170, tibia 0.778/0.107, tarsus 0.557/0.085.

Female (paratype lodged in MNA) followed by other female (where applicable): Body length 2.82 (4.21). Pedipalps: trochanter 0.552/0.294 (0.566/0.304), femur 1.034/0.335 (1.042/0.353), patella 0.904/0.340 (0.942/0.371), chela (with pedicel) 1.624/0.486 (1.635/0.506), chela (without pedicel) 1.502

(1.510), hand length 0.797 (0.698), movable finger length 0.768 (0.816). Chelicera 0.327/0.152, movable finger length 0.244. Carapace 1.040/0.944 (1.000/1.021). Leg I: femur 0.300/0.182, patella 0.540/0.146, tibia 0.56/0.108, tarsus 0.520/0.079. Leg IV: femur + patella 1.010/0.188 (1.000/0.180), tibia 0.830/0.121, tarsus 0.580/0.084.

Remarks.—As stated in the diagnosis, *H. bradybaughi* appears to be most similar to *H. riograndensis* but differs in being substantially larger and with a markedly swollen male chela, especially on the dorsal face. The only known location of *H. riograndensis* is located 670 km ESE of Parashant, and the microhabitat of both species differs with *H. bradybaughi* being found in a cave and *H. riograndensis* collected from the nest of a kangaroo rat (Heteromyidae: *Dipodomys*) (Hoff & Clawson 1952). Given the lack of eyes and eyespots, we consider *H. bradybaughi* to be a troglobite.

Tuberochernes Muchmore

Tuberochernes Muchmore 1997:206–207.

Type species.—*Tuberochernes aalbui* Muchmore 1997, by original designation.

Diagnosis.—*Tuberochernes* differs from all other chernetid genera by the combined presence of a distinct medium-sized mound on the prolateral face of the pedipalpal chela of males, and four blades in the cheliceral rallum.

Remarks.—The genus *Tuberochernes* was described by Muchmore (1997) for two species of cave-dwelling pseudo-scorpions from southwestern U.S.A., *T. aalbui* and *T. ubicki*, but the discovery of a third species, also from a cave in southwestern U.S.A., does not necessitate an alteration of the original description apart from the nature of the tactile seta of leg IV. Muchmore (1997) observed that the tactile seta of leg IV was "short, distally located" and "variably acuminate or finely denticulate". Close examination of the posterior tarsi of the new species described below does not reveal a tactile seta of this nature, and we suggest this feature appears to be variable within the genus.

The most obvious feature that distinguishes *Tuberochernes* is the presence of a medium-sized mound on the prolateral margin of the chelal hand in males (Muchmore 1997). In this respect, it resembles several other chernetid genera, including males of *Mirochernes* Beier 1930 and *Bituberochernes* Muchmore 1974, and both males and females of *Interchernes* Muchmore 1980 and *Petterchernes* Heurtault 1986, which were distinguished from *Tuberochernes* by Muchmore (1997). *Bituberochernes* further differs from *Tuberochernes* by a mound being also present on the pedipalpal patella. The function of the mound has not been ascertained, but the mound of *T. cohni* has 5 small pores, which may be responsible for discharging fluids, possibly during sexual interactions with females.

Tuberochernes cohni sp. nov.

urn:lsid:zoobank.org:act:12896B35-DD1C-4E0B-B66F-F9B30170D476

Figs. 27–37

Material examined.—*Type:* U.S.A.: *Arizona*: Mohave County: holotype male, PARA-1001 Cave, Grand Canyon-Parashant National Monument, ca. UTM 0264500 N, 4060700 E, Zone 12S, the deeper extent of the twilight zone (near the dark zone), opportunistic collecting, 13 August 2005, J.J. Wynne (MNA).

Etymology.—This species is named for the late Dr. Theodore "Ted" Cohn. Cohn was an Orthopterist and the leading authority who identified the new genus of rhaphido-phorid cricket known from PARA-1001 Cave. Dr. Cohn passed away in November 2013 at age 82. He was a passionate educator and entomologist.

Diagnosis.—*Tuberochernes cohni* differs from the other two species of the genus, *T. aalbui* and *T. ubicki*, by the more anteriorly positioned mound on the pedipalpal chela.

Description.—*Adult male:* Color: pedipalps and carapace dark red-brown, legs light red-brown, tergites yellow-brown, sternites pale yellow-brown.
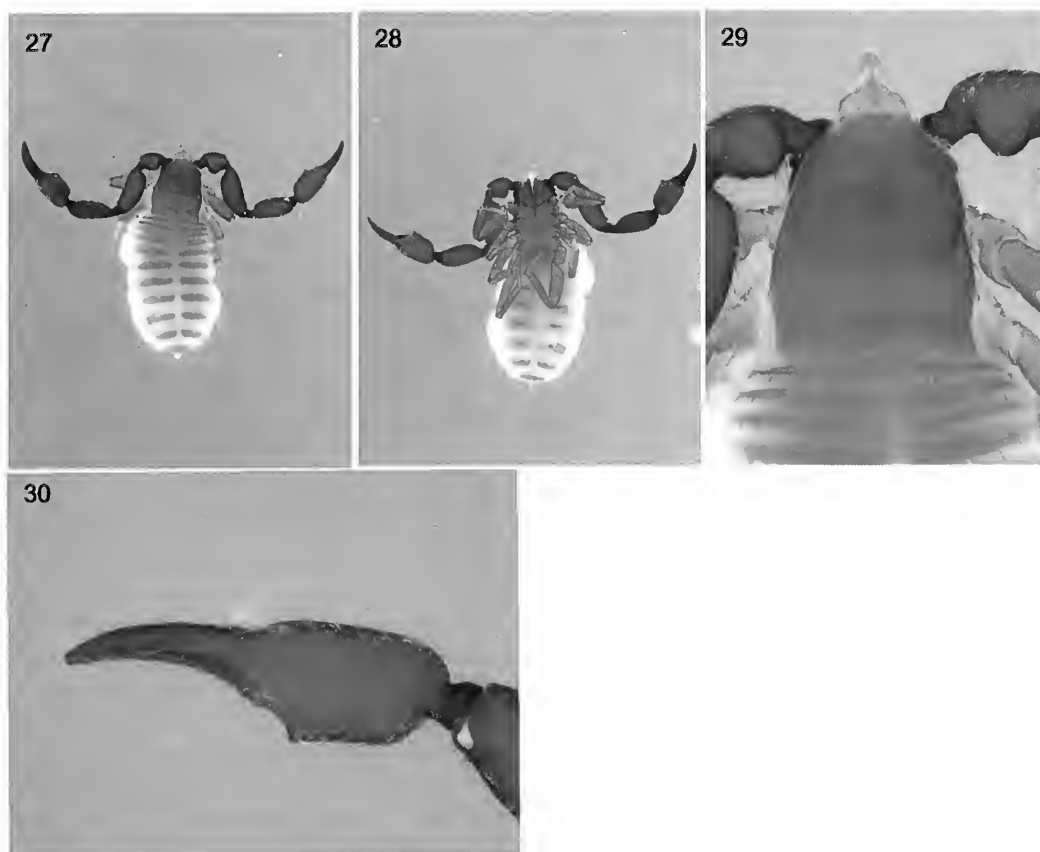
Chelicera: with 6 setae on hand and 1 subdistal seta on movable finger (Fig. 32); setae *es*, *sbs* and *bs* dentate, *ls* and *is* acuminate; with 2 dorsal lyrifissures and 1 ventral lyrifissure; galea broken; rallum of 4 blades, the most distal blade with several serrations on leading edge, other blades smooth; serrula exterior with 17 blades; lamina exterior present.

Pedipalp (Fig. 33): surfaces of trochanter, femur, patella and chelal hand coarsely granulate, chela fingers mostly smooth; patella with 5 small sub-basal lyrifissures; trochanter 1.73, femur 2.83, patella 2.88, chela (with pedicel) 3.39, chela (without pedicel) 3.11, hand 1.40 × longer than broad, movable finger 1.23 × longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 31): *eb* and *esb* situated basally, *ib* and *ist* subbasally, *est* and *isb* submedially, *et* and *it* subdistally, *isb* situated midway between *ist* and *it*, and *et* slightly distal to *it*; *t* situated subdistally, *st* situated much closer to *t* than to *sb*. Venom apparatus only present in movable chelal finger, venom ducts long, terminating in nodus ramosus midway at level of *st* (Fig. 31). Fixed finger with 3 sensillae on retrolateral face, and 1 on prolateral face; movable chelal finger with sensilla slightly distal to sb, with 2 receptors. Chela with prominent, medium-sized mound on prolateral face (Figs. 30, 34), with 5 small pores. Chelal teeth pointed and slightly retrorse, basal teeth more rounded; fixed finger with 37 teeth, plus 7 retrolateral and 3 prolateral accessory teeth; movable finger with 42 teeth, plus 4 retrolateral and 0 prolateral accessory teeth.

Carapace (Fig. 29): coarsely granulate, 1.19 × longer than broad; without eyes or eyespots; with 96 setae, arranged with 54 (including 6 near anterior margin) in anterior zone, 28 in median zone, and 14 in posterior zone; with 2 deep furrows, posterior furrow situated closer to posterior carapace margin than to anterior furrow.

Coxal region: maxillae granulate; manducatory process somewhat acute, with 2 apical acuminate setae, 1 small sub-oral seta and 25 additional setae; median maxillary lyrifissure rounded and situated submedially; posterior maxillary lyrifissure rounded. Leg coxae smooth; chaetotaxy of coxae I–IV: 13: 12: 14: 34.

Legs (Figs. 35–37): junction between femora and patellae I and II strongly oblique to long axis; junction between femora and patellae III and IV very angulate; femora III and IV much smaller than patellae III and IV; femur + patella of leg IV 4.03 × longer than broad; all tarsi with slit sensillum on raised

Figures 27–30.—*Tuberochernes cohni*, sp. nov., male holotype: 27. Body, dorsal; 28. Body, ventral; 29. Carapace, dorsal; 30. Right chela, dorsal.

mound; leg I modified with tibia thickened, tarsus slightly curved and ventral margins of patella and tibia with coarse granulation; tarsi III and IV without tactile seta, but with paired subdistal setae; subterminal tarsal setae arcuate and acute; claws simple; arolium about same length as claws, not divided.

Abdomen: tergites II–X and sternites V–X of with median suture line fully dividing each segment. Tergal chaetotaxy: 15: 20: 20: 20: 22: 22: 21: 21: 22: 17: 10: 2; uniseriate, except for medial and lateral discal seta on tergites IV–IX; setae thickened and strongly dentate. Sternal chaetotaxy: 51: (0) 8 [2 + 2] (0): (1) 8 (1): 12: 16: 17: 18: 17: 14: 8 (arranged T6T): 2; uniseriate, except for lateral discal seta on sternites IV–XI; setae of anterior sternites acicular, becoming progressively more denticulate on posterior sternites. Spiracles with helix. Anal plates (tergite XII and sternite XII) situated between tergite XI and sternite XI, anal setae denticulate. Pleural membrane longitudinally striate; without any setae.
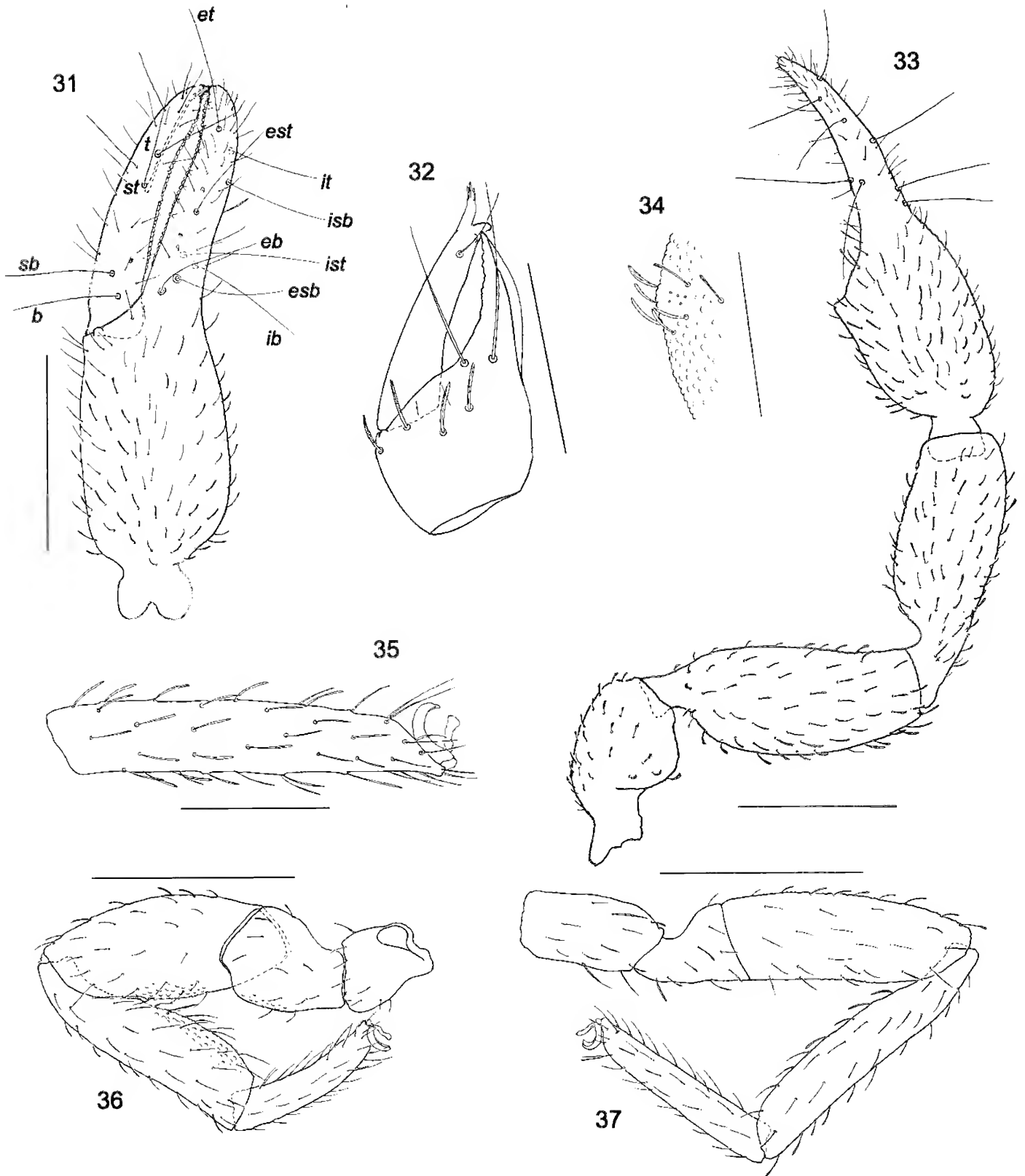
Genitalia: of the chernetid type.

Dimensions: male holotype: Body length 3.38. Pedipalps: trochanter 0.576/0.332, femur 0.944/0.334, patella 0.910/0.316, chela (with pedicel) 1.390/0.410, chela (without pedicel) 1.276, hand length 0.573, movable finger length 0.704. Chelicera 0.333/0.134, movable finger length 0.240. Carapace 1.009/0.848. Leg I: femur 0.305/0.249, patella 0.560/0.253, tibia 0.621/0.174, tarsus 0.442/0.089. Leg IV: femur + patella 0.859/0.213, tibia 0.692/0.134, tarsus 0.533/0.954.

Remarks.—*Tuberochernes cohni* possesses some very slight modifications consistent with troglomorphic adaptations of

which the most prominent is the complete lack of eyes (Fig. 29) and the slightly elongated leg segments. Thus, this animal is considered a troglobite. It appears to bear a closer resemblance to *T. ubicki* from a cave in the Santa Rita Mountains, Arizona (610 km), than to *T. aalbui* from a cave in the Inyo National Forest, California (415 km), due to the similarly expanded tibia I in males of the two Arizona species.

DISCUSSION

Our review of the pseudoscorpions detected within the caves of Grand Canyon-Parashant National Monument has revealed a modest fauna of three species: *Larca cavicola* (family Larcidae), *Hesperochernes bradybaughi* and *Tuberochernes cohni* (both in the family Chernetidae). All show modifications consistent with obligate existence in cave environments, but none show the classic signs of extreme troglomorphism found in many cave-adapted pseudoscorpions (e.g. Heurtault 1994; Harvey et al. 2000). Both species of Chernetidae lack eyes and have long slender legs, which appear to be troglomorphic modifications due to their subterranean existence, although their pedipalps do not appear to be modified compared to epigean species of the genus. Other subterranean species of *Hesperochernes* with thin legs and no eyespots—*H. holsingeri* from Indiana, *H. mirabilis* from Alabama, Georgia, Indiana, Kentucky, Ohio, Tennessee and Virginia, and *H. occidentalis* from Arkansas, Missouri, Oklahoma and Texas—appear to be more highly modified as they have elongate pedipalps. Both new species described from the Parashant may represent short-range endemic species as defined by Harvey (2002) and Harvey

Figures 31–37.—*Tuberochernes cohni*, sp. nov., male holotype: 31. Left chela, lateral; 32. Chelicera. dorsal; 33. Right pedipalp. dorsal; 34. Left chela. detail of mound, ventral; 35. Left tarsus IV; 36. Left I; 37. Left leg IV. Scale lines = 0.2 mm (Figs. 32, 34, 35), 0.5 mm (Figs. 31, 33, 36, 37).

et al. (2011) due to their highly restricted distributions. Although the junior author and colleagues sampled all known caves on Parashant, they detected these new species in only one cave (PARA-1001 Cave).

Larca cavicola appears to be less cave-adapted than the others, as it retains eyes. However, the pedipalps are noticeably thinner than epigean species of the genus, suggesting moderate morphological modifications to the cave environment. *Larca cavicola* was found in PARA-3503 and PARA-2204 Caves and has been found in Cave of the Domes, a small cave situated within Grand Canyon National Park, Coconino County (Muchmore 1981). Although this cave is also located in the Grand Canyon region, it lies on the south side of the Colorado River some 160 km from the Parashant caves, and we suggest these populations are genetically isolated from each other.

The only known locality of *Hesperochernes bradybaughi* and *Tuberochernes cohni* is PARA-1001. This is the second most biologically diverse cave, and the most biologically significant cave on the monument. It supports the largest known cricket roost in Arizona, which represents an undescribed genus of rhaphidophorid cave cricket, cf *Ceuthophilus* n. gen. n. sp., Cohn & Swanson, unpublished data; (Wynne & Voyles 2014). Its population contributes significantly to the nutrient loading via cricket guano, cricket eggs and nymphs, as well as deceased individuals at various life stages. In other regions, the ecological importance of crickets on cave ecosystems is well documented (e.g., Barr 1967; Howarth 1983; Taylor 2003; Culver 2005; Poulson 2005). Given the size of the roost, we suggest that cf *Ceuthophilus* n. gen. n. sp. represents a keystone species with the presence of this animal supporting at least four cave-adapted species including a short-range endemic and troglomorphic leiodid beetle, *Ptomaphagus parashant* (Peck & Wynne 2013), an undescribed species of troglomorphic centipede (family Anopsobiidae; Wynne, unpublished data), and the two pseudoscorpion species described here. To date, *P. parashant*, the anopsobiid centipede, and the two new pseudoscorpion species have been detected only in PARA-1001 Cave. Two other caves on the monument, with similar deep zone like conditions, were sampled using the same systematic sampling design are within a 9.7 km radius of PARA-1001; neither of these new pseudoscorpions species were detected at these caves.

Management Implications.—We recommend the same management strategies proposed by Peck & Wynne (2013) be maintained for PARA-1001 Cave. This cave should not be gated given its south-facing entrance and entrance structure, and it should remain closed to recreational use. PARA-1001 is considered the second most biologically diverse cave on the monument and supports the greatest diversity of troglomorphic arthropod species. Presently, all of these animals (including the two new pseudoscorpion species described here) are known to occur only within PARA-1001 Cave. Maintaining the management strategies suggested by Peck & Wynne (2013) should aid in the long-term persistence of these presumed short-range endemic arthropods.

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A new genus and a new species of scorpion (Scorpiones: Buthidae) from southeastern Mexico

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Abstract. *Chaneke fogoso* gen. nov. et sp. nov., are described based on specimens collected near the coast in southeastern Guerrero, Mexico. The genus is characterized by the peculiar rhomboidal shape of the subaculear tubercle, and the lack of at least one trichobothrium on the femur, patella and chela of the pedipalp, which make it the second known buthid genus with decreasing neobothriotaxy on those three pedipalpal segments, together with *Alayotityus* Armas 1973. *Tityopsis aliciae* Armas & Martin-Frias 1998, from Oaxaca, Mexico, is transferred to the new genus, resulting in *Chaneke aliciae* (Armas & Martin-Frias 1998), comb. nov. A cladistic analysis including all other New World "microbuthids" with decreasing neobothriotaxy, with 30 morphological characters, indicates that *Chaneke* is monophyletic, clearly distinct from *Alayotityus* Armas 1973 (from eastern Cuba) and *Tityopsis* Armas 1974 (from western Cuba).

Keywords: Decreasing neobothriotaxy, femur, patella, chela

The scorpion family Buthidae C. L. Koch 1837 contains approximately 90 genera (Ove-Rein 2014), approximately two-thirds of which have the β trichobothrial pattern on the pedipalp femur, and a third of which have the α trichobothrial pattern (Vachon 1975). In the New World, there are 11 buthid genera represented, one with the β pattern and the remaining 10 with the α pattern. Six of those genera are orthobothriotaxic: femur with 11 trichobothria ($=\tau$), patella with 13 τ, chela with 15 τ; and four genera have decreasing neobothriotaxy ($=$less than the "full" compliment noted above) on some or all of their species. *Alayotityus* Armas 1973 lacks femoral τ d_2 and patellar τ d_2; *Mesotityus* Gonzalez-Sponga 1981 lacks patellar τ d_2 and chela τ Eb_3; *Microtityus* Kjellesvig-Waering 1966 has variable femoral and chelal trichobothrial numbers, but the patella is always orthobothriotaxic (τ d_2 present); *Zabius* Thorell 1893 lacks femoral τ d_2 and chela τ esb, but its three species have patellar τ d_2 present, although reduced in size ($=$petite), and chela τ Eb_3 present.

The genus *Tityopsis* Armas 1974 has two species from western Cuba that are orthobothriotaxic, and a Mexican species that, although it was originally described as being orthobothriotaxic (Armas & Martín-Frías 1998), was recently redescribed and shown to be neobothriotaxic (Vidal-Acosta & Francke 2009). Another neobothriotaxic species was recently collected in the state of Guerrero, Mexico (Figs. 1, 2), which is undoubtedly congeneric with *Tityopsis aliciae* Armas & Martín-Frías 1998, from the state of Oaxaca (Fig. 3); these two Mexican species differ from *Tityopsis* in being neobothriotaxic. The objectives of this contribution are: (a) to analyze the phylogenetic relationships of the two neobothriotaxic Mexican species with other New World buthids which have an α trichobothrial pattern on the femur, (b) to describe a new genus for those two Mexican species, and (c) to describe the new species from Guerrero.

METHODS

Specimens.—The specimens used in this study are lodged in the following institutions: American Museum of Natural History, New York, USA (AMNH); Centro Oriental de Ecosistemas y Biodiversidad, Santiago de Cuba, Cuba (BIOECO); Colección Nacional de Arácnidos, Univ. Nacional Autónoma de México, México, D. F. (CNAN); Laboratorio de Entomología, Instituto de Diagnóstico y Referencia Epidemiológicos, Secretaria de Salud, México, D. F (IN-DRE); private collection Rolando O. Teruel, Cuba (ROT).

Specimens examined are listed in Appendix 1, including the first known male of *T. aliciae*. Nomenclature and mensuration for the most part follow Stahnke (1970), with the following exceptions: metasomal carinal terminology after Francke (1977), carinal terminology of pedipalp femur and patella after Acosta et al. (2008) and trichobothrial terminology after Vachon (1974, 1975). Observations, measurements and drawings were made using a Nikon SMZ800 stereomicroscope fitted with 10× ocular micrometer and camera lucida; photographs were made using a Nikon Coolpix S10 adapted to the same microscope.

Taxon sampling.—The cladistic analysis presented is based on 25 terminal taxa (Appendix 1). Trees were rooted using the out-group method (Watrous & Wheeler 1981; Farris 1982; Nixon & Carpenter 1993). The in-group includes all New World genera of the family Buthidae with non-imbricated rows of denticles on the pedipalp chela fingers and which lack supernumerary denticles along those rows. Three taxa which have supernumerary denticles are used as out-groups: *Rhopalurus junceus* (Herbst 1880); *Centruroides exilicauda* (Wood 1863), type species of the genus; and *Centruroides gracilis* (Latreille 1804), a rather divergent taxon from the type species of the genus. The tree was rooted with *Ananteris platnicki* Lourenço 1993, which is the New World genus of buthids with a femoral β trichobothrial pattern and thus distantly related.

Character matrix.—Character data were edited using WinClada, version 1.00.08 (Nixon 2002). The character matrix (Appendix 2) comprises 30 characters, eight coded into multistates and 22 coded into binary states. All characters (Appendix 3) are informative and are included in all the analyses and statistics. Multistate characters were treated as

Figure 1.—Habitat at type locality of *Chaneke fogoso* gen. nov. et sp. nov.

Figure 2.—Live habitus of *Chaneke fogoso* gen. nov. et sp. nov., dorsal view, paratype ♀ (CNAN).

unordered/non-additive (Fitch 1971), defended by invoking the principle of indifference, which asserts that if there is no apparent reason for considering one event to be more probable than its alternatives, then all should be considered equiprobable (Wilkinson 1992).

Cladistic analyses.—Analyses were conducted with parsimony and equal weighting or implied weighting with six values of the concavity constant (k) = 1, 3, 10, 30, 60 and 100, to assess the effect of weighting against homoplasious characters (as in Prendini et al. 2010). All analyses were conducted with TNT ver 1.1 (Goloboff et al. 2008), using a driven search combining three of the new technology algorithms (excluding ratchet) using a script file modified from Dimitrov et al. (2013) and Santibáñez-López et al. (in press): *hold 90000; rseed1; xm: noverb nokeep; rat: it 0 up 4 down 4 au 0 num 36 give 99 equa; dri: it 10 fit 1.00 rfi 0.20 aut 0 num 36 give 99 xfa 3.00 equa; sect:slack 20; sec: mins 45 maxs 45 self 43 incr 75 minf 10 god 75 drift 6 glob 5 dglob 10 rou 3 xss 10- 14+2 noxev noeq; tf: ron 5 minf 3 best ke nochoo swap; xm : level 10 nochk rep 50 fuse 3 dri 10 rss css noxss mult nodump conse 5 conf 75 nogive notarg upda autoc 3 xmix; xm; xmult:;.* The relative support for each node was calculated in TNT using 1000 Jackknife pseudoreplicates (for equal weighting) and symmetric resampling (for implied weighting) with heuristic searches, consisting of ten random addition sequences, followed by ten iterations of tree bisection-reconnection, retaining one tree at each iteration (Dimitrov et al. 2013), and

Bremer support (Bremer 1994), searching suboptimal trees up to six steps longer, retaining 1000 trees at each iteration. A preferred hypothesis was selected among the alternative topologies recovered by the analysis with equal weighting.

RESULTS

Cladistic analyses.—The analysis with equal weighting produced two most parsimonious trees (strict consensus tree shown in Fig. 4, Table 2). The monophyly of *Chaneke* gen. nov. was recovered by high jackknife and Bremer support values, and it was placed as sister group of the genus *Alayotityus*. *Chaneke* gen. nov. was supported by the following characters: (1) the lateral ocelli small and hidden from dorsal view by a crest (char. 2); (2) carapace without keels (char. 4); (3) one tergal carinae (char. 5); (4) male genital papillae without a distinct, fleshy point (char. 7); (5) subaculear tubercle trapezoidal, with two granules (char. 18); (6) males with basal lobe on movable finger (char. 20); (7) femoral τ i3 petite (char. 25) and (8) by the absence of chela τ Eb₃ (char. 27; see figure 11). Genus *Tityopsis* was recovered monophyletic with high jackknife and Bremer support values, and it was placed as sister of the clade formed by genera *Zabius*, *Microtityus*, *Chaneke* and *Alayotityus* (see Fig. 4).

The analyses with implied weighting under four values of the concavity constant (k = 10, 30, 60 and 100) recovered two trees,

Figure 3.—Map of Oaxaca and Guerrero area plotting known locality records for the two species of *Chaneke* gen. nov.: *Chaneke fogoso*, sp. nov. (circle), *Chaneke aliciae* (Armas & Martin-Frias), comb. nov. (square).

with the same topologies as in the analysis with equal weighting (Table 2). However, analyses with implied weighting under two values of the concav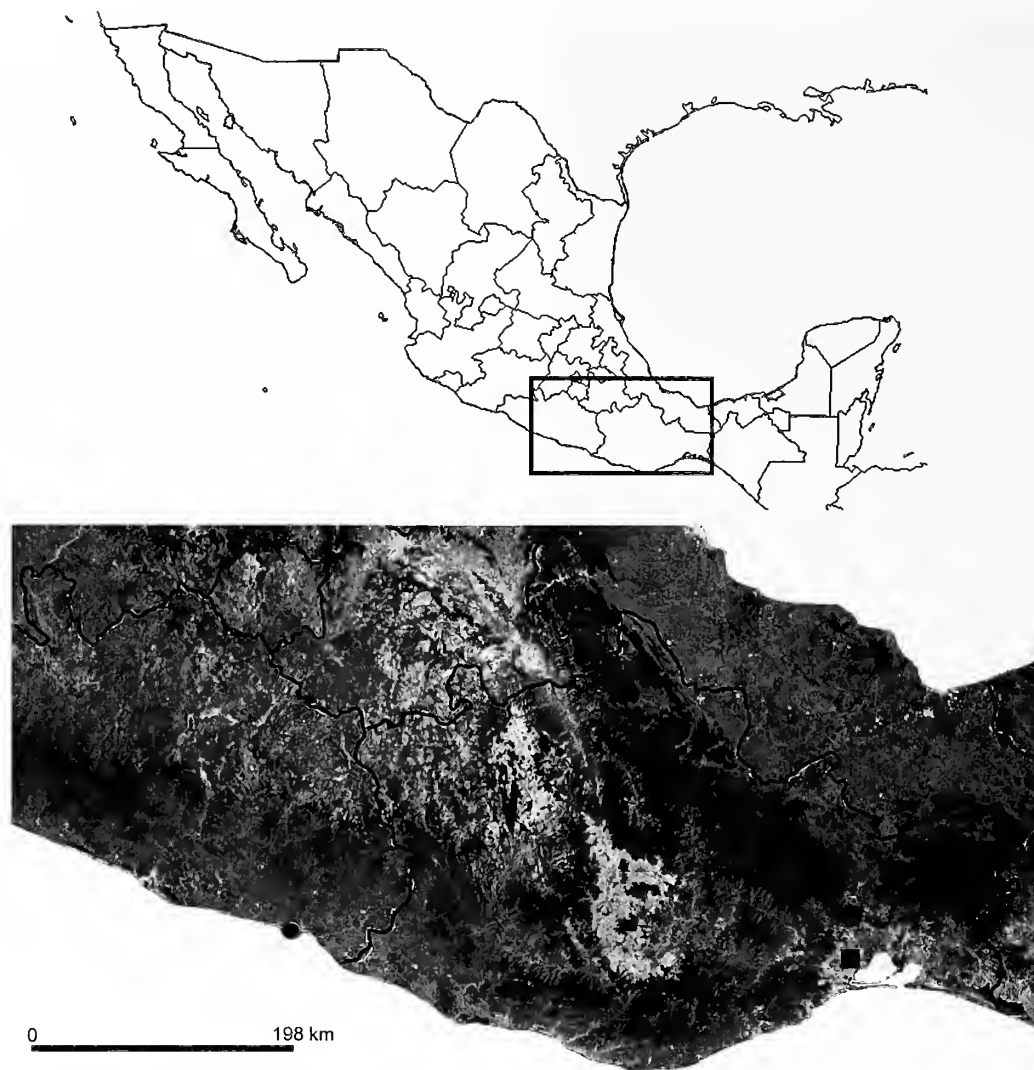ity constant ($k = 1$ and 3) reeovered three most parsimonious trees (strict consensus shown in Fig. 5; Table 2). The monophyly of *Chaneke* gen. nov. was recovered with high jackknife and Bremer support values, and it was placed as a sister group of the clade formed by genera *Tityopsis*, *Microtityus*, *Zabius* and *Alayotityus* as follows: (*Chaneke* gen. nov. (*Tityopsis* (*Microtityus* (*Zabius* + *Alayotityus*)))). Under those two analyses ($k = 1$ and 3), *Chaneke* gen. nov. was supported by the following characters (1) the trapezoidal shape of the carapace (char. 0); (2) the lateral ocelli small, dorsally covered by a crest, visible in frontal aspect (char. 2); (3) carapace without keels (char. 4); (4) male genital papillae without a distinct, fleshy point (char. 7); (5–6) males and females with a whitish patch on sternite (chars. 10; 14); (7) males with basal lobe on movable finger (char. 20).

None of these analyses recovered *Chaneke* gen. nov. as sister group of *Tityopsis*, and the creation of this new genus, along with the transfer of *Tityopsis aliciae* (= *Chaneke aliciae*, new combination) to the new genus, are well supported. The

preferred tree is the strict consensus from the analyses without weighting and those recovered with coneavity values of $k = 10$, 30, 60 and 100 (Fig. 4), which place *Chaneke* as sister group of *Alayotityus*. These two genera share: (1) males with whitish patch on sternite III (char. 10); (2) females with whitish patch on sternite III (char. 14); (3) femoral τ i_3 petite (char. 24); and (4) patella τ d_2 absent (char. 26). However, the position of *Chaneke* gen. nov. within the family remains unresolved pending a further study with the inclusion of more genera of buthids.

SYSTEMATICS

Family Buthidae C.L. Koch 1837
Genus *Chaneke*, gen. nov.

Tityopsis (in part): Armas & Martín-Frías 1998:45; Vidal-Acosta & Franeke 2009:338.

Type species.—*Chaneke fogoso*, sp. nov.

Other included species.—*Chaneke aliciae* (Armas & Martín-Frías, 1998), comb. nov.

Etymology.—"Chanekes" are legendary creatures in Mexican folklore, dating to Aztec times. They are conceived as

Figure 4.—Strict consensus tree from two equally parsimonious trees (length, 69; CI, 0.652; RI, 0.878; Fit, 24.55) obtained by the analysis of 30 morphological characters for 25 species in 11 buthid scorpion genera, with equal weighting, and with weighting concavity values of k= 10, 30, 60 and 100. Unambiguous morphological synapomorphies optimized on branches: black squares indicate synapomorphies, white squares indicate homoplasies; numbers above squares indicate characters, numbers below indicate states (see Appendix 3). Jackknife values greater than 50% indicated above branches. Bremer support values indicated below branches.

small, sprite-like beings, elemental forces and guardians of nature. It is used as a noun in apposition, and is considered masculine in gender.

Diagnosis.—Relatively small-sized buthid scorpions (adults approx. 2 cm long—Table 1) with decreasing neobothriotaxy

A α: pedipalp femur lacking τ d_2, patella lacking τ d_2, chela lacking τ Eb_3. The eight known species of *Alayotityus* lack femoral τ d_2 and patellar τ d_2, but have chelal τ Eb_3; the three known species of *Zabius* lack femoral τ d_2, but have patellar τ d_2 and chelal τ Eb_3; the two known species of *Tityopsis* are

Ananteris platnicki

Tityus bahiensis

8 12 20
0 0 1

18 19 21
1 1 1

Centruroides exilicauda

19 24
2 1
1 18

Centruroides gracilis

Rhopalurus junceus
3 0

Tityus columbianus

13 15
1 1

Tityus clathratus

18
5

22
0

Mesotityus vondangeli

99
0 2 4 7 10 14 20
1 1 1 1 1 1 1

Chaneke aliciae

28
0

Chaneke fogoso

1.00

16
1

84
3 6 8 11 19 29
2 1 0 1 2 1

0.55

95
8 12 13 19
1 3 3 3

Tityopsis inaequalis

Tityopsis inexpectata

0.50

0 9 11 18 22
1 1 0 4 0

12
3

Microtityus (M.) rickyi

Microtityus (P.) jaumei

18 24 25
3 1 2

Zabius birabeni

19 20
4 1

Zabius gaucho

Zabius fuscus

1
0

Alayotityus delacruzi

Alayotityus granma

Alayotityus juraguaensis

95
10 12 13 14 24 26
1 2 2 1 0 0

0.50

Alayotityus sierramaestrae

Alayotityus feti

28
0

Alayotityus lapidicola

Alayotityus nanus

Alayotityus pallidus

Figure 5.—Strict consensus tree from three most parsimonious trees (length, 71; CI, 0.634; RI, 0.867; Fit, 24.35; Adjusted Homoplasy, 5.65) obtained by the analysis of 30 morphological characters for 25 species in 11 buthid scorpion genera, with weighting concavity values of $k = 1$ and 3. Unambiguous morphological synapomorphies optimized on branches: black squares indicate synapomorphies, white squares indicate homoplasies; numbers above squares indicate characters, numbers below indicate states (see Appendix 3). Jackknife values greater than 50% indicated above branches. Bremer support values indicated below branches.

orthobothriotaxic. Tergites with a single, median longitudinal carina; whereas *Alayotityus* and *Zabius* have three carinae, *Tityopsis* only one, and *Microtityus* three or five. Metasomal segment V without lateral carinae; *Zabius* and *Microtityus* also lack such carinae, *Alayotityus* and *Tityopsis* always have well defined lateral carinae. Subaculear tubercle very large and

rhomboid in lateral view, considerably deeper than wide; *Alayotityus*, *Tityopsis* and *Zabius* all have a subaculear tubercle which may be obsolete to moderately developed, but is always blunt conical. Fixed finger of the pedipalp chela with 9–10 slightly imbricated rows of denticles; *Alayotityus* also has 9–10, *Zabius* and *Tityopsis* have 11–12. Dentition on

Table 1.—Measurements in mm of *Chaneke fogoso* sp. nov. L = length, W = width.

		Holotype male	Paratype male	Paratype female	Paratype female
Total	L	19.7	20.6	21.3	20.2
Carapace	L	2.8	2.9	3	2.9
	W	2.4	2.3	2.5	2.4
Mesosoma	L	6.5	6.7	7.3	7.3
Metasoma	L	10.4	11	11	10
I	L	1.5	1.6	1.6	1.5
	W	1.7	1.7	1.7	1.6
II	L	1.9	2	2	1.8
	W	1.6	1.5	1.5	1.4
III	L	2	2.1	2.2	2
	W	1.5	1.5	1.5	1.3
IV	L	2.3	2.4	2.4	2.2
	W	1.5	1.5	1.4	1.3
V	L	2.7	2.9	2.8	2.5
	W	1.5	1.5	1.4	1.3
Telson	L	2.4	2.5	2.6	2.3
	W	1	1.1	1.1	1.1
Pedipalp	L	9.4	9.7	10.3	9.7
Femur	L	2.3	2.4	2.5	2.4
	W	0.8	0.9	0.9	0.9
Patella	L	2.7	2.7	3	2.8
	W	1.1	1.2	1.2	1.2
Chela	L	4.1	4.6	4.8	4.5
	W	1.5	1.6	1.3	1.3

the fingers of the pedipalp chela without supernumerary denticles flanking the primary rows (Figs. 9B, C).

Distribution.—Known only from the Mexican states of Guerrero and Oaxaca, along the southern Pacific Coast (Fig. 3).

Chaneke fogoso, sp. nov.
Figures 1–6, 8–11 Table 1

Type data.—MEXICO: *Guerrero*: Municipio de Copala: Holotype adult ♂, Microondas Fogos (approx. 15 km ESE Copala), 16° 33.992'N, 98° 53.301'W, 103 m, 31 Aug 2008, U.V. detection, O.F. Francke, H. Montaño, C. Santibáñez & A. Valdez (CNAN T–0630). Paratypes: 19 adult ♂, 1 subadult ♂, 3 adult ♀, 3 subadult ♀, 2 juveniles, same data as holotype (1 ♂, 1 ♀ each at AMNH and BIOECO; remainder at CNAN T–0631); 1 adult ♂ (U.V.), 1 adult ♀ (sifting leaf litter), same locality, 6–7 July 2008, O.F. Francke, C. Santibáñez & A. Quijano (CNAN T–0632); 1 subadult ♂ (U.V.), same locality, 26 June 2007, O.F. Francke, L. Escalante, J. Ballesteros & H. Montaño (CNAN T–0633).

Diagnosis.—*Chaneke fogoso* has 10 primary rows of denticles on both fixed and movable fingers of the pedipalp chela, whereas *Ch. aliciae* has only nine. Pectinal tooth count on males 9–11 (mode = 10), on females 8–9 (tied); *Ch. fogoso* lacks τ *Esb* on the manus and τ *esb* on the fixed finger of the pedipalp chela, whereas *Ch. aliciae* has τ *Esb* and τ *esb* present. In addition, *Ch. fogoso* is in general smaller and has a less robust metasoma than *Ch. aliciae* (Figs. 6, 7), but also possesses the smooth, whitish patch of sternite V remarkably larger and bulkier in adults of both sexes.

Table 2.—Tree statistics for phylogenetic analysis of 25 species in 10 New World buthid scorpion genera. Length, consistency index (CI), retention index (RI), Fit and adjusted homoplasy (AH) of most parsimonious trees (MPTs) obtained by the analyses of the morphological under equal weighting (EW) and implied weighting (IW), with six concavity values (k).

		MP	L	CI	RI	FIT	AH
EW		2	69	0.652	0.878	24.55	
IW	k=100	2	69	0.652	0.878	29.76	0.24
IW	k=60	2	69	0.652	0.878	29.61	0.39
IW	k=30	2	69	0.652	0.878	29.24	0.76
IW	k=10	2	69	0.652	0.878	27.91	2.09
IW	k=3	3	70	0.643	0.872	24.6	5.4
IW	k=1	3	70	0.643	0.872	24.6	5.4

Etymology.—The specific name is a noun in apposition, "fogoso" in Spanish means "fiery", "feisty" or "lit-on-fire", befitting the generic name; in addition, it alludes to the type locality.

Description.—*Holotype male* (Figs. 6A, B): Coloration: Base color light yellow (straw-colored). Prosoma: carapace with dense, variegated fuscosity (Figs. 6A, C); venter pale yellow (Figs. 6B, D). Mesosoma: tergites I–VI with two complete, transverse fuscous bands—one on all of pre-tergite, the other on distal one-half of post-tergite; tergite VII with pre-tergite infuscate, and post-tergite with middle, posterior and lateral areas infuscate; ventrally pale yellow. Metasomal segments I–IV faintly, uniformly infuscate on ventromedian, posterior one-halves of ventrolateral and lateral inframedian, and distally on lateral supramedian intercarinal spaces; segment V and telson straw colored. Chelicerae not infuscate. Pedipalps with diffuse, uniform fuscosity, dorsally on trochanter, femur and patella; fingers on chela pale reddish brown, feebly infuscate. Legs infuscate on prolateral regions.

Carapace: Coarsely, densely granulose throughout (Fig. 8A). Anterior margin bilobed, with shallow median notch; with four short, blunt-tipped setae. Three subequal ocelli on each side. Median eyes slightly anterior to one-half the carapace length. Two moderately strong, longitudinal, submedian carinae on posterior one-fifth. Ventrally with numerous reddish setae of various sizes, some pointed, some blunt.

Mesosoma: Tergites with pre-tergite densely, minutely granulose; anterior one-half of post-tergite sparsely granulose, shiny; posterior one-half densely, coarsely granulose, matte. One coarsely granulose median carinae present on distal one-half of post-tergites I–VI. Tergite VII paramedian and lateral carinae well-developed, coarsely granulose. Sternum subpentagonal (Figs. 6B, D); with deep indentation posteromedially; three pairs of setae. Genital opercula completely separated, with five and six setae respectively; genital papillae without sharp, pointed end. Pectinal basal piece wider than long, with shallow anteromedian notch; posterior margin straight (Fig. 8B). Pectinal tooth count 9–10. Sternites moderately granulose, with scattered reddish setae throughout; stigmata small, oval-elongate. Sternite III with two anterolateral depressions underneath the pectines (where these structures presumably fit when the animal is at rest). Sternite V with a conspicuous, circular, white, shiny patch medially along posterior margin (Fig. 6B). Sternite VII submedian carinae
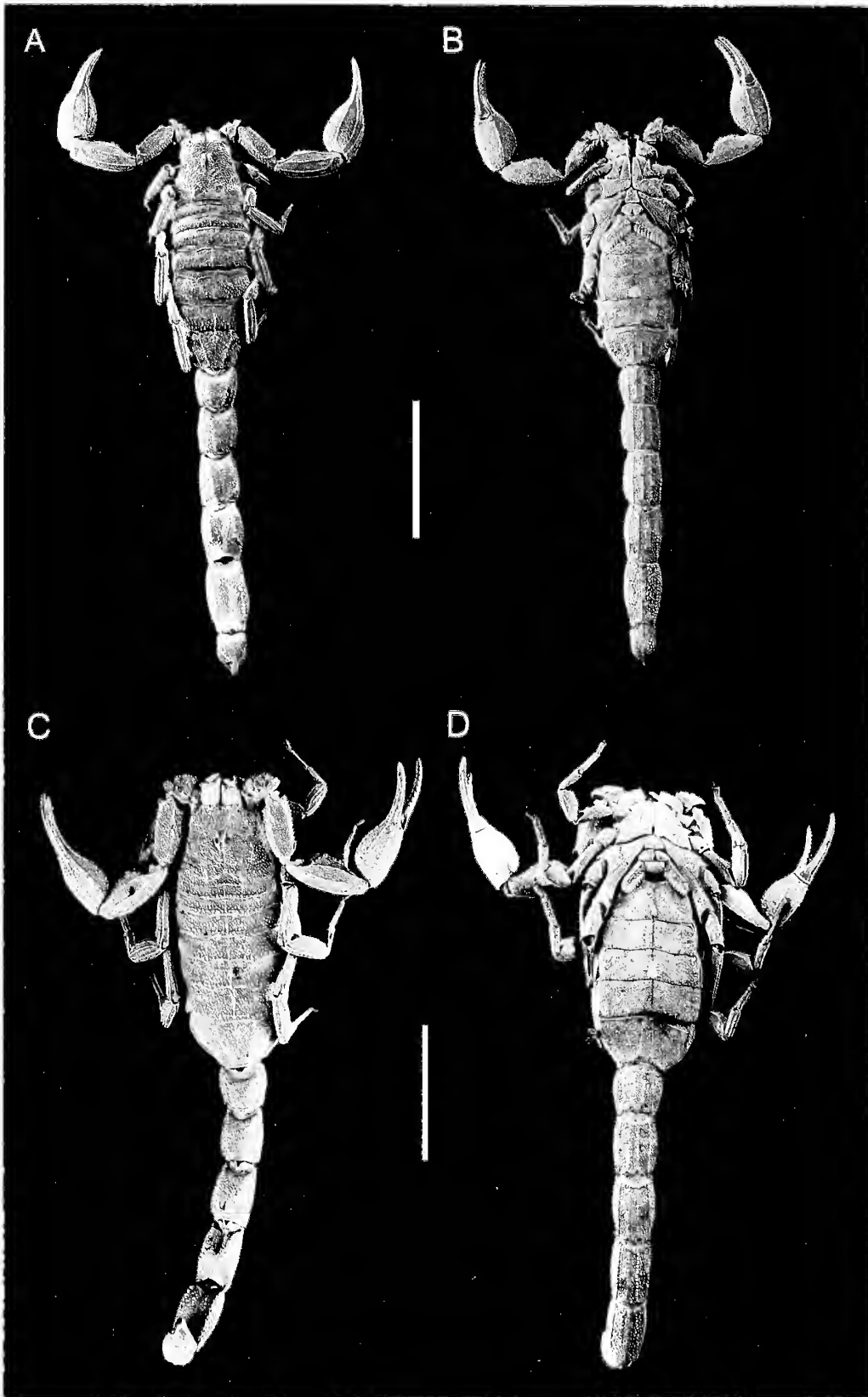
Figure 6.—*Chaneke fogoso* gen. nov. et sp. nov., habitus, dorsal aspect (A, C) and ventral aspect (B, D). A, B. Holotype ♂ (CNAN); C, D. Paratype ♀ (CNAN). Scale bar = 5 mm.
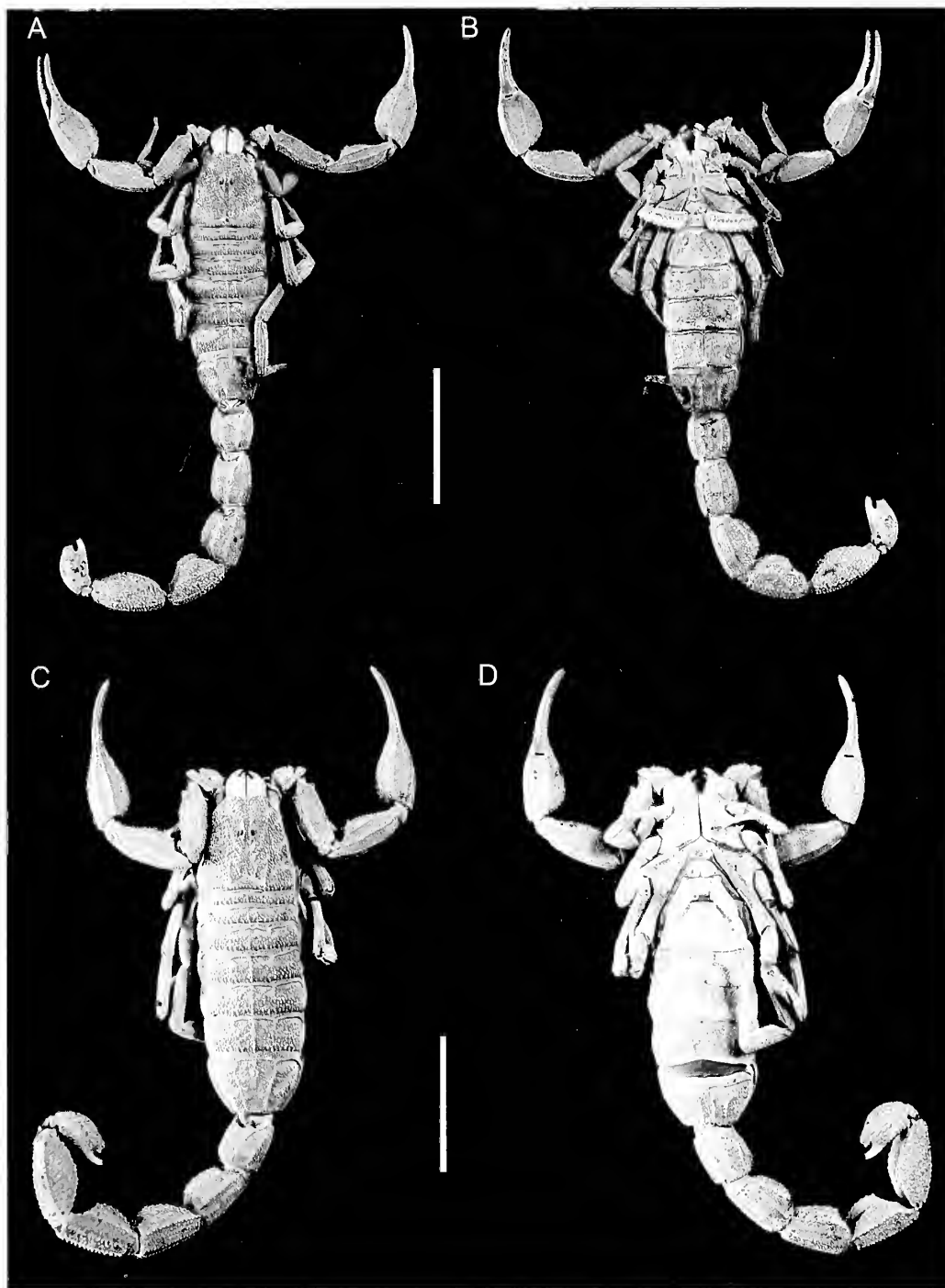
Figure 7.—*Chaneke aliciae* (Armas & Martín-Frías 1998), comb. nov., habitus, dorsal aspect (A, C) and ventral aspect (B, D). A, B. ♂ (CNAN); C, D. ♀ (CNAN). Scale bar = 5 mm.

granulose, well-defined and reaching posterior margin; lateral carinae barely discernible as short row of five granules submedially, absent on basal and distal thirds.

Metasoma: Segments I–IV with dorsolateral, lateral supramedian, ventrolateral and ventral submedian carinae strong, crenulate; lateral inframedian carinae complete, crenulate on I–II, absent on III–IV; intercarinal spaces moderately granulose. Segment V (Fig. 9A) dorsolateral, ventrolateral and ventromedian carinae strong, granulose; lateral carinae

absent; intercarinal spaces densely, coarsely granulose. Telson globose; ventrally weakly to vestigially granulose; subaculear tubercle flat, crest-like, its width same as that of base of aculeus, ending in a small finger-like projection that points towards middle of aculeus (Fig. 9A).

Chelicera: Fixed finger with three dorsal teeth; on right side basal tooth is a bicusp, on left side a sharp monocusp; ventrally with a single small tooth at level of middle dorsal tooth. Movable finger with distal tines subequal; dorsally with
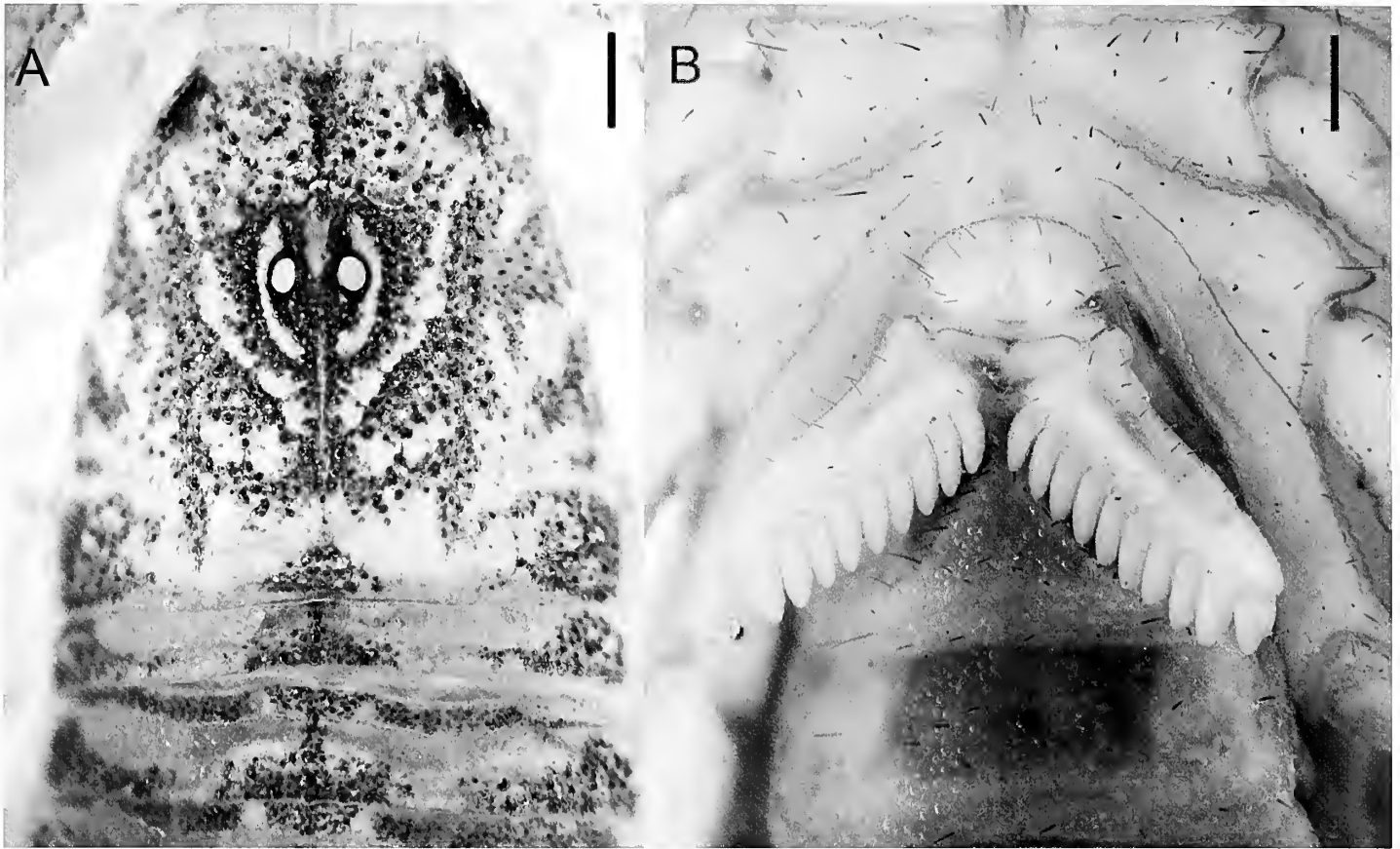
Figure 8.—*Chaneke fogoso* gen. nov. et sp. nov., holotype ♂ (CNAN). A. Carapace, dorsal aspect; B. Pectinosternal region. Scale bars = 0.5 mm.

a basal bicusp characteristic of the family; ventrally with two small teeth.

Pedipalp: Femur with prodorsal, retrodorsal, anteromedian and proventral carinae strong, granulose; intercarinal spaces moderately to densely granulose, with few clavate setae distally. Neobothriotaxia A alpha: d_2 absent, i_3 and i_4 petite (Fig. 10A). Tibia heptacarinate, all carinae strong, granulose; dorsal intercarinal spaces densely granulose, others moderately to sparsely so, with scattered clavate setae throughout. Neobothriotaxia A: d_2 absent, no petite trichobothria (Figs. 10C, D). Chela with nine carinae, smooth to feebly crenulate; intercarinal spaces with moderately dense, small granulation; with moderately dense, clavate setae throughout, including both fingers. Movable finger with 10 imbricated principal rows of granules, flanked by 11 inner and nine outer accessory granules (Fig. 9B), the apical subrow (excluded from counts) is composed by four granules located just basal to the terminal denticle. Fixed finger with 10 imbricated principal rows of granules, flanked by 11 inner and nine outer accessory granules (Fig. 9C). Neobothriotaxia A: lacking Eb_3, Esb and esb (Figs. 11A, B).

Legs: Tibial spurs absent on all legs; prolateral and retrolateral pedal spurs present on all legs. Patellae and tibiae with scattered clavate setae; tarsi with moderately dense, pointed setae.

Variability.—Pectinal tooth counts varied as follows: on males three combs with nine teeth (7.5%), 22 with 10 (55.0%)

and six with 11 (37.5%); on females six combs with eight teeth (50%) and six with nine (50%).

Variation.—Pedipalp finger dentition was analyzed on six males and six females (both right and left fingers checked for each specimen). The number of denticle rows on the fixed finger was 10 on the 24 fingers checked; the number of inner accessory granules was 10 on females (10 fingers with 10 granules, two fingers with 11) and 11 on males (two fingers with 10 granules and 10 fingers with 11 granules), and the number of outer accessory granules was 10 with no apparent sexual dimorphism (three fingers with nine granules and 21 fingers with 10). The number of denticle rows on the movable finger was 11 on the 24 fingers counted; the number of inner accessory granules was 11 on females (nine fingers with 11 granules and three fingers with 12) and 12 on males (12 out of 12), and the number of outer accessory granules was 11 with no apparent sexual dimorphism (20 fingers with 11 granules, four fingers [two male, two female] with 12 granules).

Distribution.—This species is only known from the type locality in the state of Guerrero (Fig. 3).

Remarks.—The locality where the new species was collected is a well-conserved, land-locked area; it is a small isolated hill (approx. 200 m high) along the coastal plains and has a microwave relay station on top. It is in private property, surrounded by pasture-land and scattered cultivation plots. The original vegetation on the plain and lower slopes is tropical deciduous scrub forest, whereas the upper reaches
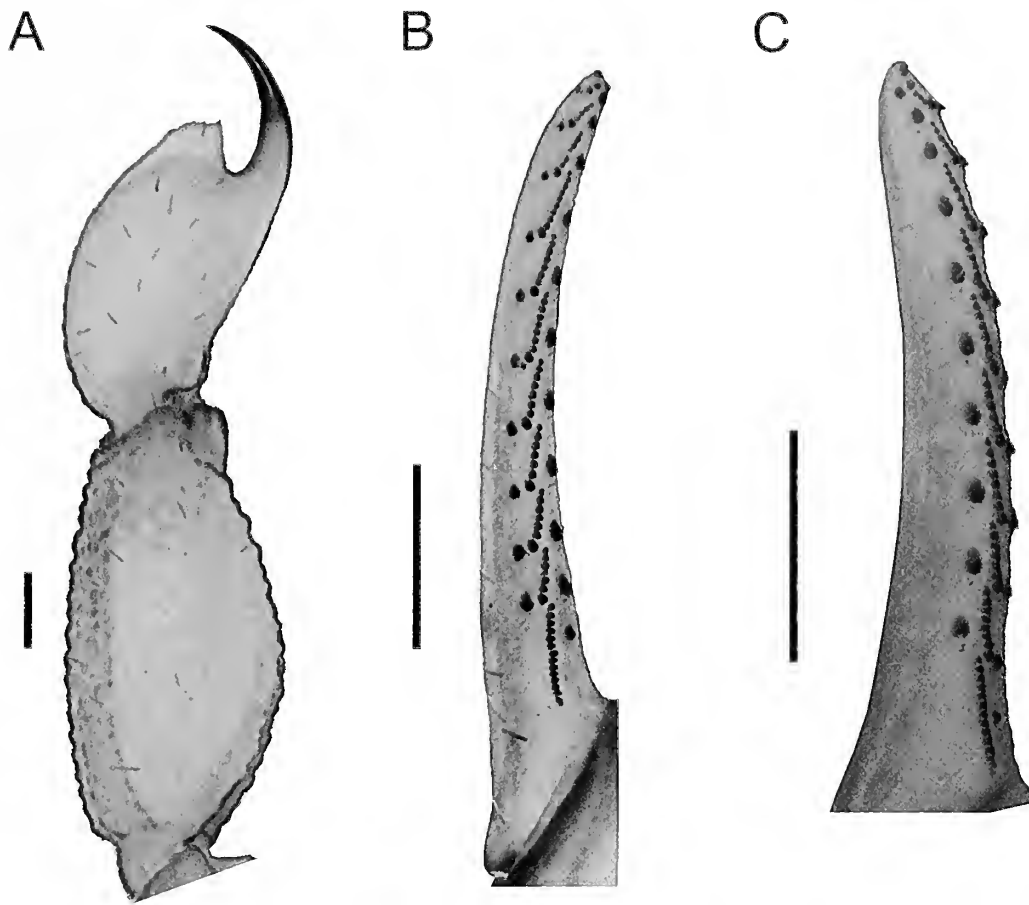
Figure 9.—*Chaneke fogoso* gen. nov. et sp. nov.: holotype ♂ (CNAN). A. Lateral aspect of distal portion of metasoma; B. Pedipalp chela movable finger showing dentition pattern; C. Pedipalp chela fixed finger showing dentition pattern. Scale bars = 0.5 mm.
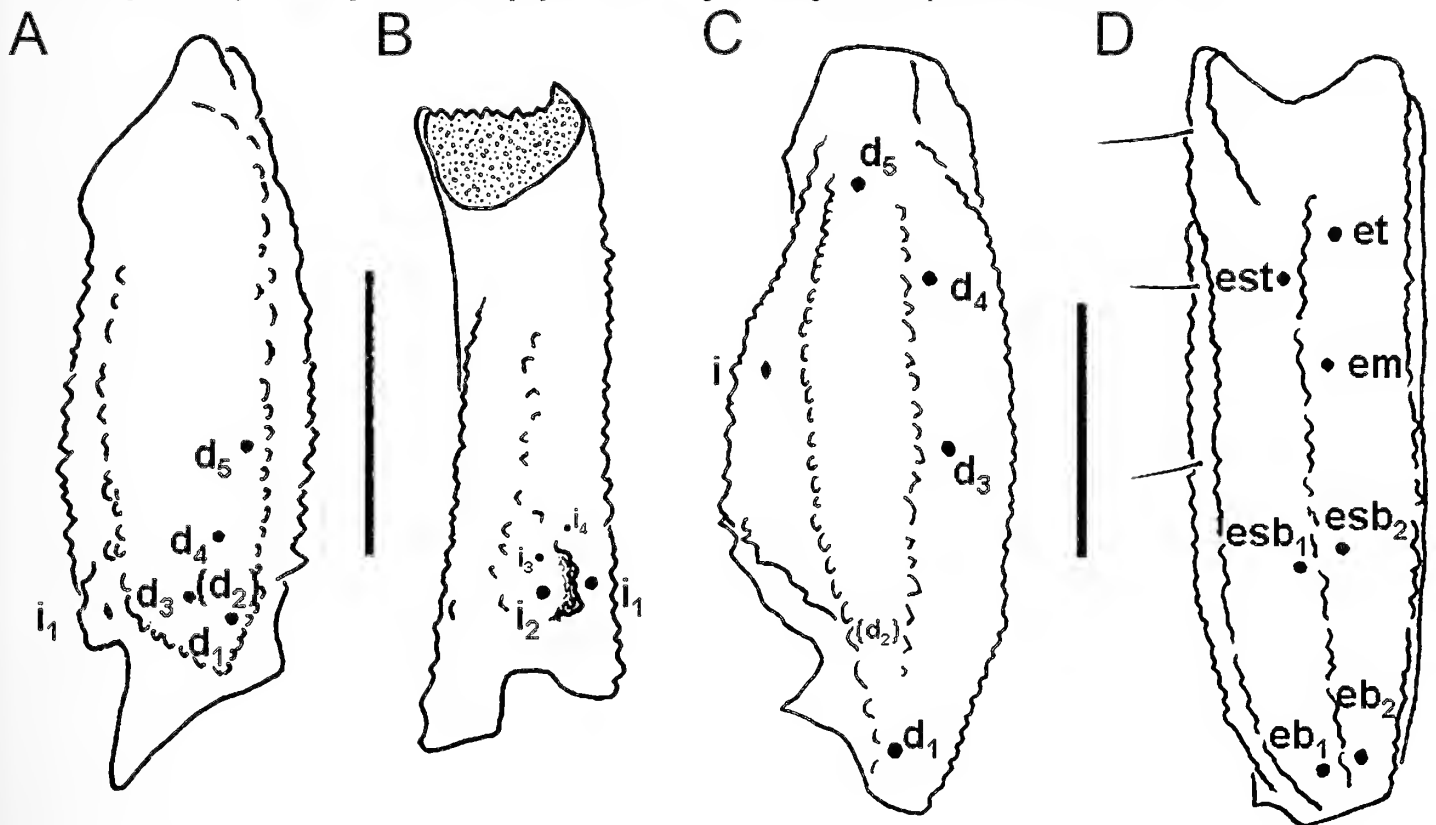

Figure 10.—*Chaneke fogoso* gen. nov. et sp. nov.: holotype ♂ (CNAN). A. Dorsal aspect of pedipalp femur, showing trichobothria (d_2 missing); B. Frontal aspect of pedipalp femur; C. Dorsal aspect of pedipalp patella; D. Posterior aspect of pedipalp patella. Scale bars = 1 mm.
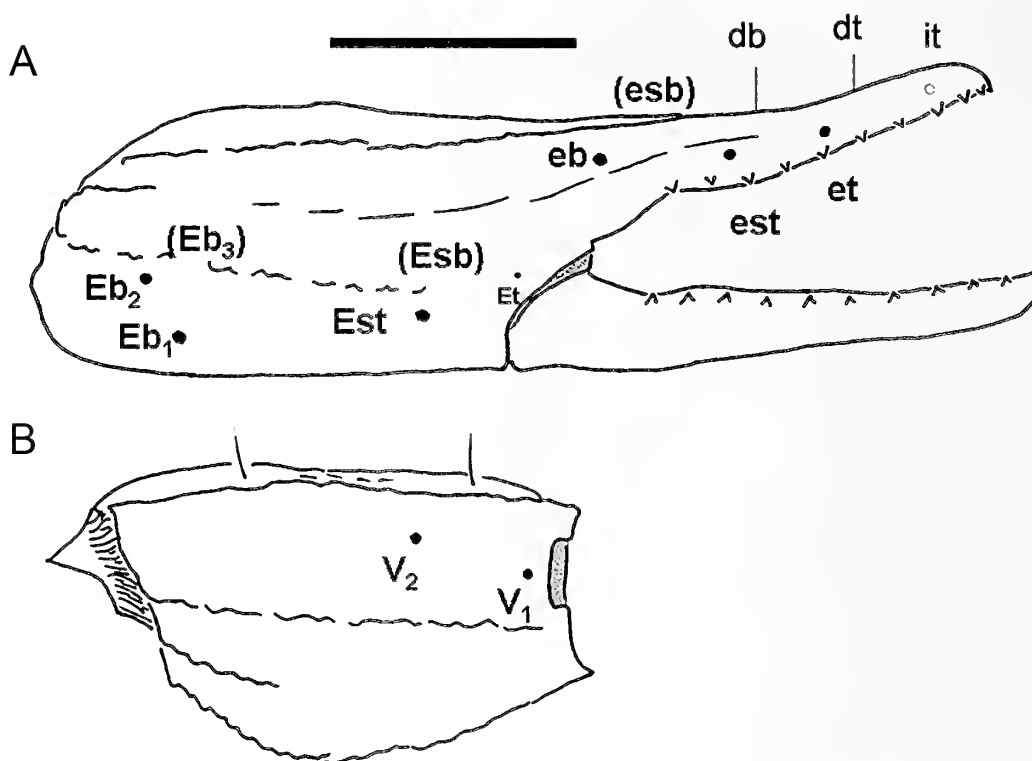
Figure 11.—*Chaneke fogoso* gen. nov. et sp. nov.: holotype ♂ (CNAN). A. External aspect of pedipalp chela showing trichobothria; B. Ventral aspect of pedipalp manus. Scale bars = 1 mm.

receive more moisture and have a mixed tropical lowland forest component. The upper habitat contains numerous large boulders, and in protected places the leaf-litter can reach 0.3–0.4 m in depth (Fig. 1). Most of the specimens were collected after abundant rains.

Chaneke aliciae (Armas & Martín-Frías 1998), comb. nov.
Figures 3–5, 7

Tityopsis aliciae Armas & Martín-Frías 1998:45–49; Santibáñez-López & Ponce-Saavedra 2009:321; Vidal-Acosta & Francke 2009:333–339.

Type data.—MEXICO: *Oaxaca*: Municipio de Santo Domingo Tehuantepec: Holotype subadult ♀, [16.31°N, 95.23°W], 30 June 1938, no collector (CNAN–T0173); 1 adult ♀, Tehuantepec, Cima street #61, under bricks [16.31°N, 95.23°W], 12 Jan 2006, no collector (INDRE); 1 adult ♂, alrededores de Colonia Emiliano Zapata, 16.32026°N, 95.27899°W, 80 m, R. Paredes, C. Santibáñez, A. Valdez (CNAN); 1 adult ♀, 2 subadult ♀, km 23.5 road Salina Cruz to La Ventosa, 16.39754°N, 95.10094°W, 20 m, C. Santibáñez, R. Monjaraz, A. Valdez, M. Fuentes (CNAN).

Diagnosis.—*Chaneke aliciae* has nine primary rows of denticles on both fixed and movable fingers of the pedipalp chela, whereas *Ch. fogoso* has ten. Pectinal tooth count on males 10–11, on females 8–9; *Ch. aliciae* bears τ *Esb* on the manus and τ *esb* on the fixed finger of the pedipalp chela, whereas *Ch. fogoso* lacks τ *Esb* and τ *esb*. The sexual secondary dimorphism is slight (as usual for the other closely-related genera): adult males can be recognized by their

more distally incrassate pedipalp chelae and metasoma, smaller mesosoma (Fig. 7), presence of genital papillae, and slight but consistently higher pectinal tooth counts.

Distribution.—This species is only known from the Santo Domingo Tehuantepec area, in the state of Oaxaca (Fig. 3).

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Appendix 1.—Specimens examined and/or references consulted during the construction of the character matrix.

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2. *Alayotityus delacruzi* Armas 1973. CUBA: *Santiago de Cuba*: Playa Siboney: Cueva de Los Majáes, 27 March 1998, R. Teruel, N. Navarro, 10 ♂, 3 ♀, 6 juv. topotypes (RTO). 18 May 2002, R. Teruel, M. Sobrino, 2 ♂, 4 ♀ topotypes (CNAN).

3. *Alayotityus feti* Teruel 2004. CUBA: *Santiago de Cuba*: La Socapa, 26 March 1999, R. Teruel, 1 ♂ holotype, 6 ♂, 8 ♀ paratypes (RTO).

4. *Alayotityus granma* Armas 1984. CUBA: *Granma*: Niquero: El Guafe, 2 km al norte de Cabo Cruz, 9–11 July 2000, R. Teruel, L. Montano, Y. Cala, R. Escalona, 8 ♂, 16 ♀, 3 juv. topotypes (RTO).

5. *Alayotityus juraguaensis* Armas 1973. CUBA: *Santiago de Cuba*: Playa Juraguá, 6–7 March 1992, R. Teruel, 1 ♂, 1 ♀, 8 juv. topotypes (RTO). Same data except 3 Juiy 1992, R. Teruel, R. Ermus, 1 ♂, 2 ♀ topotypes (RTO).

6. *Alayotityus lapidicola* Teruel 2002. CUBA: *Santiago de Cuba*: Tercer Frente: La Pimienta, 20 April 2000, R. Teruel, R. Viña, A. Fong, 1 ♂ holotype, 5 ♀ paratypes (RTO).

7. *Alayotityus nanus* Armas 1973. CUBA: *Santiago de Cuba*: Puerto Boniato, 9 March 2003, R. Teruel, Y. Pérez, 2 ♂, 5 ♀ topotypes (BIOECO). *Santiago de Cuba*: 300 m N El Cobre, 9 Sept 2000, R. Teruel, Y. Pérez, 2 ♂, 5 ♀ (CNAN).

8. *Alayotityus pallidus* Teruel 2002. CUBA: *Santiago de Cuba*: Julio A. Mella: La Cantera, 11 March 1999, R. Teruel, 1 ♂ holotype, 2 ♂, 1 ♀, 1 juv. paratypes (RTO). 26 Sept 2003, R. Teruel, L. F. de Armas, 6 ♂, 3 ♀, 8 juv. topotypes (RTO).

9. *Alayotityus sierramaestrae* Armas 1973. CUBA: *Santiago de Cuba*: Guamá: Río La Mula, 15 June 2003, R. Teruel, Y. Pérez, 2 ♀ (CNAN). 12–21 June 2005, R. Teruel, K. Blanco, A. Pupo, 6 ♂, 8 ♀, 7 juv. (RTO).

10. *Centruroides gracilis* (Latreille 1804). CUBA: *Santiago de Cuba*: Santiago de Cuba city, 28 April 2000, R. Teruel, Y. Pérez, 3 ♂, 3 ♀ (CNAN).

11. *Centruroides exilicauda* (Wood 1863). MEXICO: *Baja California Sur*: Loreto, 13 km W to San Javier, provisional dirt road, 25° 58.817′N, 111° 27.211′W, 26 June 2008 (H. Montaño, E. González). 17 ♂, 14 ♀ (CNAN).

12. *Chaneke aliciae* (Armas & Martín-Frias 1998). [see material studied above]

13. *Chaneke fogoso* Francke, Teruel & Santibáñez-López 2014. [see original description above].

14. *Mesotityus vondangeli* González-Sponga 1981. VENEZUELA: *Aragua Estate*: Henry Pittier National Park: Río Catá (± 100 m a.s.l.), night search with UVL, upstream from the dam, 6 April 2006, F. J. M. Rojas-Runjaic, 2 ♂ (IES).

15. *Microtityus (Microtityus) rickyi* Kjellesvig-Waering 1966. [see Kjellesvig-Waering, 1996].

16. *Microtityus (Parvabsonus) jaumei* Armas 1974. CUBA: *Santiago de Cuba*: Playa Siboney, 18 May 2002, R. Teruel, M. Sobrino, 3 ♂, 3 ♀ (CNAN). CUBA: *Santiago de Cuba*: Playa Verraco, 4 May 2006, R. Teruel, F. Cala, 9 ♂, 6 ♀, 1 juv. (RTO).

17. *Rhopalurus junceus* (Herbst 1800). CUBA: *Camagüey*: Sibanicú, 20 Feb 1996, R. Teruel, 2 ♂, 2 ♀, 10 juv. (CNAN). Same data except 2 Jan 1997, R. Teruel, A. Basulto, 6 ♂, 7 ♀, 5 juv. (RTO).

18. *Tityopsis inaequalis* (Armas 1974). CUBA: *Pinar del Río*: San Cristóbal: Mameyal, 16 Feb 1981, L. F. de Armas, 1 ♂ (RTO). CUBA: *Pinar del Río*: Viñales: Hoyo de Fanía, 6 Dec 1984, L. V. Moreno, J. Novo, 1 ♀ (RTO).

19. *Tityopsis inexpectata* (Moreno 1940). CUBA: *Ciudad de La Habana*: Bosque de La Habana, 8–20 Jan 2005, R. Teruel, D. Ortiz, 1 ♂, 4 ♀, 2 juv. (RTO).

20. *Tityus bahiensis* (Perty 1833). BRASIL: *Sao Paulo*: Sao Paulo, no date (no colector). 1 ♂, 3 ♀ (CNAN).

21. *Tityus clathratus* C.L. Koch 1844. VENEZUELA: *Bolívar Estate*: Cedeño: Guaniamo (6°05′N–66°02′W, 150 m a.s.l.), no further data, 4 ♂, 1 ♀ (RTO: Sco–0508).

22. *Tityus columbianus* (Thorell 1876). COLOMBIA: *Boyacá Department*: Chiquinquirá (2,550 m a.s.l.), under rocks, in sandy soil, 3 March 2007, L. F. García, 10 ♂, 9 ♀, 1 juv. (RTO: Sco–0372).

23. *Zabius birabeni* Mello-Leitão 1938. [see Acosta et al. 2008].

24. *Zabius gaucho* Acosta, Candido, Buckup & Brescovit 2008 [see Acosta et al. 2008].

25. *Zabius fuscus* (Thorell 1876) ARGENTINA: *Córdoba*: La Cumbre, February 1997, L. Coronel, 1 ♂ (RTO: Sco–0192). [see also Acosta et al. 2008].

Appendix 2.—Distribution of 30 morphological characters (0–29) scored for a cladistic analysis of 25 species in 11 new world buthid scorpion genera with α trichobothrial pattern. Characters states are scored 0–5. ? (unknown). Refer to Appendix 1 for material examined and Appendix 3 for character descriptions.

Species			
Ananteris platnicki	0200100010	1010000040	0011111110
Mesotityus voudangeli	0101000010	0011011054	0001010010
Tityus bahiensis	0101000000	0000000024	1011011110
Tityus columbianus	0101000010	0011010024	0011011110
Tityus clathratus	0101000010	0011010054	0011011110
Centruroides exilicauda	0101000000	0000000011	1111011110
Centruroides gracilis	0101000000	0000000012	1111111110
Rhopalurus junceus	0301000000	0000000001	1111011110
Alayotityus delacruzi	0002011000	1122111132	0010020111
Alayotityus feti	0002011000	1122111132	0010020101
Alayotityus gramma	0002011000	1122111132	0010020111
Alayotityus juraguaensis	0002011000	1122111132	0010020111
Alayotityus lapidicola	0002011000	1122111132	0010020101
Alayotityus nanus	0002011000	1122111132	0010020101
Alayotityus pallidus	0002011000	1122111132	0010020101
Alayotityus sierramaestrae	0002011000	1122111132	0010020111
Chaneke aliciae	1112101100	111111105	21010010011
Chaneke fogoso	1112101100	1111111052	1010010001
Microtityus (M.) rickyi	1102021001	0031011042	0001121111
Microtityus (P.) jaumei	1102011001	0011011042	0000121111
Tityopsis inaequalis	0102001010	0133011133	0011121111
Tityopsis inexpectata	0102001010	0133011133	0011121111
Zabius birabeni	0002011000	0111011134	1010121111
Zabius gaucho	0002011000	?1?1011134	?010121111
Zabius fuscus	0002011000	0111011134	1010121111

Appendix 3.—List of 30 morphological characters scored for 21 species of New World buthids with α triehobothrial pattern.

Prosoma

0. Carapace shape: trapezoidal (0), triangular (1).
1. Lateral ocelli: two pairs (0), three pairs (1), five pairs (2).
2. Lateral ocelli large, prominent, clearly visible in dorsal aspect (0), lateral ocelli small, dorsally covered by a crest, visible in frontal aspect (1).
3. Anterior margin: straight (0), V-notched (1), bilobed (2).
4. Carapace with distinct keels present (0), absent (1).

Mesosoma

5. Tergal carinae: one (0), three (1), five (2).
6. Distal granules on tergites: do not exceed posterior margin (0), do exceed posterior margin (1).
7. Males with genital papillae with a terminal fleshy, sharp, distinct point present (0), absent (1).
8. Females with basal intermediate lamella of pectines: normal (0), dilated (1).
9. Females with basal pectinal plate with posterior margin: normal (0), expanded (1).
10. Males with whitish patch on sternite III: absent (0), present (1).
11. Males with posteromedian area of sternite III: level (0), raised and granular (1).
12. Males with whitish patches on sternite V: absent (0), one posteromedian, usually oval or heart-shaped (1), two, transverse and oval (2), three, one posteromedian heart-shaped and two smaller laterally (3).
13. Females with whitish patch on sternite V: absent (0), one posteromedian, usually oval or heart-shaped (1), two, conical and widely separated (2), three, one posteromedian heart-shaped and two smaller laterally (3).
14. Females with whitish patch on sternite III: absent (0), present (1).
15. Lateral carinae on sternites IV–VI: absent (0), present [two or four] (1).
16. Respiratory stigmata: long and narrow (0), oval to round (1).

Metasoma

17. Lateral carinae on segment V: absent (0), present (1).
18. Subaculear tubercle: absent (0), smooth spine (1), spinoid with granules (2), conical (3), crest-like, (4), trapezoidal, with two granules (5).

Pedipalps

19. Number of denticle rows on pedipalp fingers: eight (0), nine or ten (1), eleven or twelve (2), thirteen or more (3).
20. Males with basal lobe on movable finger: absent (0), present (1).
21. Supernumerary denticles on fingers: absent (0), present (1).
22. Terminal macrochaeta on fingers: absent (0), present (1).
23. Femoral τ d$_2$: absent (0), present (1).
24. Femoral τ i$_3$: petite (0), normal (1).
25. Femoral τ i$_4$: absent (0), petite (1), normal (2).
26. Patella τ d$_2$: absent (0), present (1).
27. Chela τ Eb$_3$: absent (0), present (1).
28. Fixed finger τ esb: absent (0), present (1).
29. Throughout body, hollow macrochaetae with truncated apex: absent (0), present (1).

Description of *Sarax buxtoni* (Gravely 1915) (Arachnida: Amblypygi: Charinidae) and a new case of parthenogenesis in Amblypygi from Singapore

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Abstract. The type material of *Sarax buxtoni* (Gravely 1915) cannot be located and has to be considered as lost. Therefore, a description compiled from a population in Singapore is provided, including morphological and taxonomical details presented for the first time. Comparisons with closely related species are supplied. Furthermore, we describe the occurrence of parthenogenesis in a population of *S. buxtoni*, representing the first case of asexual reproduction in a member of the genus *Sarax* Simon 1892.

Keywords: Whip spiders, asexual reproduction, Southeast Asia

Amblypygi, popularly called whip spiders, are characterized by their dorso-ventrally flattened body and strong, raptorial pedipalps armed with spines. The first pair of legs is extremely elongated and antenniform. These body appendages serve important multisensory functions and play important roles during mating, hunting, and antagonistic behavior (Weygoldt 2000). According to Prendini (2011), recent Amblypygi currently include five families, 17 genera and 161 species. Harvey (2013) mentioned 186 species and at the last count (Seiter & Hörweg 2013), the group expanded by two newly described species of the genus *Heterophrynus* Pocock 1894 (Giupponi & Kury 2013) and one species of the genus *Phrynus* Lamarck 1801(Armas et al. 2013), elevating the number to 189 species. In Southeast Asia, the whip spider fauna includes four families (Charinidae, Charontidae, Phrynidae and Phrynichidae), with *Sarax* Simon 1892 (Charinidae) being the most diverse genus. Its 17 species are distributed in continental and insular Southeast Asia and Oceania with Papua New Guinea as the most eastern occurrence and India at the most western (Harvey 2003, 2013; Giupponi & Miranda 2012). Harvey (2003) further listed *Sarax mediterraneus* Delle Cave 1986 from Greece which is still included in Harvey (2013) and would, therefore, represent the most western distributed species of the genus *Sarax* Simon 1892. However, Weygoldt (2005) wrote about this doubtful record "[...] Therefore I suppose that somebody confused specimens and labels and erroneously replaced three *Charinus* specimens by three *Sarax* specimens [...]" (Weygoldt 2005: 12–13). Since then, nobody discovered the error and correctly identified these specific specimens, which are held in the SMF (Senckenberg-Museum, Frankfurt am Main, Germany). If *S. mediterraneus* is a valid species, the genus would contain 18 species.

Parthenogenesis in Amblypygi is reported from two species, both belonging to the family Charinidae: *Charinus acosta* (Quintero 1893) and *Charinus ioanniticus* (Kritscher 1959) (Armas 2000, 2005; Weygoldt 2005, 2007). *Charinus acosta* occurs in Cuba and is reported from different places through the country (Teruel 2011). *Charinus ioanniticus* is distributed around parts of the eastern border of the Mediterranean and represents the sole amblypygid occurring in Europe, if the reported occurrence of *S. mediterraneus* is truly due to a

misidentification. The European populations of *C. ioanniticus* are located on the Greek islands of Rhodes, living in subterranean passages of the ancient city of Rhodes, and Kos (Kritscher 1959; Weygoldt 2005). The population on Rhodes is an all-female population that reproduces parthenogenetically (Weygoldt 2007). *Charinus ioanniticus* has also been reported from Turkey (Kovařik & Vlasta 1996; Weygoldt 2005; Seyyar & Demir 2007), Israel (Rosin & Shulov 1960) and Egypt (El-Hennawy 2002), however all these reported populations reproduce sexually and males are present.

Sarax buxtoni (Gravely 1915) was first described under the name *Phrynichosarax buxtoni* with the type locality in Kubang Tiga cave, Perlis, Malaysia. Weygoldt (2000) considered *Phrynichosarax* to be a junior synonym of *Sarax* and transferred *P. buxtoni* to *Sarax*. Harvey (2003) transferred all of the remaining taxa from *Phrynichosarax* to *Sarax*. The diagnostic characters of the family Charinidae and the genus *Sarax* are discussed and revised in Rahmadi et al. (2010). In Singapore, two species of the genus *Sarax* occur: *S. buxtoni* and *Sarax singaporae* Gravely 1911, the latter distributed in Malaysia and Singapore (the type locality is the Singapore Botanic Garden) (Harvey 2003). Weygoldt (2002) described the sperm transfer and the mating behavior of *S. buxtoni* collected in Singapore, but without clear description of the locality ("outskirts of Singapore" mentioned as the collection site). Furthermore, the author used the moderate description and poor figures of Gravely (1911, 1915) to identify the species. The type material of this study could not be found, and the former identification is unreliable because of the incomplete description of *S. buxtoni* by Gravely (1911, 1915). Considering the incorrectly identified material of Weygoldt's study about the sexual reproduction of this species and our data about asexual reproduction in this species, here we provide (i) a detailed description of *Sarax buxtoni* from Singapore and (ii) a report of the first case of parthenogenesis in a *Sarax* species, which is the first known case of asexually reproducing amblypygids in Southeast Asia.

METHODS

Specimens of *Sarax buxtoni* were collected in Singapore, North West District, near Turf Club at 1° 19′ 29.47″N, 103° 47′ 25.97″E in a small park within the city. The specimens were found under an artificial stone cairn next to a small runlet.
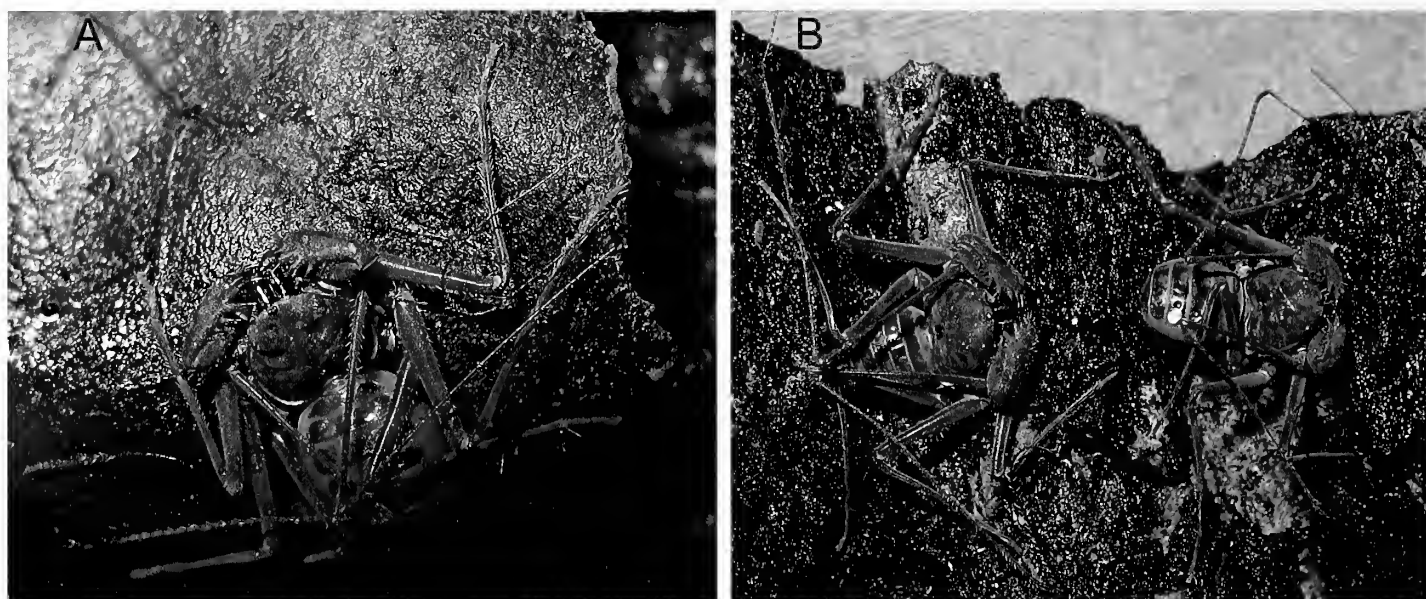
Figure 1.—Photographs of living adult *Sarax* individuals in standard plastic terraria. A: *S. buxtoni*, female. B: *S. singaporae*, female right, male left (NHMW 21893). Note the sexual dimorphism in the length of the pedipalps.

This was the only stony place in an area of one square kilometer. Here, within half of a square meter, many female specimens were found living next to each other, sitting on the underside of stones in a very humid environment, protected from the sun and rain by the vegetation. *Sarax singaporae* was found in similar microhabitats in Singapore, South West District, on the outskirts of Singapore, Jurong Bird Park at 1° 19′ 7.34″N, 103° 42′ 23.19″E. Nevertheless, this species was not found in high densities like *S. buxtoni* and was found to live mainly under stones and also in the leaf litter.

In the laboratory, we reared both species in plastic terraria of different sizes using standard methods. The enclosures contained a 2 cm deep layer of soil and pieces of bark in which the specimens could hide. Food consisted of cricket nymphs, *Acheta domestica* (Linnaeus 1758) and fruit flies, *Drosophila melanogaster* Meigen 1830. We kept all individuals under the same conditions (T = 26–27°C; RH = 65–75%) and fed them at the same intervals every seven days. Offspring were separated just after leaving the backs of the females and were raised under the same conditions as adults. All dead individuals were stored in 70% ethyl alcohol. Specimens were studied, measured and photographed under a stereomicroscope (Leica M205A) equipped with a Leica DFC420 camera, and digital images were processed using Adobe Photoshop 8.0.

The specimens were identified using the key and description of Gravely (1911, 1915) and compared with the voucher material from Weygoldt (2002). Nomenclature of the pedipalpal spines follows Quintero (1983a), modified according to Shultz (1990): pedipalps are divided into trochanter, femur, patella, tibia and tarsus (distitarsus+pretarsus or claw).

Abbreviations.—NHMW = Natural History Museum Vienna, SMF = Senckenberg Museum Frankfurt, SMNS = Staatliches Museum für Naturkunde Stuttgart, leg. = legit (collected), det. = determinavit (determined), syn = synonymized, ♂ = male / ♀ = female,

Material examined.—*Sarax buxtoni:* Holotype of *Sarax batuensis* Roewer 1962: **Malaysia:** 3 ♀, 6 juveniles, Selangor,

Batu caves (in different parts of the cave), 1959/60, leg. H.E. McClure (SMF 9913906 – RII/13906/51 – 68). **Republic of Singapore:** 4 ♀ adult (wild caught), 1 ♀ juvenile (wild caught), Singapore, North West District, near Turf Club, 1° 19′ 29.47″N, 103° 47′ 25.97″E, 14 September 2010, leg. and det. M. Seiter (NHMW 21891);1 ♀ adult (captive bred), 3 ♀ juvenile (captive bred), same data (NHMW 21892).

Sarax singaporae: **Republic of Singapore:** 1 ♂ adult (wild caught), Singapore, South West District, outskirts of Singapore, near Jurong Bird Park, 1° 19′ 7.34″N, 103° 42′ 23.19″E, 14 September 2010, leg. and det. M. Seiter (NHMW 21893); 2 ♀ adult, 1 ♂ adult (wild caught), same data except 2009, leg. S. Huber, det. M. Seiter (NHMW 21894); 3 ♀ adult, 2 ♂ adult, 2 juveniles (wild caught), same data except 27 June 1992, leg. S. Huber, det. M. Seiter (SMNS).

SYSTEMATICS

Family Charinidae Quintero 1986
Genus *Sarax* Simon 1892
Sarax buxtoni (Gravely 1915)
(Figs. 1A, 2–3)

Phrynichosarax buxtoni Gravely 1915: 439–440, Fig. 4; Mello-Leitão 1931: 52 (as *Phrynicosarax* [sic] *buxtoni*); Speijer 1937: 173; Weygoldt 1994: 244.
Sarax batuensis Roewer 1962: 519–520, Figs. 3a–b (syn. by Kraus 1970: 178).
Sarax buxtoni (Gravely): Harvey 2003: 8.

Diagnosis.—*Sarax buxtoni* can be distinguished from the closest geographical and morphological related species *Sarax singaporae* by the following characters: (i) chelicera: dorsum with five fine lateral setae in *S. singaporae* and none in *S. buxtoni* (Figs. 2I, L); (ii) moveable hand on the chelicera: in *S. singaporae* with three highly cuspid teeth not equal in size, instead of equal size and rounded (Figs. 2I, L); (iii) sternum
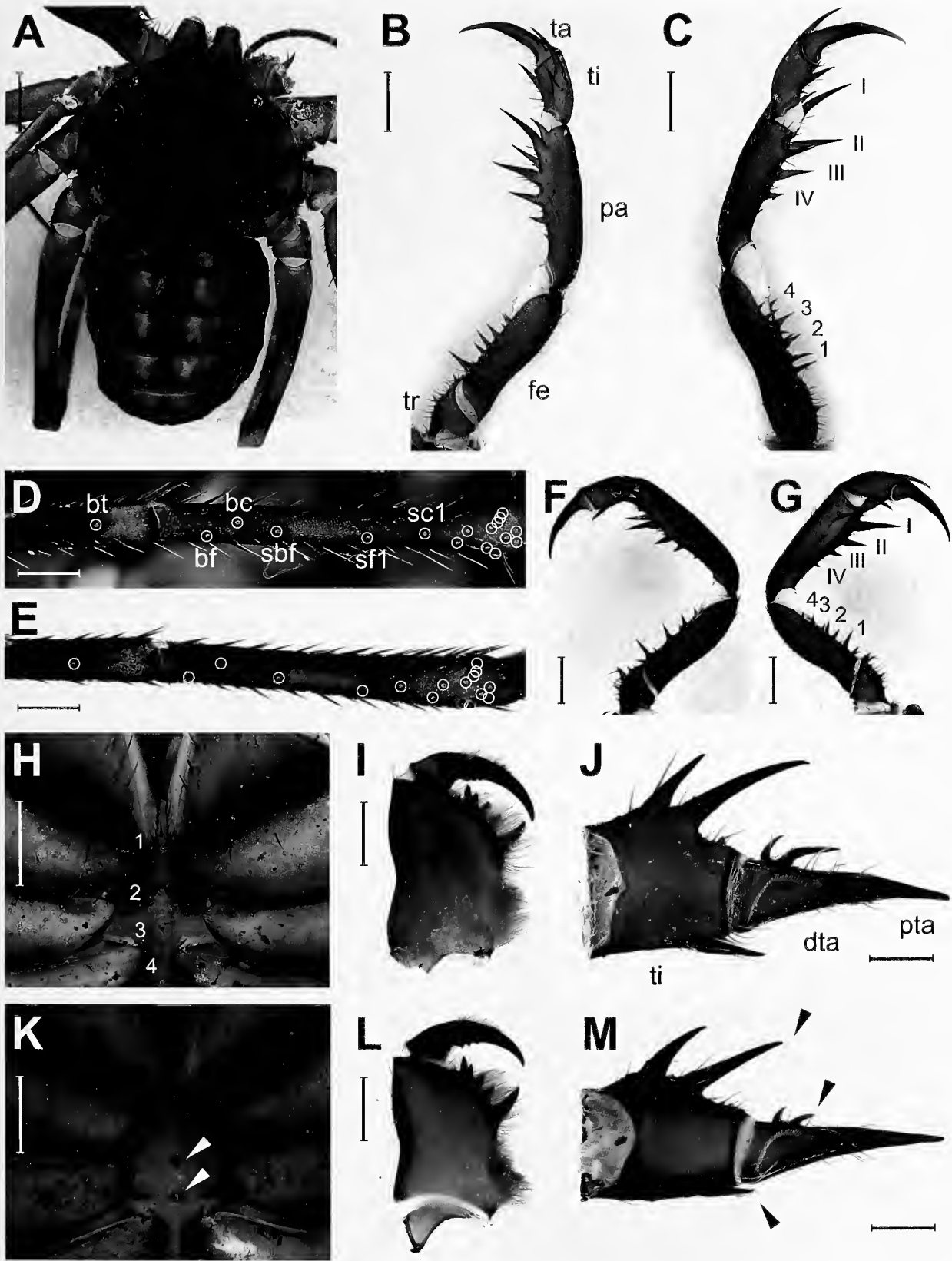
Figure 2.—A–D, H, I, J: *Sarax buxtoni*, female; E–G, K, L, M: *Sarax singaporae* (F, G, L male; K, M female). A: habitus, dorsal. B, F: pedipalp, dorsal. C, G: pedipalp, ventral; dorsal spines of femur and patella numbered, tr: trochanter; fe: femur; pa: patella; ti: ibia; ta: tarsus. D, E: Basitibia and distitibia of walking leg IV, dorsal; trichobothria marked; basitibia (bt = basitibial), distitibia (bf: basofrontal; bc: basocaudal; sbf: subbasofrontal; sc1-x: series caudal and trichobothria, sf1-x: series frontal and trichobothria). H, K: prosoma, ventral; sternae numbered in H. I, L: chelicera. J, M: pedipalp, distal parts, prolateral. Arrowheads indicate diagnostic characters in *S. singaporae*, ti: tibia; dta: distitarsus; pta: pretarsus (claw). Arrowheads indicate diagnostic characters; scale bar: A–C, F, G: 1 mm, D, E, H, I: 0.5 mm.
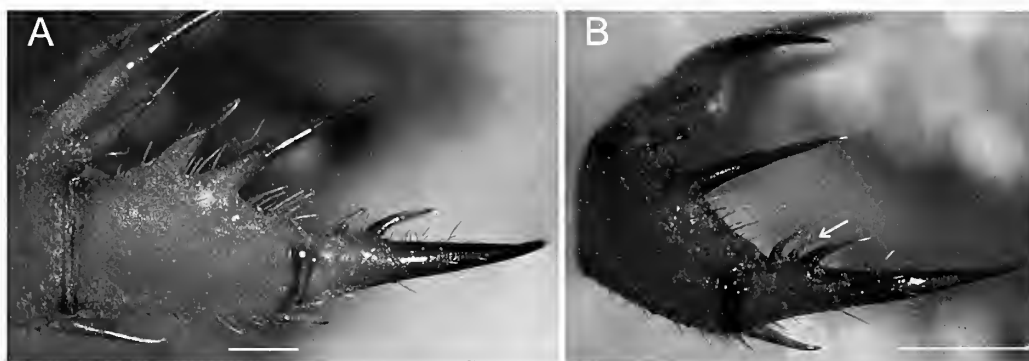
Figure 3.—Images of the distal pedipalp in *Sarax buxtoni*, adult female. Scale bars: 1 mm. A: An individual with normal spination on the right pedipalp tibia and distitarsus (SMF 9913906 – RII/13906/51 – 68). B: Another individual with an anomaly on the right pedipalp, indicated by arrow.

ventrally: with three visible sternites (second and third one rounded with apical paired setae) in *S. singaporae* instead of four (Figs. 2H, K); (iv) pedipalp tibia spination on the antero dorsal margin: proximal spine only 1/3 longer than distal one in *S. singaporae*, and without clear shared basin (Figs. 2J, M); and (v) pedipalp tarsus spination on the antero dorsal margin: about equal in size in *S. singaporae* instead of the proximal one more than half of the length of the distal one (Figs. 2J, M).

Description of adult female (from Singapore).—*Coloration (in alcohol):* Chelicerae, pedipalps and carapace yellowish. Legs light colored (Fig. 2D); *in life:* Pedipalps and carapace light reddish. Opisthosoma light brown with light lines. Legs light brown to reddish.

Carapace (Figs. 1A, 2A): Carapace ratio width to length about 2:1.4; surface finely granulated without setiferous tubercles; median sulcus present with three sulci laterally on each half of the carapace reaching to the edge of the flange; flange wide and bend upward; anterior margin rounded, with six fine large frontal setae and several small ones. Median eyes without setae, tubercle black, arranged more or less in an oval form with prominent fovea; eyes facing antero-laterally.
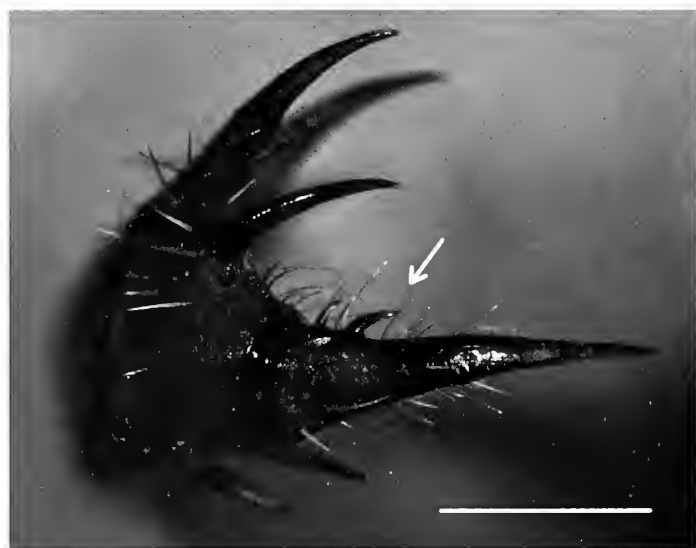
Figure 4.—*Sarax singaporae* (SMNS): adult female, pedipalp distitarsus. Arrow indicates diagnostic character, illustrating the typical spination. Scale bar: 1 mm.

Lateral eyes close to the lateral margin of carapace, distance between lateral eyes about diameter of lateral eye, normal pigmentation. Frontal process triangular, visible from above.

Prosomal sternum (Fig. 2H): First sternite (tritosternum) elongated with paired apical, median and strong basal setae; second sternite less elongated but more than the following ones, with paired apical setae and one median seta; third sternite rounded and flattened with paired apical setae; fourth sternite (metasternum) visible with 1 seta in the middle.

Opisthosoma (Figs. 1A, 2A): Light brown, each tergite with a marginal yellow line, light-brown spots on either side of middle line.

Chelicera (Fig. 2I): Dorsum smooth with one fine frontal seta. Basal segment with four teeth. Lowermost tooth largest, and uppermost tooth is bicuspid, with upper cusp larger than lower one. Outer surface with small blunt tooth opposite bicuspid tooth; moveable hand with three teeth about equal in size.

Pedipalp: short and stout. Trochanter (Figs. 2B, C) with several small setiferous tubercles on antero-dorsal margin, one spine and nine setiferous tubercles ventrally; ventral anterior apophysis equipped with several prominent setiferous tubercles. Femur (Fig. 2B, C) with four major spines antero-dorsally (length **F3 > F1 > F2 > F4**), one minor spine between F2–F3, one minor spine between F3–F4, several setiferous tubercles and small tubercles; femur with four major antero-ventral spines (length **FI > FII > FIII > FIV**), small tubercles present. Patella (Fig. 2B, C), antero-dorsal face with four major spines (length **P1 > P2 > P3 > P4**), with two minor spines, several setiferous tubercles and small tubercles; patella with three major spines (length **PI > PII > PIII > PIV**), several setiferous tubercles and small tubercles. Tarsus (Fig. 2J) with two major spines on antero-dorsal margin, length of proximal spine more than half length of distal one, one minor spine proximally, several setiferous tubercles and small tubercles; antero-dorsal margin with one major spine, several setiferous tubercles and small tubercles; distitarsus (dta) and claw (pta) divided, with two denticles on antero-dorsal margin, proximal denticle more than half length of distal one, distal one more curved towards the base as the proximal one; cleaning organ ventrally with around 30 modified hairs, several blunt setae on inner surface of tarsus, apotele present.

Legs: Tibiae II and III 4-segmented; basitibia IV 3-segmented; fourth segment with *bt* close to distal margin, *bc* in middle of *bf* and *sbf* (Fig. 2D), pulvilli present.

Table 1.—Reproductive events, dated, of consecutively numbered, wild-caught, female *Sarax buxtoni* and, in the cases of #2/1 and #2/2, captive-born female progeny of #2. Shaded entries indicate hatches of individuals from brood sacs produced after the parent had molted. Individuals #5–#9 produced brood sacs but it cannot be guaranteed that these individuals had not been previously inseminated. Most of the captive-hatched offspring died several days after molting, but two of them ultimately produced progeny without first being inseminated (#2/1 and # 2/2). E = brood sac visible; M = molted; H = hatched; nD = no data available.

Female ID	Sequence of events———→			
#1	E 09.12.2011	M 24.03.2011	E 02.06.2011	H 24.07.2011
#2	E 09.12.2011	H 22.01.2011		
#2/1	M 16.04.2011	M 16.07.2011	nD	H 03.01.2014
#2/2	M 22.04.2011	M 30.07.2011	nD	H 12.12.2013
#3	M 02.01.2011	E 03.04.2011	H 25.05.2011	
#4	E 29.10.2010			
#5	E 05.11.2010	H 06.01.2011	E 04.03.2011	
#6	E 13.11.2010			
#7	E 19.11.2010	H 12.01.2011		
#8	E 17.11.2010			
#9	E 09.12.2010			

Genitalia: Covered ventrally with genital operculum slightly concave apically, paired with two tubes projecting medially.

Measurements.—*Largest female (n=1):* Body length 7.29 mm. Carapace length: 2.78 mm, width: 4.07 mm. Median eyes to anterior margin of carapace 0.18 mm. Distance between lateral eyes 2.17 mm. Pedipalps: femur 2.49 mm, patella 2.58 mm, basitarsus 1.28 mm, distitarsus and pretarsus (claw) 1.71 mm.

Remarks.—The largest specimen of the nine specimens of *S. buxtoni* from Batu Cave (SMF 9913906 – RII/13906/51–68) has a notable anomaly. The pedipalp spination has been used extensively for systematic research and is an important character. Therefore the special spination of the right tarsus should be mentioned (Fig. 3B). Usually *S. buxtoni* has two spines on each distitarsus: large and conspicuous, the distal one about twice as long as the proximal one, the distal one is more curved near its base than is the proximal one (Fig. 3A). This especially large female bears three spines on the right pedipalp finger instead of two. The distal spines are about twice as long as the proximal one and the intermediary spine is one fifth smaller than the proximal one (length **TIII > I > II**). All three spines are equally curved. The rest of the spination is similar to the Singapore all-female population described here.

Parthenogenesis.—Nine adult females were used for this study. All of them produced at least one brood sac but only three of them can be guaranteed not to have been inseminated prior to brood sac production (Table 1). However, the possibility that only females were selectively caught is very low and can be disregarded. Three of the wild caught females molted and then produced a fertile brood. Of the hatching praenymphs (Table 1: #1, #2, #3), two individuals reached adulthood and reproduced independently, completely isolated from other individuals since birth. It should be mentioned, that several brood sacs were dropped and eaten by the females over time. Many of the praenymphs died during the first days, or did not eat *Drosophila* or small cricket nymphs.

DISCUSSION

The following discussion is subdivided into the three major parts of this paper.

Description.—Gravely (1911) reported the discovery of a new subspecies of *Sarax sarawakensis*: *S. s. singaporae*, from Singapore. Later Gravely (1915) elevated this taxon to species rank under the generic name *Phrynichosarax singaporae*. In the same paper, based on two individuals (one adult female, one immature), Gravely (1915) described a new species, *Phrynichosarax buxtoni*, with the type locality in Kubang Tiga cave, Perlis, Malaysia. The original description is rather basic with a poor figure of the distitarsus spination intended to distinguish it from other species. For the description of *S. buxtoni*, we wanted to guarantee the validity and acceptance of the specimen used here. However, since the type specimen cannot be located, we decided to provide a detailed description. As the type locality is located in mainland Malaysia, we have limited our description to our specimen from Singapore. Here, we present for the first time a complete description of *S. buxtoni* with a demonstration of basic differences from the closely related *S. singaporae*.

Parthenogenesis.—Parthenogenesis is well known among arachnids, including: mites (Acari), harvestmen (Opiliones), true spiders (Araneae: Araneomorphae), pseudoscorpions (Pseudoscorpiones) and scorpions (Scorpiones). However, so far, parthenogenesis in whip spiders has only been reported in two species: *Charinus acosta* and *C. ioanniticus* (Armas 2000; Weygoldt 2007). It is reported that during molting whip spiders lose all stored spermatozoa (Weygoldt 1999). Yet to insure that sperm storage during molting events can be ruled out, we raised *S. buxtoni* specimens for two generations isolated from one another. Based on the observation of newly collected female specimens raised in captivity for two generations, we found that *S. buxtoni* is capable of parthenogenetic reproduction. The description of sexual reproduction in *S. singaporae* [misidentified by Weygoldt (2002) as *S. buxtoni*] from Singapore is now established. We argue that the specimens used by Weygoldt (2002) were wrongly identified based on our diagnosis above (Fig. 4). So the former described sexual behavior in this study belongs to *S. singaporae* and not *S. buxtoni*. Thus there is no male known from *S. buxtoni* populations because the type material cannot be found and is unavailable for study. However, our data do not allow us to determine whether this population is facultatively or obligately reproducing asexually. The type material consisted of two specimens: one adult female and one immature specimen not sexed. This sample size is rather low, though we cannot say if

the type locality is also a parthenogenetic population or not. Nevertheless, it could be possible that the population described here is facultatively reproducing asexually with a low prevalence or absence of males. Because of the location of the presumed all-female population in a small park, completely isolated by the city and concrete roads, a possible restriction to parthenogenetic reproduction could be comparable to the "insular parthenogenesis" described by Cuellar (1977, 1994).

Testing if females from this population can reproduce with males from other populations would verify if these females are obligately parthenogenetic or not. Conversely, it would be interesting to check if isolated females of other *S. buxtoni* populations, in which both sexes occur, are able to give birth without insemination to determine if facultative parthenogenesis is a common trait of *S. buxtoni*. Of interest is the fact that many of the praenymphs died during the first few days after emergence. We can argue that it is usually very tricky to raise and breed such small species over several generations, so this case is unlikely to be associated directly with possible deficiency caused by all-female brood resulted from non-fertilized eggs (as it is usual for thelytokous parthenogenesis).

Anomaly.—A similar case of asymmetrical spine transformation in the genus *Sarax* was yet unknown and, therefore, can be considered as very uncommon. Only a few asymmetries and anomalies are documented in recent literature, e.g. *Paraphrynus aztecus* (Pocock 1894) (as *P. azteca* [sic]) has bifid spines (Quintero 1983b). Here an adult male from Oaxaca, Mexico exhibited a transformation of spines III and V of the right pedipalp patella into bifid apophyses. In contrast the left pedipalp showed normal spination. Rowland (1973) reported an unidentified *Paraphrynus* Moreno 1940 species from Mexico with different length of spines on the pedipalp. Another case was documented by Baptista & Giupponi (2002) where asymmetry in the number of pseudo-articles of the basitibia in *Charinus troglobius* Baptista & Giupponi 2002 (four in general, but five in the right leg of two males) occurred. Armas & González (2001) showed different examples of anomalies in the pedipalps of *Phrynus eucharis* Armas & González 2001, *P. hispaniolae* Armas & González 2001 and *P. marginemaculatus* C.L. Koch 1840 from the Dominican Republic. The first author (MS) observed a *Paraphrynus* species, most likely *P. mexicanus* (Bilimek 1867) from Mexico, with bifid spines similar to the documented single *P. aztecus* specimen. Nevertheless, the anomaly observed in the *S. buxtoni* specimen is very uncommon and even deviates from the basic definition of the genus *Sarax*: pedipalp tibia with two spines, the distal one larger than the proximal one (Fig. 3B).

ACKNOWLEDGMENTS

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The new spider genus *Arctenus*, an afrotropical representative of the Calocteninae (Araneae: Ctenidae)

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Abstract. *Arctenus* gen. nov. is proposed to include the type species *A. taitensis* sp. nov. from the Taita Hills in Kenya. This ctenid species appears to be the first representative of the Calocteninae in the African continent. Results of a parsimony analysis of morphological and behavioral characters indicated that the new species cannot be placed in any known genus and therefore validated the creation of the new genus whose autapomorphies are considered hypotheses for the genus synapomorphies. The phylogenetic relationships of the new genus are discussed and a distribution map of the unique species is presented.

Keywords: Kenya, systematics, Taita Hills, taxonomy, cladistic analysis, phylogenetic analysis

The family Ctenidae Keyserling 1977 is composed of small to large sized spiders (total body length of 4–40 mm), which do not build a snare web to catch prey. They are wandering and active predators, usually found in the litter layer, on tree trunks and in lower vegetation. Most of them are nocturnal, hiding during the day in the litter or in small cracks in the soil or on tree trunks. To date, the family comprises more than 480 described species in 40 genera (Platnick 2014) and are distributed mostly in tropical and temperate forests all over the world. Ctenidae can be diagnosed by the ocular arrangement 2-4-2 (Silva 2003).

The Afrotropical region holds 132 Ctenidae species, distributed in ten genera: *Africactenus* Hyatt 1954, *Anahita* Karsch 1879, *Apolania* Simon 1898, *Ctenus* Walckenaer, 1805, *Petaloctenus* Jocqué & Steyn 1997, *Thoriosa* Simon 1910, *Trogloctenus* Lessert 1935, *Viridasius* Simon 1889 and *Vulsor* Simon 1889 (Platnick 2014). *Ctenus* contains the largest number of species (more than 70). The recent redescription of the Neotropical type species, *Ctenus dubius* Walckenaer 1805, by Brescovit & Simó (2007), and results of several cladistic analyses (Silva 2003; Polotow & Brescovit 2009, 2014) indicated that the genus is polyphyletic as currently delimited.

Recent collecting expeditions in the Kenyan Taita Hills, the northernmost part of the Eastern Arc, yielded several specimens identified as Ctenidae. The species was mentioned by Jocqué (2009) as a possible member of the genus *Pseudoctenus* Caporiacco 1949, but that genus proved to belong to the Zoropsidae Bertkau 1882.

The specimens collected in the Taita Hills cannot be assigned to any of the Afrotropical Ctenidae genera. The presence of several elongated spines on tibiae and metatarsi I and II and the absence of a pair of terminal spines on tibiae I and II suggested a relationship of the Taita Hills species with *Africactenus*, *Anahita*, or *Petaloctenus*. However, the diagnostic characters of the male palp and epigynum of these three genera prove otherwise. So far, only five Ctenidae species have been described from Kenya: *Ctenus elgonensis* Benoit 1978 (Benoit 1978: Fig. 2a–c), *C. holmi* Benoit 1978 (Benoit 1978: Fig. 3a–b), *C. kenyamontanus* Benoit 1978 (Benoit 1978: Fig. 1a–c), *C. modestus* Simon 1897 (Benoit 1978: Fig. 3c; Benoit 1979: Fig. 24) and *C. noctuabundus* Arts 1912 (Benoit 1979: Fig. 10). The species collected in the Taita Hills is clearly different from all these type specimens.

Here we describe this species and include it in the most recent cladistic analysis based on morphological characters of Ctenidae (Polotow & Brescovit 2014), to test the relationships with the remaining species of the family. As a result, we propose a new genus, *Arctenus* gen. nov., to accommodate *Arctenus taitensis* sp. nov., and we discuss its phylogenetic placement in Ctenidae.

METHODS

Morphological observations and illustrations were made using Wild M10 and M5 microscopes. Photographs of the habitus were taken with a Leica MZ16 binoclar microscope using the LAS automontage software. For SEM, specimens were cleaned ultrasonically, gold coated, and then examined and photographed with a JEOL 6480 LV scanning electron microscope at the Royal Museum for Central Africa, Tervuren, Belgium (MRAC). We detached the epigynum from the abdomen and submerged it in methyl salicylate to clear the internal structures. All measurements are in millimeters. The material examined belongs to the MRAC (curator R. Jocqué).

The morphological matrix comprises 72 species and 89 characters described in detail in Polotow & Brescovit (2014). For the present analysis, we added one terminal taxon: *A. taitensis* sp. nov., male and female from Mbololo Forest, Taita Hills, Kenya, VI.1999, D. Van den Spiegel coll. (MRAC 228739). Mesquite, version 2.75 (Maddison & Maddison 2011) was used to build and edit the character matrix. Non-applicable and unknown states are presented as '–' and '?', respectively. All characters were equally weighted and all multistate characters were coded as non-additive. Character coding for the new species was as follows:

Arctenus taitensis: 00001001100100011100011000000000–000000121100000000011000001101050110002314100000001?1????0?

The parsimony analysis was performed with the same methodology described in Polotow & Brescovit (2014). The Diva-GIS version 5.2.0.2 (http://www.diva-gis.org) was used to make the maps.

The following abbreviations were used: ALE, anterior lateral eyes; AME, anterior median eyes; C, conductor; CD, copulatory ducts; CO, copulatory opening; Cy, cymbium; E,
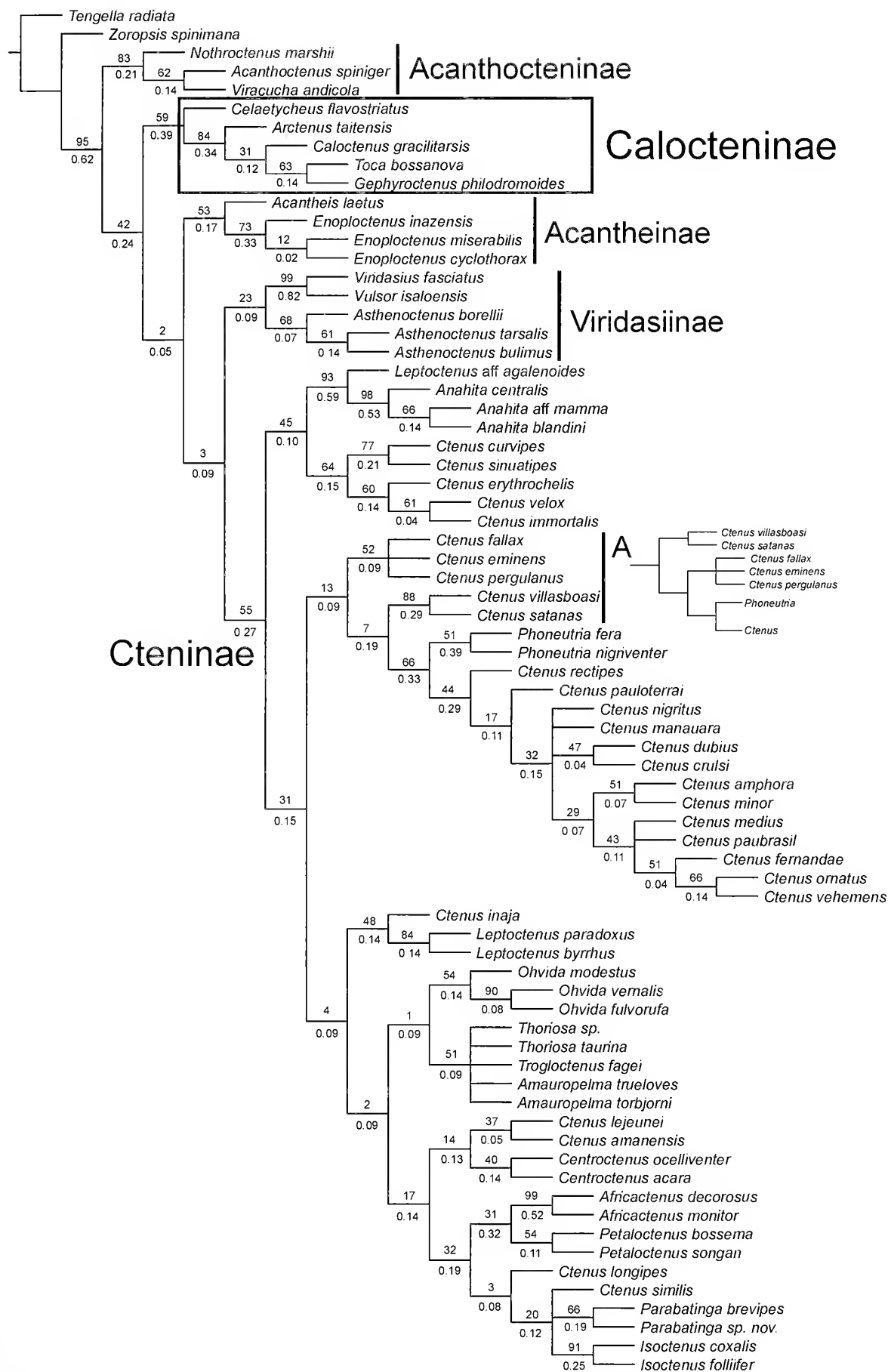
Figure 1.—Consensus tree under implied weights for constant of concavity k=3. Rectangle shows Cteninae clade. Support values for groups expressed as GC frequency differences (top) and Bremer support in units of fit × 100 (bottom).
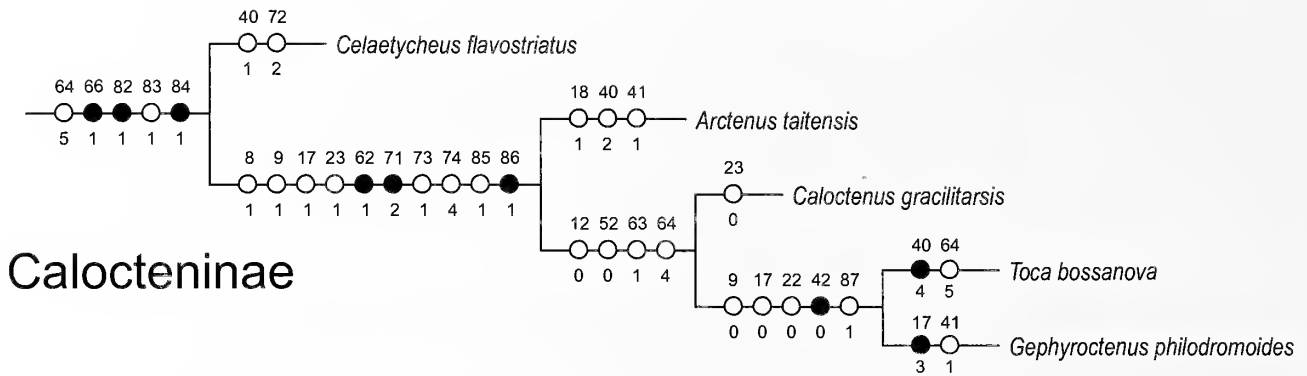
Figure 2.—Calocteninae clade of the consensus tree under implied weights for constant of concavity k=3. Character changes mapped on branches. Black circles indicate non-homoplastic synapomorphies. White circles indicate homoplastic synapomorphies.

embolus; FD, fertilization ducts; LP, lateral process; LS, lateral sector; MA, median apophysis; MS, median sector; MTP, membranous tegular process; PLE, posterior lateral eyes; PME, posterior median eyes; RCP, retrolateral cymbial process; RTA, retrolateral tibial apophysis; S, spermathecae; TF, transversal furrow; Ti, tibia; VTA, ventral tibial apophysis; VTP, ventral tibial process.

PHYLOGENETICS

The parsimony analysis under equal weight resulted in 141 most parsimonious trees, with 235 steps (CI = 50; RI = 83). In the strict consensus, 14 nodes collapsed, resulting in a tree with 295 steps (CI = 40; RI = 75). The implied weighting analyses with concavity values from 1 to 6 were performed in the data set, and we obtained the same two trees in each analysis, with 235 steps (CI = 50; RI = 83). The strict consensus of the two trees obtained by the concavities analysis resulted in one collapsed node and the same tree of 236 steps (Fig. 1; CI = 50; RI = 83).

These results are congruent with the phylogeny of Polotow and Brescovit (2014), except for the position of two clades at the base of the clade F (Polotow & Brescovit 2014: Fig. 3), with the clade formed by *Ctenus fallax* Steyn & Van der Donckt 2003, *C. eminens* Arts 1912, and *C. pergulanus* Arts 1912 in the basal part of the clade (Fig. 1A). *Arctenus taitensis* sp. nov. appears as a representative of Calocteninae, sister group of the clade formed by *Caloctenus* Keyserling 1877, *Toca* Polotow & Brescovit 2009 and *Gephyroctenus* Mello-Leitão 1936 (Fig. 1). The strict consensus of the two trees obtained by the implied weighting analysis with k=3 was chosen as the working hypothesis and these results are described below (Fig. 1). Here, we describe only the phylogenetic relationships of the Calocteninae Simon 1897 clade (Fig. 2). For detailed results of the remaining subfamilies see Polotow and Brescovit (2014).

Calocteninae (Fig. 2) is supported by three non-homoplastic synapomorphies: labium wider than long (character 66), reduced posterior median spinnerets (character 82) and presence of a row of thick anal setae (character 84). This clade is also supported by two homoplastic synapomorphies: presence of five retromarginal teeth (character 64) and posterior median spinnerets with three or fewer cylindrical gland spigots (character 83). *Celaetycheus* Simon 1897 appears as the basal clade, sister group of the remaining caloctenines (Fig. 2) and is supported by two homoplastic synapomorphies: conductor laminar and folded (character 40) and five pairs of ventral spines on tibia I and II (character 72). The clade formed by *Arctenus* gen. nov., *Caloctenus*, *Gephyroctenus* and *Toca* is supported by three non-homoplastic synapomorphies: reduced ALE lenses (character 62), the presence of three or more prolateral spines on femur I (character 71) and presence of leaf-shaped setae on the

Figures 3, 4.—*Arctenus—taitensis* sp. nov.: 3. Habitus; 4. Frontal view of the carapace. Scale bars = 1 mm.

abdominal dorsum (character 86). The clade is also supported by seven homoplastic synapomorphies: presence of ventral tibial apophysis (character 8), bifid RTA (character 9), median retrolateral cymbial process (character 17), embolus fixed by membranous region (character 23), distal pair of spines on tibia I at a distance from the apical margin of the tibia (character 73), presence of four or more ventral spines on metatarsus I and II (character 74), and presence of modified abdominal setae (character 85).

Arctenus gen. nov. appears as sister group of the clade formed by *Caloctenus*, *Gephyroctenus* and *Toca*. *Arctenus taitensis* sp. nov. presents three homoplastic autapomorphies: cymbium with scopulae (character 18), conductor laminar, wider than long (character 40) and presence of a membranous tegular process (character 41). *Arctenus* is the first representative of the Calocteninae in the African continent.

The clade formed by *Caloctenus*, *Gephyroctenus* and *Toca* is supported by four homoplastic synapomorphies: loss of ventral tibial process (character 12), loss of lateral sector processes of epigynum (character 52), cephalothorax divided into a *pars thoracica* and a *pars cephalica* by a V-shaped depression (character 63), and four retromarginal teeth (character 64).

The *Caloctenus* clade is supported by the absence of a membrane connecting the embolus and tegulum (character 23). The sister group relation of *Gephyroctenus* and *Toca* is based on the unique single folded epigynum configuration (character 42) and four homoplastic synapomorphies: conical or rounded retrolateral tibial apophysis (character 9), retrobasal cymbial process (character 17), cylindrical embolus (character 22) and abdominal dorsum with club-shaped setae (character 87).

The *Gephyroctenus* terminal branch is supported by the presence of a unique retrolateral cymbial process, covering the retrolateral surface as a laminar process (character 17) and a homoplastic membranous tegular process (character 41). The terminal branch formed by *Toca* species is supported by a unique conductor, partially covering the tegulum (character 40) and the presence of five retromarginal teeth (character 64).

TAXONOMY

Ctenidae Keyserling 1877
Calocteninae Simon 1897
Arctenus new genus

Type species.—*Arctenus taitensis* sp. nov.

Etymology.—The generic name is a combination of "arc," referring to the Eastern Arc Mountains, and "*Ctenus*."

Diagnosis.—Males of *Arctenus* gen. nov. can be distinguished from the other Calocteninae by the long hairs on the base of the RTA, the large and thick embolus with a subdistal projection and bifid tip, and presence of a dorsal cymbial scopula (Figs. 11,12) on the male palp. Females of *Arctenus* gen. nov. can be distinguished from the remaining Calocteninae by the median field with an anterior transverse furrow (Fig. 13).

Description.—Ecribellate ctenids. Total body length (males and females) 5.90–7.20. Carapace pale brown with longitudinal lighter stripe from eyes to posterior carapace margin; chelicerae, labium, endites, sternum and legs pale brown;

chelicerae with longitudinal dark markings and femur of legs with dark spots (Figs. 3,4); posterior median and lateral eyes on black tubercles (Fig. 4). Dorsum of abdomen with longitudinal white stripe (Fig. 3), venter pale brown. Eyes arranged in ctenoid pattern, 2-4-2 (Fig. 4). Chelicerae with five retromarginal teeth (Fig. 5) and three promarginal teeth. Labium short, wider than long. Fovea short, positioned in posterior third of carapace. Tarsal claws with eight teeth, four proximal teeth short and four distal teeth elongated and slight sinuous (Fig. 9). Trichobothrial base with two transversal grooves (Fig. 7). Tarsal organ rounded, projecting, with drop-shaped aperture (Fig. 8). Legs I and II with numerous pairs of elongated spines on femur, tibia, and metatarsus. Trochanters slightly notched. Abdomen oval. Male palp: tibia with RTA, ventral tibial process and additional ventral tibial projection; RTA with two distal projections and elongated hairs at base; cymbium with retrolateral median projection and dorsal scopulae; subtegulum prolateral; median apophysis hook-shaped; embolus with subdistal projection and bifid tip; hyaline projection at base of embolus; conductor short, its tip covering embolus (Figs. 6,11,12). Epigynum: divided into median field and two lateral fields; median field with anterior transverse furrow; lateral field with short lateral process; broad copulatory ducts and spermathecae rounded, situated posteriorly; fertilization ducts short, emerging from base of spermathecae (Figs. 13,14). The specimens were found with an epigynal plug covering the copulatory opening (Fig. 10).

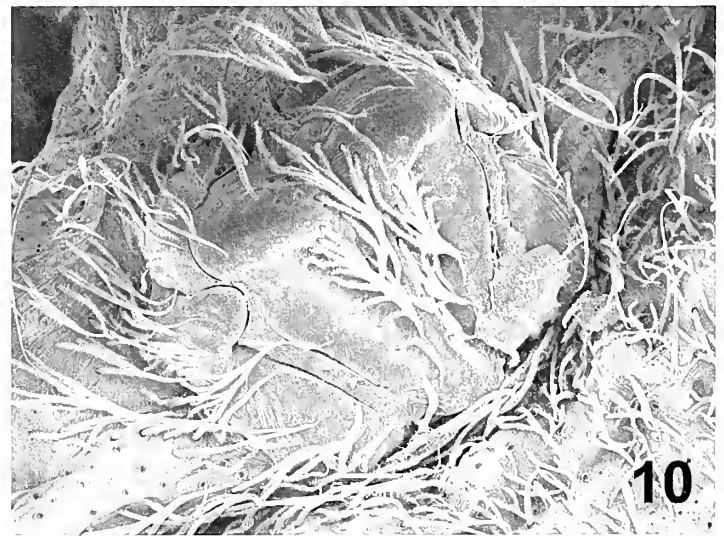**Composition.**—Only the type species, *Arctenus taitensis* sp. nov.
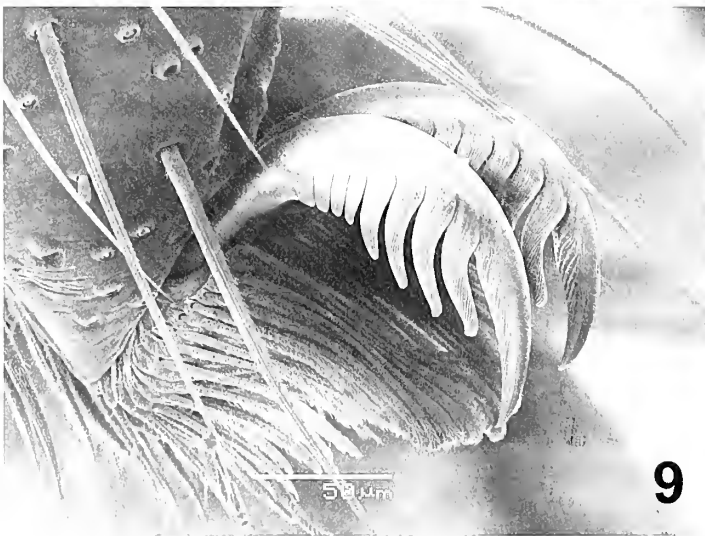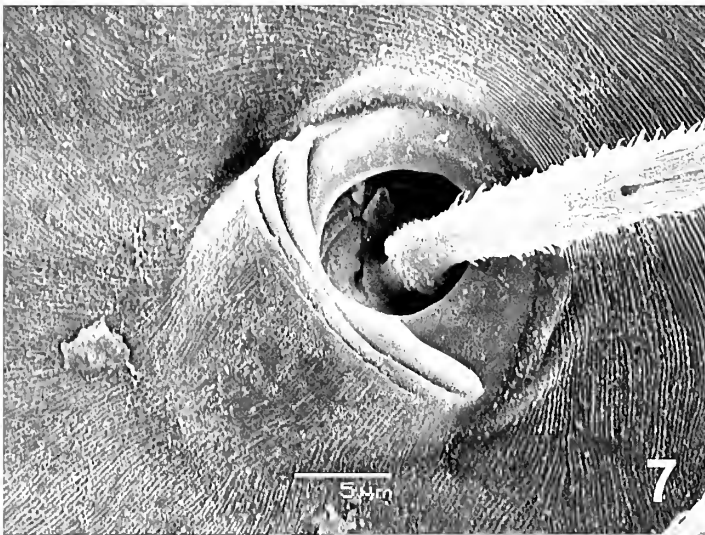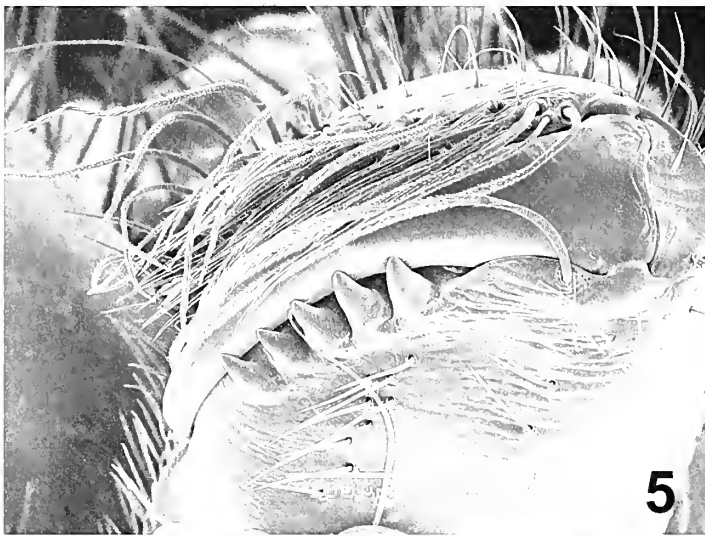
Distribution.—Kenya (Figs. 15,16). The calculated expected distribution of the species (Diva GIS) is restricted to the Taita Hills. Extensive collections in other parts of the Eastern Arc (Usambara, Ulugura and Uzungwa Mts., mainly in the Zoological Museum of the University of Copenhagen, courtesy of N. Scharff) did indeed not reveal the presence of the species there.

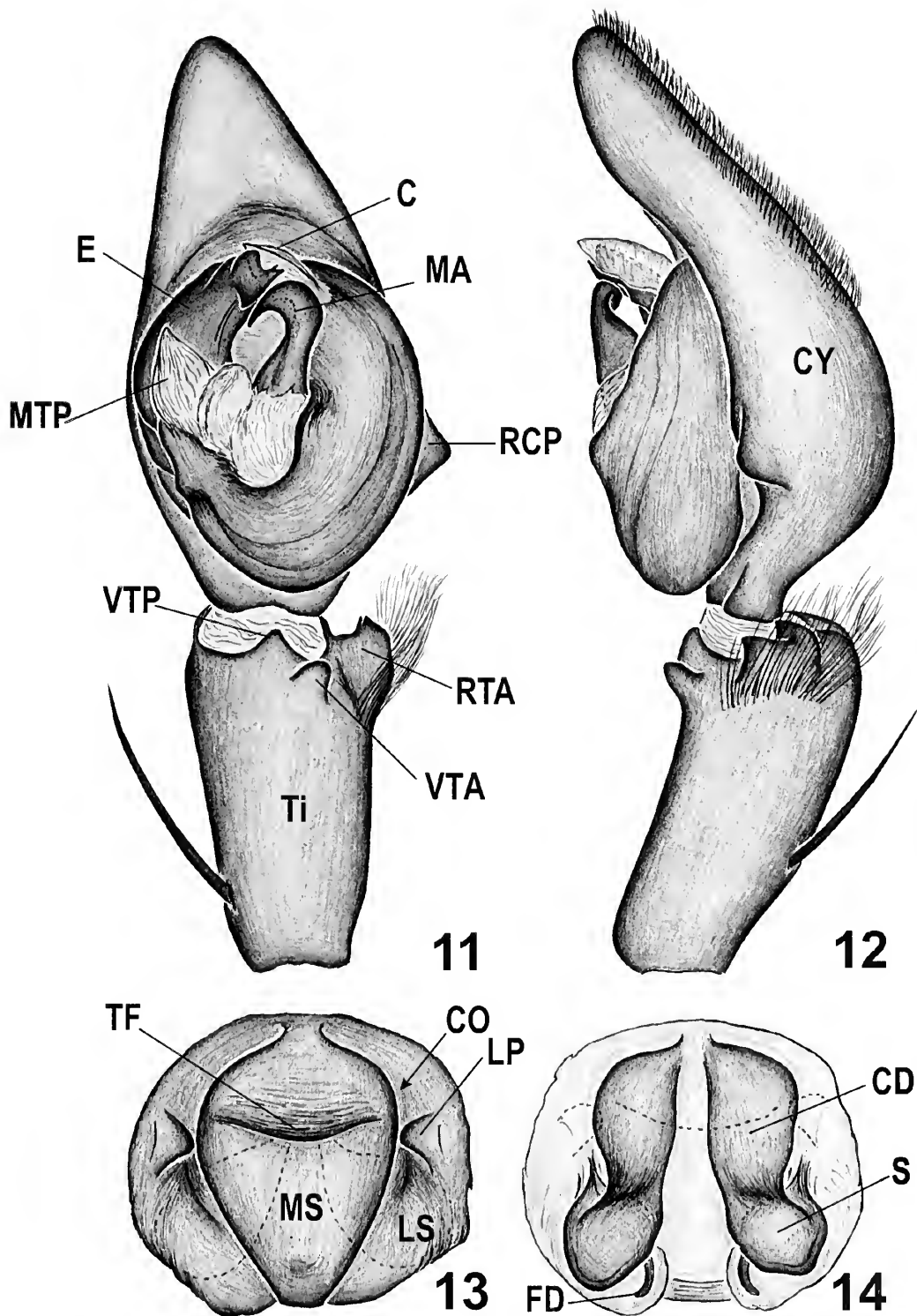### *Arctenus taitensis* new species
Figs. 3–16

Type material.—Male holotype from Mbololo Forest, Taita Hills (1580 m), 03°19′S 38°27′E, Kenya, 22.VI.1999, D. Van den Spiegel coll., (MRAC 208839); female paratype from Chawia Forest, Taita Hills (1850 m), 02°29′S 38°29′E, Kenya, 7.XII.1999, D. Van den Spiegel & J.P. Michiels coll., deposited in MRAC 209161; male and female paratypes from the same locality as the holotype (1800–1900 m), 23.VI.1999, D. Van den Spiegel coll. (MRAC 228739).

Additional material examined.—KENYA. Coast Province: Taita Taveta District, Taita Hills, Mbololo Forest, 03°19′S 38°27′E, 4 females, 23.VI.1999, D. Van den Spiegel coll. (MRAC 208808); Ngangao Forest, 03°20′S 38°22′E, 1 female, 19.VI.1999, D. Van den Spiegel coll. (MRAC 208813); Same locality, 1 female, 17–18.VI.1999, D. Van den Spiegel coll. (MRAC 208831); Same locality, 1 female, 19.VI.1999. D. Van den Spiegel coll. (MRAC 208840); Same locality, 2 females, 24.III.2000, C. Warui & R. Jocqué coll. (MRAC 209568); Fururu Forest, 1 female, 9.XII.1999, D. Van den Spiegel & J.P. Michiels coll. (MRAC 209160); Taita Discovery Center, 03°25′S 38°46′E, 1 female, 27.III.2000, C. Warui & R. Jocqué coll. (MRAC 209546).

Figures 5–10.—*Arctenus taitensis* sp. nov.: 5. Left chelicera, detail of the five teeth on retromargin; 6. Male right palp; 7. Trichobothrium, female, tarsus I; 8. Tarsal organ, female, tarsus I; 9. Tarsal claws, male, leg II; 10. Epigynum, with epigynal plug.
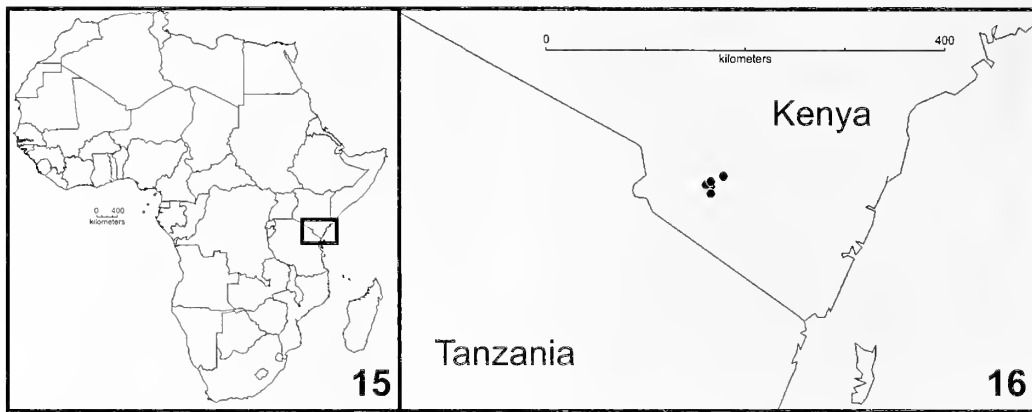
Figures 11–14.—*Arctenus taitensis* sp. nov.: 11–12. Male left palp; 11. Ventral view; 12. Retrolateral view; 13–14. Epigynum; 13. Ventral view; 14. Dorsal view. Abbreviations: C, conductor; CD, copulatory duct; CO, copulatory opening; Cy, cymbium; E, embolus; FD, fertilization ducts; LP, lateral process; LS, lateral sector; MA, median apophysis; MS, median sector; MTP, membranous tegular process; RCP, retrolateral cymbial projection; RTA, retrolateral tibial apophysis; S, spermatheca; TF, transverse furrow; VTA, ventral tibial apophysis; VTP, ventral tibial process.

Etymology.—The species epithet is an adjective derived from the type locality.

Diagnosis.—As for the genus.

Description.—*Male* (MRAC 208839): Total length 5.90. Carapace 2.90 long and 2.50 wide. Clypeus 0.11 high. Eye diameter: AME 0.15, ALE 0.12, PME 0.20, PLE 0.23. Leg measurements: I: femur 3.70/ patella 1.10/ tibia 3.95/ metatarsus 4.10/ tarsus 2.00/ total 14.85; II: 3.60/ 1.20/ 3.50/ 3.50/ 1.40/ 13.20; III: 3.20/ 1.10/ 2.70/ 2.90/ 1.10/ 11.00; IV: 3.90/ 1.10/ 3.30/ 4.30/ 1.45/ 14.05. Leg formula: 1423. Leg

Figures 15, 16.—Distribution map of *Arctenus taitensis* sp. nov. 15. African continent; 16. Detail of southern Kenya and northeast Tanzania.

spination: tibia I with eight pairs of ventral spines; tibia II with seven pairs of ventral spines; metatarsi I and II with five ventral pairs of spines; tibia III–IV 2-2-2v 1-1p 1-1r; metatarsi III–IV 2-2-2v 1-1-1p 1-1-1r. Coloration and palp: as in genus description.

Female (MRAC 228739): Total length 7.20. Carapace 3.10 long and 2.60 wide. Clypeus 0.14 high. Eye diameter: AME 0.18, ALE 0.12, PME 0.28, PLE 0.28. Leg measurements: I: femur 3.00/ patella 1.30/ tibia 3.00/ metatarsus 2.60/ tarsus 0.95/ total 10.85; II: 3.00/ 1.30/ 2.60/ 2.30/ 0.90/ 10.10; III: 2.60/ 1.10/ 2.10/ 2.30/ 0.90/ 9.00; IV: 3.20/ 1.00/ 2.60/ 3.20/ 1.15/ 11.15. Leg formula: 4123. Leg spination: tibia I and II with eight ventral pairs of spines; metatarsi I and II with five ventral pairs of spines each; tibia III 2-2-2v 1-1p 1-1r; tibia IV 2-1-2v 1-1p 1-1r; metatarsi III–IV 2-2-2v 1-1-1p 1-1-1r. Coloration and epigynum: as in genus description.

Distribution.—Kenya (Figs. 15,16).

DISCUSSION

The results indicate that *Arctenus taitensis*, from East Africa, is closely related to the Neotropical Calocteninae spiders, in a well supported clade (Fig. 1). Here we describe *Arctenus taitensis* as the first true Calocteninae from the African continent, although there is currently another species described from Ethiopia, *Caloctenus abyssinicus* Strand 1917, which was placed as *incertae sedis* within Ctenidae by Silva (2004: 13). The type specimen is lost and the original description (Strand 1917: 41) is based on an immature female, with somatic features unusual for the family. Another species, described from the Seychelles islands, *Apolania segmentata* Simon 1898, is also regarded as belonging to the Calocteninae according to Silva (2003: 30). Until the identity of *Caloctenus abyssinicus* is revealed, *Arctenus taitensis* and *Apolania segmentata* remain the only two Afrotropical Calocteninae species.

The majority, 24 out of the currently 32 species of Calocteninae (in seven genera, *Caloctenus*, *Gephyroctenus*, *Toca*, *Apolania*, *Diallomus* Simon 1897, *Celaetycheus* and *Arctenus*), were described in the last 10 years and most of the specimens were collected recently (Silva 2004; Polotow & Brescovit 2008, 2009, 2013). This is remarkable, as the shelf life between discovery and description of new species is on average 21 years (Fontaine et al. 2012, Miller et al. 2014), and

because it concerns medium sized to large spiders. It shows that at least the Neotropical and Afrotropical regions, from which these animals originate, have only superficially been inventoried even for larger invertebrates. This is particularly true for members of the family Ctenidae and *a fortiori* for the subfamily Calocteninae. Since these spiders are strictly nocturnal they were overlooked for a long time (Steyn et al. 2002). Only in recent inventories that made use of pitfalls but mainly of headlamps for night collecting, have these spiders become common in collections. That Calocteninae appear to be rare and are apparently restricted to areas with characteristics of refuges (Seychelles and Eastern Arc for Africa), is concordant with their basal position in the phylogeny of the family (Polotow & Brescovit 2014).

ACKNOWLEDGMENTS

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Manuscript received 14 April 2014, revised 17 June 2014.

Chemical defenses in the opilionid infraorder Insidiatores: divergence in chemical defenses between Triaenonychidae and Travunioidea and within travunioid harvestmen (Opiliones) from eastern and western North America

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Abstract. Live whole specimens of two species of the harvestman Superfamily Travunioidea Absolon & Kratchovíl 1932 from the eastern United States, eight species from the western United States, six morphospecies of the family Triaenonychidae Sorensen 1886 from New Zealand, and specimens of the phylogenetically early-diverging North American triaenonychid *Fumontana deprehendor* Shear 1977 were extracted in methanol, and the solvent analyzed for components from their defensive secretions. The components were then mapped on a recent phylogeny of the taxa. In both eastern cladonychiid species, *Erebomaster flavescens* Cope 1872 and *Theromaster brunneus* (Banks 1902), the major component found was anabaseine, an alkaloid related to nicotine. In the western species, *Paranonychus brunneus* (Banks 1893), *Cryptomaster leviathan* Briggs 1969, *Speleomaster lexi* Briggs 1974, *S. pecki* Briggs 1974, *Speleonychia sengeri* Briggs 1974, *Metanonychus idahoensis* Briggs 1971, *Briggsus flavescens* (Briggs 1971) and *Sclerobunus nondimorphicus* Briggs 1971, the major component was N,N-dimethylphenylethylamine, implying that the travunioids from the two regions represent different phyletic lines. The secretions of the triaenonychid species, members of the genera *Soerensenella* Pocock 1903 and *Nuncia* Loman 1902, were dominated by 4-methyl-3-hexanone, and that of *F. deprehendor* by phenol. The completely different chemistry of the two taxa, Travunioidea and Triaenonychidae, implies significant phylogenetic differences, and the presence of phenol in *F. deprehendor* may suggest a long period of separate evolution for this species.

Keywords: Nicotine, benzothiazole, 2-3′ dipyridyl, salicyl alcohol, mellein, N,N-dimethylphenylethylamine, 4-methyl-3-hexanone

Harvestmen, arachnids of the order Opiliones (also known in North America as daddy-long-legs) defend themselves chemically with secretions from paired glands in the prosoma, which open through pores on either side of the body. Information on the chemical composition of these secretions has accumulated since the initial studies of Estable et al. (1955) that identified gonyleptidine, the first defensive substance from a harvestman to be chemically determined. Developments in the field have been ably summarized in a chapter by Gnaspini & Hara (2007), which revealed that research on defensive chemistry in Opiliones has focused disproportionally on South American gonyleptids and their relatives (see also Föttinger et al. 2010). Since the 2007 review, information has been added regarding more disparate taxa for which the chemistry of the secretions was previously unknown. Raspotnig et al. (2005) published the first report on the chemistry of sironids (Cyphophthalmi Simon 1879), and Jones et al. (2009) added data for a stylocellid. Raspotnig et al. (2010) provided the first report of secretion chemistry among Dyspnoi, from *Paranemastoma quadripunctatum* (Perty 1833), and Shear et al. (2010a, b) studied two North American phalangodids, *Bishopella laciniosa* (Crosby & Bishop 1924) and *Texella bifurcata* (Briggs 1968), and a stygnopsid, *Chinquepellobunus madlae* (Goodnight & Goodnight 1967). These more recent developments have been summarized by Raspotnig (2012 [2013]), who also mentioned preliminary results for many additional harvestman species. Thus while progress has been made filling taxonomic gaps in our knowledge of harvestman defensive secretions, much remains to be done.

While these studies focused primarily on reporting the composition of secretions from individual species, some recent work has been more analytical. Rocha et al. (2013) discussed possible chemical pathways for the synthesis of secretion components. Attempts at a phylogenetic analysis of the distribution of defensive secretions include those of Caetano & Machado (2013) and Raspotnig et al. (2014). The hope has frequently been expressed that data on defensive secretions may be of value in the phylogenetics and taxonomy of Opiliones (Hara et al. 2005; Jones et al. 2009; Shear et al. 2010a, b, Föttinger et al. 2010, Raspotnig 2012 [2013]), but we see an emerging picture that may be blurred by a great deal of homoplasy. Indeed, the results of the analyses of the same data by Caetano & Machado (2013) and Raspotnig et al. (2014) came to opposite conclusions concerning the polarity of chemical transformations in Grassatores.

Traditional Opiliones taxonomic groups have now been robustly supported with genomic data sets (Hedin et al. 2012), and include the mite-like suborder Cyphophthalmi as sister to remaining harvestmen, the Phalangida Latrielle 1796. Within Phalangida, the raptorially-pedipalped Laniatores Thorell 1876 are sister to the Palpatores Thorell 1876, comprised of the often long-legged suborder Eupnoi Hansen & Sørensen 1904 and the suborder Dyspnoi Hansen & Sørensen 1904. The division of the suborder Laniatores into two infraorders, Insidiatores Loman 1900 and Grassatores Kury 2003, was proposed by Kury (2003) to taxonomically recognize two divergent phyletic lines of harvestmen. Insidiatores includes

those taxa presently grouped as Triaenonychidae Sørensen 1886, Synthetonychiidae Forster 1954, and a group of species of unsettled family-level taxonomy presently referred to as Travunioidea Absalon & Kratchovil 1932. It is not clear that Insidiatores as composed is monophyletic (but Grassatores almost certainly is). Representative Insidiatores examined here can be seen in Fig. 1.

Synthetonychiidae is a poorly studied but probably monophyletic taxon including minute harvestmen limited to New Zealand (Forster 1954, Kury 2007). In some recent phylogenies, synthetonyehiids have been resolved as an outgroup to the remaining Laniatores (Giribet et al. 2010). Triaenonychidae is composed of numerous genera and species that are important, if not dominant, elements of the harvestman fauna of the southern hemisphere (Australia, New Zealand, Madagascar, South Africa, and southern South America [Kury 2007]), but one species, *Fumontana deprehendor* Shear 1977, is known from the southern Appalachian Mountains in North America (Shear 1977, Thomas & Hedin 2008). Triaenonychid taxonomy is somewhat problematical (Mendes & Kury 2008). No triaenonychids had been examined for the chemistry of their defensive secretions prior to this study, and synthetonychiids remain unstudied.

Genera and species of the "superfamily" Travunioidea have been recorded from Europe (Kury & Mendes 2007) and Japan, but North America appears to host the most diverse and probably the best understood fauna (Fig. 1; Shear & Derkarabetian 2008, Derkarabetian et al. 2010, 2011). Only a single North American species from this phylogenetically important taxon has been examined from the viewpoint of chemical defense. Specimens from New Mexico were studied by Epka et al. (1984); at the time they referred their material to *Sclerobunus robustus* (Packard 1877), but recent work (Derkarabetian et al. 2010, 2011; Derkarabetian & Hedin 2014) has shown that at least three additional species occur in New Mexico, so the exact identity of their specimens is now unclear. Epka et al. (1984) found an extraordinary array of molecules in the secretion of *S. robustus*: N,N-dimethylphenylethylamine, nicotine, bornyl acetate, bornyl propionate, camphene and limonene.

Raspotnig et al. (2011) examined four species in the European travunioid genus *Holoscotolemon* Roewer 1915: *H. jaqueti* (Corti 1905), *H. oreophilum* Martens 1978, *H. lessiniense* Martens 1978 and *H. unicolor* Roewer 1915. They found that the secretions of *H. jaqueti* and *H. oreophilum* were dominated by nieotine, while that of *H. lessiniense* primarily consisted of the similar alkaloid anabaseine. No results were obtained from adults of *H. unicolor*.

For this study, we analyzed extracts from 15 species of Insidiatores from North America and New Zealand. While our findings for the North American species might have been predicted from the earlier examinations of *Sclerobunus ?robustus* and the European species of *Holoscotolemon*, the chemistry of the New Zealand forms was quite unexpected.

METHODS

Specimens studied were collected alive and dropped in the field into vials containing less than 1 ml of USP methanol; the vials had Teflon-lined caps. Collection localities for the specimens studied are given in Table 1. All specimens will be placed as vouchers in the collection of the Virginia Museum of Natural History, Martinsville, Virginia.

Although when it was possible to extract more than one specimen of a species separately, the results were consistent, in most cases we were restricted to a single specimen by the rarity of the species involved and the difficulties in collecting them, or analyzed extracts from several specimens collected into the same vial. For this reason, some of our results must be regarded as preliminary, and we are working to follow up with additional specimens. However, at the level we are studying, simply characterizing components without detailed quantitative analysis, previous studies have shown little variation within species in the composition of their secretions, though relative amounts of components may differ.

The analysis of the extracts was performed by HMG and THJ. Gas chromatography-mass spectrometry was carried out in the EI mode using a Shimadzu QP-5000 or QP-2010 GC/MS equipped with an RTX-5, 30 m $\times$ 0.25-mm i.d. column. The instruments were programmed from 60 ° C to 250 ° C at 10 °/min. Identification of components was accomplished using NIST/EPA/NIH mass spectral library on CD-rom, version 1.7 (1999) and the NIST/EPA/NIH mass spectral library version 2.0d (2005).

All chemicals were mapped onto a modified phylogeny based on the molecular phylogenetic analysis of Derkarabetian et al. (2010), trimmed to include only those genera with chemical data presented here. An ultrametric tree was used for character mapping, whieh was conducted in Mesquite 2.75 using the ancestral state reconstruction module using parsimony. Additionally, we mapped chemicals onto a phylogeny including triaenonychids analyzed here and the genus *Holoscotolemon*. The taxa were added aeording to their placement in the maximum likelihood phylogeny of Giribet et al. (2010).

RESULTS

Results of the analysis are presented in Tables 2–4, and struetural formulae of detected components are shown in Fig. 2. As seen in Table 2, the major component of the secretion in both eastern North American travunioid species (*Erebomaster flavescens* Cope 1872 and *Theromaster brunneus* (Banks 1902)) was the alkaloid anabaseine. Minor or trace components were anabasine (a related alkaloid), phenol, benzothiazole, salicyl alcohol, 2,3′-dipyridyl and mellein. Four individuals of *T. brunneus* were analyzed; no significant differences were found between individuals, except that salicyl alcohol was not found in two of the speeimens. A specimen of *E. flavescens* from Indiana was analyzed separately, and six specimens of the species from Ohio were extracted and analyzed as a group. The results for *E. flavescens* differed from those for *T. brunneus* in that trace amounts of 4-hydroxybenzine-ethanol were found in the *E. flavescens* extract, and that phenol, anabasine and mellein were minor components (1–10%) rather than traces (< 1%).

Table 3 summarizes the results from the analyses of extracts from eight species of travunioids from western North America. Components in common with the eastern species were phenol and benzothiazole, and as with the eastern species, these compounds were present only in trace amounts. The major component in all western species was N,N-

Figure 1.—Representatives of North American Insidiatores. High resolution images for all specimens figured here are available on Morphbank under publication ID 835667 (http://www.morphbank.net/835667). A. *Briggsus flavescens*, B. *Cryptomaster leviathan*, C. *Paranonychus brunneus*, D. *Metanonychus idahoensis*, E. *Finnontana deprehendor*, F. *Erebomaster* sp., G. *Speleomaster lexi*, H. *Speleomaster pecki*, I. *Speleonychia sengeri*.

Table 1.—Collecting localities.

Species	Voucher number	Collection localities
Cryptomaster leviathan	07-177	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp. 28 May 2007, A.Richart, C.Richart (CHR 1354)
	08-188	OR: Coos Co., Golden and Silver Falls St. Pk., 4 April 2008, S.Derkarabetian, C.Richart (CHR 2029)
	07-176	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp, 28 May 2007, A.Richart, C.Richart (CHR 1335)
Erebomaster flavescens	07-179	IN: Crawford Co., Sibert's Well Cave (near Wyandotte Cave), 3 mi NE Leavenworth, 19 Nov 2007, J. Lewis
	07-180	IN: Harrison Co., Devils Graveyard Cave, 7 mi SW Corydon, 19 Nov 2007, J. Lewis
	07-181	IN: Harrison Co., Devils Graveyard Cave, 7 mi SW Corydon, 19 Nov 2007, J. Lewis
	12-336	OH: Adams Co., Edge of Appalachia Preserve, 8 June 2011, W. A. Shear
Theromaster brunneus	08-211	NC: Haywood Co., Cullowhee Mtn. Road at Wolf Creek, 22 October 2008, W. A. Shear
Speleomaster lexi	08-172	ID: Lincoln Co., Tee Cave, 30 June 2007, A.Richart, C.Richart (CHR 1577)
	08-178	ID: Lincoln Co., Gwinn Cave, 29 June 2007, A.Richart, C.Richart (CHR 1568)
Speleomaster pecki	08-174	ID: Butte Co., Beauty Cave, 30 June 2007, A.Richart, C.Richart (CHR 1581)
Speleonychia sengeri	08-175	WA: Klickitat Co., Cheese Cave, 9 June 2007, N.Richart, C.Richart (CHR 1621)
	08-176	WA: Skamania Co., Cave #27, 9 June 2007, N.Richart, C.Richart (CHR 1622)
	08-177	WA: Skamania Co., Big Cave, 8 June 2007, N.Richart, C.Richart (CHR 1588)
	08-179	WA: Skamania Co., Slime Cave (Cave #39) 8 June 2007, N.Richart, C.Richart (CHR 1607)
Paranonychus brunneus	07-174	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp, 28 May 2007, A.Richart, C.Richart (CHR 1356)
	07-175	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp, 28 May 2007, A.Richart, C.Richart (CHR 1357)
Metanonychus idahoensis	09-248	ID: Shoshone Co., Hobo Cedar Grove, 25 July 2008, C.Richart (CHR 2361)
Briggsus flavescens	08-190	OR: Clatsop Co., Saddle Mt. Rd. near U.S. 26, 3 April 2008, S.Derkarabetian, C.Richart (CHR 2016)
Nuncia sp.	10-275	NZ: South Island, Westland, Dancing Creek, Haast Pass, 11 February 2010, M. Minor
Nuncia sp.	10-278	NZ: South Island, Buller, Aratika, 9 February 2010, M. Minor
Nuncia sp.	10-279	NZ: South Island, Buller, Springs Junction, 5 February 2010, M. Minor
Soerensenella sp.	10-271	NZ: North Island, Wanganui, Totara Reserve, 28 March 2010, M. Minor
Soerensenella prehensor	10-272	NZ: North Island, Taupo, Whakapapa Bush, 4 April 2010, M. Minor

dimethylphenylethylamine, with nicotine and N,N-dimethyli-soamylamine as minor or trace components. An exception was *Briggsus flavescens* (Briggs 1971), in which the major component was phenol, with N,N-dimethylphenylethylamine as a minor component and a trace amount of benzothiazole. This unexpected result came from one small specimen and requires confirmation.

Table 4 shows results from the analyses of extracts of triaenonychids. Three small specimens of *F. deprehendor* were extracted and analyzed together. *Fumontana deprehendor* had phenol as a major component, with traces of salicyl alcohol. Each record of a New Zealand triaenonychoid represents either one or two specimens. The major components of the New Zealand triaenonychoids were quite different from both *F. deprehendor* and the travunioids. While the travunioids and *F. deprehendor* were dominated by cyclic compounds frequently containing nitrogen, the New Zealand triaenonychoids showed linear aldehydes, alcohols and ketones. The secretions were also much less complex, with only one or two minor or trace components in *Nuncia* sp.

Results of the character mapping analyses including the triaenonychids and *Holoscotolemon* are shown in Fig. 3. This analysis indicates that if Insidiatores is monophyletic, the ancestral state for all species is phenol, with changes to 4-

Table 2.—Compounds present in eastern North American travunioids and species of *Holoscotolemon* (data on *Holoscotolemon* from Raspotnig et al. 2011). Plus sign indicates major component, "o" a minor component (<10%) and "t" a trace component (<1%). The "Unknown" is an undetermined component at m/z = 174.

Fig. 2	Component	Erebomaster flavescens	Theromaster brunneus	Holoscotolemon jaqueti[1]	Holoscotolemon lessiniense[1]	Holoscotolemon oreophilum[1]
1	Phenol	o	t			
2	Benzothiazole	t	t			
3	Salycyl alcohol	t	t			
4	4-Hydroxybenzenethanol	t				
5	Anabasine	o	t			
6	2,3'-Dipyridyl	t	t		t	
7	Anabaseine	+	+		+	
8	Mellein	o	t			
10	Nicotine			+		+
	Unknown*		t			

Table 3.—Compounds present in western North American travunioids. Plus sign indicates major component, "o" a minor component (<10%) and "t" a trace component (<1%).

Fig. 2	Component	Paranonychus brunneus	Cryptomaster leviathan	Speleomaster lexi	Speleomaster pecki	Speleonychia sengeri	Metanonychus idahoensis	Sclerobunus nondimorphicus	Briggsus flavescens
1	Phenol	t	t	o	t	t			+
2	Benzothiazole	t				t			t
9	N.N-dimethylphenylethylamine	+	+	+	+	+	+	+	o
10	Nicotine						o	o	
11	N.N-dimethylisoamylamine						o	t	

Table 4.—Compounds present in Finmontana deprehendor and six morphospecies of New Zealand triaenonychids. Plus sign indicates major component, "o" a minor component (<10%) and "t" a trace component (<1%).

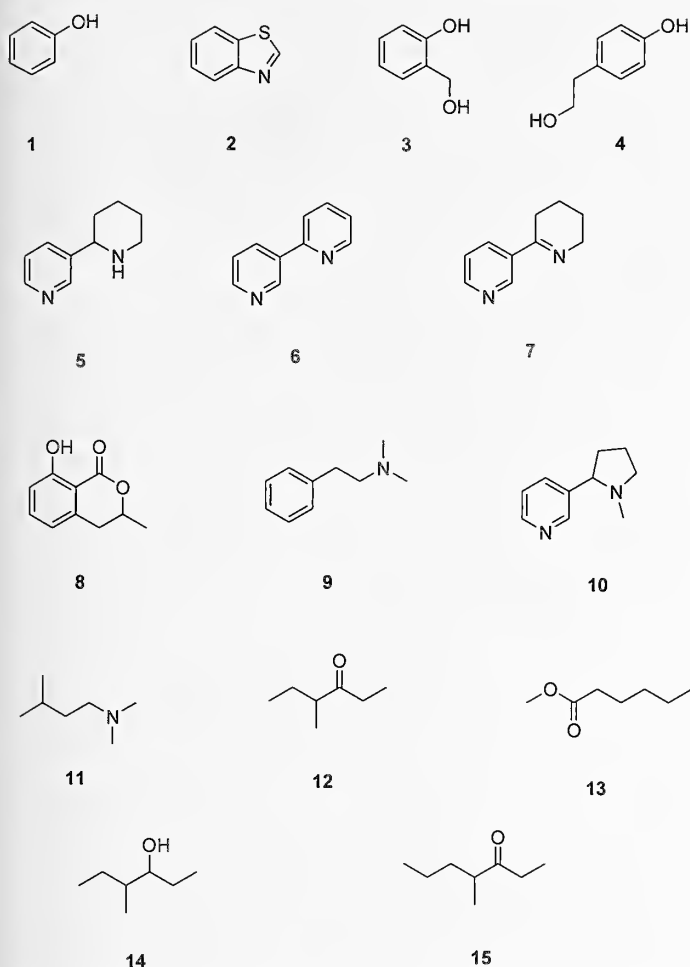| Fig. 2	Component	Soerensenella sp.	Soerensenella prehensor	Nuncia sp. Dancing Creek	Nuncia sp. Aratika	Nuncia sp. Springs Junction 1	Nuncia sp. Springs Junction 2	Finmontana deprehendor
1	Phenol							+
3	Salicylic alcohol							o
12	4-methyl-3-hexanone	+	+	+	+	+	+	
13	Methylhexanoate					t		
14	4-methyl-3-hexanol						+	
15	4-methyl-3-heptanone					t		

Figure 2.—Compounds identified in this study. 1. Phenol, 2. Benzothiazole, 3. Salycyl alcohol, 4. 4-Hydroxybenzenethanol, 5. Anabasine, 6. 2,3'-dipyridryl, 7. Anabaseine, 8. Mellein, 9. N,N-dimethylphenylethylamine, 10. Nicotine, 11. N,N-dimethylisoamylamine. 12. 4-methyl-3-hexanone, 13. Methylhexanoate, 14. 4-methyl-3-hexanol, 15. 4-methyl-3-heptanone.

methyl-3-hexanone in New Zealand triaenonychids and to N,N-dimethylphenylethylamine in travunioids.

DISCUSSION

The qualitative near-identity of the extracts from *E. flavescens* and *T. brunneus* supports the close phylogenetic relationship hypothesized on the bases of morphology and genetics by Derkarabetian et al. (2010). The strong differences between the secretions of this "eastern clade" and that of the hypothetical "western clade" of travunioids supports that distinction.

Raspotnig et al. (2011) found anabaseine as the major component in the secretion of *Holoscotolemon lessiniense,* but nicotine dominated that of *H. jaqueti* and *H. oreophilum* (Table 2). These three species appear to be closely related from morphological evidence and numerous characters, especially genitalic, place them close to *Erebomaster* Cope 1872 and *Theromaster* Briggs 1969 (Martens 1978). Trace components in these three species were pyridines with the same core structure as anabaseine and nicotine. Both chemical and morphological evidence, therefore, argue for a closer relation-

ship of the eastern North American genera with European *Holoscotolemon* than with the travunioid genera from western North America.

For the western travunioids, N,N-dimethylphenylethylamine was the major component in all species except *Briggsus flavescens. Metanonychus idahoensis* Briggs 1971 and *Sclerobunus nondimorphicus* Briggs 1971 had nicotine and N,N-diethylisoamylamine as minor components, as well as two unidentified compounds not shown. For the other species, phenol was present as either a minor component or a trace, and benzothiazole was found as a trace in *Paranonychus brunneus* (Banks 1893) and *Speleonychia sengeri* Briggs 1974. The complex mixtures found in the eastern cladonychiids and in *S. ?robustus* (Epka et al. 1984) were not recovered from the western species we studied. The complexity of the secretion extracted from the two eastern cladonychiid species is similar to that found by Epka et al. (1984) for *Sclerobunus ?robustus,* but quite different chemically. New Mexico *Sclerobunus* Banks 1893 require re-examination.

Both the complexity and the diversity of chemical composition within Insidiatores is unusual among opilionids, because in previous studies, similar classes of compounds (though different molecules) have been found in large taxonomic groupings. For example, sclerosomatids utilize a variety of ketones and alcohols, and many Grassatores produce alkylphenols and hydroquinones (Hara et al. 2007, Raspotnig 2012 [2013], Caetano & Machado 2013, Raspotnig et al. 2014). In some cases the secretion consists of a single compound (Shear et al. 2010a, b). However, in the case of the cyphophthalmids, the two species so far studied show as diverse an array of molecules as do the travunioids or even more so (Raspotnig et al. 2005, Jones et al. 2009, Raspotnig 2012 [2013]). Because cyphophthalmids are sister to all remaining Opiliones, the scanty data collected so far could be construed to suggest that early-evolving defensive secretions were complex mixtures, later winnowed down to only a few, or to single, components. Evidence against this view is that gonyleptoids, a derived group, also have complex mixtures, though the compounds are nearly all methylated and/or ethylated benzoquinones or alkylphenols (Föttinger et al. 2010, Raspotnig 2012 [2013]). However, the question that remains unexamined so far is the extent to which the method of collecting the secretions and the processing for analysis may have influenced the results; it is possible that chemical changes in some of the components could be induced during study, and this could account for the mixtures obtained.

Results of the character mapping for Travunioidea are shown in Fig. 4. The various compounds are represented by numbers that correspond to those in Fig. 2. Two major findings are seen in the parsimony reconstruction regarding the chemicals that constitute the major components. First, the major component N,N-dimethylphenylethylamine (9) was recovered as the ancestral state for all travunioid genera included in this analysis. Second, there is a transition from N,N-dimethylphenylethylamine (9) to anabaseine (7) as the major component on the branch leading to the eastern Cladonychiidae (*Erebomaster* and *Theromaster*). In addition, these two genera also possess many other minor or trace elements that are unique to this lineage, namely salycyl alcohol (3), anabasine (5), 2,3'-dipyridyl (6) and mellein (8). Also,

1. Phenol

2. Benzothiazole

3. Salycyl alcohol

6,7. 2,3'Dipyridyl and Anabaseine

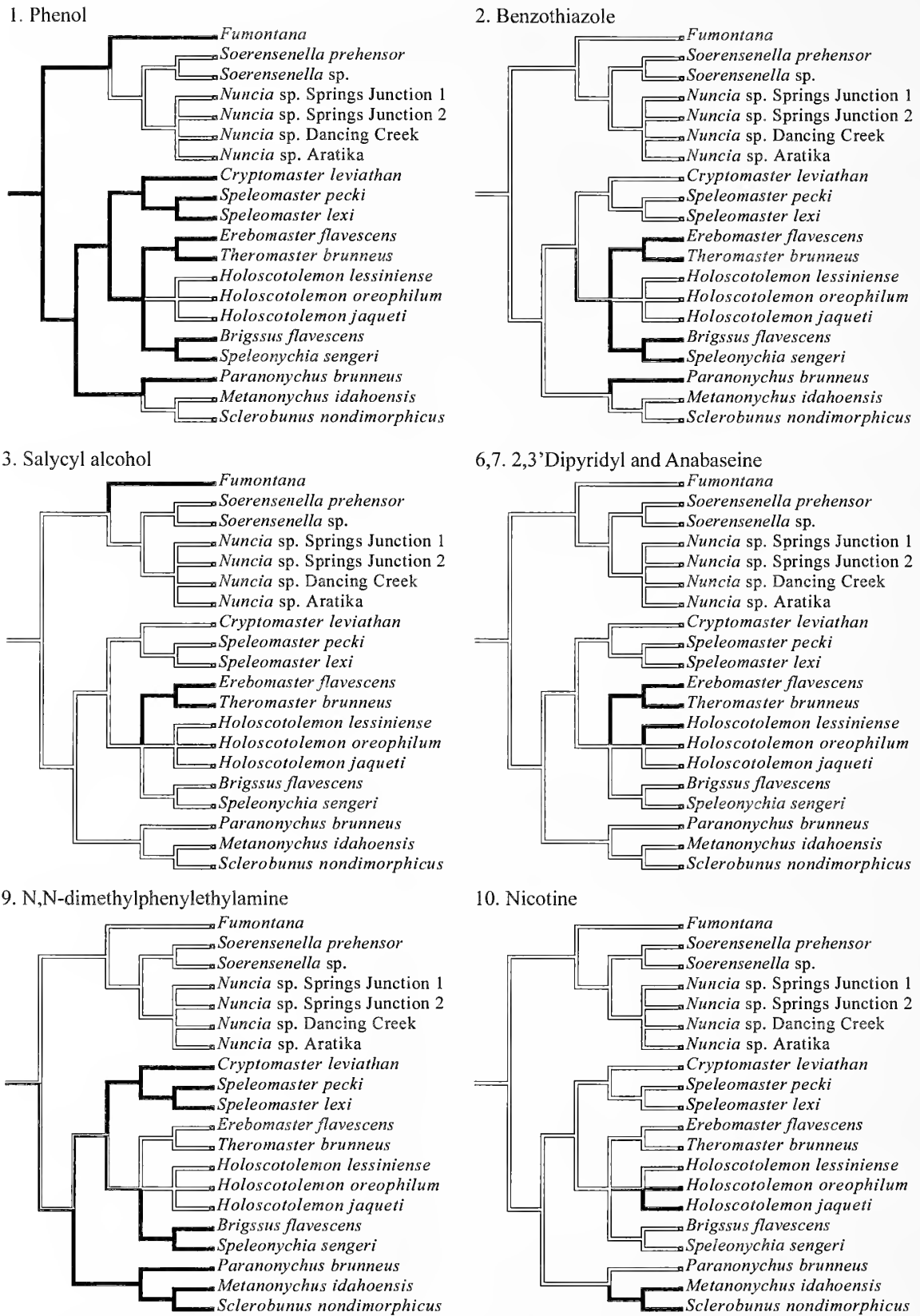9. N,N-dimethylphenylethylamine

10. Nicotine

Figure 3.—Results of chemical character mapping for Insidiatores. Only those chemicals with 2 or more steps are shown. Black = presence, white = absence.
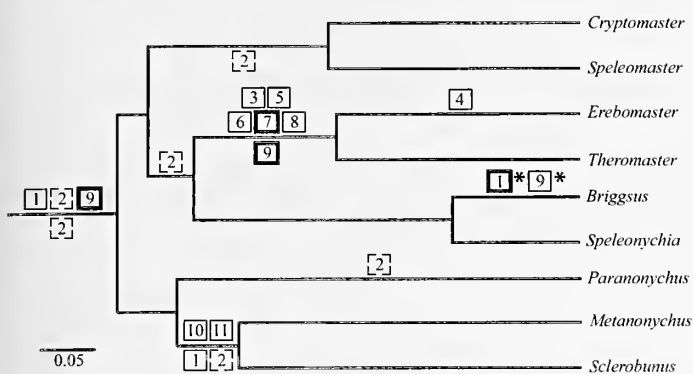
Figure 4.—Results of the chemical character mapping in Travunioidea. Numbers correspond to the chemicals listed in Tables 1 and 2. Boxed numbers above a branch are character gains, those below are losses. Bold boxes are major components and regular boxes are minor/trace components. Dashed boxes represent those chemicals that are equally parsimonious (present/absent) along the branch; but branches with definite gains for these chemicals are also included. Boxes with asterisks indicate a change in component concentration (e.g., change from major to minor).

Erebomaster is the only taxon known to possess 4-hydroxybenzenethanol (4). The sclerobunines (*Sclerobunus* and *Metanonychus* Briggs 1971) have lost phenol (1) as a component but have gained both nicotine (10) and N,N-dimethylisoamylamine (11). Interestingly, two species of *Holoscotolemon* also produce nicotine.

Raspotnig (2012 [2013]) discussed at length the possible phylogenetic and systematic implications of the diversity of defensive compounds in Opiliones. Overlooking some dissonant results, it appears that the suborder Cyphophythalmi can be characterized by methyl ketones, naphthoquinones and related compounds. Benzoquinones appear in phalangiid Eupnoi, and "sclerosomatid compounds" (noncyclic ketones, alcohols and aldehydes, such as 4-methyl-3-hexanone) are found in sclerosomatid Eupnoi. Few Dyspnoi have been examined, but naphthoquinones and anthraquinones have been found. Grassatores produce predominantly phenols, benzoquinones and hydroquinones. Insidiatores, up to the findings of this study, were characterized by nitrogen-containing alkaloids. Raspotnig (2012 [2013]) is quick to point out that taxonomic sampling within the Opiliones has been erratic and many taxa remain unsampled, or known only from unpublished or preliminary results.

Raspotnig (2012 [2013]) proposed a number of phylogenetic hypotheses that may be summarized as follows: 1) complex mixtures of secretions are plesiomorphic compared to uniform or less diverse mixtures; 2) naphthoquinones and methyl ketones, as found in cyphophthalmids, are basal; 3) naphthoquinones are synapomorphic for a clade Cyphophthalmi + Palpatores; 4) acyclic compounds in Cyphophthalmi and Sclerosomatidae may have a common origin; 5) "sclerosomatid compounds" may represent a synapomorphy for Palpatores; 6) a deep chemical divergence separates Insidiatores and Grassatores; and 7) a link between the chemistry of Cyphophthalmi + Palpatores and Laniatores remains to be found.

But the phylogenetic signal is not so clear as that. The dissonant results mentioned above seem to significantly disrupt the characterizations given. Among the anomalies Raspotnig (2012 [2013]) mentions which require explanation are the presence of naphthoquinones in some putative sclerosomatids (*Gyas* sp.), ketones in some Gonyleptidae (Grassatores), and now, as a result of our work, methyl ketones ("sclerosomatid substances") in Triaenonychidae and phenol in *Fumontana deprehendor*, a species that consistently is recovered in phylogenies as sister to remaining triaenonychids. At least these latter two make possible a tentative link between Laniatores and some Palpatores.

Caetano & Machado (2013) conducted a phylogenetic analysis of the distribution of scent gland chemistry in Grassatores, and concluded that benzoquinones were ancestral, with alkylphenols evolving independently many times. Using the same data, but a different method of analysis and a different outgroup, Raspotnig et al. (2014) concluded the opposite—that benzoquinones were derived and alkylphenols ancestral. Based on the methods used and the fact that Raspotnig et al. (2014) used a more appropriate outgroup, we agree with the latter conclusion. Our finding that phenol is probably ancestral in Insidiatores (see Fig. 3) reinforces this, although exact phylogenetic relationships between Insidiatores and Grassatores remain unclear.

Raspotnig (2012 [2013]) did not attempt to map the known characters on any established phylogenetic tree of Opiliones. However, study of his Table 2 (pp. 9–10) and our Fig. 3 seems to indicate that at least at the present state of knowledge, there is a great deal of homoplasy present, with various types of compounds being lost and then regained, or evolving independently.

In our results for Insidiatores, the most divergent observation is the presence of 4-methyl-3-hexanone as the major component in all of the New Zealand triaenonychids we studied. If we consider *Fumontana* as a plesiomorphic outgroup, we have the problem of getting from phenol to these noncyclic ketones. The travunioids stand alone with the predominant secretion of either N,N-dimethylphenylethylamine or tobacco alkaloids like nicotine and anabaseine. A major question, which by extension could be applied to the entire phylogenetic scheme of this character, is how one gets from one compound or set of compounds in a supposed plesiomorphic taxon to a chemically completely different compound further up in the tree. In other words, is it reasonable to assume a transition from phenol to 4-methyl-3-hexanone?

ACKNOWLEDGMENTS

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Species differences and geographic variation in the communal roosting behavior of *Prionostemma* harvestmen in Central American rainforests

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Abstract. Many species roost communally but the proximate causes and ultimate functions of this widespread behavior remain poorly understood. We studied the communal roosts of two undescribed species of harvestmen in the genus *Prionostemma* Pocock 1903 at a Caribbean rainforest site in southeastern Nicaragua. The species are quite similar in gross morphology but differ in body coloration, male genitalia, and roosting behavior. One species roosts primarily on spiny palms while the other species, which is darker in coloration, roosts inside buttress root cavities. In a mark-recapture study, the cavity-roosting species had higher levels of individual site fidelity than found previously in the spiny palm-roosting species, perhaps because suitable cavities are scarcer than spiny palms. The tree cavity aggregations were strongly male-biased, which our review of the literature suggests is unusual for harvestman roosts. The overall sex ratio of the spiny palm aggregations was 1:1, but some roost sites were strongly male biased while others were strongly female biased. Removing all harvestmen from 10 spiny palm roost sites shifted the overall sex ratio toward males on subsequent days, but the sites with skewed sex ratios remained skewed in the same directions despite complete turnover in roost membership. These results are discussed in relation to mechanisms of roost formation and possible sex differences in vagility, microhabitat preferences and sensitivity to disturbance. Both species also occur at La Selva Biological Station in Costa Rica but neither forms roosting aggregations in spiny palms or tree cavities there. A possible explanation for the geographic variation is that roosting patterns change over time through cultural drift.

Keywords: Aggregation, conspecific attraction, mark-recapture, Opiliones, sex ratio

Animals in diverse taxonomic groups congregate for the inactive period of the diurnal cycle, a behavior referred to as communal roosting (Eiserer 1984; Mallet 1986; Devries et al. 1987; Vulinec 1990; Alcock 1998; Bijleveld et al. 2010). Communal roosts may offer protection from predators through dilution or group defenses (Holmberg et al. 1984; Alcock 1998; Eisner 2004; Willemart & Gnaspini 2004). In some taxa, communal roosts may also provide thermoregulatory benefits (Beauchamp 1999), mating opportunities (Blanco & Tella 1999), opportunities for food sharing (Wilkinson 1984), or information about the location of food patches (Beauchamp 1999; Kerth & Reckardt 2003; Bijleveld et al. 2010). Harvestmen (Opiliones) are generally active at night and roost during the day (reviewed in Machado & Macias-Ordonez 2007). Some species roost solitarily while others form aggregations ranging in size from a few individuals to hundreds (Holmberg et al. 1984; Cockerill 1988; Coddington et al. 1990; Machado et al. 2000; Willemart & Gnaspini 2004; Machado & Macias-Ordonez 2007; Wijnhoven et al. 2007; Wade et al. 2011). The communal roosts of harvestmen can be dense aggregations, in which most individuals are clinging to other individuals, or loose aggregations in which most individuals are in contact with the substrate (reviewed in Machado & Macias-Ordonez 2007). Some species roost in caves or other dark places (Holmberg et al. 1984; Willemart & Gnaspini 2004; Chelini et al. 2011), while other species roost on the exterior surfaces of rocks or vegetation exposed to sunlight (Coddington et al. 1990; Grether et al. 2014). The most frequently proposed functions of Neotropical harvestman roosting aggregations are safety from predators, through dilution and/or chemical defenses, and protection from desiccation (Coddington et al. 1990; Machado et al. 2000;

Willemart & Gnaspini 2004; Machado & Macias-Ordonez 2007; Grether & Donaldson 2007; Wade et al. 2011; Chelini et al. 2011).

Studies of intra- and interspecific variation can provide insights into the proximate causes and ultimate functions of communal roosts (Chelini et al. 2012). In this paper, we compare the roosting aggregations of two syntopic species of *Prionostemma* Pocock 1903 (Eupnoi: Sclerosomatidae: Gagrellinae) harvestmen at Refugio Bartola, a lowland tropical rainforest site in southeastern Nicaragua. One of the species usually aggregates on the fronds and trunks of spiny palms (Arecaceae: *Bactris* spp., *Astrocaryum* spp.) in the forest understory (Fig.1; Donaldson & Grether 2007; Grether & Donaldson 2007), while the other species aggregates in cavities at the base of trees (e.g., Fabaceae: *Dipteryx panamensis*) that have buttress roots (Fig. 2). Both species form loose aggregations (Holmberg et al. 1984; Machado & Macias-Ordonez 2007) in which most individuals' legs are in contact with the substrate and the legs are flexed. The species are quite similar in body size and anatomical proportions, but the cavity-roosting species is notably darker in coloration (Fig. 3). Based on scanning electron micrographs of male genitalia (Fig. 4), the same two undescribed species occur at La Selva Biological Station in Costa Rica (69 km to the SE), although neither is known to aggregate in spiny palms or tree cavities at La Selva (see Discussion). Following Proud et al. (2012), we refer to the species that aggregates in tree cavities at Refugio Bartola as *Prionostemma* sp. 1 and to the species that aggregates in spiny palms as *Prionostemma* sp. 2.

The population of *Prionostemma* sp. 2 at Refugio Bartola has been the subject of several short studies focused on clarifying the mechanisms of roost formation. Mark-recapture studies
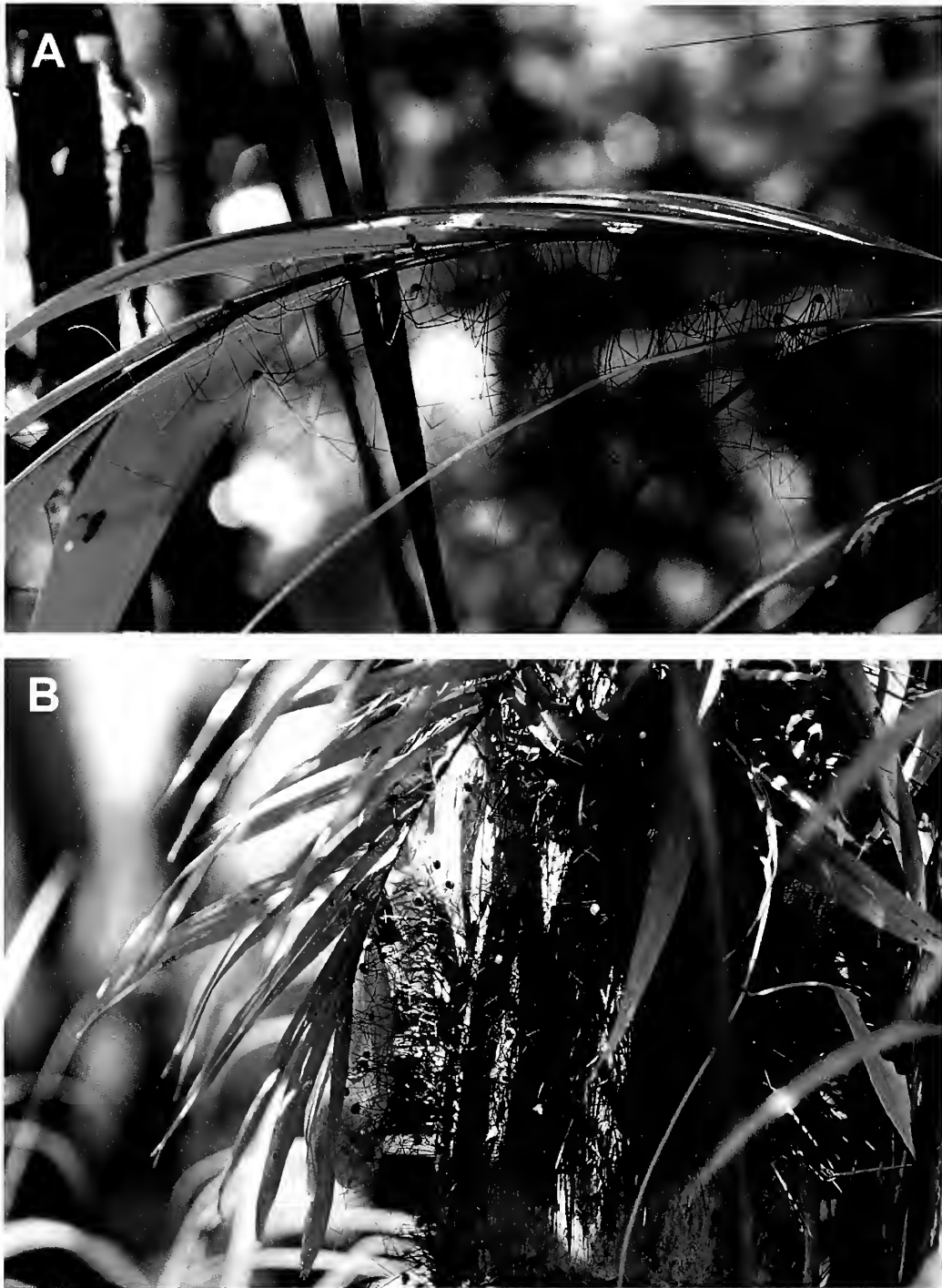
Figure 1.—*Prionostemma* roosting aggregations underneath a frond (A) and along the trunk (B) of spiny palms.

established that individual harvestmen are not roost-site faithful (Grether & Donaldson 2007; Teng et al. 2012), and yet aggregations have formed in the same locations for over 10 years (Teng et al. 2012; Grether et al. 2014). The long-term use of specific sites does not appear to be a product of habitat limitation. Most spiny palms do not attract harvestman aggregations, and those that do are not distinctive in the characteristics of the palms themselves or microclimate (Grether & Donaldson 2007; Teng et al. 2012). Based on roost site manipulations and experimental translocations, it has been deduced that these harvestmen preferentially settle in sites marked with conspecific

scent (Donaldson & Grether 2007; Teng et al. 2012). Thus, the location of the communal roosts appears to be traditional in that some sites are used in preference to others only because conspecifics roosted there in the past (Donaldson & Grether 2007). While the mechanism of roost site selection in *Prionostemma* sp. 2 may result in the repeated use of particular roosting sites for multiple years, the same mechanism could also cause populations to drift in roosting microhabitat over longer time scales. Our finding that the same species is present but does not roost in spiny palms at La Selva Biological Station provides tentative support for this cultural drift hypothesis (see Discussion).

Figure 2.—Distant (A) and close-up (B) photographs of a tree cavity with a *Prionostemma* roosting aggregation.

The aggregations of *Prionostemma* sp. 1 in buttress root cavities were first discovered at Refugio Bartola in February 2013 and have not been described previously. To begin to characterize the roosting behavior of this species, and to compare it to that of *Prionostemma* sp. 2, we made structured behavioral observations and conducted a mark-recapture study. Comparable data have already been published for *Prionostemma* sp. 2 (Donaldson & Grether 2007; Grether & Donaldson 2007; Teng et al. 2012), so we did not duplicate this work. Instead, we carried out a removal experiment at spiny palm aggregation sites (see Grether et al. 2014). In the context of the species comparison, the primary relevance of the removal experiment is that it yielded data on *Prionostemma* sp. 2 roost sex ratios, which have not been reported previously. To help place our findings into a broader context, we also analyze data on harvestman roost sex ratios reported in the literature.

METHODS

Study area.—This study was carried out in primary lowland rainforest at Refugio Bartola in southeastern Nicaragua (10.973°N, 84.339°W) from 2–20 February 2013. This private reserve is contiguous with Indio Maíz Biological Reserve, the largest remaining tract of primary rainforest in Central America (ca. 4500 km²). The climate is wet tropical, with about 4 m of rainfall per year, peak precipitation in June–August, and a dry season from February–April during which about 15% of the annual precipitation is recorded (Cody 2000). Approximately 69 mm of rain fell at Refugio Bartola during the study period.

Operational definitions.—We use the term roosting aggregation to refer to groups of two or more individuals resting in the same "site". In the case of spiny palm roosts, we consider all of the spiny palms within 1 m of each other to belong to the same site (spiny palms tend to grow in clusters with broadly overlapping fronds). In the case of tree cavity roosts, we consider a single cavity to be a site. While roosting individuals of both study species are often close enough together to have overlapping legs (Figs. 2, 3), we did not use leg overlap as a criterion for determining aggregation membership (cf. Willemart & Gnaspini 2004).

Roost measurements and behavioral observations.—Using flashlights, we searched for harvestman roosts at the base of 114 buttressed trees. At the first seven tree cavities in which *Prionostemma* roosting aggregations were found, we measured air temperature, surface temperature, and percent humidity both within the cavity and outside the cavity using a hygro-thermometer and infrared thermometer (Extech Instruments Waltham, MA USA). In addition, we measured the height, width, depth and compass orientation of the cavity, and the tree's circumference at breast height. To characterize the behavior of the harvestmen in the cavity roosts, we used scan sampling (Altmann 1974). Under red light, we observed six of the cavity roosts used in the mark-recapture study for 15 minutes, recording at 1-minute intervals the number of harvestmen that were stationary or engaged in the following behaviors: walking within the cavity; bobbing (moving body up and down, a likely anti-predator behavior; Holmberg et al. 1984; Grether and Donaldson 2007); ventral rubbing (pressing
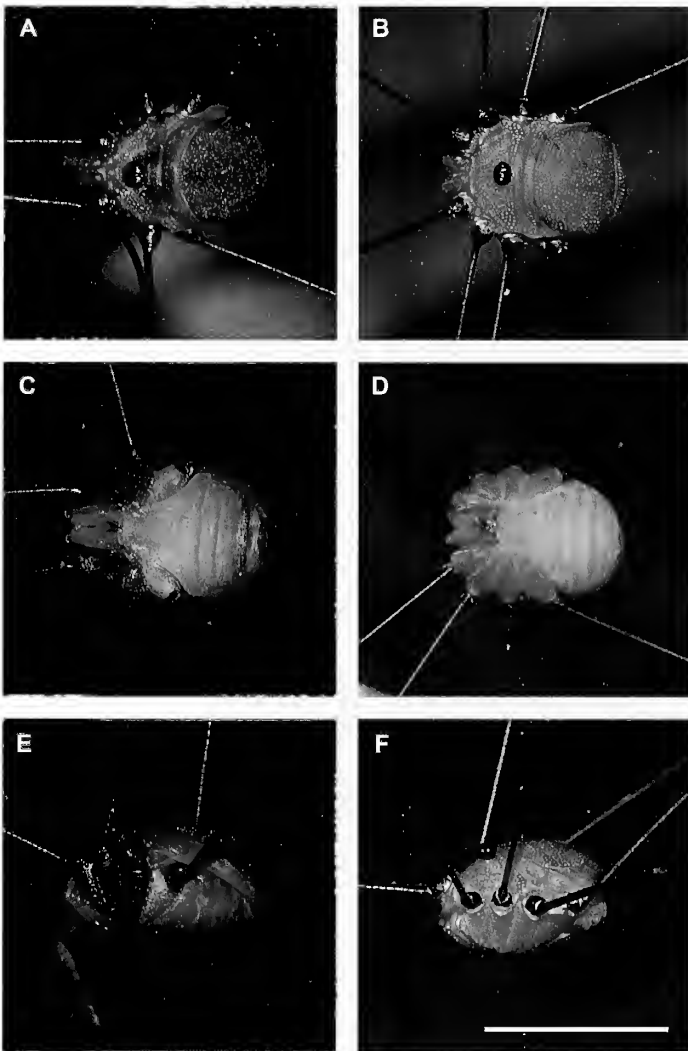
Figure 3.—Photographs of two *Prionostemma* species at Refugio Bartola, Nicaragua. Two female specimens are each shown in dorsal (A, B), ventral (C, D), and lateral (E, F) views. The female on the left was found in a tree cavity roost (*Prionostemma* sp. 1) and the female on the right was found in a spiny palm roost (*Prionostemma* sp. 2). *Prionostemma* sp. 2 is more uniform and lighter in coloration than *Prionostemma* sp. 1. The black coxae (I–III) and red and black patches on the abdomen of the *Prionostemma* sp. 1 specimen are typical of this species. Scale bar = 5 mm.

against substrate and moving body forward, a possible scent-marking behavior; Donaldson and Grether 2007; Willemart and Hebets 2011); and leg threading (moving leg through mouth parts, a self-grooming behavior; Edgar 1971; Pereira et al. 2004; Teng et al. 2012).

Mark-recapture study.—To measure daily turnover in the tree cavity aggregations, and to check for movement between nearby tree cavity and spiny palm roosts, we marked and recaptured harvestmen at seven tree cavity roosts (two other tree cavity roosts were found too late in the study period to be included in the mark-recapture study). All harvestmen in a cavity were captured by hand between 0900 and 1630 h and placed in a mesh cage (Bioquip Products). Individuals that initially were too deep inside the cavity to be captured were flushed out with a stick. The harvestmen were sexed, inspected for ectoparasitic larval mites, marked on the dorsal surface of

the abdomen with small dots of paint (Marvy Decocolor, Uchida of America, Torrance, CA) in color combinations corresponding to the capture date and location, and then released in their original cavities. This procedure was carried out on three consecutive days at each aggregation site and a final recapture was done on the fourth day. On all four days at each site, we also searched for marked harvestmen on all buttress roots and spiny palms within a 10 m radius. Recaptured individuals were given additional paint dots corresponding to the location and date of recapture. During this study, we marked 257 harvestmen.

Removal experiment.—Concurrent with the mark-recapture study, we captured and removed all of the harvestmen from 10 spiny palm roosts on at least four consecutive days and for up to six consecutive days if the site continued to attract new harvestmen. The animals were captured by hand and held temporarily in a mesh cage. Individuals that initially were too high to be captured were chased down with a wooden pole. The harvestmen were sexed, marked on the dorsal surface of the abdomen with small dots of paint identifying the capture location, and released at least 50 m away from the aggregation site on the trunk of another spiny palm. During the experiment, we removed 989 harvestmen (37–224 per site).

At each removal site, we took a standard set of measurements, including canopy cover, crown height, spine density, and trunk diameter (the first three factors have been found to correlate with the size of *Prionostemma* sp. 2 aggregations; Teng et al. 2012). Canopy cover was measured with a concave spherical densiometer (Forestry Suppliers Inc, Jackson, MS, USA). Crown height was measured with a graduated pole, and trunk diameter was measured with a ruler, on all of the spiny palms at a site. Spine density was measured by placing a 4 cm^2 wire quadrat on the trunk of the palms and counting all spines originating within the quadrat. The quadrat was placed at three different heights above the ground (0.8, 1.15, and 1.55 m) in the four cardinal directions around the trunk. If a site had more than five spiny palms within 1 m of each other, spine density was measured on half of the trees chosen at random. One observer made all measurements of a particular type. Site averages for spine density, crown height and trunk diameter were used in the analysis.

Analysis of harvestman communal roost sex ratios from the literature.—We searched the primary literature for reports of the sex ratio of harvestman communal roosts. For inclusion in our statistical analysis, a report needed to contain one of the following kinds of data on the sex ratio at communal roosts: the number of individuals of each sex, the total number of individuals and the sex ratio, or the sex ratio and its standard deviation. We did not impose our operational definitions of terms such as aggregation and roosting site (see above) on other studies but instead accepted the definitions used in the original studies. For example, some researchers define aggregations as groups of three or more individuals with overlapping legs (e.g., Willemart & Gnaspini 2004). However, we do not believe this compromised the validity of our literature review. In cases of multispecies aggregations (e.g., Machado & Vasconcelos 1998; Chelini et al. 2012), we analyzed the data for each species separately. Because a sample size of five is the minimum required to establish whether a sex ratio deviates significantly from 1:1 with a
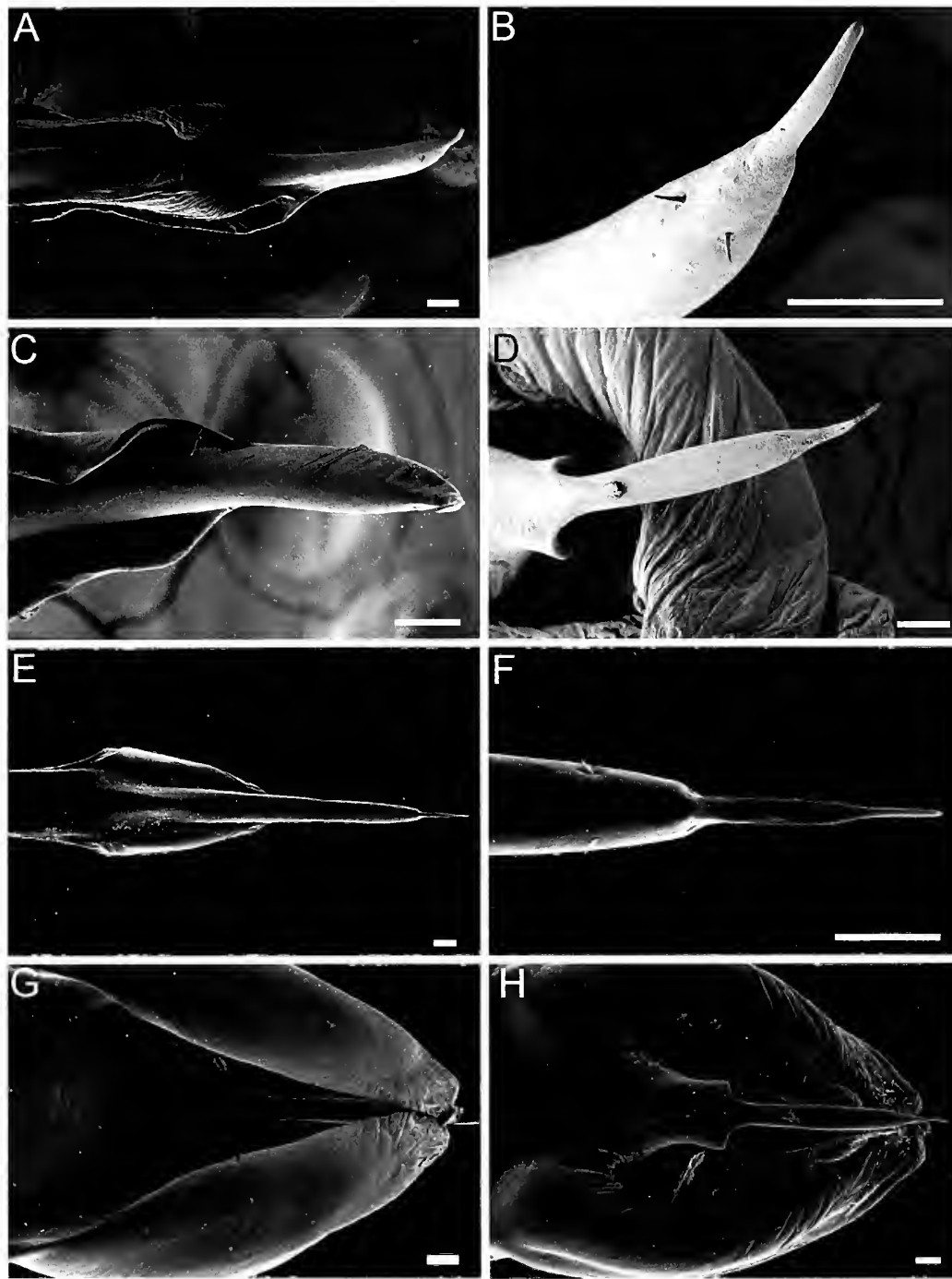
Figure 4.—Scanning electron micrographs of male genitalia. The genitalia of the species of *Prionostemma* that typically roosts in spiny palms at Refugio Bartola, Nicaragua (A, B) is very similar to that of *Prionostemma* sp. 2 (Proud et al. 2010) at La Selva, Costa Rica (E, F) in both shape and proportion. There is a small difference in the shape of the stylus – in panel F the stylus attenuates but in panel B it does not. Nevertheless, these are probably the same species. The curling of the alates (winglets) just before the glans on the Nicaraguan specimen (A) is an artifact. The species that roosts in tree cavities at Refugio Bartola (C, D) is undoubtedly the same species as *Prionostemma* sp. 1 at La Selva (G, H). The large structure at the base of the penis (best seen in panel H), the lateral expansions (alates), and the stylus are identical in size and shape, as viewed from both dorsal (C, G) and ventral perspectives (D, H), to *Prionostemma* sp. 1 at La Selva. Scale bar = 50 μm.

binomial test, we excluded sex ratios based on sample sizes smaller than five. We also excluded sex ratios based on samples that likely included solitary roosting or non-roosting harvestmen (e.g., Tsurusaki 2003). Because harvestmen can live for years as adults (Gnaspini 2007), we did not pool data from repeated visits to the same sites and instead analyzed

data from different months and seasons separately. In the case of the study of Mestre and Pinto-da-Rocha (2004), we chose one month per season that best represented the average sex ratio of all the months in that season. In the case of the study of Willemart and Gnaspini (2004), we pooled data from different aggregation sites but analyzed each collection date

Table 1.—Summary of mark-recapture study results. From left to right: the day of the study, the total number of harvestmen captured, the number that were unmarked until that day (i.e., not captured previously), the number that were marked from any previous capture, the percentage that were marked from any previous capture, the number returning on the next day, and the percentage returning on the next day.

Day	Total	Unmarked	Marked	% Marked	Returning	% Returning
1	172	172	–	–	96	55.8%
2	141	45	96	68.1%	69	48.9%
3	108	25	83	76.9%	30	27.8%
4	57	15	42	73.7%	–	–

separately because the sex ratio varied significantly within seasons.

Statistics.—Wilcoxon signed-rank tests were used to compare the microclimate inside and outside of cavities, because these data are paired by site. Skillings-Mack tests (nonparametric equivalents of repeated measures ANOVAs) were used to compare the change in harvestman numbers over time, because there were more than two time points. Binomial tests were used to compare the observed sex ratios to 0.5. Fisher exact tests were used to test for associations between nominal variables (e.g., sex and mite presence). Spearman rank correlations were used to test for correlations between continuous variables (e.g., roost sex ratio and canopy cover). For comparisons involving small sample sizes (e.g., number of roosts), we computed the P-values by permutation. All reported P-values are two-tailed. Ranges, means and standard deviations are provided to facilitate comparisons to other studies. Stata 12.1 (StataCorp, College Station, TX, USA) was used for the computations.

RESULTS

Roost characteristics and behavioral observations.—We found *Prionostemma* sp. 1 aggregations in nine (7.9%) of 114 buttressed trees examined. Solitary harvestmen of Cosmetidae species (e.g., *Cynorta* spp. Koch 1839, *Eucynorta* spp. Roewer 1912) were often found on the surface of the roots and in the gaps between them, but the *Prionostemma* aggregations were found only in cavities (i.e., holes) just above ground level. The cavities with *Prionostemma* aggregations seemed relatively narrow (mean ± sd, 0.34 ± 0.15 m, n =7) and deep (0.58 ± 0.12 m, n =7), compared to unused cavities. Trees with cavity roosts ranged in circumference from 1.25–8.14 m (mean ± sd, 3.72 ± 2.38 m, n = 7). Canopy cover readings taken at the base of the trees ranged from 92.7–96.7% (mean ± sd, 94.4 ± 1.4%, n = 7). The daytime surface temperature was consistently 1–2 °C lower inside the roosting cavities (mean ± sd, 25.0 ± 0.8 °C) than immediately outside (mean ± sd, 26.3 ± 1.4 °C; Wilcoxon signed-rank test, $T = 0$, $n = 7$, $P = 0.018$). There were no significant differences in daytime air temperature or humidity inside the roosting cavities (air temperature, 27.0 ± 0.8 °C; humidity, 86.1 ± 6.0%) compared to immediately outside (air temperature, 27.0 ± 0.8 °C, Wilcoxon signed-rank test $T = 2$, $n = 7$, $P = 0.29$; humidity, 88.7 ± 10.3%, $T = 1$, $n = 7$, $P = 0.08$). During behavioral observations made at the aggregation sites during the day,

Table 2.—Numbers of females (N_f) and males (N_m) and the sex ratio, calculated as the proportion female (P_f), at tree cavity roosts on the first day of the mark-recapture study, sorted from the most male-biased to the least male-biased. Binomial tests (BT) compare the observed sex ratio to 0.5. Two-tailed P-values are shown for samples with $n \geq 5$. With a sequential Bonferroni correction for multiple tests (Holm 1979), across the six P-values in the table, the criterion for statistical significance at $\alpha = 0.05$ is $P < 0.05$.

Tree cavity	N_f	N_m	P_f	P
1	2	18	0.1	0.0004
2	1	8	0.11	0.04
3	3	16	0.16	0.004
4	10	44	0.19	< 0.0001
5	8	28	0.22	0.001
6	8	23	0.26	0.01
7	1	2	0.33	–

most individuals were either stationary (mean of the site scan sampling means, 77.5%) or bobbing (19.2%). Some individuals were walking within the cavity (2.0%), but no leg-threading, ventral rubbing, foraging, or reproductive behaviors (e.g., mating, egg laying) were observed.

Mark-recapture study.—The maximum daily return of *Prionostemma* sp. 1 to the cavity roost where they were marked (i.e., from one day to the next) ranged from 44.4–77.4% per site ($n = 7$; mean ± sd, 59.4 ± 12.1%). Marked harvestmen were recaptured on 221 occasions and always in the same cavity where they were originally marked.

Despite the relatively high return rates, capturing and marking *Prionostemma* sp. 1 evidently reduced their likelihood of returning. The total number of *Prionostemma* found in the cavity roosts decreased from 172 on the first day to 141 on the second day, 108 on the third day, and 57 on the fourth day (Table 1). The change over time in harvestmen numbers was highly significant (Skillings-Mack test, SM = 15.3, simulation $P < 0.0001$). As the total number of harvestmen declined, the proportion of harvestmen that carried marks from any previous day's capture remained relatively stable but the proportion of harvestmen returning on the next day declined over time (Table 1).

Because recaptured individuals were given new marks on each day, we were able to infer that some individuals returned repeatedly to the same cavity. Of the 57 harvestmen found in the final recapture, 42 (73.7%) were present on a prior day, 32 (56.1%) were present on at least two prior days, and 17 (29.8%) were present on all three prior days.

Within the 10-m radii of the seven cavity roosts in the mark-recapture study, there were 35 other buttressed trees and 40 spiny palms. *Prionostemma* aggregations were found in one (2.8%) of these buttressed trees and two (5%) of the spiny palms. Only two harvestmen in the mark-recapture study were found away from the buttressed tree where they were marked. One was found on the trunk of another buttressed tree and the other was found in a spiny palm aggregation. In both cases, the marked individuals were within the 10-m radius of the cavity where they were marked (as opposed the 10-m radius of a different roost cavity).

The sex ratio at cavity roosts was strongly male-biased both overall (50 females, 207 males, proportion female = 0.24; binomial test $P < 0.0001$) and at all seven of the mark-recapture sites (Table 2; proportion female among all animals

Table 3.—Numbers of females (N_f) and males (N_m) and the proportion female (P_f) at spiny palm roosts prior to the first removal and after the first removal. Roost sites are sorted by P_f prior to the first removal, from the most male-biased to the most female-biased. Binomial tests (BT) compare the observed sex ratio to 0.5. Fisher's exact tests compare the pre-removal sex ratio to the post-removal sex ratio. Two-tailed P-values are shown. With a sequential Bonferroni correction for multiple tests (Holm 1979), across all 30 P-values in the table, the criterion for statistical significance at $\alpha = 0.05$ is $P < 0.003$.

Spiny palm	Prior to first removal				After first removal				Fisher's exact P
	N_f	N_m	P_f	P	N_f	N_m	P_f	P	
1	1	24	0.04	< 0.0001	3	9	0.25	0.14	0.09
2	5	37	0.12	< 0.0001	1	18	0.05	0.0008	0.65
3	15	91	0.14	< 0.0001	11	107	0.09	< 0.0001	0.30
4	8	34	0.19	< 0.0001	6	30	0.17	0.0007	1.0
5	17	14	0.55	0.72	28	50	0.36	0.02	0.09
6	58	36	0.62	0.03	13	46	0.22	<0.0001	< 0.0001
7	43	25	0.63	0.04	11	30	0.27	0.004	< 0.0001
8	31	7	0.82	0.0001	17	2	0.89	0.007	0.70
9	56	7	0.89	< 0.0001	41	17	0.71	0.002	0.02
10	16	2	0.89	0.001	15	7	0.68	0.13	0.15

marked, range 0.10–0.28). There was no significant variation among roost sites in the sex ratio of harvestmen marked during the first capture (Fisher's exact test, $P = 0.77$) or across all of the harvestmen marked during the study ($P = 0.27$), nor did the overall sex ratio change significantly over time from the first capture to the last recapture (Fisher's exact test, $P = 0.53$). Of the 221 recaptures, 50 (22.6%) were female, which did not differ significantly from the overall sex ratio (binomial test $P = 0.69$). Thus, males and females exhibited similar levels of individual site fidelity.
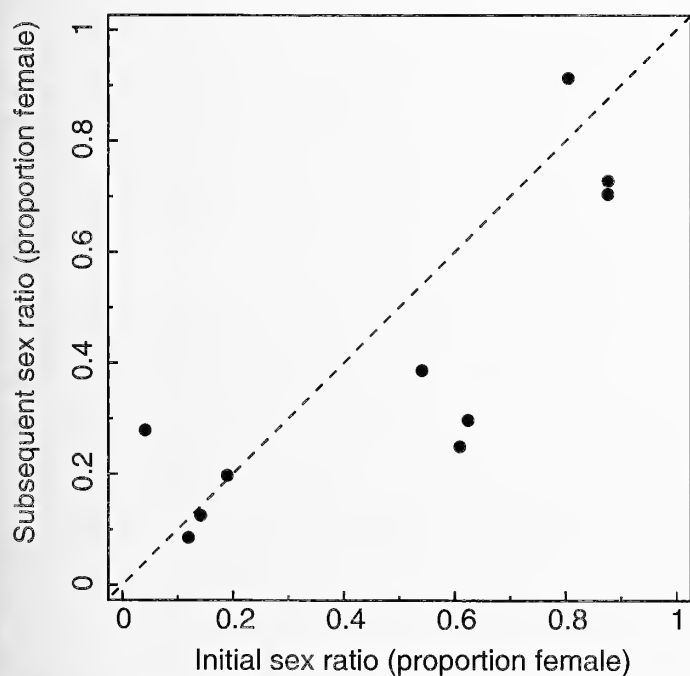

Figure 5.—Variation in, and effects of the removal treatment on, the sex ratio at spiny palm roosting sites. Each point represents the sex ratio (proportion female) before and after the removal treatment commenced at 10 established aggregation sites. The dashed line has a slope of 1 and thus points below the line indicate that the sex ratio decreased after the removal treatment began. See text for statistical results.

Red ectoparasitic larval mites were found on 21 (8.2%) of the 257 individuals marked in cavity roosts. The maximum number of mites per individual was three and most mites (24 of 26) were attached to legs. There was no significant sex difference in mite prevalence (16 of 207 males and 5 of 50 females; Fisher's exact test, $P = 0.57$). Mite prevalence varied significantly among sites (Fisher's exact test, $P < 0.0001$). No mites were found infesting harvestmen at four of the seven sites. At the site with the highest mite prevalence, 14 of 45 individuals (31.1%) had at least one mite. By comparison, mites were rare at the *Prionostemma* sp. 2 spiny palm aggregations during this study (fewer than 1 in 50 individuals; G.F.G et al., pers. obs.).

Removal experiment.—The removal treatment had an unexpected effect on the sex ratio at *Prionostemma* sp. 2 roosts. While the overall sex ratio was approximately 1:1 at the first removal (Table 3; 250 females, 277 males, proportion female = 0.49; binomial test $P = 0.26$), it was significantly male-biased in subsequent removals (total count: 146 females, 316 males, proportion female = 0.37; binomial test $P < 0.0001$). A sex ratio shift of this magnitude is very unlikely to have occurred by chance (Fisher's exact test, $P < 0.0001$). Seven of the 10 sites had strongly skewed sex ratios (female biased, $n = 3$; male biased $n = 4$), and despite complete turnover in roost membership, the initial and subsequent (i.e., post-removal) sex ratios were strongly correlated across sites (Fig. 5, Spearman rank correlation $r_s = 0.79$, $n = 10$ sites, $P = 0.008$). As shown in Fig. 5, three sites that initially had weakly female-biased sex ratios all shifted to having male-biased sex ratios, three sites that initially had strongly female biased sex ratios remained strongly female-biased, and four sites that initially had strongly male-biased sex ratios remained strongly male-biased. None of the measured site characteristics correlated significantly with the initial roost sex ratio (canopy cover $r_s = 0.22$, $n = 10$, $P = 0.53$; spine density $r_s = -0.02$, $P = 0.95$; crown height $r_s = 0.44$, $P = 0.20$; trunk diameter $r_s = -0.52$, $P = 0.14$).

All 989 of the harvestmen removed during this experiment were marked and released on other spiny palms. For the duration of the study, none of the marked harvestmen returned to the site where they were initially captured.

However, six marked individuals, from three different release sites, were found inside the same tree cavity in the mark-recapture study. The distance between the release sites and this tree cavity ranged from 28–45 m and the harvestmen were found there 1–2 days after they were released.

Harvestman communal roost sex ratios from the literature.— We found data on the sex ratios at communal roosts of 12 harvestman species in the published literature. Most of the reported communal roost sex ratios did not deviate significantly from 1:1 (Table 4). Significantly female-biased communal roost sex ratios were found in *Goniosoma albiscriptum* Mello-Leitão 1932 at one of seven sampling dates in 2000 (Willemart & Gnaspini 2004) and in a multi-year study of *Goniosoma longipes* Roewer 1931 (Machado et al. 2000), both at caves in southeastern Brazil. Tsurusaki (2003) reported significantly male-biased sex ratios in general collections of two harvestman species in Japan, but whether these species form roosting aggregations was not stated. *Prinostemma* sp. 1 appears to be the only known example of a harvestman with strongly male-biased communal roost sex ratios.

DISCUSSION

Roosting behavior (Mestre & Pinto-da-Rocha 2004; Willemart & Gnaspini 2004), sex ratios (Chelini et al. 2012), and mite infestation levels (Townsend et al. 2006) are all known to vary seasonally in harvestmen, so it cannot be assumed that the species differences that we observed hold year round. With that caveat, the preferred roosting microhabitats of the two *Prionostemma* species at Refugio Bartola during the dry season could scarcely be more distinct. All of the *Prionostemma* sp. 1 aggregations that we found were inside cavities at the base of buttressed trees, while *Prionostemma* sp. 2 aggregations are usually found several meters above the ground in spiny palms (Grether & Donaldson 2007). Some marked individuals were found moving between tree cavity and spiny palm aggregations, however, and a review of photos taken of roosting aggregation in previous years yielded three additional cases of individuals with the coloration of *Prionostemma* sp. 1 in spiny palm aggregations (G.F.G., pers. obs.). The extent to which these species intermingle at roost sites remains to be quantified. Solitary individuals of Cosmetidae harvestmen (e.g., *Cynorta*, *Eucynorta*) are often found in *Prionostemma* aggregations as well (unpublished data).

In mark-recapture studies, *Prionostemma* sp. 1 showed much higher daily return rates (up to 77%) than *Prionostemma* sp. 2 (up to 26%; Grether & Donaldson 2007). A likely explanation is that suitable tree cavities are scarce compared to spiny palms. Another possible explanation is that cavity roosts are easier for the harvestmen to relocate.

We found ectoparasitic larval mites on 8% of the *Prionostemma* sp. 1 and on less than 1% of the *Prionostemma* sp. 2. Whether this is causally related to the species difference in roosting habitat is unknown but seems possible. Species differences in larval mite infestation rates have previously been linked to species differences in foraging habitats (Townsend et al. 2008).

We have found evidence for handling effects in both species (see Grether & Donaldson 2007 for the *Prionostemma* sp. 2 evidence), but the rapid decrease over time in the number of *Prionostemma* sp. 1 at the mark-recapture sites leaves little

doubt that capturing these animals makes them less likely to return to the same site. Harvestmen have been shown to have spatial associative learning ability (dos Santos et al. 2013) and may avoid sites where they have previously been disturbed. Another possible explanation is that captured harvestmen release defensive chemicals (Machado 2002; Machado et al. 2002; Eisner 2004; Rocha et al. 2013) that persist at the site of disturbance and make it less attractive for roosting. In any case, the decreasing return rate over time (Table 1) suggests that these harvestmen would rapidly abandon a site where they were disturbed repeatedly.

Perhaps the most interesting species difference found in our study at Refugio Bartola is the difference in roost sex ratios. The *Prionostemma* sp. 1 aggregations were strongly male biased (76% male), which may be rare in harvestmen. The communal roosts of some insects are male biased (Alcoek 1998; Switzer & Grether 1999), but our review of the literature turned up no other harvestman examples (Table 4). Most harvestmen aggregation sex ratios reported in the literature do not differ significantly from 1:1, but female biases have been reported in several Laniatores species (Table 4). In some cases, the sex ratio at communal roosts may merely reflect the population sex ratio (Chelini et al. 2012), and female-biased population sex ratios may be indicative of facultative parthenogenesis (Tsurusaki 1986, 2003). Willemart and Gnaspini (2004) found that the communal roosts of *Goniosoma albiscriptum* (Laniatores: Gonyleptidae) were more female-biased than the population sex ratio and hypothesized this is because males are more aggressive and less gregarious than females. *Goniosoma albiscriptum* roosting aggregations break up during the peak reproductive season, perhaps because females become intolerant of conspecifics while guarding their eggs and males become intolerant of all other males (Willemart & Gnaspini 2004). A similar mechanism could potentially account for male-biased roost sex ratios, if males continued to roost communally while females roosted away from aggregation sites to guard their eggs. We did not encounter egg-guarding females during our study, however. Thus, the male-bias of *Prionostemma* sp. 1 communal roosts is a mystery that merits further study.

Although the overall sex ratio of *Prionostemma* sp. 2 roosts did not differ from 1:1, most of the aggregation sites were strongly sex biased. In the removal experiment, sites that initially had weakly female-biased sex ratios became male-biased while sites with strongly skewed sex ratios remained skewed in the same directions despite complete turnover in roost membership (Table 3, Fig. 5). A possible explanation for the shift in the overall sex ratio is that males are more vagile than females, as has been reported for other species of harvestmen and for arachnids generally (reviewed in Willemart and Gnaspini 2004). If removing the harvestmen from an aggregation site temporarily depletes the local pool of potential recruits, males may move into the area first, resulting in a temporary male bias in the roost sex ratio. But why would some sites attract mainly females? Sex differences in roost-site preferences could potentially explain the pattern, but none of the roost characteristics that we measured were predictive of the sex ratio. Another possible explanation is that the sexes differ in their scent-marking chemicals and are most strongly attracted to same-sex scent. The latter hypothesis could be

Table 4.—Sex ratios of harvestman roosting aggregations reported in the literature. Abbreviations in column titles: Mo., month; N_{ag}, number of aggregations sampled; N_f, number of females; N_m, number of males; P_f, proportion female; Sig., significance level of statistical test versus $P_f = 0.5$; Ref., source of data. Country codes: BR, Brazil; NL, Netherlands; NI, Nicaragua; US, United States. Season codes: Sp, spring; Su, summer; W, winter; F, fall. P-values for 2-tailed tests: NS $P > 0.1$; $ $P > 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Reference codes: 1, Machado and Vasconcelos 1998; 2, Mestre and Pinto-da-Rocha 2004; 3, Willemart and Gnaspini 2004; 4, Machado et al. 2000; 5, Machado 2002; 6, Chelini et al. 2012; 7, Cockerill 1988; 8, Wijnhoven et al. 2007; 9, this paper. Dashes indicate that the information was not provided. Binomial tests were used if harvestmen counts were provided; in lieu of counts, if means and standard deviations were provided, we tested whether the 95% confidence interval of the roost sex ratio included zero. The horizontal dashed line separates the suborders; species above the line are in the suborder Laniatores; species below the lines are in the suborder Eupnoi.

Family	Species	Location	Mo. (season)	N_{ag}	N_f	N_m	P_f	Sig.	Ref.
Gonyleptidae	*Despirus montanus*	Serra do Cipó, Minas Gerais, BR	Nov (Sp)	5	26	16	0.62	NS	1
Gonyleptidae	*Discocyrtus sp. 1*	Curitiba, Parana, BR	Aug (W)	–	8	4	0.67	NS	2
Gonyleptidae	*Discocyrtus sp. 1*	Curitiba, Parana, BR	Nov (Sp)	–	9	6	0.60	NS	2
Gonyleptidae	*Discocyrtus sp. 1*	Curitiba, Parana, BR	Dec (Su)	–	5	4	0.56	NS	2
Gonyleptidae	*Discocyrtus sp. 1*	Curitiba, Parana, BR	May (F)	–	7	6	0.54	NS	2
Gonyleptidae	*Eugyndes sp.*	Serra do Cipó, Minas Gerais, BR	Nov (Sp)	5	15	23	0.39	NS	1
Gonyleptidae	*Geraecormobius sp.*	Curitiba, Parana, BR	Jan (Su)	–	5	4	0.56	NS	2
Gonyleptidae	*Goniosoma albiscriptum*	Ribeirão Pires, São Paulo, BR	Apr (F dry)	1	24	3	0.89	***	3
Gonyleptidae	*Goniosoma albiscriptum*	Ribeirão Pires, São Paulo, BR	May (F dry)	1	9	9	0.50	NS	3
Gonyleptidae	*Goniosoma albiscriptum*	Ribeirão Pires, São Paulo, BR	Jun (W dry)	1	24	12	0.67	$	3
Gonyleptidae	*Goniosoma albiscriptum*	Ribeirão Pires, São Paulo, BR	Jun (W dry)	1	17	7	0.71	$	3
Gonyleptidae	*Goniosoma albiscriptum*	Ribeirão Pires, São Paulo, BR	Jul (W dry)	1	18	8	0.69	$	3
Gonyleptidae	*Goniosoma albiscriptum*	Ribeirão Pires, São Paulo, BR	Jul (W dry)	1	9	3	0.75	NS	3
Gonyleptidae	*Goniosoma albiscriptum*	Ribeirão Pires, São Paulo, BR	Aug (W dry)	1	4	3	0.57	NS	3
Gonyleptidae	*Goniosoma longipes*	Florestal Itapetinga, São Paulo, BR	Mar–Jun	28	–	–	0.64	*	4
Gonyleptidae	*Goniosoma aff. proximum*	Ilha do Cardoso, São Paulo, BR	–	82	–	–	0.52	NS	5
Gonyleptidae	*Ilhaia cuspidata*	Curitiba, Parana, BR	Aug (Sp)	–	131	131	0.50	NS	2
Gonyleptidae	*Ilhaia cuspidata*	Curitiba, Parana, BR	Sep (Sp)	–	159	127	0.56	$	2
Gonyleptidae	*Ilhaia cuspidata*	Curitiba, Parana, BR	Dec (Su)	–	136	149	0.48	NS	2
Gonyleptidae	*Ilhaia cuspidata*	Curitiba, Parana, BR	May (F)	–	445	390	0.53	$	2
Gonyleptidae	*Serracutisoma spelaeum*	Intervales, São Paulo, BR	Jan, Feb, Apr–Oct	28	–	–	0.55	–	6
Gonyleptidae	*Serracutisoma proximum*	Intervales, São Paulo, BR	May–Sep (W dry)	7	–	–	0.61	–	6
Leiobunidae	*Leiobunum townsendi*	Austin, Texas, US	Sep (Su)	1	157	167	0.48	NS	7
Leiobunidae	*Leiobunum sp.*	NL	–	–	349	304	0.53	$	8
Sclerosomatidae	*Prionostemma sp. 2*	Refugio Bartola, Rio San Juan, NI	Feb (W dry)	10	250	277	0.47	NS	9
Sclerosomatidae	*Prionostemma sp. 1*	Refugio Bartola, Rio San Juan, NI	Feb (W dry)	7	33	139	0.19	***	9

tested with single-sex group translocations. If females are more strongly attracted to female scent than are males, sites where only females are released should attract more female than male recruits on subsequent days.

In contrast to the sharp habitat distinction that we found at Refugio Bartola, at La Selva Biological Station both *Prionostemma* species are typically found on the vertical surfaces of medium to large tree trunks or buttresses and nearby shrubs (Proud et al. 2012). Harvestman roosting behavior can change seasonally (Holmberg et al. 1984; Chelini et al. 2011), so it is important to consider whether the reported differences between sites could be an artifact of the timing of the research conducted at the two sites. At Refugio Bartola, *Prionostemma* sp. 2 has been studied between the months of January and May, which includes the dry season (February–April) and parts of the wet season. Spiny palms are used as roosting sites throughout this period, and the observation that the locations of the communal roosts are stable from one year to the next (Teng et al. 2012; Grether et al. 2014), combined with what is known about the mechanism of roost formation (Donaldson & Grether 2007), indicates that spiny palms are used as aggregation sites year-round at this site. That is, if the communal roosts were abandoned for part of the year, they would presumably form in different spiny palms in different years, because individuals are not roost-site faithful and suitable spiny palms are not limiting (Donaldson & Grether 2007; Grether & Donaldson 2007; Teng et al. 2012). At La Selva Biological Station, harvestmen have been studied in both the dry and wet seasons, and one of us has searched for Sclerosomatidae aggregations in spiny palms and the buttresses of large trees during both seasons and encountered none (V.R.T., pers. obs.). Thus, we are confident that *Prionostemma* roosting behavior differs between the sites.

How might population differences in roosting patterns arise? We first consider a sort of null model of the roost formation process. If individual harvestmen had no microhabitat preferences and roost formation was based solely on conspecific attraction (including scent-mark detection), then the locations of roosting sites would be expected to drift randomly over time through chance colonization events. Under this null model, we would expect communal roosts to form repeatedly at the same locations but not exclusively in a specific microhabitat. Aggregations would be expected to persist longer at sites where the harvestmen survived at higher rates, however, and this could lead to a pattern in which, at any given time, most aggregations formed in microhabitats that offered protection from predators, desiccation, etc. Thus, geographic variation in roosting patterns could arise simply through chance events and variation in the factors that influence survival rates in different microhabitats (predator species, climate, etc.). A more realistic model would have individuals searching for roosting aggregations in the microhabitats where they are most likely to form, either because of associative learning or because microhabitat preferences evolve to track roosting patterns, or some combination of these mechanisms. Nevertheless, the sort of cultural drift envisioned in the null model seems likely to play some role in population differentiation.

One way to investigate the relative importance of habitat preferences versus conspecific attraction would be to seed new *Prionostemma* aggregations in different kinds of vegetation, using the group translocation method (Teng et al. 2012), and follow their fate. At Refugio Bartola, *Prionostemma* sp. 2 aggregations occasionally form on non-spiny understory plants (e.g., Rubiaceae: *Psychotria*) but not in the same places in different years (G.F.G., pers. obs.). The aggregations in spiny palms may persist longer than those in other types of vegetation simply because palm spines offer protection from predators, such as anoline lizards (Grether & Donaldson 2007). There is also evidence, however, that these harvestmen prefer spiny palms per se. When spines were experimentally removed from established roosting sites, the aggregations shifted rapidly over to previously unused spiny palms, if any were nearby (Donaldson & Grether 2007). Thus, it would be interesting to examine whether a tradition of roosting in spiny palms, once introduced, would spread through the *Prionostemma* sp. 2 population at La Selva Biological Station.

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From spiderling to senescence: ontogeny of color in the jumping spider, *Habronattus pyrrithrix*

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Abstract. The diverse colors of animals serve a variety of purposes, from acquiring mates to avoiding predators. Often, color patterns are not static throughout life, but change drastically during development, maturity, and senescence. While recent work has focused on the signaling value of vibrant colors in jumping spiders (Salticidae), we know very little about how colors change as spiders age; such information can provide a context for understanding the functions of and constraints on colorful traits. Focusing on *Habronattus pyrrithrix* Chamberlin 1924, our goals were to examine (1) the microscopic morphology of the colored body regions that males display to females during courtship (i.e., males' red faces, green legs, and white pedipalps), (2) how the colors of these regions as well as dorsal color patterns change during development prior to sexual maturity, and (3) how male condition-dependent red facial and green leg coloration changes as males age beyond sexual maturity. Although the bright white pedipalps and green legs of males appeared only upon sexual maturity, the sexes began to differentiate in facial coloration and dorsal patterning, with males developing red faces and conspicuous black and white dorsal patterning as young juveniles (ca. 2.5 mm in body length, or ca. 45% of their total mature adult body size). Even after maturity, color was not static; a male's green legs (but not red face) faded with age. Results are discussed in the context of potential functions of and constraints on color in salticids, and how they may change throughout an individual's lifetime.

Keywords: Juvenile coloration, Salticidae, sexual dichromatism, sexual dimorphism, sexual selection

Animal colors and patterns can serve a variety of functions. During courtship, they can aid in species recognition or convey information about the quality of an individual as a mate (see reviews in Andersson 1994; Hill & McGraw 2006). They also frequently keep animals hidden (i.e., camouflage) or protected (i.e., aposematism, mimicry) from predators (see reviews in Cott 1940; Ruxton et al. 2004). In many animals, color patterns are not static throughout life, but change dramatically during development, maturity, and senescence, as well as seasonally (Booth 1990). When color patterns differ between the sexes, examination of ontogenetic color change is particularly interesting because the timing and extent of sexual color differentiation can provide clues to the costs and benefits of different color patterns and their functions and constraints across contexts throughout life.

Color change from development to adulthood is typically thought to represent shifts in selection pressures as individuals change in size, mobility, vulnerability to predation, habitat use, or reproductive status (Booth 1990). In animals where bright male colors have evolved via sexual selection, sex-specific color patterns often appear suddenly upon sexual maturity, presumably because they are costly and unnecessary for juveniles (Andersson 1994). When sexually selected colors appear before sexual maturity, they are particularly interesting because they may hint at previously overlooked functional roles (e.g., Kilner 2006; Kapun et al. 2011). When the sexes differ in color due to different ecological selection pressures (e.g., Slatkin 1984), the timing of color pattern divergence can help us understand shifting selection pressures. For example, in the lizard *Eremias lugubris*, adults and older juveniles are tan and cryptic, whereas young juveniles have highly conspicuous markings, mimicking noxious oogpister beetles (Huey & Pianka 1977); in this system, subtle and changing functional roles of color would be missed by limiting study to adult stages.

Adult organisms can also change color as they age beyond sexual maturity (Booth 1990). In many birds, colors used to attract mates do not appear immediately upon sexual maturity, but are delayed until after the first breeding season (reviewed in Hawkins et al. 2012). Animals may also decline (more subtly) in color with senescence; colorful pigments or structures contained within dead tissue (e.g., feathers, scales) can fade with age as a product of abrasion, soiling, or photobleaching (Ornborg et al. 2002; McGraw & Hill 2004; Delhey et al. 2006; Kemp 2006). If maintaining colors is costly, age-based fading can have important consequences for signaling, with the ability to maintain bright colors (i.e., the ability to resist tissue/pigment damage) acting as an indicator of quality (e.g., Delhey et al. 2006). Alternatively, color fading may provide direct information about an individual's age (Manning 1985). Such information could help individuals identify more mature, viable mates (reviewed in Kokko & Lindstrom 1996). Alternatively, if older individuals are more likely to carry disease or parasite infection (e.g., Tarling & Cuzin-Roudy 2008) or if they are more likely to accumulate deleterious mutations in their germ-line (Beck & Promislow 2007), age-based color variation might enable individuals to select younger mates. A deeper understanding of how, and ultimately, why colors change with age will enable us to generate informed hypotheses about their potential signal content and evolution.

Jumping spiders (Salticidae) are an excellent group in which to examine ontogenetic color change from development through senescence. In many species, adult males are more colorful than females and display these colors to females during courtship or to other males during competitive interactions (e.g., Peckham & Peckham 1889, 1890; Lim & Li 2004; Girard et al. 2011). In addition, sexual dichromatism in dorsal color that is not displayed during courtship may

reflect different predator-avoidance strategies of males and females (LAT, unpub. data). To date, only three jumping spider species have had their colors quantified using modern color measurement techniques (i.e., speetrophotometry) (*Cosmophasis umbratica* Simon 1903 (Lim & Li 2006), *Phintella vittata* (C.L. Koch 1846) (Li *et al.* 2008a), and *Habronattus pyrrithrix* Chamberlin 1924 (Taylor et al. 2011)), and in only one study were juvenile colors measured (Lim & Li 2006). To our knowledge, no study has documented age-based changes in salticid colors as they develop from spiderlings through sexual maturity. Because species descriptions and dichotomous keys typically include details on only adults, with anatomy of mature genitalia required for proper identification (e.g., Ubick et al. 2005), the salticid literature includes few, even qualitative, descriptions of juvenile eolor patterns (but see Nelson 2010 for an exception).

The genus *Habronattus* F.O.P. Cambridge 1901, containing approximately 100 species, is one of the most highly ornamented groups; males are typically elaborately colored whereas females are cryptic (Griswold 1987; Maddison & Hedin 2003). Furthermore, patterns of juvenile coloration also vary across the genus (LAT, pers. obs.). For example, in *H. hirsutus* (Peekham & Peckham 1888) juveniles of both sexes are indistinguishable from one another to the human eye and resemble cryptic adult females until sexual maturity (LAT, pers. obs.). In *H. hallani* (Richman 1973) juveniles of both sexes are indistinguishable from one another but have striking dorsal color patterns unlike either adult males or females (LAT, pers. obs.). In *H. pyrrithrix*, juvenile males and females exhibit color patterns similar to those of sexually mature adults; males have red faces and striped dorsal patterns, whereas females are drab and cryptic throughout their life (LAT, pers. obs). This diversity in ontogenetic color change suggests that the costs, benefits, and functions of juvenile colors might be just as interesting as those of adults. Additionally, there is evidence that, after reaching maturity, adult male ornamental colors in *H. pyrrithrix* continue to undergo additional age-related changes, which could have important implications for sexual signaling (Taylor et al. 2011).

In this study, we focused on *Habronattus pyrrithrix*; males of this species are adorned with red faces, green front legs, and white pedipalps that they display to females during courtship. Our goals were to (1) examine the microscopic morphology of the elaborately colored body regions that males display (i.e., red faces, green legs, and white pedipalps), (2) examine how the colors of these regions as well as dorsal color patterns change during development leading up to sexual maturity, and (3) examine how male condition-dependent red facial and green leg coloration changes as males age beyond sexual maturity. The red facial and white pedipalp colors of *H. pyrrithrix* are contained within modified setae, or scales (e.g., Hill 1979), while the green leg coloration is present on the surface of the cuticle of the femur (e.g., Parker & Hegedus 2003; Ingram et al. 2011), which is further adorned with white scales (LAT, pers. obs.). Recent work on *H. pyrrithrix* suggests that adult male facial and leg colors are correlated with body condition in the field (Taylor et al. 2011). The red (but not green) coloration is variable among males of the same age and is positively correlated with the quality of a male's diet

(Taylor et al. 2011), and the presence of red coloration improves courtship success in certain contexts (Taylor & McGraw 2013); however, we know nothing about the role of red faeial coloration in juvenile males. We have hypothesized elsewhere that the conspicuous dorsal coloration in sexually mature adult males (combined with characteristic leg-waving behavior and high movement rates associated with mate searching) provides protection from predators through imperfect mimicry of bees and/or wasps (see Taylor 2012), yet we know nothing about the potential factors that might shape color differences in sexually inactive juveniles. Even after maturity, male colors do not appear to be static (Taylor et al. 2011). Throughout the mating season, the scales that produce the colors may undergo natural wear and degradation, which may result in predictable, post-maturity, age-related deterioration of color (e.g., Kemp 2006; Kemp & Macedonia 2006); this may allow females to use color to assess a male's age during courtship (e.g., Manning 1985).

To our knowledge, this will be the first study to quantify ontogenetic color changes throughout development in any of the more than 5000 species (Platnick 2013) of jumping spiders. Standard portable spectrophotometers used in animal coloration studies (reviewed in Andersson & Prager 2006) typically have a minimum reading area of 1 mm (e.g., Lim & Li 2006; Moreno et al. 2006; Galvan & Moller 2009); thus, precise quantifieation of color can only be done on relatively large body regions (>1 mm). Thus, using standard equipment makes the study of minute patches of color on small species of spiders challenging and makes the detailed study of color on particular body regions of juvenile salticids (e.g., faces, legs, pedipalps) impossible. Here we use a custom-designed microspectrophotometer (see Methods and also Taylor et al. 2011), allowing us to earefully measure minute patehes of color on juveniles and compare colors with those same precise areas on adult spiders.

METHODS

Study species.—*Habronattus pyrrithrix* is found throughout southern California and Arizona, USA, south to Sinaloa, Mexico (Griswold 1987). In Phoenix, Arizona, they are quite common and found at high densities in riparian areas, grassy backyards, and agricultural fields (LAT, pers. obs.). Geographic variation in coloration is common within the genus *Habronattus* (see Griswold 1987) and thus some subtleties of color pattern described in the present study for Phoenix, AZ animals may vary across the species range. Voucher specimens from our study population have been deposited in the Florida State Collection of Arthropods, Gainesville, FL, U.S.A. Additional details on the biology and courtship display behavior of *H. pyrrithrix* are provided elsewhere (Taylor et al. 2011; Taylor & McGraw 2013). Most temperate spiders live only one year in the field (see Foelix 2011); to our knowledge, nothing is known about how long *H. pyrrithrix*, in particular, live under natural conditions.

Scale morphology of adult male ornaments (Study 1).—Using five sexually mature adult specimens, we imaged the color patches on the males' red face, green front legs, and white pedipalps that they display to females, using a Leica-Cambridge Stereoscan 360 field emission scanning electron microscope (SEM) (Leica Microsystems, Wetzlar, Germany)

at an acceleration voltage of 2 kV. Prior to imaging, we allowed frozen specimens to air-dry overnight and then mounted the carapace, legs, and pedipalps onto standard SEM stubs using conductive graphite paint.

Ontogenetic color change in juveniles (Study 2).—To examine how male and female coloration changes during juvenile development in the field, we collected spiders ($n =$ 135) from a range of developmental stages (i.e., size classes) between May and October 2008 from a single, dense population within an agricultural area in Queen Creek, Arizona, USA (Maricopa County, 33.224744° N, 111.592825° W). This population was chosen because, in contrast with other sites where multiple species are abundant and interact (LAT, unpub. data), the only species of *Habronattus* that we have ever seen at this site in five years is *H. pyrrithrix*. This allowed us to be confident that all spiderlings and juveniles included in the present study were *H. pyrrithrix*. Specifically, we collected spiderlings (before they are able to be sexed, ca. 1.5–2.0 mm in length, $n = 15$), small juveniles (ca. 2.5 mm, $n = 15$ males, $n = 15$ females), large juveniles (ca. 3 mm, $n = 15$ males, $n = 15$ females), subadults (ca. 4–6 mm, $n = 15$ males, $n = 15$ females) and sexually mature adults (ca. 5–7 mm, $n = 15$ males, $n = 15$ females). Immediately after collection, we froze spiders ($-80°$ C) for later color analysis.

Post-maturity age-related changes in condition-dependent male ornaments (Study 3).—To examine how adult male color changes with age post-maturity, we collected 12 gravid adult females in July and August 2008 from the same population described above, brought them back to the lab and allowed them to lay eggs. Spiderlings were housed together until they were large enough to be sexed (ca. 2.5 mm in length), at which point the first three males from each female's egg sac were removed, housed separately in clear plastic containers (6 × 6 × 13 cm), and fed a constant diet of small crickets (*Acheta domesticus*) three times per week. Spiders ($n = 36$; three from each of 12 egg sacs) were checked daily to determine if they had molted; within each clutch, as males reached their final molt to maturity, they were randomly assigned to one of three different age groups (0, 60, and 120 days post-maturity). These age ranges were chosen because they likely represent the difference in ages of males in the field during the most active part of the mating season at this site (approximately May–August; LAT, pers. obs.). When males reached the appropriate randomly assigned age (0, 60, or 120 days post-maturity), we euthanized them and placed them in the freezer ($-80°$ C) for later color analysis.

Color measurement and analysis.—Body colors were quantified following methods described in Taylor et al. (2011). Briefly, we used a reflectance spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA) coupled to a modified Leica DMLB2 fluorescence light microscope with a 40× quartz objective lens (Leica Microsystems, Wetzlar, Germany) and illuminated with a full-spectrum Leica 75 W xenon arc lamp (Leica Microsystems, Wetzlar, Germany). This setup allowed us to quantify the minute color patches of all size classes of these spiders that are too small to measure accurately with standard spectrophotometry equipment. Unfortunately, the optics of the microscope cut out a portion of the UV spectrum, so this instrument only provides spectral data from 375–700 nm. In some jumping spider species, UV

reflectance appears to be important in communication (Lim et al. 2007, 2008; Li et al. 2008b), and thus we must use caution when excluding UV wavelengths from our analyses. However, in a previous study (Taylor et al. 2011), we confirmed that, though reflectance does extend into the UV for the green legs and white pedipalps of *H. pyrrithrix*, there are no UV peaks in either region, so the benefit of using an instrument that allows precise and repeatable measures on minute color patches of these tiny spiders far outweighs the disadvantage of excluding UV.

For Study 2, where we were interested in color changes of the faces, front legs, and pedipalps of males and females that occurred during juvenile development through maturity, we took the average of two reflectance measures of each of these three body regions. The colored areas that we measured on each specimen were 0.25 mm in diameter. For facial coloration, both measurements were taken from the same region of the face (just below the anterior median eyes). For leg coloration, one measurement was taken from the ventral side of each (right and left) femur. For pedipalp coloration, one measurement was taken from the distal segment of each (right and left) pedipalp. From these spectral data, we calculated the single color variable that captured the most sex- and age-related variation for each body region scored. Specifically, because face color among the different sex/age classes varied from white to red, the metric that captured most of this variation was 'red chroma' (i.e., the proportion of total reflectance in the red region of the spectrum, between 600 and 700 nm). Similarly, because the front legs varied from white to green, the metric that captured most of this variation was 'green chroma' (the proportion of total reflectance between 450 and 550 nm). Finally, because the pedipalps varied in coloration from gray to bright white, brightness (total reflectance over the entire spectrum) was the metric that captured most of this variation. For a detailed discussion of the rationale behind selecting relevant color variables, including those used here, see Montgomerie (2006). In addition, we qualitatively characterized the dorsal color pattern of individuals as either (1) tan and cryptic in coloration, similar to the dorsal coloration of adult females, or (2) consisting of black and white stripes and chevrons characteristic of adult males; all individuals examined fit clearly into one of these two categories (see Results). Because these categorizations were based on pattern rather than reflectance properties of the colors, we did not quantify dorsal coloration spectrophotometrically.

For Study 3, where we were interested in more subtle, age-based fading of display colors in adult males, we limited our analysis to the coloration of the red face and green legs, because previous studies showed that these two color patches were correlated with body condition in the field, presenting the possibility that such condition-dependence could be explained in part by the fading of colors as males age (Taylor et al. 2011). We took the average of two reflectance measures from each region and used these spectral data to calculate three color variables that were previously found to be correlated with body condition in the field: (1) the hue of the red face (the wavelength corresponding to the inflection point of the red curve), (2) the red chroma of the face (the proportion of total reflectance between 600 and 700 nm), and (3) the brightness
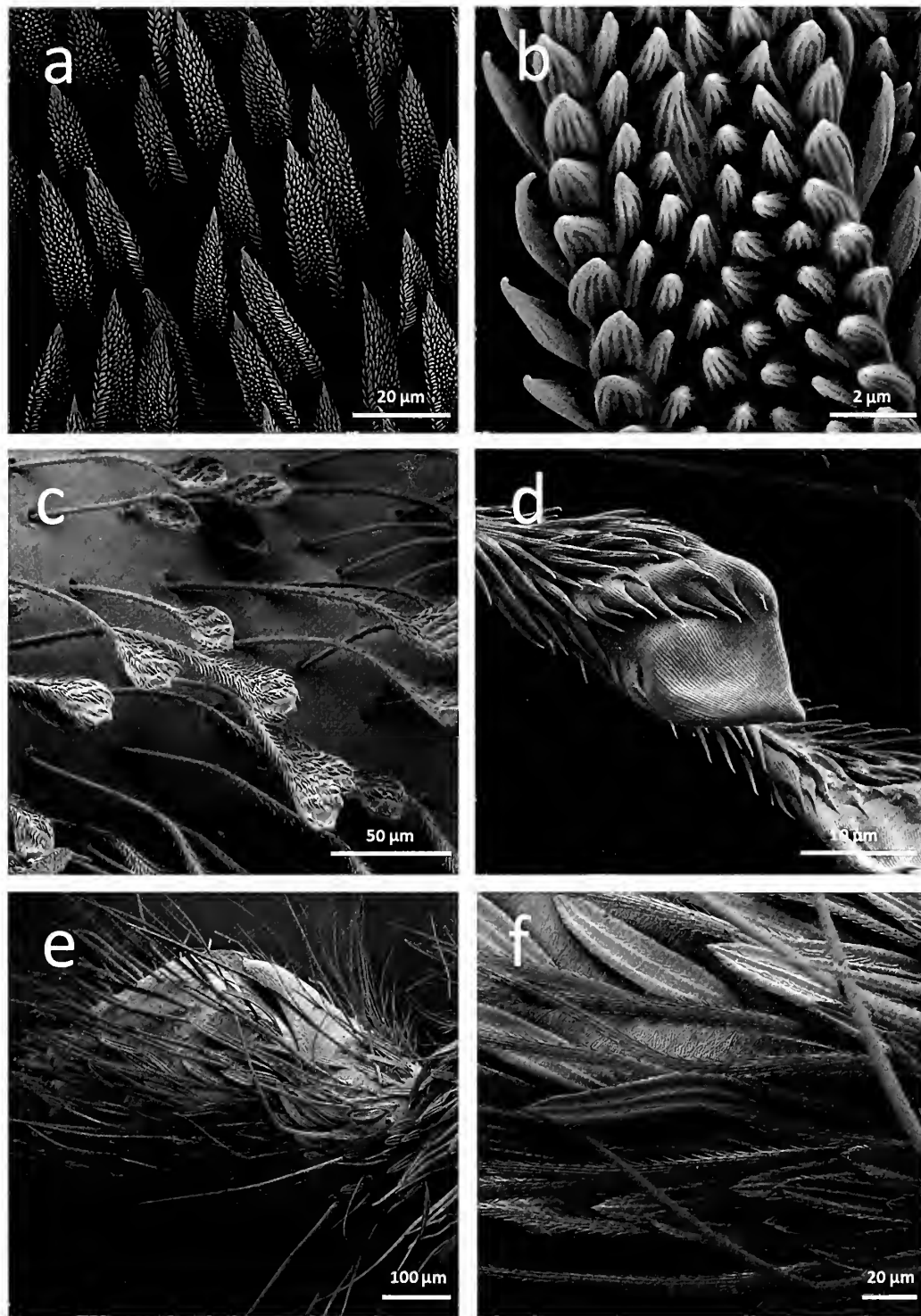
Figure 1.—Morphology of the colored body regions of adult male *Habronattus pyrrithrix*. a–b. Red scales on the face showing ridged protrusions; c–d. White spatulate scales ornamenting the green front leg (femur); e–f. Flat white pedipalp scales.

(mean reflectance) of the green front legs, following the methods described in Taylor et al. (2011). We also determined the relative size of the male's red facial patch; because larger males had larger red faces, we used the residuals of a regression of patch area on carapace width, which provides a 'relative patch size index' that is uncorrelated with body size and has previously been found to be correlated with body condition in the field (Taylor et al. 2011). Three males died

over the course of the study for unknown reasons and were thus excluded from our analyses.

Statistical analysis.—For Study 2, we used analyses of variance (ANOVA) to examine effects of developmental stage (i.e., size class), sex, and their interaction on face color (red chroma), front leg color (green chroma), and pedipalp color (mean brightness). Data did not meet normality and equal-variance assumptions and thus were rank-transformed

Table 1.—Results of ANOVA examining the effect of sex, age (i.e., size class), and their interaction on color metrics associated with the face, legs, and pedipalps during development in *H. pyrrithrix* jumping spiders. Df = degrees of freedom.

Red chroma of face	Df	F	P
sex	1,140	304.96	<0.001
age	4,140	6.30	<0.001
sex × age	4,140	20.27	<0.001

Green chroma of legs	Df	F	P
sex	1,140	0.28	0.60
age	4,140	9.37	<0.001
sex × age	4,140	5.10	<0.001

Brightness of pedipalps	Df	F	P
sex	1,140	1.43	0.23
age	4,140	41.54	<0.001
sex × age	4,140	3.33	0.01

(Conover & Iman 1981) prior to analysis. For Study 3, we used ANOVA to examine the effects of age on the hue, red chroma, and the relative size of a male's red face and on the brightness of his green legs. Because we used three males from each clutch (one assigned to each age category), we included the clutch (i.e., mother's identity) as a random factor in the model. Following ANOVA, we compared the colors among age classes using Tukey-Kramer pairwise comparisons with an alpha level of 0.05. All data from Study 3 met the assumptions of parametric statistics. All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

SEM analyses revealed varied scale structure on the three different colorful body regions of males (Fig. 1). On the red face, we found ridged protrusions covering the surface of each scale (Figs. 1a, b). The green legs were ornamented with long spatulate scales, the flattened ends of which were covered with fine ridges (Figs. 1c, d). The white scales on the pedipalps were similar in size and shape to the red facial scales, but were relatively smooth by comparison (Figs. 1e, f).

In Study 2, we found a significant effect of the age × sex interaction on all three color metrics examined (Table 1), indicating that colors developed differently between the sexes. Although spiderlings of both sexes had sparse red scales around their anterior median eyes (Fig. 2a), development of red coloration on the face was apparent in small juvenile males and increased into adulthood, whereas small juvenile females developed white facial scales (Figs. 2, 3a). Similarly, the conspicuous dorsal color pattern of males was also fully developed in small juveniles (ca. 2.5 mm), whereas spiderlings of both sexes and juvenile females had a cryptic, tan dorsal color pattern similar to adult females (Fig. 4). In contrast, the green coloration of the legs and the bright white pedipalp coloration typical of adult males showed a sudden onset at sexual maturity (Fig. 3b, c).

In Study 3, the green leg coloration of adult males was brighter (lighter) with increasing age ($F_{2,21} = 4.17$, $P = 0.03$; Fig. 5d), but we found no effect of age on any aspect of red facial coloration (hue: $F_{2,21} = 0.37$, $P = 0.69$; red chroma:
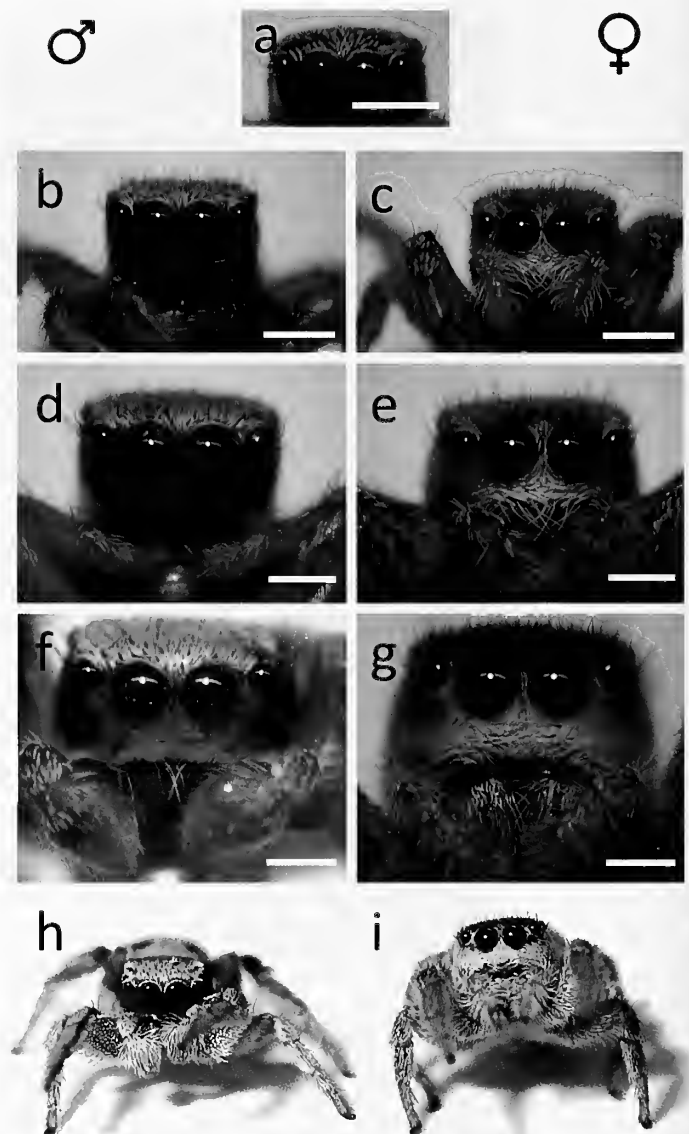

Figure 2.—Ontogenetic changes in coloration in males and females as spiders develop from spiderlings through sexual maturity. a. Spiderling stage (where sexes are indistinguishable); b. Small juvenile male; c. Small juvenile female; d. Large juvenile male; e. Large juvenile female; f. Subadult male; g. Subadult female; h. Sexually mature adult male; i. Sexually mature adult female. Scale bars represent 0.5 mm.

$F_{2,21} = 0.53$, $P = 0.60$; size of red facial patch: $F_{2,21} = 1.97$, $P = 0.17$; Figs. 5a–c).

DISCUSSION

Here we document the scale morphology associated with the three colored body regions in male *Habronattus pyrrithrix* that are prominently displayed to females during courtship. We also show how the colors of these three regions (i.e., red face, green front legs, and bright white pedipalps) develop as individuals grow from spiderlings through sexual maturity. Finally, given that the colors of two of these body regions (i.e., red faces and green front legs) were previously found to be correlated with body condition in the field (Taylor et al. 2011), we examined the possibility of age-related fading of these traits
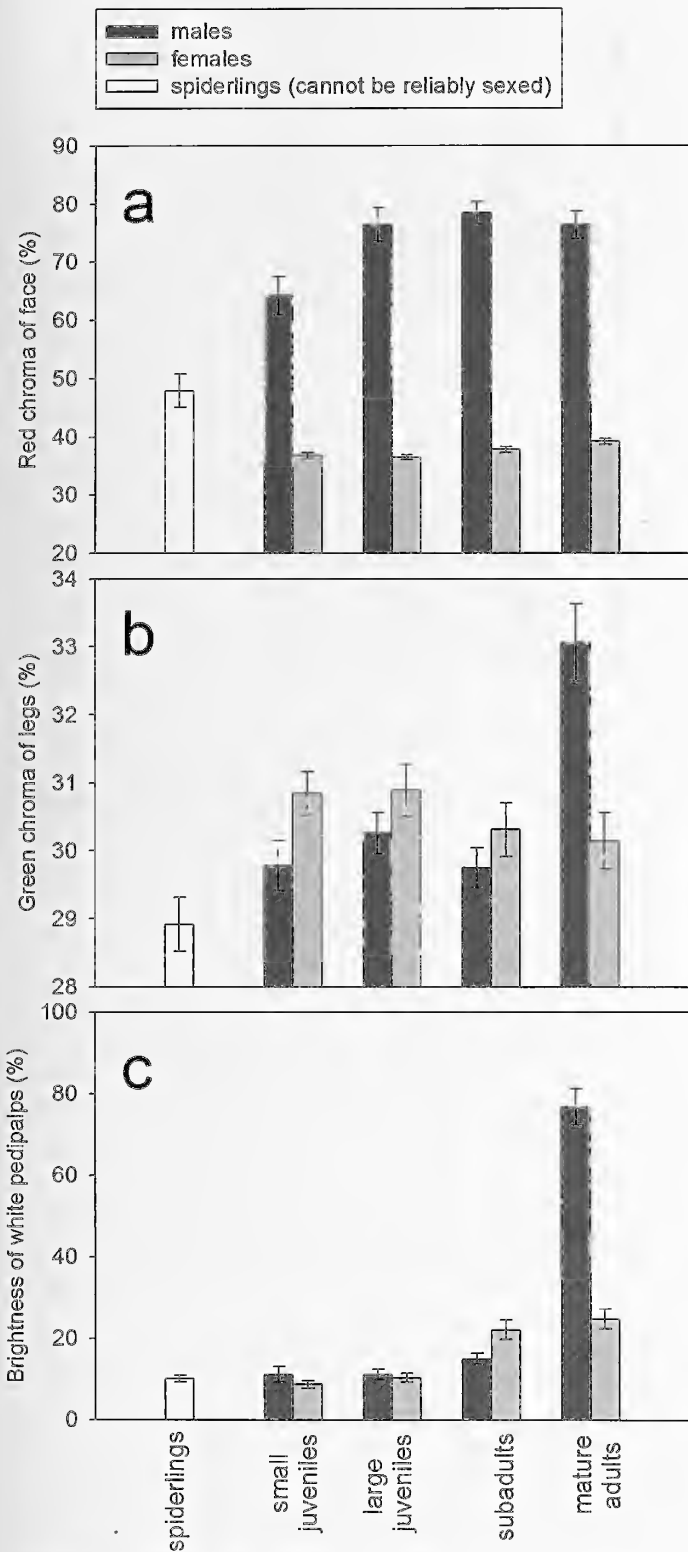
Figure 3.—Ontogenetic changes in coloration in males and females as spiders develop from spiderlings through sexual maturity (mean ± SEM). a. Facial coloration; b. Front leg coloration; c. Pedipalp coloration.
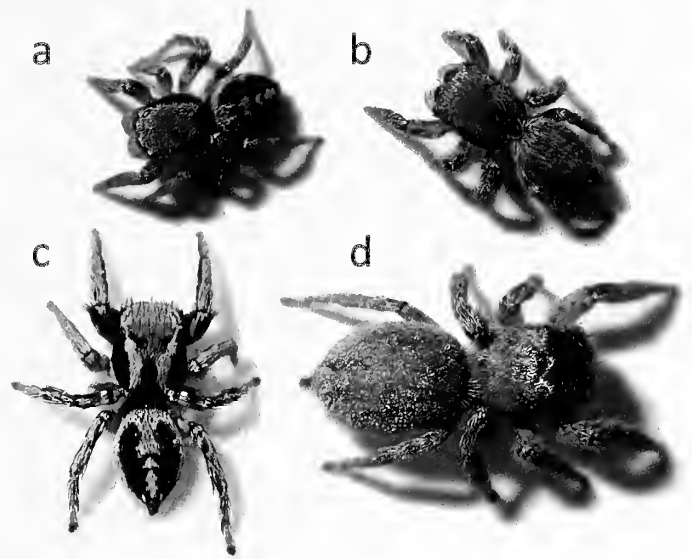

Figure 4.—Sexual dichromatism in dorsal coloration in juvenile and adult male and female *H. pyrrithrix*. a. Juvenile male; b. Juvenile female; c. Adult male; d. Adult female.

in adult males and show that green leg coloration, but not red facial coloration, fades (i.e., becomes lighter) with age.

In examining color development, we found that both the bright white pedipalps and green leg coloration of males

appeared only at sexual maturity. This is typical of many animal ornaments used in mating or aggressive competitions over access to mates; moreover, because such colors typically incur costs, it is not surprising that these ornaments are not expressed in juvenile stages (Andersson 1994). In contrast, males and females began to differentiate in red facial coloration and dorsal patterning as young juveniles (ca. 2.5 mm). During these stages, young males began to develop red facial scales and conspicuous black and white dorsal patterning typical of sexually mature adult males. The red coloration of adult males is prominently displayed in courtship and has been shown to improve courtship success in certain contexts (Taylor & McGraw 2013), yet it is unclear whether this coloration might have any functional role for juvenile males who do not engage in courtship. Red coloration has been shown to have important effects on receivers in a variety of taxa (reviewed in Pryke 2009); it could be that juvenile males use their red face for signaling in non-sexual contexts, either with conspecifics, potential predators, or prey. Regarding conspicuous dorsal patterning in adult males, this appears to be linked to higher movement rates associated with mate-searching, compared with cryptic females who spend more time at rest. Presumably, the higher movement rates of males render cryptic coloration ineffective; the pairing of conspicuous body patterns with false antennation (i.e., leg waving behavior) may help adult males avoid predators by imperfectly mimicking wasps and/or bees (Taylor 2012). Again, it is unclear what benefits, if any, this dorsal coloration might provide to young juvenile males. It is possible that, even as juveniles, males and females might face different ecological selection pressures (e.g., different dispersal or movement rates) that may drive such sex-differences in juvenile dorsal patterning (Booth 1990); in future work, such ideas should be examined in more detail. Finally, it is possible that juvenile sexual dichromatism does not have a functional role (e.g., Johnston 1967); it may simply indicate relaxed selection pressure for crypsis, compared with other species in which
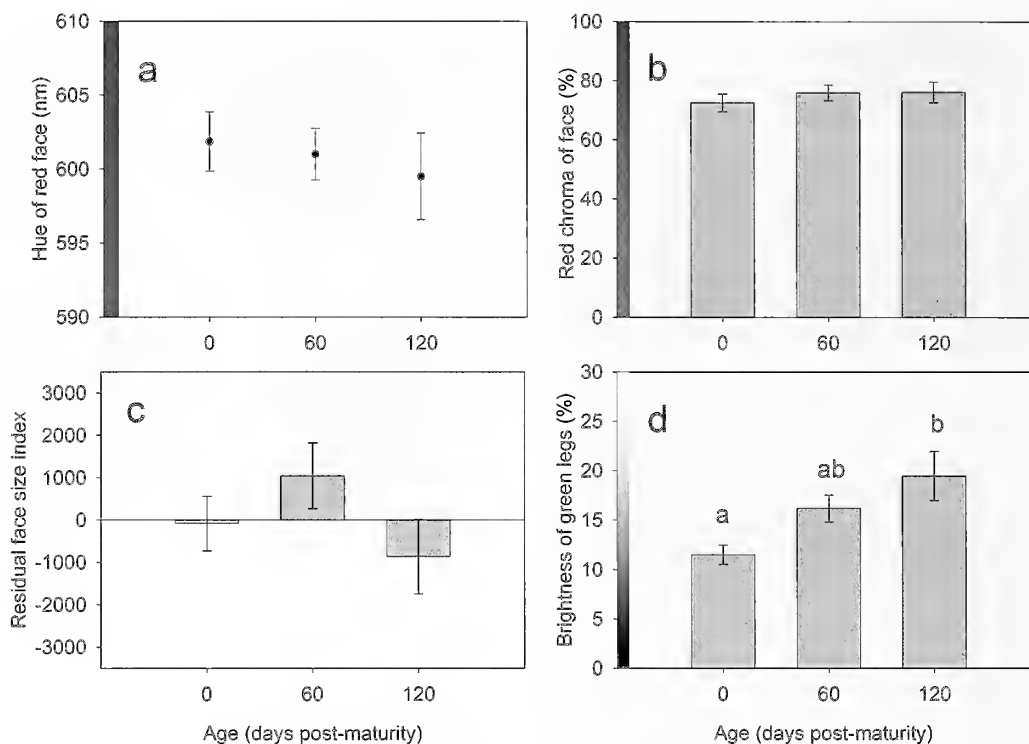
Figure 5.—Effect of adult age (post-maturity) on male display colors that were previously found to be correlated with body condition in the field (mean ± SEM). Aspects of red facial coloration (a–c) did not change with age, yet the brightness (lightness) of male green leg coloration increased as males aged (d). Different letters indicate significant differences at $P < 0.05$.

males are cryptically colored until maturity. It is interesting, however, that this species is an exception to the general pattern of salticid color development, where juveniles of both sexes typically resemble females in color pattern until reaching maturity (LAT, pers. obs.). To date, studies of any aspect of the biology of juvenile jumping spiders are rare (e.g., Nelson et al. 2005; Bartos 2008), yet they have revealed interesting aspects of life history that would have been missed by simply focusing on adults, as most studies do. *H. pyrrithrix* is a particularly good system in which to examine sex differences in juveniles because, unlike most salticid species, color patterns allow small juveniles to be accurately sexed well before reaching maturity.

In addition to age-related changes that occur during development prior to sexual maturity, our study also uncovered post-maturity, age-based color change. Previous studies have suggested that structural coloration in jumping spiders may be linked to male age (Lim & Li 2007; Taylor et al. 2011), yet both of these studies used comparisons of two groups of spiders, one that had been collected from the field and measured immediately and a second that was field-collected and measured after a certain period of time in the lab. While differences in the two groups may be due to age, we cannot rule out confounding effect of diets and captivity; in both cases, the first group experienced a field-based diet/environment for its entire life while the second group was collected from the field and then switched to a lab-based diet/environment prior to color measurement. Here we remove these confounding effects of diet and captivity to show that, even when spiders are raised entirely in the lab, the green leg coloration of adult males fades (i.e., increases in mean brightness) with age. This is also consistent with correlational

findings from a previous study (Taylor et al. 2011); this same aspect of male leg color (brightness) was correlated with body condition in the field, suggesting that younger males in better condition have darker legs, while older males in poorer condition have lighter legs.

Interestingly, this pattern of age-based fading did not hold for the males' red facial coloration, which is also correlated with body condition in the field (Taylor et al. 2011). Previous studies have shown that red facial coloration is positively correlated with the quality of a male's juvenile diet (Taylor et al. 2011). Collectively, these studies support the idea that the two different colors (red faces and green legs) have the potential to signal different aspects of male quality (reviewed in Hebets & Papaj 2005). A male's red facial coloration potentially signals a male's nutritional status and foraging ability (but not his age), while green leg coloration may signal age while containing no information about his diet or foraging ability. An interesting next step will be to examine how the mechanisms of coloration (e.g., specific pigments, structures, etc.) for these jumping spiders might facilitate or constrain the information content of a specific color and how they influence receivers (e.g., McGraw et al. 2002). Work with butterflies suggests that structural colors are more likely to fade with age than pigmentary colors (Kemp 2006). A better understanding of the detailed mechanisms of color production in *H. pyrrithrix*, including the specific pigments and structure types, will allow us to test the generality of these ideas.

Our examination of the morphology of the males' green legs offer preliminary insight into the mechanisms of age-based fading observed in our study. The green leg coloration is produced in the cuticle, while additional white light is reflected

off of the long, fragile spatulate scales (LAT, pers. obs., see Fig. 1 c,d). Fading of leg color could thus be a result of the breakdown of structures in the green cuticle, or alternatively, could be a result of damage to white spatulate scales, causing them to reflect more light. Males use these front legs in prey capture (LAT, pers. obs.), and thus damage to their scales over time may be difficult to avoid. Closer examination of the morphological changes that occur with age may help to elucidate the mechanisms behind age-based fading in *H. pyrrithrix* leg color.

Here we show that, in addition to sexually dichromatic male display colors that show a sudden onset at maturity (e.g., brilliant green legs, bright white pedipalps), males also have bright sexually dimorphic colors that begin to develop when males are still small juveniles (e.g., red faces and conspicuous black and white dorsal patterning). Furthermore, these colors are not all static at maturity; in particular, the green front legs of males are subject to age-based fading. As this is the first study to quantify age-based changes in juvenile coloration of any species of jumping spider, this work provides an important first step towards understanding the costs, benefits, and potential functions of juvenile coloration. Recent work on salticid coloration has provided some interesting and promising systems to examine general questions about color communication and evolution (Lim et al. 2007, 2008; Li et al. 2008a; Taylor et al. 2011; Taylor & McGraw 2013). Examination of ontogenetic changes in spider coloration, particularly in groups such as *Habronattus*, may help us elucidate some of the more subtle costs and benefits of color expression and change throughout an animal's life.

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Scavenging throughout the life cycle of the jumping spider, *Phidippus audax* (Hentz) (Araneae: Salticidae)

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Abstract. *Phidippus audax* (Hentz 1845), a common North American jumping spider, is a visual predator that uses its highly developed eyesight to detect and forage actively for prey. We demonstrate that *P. audax* can survive throughout its life cycle as a scavenger. We separated 600 spiderlings into eight treatments examining all combinations of three different variables: live versus dead prey, substrate present versus substrate absent, and large versus small arenas. Over the course of the study, we recorded survival rates, instar durations, and carapace widths. Our results indicate that *P. audax* can survive solely on a diet of dead prey, but at significantly lower survival rates and with longer instar durations than spiders fed on live prey. Scavenging spiders, however, exhibited no significant difference in carapace widths when compared to predators. Choice tests conducted on adults indicate that spiders raised as either predators or scavengers exhibit no significant differences in prey choice when given the option of live or dead prey.

Keywords: Dead prey, mortality, habitat complexity, development

Jumping spiders (Salticidae) are active predators that feed on a wide variety of prey. Their enlarged anterior-median eyes and secondary eyes provide them with heightened sensitivity to visual stimuli (Land 1971). Individuals first orient toward prey, then stalk or actively chase it to within a few centimeters, and then attempt a strike (Forster 1982a; Foelix 1996). Active predation is the strategy most widely studied in salticids (Givens 1978; Hill 1979; Forster 1982a; Freed 1984; Nyffeler et al. 1990; Richman & Jackson 1992; Jackson & Pollard 1996), however, alternative types of feeding behaviors do occur in this family. These alternative behaviors include araneophagy (Harland & Jackson 2000; Jackson 2000; Rienks 2000; Jackson et al. 2002; Penney & Gabriel 2009), herbivory (Meehan et al. 2009), indirect vertebrate blood feeding (Jackson et al. 2005), myrmecophagy (Jackson et al. 1998; Clark et al. 2000), nectivory (Ruhren & Handel 1999; Jackson et al. 2001), and prey stealing (Jackson et al. 2008). Our study focuses on scavenging in the salticid *Phidippus audax* (Hentz 1845).

Scavenging by spiders is not widely reported in the field; however, it has been demonstrated in the laboratory. For example, wolf spiders (Lycosidae) preferentially feed on aged, dead prey items over live prey when given the choice (Knost & Rovner 1975). Female *Theridion evexum* Keyserling 1884 (Theridiidae) collect and store dead prey in their webs, and when spiderlings emerge, they feed upon both old and newly acquired dead prey items (Barrantes & Weng 2008). The brown recluse spider, *Loxosceles reclusa* Gertsch & Mulaik 1940 (Sicariidae), also feeds on dead prey items (Sandidge 2003; Cramer 2008; Vetter 2011).

Scavenging in jumping spiders has also been demonstrated. Wolff (1986) starved 13 adult *Salticus scenicus* (Clerek 1757) females for five days and then presented them with dead house flies as prey. House flies given to starved spiders had significantly lower post-trial weights than house flies given to well-fed spiders, indicating that the starved spiders fed on the dipteran prey. Although Wolff (1986) demonstrated that starved salticids have the potential to feed on dead prey,

scavenging has never been demonstrated throughout the life cycle of any spider species. We examined scavenging in a jumping spider, *P. audax*, to determine if a highly visually-oriented predator could survive solely on dead prey throughout its life cycle.

In the present study we examined three possible variables: prey type, habitat complexity (presence or absence of substrate), and arena size. We predicted that spiders raised as scavengers would have lower survival rates than predators due to the lack of visual cues provided by dead prey. As a corollary, we hypothesized that scavengers would exhibit longer instar durations and smaller carapace widths than predators due to reduced prey consumption. We predicted that the addition of substrate and increased arena size would further hinder scavengers' ability to detect dead prey and thus further reduce their survival rate. Because the combination of added substrate and increased foraging area better reproduces the spiders' natural environment, adjusting these conditions enabled us to test the prospect of scavenging in the field, and the effects that changes within an environment might have on scavengers.

METHODS

We collected eleven gravid female jumping spiders, *P. audax*, from the Rock Springs Center for Environmental Discovery in Macon Co., Decatur, IL USA (39.817713° N, 89.00932° W) in the spring of 1998. We housed each gravid female individually in a petri dish (15 cm diameter × 1.5 cm height) until oviposition. Eight females successfully oviposited in the lab. We removed 600 spiderlings (mean = 75, SE = 14.87, range = 6–104) and housed each in a separate petri dish (10 cm diameter × 1.5 cm height) until spiderlings were randomly separated into groups.

We randomly separated the 600 spiderlings into eight groups of 75 with the following treatments: live versus dead prey, large (15 cm × 1.5 cm) versus small (10 cm × 1.5 cm) arena size, and substrate present (10 g of peat moss in large arenas and 4.5 g of peat moss in small arenas) versus substrate absent.

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Table 1.—Feeding regime for *Phidippus audax* in instars 2–8. Note that instar 1 is spent within the egg sac.

Instar	No. of prey introduced	Prey species
2	2	*Drosophila melanogaster*
3	4	*D. melanogaster*
4	6	*D. melanogaster*
5	1	*Musca domestica*
6	2	*M. domestica*
7–8	3	*M. domestica*

Spiders were kept at room temperature under a 12:12 photoperiod regime. We fed spiders three times per week, removed uneaten prey, and supplied fresh water via soaked cotton balls. We introduced prey at an approximate distance of 13 cm from the spider in large arenas and 8 cm away in small arenas. For prey, we used fruit flies, *Drosophila melanogaster*, or house flies, *Musca domestica*, depending on spider instar (Table 1). For scavenging treatments, we killed prey immediately prior to feeding. We lightly crushed fruit flies, and we killed house flies by applying pressure to the prothorax with forceps. We used organic, sphagnum peat moss as a substrate to simulate a more natural environment. The peat moss was kept dry during the course of the study and not replaced.

Throughout the life cycle of each spider, we recorded the date of every molt and the date of death when applicable. At the end of each instar, we removed exuviae and preserved them in 80% ethanol for later measurement of carapace widths. Carapace widths were recorded using a Meiji microscope fitted with an ocular micrometer. Five of the spiders were removed from the study because of unrecorded molt dates. Voucher specimens were deposited in the Millikin University Arthropod Collection.

When spiders reached maturity, we conducted a choice test to determine which prey type (live versus dead house fly) spiders would select. For these choice tests, we introduced two prey items simultaneously ≥ 7.0 cm in front of the spiders' cephalothorax in a large (15 cm $\times$ 1.5 cm) arena. We ran choice tests for approximately 20 min or until capture, and then recorded prey choice. We tested a total of 226 spiders: 144 raised as predators and 82 raised as scavengers.

Statistical analysis.—To determine the effects of scavenging on *P. audax*, we recorded survival rates, instar durations and carapace widths throughout their development, and choice of live versus dead prey as adults. To isolate differences arising from each of the 3 environmental variables (prey type,

presence or absence of substrate, and arena size), we used a Cox Regression survival analysis with survival (yes or no) as the dependent variable and prey type (live or dead), substrate (yes or no), arena size (large or small), their three-way interaction and their two-way interactions as independent predictor variables.

To determine the effects of the prey type, substrate, and arena size on instar duration, we completed a General Linear Mixed Model (LMM) with instar duration as the dependent variable and instar, prey type, substrate type, arena size, and all two-way and three-way interactions as independent variables. Spider identity was included as a random variable.

Choice test results were analyzed within each group, predators and scavengers, using the chi-square goodness-of-fit test against a null expectation of 50:50. In addition, we used a chi-square contingency test to determine whether the proportion of predators that chose live prey differed from the proportion of scavengers that preferred live prey. In all cases, *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Of the initial sample of 600 spiderlings, we successfully raised a total of 226 *P. audax* to maturity, with 144 raised as predators on live prey and 82 as scavengers on dead prey (Table 2; Fig. 1).

Survival.—There was a statistically significant three-way interaction among prey type, substrate type, and arena size with regards to survival ($\beta = 0.951$, Wald $\chi^2 = 4.714$, $df = 1$, (exp) $\beta = 0.386$, $P = 0.030$). The β is the logistic coefficient for each predictor variable (i.e. arena size, substrate type, or prey type) and represents the expected amount of change in survival when changing from one condition to the other within the predictor. The Wald test (and accompanying *P*-value) is useful in evaluating whether or not the logistic coefficient (β) is different from zero. Finally, the (exp) β represents the instantaneous relative risk of death, at any time, for a spider with one treatment for one variable compared with an individual with the other treatment for that variable. To gain an understanding of the nature of the interaction, we ran separate Cox Regression analyses within each of the two arena sizes.

Within the small arenas, differences in survival between spiders fed different prey types were dependent upon substrate type (two-way interaction of prey type and substrate type; $\beta = -1.173$, Wald $\chi^2 = 15.527$, $df = 1$, (exp) $\beta = 0.310$, $P < 0.001$). Because of the significant interaction term within small

Table 2.—Total number of *Phidippus audax* assigned to each treatment, total number of spiders raised to maturity, and percent survival in each of the eight treatments.

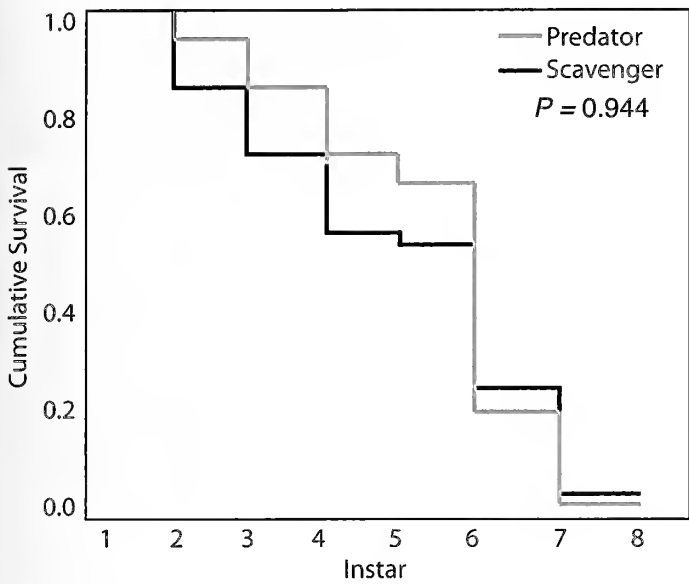| Prey type	Substrate type	Arena size	#Assigned to treatment	#Raised to maturity	% survival
Live	Empty	Large	75	43	57
Live	Empty	Small	75	34	45
Live	Substrate	Large	75	32	42
Live	Substrate	Small	75	35	46
Dead	Empty	Large	75	28	37
Dead	Empty	Small	75	42	56
Dead	Substrate	Large	75	2	0.02
Dead	Substrate	Small	75	10	13

Figure 1.—Survival curve based on Cox Regression for *Phidippus audax* raised on live prey (predator) or dead prey (scavenger). There was no significant main effect of prey type on survival ($P = 0.944$).

arenas, we ran a separate Cox Regression within small arenas with substrate and small arenas without substrate. Within small arenas with no substrate, there was greater survival to subsequent instars with dead prey ($\beta = 0.578$, Wald $\chi^2 = 8.36$,

$df = 1$, $P = 0.004$, (exp) $\beta = 1.783$; Fig. 2a). Conversely, within small arenas with substrate, there was greater survival to subsequent instars with live prey ($\beta = -0.564$, Wald $\chi^2 = 6.320$, $df = 1$, $P = 0.012$, (exp) $\beta = 0.569$; Fig. 2b).

Within the large arenas, differences in survival on different prey types were dependent upon substrate type (two-way interaction of prey type and substrate type; $\beta = 1.797$, Wald $\chi^2 = 28.077$, $df = 1$, (exp) $\beta = 6.032$, $P < 0.001$). Because of the significant interaction term within large arenas, we ran a separate Cox Regresssion within large arenas with substrate and large arenas without substrate. Within large arenas with no substrate, there was no significant difference in survival between spiders with live prey or dead prey ($\beta = 0.231$, Wald $\chi^2 = 1.285$, $df = 1$, $P = 0.257$, (exp) $\beta = 1.260$; Fig. 2c). Within large arenas with substrate, however, there was greater survival to subsequent instars among spiders with live prey ($\beta = -1.736$, Wald $\chi^2 = 34.916$, $df = 1$, $P < 0.001$, (exp) $\beta = 0.176$; Fig. 2d).

Sex comparisons in mature predators and scavengers: Of the 595 spiderlings used in this study, 117 males and 99 females successfully reached maturity. However, adding the variable 'sex' resulted in poorer models in all cases, and there was no difference in survival between males and females in the presence of the other three variables ($P > 0.198$ in all cases).

Instar duration.—There were significant three-way interactions of instar, prey type, and substrate type ($F_{1,1595} = 13.682$, $P < 0.001$; Table 3) and instar, prey type, and arena size
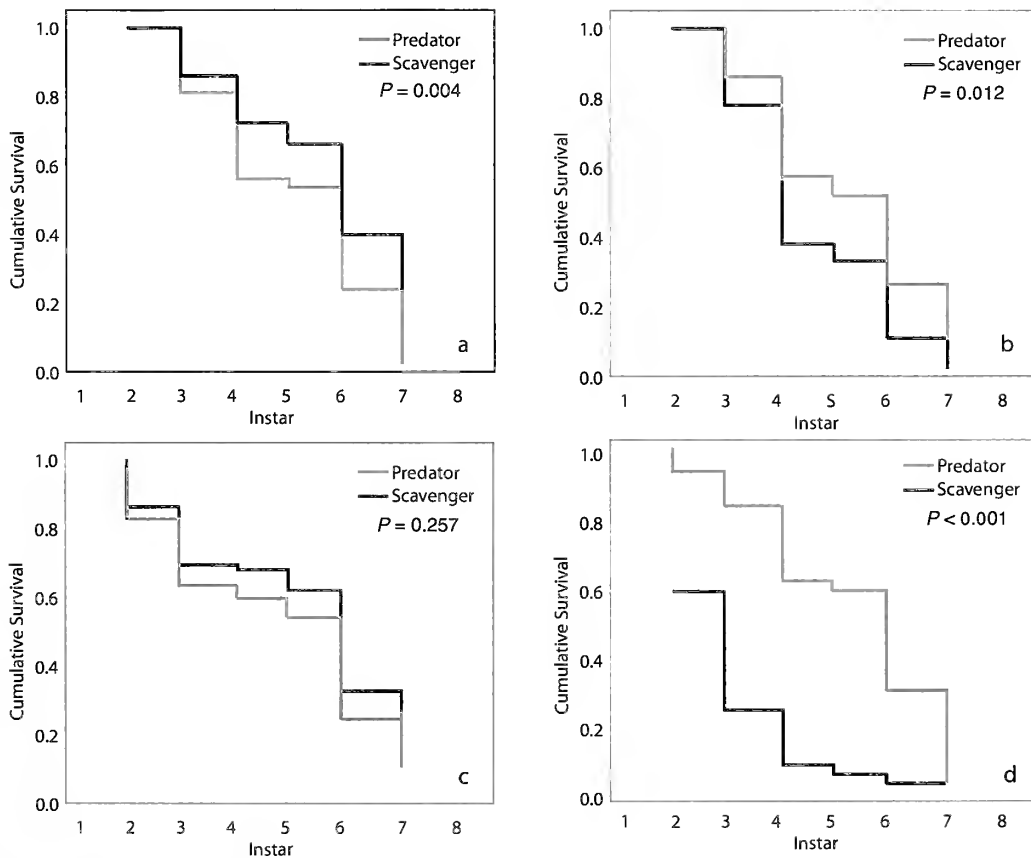

Figure 2a–d.—Differences in survival for *Phidippus audax* raised on live prey (predator) or dead prey (scavenger) in a) small arenas without substrate ($P = 0.004$); b) small arenas with substrate ($P = 0.012$); c) large arenas without substrate ($P = 0.257$); and d) large arenas with substrate ($P < 0.001$).

Table 3.—Results from a General Linear Mixed Model with instar duration as the dependent variable and spider identity as a random variable. Random variable (Spider ID): Wald Z = 28.249, P < 0.001 (retained in all models).

Variable	df	F	P
Instar	5, 1595	148.863	<0.001
Prey type	1, 1595	81.492	<0.001
Habitat	1, 1595	45.681	<0.001
Arena size	1, 1595	4.351	0.178
Instar*Prey	5, 1595	18.913	<0.001
Instar*Habitat	5, 1595	3.835	0.137
Instar*Arena	5, 1595	1.083	0.247
Prey*Habitat	1, 1595	53.919	<0.001
Prey*Arena	1, 1595	2.352	0.577
Habitat*Arena	5, 1595	6.799	0.146
Instar*Prey*Hab	1, 1595	13.682	<0.001
Prey*Hab*Arena	5, 1595	0.149	0.7
Instar*Prey*Arena	5, 1595	6.006	<0.001

($F_{5,1595}$ = 6.006, $P < 0.001$; Table 3). The significant three-way interactions of instar and prey type with substrate type and arena size indicate that instar duration is dependent upon multiple variables; therefore, to determine the nature of the interactions, we used subsequent LMM's to analyze the effects of instar and prey type as well as the two-way interactions of instar and prey type within each of the possible combinations of arena size and substrate type. The random variable, spider identity, was also significant (Wald Z = 28.249, $P < 0.001$), therefore, it was used in all subsequent analyses.

Within small arenas and no substrate, there was a significant interaction between instar and prey type ($F_{5,511}$ = 6.473, $P < 0.001$); therefore, we ran a separate LMM within those with dead prey and found a significant difference in instar duration among instars with a general pattern of increasing instar duration from instar 2 (14.94 days) to instar 7 (63.95 days; Fig. 3a). The second LMM, within live prey, revealed a similar pattern, with an increase in instar duration from instar 2 (12.46 days) to instar 6 (52.6 days), however, instar 7 was slightly lower than instar 6 (51.7) creating the significant interaction term. In general, instar duration is shorter with spiders given live prey within small arenas and no substrate (Fig. 3a).

Within small arenas with substrate, there was again a significant interaction between instar and prey type, and a subsequent LMM within spiders given dead prey revealed a significant difference in instar duration among instars, with a general pattern of an increase in instar duration from instar 2 (23.6 days) to instar 7 (91.5 days; Fig. 3b). Exceptions were an increase in instar duration in instar 4 to 64.59 days, followed by a decrease in duration to 52 days in both the 5th and 6th instars. The second LMM, within live prey, again showed a general increase in instar duration from instar 2 (13.8 days) to instar 7 (47.15 days; Fig. 3b). The interaction term, then, is a product of the increase in instar duration to 64.59 days in the dead prey group's 4th instar. Again, overall, spiders given live prey had shorter instar durations than those given dead prey within small arenas with substrate (Fig. 3b).

Within large arenas without substrate, there was a significant interaction between instar and prey type, and a subsequent LMM within spiders given dead prey revealed

a significant increase in instar duration from instar 2 (17.46 days) to instar 4 (43.68 days). However, there was a plateau in instar duration for the subsequent instars (Fig. 3c). From an LMM within spiders given live prey, we found a significant increase in instar duration from instar 2 (11.2 days) to instar 7 (51.85 days). Again, spiders given live prey, in general, had shorter instar durations than those given dead prey (Fig. 3c).

Within large arenas with substrate, there was another significant interaction between instar and prey type. Therefore, we ran a separate LMM within spiders with dead prey and found a significant increase from instar 2 (17.34 days) to instars 4 and 5 (89.25 days and 76.5 days, respectively). Only one spider in this group survived to instar 6 (instar duration of 37 days) and no spiders in this group survived to instar 7. From the second LMM within spiders given live prey, there was a significant increase from instar 2 (14.76 days) to instar 7 (70.64 days; Fig. 3d). Again, overall, spiders given live prey consistently had shorter instar durations than those given dead prey (Fig. 3d).

Sex comparisons: We initially used a LMM that included sex as an independent variable, but there was no significant interaction between other independent variables and sex ($P >$ 0.114 in all instars) nor was there a significant difference between males and females with regards to instar duration ($P > 0.182$). Given the low percentage of spiders surviving to an instar where sex could be determined and that there were no significant interactions or main effects of sex, adding sex to the LMM substantially reduced the power of the analysis. Therefore, sex was not included in the final analyses of the differences in instar durations.

Carapace widths.—Overall, as spiders matured, carapace widths were not significantly different among the eight treatments in any of the instars ($P > 0.05$ in all cases).

Choice tests.—Whether raised as predators or scavengers, spider choice of prey type differed from random (i.e., 50:50). Among predators, 117 chose live prey, while 27 chose dead prey ($\chi^2 = 56.25$, $df = 1$, $P < 0.001$). Among scavengers, 62 chose live prey, while 20 chose dead prey ($\chi^2 = 38.03$, $df = 1$, $P < 0.001$). There was no significant difference in the proportion of predators (117/144) and scavengers (62/82) that preferred live prey ($\chi^2 = 0.283$, $df = 1$, $P = 0.595$).

DISCUSSION

Spiders can survive on dead prey alone but face costs, such as lower survival rates and longer instar durations. Additionally, the two independent variables of substrate/no substrate and large/small arenas had significant effects on scavenging spiders.

Survival.—With the addition of substrate in both small and large arenas, scavengers exhibited lower survival rates. Our results are consistent with those of previous studies. *Phidippus audax* has been observed to hunt mainly on upper, well-lit areas of vegetation, such as leaves and branches, as well as on the sides of houses and fence posts (Givens 1978; Carducci & Jakob 2000). It therefore stands to reason that the dark substrate color and the lack of visual stimuli from dead prey hindered the spiders' ability to find dead prey items and would both have a significant, negative impact on the spiders' survival rates and instar durations. This indicates a low
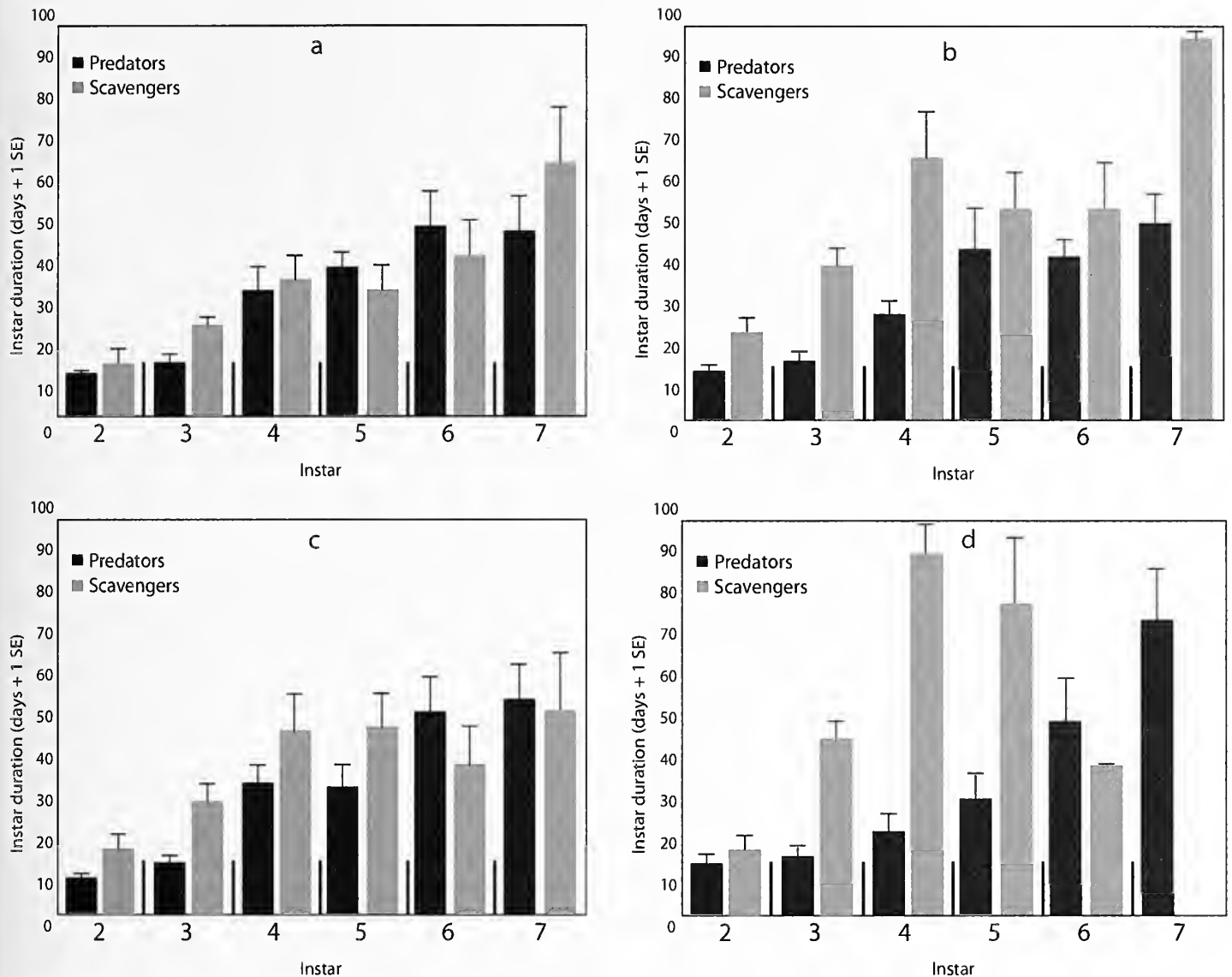
Figure 3a–d.—Differences in instar duration for *Phidippus audax* raised on live prey (predator) and dead prey (scavenger) in a) small arenas without substrate; b) small arenas with substrate; c) large arenas without substrate; and d) large arenas with substrate.

probability of successful scavenging by *P. audax* in nature, where the foraging area is substantially larger and substrate is varied and abundant. In the smaller foraging area, spiders had a greater likelihood of finding dead prey by chance.

We found an interesting exception to the trend of lower scavenger survival rates for treatments involving empty arenas. While predators and scavengers in large, empty arenas had statistically similar survival rates, scavengers had significantly greater survival to subsequent instars than predators in small, empty arenas. These results are somewhat counterintuitive, but a possible explanation is that scavenger *P. audax*, within a smaller foraging area, could have encountered and began feeding upon dead prey items more quickly than predator *P. audax* could capture and begin feeding on live prey. In accord with our results, when predatory waterbugs *Microvelia macgregori* Kirkaldy (Hemiptera: Veliidae) held in water-filled arenas, were given dead prey items, *D. melanogaster*, the waterbugs began feeding when they came across a dead prey item (Jackson & Walls 1998). Wolf spiders often

took dead prey as a meal if given the option, even if live prey items were present (Knost & Rovner 1975). The jumping spider, *Trite planiceps* Simon 1899 fed on freshly killed squashed flies, if left overnight in their arenas (Forster 1982b). In the latter case as well as in our study, the scavenging spiders may have detected minor residual movements from the freshly killed flies that prompted them to attack and feed.

Instar duration.—On average, scavengers had longer instar durations. Scavengers raised in substrate-filled arenas, both large and small, exhibited the longest instar durations, presumably due to difficulty in finding prey. Our results are consistent with the literature. Pholcid spiders, *Holocnemus pluchei* (Scopoli 1763), developed significantly faster and often underwent fewer molts when they were given a prey diet that allowed them to reach their satiation point (Jakob & Dingle 1990). Alternatively, when prey were limited, the orb-weaving *Zygiella-x-notata* (Clerck 1757), had longer instar durations, a correspondingly longer development time, and reduced adult

weight (Mayntz et al. 2003). In addition, spiders reduce their metabolic rates during long periods of food deprivation and consequently survive longer (Anderson 1974; Greenstone & Bennett 1980), which in turn may result in longer instar durations.

Although *P. audax* are naturally active predators feeding on a wide variety of live prey, we have shown for the first time that these spiders are capable of surviving from egg sac emergence to maturity solely on a diet of dead prey, albeit with lower survival rates and longer instar durations. In addition to acquiring nutrients from the dead prey, spiders raised as scavengers may have also used metabolic defense mechanisms to survive. For example, in a time of prey shortage, spiders will exhibit a high tolerance to starvation by lowering metabolic rates and using their abdomens to store large quantities of lipids that can be used slowly until the prey shortage ends (Anderson 1974; Greenstone & Bennett 1980; Iida 2005). Further research should be conducted to better understand the types of nutrients being obtained from freshly killed or desiccated prey items. Whatever the nutrients are, our results indicate that at least some jumping spiders were able to survive by further breaking down dead prey items (Givens 1978; Cohen 1995; Foelix 1996; Morse 1998).

Carapace widths.—Overall, as spiders matured, we found that carapace widths were not significantly different among the eight treatments from instar to instar. Predators and scavengers grew comparably, regardless of their prey type. With regard to scavenging, these results may indicate that even though we reported significant differences in mortality and instar duration, individuals were able to reach average size. Correspondingly, the orb-weaver, *Zygiella x-notata*, experienced longer instar durations when prey was limited, but these prey shortages did not negatively impact growth within an instar. Additionally, spiders fed low quality prey experienced higher instar growth ratios by utilizing the longer instar durations to gain more weight (Mayntz et al. 2003). The wolf spider *Pardosa prativaga* (L. Koch 1870) experienced longer instar durations when food restricted or fed nutritionally insufficient prey items. However, when available prey was more abundant, spiders were able to catch up on any lack in growth and development (Jespersen & Toft 2003). Although the ability to stay within an instar for longer periods of time to grow to average size may be beneficial in the long run, in the short run it would make seavengers more susceptible to predators in the wild.

Choice tests.—Because spiders raised both as predators and scavengers preferred live prey as adults, *P. audax* exhibited its instinctive predatory behavior regardless of the diet on which it was raised. However, it is important to note that 47 spiders did choose dead prey. This result could simply be due – at least in part – to spiders finding and feeding on dead prey before detecting live prey. Corroborating this hypothesis, wolf spiders (Knost & Rovner 1975) and jumping spiders (Forster 1982b) will feed on dead prey if they happen to come into contact with it while foraging.

Our results indicate that *P. audax* can be reared as a scavenger throughout its entire life cycle, but at certain costs to the organism. Whether or not scavenging occurs in the field is largely unknown. Much of the research conducted on scavenging has been carried out in a controlled laboratory setting (Knost & Rovner 1975; Wolff 1986; Cramer 2008), where many of the variables can be restricted to much narrower ranges than those that prevail in the natural world. Because *P. audax* is a highly visual predator that actively hunts for prey, scavenging may be a way to supplement food intake during times of prey shortage. Further research should also be conducted to determine the effects of a multi-prey diet on scavenging as an alternative feeding strategy.

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Removal of genital plugs and insemination by males with normal and experimentally modified palps in *Leucauge mariana* (Araneae: Tetragnathidae)

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Abstract. Both males and females of the spider *Leucauge mariana* (Taczanowski 1881) contribute material to the plugs that often occlude the genital openings of females in the field. Males were sometimes unable to remove or penetrate these plugs, but overcame others using three different mechanical mechanisms: snag the plug and pull it off; break and penetrate through it; and break its adhesion to the epigynum by injecting material under it. They used their genitalia to accomplish these tasks, despite the fact that the genital bulb lacks muscles and innervation, thus limiting the male's ability to guide genital movements precisely. The effects of two male genital structures, the conductor tip and the conductor hook on sperm transfer and genital plug removal were tested by direct observations of their morphology and behavior, and by experimental removal of structures from one but not the other palp of the same male. Removal of the conductor tip reduced sperm transfer, while removal of both the hook and the conductor reduced plug removal. A preliminary characterization of palp movements and their sequences did not reveal any behavior that seemed especially designed for removing plugs, as opposed to inseminating the female.

Keywords: Copulatory plugs, genitalic function, cryptic female choice, plug removal

Genital plugs in female genitalia occur in many animals, and are generally formed from male seminal products or parts of the male's own genitalia (Smith 1984; Birkhead & Møller 1998; Simmons 2000; Uhl et al. 2010). Some plugs prevent subsequent males from gaining access to the female's reproductive tract, and plugs are often included in lists of sperm competition devices of males (Parker 1970; Thornhill & Alcock 1983; Smith 1984; Birkhead & Møller 1998; Simmons 2000). Active female participation in making plugs occurs, however, in some spiders (Knoflach 1998; Uhl et al. 2010; Aisenberg & Barrantes 2011) and insects (Markow & Ankney 1988; Hosken et al. 2009).

In several groups, plugs do not consistently exclude subsequent males (reviewed in Eberhard 1996; Uhl et al. 2010), and males of some species remove at least some copulatory plugs from the female (Milligan 1979; Masumoto 1993; Eberhard 1996; Knoflach 1997). The male's genitalia often seem to be active during the process of plug removal, but details of the mechanisms by which plugs are removed have been little studied. Most data involve only extrapolations from the probable mechanical properties of male genital structures. For instance, penile spines in microtine rodents and eversion movements of the hemipenes in lizards have been hypothesized to function to remove plugs (Milligan 1979; In den Bosch 1994), but direct observations and experimental evidence are lacking. The thin pointed shape of the distal portion of the aedeagus of a papilionid butterfly has been hypothesized to allow the male to tunnel through or to slip past soft, recently formed or small plugs (Matsumoto & Suzuki 1992). The male of the linyphiid spider *Dubiaranea* (?) apparently dissolves plugs *in situ*, perhaps with liquid from either his mouth or his palps, and he then removes the pieces with undetermined portions of his palps (Eberhard 1996). Male *Agelena limbata* Thorell 1897 spiders also use unspecified portions of their palps to pry plugs from the female (Masumoto 1993). To our

knowledge, no male morphological structure has ever been demonstrated experimentally to be specialized for plug removal.

Given the selective importance to males of gaining access to internal female genitalia, it seems likely that male structures specialized for plug removal exist. Male genitalia seem particularly likely to have plug removal structures, as they probably often contact plugs. Plug removal devices could evolve under sexual selection by male-male competition (sperm competition), female choice (if females influence plug deposition, the necessity for plug removal, or the effectiveness of removal attempts), male-female conflict (if the female's best interests involve maintaining a plug), or combinations of these factors (e.g. Wiley & Posten 1996; Arnqvist & Rowe 2005; Eberhard 2010).

The present study documents female effects on plug deposition and removal, and a male genital structure whose form, mechanical properties and behavior suggest that it represents an adaptation to remove plugs in the tetragnathid spider *Leucauge mariana* (Taczanowski 1881), a member of the large cosmopolitan genus *Leucauge* White 1841 (>150 species; Platnick 2013) that is abundant in early second growth and secondary forest in the Central Valle (San José Province) of Costa Rica. Copulation and sperm transfer have been studied in detail in this species (Eberhard et al. 1993; Eberhard & Huber 1998a; Méndez 2002; Aisenberg 2009; Aisenberg & Eberhard 2009; Barrantes et al. 2013), but nearly exclusively in virgin females.

As in other spiders (Eberhard & Huber 2010), the sperm of *L. mariana* are encapsulated when they are transferred from the male's palp to the female's internal spermathecae in a viscous liquid matrix (Figs. 1, 2c). Once inside the female, the sperm emerge from their capsules (Eberhard & Huber 1998a), as in the related *Nephila clavipes* (Linnaeus 1767) (Brown 1985). Sperm precedence patterns are not known in *L.*
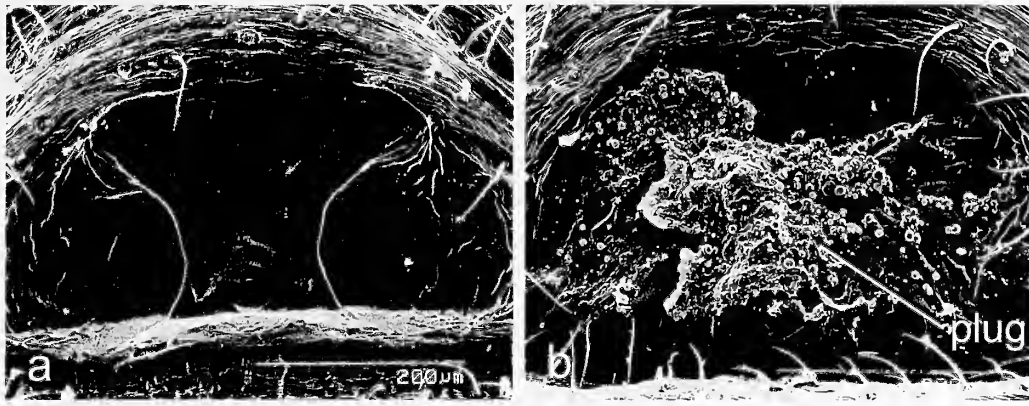
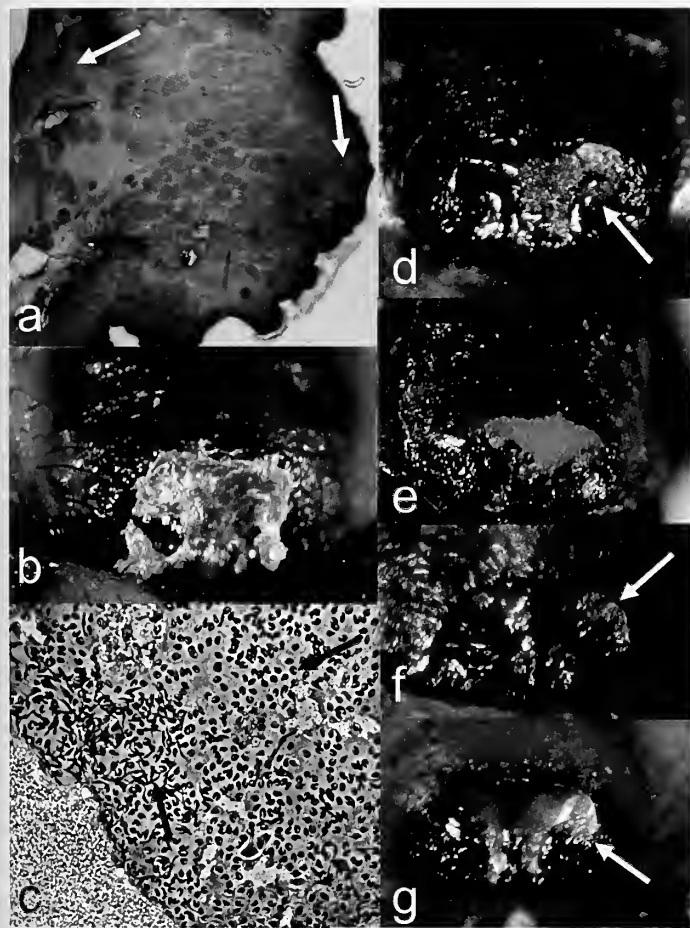Figure 1.—Epigynum without a plug (left) and with a partial, asymmetrical plug (right).

Figure 2.—Ventral and microscopic views of plugs of *L. mariana*. a) a yellow plug containing spheres (arrows) but no sperm; b) a large mixed white and yellow plug overflowing the central cavity, with an irregular surface; c) contents of a plug stained with acetocarmine which contained both encapsulated sperm (right arrow) and decapsulated sperm (left arrow); d) a white plug covering the lower portions of one side of the epigynum (arrow indicates a portion of the epignal curved ridge that was not covered; e) a yellow-orange plug with a granular surface; f) a small yellowish plug with a smooth surface at the anterior corner of the left side of the central cavity (arrow); g) a white plug with a smooth surface that covers most of the central cavity.

mariana, but the fact that males in the field occur preferentially with penultimate instar females rather than mature females (Eberhard et al. 1993), indicates that the first male to mate with a female often sires at least some of her offspring. On the other hand, the following combination of observations indicates that first male sperm precedence is not complete: males mate with non-virgin females both in the field and in captivity (Méndez 2002; W. Eberhard unpub. obs.); distinctive behavior of the male's genitalia results in deposition of one component of the plug during the latter stages of copulation (Eberhard & Huber 1998a); females in some cases add a second component to the plug (Eberhard & Huber 1998a; Aisenberg 2009; Aisenberg & Eberhard 2009); and males push and scrape at some plugs with their genitalia without dislodging them, but dislodge others and then apparently succeed in inserting their genitalia in the female (Méndez 2002; the present study). Mixed first and last male paternity has been observed in the related genus *Tetragnatha* Latreille 1841 (Danielson-François & Bukowski 2005).

The female's epigynum, where all male insertion, plugging, and unplugging attempts occur, is a sclerotized plate on the ventral surface of her abdomen, with a central cavity that is bounded anteriorly by an overhanging wall (Fig. 1); access to the entrance of each of the two insemination ducts, which lead to the two spermathecae, is through slits at the base of the rounded lateral wall of the central cavity. Plugs consist of masses that vary in size, shape, consistency and texture that are located at variable sites on the epigynum (Figs. 1b, 2b, d–g) (Méndez 2002).

During copulation, the palps are extended, and contact the female's abdomen in alternation. The subapical cymbium of the palp (Fig. 3) is first placed on a featureless region of the ventral surface of the female's abdomen just anterior to her epigynum. Then the basal hematodocha inflates ("primary inflation"), causing the distal bulb to rotate so that its terminal portion, which includes the intromittent embolus and the tip and hook of the conductor sclerite, moves ventrally away from the cymbium and then dorsally toward the entrance of the insemination duct on the female's epigynum. If the entrance is unobstructed and the palp is correctly aligned, the conductor hook sweeps antero-laterally across the female's epigynum until it is arrested by the anterior wall, and the basal hematodocha then swells further (a "secondary inflation"), causing further rotation that drives the conductor tip and the
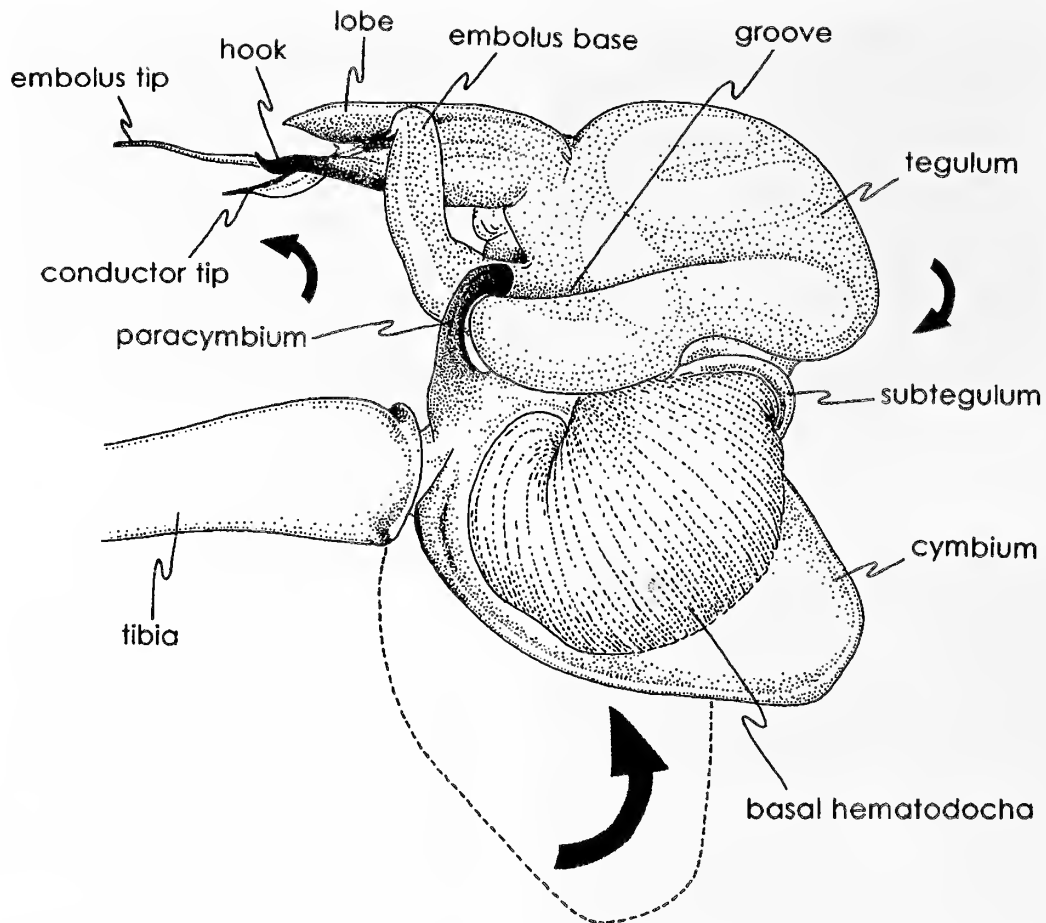
Figure 3.—Movements (small arrows) of embolus base and tegulum that resulted from inflation of the basal hematodocha (partially collapsed in this preparation; the approximate position of the cymbium in life is indicated by the dotted lines). The tegulum rotated against the paracymbium, whose tip slid along the groove in the tegulum as the expansion of the basal hematodocha drove the embolus distally from the conductor (from Eberhard & Huber 1998a).

embolus into the insemination duct (an "insertion") (Eberhard & Huber 1998a). Substantial force is applied to the female during primary and secondary inflations, sometimes displacing her entire abdomen laterally.

Two types of palpal insertion occur in copulations with virgin females (Eberhard & Huber 1998a). "Long" insertions (when sperm transfer probably occurs, at least in copulations with virgin females) last on the order of 1 min. Repeated secondary inflations of the basal hematodocha alternate with brief collapses; each inflation drives the embolus tip into the insemination duct. "Short" insertions last on the order of 1s and involve only a single secondary inflation, and both the embolus and conductor are then pulled away from the epigynum when the basal hematodocha collapses. Short insertions usually occur in bouts, and later in copulation. A small mass of white material emerges from the tip of the embolus and is deposited on the surface of the epigynum during many short insertions. Many apparent insertion attempts fail (44% in copulations with virgin females; Eberhard & Huber 1998a), when the conductor tip and or the hook snag the epigynum only momentarily or miss it completely during a primary inflation ("flubs" in the terminology of Watson 1991). On average, copulation with virgin females lasted 17.3 ± 6.1 min; there were 3.5 ± 2.0 long insertions, averaging about 108–120 s in duration, and 6.2 ± 5.2 bouts of short insertions with a mean of 14.6 ± 7.0 inflations per bout. Copulations with unplugged non-virgin females were shorter (9.9 ± 13.3 min), and had fewer long insertions (0.2 ± 0.6).

It is important to keep in mind that insertion attempts by male *L. mariana* are "blind" in two senses. The male's eyes are on his dorsal side, so he cannot possibly see his palps, copulatory plugs, or the female's genitalia during copulation. In addition, his palpal bulb is not innervated (Eberhard & Huber 1998b, 2010), so he has no direct sensory feedback from the bulbal structures (conductor tip, hook, embolus) that contact the female's genitalia. Movements of bulb sclerites are produced by changes in internal pressure and expansion of hematodochal membranes, rather than by contractions of muscles. The only sensory feedback that may be available to the male is from more basal structures such as his cymbium, which is innervated and has abundant setae on its surface that contact the female's abdomen during intromission attempts, or other segments of his palp.

METHODS

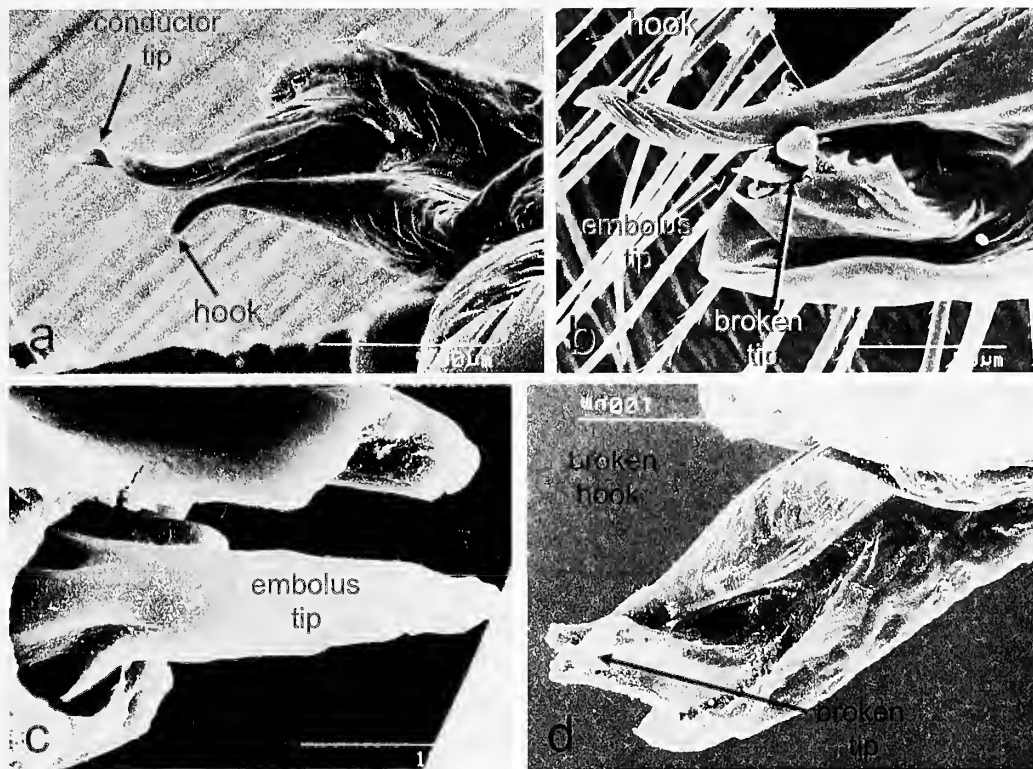Spiders were readily induced to copulate ventral side upward under a dissecting microscope, where details of the

Figure 4.—SEM images of the distal portions of the male palpal bulb of *L. mariana*. a) intact palp; b both the hook and the conductor tip removed; c only the tip of the conductor removed; d) close-up of embolus tip at the site where the conductor tip was cut off.

behavior of the male's palps and their mechanical interactions with the female's epigynum were observed and recorded. An orb of a mature female in the field was mounted on the raised edges of a plastic plate about 30 cm in diameter, and the female to be observed and then the male were induced to climb onto the web. The plate was then placed under a microscope. We captured each mature male in the field the day he was observed. All spiders were collected on the campus of the Universidad de Costa Rica in San Pedro de Montes de Oca, San José Province, Costa Rica (el. 1100 m).

Each palp introduces sperm into only one of the female's two spermathecae, so paired tests were possible to test for effects of modifying one of the male's palps but not the other on plug removal, sperm transfer, and palp behavior (see Discussion for limits on details of the replications). We modified the palps of some males experimentally by first clamping the male gently between the foam-rubber covered tips of a fine forceps with one palp exposed, then cutting palpal sclerites with a fine scissors under a dissecting microscope (Fig. 4). We made two types of cut: both the conductor tip and the conductor hook of one palp were cut near the tip of the conductor lobe (Fig. 4d); or the conductor tip was cut leaving the hook intact (Fig. 4b). The tip of the male's intromittent organ (the embolus) (Fig. 4c) was enclosed in a slot in the conductor tip, basal to the tip of the lobe; it was thus not affected by cuts at the level of the hook, and little affected by more basal cuts. No fluid was seen to leak from these injuries, either when the cuts were made or subsequently during copulation. Incidental contact with the sclerites during these operations revealed that the tip of the conductor was

flexible and bent easily when contacted; the hook, in contrast, was more rigid and bent little if at all.

We left the male's other palp intact as a control. Thus, in contrast to other well-known tests of the effects of experimental modifications of male morphology on female responses (e.g. Andersson 1982; Møller 1988; Basolo 1990), we controlled at least partially for the possibility that modification of the male's morphology affected him in additional ways (e.g., his courtship behavior) that could affect his reproductive success. The asymmetric nature of some plugs (e.g., Fig. 1b) meant, however, that the conditions encountered by the male's two palps were not always identical (see Discussion). The plugs in all plugged females that were mated to males with modified palps were white and apparently hard. Nearly all operated males were observed copulating with only one female; one male was observed with one female with a plug and another female that was virgin.

We checked insemination success in matings with virgin females by dissecting the epigynum and the spermathecae from the female, placing the pair of spermathecae on a microscope slide in a drop of saline and squashing them under a coverslip. The areas of the separate sperm masses that were expelled from each of the membranous first spermathecal chamber (where sperm are deposited by the male; Eberhard & Huber 1998a) were compared for the spermatheca that corresponded to the experimentally modified palp versus the spermatheca that was inseminated by the control palp. While the pressure of the squash with the cover slip was not standardized, the two spermathecae were squashed simultaneously and with enough pressure to extrude whatever sperm

they contained, so meaningful comparisons of their contents could be made.

Sperm from the male genitalia, complete plugs, and white masses that we collected from the male's palp on the tip of a fine needle without allowing the material to contact the epigynum were mounted on microscope slides and stained with acetocarmine, a DNA stain that colored the sperm nuclei bright red while leaving the other material relatively transparent (Fig. 2c). We assessed plug consistency by gently poking and prying at plugs on the epigyna of live spiders with a small needle under a dissecting microscope.

We captured plugged females in the field, and obtained virgin females by allowing field-captured penultimate instar females to molt in isolation in captivity. We recorded copulation behavior using a Sanyo VDC-2950 video camera that was attached to a dissecting microscope and focused tightly on the female's epigynum, so that its width occupied about 75% of the width of the screen. Male palp behavior was classified in video recordings as follows: flub with a brief snag on plug or epigynum; flub without a snag; reposition cymbium on female abdomen; secondary inflation without insertion (of at least the conductor – see below); secondary inflation with insertion; palp immobile (motionless for > 1 s); and withdraw palp from abdomen (usually to change palps).

RESULTS

Origin and composition of genital plugs.—Genital plugs on the epigyna of mature field-collected females varied in size, color, surface texture, site, and contents (Fig. 2). Yellowish plugs (Fig. 2e) were rare (2.5% of 200 females checked in January 2007), and often lacked sperm (56.7% of 33), but sometimes contained spheres (Fig. 2a) (39.4% of 33). All broke easily into flakes when poked with a needle. Silvery-white plugs (Fig. 2b, d, f, g), in contrast, all contained sperm (100% of 57) (Fig. 2c), never contained spheres (0% of 57), were hard, did not break into flakes when poked (although they occasionally broke into large chunks), and adhered more tightly to the epigynum than did yellowish plugs. Some field-collected genital plugs were heterogeneous, possibly the result of the mixture of new plug material and partially dislodged previous plugs; mixing of this sort occurred in matings in captivity. Plugs that were not disturbed by subsequent matings were long-lasting. Each of ten wild-caught females that were kept isolated from males for 22 days in captivity had the same type of plug at the end that she had had when captured.

All sperm inside the palpal bulbs of two males were encapsulated (right arrow in Fig. 2c). The small masses of white material deposited by the male on the epigynum and collected directly from the palps also contained abundant sperm that were almost exclusively encapsulated (all sperm in ten masses were encapsulated; all but a single sperm among many sperm in one other mass were encapsulated). No spheres were present in the material collected directly from the palps or the white masses.

We confirmed previous suggestions that females contribute material to plugs (Eberhard & Huber 1998a; Aisenberg 2009; Aisenberg & Eberhard 2009) in three ways. Direct observations of copulating pairs under the dissecting microscope showed, in a few cases in which visibility was good, that liquid welled up into the atrium from inside the female's insemination

duct during copulation, replicating previous observations (Eberhard & Huber 1998a). This liquid appeared to cause the white masses from the male to dissolve or disperse, forming a silvery-white or transparent plug. In two cases, a plug that was composed of both new material and parts of a previous plug that was not completely dislodged apparently hardened rapidly and blocked further insertion attempts; but more often the male easily penetrated the apparently liquid plug repeatedly during copulation. Some females may have also added liquid soon after copulation ended and the spiders separated, as the material on the epigynum generally acquired a more liquid appearance following the end of copulation. When no liquid emerged from the interior of the female during copulation, as was common in copulations with virgin females in captivity (Eberhard & Huber 1998a; Aisenberg & Eberhard 2009), the male removed nearly all or (more often) all of the white masses that he deposited; the small masses adhered to his palps during subsequent insertions, and were withdrawn adhering to them and then fell or were lost.

Plug composition gave a second indication of active female participation in the formation of both yellow and white plugs. Of 57 white plugs, 18 contained multiple decapsulated sperm (left arrow in Fig. 2c). Because sperm in the spermatheca become decapsulated following insemination (Eberhard & Huber 1998a), while all or nearly all of the sperm in the male's genitalia prior to copulation and in the white mass that he deposited on the female epigynum were encapsulated (above), the abundant decapsulated sperm in these plugs suggest that the plugs contained material from the female, probably from her spermatheca. Yellow plugs, on the other hand, are probably often produced by only the female; 56.7% of 33 contained no sperm at all. In contrast, all material we collected from male palps, as well as material seen in sections of the distal portions of the sperm ducts inside intact palps (Eberhard & Huber 1998a) contained numerous sperm (all of which were encapsulated).

Finally, the sites of some small plugs that did not cover the entire central cavity were consistent with female contributions. They were along the sides of the cavity or at its anterior-lateral corners, and covered the lower rather than the more salient portions of the epigynum (Fig. 2, d f, g); these are sites where liquid ejected from the insemination ducts would be expected to first accumulate. This evidence does not clarify which sex produced the plug substance because male contributions could not be ruled out, but they are compatible with female participation.

Copulations that fail to result in plugs may be common in the field. Of 64 females collected with no plugs, 82.8% nevertheless had sperm in their spermathecae. Plug removal by the female with her legs could not be ruled out in these cases, but only infrequent removal is seen in captive females (above) so this is probably not the sole explanation. Field populations of *L. mariana* showed strong seasonal peaks of abundance, and unplugged females were more common in the field early in population peaks than later (Méndez 2002).

Plug removal.—Intact males attempting to copulate with a female with a white plug were only sometimes (68% of 28 pairings) able to dislodge it enough to allow insertion of the conductor into at least the outer portion of the insemination duct on at least one side of the epigynum ("plug removal"

hereafter) (in these and other "insertions" described below, direct determination of whether deeper penetration by the embolus occurred was not possible, because the tip of the conductor was out of sight). Plugs were dislodged by the palps in three different ways. In each case removal occurred after the palp had "snagged" against the plug (its movement was interrupted at least briefly by contact with the plug). In 21 pairs, the mechanism of removal was determined: pulling or prying the plug away as a single piece from the epignum (14%); breaking the plug and then either prying away the pieces or penetrating past them (33%); and injecting material under the plug and then pulling it off as a unit (53%). In pulling a plug off as a unit, the conductor tip or hook scraped across the surface of the epignum, snagged the plug, and then pulled or pried it free. No material emerged from the palp during these movements. In perforating or inserting the tip of his palp through a crack in the plug, the male apparently drove the conductor tip toward or into the insemination duct. Some broken pieces of these plugs were pulled from the epignum during subsequent inflations. In removing a plug by injecting material under it, the conductor tip and probably the hook (it was not possible to resolve this detail in direct observations) penetrated through the plug, but did not appear to enter the insemination duct. The palp ejected material that accumulated between the plug and the surface of the epignum and broke the plug free from the epignum; it was then pulled away during subsequent inflations. We did not discern differences in the movements of the male's genitalia that seemed to be specially designed to utilize these different mechanisms.

In some cases, when the plug consisted of more than one mass or was broken into pieces but not all the pieces were removed, the male nevertheless succeeded in inserting one or both of his palps into at least the entrance of the female's insemination ducts. In some video sequences it was clear that the conductor tip was bent back sharply as it scraped across the surface of the plug, suggesting that the more rigid hook was more effective than the conductor tip in applying force to the plug. In all copulations in which a plug was removed the male subsequently deposited new plug material.

The basic movements of the palp before and after the plug was dislodged were compared in ten intact males that were paired with females with white plugs. Cymbium placement, and primary and secondary basal hematodochal expansions that swung the conductor tip and hook across the epignum were at least qualitatively similar before and after the plug was dislodged.

Effects of experimental modifications on plug removal and sperm transfer.—The frequency of plug removal was only barely significantly reduced when both the hook and the conductor tip of one palp were removed compared with intact males (41% of 17 pairs) ($P = 0.04$ with one-tailed χ^2); there was no significant reduction when only the conductor tip was removed (52% of 21 pairs ($P = 0.27$ with χ^2). Comparisons between the modified and unmodified palps of the same male gave more dramatic differences in some respects. Of seven cases in which a plug was broken by a male that had lost both hook and conductor tip, all breaks were produced by the intact rather than the modified palp ($\chi^2 = 7.0$, df = 1, $P = .008$); in contrast, of 20 cases in which the plug was broken when the male had lost only the conductor tip, half were produced by the intact palp and half by the modified palp. Of

five cases in which a plug was removed as a unit from both sides of the epignum at once in experiments in which both the hook and the conductor tip were removed, the trend was in the expected direction: the intact palp removed the plug in four of them ($\chi^2 = 1.8$, df = 1, one-tailed $P = 0.09$). Summing the two modification experiments, the plug was dislodged as a unit by the intact palp in seven of eight cases ($\chi^2 = 4.50$, df = 1, one tailed $P = 0.017$).

In contrast, both modified and control palps were effective once a plug was broken. When the plug was broken and at least one piece was removed, the intact palp removed a piece of the plug on its side of the epignum in eight cases and the modified palp in seven. The frequency with which a palp snagged the plug at least once was not altered (59% for palp lacking both the hook and the conductor tip, 76% for palp lacking only the conductor tip, 71% for the intact palp).

Insemination of virgin females was reduced when the palps were modified. The spermatheca on the side into which the intact palp was inserted (the "control" spermatheca) was full in all 19 females that were dissected after being mated to males with both conductor tip and hook removed, while the "experimental" spermatheca (into which the modified palp was inserted) was uninflated and apparently empty of sperm in 53% of these females ($\chi^2 = 13.6$, df = 1, $P = 0.0002$, comparing empty and non-empty spermathecae). The control spermatheca was more full than the experimental in 17 (90%) of these females ($\chi^2 = 13.5$, df = 1, $P = 0.00024$). Corresponding data when only the conductor tip was removed were 11 of 11 control spermathecae full, and 64% of the experimental spermathecae not inflated ($\chi^2 = 10.3$, df = 1, $P = 0.0014$, comparing empty and non-empty spermathecae). The control spematheca contained a greater amount of sperm than the experimental spermatheca in nine of 11 (82%) cases ($\chi^2 = 4.45$, df = 1, $P = 0.035$). The differences in the frequency of uninflated spermathcae between the two experimental treatments with respect to the control spermathecae were not significant ($P = 0.71$ with a two-tailed Fisher Exact Test).

The total durations of attempts to intromit (including both primary and secondary inflations) in 39 matings with modified males were not significantly shorter than in 29 matings with intact males ($P = 0.39$ with Wilcoxon/Kruskal Wallis Rank Sums Test). The total numbers of primary inflations (with and without subsequent secondary inflations) of control and modified palp were nearly equal in 37 copulations (2056 inflations by control palps, 2098 by modified palps; respective means = 69.4 ± 59.3 and 69.3 ± 58.5; $P = 0.92$ with Mann-Whitney U Test). The proportion of flubs in which control and modified palps snagged at least briefly on the plug or the epignum also did not differ (respective means = $55 \pm 34\%$ and $58 \pm 34\%$; $P = 0.99$ with Mann-Whitney U Test).

The female pushed the male's palp away from her genital opening with her legs in two pairs in which the male lacked both conductor tip and hook, but also pushed the male's palp away in two matings with intact males; in one additional case, the female pushed the plug material out of her epignum with her leg.

DISCUSSION

Some genital plugs impeded subsequent mating attempts, and such exclusion presumably benefits the male that made

the plug. Females also participated actively in the formation of successful plugs, so they presumably also benefit, but their benefits are less clear. One possible female benefit is biasing the paternity of her offspring in favor of males with certain traits (cryptic female choice). By helping some males but not others to form a plug, the female could favor paternity for subsequent males better able to remove plugs. Other female behaviors, such as pushing the male's palp or plug material from her epigynum with her tarsus, may also influence paternity. It is not known in most of these cases, however, whether these cooperative or resistant processes of the female are biased toward males with certain traits. An exception is the association between larger numbers and durations of bursts of one type of male copulatory courtship (gentle pushing with his legs on those of the female) and a greater frequency of plug production (Aisenberg & Eberhard 2009). Thus cryptic choice involving plug production and removal is feasible, but so far strong support has been demonstrated only with respect to male leg pushing.

Females also apparently occasionally formed some epigynal plugs without male participation. These yellowish and orange plugs crumbled easily when poked with a pin, and it seems very unlikely that they could exclude forceful intromission attempts by subsequent males. Presumably they have some other, as yet undetermined function.

Despite the limited mobility of genital sclerites in the male palpal bulb and their inability to provide the male with sensory feedback, male *L. mariana* frequently penetrated or dislodged even hard, firmly-attached epigynal plugs. They were also able to insert their genitalia at least in the entrance of the insemination duct, even when the contours of the epigynal surface were substantially altered by remaining pieces of plugs. The male's ability to adjust to striking variations in female morphology contrasts strongly with the tight mechanical fit between male and female morphology that is typical of many other spiders (Gering 1953; Grasshoff 1973; Huber 1995; Eberhard & Huber 1998b, 2010). The relative simplicity of the morphology of the male genitalia of *Leucauge* and other tetragnathids is apparently derived (Griswold et al. 1998); perhaps this simplicity (especially of the relatively small fraction of the *Leucauge* palp that physically contacts the female) increases this ability to adjust. Similar flexibility, in the form of an ability to inseminate both sides of the female with a single palp, has been demonstrated in two other, distantly related spiders (Costa et al. 2000; Knoflach & van Harten 2000), one which also has a very simple palp design. Tetragnathid spiders have changed the sides of the female that are inseminated by the male palps (Huber & Senglet 1997), also suggesting flexibility at some point in their evolutionary history.

Male genital movements in a species like *L. mariana* may be under two types of selection—to couple mechanically with the female genitalia in order to inseminate (and perhaps stimulate) her, and to remove plugs that impede such coupling. Nevertheless, male *L. mariana* used the same or similar basic genitalic movements in copulations with plugged and unplugged females. The relative frequencies of different types of palp movement changed, but it was uncertain whether these changes were simply consequences of greater difficulty in mechanically engaging the palp with the epigynum when it was

plugged, or the changes in male behavioral tactics were designed to remove plugs. Our behavioral categorizations were only general, however, and more detailed observations might reveal differences. It is at least possible that a male could sense the presence of a plug. The more frequent withdrawal of the palpal bulb following a flub seen by Eberhard & Huber (1998a) suggests that a male obtains enough sensory feedback from his palps to sense whether mechanical coupling has occurred. Males of some other spiders appear to use their palps to search for the female's genitalic openings (Huber 1995), also implying some sensory feedback.

The conductor hook may be especially important for plug removal. Its rigidity combined with its hooked design probably improves its ability to snag and pull or pry off plugs, and perhaps also to perforate them. The results of copulations when the hook was experimentally removed, however, showed only a weak trend toward less frequent plug removal. The plugs in *L. mariana* vary in many ways, however, that could affect removal, including composition, size, the portion of the epigynum that is covered, left-right asymmetry, and the roughness of the outer surface; none of these traits was standardized in these experiments. Thus even in comparisons between the intact and modified palps of the same male, the experimental results can at best be only suggestive. Our ability to determine whether it was the modified or unmodified palp that originally dislodged the plug may also have been imperfect. Many plugs consisted of a mass of material that extended to both sides of the epigynum, and it was not always possible to eliminate the possibility that a minor, difficult to perceive preliminary dislodgement with one palp could have led to a subsequent removal by the other. In sum, the intramale differences observed in plug removal by intact and modified palps are compatible with the hypothesis that the hook functions to remove plugs, but are not conclusive.

The flexibility of the conductor tip makes it poorly designed to remove plugs by hooking and prying, but well designed to slip along the curved external wall of the epigynum and of the insemination duct. We speculate that it may facilitate deeper intromission by the embolus, slipping between the plug and the epigynum wall to inject material below the plug, allowing the male to dislodge the plug as a unit. This facilitation of embolus insertion could explain its positive effects on sperm transfer documented here. Our experimental modifications of palpal morphology were crude, however, and cannot illuminate the functional significance of details of their forms.

Details of the forms of both hooks and conductor tips vary interspecifically in *Leucauge*. Hooks that are similar in shape to that of *L. mariana* occur in *L. venusta* (Walckenaer 1841) (Levi 1980), *L. wulingensis* Song & Zhu 1992 (Song & Zhu 1992), and *L. argentata* (O.P.-Cambridge 1869) (Chrysanthus 1975). In contrast, the hooks have quite different forms in *L. decorata* (Blackwall 1864) (Chrysanthus 1975; Tanikawa 1990) and *L. tessellata* (Thorell 1887) (= *termistica*) (Song & Zhu 1992), while conductor hooks are missing in still others, such as *Opadometa* (= *Leucauge*) *grata* (Guérin 1838) (Chrysanthus 1963), *L.* (= *Plesiometa*) *argyra* (Walckenaer 1841) (Barrantes et al. 2013), and possibly *Tylorida* (= *Leucauge*) *mornensis* (Benoit 1978) (Benoit 1978). Epigynal plugs occur in at least one of the species (*L. argyra*) in which the conductor hook is missing (Barrantes et al. 2013). The genus *Leucauge* has

apparently never been revised, and no phylogeny is available which could clarify the order in which different forms and functions for the hook and conductor tip evolved. It seems likely that the hook was favored by sexual selection, but the data do not permit discrimination among possible (non-exclusive) types of selection such as sperm competition, cryptic female choice, or sexually antagonistic coevolution.

This is to our knowledge the first experimental demonstration of effects on plug removal for any particular male genitalic structure, and also the first demonstration of multiple functions for genitalic structures and the behavior patterns which they execute. The evolutionary interactions between male and female genitalia in *Leucauge* are obviously complex and merit further study.

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Burrow structure and microhabitat characteristics of *Nesiergus insulanus* (Araneae: Theraphosidae) from Frégate Island, Seychelles

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Abstract. The burrow structure and microhabitat variables of the little known theraphosid *Nesiergus insulanus* Simon 1903 were determined on Frégate Island, Seychelles. The species constructed burrows in fossorial substrates, including rocks, leaf litter and bare soil as well as on the trunks of decaying trees, both recumbent and standing. The majority of burrows were predominantly found in sandy loam soil with partial protection from the sun. The density of burrows was determined to be weakly positively correlated to soil and substrate type and strongly negatively correlated to degree of exposure to the sun. The pH of the soil in which burrows are found was not significantly related to burrow sites, and variability in burrow structure was revealed. Burrow aggregations vary from single burrows to aggregations exceeding 100, distributed randomly.

Keywords: Tarantula, habitat, generalist

Little is known of the theraphosids of the Seychelles archipelago and published reports consist of little more than taxonomic descriptions and brief observations of their natural history (Simon 1903; Hirst 1911; Benoit 1978; Guadanucci & Gallon 2008; Saaristo 2010). More generally, despite numerous recently published papers on the behavior of tarantulas (Kotzman 1990; Fernandez-Montraveta & Ortega 1991; Costa & Pérez-Miles 1998, 2002; Quirici & Costa 2005), the biology and ecology of many tarantulas is poorly known (Carter 1997; Yáñez et al. 1999; Machkour M'Rabet et al. 2005).

Three species of *Nesiergus* are recognized and are likely endemic to Seychelles. *Nesiergus insulanus* Simon 1903 is the type species for the genus and is known from Frégate and L'Îlot Frégate Islands (Canning et al. 2013), with anecdotal and photographic evidence from naturalists on Cousine Island indicating that it may be more widely distributed than currently recognized. *Nesiergus halophilus* Benoit 1978 is known from Frégate, Récife, Silhouette and Curieuse; *Nesiergus gardineri* Hirst 1911 is known from Mahé, Felicite, Praslin, Silhouette and The Sisters (Guadanucci & Gallon 2008).

The burrows of these spiders, as with other members of the family, are used for protection against predators and parasites, for the protection of eggs and developing spiderlings, protection during ecdysis, for the capture of prey and for the control of thermal stress (Dippenaar-Schoeman 2002). Studies of habitat use by spiders have found that there are strong associations with abiotic factors such as structural features, temperature, wind, rain and humidity. Temperature and humidity have been shown to be critical factors influencing microhabitat selection for a number of spider species (Norgaard 1951; Williams 1962; Cherrett 1964; Sevacherian & Lowrie 1972; Riechert & Tracy 1975) and similar associations have been found with areas of high prey availability (Riechert & Gillespie 1986). Spiders are known to select high quality habitats (Morais-Filho & Romero 2008), and the structure of the burrows and the environmental parameters necessary for their construction must be known to provide a better understanding of a poorly known species, its role in the community, and even as a potential indicator of habitat change.

METHODS

Study site.—Frégate Island (04° 35′ 19″S, 55° 56′ 55″E) is the most isolated of the Seychelles granitic islands (Ferguson & Pearce-Kelly 2004) and is situated 55 km east of Mahé Island (Skerrett et al. 2001). It is 219 ha in area, has an altitude of 125 m at its highest point and overlies oceanic basalt. Phosphatized granite and phosphate-cemented sandstone are associated with guano deposits on the plateau. The low-lying areas of the island were marshy in the past and are now characterized by sediments of fine clay and quartz (Braithwaite 1984). However, these marshy areas have been replaced, to the detriment of many species, by cultivated fields, gardens and a marina development.

Field methods.—Field sampling sites were determined by initially conducting a pilot study. The island was stratified into habitat types based on the vegetation map of Henriette & Rocamora (2009). Vegetation types were clearly distinguishable as a result of large-scale anthropogenically-induced vegetation changes. Ground truthing determined the precise location of these various habitats and in each described habitat an extensive search was conducted on three separate occasions. In each habitat type, we extensively searched leaf litter, overturned rocks and logs and searched all other litter to find burrows. This allowed us to determine the habitat types in which spider burrows occurred. These sites were exhaustively searched to ensure that burrows or signs of spiders were not missed. The habitat types that were found in the pilot study to support these spiders were the following (with number of sample sites per habitat type determined by random selection in parentheses): Coconut-dominated woodland (8), *Ficus benghalensis* (3), Mixed exotic woodland (7), Native woodland (6), Replanted native woodland (6), Hotel area native planted (4), Exotic scrub (8), Grassland (3) Coconut woodland planted with natives (3). Those habitats in which no burrows or other

signs of spiders were found included bamboo, coconut plantations with grassland, cultivated areas, orchards and *Scaevola*; these sites were not sampled further.

Subsequent to the pilot study, the island was stratified into numbered quadrats, each measuring 100 × 100 m. From these quadrats, a random integer generator (www.random.org) was used to obtain random sample quadrats in each vegetation type in which spiders were found in the pilot study, ensuring that approximately 25% of the island was represented. Sampling sites within these quadrats consisted of a 100 m × 2 m transect at right angles to the contour. Those vegetation types in which no burrows were found in the pilot study were excluded from the selection of sampling quadrats. Forty-eight quadrats were generated in this way and sampled, of which 38 sites contained burrow aggregations. We define an aggregation as a cluster of burrows within a distance of less than one meter from one another.

Burrow structure: Burrows were examined at the 38 sample sites. At each sample site, an individual spider was extracted from a burrow to confirm the identification of the species. This was completed after data from the particular burrow had been quantified. At each sample site in which burrow aggregations were found, we measured the diameter of the largest burrow and determined the orientation of all burrows. We noted whether each burrow's entrance was flush with the ground and whether debris was incorporated in the burrow entrance. The depth of the burrow could not be accurately determined without digging them up due to their varying shapes. To determine dimension and shape, five burrows were randomly selected, spiders were extracted and Plaster of Paris was poured down burrows to create an impression of the burrow. The volume of each of these burrows was determined by immersing the casts in a measuring cylinder of water and measuring the displacement. The dimensions and shape of burrows were also established by actively seeking burrows adjacent to rocks or other objects, such as coconuts or large fallen branches. At ten of these sites, objects were removed to expose a cross section of the burrow. These burrows were closely examined, measured and photographed to confirm shape, number of chambers, number of spiders within each burrow, use of silk and dimensions.

Microhabitat characteristics: Each sample site was visited in the early morning, at midday and late afternoon on at least three separate occasions only on sunny days for a three-month period to determine the temporal exposure of burrows to the sun. Burrows were considered to have full protection from the sun if they were in shade at each visit, partially protected if they were in sun on at least one visit and having no protection if they were exposed to the sun on each visit. We recorded a description of the habitat surrounding the burrow aggregation. The substrate was characterized as leaf litter, bare soil, woody vegetation, grass or other. Leaf litter (Fig. 1a) consisted of soil substrate covered with a complete layer of leaf litter with minimal or no soil exposed. The leaf litter varied from a single layer of leaves covering the soil to three to four layers of leaves. Bare soil (Fig. 1b) consisted of a substrate of exposed soil, with leaves sporadically scattered over the substrate, but not to the extent that they entirely covered the substrate. Woody vegetation represented burrow sites where the burrow had been constructed in living plant

material such as roots. Grass (Fig. 1c) consisted of the substrate being covered in a layer of living grass. Other represented burrows were constructed in rocks (Fig. 1d), coral remnants or decaying tree trunks (Figs. 1e, f).

Ambient and burrow temperature and humidity were recorded using a thermistor digital instrument with penetration probe. Soil characteristics were determined by collecting three soil samples of approximately 500 grams each from each site and the basic soil texture, pH, soil type and soil moisture were determined. Soil texture was determined by using the United States Department of Agriculture soil triangle (online at   http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/ ?cid=nrcs142p2_054311). Soil moisture was determined by using the soil moisture content standard test method of the Australian Department of Sustainable Natural Resources. Each soil sample was weighed, dried in an oven at a constant temperature of 110 °C for 4 hours and then weighed again after cooling. The moisture content was determined as weights compared before and after drying expressed as a percentage. Soil sampling was conducted in the dry season to discount the influence of rain on moisture content. The pH of the soil samples was determined with the use of a Bluelab combometer calibrated to pH 7.0 before the testing of each soil sample.

Spiders were also observed under captive conditions. Twenty females, including four mature specimens, were kept in a confined situation in a communal polystyrene box (63 × 29.5 × 17 cm) and with a layer of soil 8 cm deep. The top of the box was covered with a glass sheet to prevent escape and for observation purposes. Spiders were provided with fresh water daily and food once a week. Burrowing behavior was observed under these conditions.

Analyses: One-way ANOVA was used to compare the number of burrows found in the sample sites with the microhabitat characteristics to determine whether there were any statistically significant differences. The analyses included those sample sites in which no burrows were found in the habitat types that were found to include burrows in the pilot study. Correlations were used to determine relationships between burrow densities and various microhabitat variables. Nearest neighbor analysis was adapted for this study to determine the patterning of burrows within an aggregation. As the method eliminates the effect of scale, the patterning within the distribution of the burrows in a cluster was determined (Rossbacher 1986). The formula used to determine aggregation distribution was $Rn = 2\bar{d}\sqrt{n/a}$ where the value of Rn represents the degree to which an observation departs from a predicted random distribution and $\bar{d}$ = the mean distance between the nearest neighbors, n = total number of points and a = area under study. Rn ranges between 0 for a clustered distribution, 1.00 for a random distribution and 2.15 for a regular distribution (Clark & Evans 1954). Nearest neighbor analysis was used only at sites where there were more than 30 burrows in the aggregation (n = 15 sites). A Rayleigh test was used to determine whether the direction of the burrows was random or non-random.

RESULTS

Microhabitat characteristics.—The number of burrows in each sample site varied between habitats from no burrows to 134 burrows in aggregations. The mean aggregations and

Figure 1a–f.—Substrate types in which burrows of *Nesiergus insulanus* are found on Frégate Island, Seychelles. a. Leaf litter; b. Bare soil; c. Grass; d. Rock; e. Tree trunk with arrows indicating position of burrows approximately 1.5 and 1.7 m above ground level; f. Recumbent rotting log.

densities per square meter combined from sample sites in each habitat type were as follows: Exotic scrub 8.4 at $0.042/m^2$, native woodland 36 at $0.18/m^2$, coconut-dominated woodland 11.4 at $0.057/m^2$, *Ficus benghalensis* 36.5 at $0.1825/m^2$, mixed exotic woodland 6.5 at $0.01/m^2$, grassland 3.5 at $0.0175/m^2$, hotel area native planted 15.4 at $0.0775/m^2$, coconut woodland planted with natives 1.7 at $0.0085/m^2$ and replanted native woodland 21 at $0.105/m^2$. Microhabitat variables varied between sample sites (Table 1) with the mean ambient temperature found to be 2.13°C higher than the temperature within the burrows across habitat types. In contrast, the humidity within the burrows was found to be an average of 9.93% higher than the ambient humidity. Open grassland was the only habitat in which at least some burrows were found to be fully exposed to the sun. The mean ambient temperature for grassland was 2.5° C warmer than the mean across all habitat types. The mean seasonal change in temperature is in a very narrow band and this is reflected in the mean temperature across habitat types.

An ANOVA showed a significant difference in number of burrows between substrates across sample sites ($F_{3, 32} = 3.42$,

$P = 0.03$) with leaf litter and bare soil being preferred over other substrates. A follow-up test to determine differences between these two substrates showed that there is no significant difference in choice between bare soil and leaf litter ($F_{1, 16} = 0.09$, $P = 0.77$) as the more frequently used substrates. Few burrows were found in grass-covered areas and in the cracks and holes of rocks. Those burrows dug in bare soil were found among vegetation and often close to rotting logs that provided protection and a supply of prey in the form of termites or other invertebrates. There was a significant difference in soil types in which burrows occurred ($F_{7, 64} = 5.66$, $P < 0.0001$) with the majority being found in sandy loam. Protection from full exposure to the sun was statistically highly significant ($F_{2, 24} = 11.13$, $P = 0.0003$) with spiders preferring partial protection from the sun. There was a non-significant correlation between the soil types and the density of burrows (Spearman Rank correlation, $r = 0.167$, $P = 0.157$), a significant correlation between choice of substrate and burrow density ($r = 0.357$, $P = 0.013$) and a very strong correlation between protection from the sun and burrow densities ($r = 0.9995$, $P = 0.001$). The soil pH varied

Table 1.—Summary of microhabitat variables across habitat types for burrows of *Nesiergus insulanus* on Frégate Island. Figures given are the percentages of the total number of burrows displaying that particular variable for the habitat type. Moisture content, pH and temperature measurements are from the lowest to the highest recorded measurement at each site in the specific habitat. FB = *Ficus benghalensis*, CWPWN = coconut woodland planted with natives, MEW = mixed exotic woodland, ES = exotic scrub, NW = native woodland, RNW = replanted native woodland, HANP = hotel area nativeplanted, CDW = coconut dominated woodland, GL = grassland.

	FB	CWPWN	MEW	ES	NW	RNW	HANP	CDW	GL
Sampling sites (N)	3	3	7	8	6	6	4	8	3
Wind Protection									
none	66								50
partial	33	33		57.1	25	80	50	37.5	50
full		66	100	42.8	75	20	50	62.5	
Sun Exposure									
none		33	75	14.2	25			50	
partial	100	66	25	85.7	75	100	100	50	50
full									50
Substrate									
bare soil	33	33	50	71.4		60	50	37.5	
leaf litter	66	33	50	14.2	100		50	37.5	
grass									100
other(vegetation)		33		14.2		40		25	
Soil Characteristics									
silt loam				28.5					50
loam			25				100	12.5	
loamy sand		33	25	14.2		40		25	
silt	33								
sandy loam	33	66	50	42.8		20		62.5	50
sandy	33								
other (rock)						20			
moisture Content	10–71%	5–6%	6–23%	3–23%	12–27%	1–25%	10–15%	4–25%	6–10%
pH	4.2–4.9	6.1–8.9	5.7–8.2	3.7–7.4	4.9–8.5	5.1–8.3	5.2–8.4	5.7–8.9	7.3–8.2
Ambient Temp. (°C)	30.8–32.9	30.4–30.5	27.1–30.4	30.1–34.1	29.9°–31.8°	29.5°–31.5°	31.6°–32.1°	29.8°–31.5°	32.9°–33.5°
Burrow Temp. (°C)	28.0–31.2	27.9–29.1	25.7–27.3	27.6–32.3	27.1°–28.7°	27°–30.8°	28.4°–30.4°	26°–29.7°	31.8°–32.2°

considerably between habitat types and between sample sites, from 3.7 to 8.9 with a mean of 6.45 (UCL = 6.92, LCL = 5.98). A linear regression analysis determined that the relationship between pH and spider densities was nonsignificant ($r = 0.9815$, $P = 0.33$) and therefore plays no role in burrow site selection.

Burrow structure.—Nearest-neighbor analysis showed that the distribution of burrows within an aggregation was random, (average Rn = 1.17). These spiders make use of both fossorial substrates (Figs. 1a–c) and the trunks of decaying trees. The trunks of rotting trees, both standing (Fig. 1e) and recumbent (Fig. 1f) were used. The decomposing wood likely provides a regular supply of food to prey on such as termites and other invertebrates as well as providing a stable microclimate. Hollows and cracks in rocks were exploited on occasion (Fig. 1d). Spiders were also found under rocks where either a silk-lined depression or a burrow was constructed.

Captive specimens of *N. insulanus* were able to excavate their own retreats and were able to burrow through wood and roots, despite lacking a rastellum. When disturbed or if their burrow was damaged or destroyed, they excavated a new burrow. Chelicerae were used in loosening the soil and the first pair of legs was used to pass the soil to the side of the burrow entrance or under the body where the third and fourth pairs of legs pushed the soil from the burrow. The first one-third to one-quarter of the inside of the burrow was lined with silk. There were no silk mats or trip lines around the burrow entrance for prey detection. Silk was used in bends in burrows to support the walls at these bends (Fig. 2).

The majority of the burrow entrances lay flush with the surface and had no debris, although some debris in the form of small stones, sticks and millipede droppings were on occasion attached to the silk around the lip of the burrow. Burrows in the cracks of rocks were fully constructed of silk with debris, including feathers, attached along the full length of the burrow. A single entrance was observed at all burrows. These entrances were closed with silk with soil attached when the spider was in the process of ecdysis, incubating, when there were pre-emergent spiderlings in the maternal burrow, or under adverse weather conditions, such as during heavy rain. The entrances were completely camouflaged with soil during this period.

The largest burrow diameter found at sample sites was 13.59 mm with a mean diameter for all sampled burrows of 6.42 mm. Orientation of the burrow entrance of 116 burrows in all habitat types was determined and a Rayleigh test indicated that there was no particular prevailing orientation of burrow entrances ($Z = 0.282$, $P = 0.50$). Burrow shape was widely diverse and a single distinguishing shape cannot be

Figure 2.—Burrow of *N. insulanus* with resident spider in first chamber. Arrows indicate use of silk below burrow entrance, at curve above first chamber and on roof of second chamber.
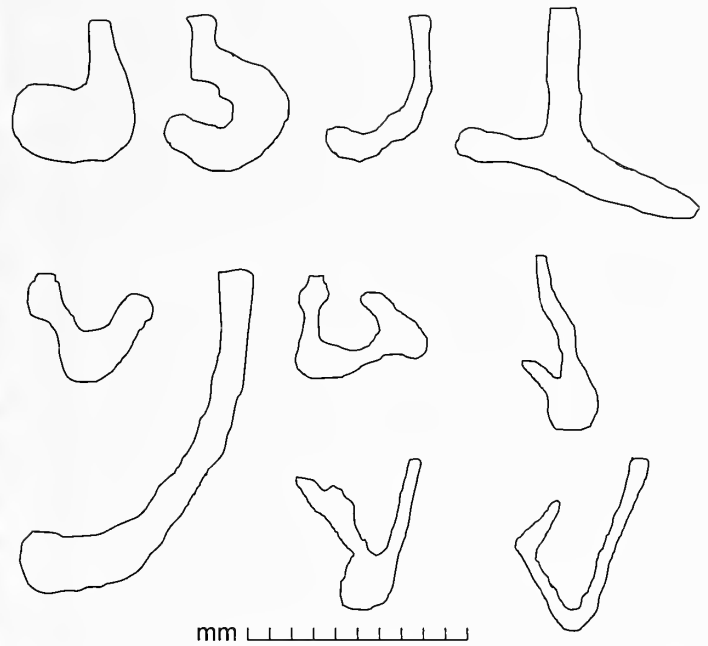

Figure 3.—Burrow shapes of *N. insulanus* indicating diverse shapes, including basic burrow shapes, as well as variations thereof, with additional chambers and shafts.

attributed to this species. Burrows were J-shaped, U-shaped and V-shaped with variations of these basic profiles. Variations included additional chambers or shafts. Variations were sometimes due to an obstruction that the spider could not burrow through or around and sometimes appeared to be random. U-shaped variations included burrows recumbent with an extended burrow entrance. V-shaped burrow variations included additional horizontal arms or supplementary arms giving the burrows a Y-shape, and the dimensions of observed burrows varied widely (Fig. 3). The displacement volume of the five burrow molds were 22 ml, 41 ml, 53 ml, 10 ml and 7 ml.

DISCUSSION

Nesiergus insulanus makes use of a number of available substrate types including soil, tree trunks and cracks in rocks in which to create burrows. The exploitation of these substrates indicates adaptability that allows the species to exploit a wider range of habitats than would be available to more specialized species. This behavior could be considered an obligatory adaptation to their occurrence on small and isolated islands with limited resources, thus restricting their ability to occupy a more specialized niche.

Machkour M'Rabet et al. (2007) found that densities of the tarantula *Brachypelma vagans* Ausserer 1875 were dependent on soil type. This study also found significant associations with soil types, the type of soil apparently being important in burrow construction because of the possibility of collapse when these spiders only partially line their burrows with silk. The variation in burrow structure from simple, single-chambered structures to fairly complex constructions that are found in high densities in suitable habitat has also been recorded in *B. vagans* (Machkour M'Rabet et al. 2007).

The combination of a number of suitable microhabitat variables appears to be necessary to support a population of these spiders and where these variables are absent, so too were the spiders. They were commonly found adjacent to rocks and decaying logs, as well as on pathways. These logs and rocks as well as roads and pathways provide ecotones that support increased biodiversity and productivity (Risser 1995). Arthropods have been found to be greatly influenced by changes in temperature and humidity (Cady 1984) and we found that sites of burrows that were at least partially protected from sun exposure, thus limiting fluctuations, were preferred over sites that offered little protection from the elements. Burrows found in exposed areas were few in number and even these were offered a degree of protection close to the ground.

The disturbance and alteration of natural habitats and the introduction of alien plant species is detrimental to the distribution of the species. Large-scale changes to the native vegetation on the island limits the opportunity for dispersal to new habitats and is cause for concern for a species with a limited distribution. Frégate Island has been severely degraded and large areas of the island are covered in alien species. In particular, monospecific stands of coconuts, *Cocos nucifera*, cover vast areas of the island, severely reducing available native habitat. The occurrence of these spiders in such degraded habitats is limited or absent and is of concern for the long term welfare of the species. As tarantulas do not balloon as a means of dispersal (Jankowski-Bell & Horner 1999) and spiderlings do not wander greatly if a suitable patch is found in which the spiderlings are able to burrow (Cutler & Guarisco 1995) their dispersal capabilities are reduced. The restoration of habitat and the creation of corridors between restored habitat and habitats in which this species is to be found are essential for the long term viability of the species.

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Thermal preference of *Dysdera crocata* C. L. Koch 1838 (Araneae: Dysderidae)

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Abstract. Body temperature is the most important ecophysiological variable affecting all aspects of the performance of ectotherms. However, thermal preferences and tolerances of spiders have been studied only in 0.1% of spider species. Knowledge of thermal preferences and tolerances is necessary to describe the ecology of these animals, defining the preferred foraging sites or preferred shelters and reproductive sites. In this study we report for the first time the preferred temperature of *Dysdera crocata* C.L. Koch 1838 in the laboratory. This is an epigean spider of Mediterranean climates with large temperature fluctuations. The preferred temperature was low: 9.12° ± 5.12 °C, and actively searched. It did not vary throughout the day.

Keywords: Woodlouse spider, micro-environments, Chile

Spiders are ectothermic animals; their energetic processes are highly correlated with the temperature of their surroundings, which has consequences in energy conservation, reproduction and prey capture. However, thermal preferences and tolerances of spiders have been studied only in 0.1% of spider species (Humphreys 1987; Schmalhofer 1999; Hanna & Cobb 2007). Knowledge of thermal preferences and tolerances is necessary to describe the ecology of these animals (Hertz et al. 1993), defining the preferred foraging sites or preferred shelters and reproductive sites (Hanna & Cobb 2007).

By analyzing thermal preferences and tolerances we can estimate the thermal niche, which is one of the niche dimensions. This may be assessed by means of mechanistic biophysical ecological methods not using the environment per se but rather the state of the organism, for example body temperature (Tb). Tb drives an organism's physiological state; thus it is crucial to quantify patterns of body temperature if we are to link controlled laboratory conditions with those in the field. The principles of these models provide a robust approach to determining niches of organisms mechanistically (Kearney 2006; Kearney & Porter 2009; Kearney et al. 2010; Kearney 2012).

Spiders of the genus *Dysdera* Latreille 1804 (Family Dysderidae) are ground dwellers characteristic of xerothermic forests of the Mediterranean and adjacent areas. During the day, they shelter in gravel covered by organic material or under stones, and it has been reported that at night these wandering nocturnal hunters search for woodlice (terrestrial isopods), their principal prey (Cooke 1965; Bradley 2013).

The woodlouse spider, *Dysdera crocata* C. L. Koch 1838 is originally from the Mediterranean and eastern European region (Cooke 1965) but has spread throughout the world. There are over 240 species of *Dysdera* (Platnick 2013) however only *D. crocata* is cosmopolitan. It is amenable to a wide spectrum of environmental conditions, being a common spider in regions with cold temperatures and winter snow cover (e.g., Illinois, Ohio, Great Britain, Tasmania) as well as regions of hot, dry summers and mild winters of a Mediterranean climate (e.g., southern California, Greece) (Southcott 1976; Roberts 1995; Bradley 2004; Bosmans & Chatzaki 2005; Vetter & Isbister 2006).

In Chile, *D. crocata* is mostly limited to urban areas in the central region (Mediterranean climate); it is considered to be a synanthropic spider (Taucare-Rios et al. 2013). It is an epigean species which can be captured under stones, rocks and rotting logs that also support isopods; it is active throughout the year in a micro-environment practically isolated from light, and with constant high humidity. The temperature in this environment is affected by the fluctuations characteristic of the Mediterranean climate, varying in the year more than fifteen degrees at a depth of 10 cm in the soil (Villaseca 1990). There are no studies of the thermal biology of *D. crocata*. Because body temperature is the most important ecophysiological variable affecting all aspects of the performance of ectotherms, including locomotion, immune function, sensory input, foraging ability, courtship and rates of feeding and growth (Angilletta et al. 2002; Portner et al. 2006; Angilleta 2009; Hazell et al. 2010), the objective of this study was to determine the preferred temperature of this species.

METHODS

Animals and study area.—Twenty individuals of *D. crocata* were collected in the peri-urban zones of Santiago, Chile (32°S, 70° 40′ W). They were transferred to the laboratory in the Faculty of Science of the University of Chile. Each spider was introduced into a plastic box with moist soil and isopods obtained in the capture site and maintained for one week at room temperature (20° ± 5 °C, 60 ± 5% RH) and at 10L (8:00–18:00):14D (18:00–8:00) photoperiod. All experiments were conducted in this laboratory during March 2012–October 2012.

Preferred temperature.—After a week of acclimation, twenty individuals (11 females and nine males; m_b= 102.29 ± 60.88 mg) were exposed to a temperature gradient between 2° ± 2.56 °C and 50° ± 0.89 °C established in a plastic cylinder oriented horizontally with the extremes halfway submerged in a thermoregulated chamber 1.20 m long × 0.25 m wide × 0.25 cm high. This chamber had a thermoregulated heater in one end and a cold point in the other, generating a thermal gradient between the end points (Fig. 1). The gradient was closely linear with a temperature of 19.3° ± 5.10 °C in the
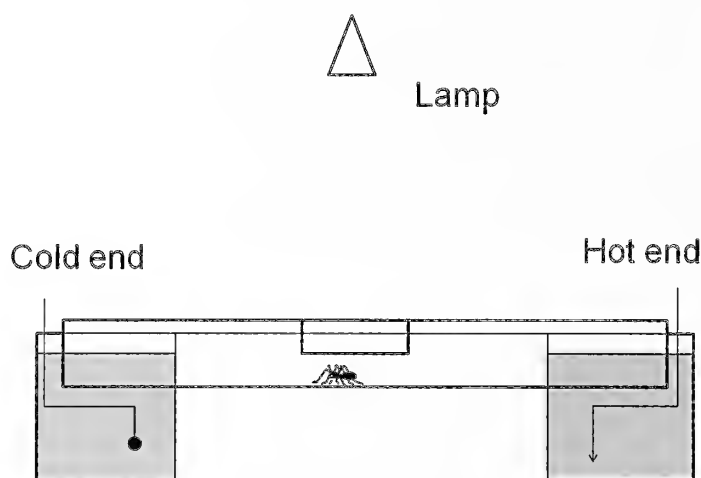
Figure 1.—Experimental temperature gradient apparatus used to measure preferred temperatures of *Dysdera crocata*. Thermoregulated water baths in grey. Infrared lamp at 2.5 m.

Figure 2.—Frequency histogram of preferred temperatures for *Dysdera crocata*.

center. Prior to the beginning of the experiments, the thermal gradient was calibrated with thermocouples installed every 5 cm. The hot/cold ends of the gradient were always switched between trials to account for potential side biases.

The spiders were exposed individually for 65 min. This was repeated twice in the morning (09:00, 12:00) and twice in the twilight-night period (18:00, 20:00). The experiments were conducted in an isolated room illuminated with artificial light in the two morning experimental hours and with only an infrared lamp positioned perpendicular to the gradient at a distance of 2.5 m in the two twilight-night periods. Individuals were deposited in the center of the chamber and allowed five minutes of settling. Then the temperature of the spiders was measured at the midpoint of the cephalothorax with an infrared thermometer every five minutes for one hour. Prior to the experimental trial, the body mass of spiders was measured with an analytical balance (Shimadzu, AUX 220, ± 1 mg).

For each individual a record of the 12 temperatures chosen by the spiders (t_i: one every 5 minutes) in the periods was obtained. These were recorded starting at 9:00, 12:00, 18:00 and 20:00. With these temperature records, frequency histograms of the chosen temperatures were constructed. For each individual the mean preferred temperature (Tp) in each hour was calculated (the average of the 12 values).

Analysis and statistics.—The temperatures chosen by the spiders (t_i) were characterized with frequency histograms. The normality of the distributions was tested with the Shapiro-Wilks test (W). The initial and t_i temperatures were compared using the Friedman test (Fr), with *a posteriori* multiple comparisons. Sex differences were analyzed with the Mann-Whitney test (U), and the correlation between initial temperature and the temperature at the end of the trials were evaluated with the Spearman correlation coefficient (R).

To analyze differences in thermal preferences of the species, the temperatures chosen were averaged for each hour so that each experimental time was represented by a single value (Tp). Considering that each individual was studied at four different hours (repeated measures design) and the non-normal distribution of the data, a non-parametric Friedman test for dependent samples was performed, with Tp the response
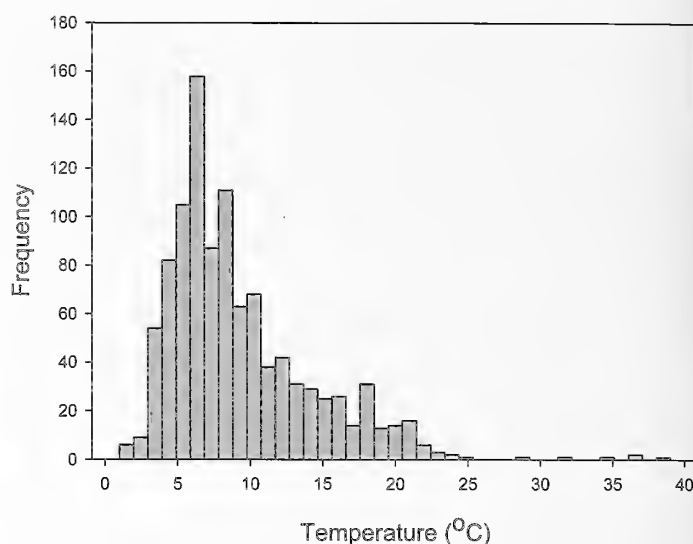
variable and the four experimental times (9:00, 12:00, 18:00 and 20:00) as the factors.

RESULTS

The preferred temperature over all individuals and experimental hours was 9.12° ± 5.12 °C, with median and mode 8.0° and 6.0 °C, respectively. This was not related to sex (U = 35, $P = 0.29$) or to the body mass of the spiders (R = 0.11, $P > 0.05$). The distribution had a skewness of 1.47 and a kurtosis of 0.32 (Fig. 2) and was different from a normal distribution (W = 0.884, $P \ll 0.001$). The body temperature at the end of the experiment was correlated with the body temperature at the beginning of each experimental trial (R = 0.299, $P < 0.05$), but the variance explained was very small ($R^2 = 0.09$) and preferred temperatures changed quickly with respect to the initial preference. Initial body temperature was different than temperatures chosen in the following minutes in the experimental trials ($Fr_{80,13} = 146$, $P \ll 0.001$) (Fig. 3). There was a mean displacement of 5.9 ± 5.7 cm every five minutes.

No differences among Tp were found comparing the four experimental hours ($Fr_{20,3} = 5.82$, p = 0.121) (Fig. 4).

DISCUSSION

Thermal preferences facilitate the description of the ecology of a species and assessment of the suitability of the habitat (Hertz et al. 1993). According to Sevacherian & Lowrie (1972), individual limits and physiological processes determine the conditions in which an organism can survive and adapt successfully to a particular environment.

Preferred temperatures for *D. crocata* were low compared to the range described for other araneomorph species. For example these temperatures are between 23° and 23.5 °C in Agelenidae (Pulz 1987), between 16° and 22.3 °C in Clubionidae (Almquist 1970) and between 19.2° and 26.2 °C in Lycosidae (Almquist 1970; Sevacherian & Lowrie 1972; Pulz 1987). However there are reports of low preferred temperatures in other species. For example, preferred temperatures of some species of Linyphiidae have been reported; 4.1
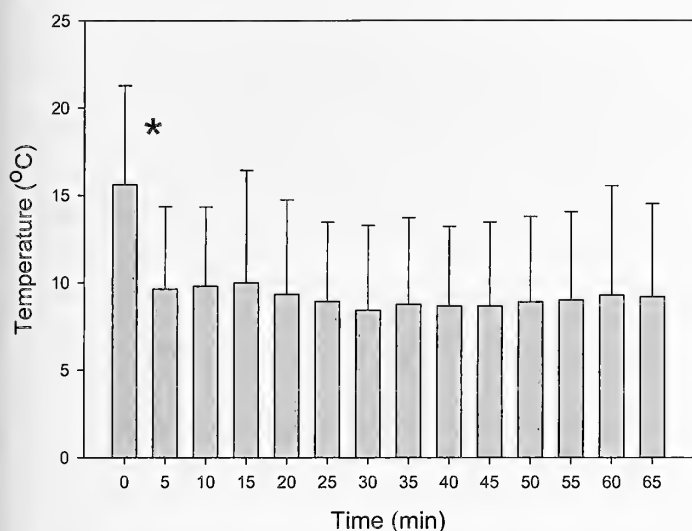
Figure 3.—Changes in body temperature over time in all experimental series. The asterisk indicates that the initial temperature was different than all others in a posteriori multiple comparisons.

Figure 4.—Preferred temperatures of *Dysdera crocata* at different times of the day (mean and standard deviation).

°C in *Bolephthyphantes* (=*Bolyphantes*) *index* (Thorell 1856) (Pulz 1987) and 1.2 °C in *Macrargus rufus* (Wider 1834) (Almquist 1970). The preferred temperature of *D. crocata* is probably associated with temperatures that are usually found in their habitat under stones, dried leaves and organic material.

In Santiago, Chile, in the location where the specimens were captured, the soil temperature can vary more than 15 °C, with the lowest temperatures in the months of April to September (winter), where temperature at 10 cm depth can reach 8 °C. The lowest temperatures are reached at night, which coincides with the activity period of *D. crocata*. Also, this time range of low temperatures coincides with the time when the experiments were performed. It has been reported that this species feeds on the isopods with which they coexist (Cooke 1965, Bradley 2013). In Chile, it is common to find *D. crocata* sharing its habitat with the common woodlouse *Porcellio laevis*, which would be its usual prey. A study of preferred temperatures of this isopod demonstrated that it is variable at different locations in Chile and according to the time spent in the measurement system (Castañeda et al. 2004). Interestingly, the preferred temperature for specimens of *P. laevis* in Santiago was 9.4° ± 1.1 °C using a measurement period similar to the time that we ran our experiments, and varied between 9.4° ± 1.1 °C and 12.2° ± 1.1 °C in the total experimental range of this study, which is fully consistent with our results. Thus two species that share a habitat in the field, one a predator and the other its prey, have similar preferred temperatures. A similar result was reported for the spider *Loxosceles laeta* (Nicolet 1849) and its predator *Scytodes globula* Nicolet 1849 (Canals 2004; Canals & Solis 2013), in which the preferred temperatures, the critical temperatures and desiccation tolerances have a large overlap (Alfaro et al. 2013; Canals et al. 2013). The body temperature of *D. crocata* varied from an initial temperature of 15.6° ± 5.6 °C to 9.6° ± 4.6 °C in 5 minutes, and afterwards remained close to their preferred temperature (Fig. 3), with an average displacement
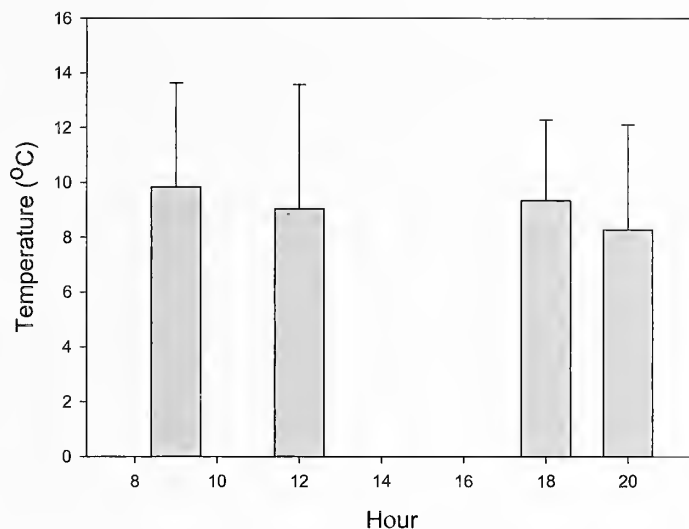
of about 6 cm in 5 min suggesting that spiders actively sought their preferred temperature.

D. crocata did not present hourly variations in preferred temperature throughout the experimental hours, contrasting with those reported in other arthropods (Canals et al. 1997), mygalomorph spiders with crepuscular and nocturnal activity (Alfaro et al. 2012), and other nocturnal araneomorph spiders (Alfaro et al. 2013).

Regarding thermal preferences, *D. crocata* had a standard deviation of ± 5.12 °C, a value that is nearly 1 °C low than other spiders such as *L. laeta* and *S. globula*, suggesting a more narrow range of thermal microenvironment preference than these species. The election of low temperatures and a relatively narrow range may be explained by phenotypic plasticity as an adaptation to the particular environmental conditions present in Chile. This plasticity in preferred temperatures has been reported in *Paraphysa parvula* Pocock 1903 and *Grammostola rosea* (Walckenaer 1837), two mygalomorph spiders of central Chile (Alfaro et al. 2013). Species from different environments typically also have different thermal preferences (Pulz 1987; Schmalhofer 1999) and these may vary seasonally (Schmalhofer 1999), with the breeding season (Hanna & Cobb 2007; Veloso et al. 2012) or during the day, as in other ectotherms (Canals et al. 1997; Alfaro et al. 2013).

The woodlouse spider, *Dysdera crocata*, originated from the Mediterranean and eastern European region (Cooke 1965) but has spread throughout the world; it is considered to be a cosmopolitan spider. Its distribution is mainly in the holartic region and it is more common near the coast. It is a common spider in regions with cold temperatures and winter snow cover (e.g., Illinois, Ohio, Great Britain, Tasmania) as well as in regions with the hot, dry summers and mild winters of a Mediterranean climate (e.g., southern California, Greece) (Southcott 1976; Roberts 1995; Bradley 2004; Bosmans & Chatzaki 2005; Vetter & Isbister 2006). The projection of our results from the micro scale to the temperature conditions associated with its world distribution would be not correct because preferred temperatures indicate the suitable environments for *D. crocata*. These preferred temperatures may be

different in hotter environments (phenotypic or physiologic plasticity), or this spider has a great ability to find its preferred microenvironments, probably associated with its prey: isopod populations.

ACKNOWLEDGMENTS

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Natural history of *Phoneutria boliviensis* (Araneae: Ctenidae): habitats, reproductive behavior, postembryonic development and prey-wrapping

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Abstract. *Phoneutria boliviensis* (F.O.P.-Cambridge 1897) is a medically important wandering spider distributed from Central America to northern South America. This study is the first description of the natural history of this species, and presents data on several aspects of its natural history: reproductive and prey wrapping behavior, postembryonic development, and habitats in the departments of Valle del Cauca and Quindio, Colombia. Prior to copulation, the male did not engage in any courtship from a distance, but instead climbed onto the female, adopting the typical copulation position of "modern wandering spiders" (position III). Females laid up to four egg sacs; between 430–1300 hatchlings emerged after 28–34 days. After hatching, spiderlings had a third claw on all their legs and built an irregular web, where they remained until the next molt. Sexual maturity occurred after 14–17 molts, and spiders matured 300–465 days after emerging from the egg sac. The species was found in disturbed habitats associated with both dry and wet tropical forests, usually on the ground with little litter. Spiders wrapped prey in silk, moving in a stereotypically circular pattern around the prey without manipulating threads with their legs. Attachments to the substrate involved rapid movements of the anterior spinnerets, while the others remained immobile.

Keywords: Mating, maternal behavior, Colombia, banana spider

The family Ctenidae is well represented in the Neotropics by medium to large wandering spiders that usually inhabit the forest floor and low vegetation; few are arboreal. In this family, the genus *Phoneutria* currently comprises eight large (17–48 mm) nocturnal wandering spider species that are widely distributed in Central America (Costa Rica) and South America east of the Andes into northern Argentina (Simó & Brescovit 2001; Martins & Bertani 2007). They are generally known as "banana spiders" because they often inhabit this crop. They are considered aggressive, and among the most medically important spiders in the world (Foelix 2010). Their venom has a neurotoxic action (Foelix 2010) and many researchers have analyzed its components and the epidemiology of bites (Bücherl 1953a, b, 1956; Cruz-Höfling et al. 1985; Marangoni et al. 1993; Pineda & Florez 2002; Florez et al. 2003; Garcia et al. 2008; Maguiña et al. 2008).

The natural history of several species in the genus *Phoneutria* has been examined in some studies. Bücherl (1969), Ramos et al. (1998), and Almeida et al. (2000) presented data on the development, activity, reproduction seasonality, and habitat use of *Phoneutria nigriventer* (Keyserling 1891). Simó & Bardier (1989) described the postembryonic development of *P. keyserlingi* (F.O.P.-Cambridge 1897). In the Amazon region, Gasnier et al. (2002) and Torres & Gasnier (2010) offered data on the adult size, sexual dimorphism, habitat use, and temporal changes in body size structure of *P. fera* Perty 1833 and *P. reidyi* (F.O.P.-Cambridge 1897). Dias et al. (2011) modeled the potential geographical distribution of *P. bahiensis* Simó and Brescovit 2001, a threatened species endemic to Brazil.

Phoneutria boliviensis (F.O.P.-Cambridge 1897) is widely distributed in Central America (Costa Rica) to northern South America (Simó & Brescovit 2001). Except for the brief mentions by Valerio (1983) and Hazzi et al. (2013) on geographical distribution expansions, Florez et al. (2003) on the epidemiology of bites and Jäger & Blick (2009) on the

introduction into other countries via commerce in banana products, nothing is known regarding its general biology. The following study presents data about the natural history of *Phoneutria boliviensis*: reproductive and prey wrapping behavior, postembryonic development, and habitat. It is based on animals kept in captivity and on complementary field observations.

METHODS

Habitat.—Collections and nocturnal observations were made in localities in the Quindío and Valle del Cauca departments in Colombia (Fig. 1). With the help of a headlamp, I located spiders by the reflection of the light in their eyes. Daylight observations were also performed by turning over rocks and tree trunks and by visual searches of the vegetation.

Reproductive behavior.—Six females and three males collected from Cali, Aguacatal (Table 1, Fig. 1) were kept about 200 m from the collection locality in 30 × 20 cm terraria, with soil as substrate and wet cotton wool moistened daily. The spiders were maintained under ambient conditions of temperature (day/night approximately 27/25°C), humidity, and lighting. The spiders were fed cockroaches, *Periplaneta americana*, two times a week. This methodology allowed observations of egg sacs, time of the emergence of spiderlings, maternal care, and spiderling behavior in the first days. In addition, one female found with an egg sac was left in the field and was monitored daily to compare her maternal and spiderling behavior with those observed in captivity. This female was found in a cleft formed by two rocks at the side of a road.

Matings were observed at night involving three males and four females (two females were immatures that were raised to maturity, and thus virgins) in the same terraria and conditions describe above. For each encounter, I carefully introduced the male into the larger container housing the female's terrarium
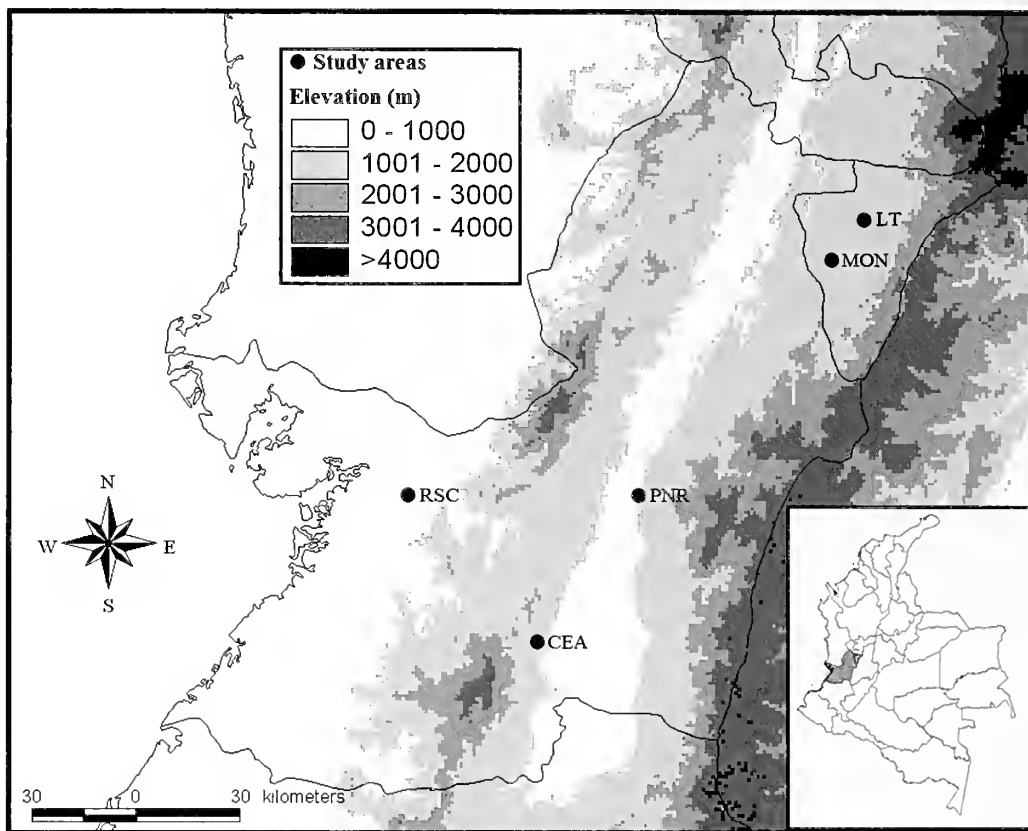
Figure 1.—Study areas in the Valle del Cauca and Quindio departments in Colombia. RSC = Reserva Natural San Cipriano; PNR = Parque Natural Regional "El Vinculo"; CEA = Cali "El Aguacatal"; LT = La Tebaida; MON = Montenegro.

about 20–25 cm from the female. I performed 12 male-female pairings in all combinations, and both males and females were given four possible mating opportunities. Male pre-copulatory and copulatory courtship behavior and copulation are defined as in Eberhard & Huber (1998). Male courtship refers to those behaviors that induce the female to respond in a way that favors the male's reproduction (Eberhard 1996). Copulation consists of all genitalic contact between a particular male-female pair, including the insertion of the embolus into the epigynal opening (Eberhard & Huber 1998).

Post-embryonic development.—In order to determine inter-molt period in each instar and number of molts and necessary time to reach to sexually maturity, I raised 43 spiderlings taken at random from two egg sacs obtained from two of the six females. The spiderlings were housed individually in plastic cylinders (4 cm diameter × 6 cm high) until the fifth instar, when they were transferred to larger plastic cylinders (10 cm diameter × 15 cm high). A moistened cotton ball was supplied weekly. Juveniles up to the fifth instar were fed with *Drosophila melanogaster* larvae and adults raised in the

Table 1.—General characteristics of the areas studied and ctenids living sympatrically with *P. boliviensis*. Forest types were classified follow Holdridge's life zone. A.M.T. = annual mean temperature; A.M.P. = annual mean precipitation.

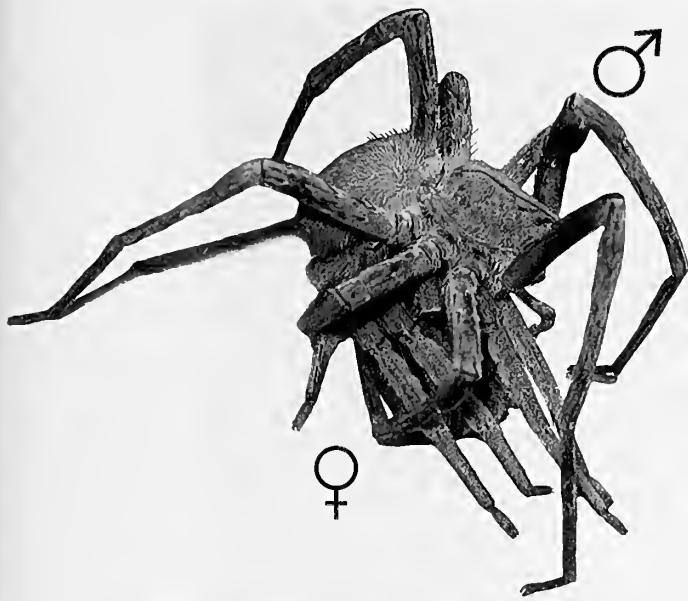| Locality	Forest type	Coordinates (Lat. N; Lon. O)	Elevation range (m)	A.M.T. (°C)	A.M.P. (mm)	Sympatric ctenids
Reserva Natural San Cipriano	Tropical rainforest (bp-T)	3° 50′ 20″; 76° 53′ 52″	0–80	26	5200	*Acanthoctenus* sp, *Ancylometes bogotensis*, and *Cupiennius granadensis*
Parque Natural regional "El Vinculo"	Tropical dry forest (bs-T)	3° 50′ 23″; 76° 18′ 07″	950–1100	25	1400	*Cupiennius bimaculatus*
Cali "El Aguacatal"	Tropical dry forest (bs-T)	3° 27′ 31″; 76° 33′ 45″	1000–1100	25	1300	*C. bimaculatus*
La Tebaida	Premontane wet forest (bh-PM)	4° 26′ 59″; 75° 48′ 01″	1200–1300	22	1700	*Cupiennius bimaculatus* and *C. coccineus*
Montenegro	Premontane wet forest (bh-PM)	4° 33′ 13″; 75° 43′ 03″	1300–1400	21	2100	*Cupiennius bimaculatus* and *C. coccineus*

Figure 2.—Mating position of *P. boliviensis*.

laboratory. Older spiderlings were fed field-collected crickets and juvenile *Periplaneta americana* raised in the laboratory. Spiderlings were fed and checked for molting three times a week. I consider spiderlings that recently emerged from the egg sac as second instar individuals (Foelix 1996).

Vouchers specimens are deposited in the arachnological collection of Museo de Entomología de la Universidad del Valle (MUSENUV), Cali, Colombia.

Prey-wrapping behavior.—Previous studies have shown that wrapping behavior varies both qualitatively and quantitatively depending on prey size and species (see references in Barrantes & Eberhard 2007). In this study, I focused on determining whether or not *Phoneutria boliviensis* exhibited a given general behavior pattern, rather than whether or not this behavior was omitted under certain conditions. I always used adults of *Periplaneta americana*, a difficult prey for the spiders. In four of the six females collected, I observed eight prey-wrapping episodes (two for each female). Video recordings were made with a digital Canon PowerShot ELPH 100 HS camera.

Behavioral and postembryonic developmental data are presented as mean ± SD (range: min-max). Because of the small samples, they are meant only to provide general descriptions of magnitudes, rather than to characterize the behavior of this species.

RESULTS

Habitat observations.—Sixty-nine individuals were found in remnants of dry forests, premontane wet forests transformed into banana plantations and rain forests (Table 1). In the dry and wet premontane forest, spiders were always associated with synanthropic environments. I also observed the spiders in forest edges or adjacent habitats (roadsides). During the day, I found spiders (n = 20) under rocks, piles of banana leaf litter, and building rubble (tiles and bricks) near the forests or banana plantations. At night, I observed spiders (n = 49) on the ground with scattered litter (n = 40) and a few in low vegetation, usually below 40 cm above the ground (n = 9).

Mating behavior.—Mating occurred in four of 12 couples that were placed together. In no case did the male court the female from a distance. The males reacted to contact with female silk using palpal movements and began to search for the female by keeping their palps near the substrate, maintaining contact with female silk and slowly tapping in different directions with their first legs. When the male contacted the female, he turned until they were head-to-head, and touched her very quickly (less than 2 s) with his forelegs. If the female was not receptive (n = 8), she rapidly ran away. However, if she was receptive (n = 4), the male climbed over her so that they faced opposite directions and she drew in her legs close to her body so that the patellae of all her legs almost touched each other above her carapace. The male moved laterally to the sides of the female's body and contacted her epigynum with one palp. The mating position was type III, as in Foelix (2010) (Fig. 2). Copulation lasted less than 15 s and the male's extended palp moved rapidly to touch the epigynum briefly. In three pairs, it was possible to record palpal insertions; in two, there was only one insertion and in the other there were two, one on each side of the epigynum. In the video recording, I observed that the spines on the male's legs became erect momentarily at the beginning of each palpal insertion. After mating completion, the male ran away.

Post-embryonic development.—Five of the six females attached egg sacs (n = 10) to the terrarium wall, always above the ground. Egg sacs were white, with a flat face of an average diameter of 28 mm ± 4 (range 22–33, n = 10) against the wall and a convex face (Fig. 3). Spiderlings emerged on average 30 ± 2 days (range 28–34, n = 5) after the egg sacs were produced. The average number of offspring per egg sac was 836 ± 436 (range 430–1300, n = 5). Before hatching, females only left the egg sacs for short periods, moving down in the terrarium to drink. However, they still preyed on food that was placed in the terrarium away from the egg sacs. They were more aggressive while guarding, lifting their first pair of legs, opening their fangs, and making lateral movements of the body as is characteristic of the genus (Simó & Brescovit 2001) (Fig 4.).

Twice I observed the emergence of spiderlings, one hour after the females began to bite the egg sac with her chelicerae repeatedly in different parts about once per minute. After hatching, spiderlings emerged and built an irregular web where they remained until their second molt (Figs. 5, 6). Spiderlings began to leave the communal web 15 days after emerging from the egg sac when all had molted, and they then began to feed.

Spiderlings in the second instars had a third claw on all their legs that in the following instars was lost and replaced by a dense claw tuft.

The mother and offspring behaviors just described were also observed in the field. The female stayed near the egg sac, while the spiderlings built the irregular web; when they dispersed a day after their second molt, she was still nearby. After the spiderlings had dispersed, the female also vanished.

When the communal web made by the spiderlings was disturbed by strong vibrations applied with a brush, most of them moved away a short distance, but returned to the web after 10 min. When some spiderlings were removed from the communal web and placed in another terrarium, they soon formed a group. This behavior ceased within a few days after the second molt.

Figure 3–6.—Maternal behavior and communal web of the spiderlings. 3, Female above the egg sac protecting it, white arrow indicates attachment threads; 4, female defending the egg sac; 5, Communal web of the spiderlings, white arrow indicates the group of spiderlings; 6, Detail of the communal web.

Number and duration of the molts.—Four females reached maturity after 14, 15, 16 and 17 molts respectively. There was no pattern of increase or decrease in the instar duration (Table 2). However the duration of the first instar was always the shortest and presented less variation than the others. The mean time from emergence of the spiderlings until maturity was 396.7 ± 72 days (range 300–465).

Table 2.—Duration (days) of *Phoneutria boliviensis* nymphal instars.

Instar	n	Mean ± SD	Range
II	43	11 ± 3.0	7–16
III	35	20 ± 2.7	16–26
IV	26	21.1 ± 9.5	9–47
V	19	26 ± 8.3	14–39
VI	16	30 ± 11.7	17–53
VII	6	31 ± 13.8	16–52
VIII	6	25 ± 2.4	22–28
IV	6	23 ± 3.7	20–29
X	5	24.2 ± 2.6	21–28
XI	4	23.7 ± 4.2	18–28
XII	4	27.5 ± 3.3	23–30
XIII	4	41.2 ± 13.2	29–60
XIV	4	28.2 ± 5.6	23–34
XV	3	39.3 ± 10.8	27–47
XVI	2	39 ± 15.5	28–50
XVII	1	29 ± 0	29

Prey-wrapping behavior.—When a spider captured and bit a cockroach, it waited a few minutes until the insect finished moving. If the spider was on the floor, she climbed the terrarium wall (no more than 15 cm) and turned to face down (Fig. 7A) after the cockroach stopped moving (the antennae sometimes still moved). The spider inclined her abdomen toward the wall to attach silk, and then turned in a semicircular path around the prey keeping the cockroach in her chelicerae (Fig. 7B), while she made a third attachment to the surface. The silk from the first attachment still remained on the spinnerets so that a sheet of silk covered the prey (Fig. 7C). Holding the cockroach in her chelicerae, the spider continued this stereotyped circular motion, adding more silk to the prey. As the cockroach became more tightly attached to the substrate, the spider sometimes released its hold with the chelicerae while continuing to wrap it. The attachments disks were never on the prey, but on the surface around on it. Throughout this process, the palps repeatedly contacted the prey. The average number of attachments per turn was 2.4 ± 0.7 SD (range 1–3) and the average total number of attachments was 9.6 ± 2.1 SD (range 7–13). At the conclusion of prey wrapping, the spider lifted the cockroach with its chelicerae and moved slightly forward, causing the threads to the substrate to tighten. The mean duration of the prey wrapping was 81 s ± 13 SD (range 65–100).

While the spiders fed, they sometimes repeated prey wrapping several times, but with shorter durations and fewer attachments than the initial wrap. In seven of the eight observations, the wrapping lines formed from the first two
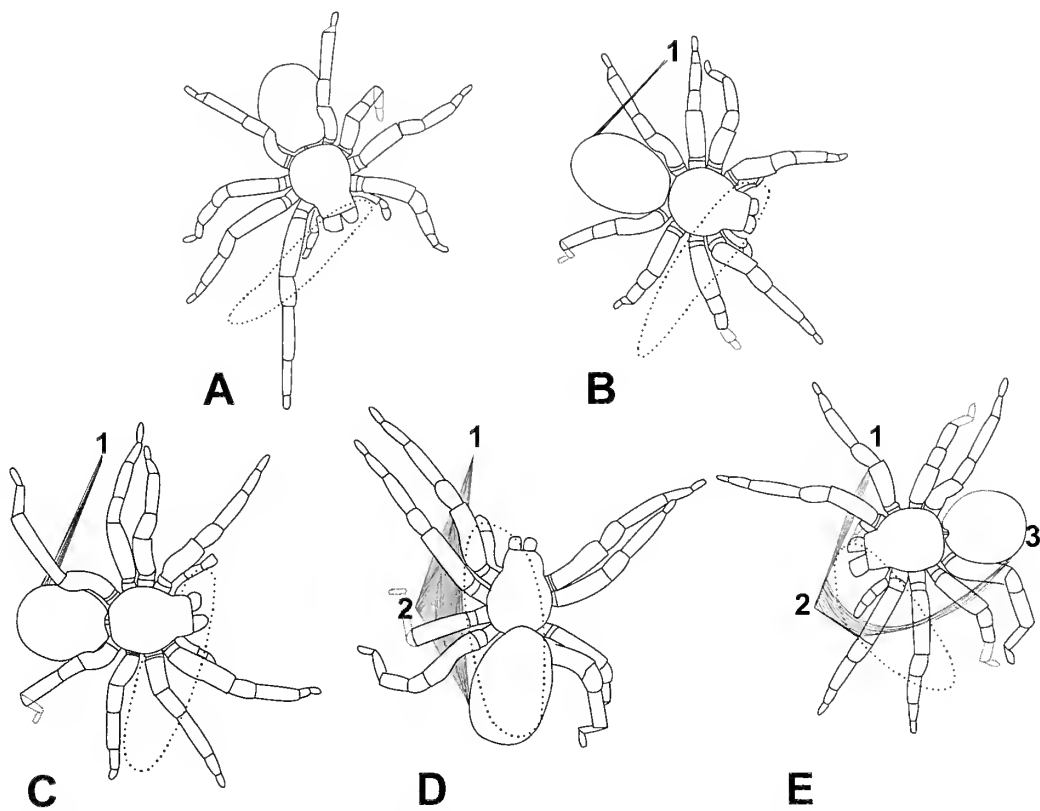
Figure 7.—Prey wrapping behavior sequence of *Phoneutria boliviensis* (the numbers indicate the order of the attachments). After the third attachment, the cockroach is fixed to the substrate.

attachment disks that the spider made did not contact the cockroach. The general pattern of attaching wrapping lines on prey was in one direction (Fig. 9). When the spider began to make this circular motion, it was always performed in a clockwise direction without changing course. I observed that the silk was slack and consisted of numerous threads. In no case did any leg hold any line which was being produced or to which the spider was attaching.

Because the spiders wrapped the prey while on the vertical glass wall of the terrarium, it was possible to observe the movement of the spinnerets as they produced silk. Silk emerged from all three pairs of spinnerets. Only the anterior

Figure 8.—Patterns of attachments of wrapping lines by two individuals of *Phoneutria boliviensis* (the numbers indicate the order of the attachments and arrows the direction taken by the spider). The silk is slack and due to the circular movement of her body, the threads do not pass straight over the prey as schematized, but instead are curved around it.

spinnerets moved when an attachment was made in alternated fashion. The immobile posterior lateral spinnerets (PLS) were usually in an asymmetric position, depending on the direction taken by the spider in the circular motion pattern; for instance, if the spider was moving to the left, the right PLS was always raised and the left PLS was lowered touching the substrate. These asymmetric positions of the spinnerets created the silk sheet shown in Fig. 7D.

DISCUSSION

Phoneutria boliviensis has been associated with wet and very wet forest ecosystems with annual precipitation > 2500 mm. Valerio (1989) indicates that in Costa Rica this species is restricted to wet and very wet forests in tropical life zones system (Holdridge system) and altitudes not exceeding 600 m; Florez et al. (2003) recorded this species in the Urabá region, Colombia, an area known for its high precipitation; Martins & Bertani (2007) consider it to be a typical species of the Amazon region. In this study, I also found *P. boliviensis* in rainforests, and also in remnants of dry forests with annual precipitation of 1300–1400 mm and at elevations of up to 1400 m. Thus this species is not restricted to lowland rainforests.

In the mating process, male *P. boliviensis* made palpal movements when contacting female silk. These movements apparently are similar to those described in some species of lycosids (Tietjen & Rovner 1980), and allow the male to locate the female by following her silk (Tietjen 1977; Tietjen & Rovner 1980). Male *P. boliviensis* did not court from a distance prior to mating. Folly-Ramos et al. (2002) found that *P. nigriventer* also lacks courtship. It appears that the female recognizes the male when he contacts her because if the female is receptive, she adopts a passive posture when touched. In contrast, *Cupiennius* spp. Simon 1891 and *Ancylometes bogotensis* (Keyserling 1877) have elaborate courtship before mating, involving rhythmic movements of the first pairs of legs and palpal drumming which sends vibrations through the substrate (Merrett 1988; Barth 2002). In addition, *A. bogotensis* and *C. coccineus* F.O. Pickard-Cambridge 1901 are unique among ctenids in wrapping the legs of the female with silk during mating (Merrett 1988; Schmitt 1992). Other ctenid species like *Ctenus medius* Keyserling 1891 and *Isoctenus* sp. Bertkau 1880 have less elaborate courtship involving only vibrational motions of the first pair of legs (Folly-Ramos et al. 2002; Pellegati-Franco 2004). The erection of the leg spines at the beginning of each palpal insertion by the male is due to increased body pressure during insertion and expansion of the hematodocha (Foelix 2010).

The mating position of *P. boliviensis* was type III (Foelix 2010), typical of the "modern wandering spiders" such as Anyphaenidae, Clubionidae, Lycosidae, Pisauridae, Salticidae, Tengellidae, Trechaleidae and Thomisidae (Sierwald & Coddington 1988; Costa 1993; Huber 1995; Barrantes 2008; Foelix 2010). The ctenids species mentioned above, except *C. medius*, use this same mating position. Thus this behavior could be a tentative synapomorphy as families sharing this trait belong to the monophyletic RTA (retrolateral tibial apophysis) clade.

According to my observations made of this species both in captivity and in the field, *P. boliviensis* demonstrated effective maternal care, consisting mainly of her remaining with the egg sac and defending it until the spiderlings emerged and dispersed within a few days after molting. This behavior by mothers could prevent predation on spiderlings because females were more aggressive during this period. Toyama (1999) reported a similar maternal behavior in *Cheiracanthium japonicum*, which greatly improved survival and development of eggs as well as spiderlings in the field.

The shape of the egg sac, maternal behavior, the construction of a communal web by the spiderlings, and dispersal following the second molt are all traits shared with some other ctenids such as *Phoneutria keyserlingi* (Simó 1989); *Parabatinga brevipes* (Keyserling 1891); *Asthenoctenus borellii* Simon 1897 (Simó et al. 2000); *Ctenus medius* (Folly-Ramos et al. 2002); *Ctenus fasciatus* Mello-Leitão 1943 and *Enoploctenus cyclothorax* (Bertkau 1880), however *C. fasciatus* usually put grains of dirt on the egg sac, apparently for camouflage (Pellegatti-Franco 2004). Other ctenids, such as *Cupiennius* spp., differ by carrying the egg sac on spinnerets (Barth 2002) or with the chelicerae, as in *Ancylometes bogotensis*, *Ctenus amphora* Mello-Leitão 1930 and *C. crulsi* Mello-Leitão 1930 (Merrett 1988; Höfer et al. 1994).

It is well known that the middle claw is important for web spiders because they use it to catch hold of the silk threads of their webs (Foelix 2010). In *Phoneutria boliviensis* and maybe other ctenids mentioned above, the presence of this claw in early instars is necessary because the spiderlings build a communal web after emergence. Homann (1971) also mentioned the presence of a middle claw in early instars of *Ctenus medius*, *Cupiennius salei* (Keyserling 1877), and *Phoneutria keyserlingi*. Others ctenids species of *Ancylometes* and *Cupiennius*, also have a third claw. The *Cupiennius* adults have a much reduced middle claw (Barth 2002; Höfer & Brescovit 2000). According to Silva (2004), the occurrence of a middle claw could be an ancestral condition for the ctenoid spiders.

There are two general contexts in which the spiders wrap their prey: to restrain active prey and prevent their escape ("immobilization wrapping") and to form more compact and manageable packages ("post-immobilization wrapping") (Eberhard 1967; Robinson et al. 1969; Rovner & Knost 1974; Barrantes & Eberhard 2007). *Phoneutria boliviensis* performed only "post-immobilization wrapping." The cockroach became more compact during the wrapping process, and became more securely fastened to the vertical substrate. This allowed the spider to occasionally release the prey with her chelicerae and chew on another part without falling. The circular pattern of wrapping and the movement of the anterior spinnerets of *P. boliviensis* was similar to observations of *Rabidosa* (=*Lycosa*) *rabida* (Walckenaer 1837) and *R. punctulata* (Hentz 1844) (Rovner & Knost 1974), which also perform this behavior while above the ground (in vegetation).

While wrapping, *P. boliviensis* does not manipulate threads with any legs, but rather attaches threads to the substrate through body movements. Pulling wrapping silk using movements of the body is ancestral in araneomorph spiders and its homology is supported by the similarity in their asymmetrical use of PLS described in several families (Barrantes & Eberhard 2007). Such asymmetry alters the distribution of lines on the prey package from that expected if the spinnerets were used symmetrically. In the case of the prey-

wrapping behavior of *P. boliviensis*, the asymmetric position of the PLS create a silk sheet that encases the prey more efficient than if the PLS were in a symmetric position, creating only a swath of lines.

In *Rabidosa rabida*, Rovner & Knost (1974) sealed each pair of spinnerets separately with paraffin to identify the functions of the types of silk they produced during prey wrapping. The anterior spinnerets produced attachment discs with lines from the pyriform glands. In addition, the anterior spinnerets produced drag lines from the ampullate glands. The median and posterior spinnerets produced wrapping silk from the aciniform glands. The movements of the anterior spinnerets of *P. boliviensis* while attaching lines to the wall presumably resulted in the zigzag pyriform lines typically seen in attachment discs.

ACKNOWLEDGMENTS

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SHORT COMMUNICATION

The mechanism behind plasticity of web-building behavior in an orb spider facing spatial constraints

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Abstract. Orb spiders demonstrate an impressive ability to adapt their web-building behavior to a wide range of environmental and physiological factors. However, the mechanisms behind this plasticity remain poorly understood. Behavioral plasticity can be categorized as either developmental, where new neural pathways arise from learning, or activational, which rely on more costly pre-existing neural pathways. Here I argue that orb spiders and their webs in general and their response to spatial constraints in particular make an ideal model system in which to explore these two mechanisms further. I show that the spider *Eustala illicita* (O. Pickard-Cambridge 1889) immediately modifies its first orb web after being placed in spatially confined experimental frames without showing subsequent improvements in design of the second web. Thus, these data are in accord with the hypothesis that this spider relies on activational behavioral plasticity, which might be linked to its preferred habitat in the wild.

Keywords: Behavioral flexibility, learning, experience, web geometry, *Eustala illicita*

The ability of an animal to rapidly adapt its behavior to changes in its environment, so-called behavioral plasticity or behavioral flexibility, has been described from a wide range of vertebrate and invertebrate taxa. Phenotypic plasticity in general, and behavioral plasticity in particular, has previously been recognised as arising either from an innate pre-programmed pathway or from internal physiological or external environmental changes including developmental changes and learning (West-Eberhard 2003; Mery & Burns 2010). Most studies focus on the interaction between environmental change and the evolution of learning. Initially it was assumed that learning was always favored in variable environments, but more detailed experimental and theoretical studies show that learning is only favored when the environment changes relatively little within an individual lifetime and shows predictable changes between generations (so-called coarse-grained environmental variation). Innate behavior is favored when the environment changes randomly and unpredictably within generations (so-called fine-grained environmental variation) (Stephens 1991; Dunlap & Stephens 2009).

The above and similar studies have significantly increased our understanding of the evolution of learning, but the relationship between behavioral plasticity and learning remains poorly defined. However, this relationship has recently been the subject of a review by Snell-Rood (2013), in which she defined two different kinds of behavioral plasticity based on separate costs and benefits. Developmental behavioral plasticity is the slower process that requires a physical re-organisation of the underlying neural pathways caused by, for example, learning, which is hypothesised to be favoured in environments that show coarse-grained variation. Activational behavioral plasticity, which is an immediate reponse that relies on pre-configured neural pathways, is favoured in environments that show fine-grained variation. Both require significant initial investment in costly neural tissue, but developmental behavioral plasticity allows animals to prune and optimize the neural network over time, while activational behavioral plasticity relies on a constant amount of neural tissue (Snell-Rood 2013). However, the two mechanisms do not necessarily operate completely separately. What may look like activational behavioral plasticity in the adult animal may have arisen through interactions between the genes and the environment including learning processes in the juvenile animal. Thus activational

behavioral plasticity that does not involve any learning in the present may be the result of neural pathways that were fixed through developmental behavioral plasticity in the past. More experimental data is required to investigate the prevalence and interaction of these two types of behavioral plasticity.

Here I propose that orb spiders and their webs constitute an ideal model system in which to study behavioral plasticity. Orb spiders show an impressive ability to modify their webs to a range of environmental and physiological factors including temperature (Vollrath et al. 1997), wind (Vollrath et al. 1997; Liao et al. 2009), prey size and type (Nakata 2007; Blamires et al. 2011), silk availability (Eberhard 1988; Vollrath et al. 1997), leg loss (Pasquet et al. 2011) and spatial constraints (Ades 1986; Vollrath et al. 1997; Harmer & Herberstein 2009). However, the majority of these studies tested either only the first web or allowed the spiders a week or more to acclimatize to experimental conditions before testing them, and so do not allow us to unravel whether spiders immediately adapt their webs to the new condition (i.e., activational behavioral plasticity) or improve their webs gradually as they gain more experience with the condition (i.e., developmental behavioral plasticity). Given that inexperienced spiders build perfectly normal webs (Reed et al. 1970) and that spiders do not improve webs with age or size (Eberhard 2007; Hesselberg 2010), a reliance on developmental behavioral plasticity is perhaps less likely. However, orb spiders readily learn to avoid dangerous and distateful prey (Hénaut et al. 2014); gradually alter their sticky spiral mesh size, web size and web asymmetry based on recent prey capture experiences (Heiling & Herberstein 1999; Venner et al. 2000); improve the size, planarity and verticality in subsequent webs built at the same site (Zschokke & Vollrath 2000; Nakata & Ushimaru 2004); and also seem to gradually improve their locomotory and web-building skills under weightless conditions in space (Witt et al. 1977).

Here I propose that spiders' adaptation to building webs in spatially constrained spaces is particularly useful for studying behavioral plasticity as it is ecologically relevant and has been studied in a number of different species (Ades 1986; Vollrath et al. 1997; Krink & Vollrath 2000; Harmer & Herberstein 2009; Barrantes & Eberhard 2012; Hesselberg 2013). I re-analysed previously collected data on behavioral flexibility in *Eustala illicita* (O. Pickard-Cambridge 1889), which successfully built webs in size-limited experimental frames (Hesselberg 2013). Late instar female spiders were collected in a dry tropical rain forest in Panama City, Panama (9°N, 80°W) and

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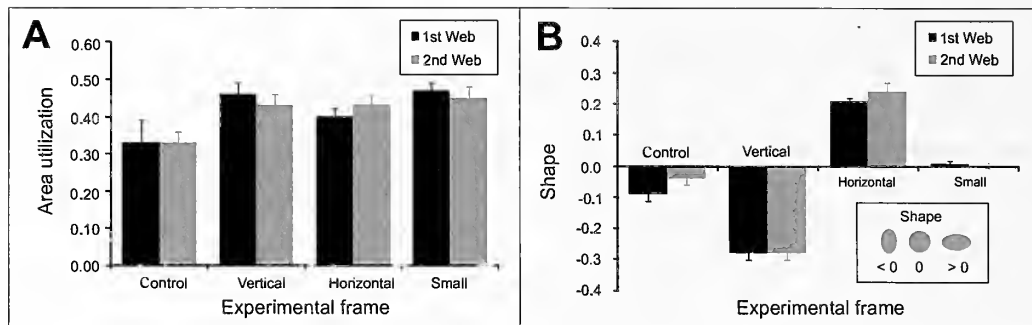
Figure 1.—Differences in area utilization (A) and shape (B) between first (dark grey bars) and second (light grey bars) webs of *Eustala illicita* built in experimental frames (Control (N = 5): 30 × 30 cm; Vertical (N = 8): 15 × 30 cm; Horizontal (N=6): 30 × 15 cm; Small (N = 10): 15 × 15 cm). The error bars indicate the standard error of the mean. The inset on figure B gives an interpretation of the numerical shape values with a value of 0 indicating a perfect circle. Shape was calculated using the following equation ($d_h − d_v$)/($d_h + d_v$), where d_h and d_v is the horizontal and vertical diameter of the web.

were given a week to acclimatize to building webs in standard frames (30 × 30 × 5 cm) in the laboratory during which they were watered and fed fruit flies regularly after which their webs were cut into a single strand with a soldering iron (see Hesselberg 2013 for a more detailed description of methods). Spiders that built normal-looking webs nearly daily were included in the experiment, which consisted of transferring spiders to experimental frames (control frames: 30 × 30 × 5 cm, vertical frames: 15 × 30 × 5 cm, horizontal frames: 30 × 15 × 5 cm and small frames: 15 × 15 × 5 cm), where they were kept for three days with any webs being photographed and subsequently destroyed with a soldering iron as described above. Spiders were given water but not fed throughout the three-day experimental period. In the present study I used the spiders that built multiple webs in the three day period to compare the first web built on day 1 with the second web built on day 2 or day 3. As only about half the spiders built three webs, I decided to compare only web 1 and 2. A range of web parameters were measured from digital photographs using ImageJ (v1.41, National Institute of Health, USA) and were analysed with IBM SPSS v. 20 (IBM Corporation 2011) using a significance level of 5%. The tests performed were either a repeated measures ANOVA with web number as the within-subject factor and experimental frame as between-subject factor or a paired t-test.

The main parameters of interest were the area utilization (i.e. the proportion of the available area in the frame taken up by the capture spiral) and the shape of the web (Vollrath et al. 1997; Krink & Vollrath 2000; Hesselberg 2013). As shown in Fig. 1A, this study found only minor and non-significant differences in area utilization between first and second webs across all four experimental treatments (repeated measures ANOVA: $F_{(1,25)}$ = 0.12, P = 0.915) but, as expected, spatially constrained spiders utilized a significantly higher proportion of the available area than the control spiders (repeated measures ANOVA: $F_{(3,25)}$ = 5.56, P = 0.005). Similarly, there were no differences in shape between the first and the second web across the different experimental frames (repeated measures ANOVA: $F_{(1,25)}$ = 2.17, P = 0.153) but, as expected, there were significant differences in shape between webs in the different treatments (repeated measures ANOVA: $F_{(3,25)}$ = 173.39, P < 0.001), with control and small frames resulting in almost round webs while the vertical frames had vertically elongated webs and the horizontal frames had horizontally elongated webs (Fig. 1B). The repeated measures ANOVA found no significant interactions between web number and frame shape for either area utilization or shape (test results not shown). The similarity of first and second webs across all the experimental frames was further supported by the lack of differences between first and second webs in a range of web parameters for all four treatments (Table 1), except that mesh height in the horizontal frame was slightly larger in the second web.

In conclusion, the data presented here suggest that *E. illicita* immediately adjusts its first orb web to match the experimentally

constrained space with no improvements in shape or area utilization in the second webs built under the same conditions. Although the present lack of statistical differences could be attributed to the relatively small sample size, none of the measured parameters show any consistent trends towards better adapted, larger or denser second webs. *Eustala illicita* therefore appears to rely on activational behavioral plasticity to adapt its web to spatial constraints, which the spider probably frequently encounters in its natural habitat. It is almost exclusively found in relative high densities within the branches of the ant acacia *Acacia collinsii*, which might give rise to competition for available space (Hesselberg & Triana 2010; Styrsky 2014). As the individual spiders grow larger, they are therefore likely to be subject to fine-grained environmental variation as they move around on the acacia in search of suitable web-building sites. Since the spiders used in this study were caught in the wild, however, it is possible that the present behavior is the result of earlier developmental behavioral plasticity that has resulted in fixed neural pathways for dealing with spatial constraints. In this regard the present behavior can be viewed as an example of context-dependent behavior in that spiders utilize earlier learning to adapt their web-building behavior when facing similar constraints. Such context-dependent learning has previously been found in spiders (Skow & Jakob 2006), although the two very different contexts in this study in terms of learning in the complex natural environment and using this learning in the much simpler artificial environment in the laboratory renders this less likely. Finally, there is also the possibility that no learning or plasticity takes place and that the ability to adapt their webs to spatial constraints is a passive emergent property of the spider's web-building behavioral rules. This, however, is unlikely for the following reasons: the spiders in this experiment and in others (Vollrath et al. 1997) readily adapt their webs to many different types of spatial constraints; orb spiders in general match the size and shape of their webs to their available silk resources (Eberhard 1988) and therefore probably gather information during their exploratory behavior relevant to the size and shape of their future webs (Vollrath 1992); and other species of orb spiders, likely using similar behavioral rules, are unable to adapt their webs to limited space (Hesselberg 2013). Given the discussion above and because the present study only investigates learning over a short period of time for only one situation, that of web-building behavior in spatial constraints, this study provides a relative weak test for the role of learning in behavioral plasticity of web-building behavior generally. However, the activational behavioral plasticity hypothesis is further supported by the strong either-or response in web-building frequency between spiders that match their webs to available space (Vollrath et al. 1997) and those that do not (Hesselberg 2013) as well as the immediate response in web parameters observed in *Cyclosa octotuberculata* (Karsch 1879) to feeding and prey detection experiences (Nakata 2007, 2012).To determine whether orb spiders

Table 1.—A comparison between first and second webs of *Eustala illicita* facing spatial constraints. Measures are given as mean ± standard error.

	First web	Second web	Paired *t*-test	*P-value*
CONTROL FRAME				
Number of webs	5	5		
Number of radii	28.8 ± 3.3	31.0 ± 2.2	−1.77	0.151
Number of spirals	32.5 ± 4.7	33.1 ± 4.4	−0.58	0.591
Mesh height (cm)	0.25 ± 0.03	0.24 ± 0.04	0.37	0.733
Vertical assymetry[1]	−0.51 ± 0.04	−0.54 ± 0.02	0.82	0.458
VERTICAL FRAME				
Number of webs	8	8		
Number of radii	31.4 ± 1.6	33.1 ± 1.5	−1.07	0.320
Number of spirals	31.9 ± 1.7	29.2 ± 1.8	1.52	0.173
Mesh height (cm)	0.20 ± 0.01	0.20 ± 0.01	−0.73	0.487
Vertical assymetry[1]	−0.44 ± 0.03	−0.40 ± 0.06	−0.70	0.506
HORIZONTAL FRAME				
Number of webs	6	6		
Number of radii	33.5 ± 1.4	35.7 ± 1.7	−1.23	0.273
Number of spirals	33.9 ± 2.4	31.7 ± 1.4	1.49	0.193
Mesh height (cm)	0.18 ± 0.01	0.19 ± 0.01	−3.10	0.027*
Vertical assymetry[1]	−0.45 ± 0.02	−0.47 ± 0.04	Z = −0.67	0.500
SMALL FRAME				
Number of webs	10	10		
Number of radii	29.2 ± 1.4	29.4 ± 1.3	−0.12	0.907
Number of spirals	25.4 ± 1.6	24.7 ± 1.3	0.49	0.639
Mesh height (cm)	0.17 ± 0.01	0.18 ± 0.01	−1.44	0.184
Vertical assymetry[1]	−0.42 ± 0.05	−0.37 ± 0.07	−0.85	0.419

[1] Vertical asymmetry was calculated using the following equation: $(r_u - r_l)/(r_u + r_l)$, where r_u and r_l are the upper (above hub) and lower (below hub) web radii. The Wilcoxon Signed Rank test (Z) was used where data could not be normalized.

generally rely exclusively on activational behavioral plasticity, or on a combination of the two behavioral plasticity mechanisms, to adapt their behavior to changes in the environment requires further comparative studies in a range of situations including naturally occurring ones such as leg loss and experimental ones such as changes in the magnitude or direction of gravity.

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SHORT COMMUNICATION

Development of novel microsatellite markers for the spider genus *Loxosceles* (Sicariidae) using next-generation sequencing

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Abstract. We report the step-by-step process of developing *de novo* microsatellite (SSR) loci in two *Loxosceles* spider species. We used reads obtained with next-generation sequencing (Roche 454) to select hundreds of potentially-amplifiable SSRs. After testing amplification and cross-amplification, we characterized 18 SSRs, 11 of which were polymorphic in *Loxosceles rufescens* (Dufour 1820) and seven of which were polymorphic in *L. sp.* Fuerteventura - Lanzarote. This method is a relatively fast and economic procedure for the development of fast-evolving nuclear markers in spiders.

Keywords: 454, nuclear markers, cross-amplification, Mediterranean, Canary Islands

Microsatellites (SSRs: simple sequence repeats) are popular codominant genetic markers used in many areas of research, including molecular ecology and population genetics. They consist of tandem repeats of very short nucleotide motifs (1–6 bases long). One property that makes SSRs attractive for evolutionary studies is their high mutation rate (Guichoux et al. 2011). However, the technical and economic effort required for developing *de novo* SSRs in organisms for which no or few genomic resources are available (the so-called non-model organisms) has, until recently, prohibited wide implementation. The recent emergence of next-generation sequencing technologies has reduced the economic and technical difficulties associated with developing SSRs (Santana et al. 2009), and has boosted their usage in a wide range of organisms, including spiders (Esquivel-Bobadilla et al. 2013; Parmakelis et al. 2013), a group in which the development and application of microsatellites has been limited (Brewer et al. 2014).

In this study, we focused on spiders of the genus *Loxosceles* Heineken & Lowe 1832 (Araneae: Sicariidae) from the Mediterranean Basin and the Canary Islands. *Loxosceles rufescens* (Dufour 1820) is considered cosmopolitan (World Spider Catalog 2014), but is native to the Mediterranean (Gertsch 1967; Duncan et al. 2010; Planas et al. 2014). In this region, several deep mitochondrial lineages have been detected (Duncan et al. 2010; Planas et al. 2014), some of which lack geographic structure as a consequence of the confounding effects of human-mediated transportation. Recently, Planas and Ribera (2014) discovered an endemic group of seven lineages of *Loxosceles* spiders in the Canary Islands. The two easternmost islands in this archipelago, Fuerteventura and Lanzarote, harbor one of these identified lineages. Despite the relatively impoverished fauna of Fuerteventura and Lanzarote, these two islands, together with the surrounding islets, have been shown to be ideal systems to study phylogeographical processes (i.e., Bidegaray-Batista et al. 2007; Macías-Hernández et al. 2013). Here, we acquired fast-evolving nuclear loci for the study of fine-scale evolutionary processes in the *Loxosceles* species endemic to Fuerteventura - Lanzarote (hereinafter *Loxosceles* sp. FV-LZ), and for contrasting the mitochondrial patterns observed within *L. rufescens* across the Mediterranean Basin (Planas et al. 2014).

We used next-generation sequencing to obtain SSRs and describe the step-by-step process from DNA extraction to characterization of selected markers. Genomic DNA was extracted from the legs of three specimens of *Loxosceles*, two of which belong to two different evolutionary lineages (A6 and B3; Planas et al. 2014) within *L.*

rufescens, and a third belonging to *Loxosceles* sp. FV-LZ, using the SpeedTools Tissue DNA Extraction Kit (Biotools) following manufacturer's protocols. We conducted pyrosequencing on a Roche Life Science 454 GS-FLX System at the University of Barcelona's Scientific-Technical Services. Roche 454 is a next-generation sequencing technology that obtains larger average fragment sizes, thus increasing the probability that the fragments containing SSRs have flanking regions to enable primer design. We pooled samples using individual multiplex identifiers (MIDs), together with an *Echinaster sepositus* sample (Garcia-Cisneros et al. 2013), within half a plate because physical separation decreases the overall number of sequences obtained. We acquired a total of 143,708 reads with a mean length of 341.86 bp for *Loxosceles* sp. FV-LZ, 45,377 (mean length 313.91 bp) for *L. rufescens* A6, and 195,081 (mean length 346.24 bp) for *L. rufescens* B3.

Raw data were processed with the Roche's 454 pipeline using default settings for quality control and with seqclean (https://sourceforge.net/projects/seqclean/) to remove low quality sequences and contaminants. Sequence reads from duplicated loci and mobile elements were identified in iQDD (Meglécz et al. 2010) using default parameters and were excluded from further analyses. We searched for reads with SSRs using iQDD, and retained those meeting a series of requirements suggested by Guichoux et al. (2011). Specifically, we looked exclusively for SSRs with perfect motif repetition, improving the probability that the SSRs follow a stepwise mutation model. We searched for SSRs with a minimum of 11 repeats in dinucleotides and eight repeats in tri-, tetra-, penta- and hexanucleotides, but no more than 16 repeats in both cases. Primers for selected SSRs were designed with the software PRIMER 3 (Rozen & Skaletsky 2000) included in iQDD. We avoided designing primers in flanking regions containing short repeats (e.g., nanosatellites), and we selected putative PCR products between 90 and 500 bp in length. Among all possible primer combinations for each SSR, we kept those with better evaluation based on the penalty score of the primer pairs after applying stringent parameters to ensure amplification (i.e., no primer-dimer interaction, similar annealing temperature, GC primer end content, and primer end stability). The number of reads containing SSR and the number of those with suitable flanking PCR-primer sites are shown in Table 1. Dinucleotides were the most frequent SSR, followed by tri-, tetra-, penta- and hexanucleotides (Table 1). Even after applying stringent parameters for SSR selection, we obtained over 800 SSRs that met the requirements specified above. We should note that relaxed selection criteria rigor (e.g., allowing for a minimum number

Table 1.—Number of reads containing SSRs and number of potentially amplifiable SSRs. Individual cells in the table record the number of reads obtained from each individual (*Loxosceles* sp. FV-LZ / *L. rufescens* A6 / *L. rufescens* B3).

	Dinucleotides	Trinucleotides	Tetranucleotides	Pentanucleotides	Hexanucleotides
Reads containing SSRs	3525/961/4261	334/141/673	38/34/181	3/0/6	0/0/1
Reads with potentially amplifiable SSRs	327/107/206	37/21/87	1/1/7	1/0/5	0/0/0

of eight tandem repeats) would have increased substantially the number of yielded SSRs. We selected 58 among the hundreds of candidate SSRs, considering the length of the expected PCR product. We then tested their amplification and cross-amplification success in eight individuals, four from *L. rufescens* and four from *L.* sp. FV-LZ. That is, we tested SSRs obtained from reads of *L. rufescens* for amplification in *L.* sp. FV-LZ individuals and *vice versa*. Of the 58 SSRs tested, 40 were rejected because PCR amplification was unsuccessful.

We retained the 18 SSR loci with higher amplification success and labeled the forward primers with fluorescent dye. We tested for polymorphism using 38 *L. rufescens* individuals from four different localities, and 16 *Loxosceles* sp. FV-LZ individuals from four

different localities. We conducted PCR reactions in a final volume of 10 μL using Biotools *Pfu* DNA Polymerase (Biotools). Annealing temperatures ranged between 42° and 58° C for all primer pairs. We pooled PCR products according to dye type and expected allele size ranges, and genotyped them in an ABI 3730XLs automated sequeneer at Macrogen (Seoul) with the internal size standard 500 LIZ. We used the Microsatellite Plugin 1.3 in Geneious 6.1.6 (Biomatters) for allele calling. For each locus, the primer sequences, number of alleles (N_a), and observed (H_O) and expected (H_E) heterozygosity are listed in Table 2.

All but one SSR were polymorphic for at least one of the two species analyzed. One SSR (ME083) obtained from *L. rufescens* reads

Table 2.—Characteristics of 18 microsatellite loci, tested with 38 samples of *Loxosceles rufescens* from four different localities, and 16 samples of *Loxosceles* sp. FV-LZ from four different localities. Locus name, accession number, repeat motif and primer sequences (F: forward, R: reverse) are listed for each locus. In the last four columns of the table, *L. rufescens* data are presented in the first row for total number of alleles, allele size (bp), expected heterozygosity (He) and observed heterozygosity (Ho), and *Loxosceles* sp. FV-LZ in the second row.

Locus	Accession number	Repeat motif	Primer sequence (5'–3')	Total number of alleles	Allele size (bp)	Ho	He
ME012	KM879453	(AGAT)	F: GTGGGTGGTCCATTGATAGG	8	137–165	0.57	0.77
			R: TTTAACAAGACGCAGCGAAA	-	-	-	-
ME031	KM879448	(AAAT)	F: AAACTTCGATTTATTTTGTTTCTTG	4	89–109	0.19	0.66
			R: AAATGTCTGGCGGATCAGAA	-	-	-	-
ME034	KM879450	(AAAT)	F: CGTCTGCAGTGTGAACGG	6	93–149	0.47	0.71
			R: ATATGTGCTTTTGCGCCTGT	-	-	-	-
ME064	KM879451	(AAAT)	F: TCTGTAAATGGATTCTCATCTGTTG	2	151–155	0.13	0.12
			R: TCGTCCAACCATCCTCTTTC	-	-	-	-
ME067	KM879446	(AGAT)	F: TGTGATGTACCTGCGTTCGT	4	142–160	0.11	0.10
			R: GCAAGATCAACCCACAACCT	-	-	-	-
ME077	KM879454	(AAACT)	F: TATGTAATCACCGGGGTTGG	3	152–177	0.21	0.55
			R: CGTGCAATCTGGTTAACTTCG	-	-	-	-
ME083	KM879445	(ACACT)	F: TAGGGAATGGAATGGCAGAC	1	160–160	0	0
			R: TTTGCAGATTTGATCTGGGAC	1	163–163	0	0
ME088	KM879449	(AAAT)	F: AGCGTTGATACAGGTGGTCC	3	208–254	0.10	0.59
			R: TCACTGCACAGTGTAAAGCCA	-	-	-	-
ME103	KM879452	(AAT)	F: TTAGCGACCTTCCCTGTCAC	6	262–280	0.34	0.73
			R: TGGTAAACGGGAGGACTAGG	-	-	-	-
ME113	KM879447	(AAT)	F: AACCTGAAGGGCTGATGAAT	6	75–96	0.37	0.78
			R: CAGGAGCAGGATGCCATATT	-	-	-	-
CA001	KM879461	(AAT)	F: ATGTATCACGCGCCTTTTG	-	-	-	-
			R: GTTGTCTGGAGCAAACAGCA	5	75–93	0.60	0.72
CA003	KM879460	(AAT)	F: TGTACCAGGGGCTGGTCTAA	-	-	-	-
			R: CATACGTGGTGGCAGCATAC	5	66–92	0.28	0.73
CA027	KM879457	(AAGTG)	F: TACCACAAGGGGAGAATCCA	3	103–113	0.39	0.32
			R: AAGCCAGAGGTGCAATTGTT	10	132–182	0.40	0.79
CA030	KM879462	(AAT)	F: AGGTGTGGCACTACCGTTTT	-	-	-	-
			R: CAAATGAGCATTCAACCTCG	7	133–157	0.46	0.70
CA038	KM879458	(AAT)	F: ATGTTTGAGGGGTCTCGTTG	-	-	-	-
			R: ACATGATGCCCCACGATAAT	4	272–284	0.93	0.69
CA105	KM879455	(AC)	F: TAAATAACCTGATATCGGATCTATGAC	-	-	-	-
			R: AAAGTATATCGGACAAACATCCAACC	5	255–267	0.75	0.75
CA238	KM879459	(AG)	F: GGCACCCCAGACTAACAAGA	1	233–233	0.00	0.00
			R: ACCTCTGGCACGAATACACC	4	221–231	0.93	0.69
CA243	KM879456	(AT)	F: AATAACGGAGACCGTGCAAC	5	225–279	0.68	0.64
			R: CCTCCAGTATCCGAAGACGA	-	-	-	-

amplified successfully in *Loxosceles* sp. FV-LZ individuals, although it was monomorphic in both species. Three SSRs obtained from *L.* sp. FV-LZ reads amplified successfully in *L. rufescens*, and was monomorphic in one locus (CA238) and polymorphic in the other two loci (CA027 and CA243). In total, 11 polymorphic SSRs were developed for *L. rufescens* and seven for *L.* sp. FV-LZ.

Results from this study suggest that next-generation sequencing is an efficient and cost-effective procedure for the fast development of microsatellite loci in spiders. Despite the close phylogenetic relationship of the two species used in this study (Planas & Ribera 2014), the cross-amplification rate for the microsatellites was low. The few SSRs that cross-amplified successfully were found to be monomorphic or less polymorphic in the species from which they were not initially obtained (except for CA243). Thus, we advise developing specific microsatellites for each target species. We obtained thousands of reads by sequencing three *Loxosceles* specimens in half a Roche 454 plate, and we used a fast bioinformatic pipeline applying stringent selection criteria to identify hundreds of potentially amplifiable SSRs. Although 454 sequencing was preferred for the longer read lengths obtained which facilitates the design of PCR primers, a similar approach for SSR development has been successfully implemented using alternative, more cost-effective sequencing technologies (i.e., Illumina) (Castoe et al. 2012, but see Drechsler 2013).

ACKNOWLEDGMENTS

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SHORT COMMUNICATION

Pre-ballooning in *Ummidia* Thorell 1875 (Araneae: Ctenizidae) from the Interior Highlands, USA: second account from the region and review of mygalomorph ballooning

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Abstract. The present study represents the second record of pre-ballooning behavior in Arkansas *Ummidia* Thorell 1875 (Ctenizidae). Mygalomorph ballooning is discussed and our observations are compared with previous authors' observations. Photographs and video of the behavior are included. Images and discussion are provided detailing genus-level identification of the spiderlings.

Keywords: Trapdoor spider, aerial dispersal, videography

Although most non-araneomorph spiders do not disperse aerially like many araneomorphs, ballooning has been recorded from mygalomorphs in three families. Bell (2005) reviewed ballooning accounts across arthropods and listed five mygalomorph ballooners: *Missulena insignis* (Cambridge 1877) (Actinopodidae); *Atypus affinis* Eichwald 1830 and *Sphodros atlanticus* Gertsch & Platnick 1980 (Atypidae); and *Conothele malayana* (Doleschall 1859) and *Ummidia* Thorell 1875 (Ctenizidae). This list combined accounts of confirmed ballooning (*M. insignis* [Main 1976, 1981; Brunet 1994]; *S. atlanticus* [Coyle 1983, Coyle et al. 1985]; and an unidentified *Ummidia* [Coyle 1985]), as well as pre-ballooning accounts without observed ballooning (*A. affinis* [Enock 1885; Bristowe 1941]; *S. rufipes* (Latreille 1829) [Muma & Muma 1945]; *C. malayana* [Main 1957, 1976]; and *U. carabivora* (Atkinson 1886) [Baerg 1928]). Additionally, Eberhard (2005) described ballooning in Costa Rican *Ummidia*. Main (1981) noted the oddity that *Missulena* Walckenaer 1805 and *Actinopus* Perty 1833 have similar distributions across Australia, even though *Actinopus* was not known to balloon. Solving this mystery and adding to the list of ballooning mygalomorphs, Ferretti et al. (2013) confirmed ballooning in an unidentified *Actinopus*. Most of these species have in common large ranges that cross water barriers, including accounts on islands (e.g., *Ummidia* from St. Vincent [Simon 1891]; *Conothele* from Pacific Islands and Seychelles [Pocock 1898; Berland 1938; Roewer 1963, Saaristo 2002]).

The present study provides information for *Ummidia*, which are the most common ctenizids in the eastern U.S. They are immediately identifiable by a dorsal saddle-shaped indention on the third tibiae, which has been suggested to aid in securing them in the burrow (Coyle 1981). There are 25 described species of *Ummidia*, with 18 in the New World (10 USA, five Central America, three South America) and seven in the western Mediterranean (Platnick 2014). However, perhaps as many as 100 (Platnick, via Bond & Coyle 1995) are left undescribed (Bond & Hendrixson 2005). The trans-Atlantic distribution has traditionally been considered the result of human introduction, but this hypothesis was recently rejected with molecular evidence (Opatova et al. 2013). Instead, *Ummidia* were likely widespread in Laurasia, rendering Old World species much older than previously suspected. Interestingly, unlike other organisms, New and Old World lineages diverged later than the breakup of Laurasia, which the authors attributed to gene flow on either side of the newly opening Atlantic ocean due to the ability of *Ummidia* to disperse by ballooning. A related genus, *Conothele*, has a non-overlapping Australasian distribution and the characters differentiating *Conothele* from *Ummidia* are variable (Main 1985), leaving geography and burrow construction as the best distinguishers (Haupt 2005; Decae

2010). Further, the molecular analyses of Opatova et al. (2013) showed only a small amount of divergence between *Conothele* and *Ummidia*. In short, there is great need for a revision of New World *Ummidia*, as well as phylogenetic investigation of the generic complex (*Conothele* + *Ummidia*), at which point, *Conothele* will likely be lumped with *Ummidia*. Ballooning by *Conothele* spiderlings is currently unknown.

Field photographs and video were taken with an iPhone 4S, which was the only camera available at the time, highlighting an example of such technology used for natural history. Video taken of this behavior can be found at https://www.youtube.com/watch?v=gleB4sIrDQw. The video was compiled with Adobe Premiere Pro CS6. Morphological images were montaged from many stereomicrographs (20–30 for habitus and 8–14 for appendages) using Helicon Focus 6.

Following the accounts of Baerg (1928), this study presents the second record of pre-ballooning behavior of Arkansas *Ummidia*. On the afternoon (15:00–16:00) of 22 March 2014, on a trail that paralleled a paved road (100–200 m away) at Devil's Den State Park, Arkansas (35° 46′ 51.54″N 94° 15′ 22.74″W; elev. 395 m), six *Ummidia* spiderlings were observed climbing an oak (*Quercus*, Diameter at Breast Height (DBH) approx. 24 cm) on a vertical 2–6 mm wide silk band. The silk band rose approximately 6 m along the trunk and then continued along a horizontal limb where it bifurcated several times and was eventually lost after about 2 m (Fig. 1A, C). The observation spanned approximately 30 min, although all six spiderlings were discovered in the first 5 min. The oak was atop a steep slope that overlooked a valley and was therefore exposed to wind gusts (Fig. 1B). Other conditions were as follows: 13°–15°C; wind 1.8–4.5 m/s; recently turned cloudy, prior to light rain. At the base of the tree the silk band apered to a few strands, where it was soon lost; not even single-strands could be found (Fig. 1E). Although we have previously found *Ummidia* burrows on steep slopes in the area, careful examination of the area surrounding the ballooning tree failed to uncover either the maternal burrow, or ground silk trails. Ballooning spiderlings were not directly observed, but this silk band-making behavior as an antecedent to ballooning in *Ummidia* is well known (Coyle 1985, Eberhard 2005).

Most of these observations conform to what has been previously described for *Ummidia* by Baerg (1928) and Coyle (1985). Compared with most observations of mygalomorph ballooning, the pre-ballooning bands observed by Baerg (1928) in Arkansas were much longer both horizontally on the ground leading from the maternal burrow to the ballooning tree (3–21 m; 8.5 m avg.) and vertically along the ballooning tree (4–9 m). For example, the band observed by Coyle (1985) was only 0.9 m vertically on a tombstone and 1.5 m
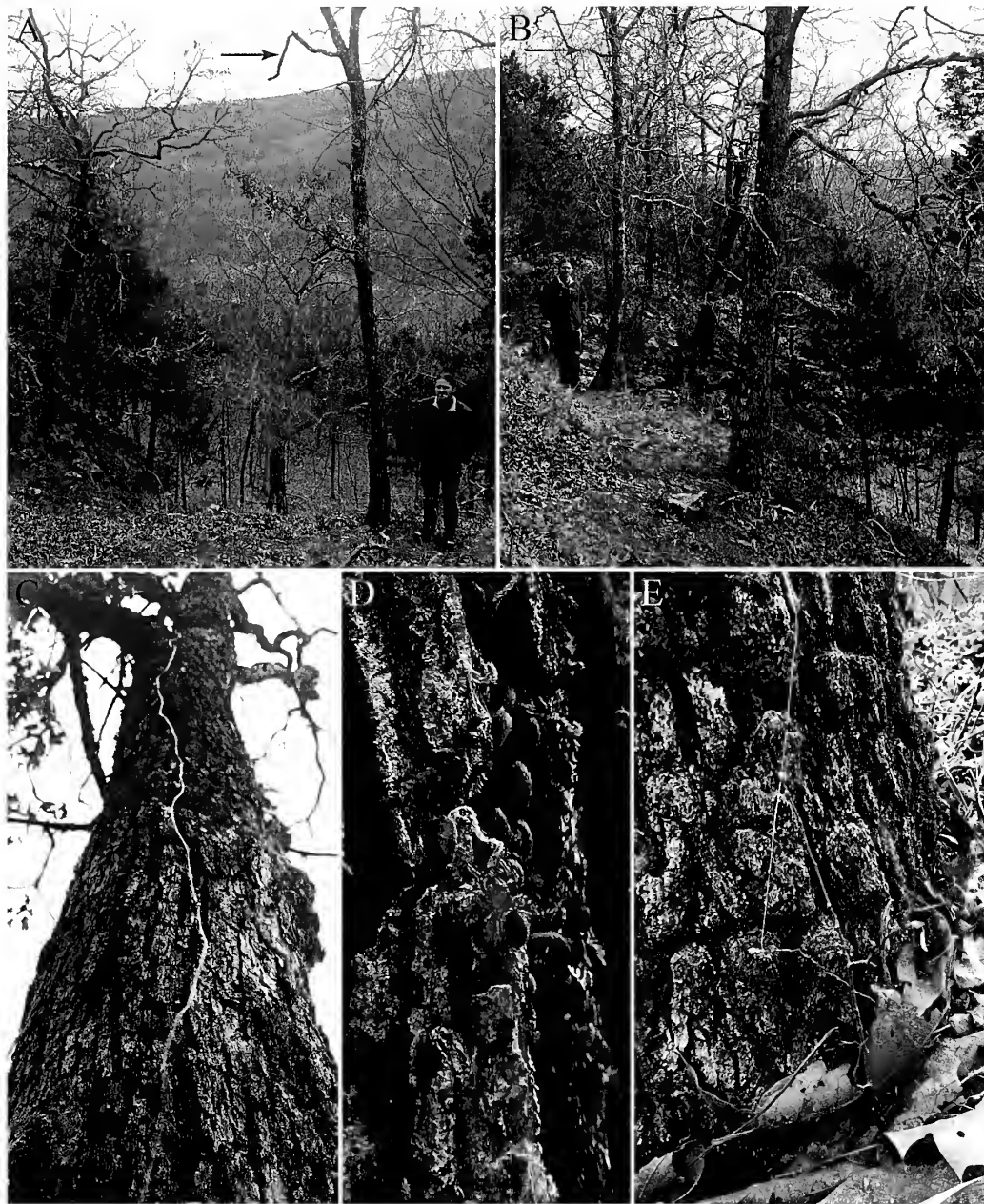
Figure 1.—Ballooning site. A. south-facing view of ballooning tree overlooking the valley; B. east-facing view of ballooning tree depicting steep slope; C. pre-ballooning silk band on trunk, leading to horizontal limb; D. spiderling climbing pre-ballooning band; E. base of tree showing pre-ballooning band diffusing quickly into litter. Red arrows indicate ballooning limb.

horizontally on the ground. Our observations are similar to Baerg's in the following ways: emergence date (15–22 March); silk band width (2 mm) and height (4–9 m); silk band ending on a horizontal limb; and tree size (not less than 15 cm DBH). The present observation occurred in the afternoon and Baerg (1928) described primarily morning activity ending mid-day. However, given the sparse activity and well-developed silk band, we suspect these individuals represented the last members of a ballooning event.

Steep slopes of sparsely wooded, disturbed habitats are commonly noted as preferred *Ummidia* habitat. Baerg (1928) made many pre-ballooning observations over several years on the University of Arkansas (UA) campus, which certainly was disturbed and sparsely wooded, but lacked steep slopes. Despite continued searching over five years, we failed to find either burrows or ballooning spiderlings in the area surrounding campus, which suggests a significantly

diminished population since 1928. However, we have found many *Ummidia* burrows on steep slopes in second-growth oak/hickory forest of northwestern Arkansas, including at Devil's Den State Park. The presence of pre-ballooning behavior similar to Baerg's observations (i.e., long silk trails) in naturalized forest, which is much less open than UA's campus, confirms the use of this method outside of an urbanized setting. That said, because of the position of the ballooning tree (Fig. 1A, B) and time of year (i.e., pre-bud-break), once acquiring their position on the horizontal limb, the spiderlings were functionally in an open area for ballooning.

With regard to identification, Baerg (1928) suggested that *U. carabivora*, known from the east coast, was actually widespread in the U.S. and identified his Arkansas specimens as this species. Indeed, as discussed above, aerial dispersal does enable large distributions. However, several pieces of evidence suggest that the spiderlings we

Figure 2.—Spiderling genus-level identification. A. dorsal habitus; B. lateral prosoma (right appendages removed), note ocular tubercle (arrow); C. pedipalp, note single clavate trichobothrium (dotted arrow) and ventral curvy spines (arrows); D. leg III, note trochanteral apophysis (dotted arrow) and tibial depression (arrow); E. eye group. Not to scale.

observed may not be *U. carabivora*. First, *Ummidia* is already suspected to contain considerable undescribed diversity. Second, specimens from this region exhibit longer pre-ballooning bands than what has been previously described for the genus. And third, the Interior Highlands are known to have a high rate of endemism (Redfearn 1986; Allen 1990; Robison & Allen 1995; Skvarla et al. 2013), but these endemics are often overlooked by surveys and specimens from the region are rarely included in phylogenetic analyses. Ultimately, a large-scale, integrative investigation of the genus in the New World is needed before confidence in species identification is warranted. Therefore, we refrain from suggesting species identification at this time, but offer the following discussion on genus-level identification of juvenile *Ummidia*.

Juvenile morphology in most animals (including spiders) is regularly overlooked, despite juveniles of certain taxa being frequently noticed and collected. This is evidenced by the prevalence of amateur naturalists not only photographing spiderlings, but also documenting pre-ballooning behavior in *Ummidia* on websites like Flickr.com and Bugguide.net. However, we are not aware of primary literature containing useable information on the identification of *Ummidia* immatures.

Decae (2010) lists five characteristics that differentiate ummidiines (*Ummidia* + *Conothele*) from other ctenizids as follows: 1) proximal dorsal glaborous depression on tibia III; 2) sharp apophysis on dorsolateral trochanter III; 3) dorsal clavate trichobothria on tarsi; 4) lateral curvy spines on distal podomeres of leg I, II, and pedipalps; and 5) compact eye-group (Fig. 2E) on an ocular tubercle (Fig. 2B). Each of these characters is apparent in the spiderling, although the trochanteral apophysis (Fig. 2D) and curvy spines (Fig. 2C) are not fully developed. Additionally, clavate trichobothria are absent from the legs, but a single clavate trichobothrium (Fig. 2C) is present on pedipalpal tarsi and is proportionally larger than on adult specimens. Noteworthy are the readily apparent tibial depressions commonly implemented as a diagnostic character for adults (Fig. 2D).

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General: The *Journal of Arachnology* publishes scientific articles reporting novel and significant observations and data regarding any aspect of the biology of arachnid groups. Feature articles and short communications must be scientifically rigorous and report substantially new information. Submissions that are overly narrow in focus (e.g., local faunal lists, descriptions of a second sex or of a single species without additional discussion of the significance of this information), have poorly substantiated observational data, or that present no new information will not be considered. Book reviews will not be published.

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Title page.—The title page includes the complete name, address, and telephone number of the corresponding author; a FAX number and electronic mail address if available; the title in sentence case, with no more than 65 characters and spaces per line in the title; each author's name and address; and the running head.

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