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A REVISION OF *DIOON TOMASELLII* (ZAMIACEAE) FROM WESTERN MEXICO, A RANGE EXTENSION OF *D. MEROLAE*, AND CLARIFICATION OF *D. PURPUSII*

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ABSTRACT

Dioon tomasellii was treated by De Luca *et al.* 1984 as two varieties; var. *tomasellii* and var. *sonorense*. To quote from the description, "Both the vegetative and reproductive characters show in general continuous variation patterns that do not support specific segregation within the range of *D. tomasellii* but the variation in the populations of Sonora and northern Sinaloa is such to warrant segregating them as a distinct variety." Apparently the reference to a continuous gradient of patterns was based on conjecture and not observations in the field, as we have examined both living plants and/or herbarium vouchers of nearly all known populations and have found no pattern of continuous variation. Our studies show that the two varieties of *D. tomasellii* merit recognition at the species level based on: a host of distinct morphological characters that are maintained even in cultivation; the lack of continuous variation between the two varieties; different habitat preferences; and an RFLP analysis by Moretti *et al.* 1993. A nomenclatural recombination (***Dioon sonorense***) is proposed. Some comments follow on *Dioon merolae* and *D. purpusii*.

KEY WORDS: *Dioon*, Zamiaceae, México, systematics

Populations of *Dioon* occurring on the west coast of México inhabit the foothills of the Sierra Madre Occidental ranging from Sonora in the north to Chiapas in the south. The ranges of *D. sonorense* (De Luca *et al.*) J. Chemnick, T. Gregory, & S. Salas-Morales and *D. tomasellii* De Luca, P., S. Sabato, & M. Vazquez Torres are detailed below. *Dioon holmgrenii* occurs in southern Oaxaca and *D. merolae* De Luca, P., S. Sabato, & M. Vazquez Torres ranges from south-eastern Oaxaca to southwestern Chiapas. The population of *D. tomasellii* closest to *D. sonorense* occurs in Durango but is decidedly within the described morphological range of other populations of *D. tomasellii* further south. Though the Durango plants are much closer geographically to

the Sonoran populations of *D. sonorense* than the next closest populations of *D. tomasellii* found in Nayarit, no intermediate forms are known to exist. All currently known populations of *D. tomasellii* are sufficiently similar to each other to be treated as a single species and are very different from the known populations of *D. sonorense*.

A SUMMARY OF CHARACTER DIFFERENCES

Dioon tomasellii can be readily distinguished from *D. sonorense* by: its fewer but longer, arching leaves; a thicker rachis and petiole which is densely tomentose when emerging and occasionally yellow with age; wider, deflexed, falcate, nearly entire, glabrous dark-green leaflets with conspicuously persistent tomentum on the abaxial side; and almost no spacing between the margins of the leaflets at the widest point. *Dioon sonorense* is distinguished from *D. tomasellii* by: its crowns of more numerous leaves which are shorter, upright, sometimes twisted and spirally ascending; a more slender rachis and petiole which emerge with dense pubescence and generally remain green with age; considerably narrower, linear-lanceolate leaflets often armed with one to three small spines on the distal edge of the leaflet; and leaflets that are generally flat, but occasionally slightly deflexed, or slightly keeled on the petiole and widely spaced between the margins by almost the width of the leaflets. The newly emerging leaves of *D. tomasellii* are covered entirely by a dense golden-brown tomentum and taper inwardly at the tip. The newly emerging leaves of *D. sonorense* are light green and taper outwardly at the tip; the leaflets are only lightly tomentose while the rachis is covered by a silvery pubescence.

DISTRIBUTION, HABITAT, AND NOTES

Dioon tomasellii is widely but sporadically distributed in oak and oak-pine forest in the states of Durango, Nayarit, Jalisco, Michoacán, and Guerrero in canyons and woodlands at elevations ranging from 600-1850 m with an annual rainfall of 1000-1500 mm. *Dioon sonorense* is currently distributed entirely within the state of Sonora (though it has been reported from northern Sinaloa) growing in oak woodland and the transition zone between high desert (deciduous wet/dry thorn/caudiciform forest) and oak woodland at an altitude of 615-1200 m. Plants are usually found on steep terrain growing under extremely dry conditions with an annual rainfall of 250-500 mm.

The taxonomy of *Dioon* has historically been and still is based almost entirely on vegetative characters. We are currently examining the megasporophylls of various species within the genus in order to find other useful characters. We have developed a process that completely removes the hairs from the megasporophylls and thus reveals texture, structure, and color beneath. The removal of cone hair is achieved by soaking the scales in an aqueous solution of 10% w/v sodium hypochloride for 12 hours and then gently washing them in a steady stream of fresh water. Hopefully other workers will find this process useful in their search for meaningful cone characters. Because the systematics of *Dioon* is not well-understood, we eagerly await the advent of an accurate and useful DNA fingerprinting process to help determine interspecific

relationships and genotypic mutative distance that is invisible to the observer relying solely upon morphology to determine the disposition of a group of closely allied taxa. RAPDs hold great promise but are still in the initial stages of application. However, molecular analysis has already provided some useful insights.

A phylogenetic analysis of all taxa in the genus *Dioon* was undertaken by Moretti *et al.* in 1993 using chloroplast DNA restriction fragment length polymorphism. Careful examination of their 187 character matrix, drawings, and conclusions support the separation of *D. sonorense* and *D. tomasellii* based on a phenetic approach as Moretti's results illustrate (see Moretti *et al.* 1993, Figure 3). We scored the number of differences within their character matrix between selected pairings of taxa and found the following:

tomasellii-sonorense	10 differences	*merolae-califanoi*	9 differences
merolae-purpusii	8 differences	*purpusii-caputoi*	9 differences
holmgrenii-caputoi	8 differences	*purpusii-califanoi*	6 differences
holmgrenii-purpusii	8 differences	*edule a.-edule e.*	6 differences
holmgrenii-merolae	1 difference	*spinulosum-rzedowskii*	3 differences

There are more differences in the 187 character matrix between *Dioon tomasellii* and *D. sonorense* than between the other pairs above (within the genus *Dioon*). Thus, there is molecular evidence to support conferring specific status upon *D. tomasellii* and *D. sonorense*. It is interesting to note that the interspecific comparisons of *spinulosum-rzedowskii* and *holmgrenii-merolae* yielded fewer differences than the intraspecific comparison of *edule angustifolia-edule edule*. Perhaps some further revision of the genus is indicated by these results. We also looked at other pairs of taxa that we considered more distantly related based on gross morphology to see whether the RFLP character matrix would support our assessment of those taxa and scored the differences as follows:

merolae-tomasellii	18 differences	*purpusii-sonorense*	21 differences
caputoi-edule a.	20 differences	*merolae-sonorense*	22 differences
sonorense-edule	29 differences	*sonorense-spinulosum*	82 differences

The results conform with our morphological analyses of the above taxa and yield nothing that is counter-intuitive to the apparent relationships within the genus *Dioon* except the surprising lack of differences between *D. holmgrenii* and *D. merolae*; two taxa that are distinct based on gross morphology. In general, the cpDNA RFLP analysis seems to be a reliable method for examining interspecific relationships as it corresponds well with our systematic sense of *Dioon* based on morphological and ecological evidence from plants in the field and in cultivation.

In consideration of the ecological, geographical, morphological, and molecular evidence, this paper therefore confers specific status on:

Dioon sonorense (De Luca *et al.*) J. Chemnick, T. Gregory, & S. Salas-Morales, *comb. nov.* BASIONYM: *Dioon tomasellii* De Luca, P., S. Sabato, & M. Vazquez Torres var. *sonorense* De Luca *et al.*, Brittonia 36:223-227. 1984.

A RANGE EXTENSION FOR *DIOON MEROLAE*

In their description of *Dioon merolae*, De Luca *et al.* (1981) report the distribution of the species as endemic to the state of Chiapas. They observe that, "It is noteworthy, furthermore, that *D. merolae* is well separated orographically from the other Mexican species by the Isthmus of Tehuantepec." We wish to report the existence of two populations of *D. merolae* in the state of Oaxaca in the Sierra de Juárez and the Sierra Madre del Sur within the drainage of the Río Tehuantepec. These Oaxacan *D. merolae* are noteworthy because they are quite similar to plants found in western Chiapas 160 km to the east, yet occur only 30 km east of populations of *Dioon* sp. of uncertain affinity, confirming that the Isthmus of Tehuantepec is not necessarily a geographic barrier to the distribution of the species. We observed Oaxacan populations of *D. merolae* during a field trip in May, 1997 and again in December, 1997. A third population of Oaxacan *D. merolae* was discovered by S. Salas-Morales in the eastern region of the Chimalapas growing in oak-pine forest at an altitude of 810 m. The population in the Sierra Madre del Sur was growing at an elevation of 1150 m in soil derived from sedimentary rock along a ridge with both SE and NE exposure in oak/pine forest. The phenology of this population was complex: mature plants with dried microstrobili and developing megastrobili; active recruitment of younger but decidedly post-juvenile plants of varying size and several seedlings. Domestic pigs eat the fruit but, fortunately, pass the seed unharmed. The paucity of seedlings was probably the result of grazing goats. More than 400 mature plants were observed. The population in the Sierra de Juárez was growing at an elevation of 1080 m in limestone kaarst with an E/SE exposure. The plants were in association with *Beaucarnea recurvata, Chamaedorea elegans, Agave* spp., *Plumeria rubra, Bilbergia* sp., *Hechtia* spp., and *Tillandsia brachycaulus*. The phenology of the population was likewise complex; mature plants with dried microstrobili and developing megastrobili; female plants with recently dehisced cones, and seedlings were observed. The total number of mature plants observed was in excess of 100 individuals. Herbarium vouchers from the above localities have been deposited at the Instituto de Ecología de Xalapa.

The ethnobotanical use of Oaxacan Dioons is widespread. The leaves are commonly seen ornamenting windows, doorways, and religious figures during holidays, especially Easter. Churches often display wreaths of *Dioon* leaves and occasionally cultivate plants in the garden to provide a ready source of plant materials as the closest population of Dioons is often a distant and difficult journey. The sarcotesta is sometimes a source of food. The sclerotesta is used for games, bracelets, and necklaces. *Dioon* leaves are occasionally used in religious and festive ceremonies. The local common names in the Sierra Madre del Sur are *mais viejo* (oldtime corn) and *palma espinuda* (spiny palm). The sarcotesta has historically been utilized as a food source in periods of diminished corn supplies. Though none of the authors has sampled the fleshy yellow sarcotesta, assurance was given that it remains a popular food item today, prized for its rich flavor. The leaves are used as Christmas party ornaments and as such, are sold in the markets of Tehuantepec. Occasionally the mature megastrobili are harvested, apparently by outsiders who sell the cones to a broker in the port city of Salina Cruz for unspecified uses and destinations but most likely to meet the foreign demand for propagation. The plant is known locally in the Sierra de Juárez as *palmilla* (little palm). It is harvested solely for the leaves which are

used in all manner of festivities, Christmas decorations, weddings, and during the week of Easter. Extreme caution should be exercised whenever traveling or doing field work in rural mountainous areas of Oaxaca due to the widespread cultivation of illegal crops. As such, it is imperative to work with a local guide.

CLARIFICATION OF *DIOON PURPUSII*

Much confusion persists in the proper identification of *Dioon purpusii* (see Rose 1909) due largely to the brevity of the original description and the remoteness of the known populations. In spite of the efforts of De Luca *et al.* (1978) to clarify the description of *D. purpusii*, an error persisted in the description of the fronds as "flat in adult plants, keeled in young plants" and in the comparison between *D. purpusii* and *D. califanoi* which claimed that "It (*D. purpusii*) differs from the former (*D. califanoi*) because its fronds are flat and not keeled (with the exception of the juvenile ones which are very similar to the fronds of *D. califanoi*)" (De Luca *et al.* 1979) .

We visited the type locality in Santa Catarina, Oaxaca as well as three other populations within the drainages of the Río La Hondura and the Río Santo Domingo. Our awareness of previously unknown populations of *Dioon purpusii* is the result of extensive field work by Silvia H. Salas. We examined a number of leaves on various plants and found that the mature leaves on adult plants are rarely flat; instead they are moderately to strongly keeled. The leaflets are inserted obliquely on the rachis, angled forward and usually *held at an angle above the rachis of 20- 45 degrees* in adult plants as well as juvenile plants. Unfortunately this misapprehension about flat leaves in *D. purpusii* has persisted and undoubtedly contributes to the misidentification and confusion within this taxon. Consequently, misidentified plants are common in cultivation. Many Dioons labeled as *D. purpusii*; with spines on the margin of the leaflets are likely to be *D. merolae, D. holmgrenii, D. caputoi, D. sonorense,* or *D. tomasellii*, especially if the leaves are flat or deflexed. Dioons with keeled leaves and spines on the margins of the leaflets are likely to be *D. purpusii* though *D. califanoi* occasions the same habit. De Luca *et al.* (1980) correctly addressed the leaf aspect of *D. purpusii* in the notes of their description of *D. caputoi* as follows: "*Dioon purpusii*, to which were erroneously attributed specimens of *D. caputoi*, differs in its lightly keeled fronds. . . ." Sabato & De Luca (1985) amended the matter further in the Key to Species separating *purpusii* from *califanoi* on the basis of "leaf flat or slightly keeled" rather than "strongly keeled".

We have recently examined populations of plants in central Oaxaca that apparently have an affinity with *Dioon purpusii*, but which produce leaves that are flat to slightly keeled and moderately to densely tomentose. We are currently cultivating plants of these populations from seed to compare leaf morphology under uniform conditions. Further examination of both known and newly discovered populations in what seems to be emerging as a *D. purpusii* complex is required to comprehensively determine the disposition of Oaxacan Dioons.

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REVIEW: "A REVISION OF *HETEROTHECA* SECT. *PHYLLOTHECA* (NUTT.) HARMS (COMPOSITAE: ASTEREAE)" BY J.C. SEMPLE

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ABSTRACT

A summary and overview are provided for the recent monograph of *Heterotheca* sect. *Phyllotheca* by John Semple (1996), with emphasis on taxonomic concepts and implications of the formal taxonomic recognition of sympatric infraspecific taxa. Aspects of the taxonomy of sect. *Heterotheca* and sect. *Ammodia* also are discussed. Three new combinations allow a more evolutionarily congruent taxonomy for the *H. sessiliflora* complex: ***H. sessiliflora*** var. ***thiniicola*** (Rzed. & Ezc.) Nesom; ***H. echioides*** var. ***bolanderioides*** (Semple) Nesom; and ***H. echioides*** var. ***bolanderi*** (A. Gray) Nesom. Two combinations necessary in the same group remain to be formally completed by Semple. Rationale regarding the taxonomic status of *Bradburia* (independent genus vs. subgroup within *Chrysopsis*) is examined.

KEY WORDS: *Heterotheca, Bradburia, Chrysopsis*, Astereae, Asteraceae, nomenclature

John Semple (1996) has published "the first comprehensive monograph of the prairie and montane goldenasters, *Heterotheca* sect. *Phyllotheca* (Nutt.) Harms." "This study was based on more than 10,300 herbarium specimens (6,844 separate collection numbers)" and includes specimen citations, typification, and a detailed illustration and distribution map for every taxonomic entity recognized in the treatment. An intuitive phylogenetic diagram (p. 6), drawn from molecular and morphological information, shows Semple's view of relationships among the goldenaster genera (subtribe Chrysopsidinae, sensu Nesom 1994) and provides a summary of the taxonomy and species relationships within sect. *Phyllotheca*. There are taxonomic rearrangements, and two new species are described; one species is newly raised from varietal rank. The treatment also provides background for understanding nomenclatural combinations in sect. *Phyllotheca* that were published earlier (Semple 1987, 1992, 1994). Most immediately, the value of the treatment is evident to anyone needing to identify plants of *Heterotheca*, but details of the nomenclature,

morphological accounts, and maps make it much simpler to comprehend the genus at all levels.

A synopsis of the history, morphology, and distribution of the goldenaster genera is presented at the beginning of the *Phyllotheca* monograph. The segregation of *Heterotheca*, *Chrysopsis*, and *Pityopsis* makes sense morphologically, cytologically, and phyletically, and those generic delimitations have gained increasing acceptance over the 20 years since publication of evidence for this system (Semple 1977; Semple et al. 1980). Among the goldenaster genera, *Heterotheca* is the largest and most taxonomically difficult. The complexity of the variation patterns apparently has long postponed a treatment of the largest part of the genus (sect. *Phyllotheca*), and in the wake of Semple's comprehensive study, it seems unlikely that anyone will be eager to begin any detailed process of reevaluation of the whole group.

An earlier review of Semple's treatise (Burk 1996, p. 219) speculated, however, that "because of its inherent variability, sect. *Phyllotheca* will continue to present difficulties for field biologists." The treatment will be subject to "the inevitable revisions of the 21st Century," and "if [Semple's taxonomic] structure is in time dismantled, he has nonetheless brought together here the building blocks to shape another." As with any study that pulls together such a large amount of information, unresolved problems also are brought to clearer focus, and the treatment provides an invaluable basis for further studies of the biology and evolution of these species. The present review provides an overview and perspective for some of the more interesting conclusions and questions that arise from the *Phyllotheca* monograph.

The 20 species (as recognized by Semple) of sect. *Phyllotheca* are a mixture of narrow endemics (e.g., *Heterotheca rutteri*, *H. marginata*, *H. jonesii*, *H. brandegeei*, *H. pumila*, *H. barbata*, *H. shevockii*, *H. monarchensis*, *H. mexicana*) and entities more widespread to varying degrees (e.g., *H. villosa*, *H. canescens*, *H. stenophylla*, *H. camporum*, *H. mucronata*, *H. zionensis*, *H. fulcrata*, *H. viscida*, *H. echioides*, *H. sessiliflora*). The most complex taxa are *H. villosa* (nine varieties, no subspecies) and *H. sessiliflora* (four subspecies, seven basic entities). *Heterotheca mucronata*, *H. camporum*, and *H. stenophylla* have two varieties each and *H. fulcrata* has four varieties.

Semple has dealt with the complex variation and difficulties in identification in a forthright way by separating specimen citations for collections that deviate from the typical form of the taxon. These are given in paragraphs (often several) after citations of "typical" collections with the heading of "aff. [the taxon under consideration]" followed by a parenthetical explanatory expression (e.g., "approaching var. *minor*" or "possible hybrids with *H. zionensis*"). A commentary on unusual variation for each taxon also is provided, and the indications of "aff." status are shown by distinct symbols on the distribution maps.

Taxonomic delimitations in sect. *Phyllotheca* are based in part on multivariate morphometric analyses "on more than 600 specimens including 76 type specimens," to be published separately (p. 2). Their publication will correspondingly contribute to an understanding of variation in sect. *Phyllotheca* and its taxonomic treatment. And "a cytogeographic study of the whole genus with a review of all previously published counts and new reports for several hundred individuals is in preparation" (p. 23).

Discussion of evolutionary processes underlying the variation patterns are found in the commentaries by Semple on individual species.

Among the most interesting features of the variation patterns described by Semple are the strongly overlapping geographic ranges in infraspecific taxa of most of the widespread species (especially see *H. sessiliflora*, *H. villosa*, *H. fulcrata*, and *H. mucronata*). Are these now sympatric entities recently spread from originally allopatric, more restricted ranges, with extensive hybridization resulting in blurred morphological boundaries in regions of overlap? Or, do these sympatric entities maintain their evolutionary independence to a significant degree? Evidence suggests that both situations may exist in sect. *Phyllotheca*.

"Within species, intervarietal hybrids are common in areas of sympatry" (p. 24), but these are usually between plants at the same ploidy level. Interspecific hybridization, however, is generally uncommon between diploids of sect. *Phyllotheca* but more common among tetraploids, suggesting that the difference in ploidy (between diploids and tetraploids) provides an effective isolating mechanism (see various comments below). Triploids are rarely encountered.

Taxonomic concepts

Indication of Semple's general approach toward fitting a nomenclatural system to the variation patterns is provided in commentary regarding *Heterotheca villosa*. "The races [= varieties, of *Heterotheca villosa*] fit well with the concept of variety in that each occurs in pure form in some populations, and the overall ranges are sympatric to a considerable degree with at least one other variety. Some taxa have sufficiently non-overlapping ranges that subspecies status might be considered. . . . Each variety most likely evolved in isolation and adapted to a different set of habitat parameters, but by and large no variety now occurs in isolation" (1996, p. 108). The biology and taxonomy of *H. villosa* and others (where the only infraspecific category is "var.") contrast in the *Phyllotheca* monograph with that of *H. sessiliflora* (where both "subsp." and "var." are used).

For a more detailed explanation of his concepts of subspecific and varietal categories, Semple refers to an earlier study of the genus *Xanthisma*: "A subspecies is characterized by all members exhibiting a particular morphology distinct from other individuals in the species and by the allopatric distribution of these members from the rest of the species" [citing various references] (Semple 1974, p. 4). "The variation between subspecies can be described as discontinuous, except for the few hybrids" (1974, p. 8). "A variety is characterized by all members of a population exhibiting a particular morphology distinct from other individuals in the species. The distribution of these populations is sympatric with populations whose members are not within the same variety, and also many populations of morphological intermediates exist [citing various references]. . . . Van Steenis described varietal level variation as being continuous with other varieties, although the continuum would have pronounced modes" (Semple 1974, p. 8-9).

Another perspective on Semple's varietal concept is found in his comments on *Heterotheca brandegeei*, which is markedly variable in glandularity and density of indument. The species is narrowly endemic to Sierra San Pedro Martir in Baja

California and is not suspected of intergrading with any other. "Even in a species with a limited distribution and a relatively few number of populations the full range in indument variation can be encountered. In other species with a greater range in [geographic] distribution than *H. brandegeei* (e.g., *H. sessiliflora*, *H. fulcrata*, *H. mucronata*, and *H. villosa*) past periods of isolation apparently have allowed fixation of different alleles controlling indument features in different portions of the range resulting in morphologically more well-defined races (generally labeled in this treatment as varieties)" (p. 66).

The taxonomic approach taken by Semple (recognition of numerous sympatric varieties) is perhaps by necessity a first step simply in providing a documented phenetic framework for the variation in this biologically complex group. Semple notes that this approach serves a related practical purpose. In discussing the strong similarity between *Heterotheca villosa* vars. *ballardii* and *foliosa* (both mostly tetraploid), he observes that if the diagnostic distinction of the former provides insufficient grounds for its formal recognition, "it then would be logical to merge all other varieties together with no infraspecific taxa being recognized in *H. villosa*. This would result in the loss from the formal nomenclature of a great deal of information on variation and distribution in what is admittedly a difficult species complex. Splitting seems justifiable in this case, and it maintains a nomenclature that parallels what has been adopted with less hesitation for other species in the section" (p. 114).

Still, if entities can be identified with some degree of consistency (as implied by the maps and specimen citations), and if they are sympatric and similar in habitat and phenology, some degree of internal reproductive isolation might be inferred to exist. Alternatively, segregation of linked genes controlling the character suites by which these taxa are identified may have a large effect on the variation patterns. Needed for interpretation, but missing in most cases, are observations on variation within populations of the taxa concerned. For those species where isolation does exist among the infraspecific taxa, the taxonomic approach could be shifted more toward an evolutionary perspective. Alternative taxonomic interpretations are possible, based on the same evidence and information.

Semple's approach to variation patterns and taxonomic applications in various species of *Heterotheca* is discussed below.

Heterotheca villosa/stenophylla var. *angustifolia*

Semple has transferred var. *angustifolia* of *Heterotheca villosa* to *H. stenophylla*. The latter species then becomes "divided into two seemingly quite distinct [and strongly sympatric] varieties that differ in gland and hair density" (p. 88). The transfer of var. *angustifolia* was made on the basis of "field experience and the results of multivariate analyses" showing that "the type of var. *angustifolia* is morphologically closer to many individuals of var. *stenophylla* than it is to either *H. canescens* or typical *H. villosa*" (p. 94).

Semple speculates that "tetraploid var. *angustifolia* originated from diploid var. *stenophylla* and subsequently converged toward tetraploid *H. canescens* due to putative occasional hybridization with the latter. . . . Alternatively, var. *angustifolia* might have originated via allopolyploidy from more hairy and less glandular diploid *H.*

stenophylla var. *stenophylla* and *H. canescens*" (p. 94). *Heterotheca stenophylla* and *H. canescens* are shown as sister species in Semple's phylogram.

Based on Semple's estimate of its evolutionary origin, var. *angustifolia* could justifiably be treated within or close to either of the two contributors to its genome: *(a) Heterotheca stenophylla* and (b) *H. canescens*.

(a) "The range of var. *angustifolia* is generally the same as that of var. *stenophylla* from Oklahoma northward, except that var. *angustifolia* occurs over a slightly greater area and in the gaps between the disjunct populations of var. *stenophylla*" (p. 94). "The two varieties occur in pure and mixed populations throughout the range of the species" (p. 53). Cytological evidence seems unequivocal in suggesting that var. *angustifolia* is genetically isolated from var. *stenophylla*. Most chromosome number reports for var. *stenophylla* have been of diploids, while all of many reports for var. *angustifolia* have been of tetraploids. "Several triploid counts [have been reported] from putative intervarietal hybrids" (p. 92).

(b) *Heterotheca canescens* also is mostly diploid over its range and also is broadly sympatric with var. *angustifolia*. The only intermediate collections cited by Semple for *H. canescens* are those "aff. *H. canescens* (close to *H. stenophylla* var. *angustifolia*)" (p. 100). Presumably, var. *angustifolia* - *H. canescens* hybrids are triploid.

Because var. *angustifolia* is broadly sympatric with both of its putative parents and apparently genetically isolated from them to a significant degree, its treatment at specific rank also is a possibility. It presumably is an evolutionarily distinct entity and its morphogeographic circumscription is the same regardless of its taxonomic placement.

If Semple's hypothesis of origin for var. *angustifolia* is correct, placement of it within *Heterotheca stenophylla* is better than within *H. villosa*. Inclusion of var. *angustifolia*, however, only slightly increases the morphological complexity of *H. villosa*, as defined by Semple, and occupies a part of the overall geographic range where its sympatry with conspecific varieties is relatively less (Figs. 39 and 40).

Heterotheca villosa

Heterotheca villosa is "highly variable in diagnostic features" and is "difficult to define as a species, although each infraspecific taxon has a diagnostic suite of traits." The species is "very variable in stem height, leaf base shape, stem and leaf indument traits, numbers of heads per capitulescence and florets per head" (p. 105). It is "defined by what it lacks rather than what it possesses" (p. 108).

Nine varieties are recognized within *Heterotheca villosa* in 1996, but Semple's concepts of these taxa have fluctuated. In 1990, he placed a number of names as synonyms of *H. villosa* var. *hispida* (= *H. villosa* var. *minor* of 1996) with the following comment: "Included are morphotypes that I have previously accepted as species or subspecies (Semple 1987), but have come to view as sometimes semi-distinct regional 'races' that grade into each other to such an extent that continued recognition cannot be justified with the data available to me at present." Later (1994, 1996), apparently based on multivariate studies, he returned to his earlier position of

formally recognizing these races, including four varieties within *H. villosa* from the same 1990 list of synonyms. "A number of morphotypes [of *H. villosa*] appear sufficiently distinct to warrant recognition. . . . All races have well defined geographic distributions which overlap to a considerable degree in some cases (Figs. 39-40). The highly plastic nature of the species and undoubted hybridization make identification to variety difficult in numerous cases" (1996, p. 108).

"The diploid races [of *Heterotheca villosa*] are usually distinct from each other, but each has given rise to one (or more) tetraploid lines [exception noted below]. Tetraploids ['more common than diploids in *H. villosa*'] tend to look more alike because the diploid traits are less pronounced and because the tetraploids are more likely to have hybridized, thus further blurring the distinctions between the races. Possible occasional hybridization with tetraploids in other species may also have further buffered the distinctive morphology of the tetraploid level of the pillar complex" (p. 108).

Some infraspecific taxa of *Heterotheca villosa* are more distinct than others. Two have been regarded as species in recent floristic treatments: (a) var. *nana* (as *H. horrida*, e.g., Correll & Johnston 1970; Dorn 1988) and (b) var. *depressa* (as *H. depressa*, Dorn 1988). The distinctiveness of these entities is further emphasized by the relatively few collections cited for them as "aff." Treatment of var. *nana* and var. *depressa* at species rank appears to be a reasonable alternative potentially providing a closer match between taxonomy and the evolutionary pattern.

(a) Var. *nana* (diploid, many counts, without tetraploid populations) is almost completely overlaid in its geographic range by var. *foliosa* (diploid and tetraploid but tetraploid in its area of overlap with var. *nana*, many reports) and by var. *minor* (diploid and tetraploid, numerous reports). Intermediates between var. *nana* and var. *scabra* occur in the Four Corners area; the closest relative of var. *nana* is the narrow endemic var. *sierrablancensis* (diploid), which occurs at the southeastern corner of the range of var. *nana*.

(b) Var. *depressa* (tetraploid, several counts, with only a speculative evolutionary connection to diploids) is endemic to habitats connected with hot springs and geyser basins mostly in the area of Yellowstone National Park. Putative hybrids have been observed between var. *depressa* and var. *minor*, which is sympatric but different in habitat.

Heterotheca villosa var. *pedunculata* also has distinctions that set it apart from other taxa within *H. villosa*. "Semple (1990) included it among tentative synonyms of var. *villosa*, but the results of multivariate analyses conducted since then indicate that it is sufficiently distinct from var. *villosa* to warrant recognition even when only non-diagnostic traits are used in the discriminant analysis. It is the only usually tetraploid taxon in sect. *Phyllotheca* that has very densely pubescent leaves" (1996, p. 124). Intergrades occur between var. *pedunculata* and var. *minor* (tetraploid) and var. *scabra* (tetraploid).

Var. *pedunculata* "is similar to the recently described *Heterotheca mexicana*, which has achenes with a weakly developed short outer pappus whorl. If the more pubescent forms of the Mexicana complex [*H. mucronata*, *H. gypsophila*, *H. mexicana*] are primitive in the section, then var. *pedunculata* is likely to be similar to the ancestral

form of *H. villosa* from which other taxa evolved . . ." (1996, p. 124). These comments seem to imply that var. *pedunculata* is closely related to the Mexicana species, but there apparently is no further development of the hint that the Mexicana complex may be primitive within sect. *Phyllotheca*. Nor does the 1996 phylogram support this point of view. The phylogram also places *H. villosa* in a position widely separated from the Mexicana complex.

Heterotheca mucronata

Semple has described *Heterotheca mucronata* var. *harmsiana* (var. nov.) from the northeast Mexican states of Tamaulipas, Nuevo León, San Luis Potosí, and Coahuila. Var. *harmsiana* differs from the typical variety in its leaves with "fewer hairs and more glands," illustrating Semple's observation (p. 94) that "most other species [of sect. *Phyllotheca*] include both more glandular and more hairy races." Var. *mucronata* and var. *harmsiana* have essentially congruent geographic distributions and both have been collected from at least six of the same localities or localized areas from a relatively small region within Nuevo León and Coahuila (see specimen citations for the two taxa): the Peña Nevada area; east of Iturbide; Chipinque; Sierra de la Viga; Sierra de Arteaga; and Cañon de San Lorenzo.

My own field and herbarium experience have indicated that only a single evolutionary entity exists among plants identified as *Heterotheca mucronata*. Plants from Tamaulipas and near Linares in southeastern Nuevo León have eglandular leaves and a more densely sericeous vestiture of thinner-based trichomes than those in the remainder of the Mexican range of the species (pers. observ.), but the distribution of these variants does not match the distribution of var. *mucronata* described by Semple. Putative intergrades with *H. fulcrata* (see below) have been collected around Saltillo, Coahuila, and slightly to the south in northern Zacatecas.

The recognition within *Heterotheca mucronata* of closely sympatric varieties with no apparent difference in habitat or phenology suggests that the taxa recognized are inter- or infra-populational variants differing in the expression of two types of trichomes. Local adaptation and genetic segregation could account for differentiation among and within populations. An independent evaluation would be useful to resolve the differences in perception of these variation patterns, but differences in our concepts of the varietal category apparently preclude any chance of taxonomic agreement.

Heterotheca fulcrata

The distinctive species *Heterotheca fulcrata* comprises four varieties in Semple's concept. Numerous reports of diploid chromosome numbers have been reported for all of them (plus one "unconfirmed" tetraploid count for var. *fulcrata*). Vars. *fulcrata*, *arizonica*, and *senilis* are sympatric with nearly congruent ranges in the montane habitats of the Chihuahuan Desert region in northeastern México and trans-Pecos Texas and from there into southern New Mexico and Arizona. I have identified these plants in México as a single evolutionary entity (= *H. fulcrata*). The overall geographic distributions of var. *fulcrata* and var. *amplifolia* (sensu Semple) also are remarkably similar, as are those of var. *arizonica* and var. *senilis*. In fact, given the apparent cohesiveness of the species, evidence suggests that the varieties (sensu

Semple) of *H. fulcrata* are better regarded as local variants in the sense of most current botanists, perhaps treated with taxonomic status as "forma," which would retain the formal nomenclature desired for these entities by Semple (see "Taxonomic Concepts," above).

Heterotheca sessiliflora complex

Within the primarily Californian *Heterotheca sessiliflora* complex, Semple has fashioned an amalgum of greatly increased complexity by combining *H. echioides*, *H. camphorata*, *H. bolanderi*, *H. fastigiata*, and *H. sessiliflora* into a single species (*H. sessiliflora*). Four of these are treated at subspecific rank (subspp. *echioides*, *bolanderi*, *fastigiata*, and *sessiliflora*); *H. camphorata* is treated as a variety and placed within subsp. *echioides*. Varieties are recognized within subsp. *fastigiata* (2 vars.) and subsp. *echioides* (3 vars.); subspp. *sessiliflora* and *bolanderi* are monotypic. *Heterotheca monarchensis* is a narrow endemic from the Kings River canyon in Fresno County. It is similar to *H. echioides* but is morphologically distinct and geographically separated from other members of the *H. sessiliflora* complex.

Semple's Figure 14 maps the geographic distribution of the basic taxa of *Heterotheca sessiliflora* as he has defined that species. Four varieties of *H. sessiliflora* are sympatrically overlaid in Los Angeles County, three each in San Bernardino and Ventura counties. Var. *camphorata* is closely sympatric with var. *echioides* in Monterey, Santa Clara, and Santa Cruz counties.

Without disagreement regarding delimitation of the basic evolutionary units of the *Heterotheca sessiliflora* complex, they can be positioned in a way that more closely matches the evolutionary situation by essentially eliminating sympatric entities within a single species. A taxonomic arrangement to accomplish this is suggested below (Fig. 1), contrasted with Semple's arrangement of the same basic entities (Fig. 2). Continuing elimination of natural habitats and creation of hybrid habitats by human activities might drive this whole complex toward a genetic swarm, but the suggested alternate arrangement preserves the morphological coherence of the taxa involved and provides a more comprehensible tool for dealing with the current morpho-geographic pattern of variation. Based on the information presented by Semple, and in my experience, the *H. sessiliflora* complex (sensu Semple) is significantly different from most other *Heterotheca* species of this treatment that are divided into sympatric varieties.

Semple's basic units in the *Heterotheca sessiliflora* complex are a mix of subspecies and varieties. He did not treat the entities subsp. *sessiliflora* and subsp. *bolanderi* at varietal rank, apparently because both are restricted to coastal strand habitats and neither is geographically overlapping with any other taxa (see definitions above of variety and subspecies). Formal varietal combinations were not provided for "var." *fastigiata* and "var." *echioides*, although it appears that this was intended, as they are repeatedly referred to as "var. *fastigiata*" and "var. *echioides*" and shown on the phylogram as entities coordinate with other varieties. The count of "24 varieties" in the Abstract also must include "var. *fastigiata*" and "var. *echioides*."

Heterotheca sessiliflora (Nutt.) Shinners
var. *sessiliflora*
var. *fastigiata* (Greene) Semple, ined. [nom. nud. in Semple 1996]
var. *sanjacintensis* Semple
var. *thiniicola* (Rzed. & Ezc.) Nesom

Heterotheca echioides (Benth.) Shinners
var. *echioides*
var. *bolanderioides* (Semple) Nesom
var. *bolanderi* (A. Gray) Nesom

Heterotheca camphorata (Eastw.) Semple

Heterotheca monarchensis York, Shevock, & Semple

Figure 1. Alternate taxonomy for the *Heterotheca sessiliflora* complex. Except for var. *fastigiata*, nomenclatural combinations to formally complete this are provided below.

Heterotheca sessiliflora (Nutt.) Shinners
subsp. *sessiliflora*
subsp. *fastigiata* (Greene) Semple
var. *fastigiata* (Greene) Semple, ined. [nom. nud. in Semple 1996]
var. *sanjacintensis* Semple
subsp. *echioides* (Benth.) Semple
var. *echioides* (Benth.) Semple, ined. [nom. nud. in Semple 1996]
var. *bolanderioides* Semple
var. *camphorata* (Eastw.) Semple
subsp. *bolanderi* (A. Gray) Semple

Heterotheca thiniicola (Rzed. & Ezc.) B.L. Turner

Heterotheca monarchensis York, Shevock, & Semple

Figure 2. Semple's taxonomy for the *Heterotheca sessiliflora* complex. See comments in text regarding "ined." nomenclature.

The alternate arrangement adopts Semple's suggestions in combining *Heterotheca fastigiata* with *H. sessiliflora* and *H. bolanderi* with *H. echioides*, adding a newly described variety to each species. *Heterotheca bolanderi* (diploid) is discrete in geography and habitat and might be kept as a distinct species, but it is closely similar to var. *echioides* and var. *bolanderioides* and may have been directly involved in the parentage of the latter, which is primarily tetraploid (fide Semple, p. 49). *Heterotheca camphorata* (mostly diploid) is kept as a separate species (with a combination made earlier by Semple) and *H. thiniicola* is brought within *H. sessiliflora* (comments below). This arrangement does not eliminate difficulties in identifying hybrids, introgressants, and other intermediates for whatever reason, but such problems exist no matter what taxonomic superstructure is laid over the basic evolutionary units. The most common interspecific hybrids in the suggested alternate arrangement appear to be between *H. echioides* (var. *echioides*) and *H. sessiliflora* (var. *fastigiata*) where they are sympatric in San Bernardino, Los Angeles, and Ventura counties.

Heterotheca sessiliflora (s. str.), like *H. bolanderi*, is a coastal strand entity discrete in geography and habitat, but Semple's proposal to unite it with *H. fastigiata* is a good one. The four varieties of *H. sessiliflora* (as suggested here) are exclusively diploid and distributed allopatrically in southwestern California and northwestern México (Baja California and Sonora). A sericeous indument of short hairs and leaves with distinctly wavy margins unite this group of plants and give it an immediately recognizable appearance.

In a treatment of Mexican *Heterotheca* (Nesom unpublished), *H. thiniicola* (a desert habitat population from northwestern Sonora) has been included in the same circumscription as the type of *H. fastigiata*. Semple, in contrast, has maintained *H. thiniicola* at specific rank, noting (p. 54) that "While similar to var. *fastigiata*, *H. thiniicola* is sufficiently different to warrant recognition as a separate taxon. Its unique habitat indicates that it is more than just a disjunct population of the montane var. *fastigiata*."

The only differences I can confirm to separate *Heterotheca thiniicola* from *H. sessiliflora* var. *fastigiata* are those noted by Semple: the absence of osteiform (Type A) trichomes on the disc corollas of the former, its distinctly desertic habitat at 110 meters elevation, and a geographic disjunction of about 200 miles from other *H. sessiliflora*. Var. *fastigiata*, however, occurs in habitats at "(150) -300-1800- (2200)" meters elevation, low enough to include "desert washes," although its primary habitat is higher in "pine forests and transition chaparral" (p. 40). Semple has made the useful observation that the consistent occurrence of osteiform trichomes on the disc corollas is evidence for monophyly of the *H. sessiliflora* complex (sensu Semple, including *H. monarchensis*) --- the absence of these trichomes on *H. thiniicola* corollas almost certainly has resulted from a recent evolutionary loss (vs. primitive absence) and does not suggest the species should be considered apart from var. *fastigiata*, to which it is otherwise nearly identical. To formally recognize the evolutionary independence (via geographic isolation) of the Sonoran population and its small degree of morphological divergence, it is treated here at varietal rank within *H. sessiliflora*, coordinate with the other three varieties.

Heterotheca sessiliflora (Nutt.) Shinners var. ***thiniicola*** (Rzed. & Ezc.) Nesom, *comb. nov.* BASIONYM: *Haplopappus thiniicola* Rzed. & Ezc., Cienc. Interamer. 26:16. 1986. *Heterotheca thiniicola* (Rzed. & Ezc.) B.L. Turner, Phytologia 63:128. 1987.

Heterotheca echioides (Benth.) Shinners var. ***bolanderioides*** (Semple) Nesom, *comb. nov.* BASIONYM: *Heterotheca sessiliflora* (Nutt.) Shinners var. *bolanderioides* Semple, Phytologia 73:450. 1992.

Heterotheca echioides (Benth.) Shinners var. ***bolanderi*** (A. Gray) Nesom, *comb. nov.* BASIONYM: *Chrysopsis bolanderi* A. Gray, Proc. Amer. Acad. Arts 6:543. 1866. *Heterotheca bolanderi* (A. Gray) Harms, Brittonia 26:61. 1974.

Species concepts in sect. *Heterotheca*

Semple recognizes seven species of sect. *Heterotheca* (see p. 25: "Key to *Heterotheca* sect. *Heterotheca* [after Wagenknecht, 1960, with modifications]"), noting that my approach (Nesom 1990) contrasted with that of Wagenknecht. He adopted Wagenknecht's definitions of taxa without commenting on the suggestion that *H. subaxillaris* be broadened to include *H. latifolia* (including varieties), *H. psammophila*, and *H. chrysopsidis*. Regional morphological tendencies in *H. subaxillaris* can be recognized, but my brief study was unsuccessful in sorting out morpho-geographic "nodes" in this phenotypically malleable complex that could be unarbitrarily recognized. Nor has anyone provided a documented (specimen-based) map showing the distribution of these taxa. Commenting on previous studies of sect. *Heterotheca*, including Wagenknecht's, Harms (1968, p. 9) observed that "Perhaps this entire [*H. subaxillaris*] complex should still be accepted as a single, polymorphic, polytypic species." Lammers (1997), in contrast, apparently has identified *H. latifolia* (as distinct from *H. subaxillaris*) with confidence and is able to distinguish all three varieties of *H. latifolia*.

Semple's key to sect. *Heterotheca* gives an overview of the typological concepts in the *H. subaxillaris* complex that may be applied to indicate that one or another plant approaches the typical morphology of a named taxon, but either extensive interregional gene flow or weak primary differentiation, or both (see comments by Burk 1961, 1966), have not made it simple to find geographic patterns to which a meaningful (predictive) taxonomy can be applied. Field and lab study may yet show that such patterns and evolutionary entities exist, but as indicated earlier (Nesom 1990), it will be a considerable challenge to provide this evidence.

Taxonomy of *Heterotheca* sect. *Ammodia*

A taxonomic study of the single species of sect. *Ammodia* (*Heterotheca oregona*) was published earlier (Semple *et al.* 1988). The treatment of infraspecific variation there is similar to that in sect. *Phyllotheca*. Four partially sympatric entities with "no

indication of any pronounced differences in habitat preference" (p. 554) were found to separate with little or no overlap in a multivariate analysis. Following earlier criteria (e.g., Semple 1974), "varietal rank was determined to be most appropriate. The ranges of the four races overlap to a great extent in California, which precludes subspecies status, although each has a unique range" (1988, pp. 549-550). Chromosome numbers have been reported for three of the varieties: all are diploid.

"The varietal differences [within *Heterotheca oregona*] are thought to have evolved as a consequence of geographic isolation. During the Holocene, migration and range expansions have eliminated the spatial isolation and the sympatric races now hybridize" (1988, p. 553). Non-overlap in multivariate analyses, however, and a low frequency of intermediacy ("about 10% of all herbarium specimens . . . studied") seem to indicate that the infraspecific taxa may be separated by substantial internal reproductive isolation. As presented by Semple *et al.* (1988), information suggests that these closely sympatric but little intergrading entities with small morphological differences may be biological microspecies.

Status of *Bradburia*

Semple notes that "circumscription of all the generic limits of the goldenasters remains in turmoil" (p. 7). His only example, however, of problematic generic limits is the question of taxonomic rank for *Bradburia* (as a separate genus vs. a subgroup within *Chrysopsis*). Turmoil is not evident, and given increasing agreement with Semple's arrangement of *Heterotheca*, *Chrysopsis*, and *Pityopsis*, the only controversy appears to involve the *Bradburia* question and what it may imply (for consistency) about the relationship of sect. *Heterotheca* to the rest of the genus (sensu Semple).

In contrast to my decision to merge the genus *Bradburia* with *Chrysopsis* as sect. *Bradburia* (enlarged to two species with the addition of *Chrysopsis pilosa*, Nesom 1991a), Semple has decided to retain *Bradburia* as a separate genus including the same two species. He has observed the close similarity and relationship between *B. hirtella* and *C. pilosa* (Semple & Chinnappa 1984) and accepts the results of recent morphological analyses (Nesom 1991a) and molecular analyses (Lane *et al.* 1996) that place them as sister species. These two, in turn, are the sister group to the rest of *Chrysopsis* in phyletic analyses including other taxa of goldenasters (Nesom 1991b; Lane *et al.* 1996) as well as in Semple's own diagram of goldenaster relationships (1996, p. 6).

Semple's published justification for maintaining *Bradburia* at generic rank is solely his view that a ditypic *Bradburia* could serve as an "alternative solution to the generic limits problem surrounding the goldenasters" (p. 7). He has neither indicated on what grounds he prefers one alternative rather than the other nor provided any discussion of the relative merits or problems regarding the choice of options. Based on his comments and distribution maps, the distinctions between the two genera are summarized as follows.

1. Perennial; leaves and stems with "distinctly flagelliform hairs"; cells of disc corolla throat with elongate crystals; Florida to Mississippi, Louisiana, and Texas, but mostly east of the Mississippi River..... *Chrysopsis*

1. Annual or perennial; leaves and stems with "less to non-flagelliform hairs"; cells of disc corolla throat without crystals or crystals reduced in size; Texas and Louisiana to Missouri and Kansas, Tennessee, Mississippi, and Alabama, but mostly west of the Mississippi River. ..... *Bradburia*

Additionally (Nesom 1991a), these two species differ as a pair from other *Chrysopsis* in longer flowering branches, scarious-margined phyllaries, sharp-pointed sweeping hairs on the style branches, and karyotype.

If the characterization of *Bradburia hirtella* and *Chrysopsis pilosa* as sister species is correct, and if these two are phylogenetically coordinate with the rest of *Chrysopsis*, taxonomic treatment of a ditypic *Bradburia* at either rank (within or distinct from *Chrysopsis*) is consistent with the phylogeny. My study also noted that the enlargement of an independent *Bradburia* was an alternative solution (Nesom 1991a, p. 111): "*Chrysopsis pilosa* and *Bradburia* are so distinct as a pair that *C. pilosa* might justifiably be transferred to *Bradburia*." Does available evidence support a decision regarding the taxonomic placement of ditypic *Bradburia*? And which treatment is more consistent with existing taxonomic arrangements within the Chrysopsidinae?

Within the goldenaster group (subtribe Chrysopsidinae), ditypic *Bradburia* is united with *Chrysopsis* (sensu Semple) by a set of cytological and morphological features: reduced base chromosome number (x=5 or 4; shared with the genus *Osbertia*); long, smooth-walled osteiform trichomes often conspicuously drawn out into flexuous, filamentous extensions; achene shape obovate and asymmetric (shared with sect. *Heterotheca*); achene surfaces with thick, rounded ridges, the nerves completely below the epidermal surface; and pappus insertion inset from the shoulder rim of the achene apex. A significant degree of genetic similarity between the two segments of *Chrysopsis* was demonstrated by hybrids between *C. pilosa* (sect. *Bradburia*) and *C. gossypina* (sect. *Chrysopsis*) synthesized by Semple (1981), who then viewed *C. pilosa* as the sister species to *C. gossypina* and justifiably treated within *Chrysopsis*. As noted above, molecular data also indicates that *Bradburia*/*Chrysopsis* is monophyletic.

The relationship of ditypic sect. *Bradburia* to the rest of *Chrysopsis* appears to be analogous to the relationship of sect. *Heterotheca*, and perhaps of sect. *Ammodia*, to the rest of the genus *Heterotheca* (sect. *Phyllotheca*). Semple (1996) considers sect. *Heterotheca* to be the sister group to rest of the genus, sect. *Ammodia* phyletically coordinate with sect. *Heterotheca*. My cladistic analysis (Nesom 1991b) placed sect. *Ammodia* basal within the genus and sect. *Heterotheca* among other clades, but only weak characters supported this. Sect. *Heterotheca* is a distinct and clearly monophyletic group, but Harms (1965) synthesized viable hybrids between *H. subaxillaris* (sect. *Heterotheca*) and *H. canescens* (sect. *Phyllotheca*). The option ("alternative solution") of segregating sect. *Heterotheca* as a small genus within the goldenasters has often been followed, with the remainder of the *Heterotheca* species placed into an expanded *Chrysopsis*. If sect. *Heterotheca* were segregated today, however, the generally accepted redefinition of *Chrysopsis* would necessitate recognition of a new genus to accommodate the species of sects. *Phyllotheca* and *Ammodia*. Nevertheless, Semple's rationale for segregrating *Bradburia* as a genus

provides a similar one for the treatment of *Heterotheca* s. str. The monotypic sect. *Ammodia* also has been treated as a separate genus (Nuttall 1841) and could be again.

Available evidence and the current taxonomy of the Chrysopsidinae indicate to me that ditypic *Bradburia* (in the current view of its phylogeny) is better viewed as a well-defined subgroup of *Chrysopsis* rather than a weakly separated genus.

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RAPD ANALYSIS OF GENETIC DIVERSITY AMONG AND WITHIN POPULATIONS OF *BALDUINA ATROPURPUREA* AT FORT STEWART, GEORGIA

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ABSTRACT

Compared with other federal land management agencies, the Department of Defense (DoD) has a disproportionately large number of threatened, endangered, and sensitive (TES) plant species known to occur on its lands (Flather *et al.* 1994). In some instances, this has resulted in a conflict between measures necessary to meet conservation requirements for TES species and the ability of the installation to train troops and test weapons and equipment to assure military readiness. To support the mission of the U.S. Army and Fort Stewart, researchers at the U.S. Army Construction Engineering Research Laboratories (USACERL) undertook a multi-scoped project to investigate various aspects of *Balduina atropurpurea*, a federal 'species of concern' (formerly, category 3C under the Endangered Species Act) that is state listed as 'rare' in Georgia (Smith 1994). This particular portion of the project was undertaken to determine relative levels of genetic diversity among and within on-post populations of *B. atropurpurea*. Seedlings from five representative on-post populations were evaluated using Random Amplified Polymorphic DNA (RAPD) marker analysis. Very little genetic variation was detected among or within the on-post populations evaluated. The variation observed was randomly and approximately equally distributed among populations and among individuals within populations.

KEY WORDS: *Balduina atropurpurea*, RAPD analysis, genetic diversity

INTRODUCTION

Compared with other federal land management agencies, the Department of Defense (DoD) has a disproportionately large number of threatened, endangered, and sensitive (TES) plant species known to occur on its lands (Flather *et al.* 1994). In some instances, this has resulted in conflict between measures necessary to meet conservation requirements for TES species and the ability of the installation to train troops and test weapons and equipment to assure military readiness. There is, therefore, great interest in pursuing innovative ways to manage and monitor TES species on DoD lands. To support the mission of the U.S. Army and Fort Stewart, researchers at the U.S. Army Construction Engineering Research Laboratories (USACERL) undertook a multi-scoped project to investigate various aspects of *Balduina atropurpurea* Harper, a federal 'species of concern' (formerly, category 3C under the Endangered Species Act) that is state listed as 'rare' in Georgia (Smith 1994) and known to occur on Fort Stewart.

Balduina atropurpurea (Asteraceae) is a perennial herb that occurs in wet areas of peaty pitcher plant bogs, pine flatwoods, and pine savannas with seasonal standing water (Patrick 1994). The species is endemic to the southeastern Coastal Plain area of the United States (Lutz 1995). Extant populations are known to occur in scattered locations in south to south-central Georgia, northeastern Florida, southeastern Alabama, and southern Mississippi (Helton 1995; Mississippi Natural Heritage Program 1991).

The largest, healthiest known populations of *Balduina atropurpurea* are thought to occur on the U.S. Army's Fort Stewart, Georgia, where 21 populations, distributed across five training areas, have been identified. These populations range in size from < 10 to > 2,000 individuals and cover areas of approximately 1 m^2 to 19,500 m^2 (Helton 1995; Fort Stewart Natural Resources Office, FSNRO 1996, pers. comm). Prior to 1995, only six populations of *B. atropurpurea* were known to occur at Fort Stewart. These populations were identified by The Nature Conservancy (TNC) during a survey of the installation conducted between March 1992 and October 1994 (U.S. Department of Defense 1994). An additional fourteen populations were discovered in 1995 during a survey of the installation by C. Helton (1995) and one population was identified in 1996 by Fort Stewart Personnel (FSNRO 1996, pers. comm.). These populations potentially double the number of known individuals of *B. atropurpurea* in the state of Georgia. The Fort Stewart populations are, therefore, of particular significance to the recovery and future listing status of the species.

Throughout its range, potential threats to the survival of *Balduina atropurpurea* include: alterations to the hydrological regime; loss of habitat to agricultural, commercial, and residential development; and inappropriate site management, particularly fire suppression, resulting in increased shading by shrubs and trees (Smith 1994). Military training exercises that alter the hydrological regime, cause excessive soil disturbance, or suppress the occurrence of fire could negatively impact the Fort Stewart populations. At least 43% of the Fort Stewart populations show significant impacts from tank maneuvers and/or off-road vehicle traffic. Most (>71%) of the populations are in need of prescribed burning to reduce the encroachment of shrubs and woody vegetation and to encourage the establishment of a healthy herbaceous layer. Currently, no U.S. Fish and Wildlife Service recovery plan has been prepared

for *B. atropurpurea*, and there are no existing management plans in place at Fort Stewart specifically designed for this species.

Development of a recovery plan for *Balduina atropurpurea* will be especially challenging as very little is known about the reproductive biology of this species. In the field, individuals typically produce a rosette the first year, with inflorescences produced in the second and subsequent years. Under greenhouse conditions, we observed individuals flowering during their first year of growth. Parker & Jones (1975) reported that *B. atropurpurea* is self-incompatible, and that interspecific hybridization does not occur among species of *Balduina*. They also reported the occurrence of vegetative reproduction from root stocks. R. Determann, Atlanta Botanical Garden, successfully propagated seeds of *B. atropurpurea* following four weeks of cold stratification; the majority of the seeds germinated and produced robust rosettes (R. Determann 1996, pers. comm.). Investigations into the phenology, reproduction, seed dispersal, and seedling establishment of *B. atropurpurea* are needed. Studies evaluating the effects of disturbance, as well as fire frequency and intensity, on the reproduction and health of this species are also necessary.

Knowledge regarding relative levels of genetic diversity among and within populations of *Balduina atropurpurea* at Fort Stewart would aid in determining whether any on-post populations contain unique genetic characteristics. Such populations should be given priority for conservation as their destruction would lead to the potential loss of genetic diversity necessary for adaptation to environmental changes or habitat disturbances. In addition to genetic diversity, other factors such as population health and community structure should be considered when determining the overall biological value of each on-post population.

The objectives of this study were (1) to evaluate the relative levels of genetic diversity among and within a representative sample of on-post populations of *Balduina atropurpurea*; and (2) to examine the relationship among genetic diversity, morphological diversity, and habitat diversity for this species. The information obtained from this study will aid in the development of a management plan for *B. atropurpurea* at Fort Stewart.

METHODOLOGY

Seedlings from five Fort Stewart populations of *Balduina atropurpurea* were obtained from R. Determann, Atlanta Botanical Garden. Seeds collected from the remaining populations were either immature or non-viable and failed to germinate. The five populations evaluated represent a diversity of habitats among the Fort Stewart sites in which the species is found. The seedlings were transported to Colorado State University, transplanted into pots containing a commercial, soilless potting medium (Metro Mix) and placed in a greenhouse. Six individuals each from populations '1', '2', '3', and '4', and four individuals from population '5' were included in the genetic analysis.

DNA was extracted from fresh leaf tissues according to procedures adapted from

Stewart & Via (1993) (Appendix 1). Random Amplified Polymorphic DNA (RAPD) analysis was conducted on the DNA extracts according to procedures adapted from T. Lowrey (unpubl.) (Appendix 2). When the amplification process was complete, 10 µl of electrophoresis tracking dye was added to each reaction tube and the reactions loaded into individual wells on 2.0% agarose gels. The first well on each half of the gel (upper and lower) contained a molecular weight marker of known band sizes. A negative control, without DNA, was included with each set of reactions. Gels were electrophoresed at 80 - 120 mA for approximately 16 - 18 hrs, stained with ethidium bromide for 1 - 2 hr, destained with ddH_2O for 2 - 3 hr, and photographed over UV light using Polaroid Type 665 positive/negative film. Variations in banding pattern among amplification products were analyzed from the resulting photographs.

DNA samples from four randomly chosen individuals were used for initial primer screening. Among 100 primers screened, 59 successfully amplified the DNA samples and were subsequently used in RAPD analysis on the entire set of individuals described above. Individual bands produced with each primer were numbered and scored as present or absent in each amplification product. Comparisons among and within populations were based on the presence or absence, in a given individual, of specific bands produced during amplification. To minimize potential scoring errors resulting from any uncontrollable variation in the amplification environment, only bands of moderate to high intensity (major bands) were scored for evaluation. Faint (minor) bands were excluded from the analysis. Due to the low level of variation in RAPD banding patterns detected among amplification products, quantitative analysis was not deemed appropriate, and the results were evaluated qualitatively.

RESULTS

Fifty-nine of the 100 primers initially screened produced strong amplification products and were evaluated across all samples. Of these, only seven (11.9%) revealed genetic differences among the individuals evaluated, producing a total of 25 scorable bands. Only ten of the 25 bands showed variability among samples (Figure 1). The slight differences in banding patterns produced by these primers were randomly, and approximately equally, distributed among populations and among individuals within a population. No particular individual(s) showed an especially high level of variation for RAPD banding patterns and no particular population(s) contained an especially high proportion of variable individuals (Table 1).

Thirty-one (52.5%) of the primers evaluated revealed no variation for RAPD banding patterns among samples, producing a total of 78 scorable bands (Figure 2).

Despite producing strong amplification products with the four samples evaluated during primer screening, 21 (35.6%) of the primers tested repeatedly produced a large number of non-scorable amplification products when evaluated over all samples. Fifteen (71.4%) of the non-scorable primers failed to amplify a large number of the samples and produced very faint bands among many of the samples that did amplify. High levels of non-specific amplification occurred among a majority of the samples

with two (9.5%) of the non-scorable primers. This resulted in a considerable amount of background smearing, thus preventing definitive scoring of bands. It could not be determined whether either of these two classes of non-scorable primers would have revealed variability among individuals if band scoring had been possible across all samples. No variation was observed, however, among those samples that were successfully amplified with any of these primers.

Four primers (19.0%) produced unique, repeatable banding patterns for each of the samples evaluated. These were also considered non-scorable, as they could not be used to identify relative levels of genetic variation among individuals or populations.

DISCUSSION

Despite showing strong amplification during primer screening, several primers produced non-scorable banding patterns when evaluated across populations. This was most likely due to high levels of contaminants, particularly polysaccharides, complexed with many of the extracted DNA samples (Demeke & Adams 1992; Fang *et al.* 1992). Several methods for removing the contaminants were attempted (Demeke & Adams 1992; Fang *et al.* 1992; Maniatis *et al.* 1982; Murray & Thompson 1980; Ranu 1996, pers. comm.), with varying degrees of success. To compensate for the high levels of complexed contaminants, the concentration of template DNA in each reaction mixture was reduced to 0.5 - 1.0 ng. This allowed for adequate amplification of template DNA with most of the primers evaluated, while reducing interference from the complexed contaminants. Several primers still failed to yield consistently clean amplification products across samples, resulting in either a large number of non-amplified extracts or a high degree of background smearing.

The results of the RAPD analysis indicate the Fort Stewart *Balduina atropurpurea* populations evaluated are quite similar in genetic composition. While only 25% of the Fort Stewart populations were evaluated in this study, these populations represent a diversity of the habitats found among on-post populations. The five populations chosen for genetic diversity analysis also had previously been sampled for morphological variation (D. Lincicome, unpubl. data). Once morphological analysis is complete, these two measures of diversity will be compared. An in-depth study of habitat characteristics also should be conducted for these populations and the results evaluated against morphological and genetic diversity. Site and population data obtained thus far include population size; approximate number of individuals per population; associated herbaceous, shrub, and tree species; soil type and nutrient content; evidence of fire or disturbance; and general site quality (Helton 1995; unpubl. data). The majority of the site and population data have not yet been analyzed. Soil sample analyses revealed similarities among the on-post sites. The Fort Stewart populations occur on slightly acidic (pH 3.9 - 5.0) soils with a sandy loam texture and a relatively low organic matter content (1.0 - 6.5%). Soil nutrient content varied considerably from site to site, particularly levels of phosphorus, potassium, and iron (unpubl. data).

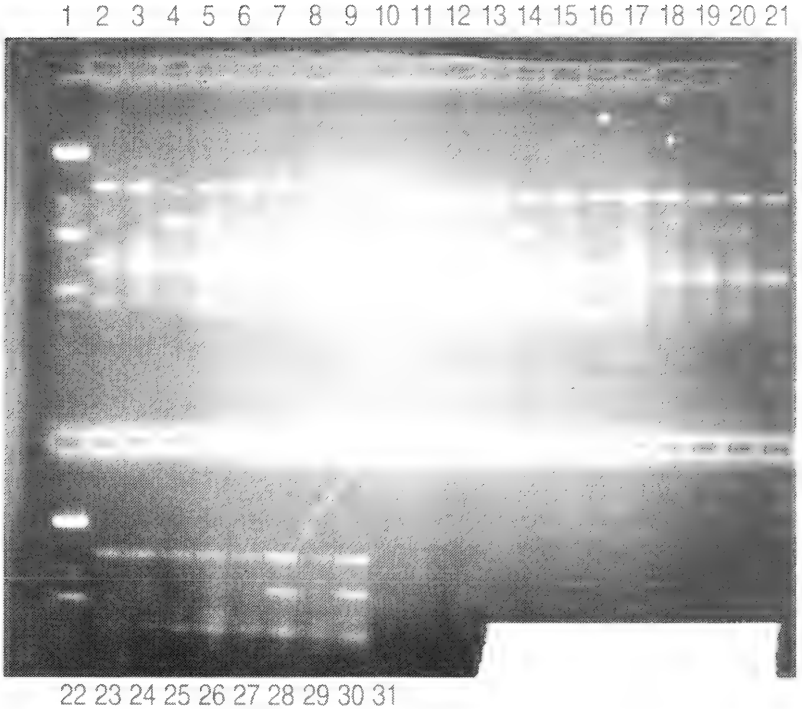

Figure 1. Banding patterns from amplification of *Balduina atropurpurea* DNA using RAPD analysis. Amplification products show variation among DNA extracts. Both inter- and intra-population variation is evident. Lanes 1 and 22: molecular weight marker; Lanes 2-7: DNA extracts from population 1; Lanes 8-13: DNA extracts from population 2; Lanes 14-19: DNA extracts from population 3; Lanes 20-21 and 23-26: DNA extracts from population 4; Lanes 27-30: DNA extracts from population 5; Lane 31: negative control (no DNA).

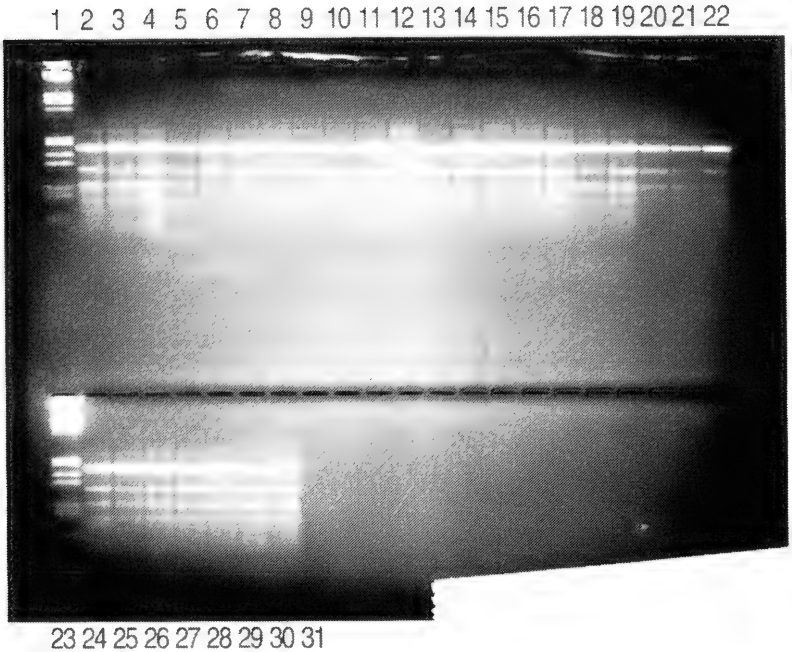

Figure 2. Banding patterns from amplification of *Balduina atropurpurea* DNA using RAPD analysis. Amplification products show no variation among DNA extracts. Lanes 1 and 23: molecular weight marker; Lanes 2-7: DNA extracts from population 1; Lanes 8-13: DNA extracts from population 2; Lanes 14-19: DNA extracts from population 3; Lanes 20-22 and 24-26: DNA extracts from population 4; Lanes 27-30: DNA extracts from population 5; Lane 31: negative control (no DNA).

Although the morphological analysis is not yet complete, initial evaluations revealed differences among the on-post populations for several vegetative and seed characters (D. Lincicome, unpubl. data). The variance observed for those characters, however, is high, thereby reducing differences among character means for the populations (D. Lincicome 1996, pers. comm.). Thus, levels of morphological variation within and among populations may be similar. This is consistent with the results from the RAPD analysis. While little genetic variation was detected overall with RAPD analysis, the variation that was observed occurred with similar frequency both among and within populations.

If considerable inter- or intra-population variability for morphological characters should ultimately be found, this would not necessarily reflect the presence of high levels of genetic variation among or within populations. Morphological variation is a product of differential gene expression and may not be correlated with underlying levels of genetic variation. Populations may be very similar in genetic composition, yet show considerable morphological variation. This can result from differences in phenotypic expression in response to environmental differences (Williams *et al.* 1995) or from interactions among a small number of genes controlling morphological characters (Kochert *et al.* 1991).

Given the out-crossing nature of *Balduina atropurpurea*, it was not unexpected to observe genetic variation within populations, as well as among populations. Many questions remain unanswered regarding the reproductive biology of this species including identification of the primary pollinator(s), the distance viable pollen can "travel" between populations, and the origin(s) of the on-post populations. Thus, the degree to which populations might be genetically differentiated from one another cannot be predicted. Among the populations evaluated, population '5' exists in a distinct location relative to the others and would have the lowest probability for gene exchange with surrounding populations. Population '5', however, did not show a high proportion of genetic variation relative to the other populations evaluated nor was it genetically distinct from the remaining populations based on RAPD banding patterns.

It would be beneficial to evaluate the remaining sixteen on-post populations using RAPD analysis to determine whether any of these populations contain unique genetic characteristics. Such analyses would be time-consuming and expensive given the low levels of genetic variation detected among the populations evaluated thus far. In the short term, conducting in-depth habitat characterizations for each population, in combination with evaluations of reproductive success and population sustainability, would aid management decisions. Ideally, genetic evaluations should occur simultaneously with habitat characterization studies since long-term survival of the species is ultimately dependent upon maintaining adequate genetic diversity among and within populations. Genetic diversity provides a species with a means for better adaptability to environmental changes and/or habitat disturbance.

To obtain a better understanding of the relative value of the Fort Stewart populations compared to surrounding populations, we recommend that habitat characteristics, population parameters, morphological variation, and genetic diversity also be evaluated for several off-post populations and compared to results obtained from the on-post populations. A diverse range of populations should be sampled, including nearby off-post populations in Tattnall and Bulloch counties, Georgia, as

well as more distant populations known to occur in Georgia, Florida, Alabama, and Mississippi. This would aid in the development of a recovery plan for *Balduina atropurpurea* within the region as a whole, in addition to a management plan for the species at Fort Stewart.

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APPENDIX 1
Methodology used for DNA extractions.
Adapted from Stewart & Via (1993).

For each sample, approximately 0.1 g of fresh leaf tissue was harvested and mechanically ground in an individual tissue grinder. Five µl β-mercapto-ethanol and 1 ml warm (60°C) CTAB extraction buffer (2% w/v CTAB, 1.42 M NaCl, 20 mM TRIS-HCl pH 8.0, 2 % w/v PVP, and 5 mM ascorbic acid) were added, and the mixture incubated in a 60°C water bath for 30 min. Each sample was transferred to a clean eppendorf tube and 500 µl chloroform:isoamyl alcohol (24:1) was added. The samples were placed on a mechanical shaker at 100 rpm for 15 min., followed by centrifugation at 10,000 rpm for 5 min. The upper phase of each sample was transferred to a fresh eppendorf tube using a pasteur pipette and re-extracted with chloroform:isoamyl alcohol. The DNA was precipitated out of each sample by adding an equal volume of ice-cold (approximately 0°C) isopropanol to the tube and gently inverting the mixture. Samples were placed in a -20°C freezer overnight to further precipitate the DNA. This was followed by centrifugation at 5,000 rpm for 5 min. The supernatant was discarded and the pellets washed with 500 µl of a 0.2 M sodium

acetate/70% ethanol solution. The sodium acetate/ethanol mixture was added to the tube, and the pellet was dislodged and allowed to soak for 10 min. The samples were briefly centrifuged and the supernatant discarded. The pellets were air-dried and resuspended in 200 µl Tris-EDTA. Extracted DNA samples were stored at -20°C.

The quantity of DNA obtained per sample was measured using a spectrophotometer, based on relative absorption of 260 and 280 nm wavelengths UV radiation passing through the sample. The quality of the DNA extracted from each sample was determined by electrophoresing a subsample through a 1.0% agarose gel, staining the gel with ethidium bromide, and exposing the gel to UV light.

APPENDIX 2
Methodology used for RAPD analysis.
Adapted from T. Lowrey, University of New Mexico (unpubl.).

Each RAPD reaction mixture was prepared by adding the following reagents to a sterile microcentrifuge tube: 17 µl sterile ddH_2O, 5 µl Master Mix [10× Electrophoresis Reaction Buffer (Boehringer Mannheim); 10 mM each dATP, dCTP, dGTP, and dTTP; ddH_2O; and 1 M $MgCl_2$ (magnesium chloride), (bringing the total $MgCl_2$ concentration to 2 mM)], 1 µl (5 picamoles) primer (Operon Technologies, Inc., Alameda, California), and 1 µl diluted DNA sample (0.5 - 1.0 ng). The reaction mixture was gently vortexed, then briefly centrifuged to collect the mixture at the bottom of the tube. Each reaction mixture was overlain with approximately 50 µl electrophoresis grade mineral oil to prevent evaporation during amplification. The reaction tubes were placed into individual wells, to which one drop of mineral oil had been added, in a DNA thermal cycler (MJ Research Inc. PTC 100 Programmable Thermal Controller). The amplification program used was as follows: (Step 1) 'Hot Start' of 2 min. @ 94°C; (Step 2) addition of 0.5 unit Taq DNA polymerase (Boehringer Mannheim) to each reaction tube, @ 80°C (held for 20 min.); (Step 3) time delay of 3 min. @ 94°C; (Step 4) 35 cycles, each consisting of 1 min. @ 94°C (denaturing), 1 min. @ 38°C (first annealing), 30 sec. @ 54°C (second annealing), 2 min. @ 72°C (elongation); (Step 5) 15 min. @ 72°C (final elongation); (Step 6) indefinite soak @ 4°C.

Table 1. Variation in RAPD Banding Patterns Among and Within Populations of *Balduina atropurpurea*.[1,2]

Primer # OPZ-7	
Pattern (+++)	I (B1, B7, B11, B13); II (B22, B35, B38); III (B41, B58, B67); IV (B70, B75, B79); V (B85, B88)
Pattern (+-+)	I (B3, B14); II (B26, B34, B39); III (B46, B49, B64); IV (B71,

B74, B76); V (B87)

Primer # OPZ-9*
Pattern (++) I (B1, B14); II (B34); III (B58, B64); IV (B76, B79); V (B82)
Pattern (+-) I (B3, B7, B11, B13); II (B22, B26, B35, B38, B39); III (B41, B46, B49, B67); IV (B70, B71, B74); V (B85, B87, B88)
* B75 missing (did not amplify with this primer)

Primer OPT-20*
Pattern (++++++) I (B1, B3, B7, B14); II (B26, B35, B38, B39); III (B58, B67); IV (B71, B75, B79); V (B85, B87, B88)
Pattern (++++-+) I (B11, B13); II (B34); III (B41, B46, B49, B64); IV (B70, B74, B76); V (B82)
* B22 missing (did not amplify with this primer)

Primer OPAL-18*
Pattern (+++) I (B7); II (B26, B34); III (B46, B64, B67); IV (B71); V (B85, B88)
Pattern (+-+) I (B3, B11, B13, B14); II (B35, B38, B39); III (B41, B49, B58); IV (B70, B74, B75, B76, B79); V (B87)
* B1, B22, and B82 missing (did not amplify with this primer)

Primer OPA-7*
Pattern (+++) I (B13); III (B46, B58); IV (B76)
Pattern (++-) I (B1, B3, B7, B14); II (B22, B26, B34, B35, B38, B39); III (B41, B49, B64, B67); IV (B70, B71, B74, B75, B79); V (B82, B85, B87, B88)
* B11 missing (did not amplify with this primer)

Primer OPJ-13
Pattern (+++) I (B1, B3, B7, B11, B13, B14); II (B22, B26, B34, B35, B38, B39); III (B41, B46, B49, B58, B64, B67); IV (B70, B71, B74, B79); V (B82, B85)
Pattern (+-+) IV (B76); V (B87)
Pattern (-++) IV (B75); V (B88)

Primer OPJ-10*
Pattern (+++++) III (B46, B58); IV (B70, B74, B76)
Pattern (++++-) I (B3, B7, B11, B13); II (B22, B26, B35, B39); III (B41, B49, B64); IV (B71, B75, B79); V (B82, B85, B87)
Pattern (+++-+) I (B1)
Pattern (++-+-) II (B38)
* B14, B34, B67, and B88 missing (did not amplify with this primer)

1 I - V refer to population numbers.
2 B1, B3, etc. refer to DNA extracts from individual plants evaluated in RAPD analysis.

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SABAZIA LAPSENSIS, A NEW NAME FOR *S. BREEDLOVEI* B.L. TURNER (1997) (ASTERACEAE, HELIANTHEAE), NON *S. BREEDLOVEI* B.L. TURNER (1976)

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ABSTRACT

A new name ***Sabazia lapsensis***, is provided for a later homonym of *Sabazia breedlovei*.

KEY WORDS: *Sabazia*, Heliantheae, Asteraceae, nomenclature, later homonym

K. Gandhi, "keeper" of the Gray Card Index, Harvard University, has called to my attention a grievous lapse in my recent description of *Sabazia breedlovei,* a proposed new species from Guerrero, México, noting that I had already used the latter name (based upon a different type, Turner 1976) for a Chiapan species, although the latter was subsequently transferred to the genus *Alepidocline* (Turner 1990). In short, *Sabazia breedlovei* B.L. Turner (1997) is a later homonym of *S. breedlovei* (1976) and is therefore illegitimate.

To correct this embarrassing error I propose the following:

SABAZIA LAPSENSIS B.L. Turner, *nom. nov.* Based upon *Sabazia breedlovei* B.L. Turner, Phytologia 82:278. 1997. (TYPE: *Breedlove & Almeda 65204*); non *S. breedlovei* B.L. Turner, Wrightia 5:303. 1976. (TYPE: *Breedlove & Smith 77632*).

The specific name is obviously derived from Latin *lapsis,* meaning "slip" or "sleep", a name which I have opted to pin on the taxon for the lapsitic error concerned.

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EFFECT OF FARMYARD MANURE AND MINERAL FERTILIZERS ON COLORATION, GROWTH, AND BIOMASS PRODUCTION OF *AZOLLA PINNATA*

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ABSTRACT

An experiment was conducted in ponds to determine the effect of farmyard manure and mineral fertilizers on the coloration, growth, and biomass production of *Azolla pinnata*. Dark green color was observed in the treatment supplied with farmyard manure and brilliant green color in the treatments supplied with phosphorus, and phosphorus plus nitrogen. Plants without any fertilizer were light green in color. The number of plants, and fresh and dry weight of *A. pinnata* were significantly higher ($P \leq 0.05$) in farmyard manure than other treatments. Addition of phosphorus, and phosphorus plus nitrogen also significantly (≤ 0.05) increased the number of plants, and fresh and dry weights of *A. pinnata* as compared to the control treatment. Results suggest that farmyard manure is more effective and cheaper than phosphorus, or phosphorus plus nitrogen for propagation and multiplication of *A. pinnata*.

KEY WORDS: *Azolla*, Azollaceae, fertilizer, ecology

INTRODUCTION

Azolla is a free floating aquatic fern belonging to the cryptogamic family Azollaceae. It forms symbiosis with the nitrogen-fixing cyanobacterium *Anabaena azollae* (Watanabe 1982). The importance of *Azolla* as an organic input in rice cultivation is extensively reported (Yanni 1991; Kumarasinghe & Eskew 1993; Yanni *et al.* 1994; Kundu & Ladha 1995). The genus is widely distributed throughout temperate and tropical regions with *A. pinnata* as the most prevalent species in Asia (Lumpkin 1987; Liu & Zheng 1992; Kundu & Ladha 1995).

Azolla varies in color under different environmental conditions. Growth of *Azolla* is also greatly affected by physico-chemical factors (Lumpkin 1987; Siddiqi *et al.* 1987; Bonetto & Carcano 1995). The *Azolla-Anabaena* symbiosis has been observed both in the presence of combined nitrogen and in a nitrogen free medium (Lumpkin 1987; Watanabe 1982). But the optimum growth of *Azolla* requires fertilization with phosphorus and sometimes with nitrogen (Watanabe *et al.* 1980; Aziz & Watanabe 1983; Watanabe & Ramirez 1990; Yanni *et al.* 1994). However, use of chemical fertilizers for *Azolla* culture is limited due to economic reasons and environmental restrictions (Kundu & Ladha 1995).

Azolla is found naturally in ditches, ponds and roadside streams in central Punjab, Pakistan. It is gaining importance in rice growing areas due to its nitrogen-fixing ability and green manuring property (Kumarasinghe & Eskew 1993; Kundu & Ladha 1995). The *Azolla-Anabaena* symbiosis can produce 1 ton of green manure per hectare per day containing 3 kg of fixed nitrogen which is equivalent to 15 kg of ammonium sulphate of 7 kg of urea (El-Bassel *et al.* 1994). The benefit of *Azolla* incorporation on the yield of rice has been well demonstrated. *Azolla* has been used as green manure for rice in China, Philippines, and northern Vietnam (Watanabe *et al.* 1989). The use of *Azolla* as green manure in wetland rice was summarized by Watanabe (1987) from internationally coordinated work by the International Network on Soil Fertility and Fertilizer Evaluation for Rice (INSFFER). Watanabe (1987) mentioned that incorporation of one crop of *Azolla* gave an increased yield equivalent to that given by 30 kg urea N per hectare. Siddiqi *et al.* (1985) and Bonetto & Carcano (1995) reported 16-19% grain yield increase over control when *Azolla* was incorporated into rice fields. The use of *Azolla* by farmers is limited by a number of constraints. The most important constraint faced in the use of *Azolla* is the large size of inoculum needed for a limited field area, especially with the difficulties of preservation and transportation (Kulasooriya 1991). Besides, *Azolla* must be produced and distributed fresh among rice farmers just before use. Attempts have been made to identify factors affecting growth and nitrogen-fixing ability of *Azolla-Anabaena* and to develop conditions suitable for its propagation, transportation and utilization in rice (Siddiqi *et al.* 1987; Kumarasinghe & Eskew 1993; Kulasooriya *et al.* 1994; Yanni *et al.* 1994). The current studies were designed to determine the effect of farmyard manure and mineral fertilizers on the coloration, growth, and biomass production of *Azolla*.

MATERIALS AND METHODS

The experiment was conducted in ponds constructed at the National Agricultural Research Center, Islamabad (33° 42′ N, 73° 7′ E, elevation approximately 518 m). *Azolla pinnata* was obtained from the Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan. *Azolla* plants were cultured in ponds measuring 3 × 3 × 1 m filled with irrigation water from a nearby well. The bottom and side walls of all ponds were lined with polyethylene sheets. There were four treatments replicated four times in a completely randomized design. T-1 was control without addition of any fertilizer. T-2 received farmyard manure (FYM) at the rate of 10 ton/ha fresh weight. Farmyard

manure used in this experiment contained buffalo and cow dung, liquid animal excreta, and organic matter including rice and wheat straw. Farmyard manure contained 1.24% nitrogen, 0.75% phosphorus, and 1.07% potassium on a dry weight basis. T-3 was supplied with phosphorus as single super phosphate (SSP) at the rate of 5 kg/ha. T-4 was ameliorated with 5 kg/ha of phosphorus from single super phosphate plus one kg/ha nitrogen from urea. One kg (fresh weight) of healthy *Azolla* plants was added to each pond during late March. Population was recorded after two and five months in each pond. Sampling was done with a wooden quadrat measuring 50 × 50 cm taking five samples from each pond. Change in the color of *Azolla* was observed visually and population was recorded by counting the total number of plants per quadrat at each sampling. Biomass production per quadrat was expressed both as fresh and dry weight. Dry weight was determined by drying the plants in an oven at 60° C for 48 hours. Results were analyzed statistically by the method of Steele & Torrie (1980). Means were compared with the LSD multiple comparison test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Azolla pinnata changed color from green to red within two weeks after application of treatments and remained red until the first week of April when the plants were four weeks old. With the increase in average day and night temperature from 20°/12° C during March to 26°/15° C during April, the color began to change to green. The average humidity was 85% during March and 61% during April. *Azolla* in all the four treatments was green in May, when average day/night temperature was 29°/20° C and average humidity was 71%. The color was again red in June/July when average day/night temperature was 37°/22° C and 36°/25° C, and average humidity was 54% and 70%, respectively.

The color of *Azolla pinnata* was light green in the control treatment but it was dark green when treated with farmyard manure. *Azolla* plants in the phosphorus treatment, and in phosphorus plus nitrogen treatment were brilliant green. Similar observations were made by Watanabe *et al.* (1980, 1989), Siddiqi *et al.* (1985) and Watanabe & Ramirez (1990) who reported that deficiency of phosphorus activates the production of anthocyanin which causes the appearance of red color in *Azolla*.

The research on *Azolla* culture points out several constraints to its universal use (Kulasooriya 1991; Kulasooriya *et al.* 1994; Bonetto & Carcano 1995). Among them, excessive light and temperature have been mentioned (Chung 1987; Fiore & Gutbrod 1987; Watanabe *et al.* 1989). It has been reported that red coloration in *Azolla* due to anthocyanin formation does not affect its growth and nitrogen-fixing ability (Chung 1987; Lumpkin 1987). The change in the color of *Azolla* at the beginning of the experiment may be due to sudden change in environment including low temperature which produced physiological stress in the plants. The average temperature increased from 26°/15° C during April to 29°/20° C during May, and humidity also changed from 61% during April to 71% during May providing optimum conditions for the

growth of *Azolla*. *Azolla* showed green coloration and grew vigorously during this period. The humidity for the normal growth of *Azolla* is above 60% but optimal values range from 85% to 90% (Watanabe 1982). Average temperature increased to 37° C in June reducing the humidity by as much as 54%. Plants developed red color indicating stress conditions. The next change in color was observed during September/October when average humidity was 50% and average day/night temperature was 33°/21° C and 25°/15° C, respectively. The color again changed to red with a drop in temperature below 22°/10° C during the later months.

The number of plants and biomass production of *Azolla pinnata* in various treatments is presented in Table 1. The number, fresh and dry weight of *Azolla* plants two months after treatment were significantly higher ($P \leq 0.05$) with farmyard manure than with other treatments. Addition of phosphorus, and phosphorus plus nitrogen also significantly ($P \leq 0.05$) increased the growth and biomass of *A. pinnata* as compared to the control treatment. Similar trends of growth and biomass production were obtained in all treatments after five months. Fertilizing *Azolla* with compost can be a good practice for the mass cultivation of *Azolla* but conclusive results are not available (Liu & Zheng 1992; Bonneto & Carcano 1995). Phosphorus is also essential for the optimal growth of *Azolla* (Watanabe *et al.* 1980; Aziz & Watanabe 1983; Watanabe & Ramirez 1990; Yanni 1991; Yanni *et al.* 1994). Best results can be obtained if phosphorus is supplemented with some nitrogenous fertilizer because synergistic effects of amelioration promote multiplication and enhance growth of *Azolla* (Yanni 1991; Yanni *et al.* 1994; Bonneto & Carcano 1995).

Table 1. Effect of various fertilizers on the population and biomass production in *Azolla pinnata*.

Treatment	May			August		
	No. of Plants	Fresh Wt. (g)	Dry Wt. (g)	No. of Plants	Fresh Wt. (g)	Dry Wt. (g)
Control	6066a	294.3a	17.9a	6233a	301.6a	15.5a
FYM	9293d	468.0d	25.5c	14800d	660.3d	30.7d
SSP	7000b	337.0b	20.1b	7300b	383.6b	19.6b
SSP + Urea	7830c	390.0c	23.3b	8000c	405.0c	25.3c

Values in each column followed by the same letters are not significantly different at $P \leq 0.05$.
FYM stands for farmyard manure and SSP for single super phosphate.

Results of this experiment suggest that farmyard manure is more effective and cheaper than phosphorus, and phosphorus plus nitrogen for multiplication and

biomass production of *Azolla pinnata*. *Azolla* may be a promising crop for vast areas of rice growing regions and it can also be a good substitute for substantial amounts of expensive nitrogen without environmental damage. *Azolla* can either be repeatedly harvested and incorporated as green manure or intercropped with rice to meet the fertilizer requirements of rice. However, further experiments are required to identify the stress factors affecting growth and multiplication of *Azolla*. Appropriate techniques which are agronomically feasible and socially acceptable also need to be developed for its economical propagation and use in rice. Future studies will be directed along these lines to work out solutions to these problems for sustainable farming systems.

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STUDIES ON GEOCALYCACEAE (HEPATICAE). X. NEW TAXA AND NEW COMBINATIONS IN *CHILOSCYPHUS CORDA* FOR AUSTRALASIA

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ABSTRACT

Chiloscyphus subg. ***Lophocolea*** is a new combination. ***Chiloscyphus erosus***, ***C. fertilis***, ***C. subopposítus***, ***C. edentatus***, ***C. tuberculatus***, ***C. connatifolius***, ***C. parvispinus***, ***C. semiteres*** var. ***retusus***, ***C. mittenianus*** var. ***obtusus***, and ***C. mittenianus*** var. ***symmetricus*** are described as new species and varieties from Australasia. ***Chiloscyphus subporosus*** var. ***inflexifolius*** is a new combination.

KEY WORDS: *Chiloscyphus,* Geocalycaceae, Hepaticae, Australasia

The following new taxa and new combinations are the result of a systematic study of the Australasian species of the genus *Chiloscyphus*, and a treatment of the genus for a second volume of a Manual of New Zealand, Hepaticae. The names are here published separately to make them immediately available for use.

1. ***Chiloscyphus*** subg. ***Lophocolea*** (Dum.) Engel & Schust., *comb. nov.* BASIONYM: *Jungermannia* sect. *Lophocolea* Dum., *Syll. Jungerm. Eur.* 59. 1831. [TYPE: *Jungermannia bidentata* L.]. *Lophocolea* (Dum.) Dum., *Recueil d' Observ. Jungerm.* 17. 1835.

2. ***Chiloscyphus connatifolius*** Engel, *spec. nov.* HOLOTYPE: AUSTRALIA. Tasmania: Gordon River, Gorge Creek, near Pine Landing, sea level, *Engel 14648* (F); Isotype: HO.

   Folia dorsaliter connata. Amphigastrium bidentatum vel usque 0.30-0.45 mm longitudinis bilobum, segmentis semper magnioribus quam armaturis foliorum ceteris praeditum, apicem cetera armatura adjecta carentem evolutum; margines laminarum utrinsecus dente vel cilio armati. Tuberculae cellulorum foliorum conspicuae perevolutaeque. Cellulae amphigastriorum grandes, laeves, etuberculatae.

A combination of features will separate this species from all other species in sect. *Leucophylli*. The leaves are dorsally connate and conspicuously tuberculate. Underleaves are bidentate to bilobed to at most 0.45 mm, and the segments are uniformly larger than any other underleaf armature. Underleaf cells, however, are devoid of tuberculae.

3. ***Chiloscyphus edentatus*** Engel, *spec. nov.* HOLOTYPE: AUSTRALIA. Tasmania: Cradle Mtn.-Lake St. Clair Natl. Park, Ballroom Forest, SW side of Lake Dove, 950-1050 m, *Engel 13993* (F); Isotype: HO.

Plantae ad 2.2 mm latae. Ramificatio precipue vel omnino terminalibus. Folia dorsaliter discreta, verticale vel subverticale, valde dorsaliter assurgentia et precipue erecta vel suberecta, transversa vel subsuccuba. Apex foliorum interdum anguste rotundatus, retuse-bidentatus, symmetrice vel asymmetrice breviter bifidus vel 1-dentatus. Corpora oleosa 2(3), elliptica ad longe linearia.

Chiloscyphus edentatus is allied to *C. subopposítus*, but differs from that species by the predominantly to exclusively *Frullania* type branching; the variable leaf apices, which are narrowly rounded to retuse-bidentate to symetrically or asymmetrically short bifid; the vertical to subvertical, strongly dorsally assurgent and mostly erect to suberect leaves which are consistently free dorsally; and the fewer number of oil-bodies per cell, being 2(3) vs. 3-5 per cell in *C. subopposítus*.

4. ***Chiloscyphus erosus*** Engel, *spec. nov.* HOLOTYPE: NEW ZEALAND. North Is., South Auckland Prov., Plateau E of Waiotapu Valley, ca. 1800 ft., *Allison 3569* (CHR!).

Caules demum plerumque flagelliformes; cellulae caulis parietibus distincte tenuibus praeditae. Amphigastria aspectu ventrali convexa, bifida usque 0.9 longitudinis, marginibus laminae utrinque omnino vel maximam parte appendiculo dentiformi vel laciniiformi armatis. Gemmae abundantes.

This species is related to *Chiloscyphus perpusillus* (Hook. *f.* & Tayl.) Engel, but differs in several respects. The leaf apex (gemmiparous leaves) becomes progressively more erose, and with continued gemmae formation the lobes disappear altogether (the leaf apex then ± broadly rounded). The leaves often with 1-2 accessory lobes at apex lending a ragged appearance. Vegetative branches are all or mostly intercalary (both lateral and ventral).

5. ***Chiloscyphus fertilis*** Engel, *spec. nov.* HOLOTYPE: AUSTRALIA. New South Wales: Lane Cove, *Forsyth 60* as *L. bridellii*--c. sporo.+ (male) (NSW!).

Ramificatio tantum lateri-intercalaris, ramis terminalibus carens. Caules cellulis corticalibus medullaribusque parietibus percrassis praeditis vestiti. Apex marginesque foliorum integri; trigonae gangliiformes. Bracteola foeminea 0.2-0.4 areae bracteae occupans. Lobi perianthii non divisi et integri

vel repandi vel subdenticulati vel usque 1/2 numerii toti loborum parvi-bifidi. Plantae fructibus persaepe adsunt.

The species is related to *Chiloscyphus semiteres*, but differs in 1) branching strictly lateral-intercalary; 2) stems with cortical and medullary cell walls very thick; 3) perianth lobes entire or repand-sparsely denticulate, or with 1-2 of lobes short-bifid, but never with all 3 lobes bifid; and 4) female bracteole 0.2-0.4 bract area.

6. *Chiloscyphus mittenianus* (Col.) Engel

Chiloscyphus mittenianus (Col.) Engel var. ***obtusus*** Engel, *var. nov.* HOLOTYPE: NEW ZEALAND. South Is., Otago Prov.: Mt. Maungatua, W of Mosgiel, 760 m, *Engel 17768* (F); Isotype: CHR.

A varietate typica foliis plerumque dorsaliter connatis, apice non diviso, integro, angustate vel interdum late rotundato differt.

Chiloscyphus mittenianus (Col.) Engel var. ***symmetricus*** Engel, *var. nov.* HOLOTYPE: NEW ZEALAND. South Is., Westland Prov.: Westland Natl. Park, track to Alex Knob, off track to Louisa Peak, 1170 m, *Engel 18973* (F); Isotype: CHR.

A varietate typica foliis uniformiter dorsaliter liberis, apice subaeque vel aeque bifido segmentis piliferis praedito, segmento ventrali in stratum uniseriatum e 5-7(-8) cellulis compositum terminanti differt.

7. ***Chiloscyphus parvispinus*** Engel, *spec. nov.* HOLOTYPE: NEW ZEALAND. South Is., Otago Prov.: S side of Mt. Cargill, just below summit, N of Dunedin, ca. 2200 m, *Engel 17563* (F); Isotype: CHR.

Plantae dioecae. Folia tota bifida, pagina dorsali hispida, supra luminem cuiusque cellulae prominenta brevi-conica e 1(2) cellulis composita obsita, pagina ventrali perlaevi. Segmenta amphigastriorum integra vel 1-2 dentibus armata, armatura nullo modo regulariter opposita. Perianthium et paginae utrinque bractearum gynoicialium hispidae.

Chiloscyphus parvispinus differs from the related *C. gippslandicus* Engel & Schust. of Tasmania and Australia by the 1(2) celled surface teeth, which are juxtaposed over the lumen of most lamina cells; the uniformly hispid, consistently bifid leaves; and the hispid perianth and gynoecial bract surfaces.

8. *Chiloscyphus semiteres* (Lehm.) Lehm. & Lindenb.

Chiloscyphus semiteres (Lehm.) Lehm. & Lindenb. var. ***retusus*** Engel, *var. nov.* HOLOTYPE: AUSTRALIA. New South Wales: Murrumbidgee River, Rules Point, 37 km NW of Adaminaby, *Streimann 7482* (CBG!).

A varietate typica apicibus saepe retusis vel curto-bifidis, lobis plerumque rotundatis differt.

The *Chiloscyphus semiteres* complex also includes the following:

Chiloscyphus platensis (Mass.) Engel, *comb. nov.* BASIONYM: *Lophocolea platensis* Mass., Atti Accad. Sci. Mediche Natur. Ferrara 80(3/4):12. 1906 of NE Argentina and SE Brazil.

9. ***Chiloscyphus subopposítus*** Engel, *spec. nov.* HOLOTYPE: AUSTRALIA. Tasmania: Cradle Mt.-Lake St. Clair Natl. Park, Pine Valley, Cephissus Falls, NNW of L. St. Clair, 850 m, *Engel 14247* - c. sporo. (F); Isotype: HO.

Plantae dioecae, magnae, usque 5 mm latae. Ramificatio maximam partem intercalaris. Folia subopposita horizontalia, late patentia, vulgo dorsaliter connata; apices non congruente breviter bifidi segmento dorsali perparviori; segmentum ventralis folii varium, acutum vel acuminatum interdum apiculatum; margines folii dorsales ventralesque integri. Amphigastria usque 0.5 longitudinis divisa.

Chiloscyphus subopposítus is a close relative of *C. trialatus* (Gott.) Engel & Schust., but may be distinguished from that species by 1) the shallowly divided underleaves, divided to at most 0.35 mm; 2) the dioecious condition; and 3) the opaque and rigid texture.

10. ***Chiloscyphus subporosus*** var. ***inflexifolius*** (Steph.) Engel, *comb. & stat nov.* BASIONYM: *Lophocolea inflexifolia* Steph., Spec. Hep. 6:278. 1922.

11. ***Chiloscyphus tuberculatus*** Engel, *spec. nov.* HOLOTYPE: NEW ZEALAND. South Is., Southland Prov.: Fiordland Natl. Park, Tutoko River, W. of Milford Sound, 50 m, *Engel 18844* (F); Isotype: CHR.

C. aculeato Mitt. similis, sed ramificatione frullanioidea plerumque terminali, tuberculis folii bene evolutis in paene toto celularum conspicuis, foliis dorsaliter liberis usque 0.30-0.35 longitudinis bifidis, cellulis folii medianis 17-23 μm latis × 20-25 μm longis, lobulis bractearum masculorum saccati, a latere viso sacculi verrucoso-mamillato differt.

The species is closely allied to *Chiloscyphus aculeatus* Mitt., but may be distinguished from it by predominately terminal, *Frullania*-type branching; the well-developed tuberculae, which are conspicuous on nearly all leaf cells; the more deeply bifid leaves (divided to 0.30-0.35 mm), which are free dorsally; and the smaller leaf cell size.

The following combination also is required:

Chiloscyphus profundus subsp. ***cladogynus*** (Schust.) Engel, *comb. nov.* BASIONYM: *Lophocolea heterophylla* subsp. *cladogyna* Schust., Hep. Anthoc. N. Amer. 4:223. 1980.

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CERATOZAMIA MIXEORUM (ZAMIACEAE), A NEW SPECIES FROM OAXACA, MEXICO WITH COMMENTS ON DISTRIBUTION, HABITAT, AND SPECIES RELATIONSHIPS

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ABSTRACT

Ceratozamia mixeorum *spec. nov.*, from Oaxaca, México is described and illustrated. The species differs from others in the genus by the presence of both remarkably long peduncles bearing the megastrobili and microstrobili, and large, arching leaves with numerous, wide leaflets. Its affinity is unresolved at present, but it is likely to be close to *C. matudae*. *Ceratozamia mixeorum* is known only from cloud forest on montane peaks of the Sierra Mixes in central Oaxaca, ranging in elevation from 1440 m to 1895 m.

KEY WORDS: *Ceratozamia*, Zamiaceae, Mexico, Oaxaca, systematics

Ceratozamia mixeorum Chemnick, Gregory, & S. Salas-Morales, *spec. nov.* TYPE: MEXICO. Oaxaca: Vicinity of Juquila Mixes, May 1997, *Chemnick, Gregory, & S. Salas-Morales.* HOLOTYPE: HNT; Isotypes: to be distributed to FTG and XAL.

Truncus semihypogaeus, ad 48 cm altus; folia pauca, usque 8, glabra; petiolus teresve, 61-81 cm longus, parte infima dilatatus, pauca spinis armatus; rachis subteres, supra bisulcata, in dimido inferiore, paucis spinis armata, supra fere inermis vel inermis, in cuspidem 10-25 mm longam excurrens; foliala opposita vel subopposita, 30-40 juga, lanceolata vel falcata, 27-37 cm longa, 23-27 mm lata, coriacea, falcata, basi attenuata, apicam lanceolata acuminata, margine integerrima, revoluta; strobilus microsporangiatis linear-cylindricus, 22-24 cm longus, 70-75 mm latus; pedunculus tomentosus, 13.5-15.0 cm longus, 18-20 mm latus; strobilus megasporangiatis cylindricus pendulus, apice mucronatus, 24.0-30.6 cm longus, 12.2-15.2 cm latus; pedunculus tomentosus, 12.5-23.0 cm longus, 1.5-1.9 latus.

Stems mostly solitary, semihypogeous, cylindric 34-125 cm long, 14-18 cm in diameter, smooth, medium brown, with no protruding leaf bases, approximately 20-25% of the mature plants bifurcate, some individuals with up to 4 branches of nearly equal length originating below grade, branches originate from subterranean procumbent stems that are often gnarled and in varying degrees of decomposition; leaves 1.46-1.98 m long, usually in whorls of 5-11, ascending pendulous, recently-emerged and juvenile leaves bright pea-green, turning dark green with age, glabrous, slightly lighter in color on abaxial surface, adult plants with up to 3 crowns of leaves; petiole 45-85 cm long, green, round with an expanded base that is dark reddish brown and forms a distinct ridge at junction with the petiole, 25 mm in diameter at petiole base tapering to 10 mm in diameter at the mid-way point, moderately armed with simple spines 3-5 mm long gradually decreasing in frequency distally, adaxial surface shallowly bisulcate with grooves arising just above the petiole base and extending distally to the first pair of leaflets; rachis round, arching, 50-85 cm, sparsely armed with spines 3-5 mm long gradually decreasing in frequency distally, nearly unarmed on the distal 25%; leaflets linear-lanceolate, acuminate, often falcate, moderately coriaceous with margins slightly revolute and turned upward, with veins neither conspicuously raised nor visible, flat to deflexed on rachis except basal 3-5 "pairs" that are keeled, the median leaflets 24-39 cm long and 21-29 mm wide decreasing slightly in length towards apex, total number of leaflets 49-77 arising opposite to subopposite along rachis inserted 3-4 cm apart; microsporangiate strobilus elongate-conical, solitary, 22-24 cm in length, 7.0-7.5 cm in diameter, tapering gradually towards apex, microsporophylls 14-15 mm wide and 7-8 mm long, yellow green, peduncle 13.5-15.0 cm in length and 18-20 mm in diameter, green but covered with reddish-brown tomentum; megasporangiate strobilus cylindrical, 23.5-30.5 cm in length and 12-15 cm in diameter with mature megasporophylls arranged in 12 vertical "columns" and 8 horizontal "rows", solitary, apiculum truncate, megasporophylls 24-28 mm long and 42-50 mm wide, green suffused with yellow, horns 5 mm long and inserted 10 mm apart, decumbent at tip, with a 3 mm long by 3 mm wide triangulate process evident on the upper facet of the megasporophyll between the horns; megastrobilus horizontal at receptivity and pendant at maturity; peduncle 12.5-23.0 cm in length and 15-20 mm in diameter, green with reddish-brown tomentum; sclerotesta ovoid, smooth, tan, 25-32 mm in length and 18-20 mm in diameter; initial seedling leaf usually with 4 leaflets. Developing strobili of both sexes appear orange in color from a distance due to the concentration of dark red tomentum on light yellow green scales and are borne on peduncles of nearly mature length even when the immature strobili are only 6-7 cm long.

Etymology: The species is named for the people inhabiting the region of distribution.

DISTRIBUTION AND HABITAT

Ceratozamia mixeorum is known only from cloud forest covering the peaks of two adjacent mountains in the extreme eastern Sierra Norte de Oaxaca (Sierra Mixes), ranging in elevation from 1440 m to 1895 m. Precipitation occurs throughout the year.

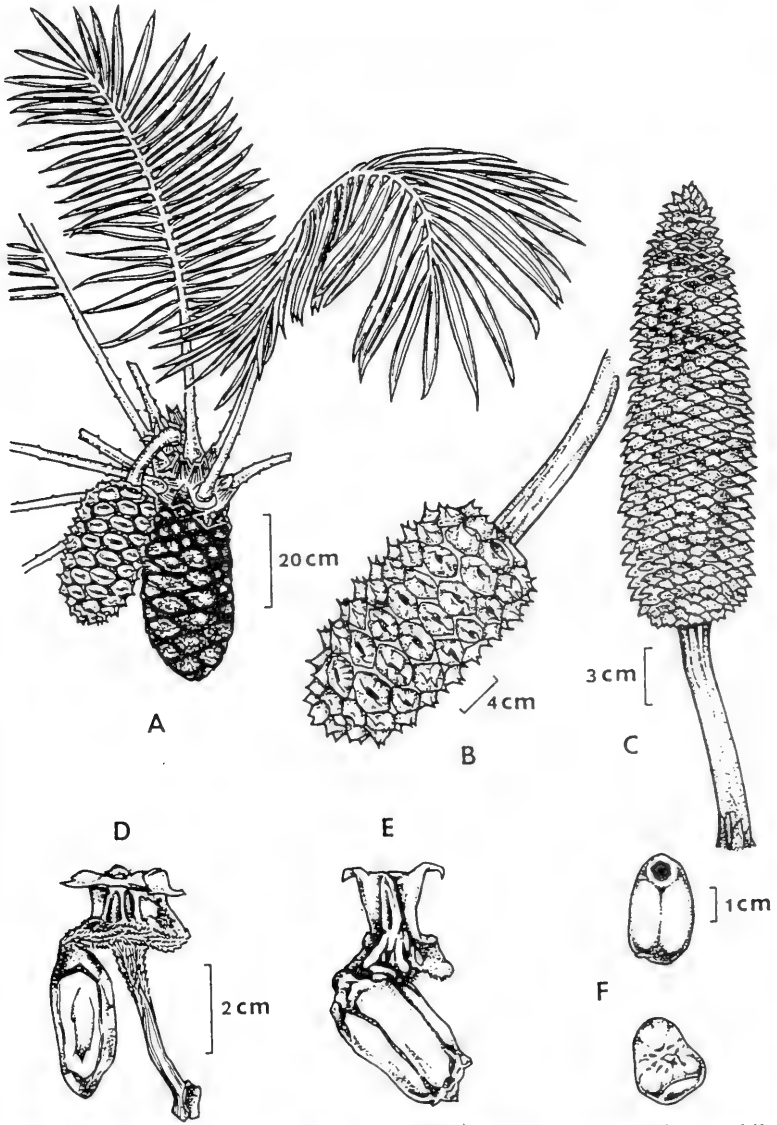

Figure 1. *Ceratozamia mixeorum*. A, Habit of plant with megasporangiate strobilus. B, Megasporangiate strobilus. C, Microsporangiate strobilus. D & E, Megasporophyll with attached seed at maturity in two aspects showing details of sarcotesta. F, Seed showing details of sclerotesta in two aspects.

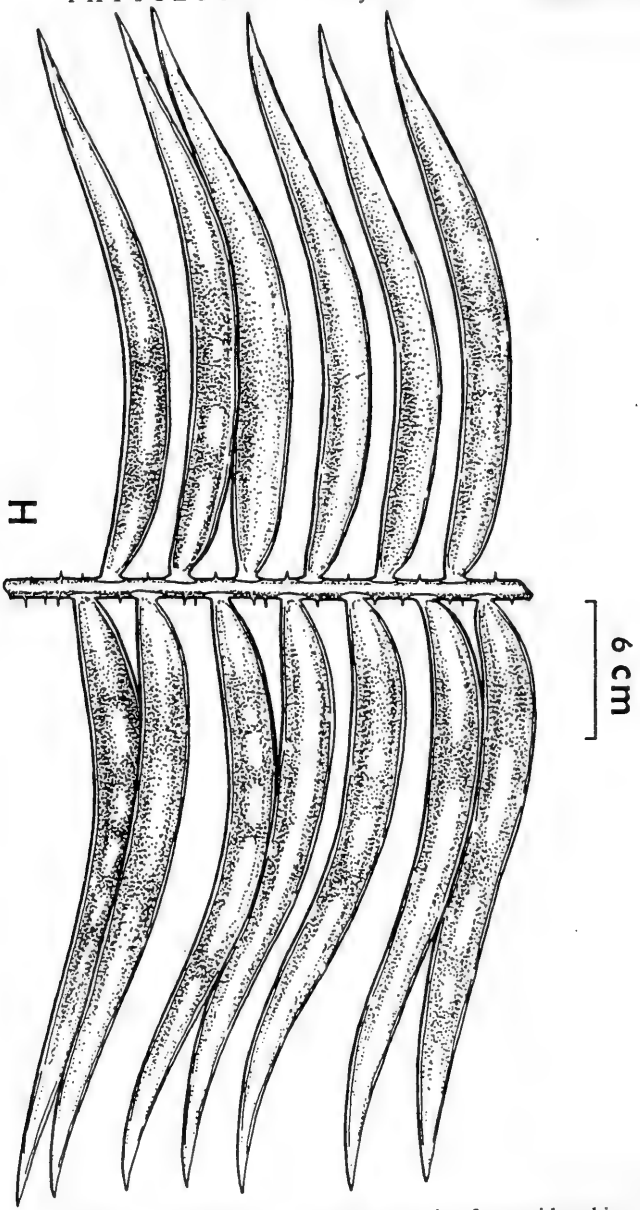

Figure 2. *Ceratozamia mixeorum*. H, Leaf detail; section from mid-rachis.

Habitat consists of very steep slopes with small pockets of remnant primary forest now interdigitated with coffee and secondary growth. The cloud forest consists of two arboreal strata. The upper canopy is comprised chiefly of *Weinmannia pinnata, Liquidambar styraciflua, Cyathea mexicana, Alchornea latifolia, Ticodendron incognitum, Clethra mexicana, Oreopanax xalapensis, Quercus excelsa,* and *Dendropanax.* The understory consists mainly of *Hedyosmum mexicanum, Phyllonoma laticuspis, Rondeletia,* and *Ternstroemia oocarpa* among others. *Ceratozamia mixeorum* is the dominant bushy plant accompanied in the higher elevations of its range by *Eugenia.* Though there is a paucity of herbaceous ground cover, the overstory is laden with an abundance of epiphytes, predominantly orchids and bromeliads. *Ceratozamia mixeorum* occurs on heavily shaded east- and west-facing slopes in primary forest with *Chamaedorea, Geonoma, Melastoma, Acanthus, Ficus, Begonia,* and *Selaginella.* The substrate consists of a light-colored crumbly, rocky clay soil with a pH of 5 and outcroppings of sedimentary rock.

The entire locality is rapidly being cleared and planted almost to the tops of the peaks and thus this cycad must be considered threatened. Local prohibition of further deforestation to protect the watershed is a likely benefactor for this species as well. Since *ex situ* specimens of *Ceratozamia mixeorum* are unknown, it appears that habitat destruction is currently the sole threat to its existence. We have withheld the exact locality to protect it from the depredations of collectors. In our most recent survey of the locality in May, 1997, we observed approximately 500-1000 plants during one day of field work. Seedlings were abundant. Continuous recruitment into the population was evidenced by the occurrence of many juvenile and older plants in a gradation of size up to coning plants. Nearby peaks of the surrounding mountains are likely to contain additional populations of *C. mixeorum* but their existence is yet to be determined because accessibility is difficult. It is noteworthy that numerous individuals persist in the dense scrublike secondary growth just below the primary forest of the mountain tops. The local name of *C. mixeorum* is "carrete" (ox cart) because the children play with the microstrobilus in a related manner.

RELATIONSHIP TO OTHER SPECIES OF *CERATOZAMIA*

Ceratozamia mixeorum is most likely allied to *C. matudae* Lundell (1939) because both taxa possess a long peduncle that is atypical for the genus. Apparently there are several populations of *C. matudae*-like plants with long peduncles that occur in Chiapas currently under investigation (Miguel A. Perez Farrera, pers. comm.). The occurrence of various populations of *Ceratozamia* with elongated peduncles suggests a complex that ranges throughout Chiapas and into central Oaxaca. The Sierra Madre Sur contains several peaks of a similar elevation between the known localities of *C. mixeorum* and *C. matudae* and further field studies will undoubtedly uncover new populations of plants in what is emerging as the "*C. matudae* complex".

Ceratozamia matudae occurs in cloud forest and is characterized by pendant cones borne on elongated peduncles as in *C. mixeorum.* However, the leaves are much shorter and the leaflets narrower in *C. matudae* and the cones are much smaller. *Ceratozamia zaragozae* Medellin-Leal (1963) is characterized by a small, pendant

megastrobilus borne on an elongated peduncle and its leaves are small, spirally ascending, and unarmed. Additionally, *C. zaragozae* occurs in a much drier habitat far to the north. The other known *Ceratozamia* with elongated peduncles have larger leaves than does *C. matudae* but are still much smaller than the leaves of *C. mixeorum*. Vegetatively, *C. mixeorum* is similar to the various *C. mexicana* Brongniart (Vovides *et al.* 1983; Stevenson *et al.* 1986) ecotypes by the presence of large, arching leaves. However, the cylindrical-long shape, smooth texture, and branching habit of the stems are distinct. The peduncle length, combined with the size of the leaf and character of the stem, are diagnostic for *C. mixeorum* and thus it is easily separated from the other similar species of *Ceratozamia* that occur in Oaxaca, *C. robusta* Miquel (Vovides *et al.* 1983; Stevenson *et al.* 1986), and *C. whitelockiana* Chemnick-Gregory (1995).

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THE STATUS OF *PARNASSIA* (SAXIFRAGACEAE) IN THE WEST GULF COASTAL PLAIN

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ABSTRACT

A recent find of *Parnassia grandifolia* DC. from central Louisiana led to a review of the status of *Parnassia* in the West Gulf Coastal Plain.

KEY WORDS: *Parnassia grandifolia* DC., *Parnassia asarifolia* Vent., Saxifragaceae, Louisiana, Texas

Parnassia asarifolia Vent., a southeastern species, is found in only one area of east Texas: northern Nacogdoches and southern Rusk counties (Correll & Johnston 1970; Correll & Correll 1972; Godfrey & Wooten 1981; Johnston 1990; Jones *et al.* 1997). It is not known from Louisiana, Oklahoma, Arkansas, or Mississippi. The closest known occurrence to the Texas site is in Coosa Co., Alabama, 775 km east (Alfred Schotz, pers. comm.).

Parnassia grandifolia DC., a southeastern species, is considered to be rare, threatened, or of special concern throughout most of its range - Virginia south to Florida and westward to Texas and Oklahoma (Correll & Johnston 1970; Correll & Correll 1972; Eakes 1989; Godfrey & Wooten 1981; Johnston 1990; Jones *et al.* 1997). It is found in northern Arkansas (Smith 1988). There are several *P. grandifolia* sites in southern Mississippi (Stone, Perry, Forrest, Newton, and Pearl River counties) (Lowe 1921; Eakes 1989; Steve Leonard, pers. comm.; Ken Gordon, pers. comm.) and in Alabama (Freeman 1978). Although there are numerous seeps in the coastal plain area of Oklahoma, Taylor & Taylor (1978) found *P. grandifolia* in only one, where it was common (Connie Taylor, pers. comm.). It has been reported

from two sites in Texas. Reverchon found *P. grandifolia* in 1902 near Swan, Texas, in Smith County; Bob O'Kennon saw it in northern Newton County in 1985 (Lindsay Woodruff, pers. comm.; Geraldine Watson, pers. comm.; Bob O'Kennon, pers. comm.), but there is no voucher for the Newton Co. locality. It was reported from Louisiana by Riddell in 1852, a report that has been overlooked by subsequent collators of Louisiana plant taxa (Thomas & Allen 1982; MacRoberts 1984, 1989; Louisiana Natural Heritage Program 1995). We searched for Riddell's collection of *Parnassia* from Louisiana but were unable to locate it at US or NO, two repositories where his specimens and those of other early Louisiana botanists may have been housed (MacRoberts 1984). Riddell referred to the specimen as "Parnassia Caroliniana, Michx. var. grandifolia." Where in Louisiana it was found remains unknown.

On March 21, 1997, while surveying for rare plants on the Winn Ranger District of the Kisatchie National Forest, Natchitoches Parish, Louisiana, we found dozens of *Parnassia grandifolia* growing in a small (5 m × 3 m) seepage area in a pine-hardwood forest ravine at the base of a 20° west-facing slope, approximately 200 feet from the top of the adjacent ridge and approximately 20 feet from a 2nd order perennial stream. The plants were growing in sandy, mucky soil at the edge of slow moving water that was surfacing at the seep. The plants were not in flower, but from leaf form and dried scapes of the previous year they were unmistakably *Parnassia*. To confirm our initial identification, we monitored them until they flowered in mid-October. On October 29, 1997, the population had 32 flowering stems: 2 in flower and 30 buds.

Soil information is given in Table 1. Soil at two places in the seep was taken from the upper 10 cm next to the *Parnassia* and analyzed by A & L Laboratories, Memphis, Tennessee.

Table 1. Soil characteristics of *Parnassia grandifolia* site.

		Exchangeable Ions (ppm)				
Sample	pH	P	K	Ca	Mg	OM%
1	5.8	4	39	316	84	2.1
2	5.2	5	51	438	134	3.7

The Louisiana *Parnassia* soils are essentially the same as those analyzed by Eakes (1989) for southern Mississippi *Parnassia* sites except that there may be slightly more calcium and magnesium in the Louisiana samples.

Associated species were *Aster* sp., *Athyrium filix-femina* (L.) Roth, *Callicarpa americana* L., *Carex atlantica* Bailey, *Carex crinita* Lam., *Carex debilis* Michx., *Chasmanthium laxum* (L.) Yates, *Dichanthelium sphaerocarpon* (Ell.) Gould,

Eupatorium fistulosum Barratt, *Gelsemium sempervirens* (L.) St. Hil., *Hypericum hypericoides* (L.) Crantz, *Lyonia ligustrina* (L.) DC., *Melanthium virginicum* L., *Mitchella repens* L., *Myrica cerifera* L., *Myrica heterophylla* Raf., *Osmunda cinnamomea* L., *Osmunda regalis* L., *Oxypolis rigidior* (L.) Raf., *Platanthera clavellata* (Michx.) Luer, *Rhododendron canescens* (Michx.) Sw., *Rhododendron oblongifolium* (Small) Millais, *Rhynchospora glomerata* (L.) Vahl., *Rhynchospora gracilenta* A. Gray, *Rubus* spp., *Scleria triglomerata* Michx., *Smilax glauca* Walt., *Smilax laurifolia* L., *Solidago patula* Muhl. *ex* Willd. var. *strictula* Torrey & A. Gray, *Solidago rugosa* P. Mill., *Toxicodendron vernix* (L.) Kuntze, *Vaccinium* spp., and *Viola primulifolia* L. Canopy species included *Acer rubrum* L., *Magnolia virginiana* L., *Nyssa sylvatica* Marsh., and *Pinus palustris* P. Mill., with a midstory of *Nyssa sylvatica* and *Persea borbonia* (L.) Spreng. The seep was well shaded with total canopy cover.

The ravine in which the seep occurred and surrounding ravines had numerous seeps and three hillside pitcher plant bogs, but none had *Parnassia grandifolia*. However, very few sites appeared to be permanently flowing like the *P. grandifolia* site.

The distribution of *Parnassia asarifolia* and *P. grandifolia* in Arkansas, Texas, Oklahoma, Mississippi, and Louisiana is shown in Figure 1.

DOCUMENTATION

Parnassia grandifolia: LA: Natchitoches Parish, *MacRoberts, MacRoberts, Stacey, Moore s.n.* [NLU]; *MacRoberts & MacRoberts 3341, 3736* [LSU]. OK: Choctaw Co., *Taylor & Taylor 23223, 23596, 23797, 27365* [DUR-BRIT]. TX: Smith Co., *Reverchon s.n.* [BRIT].

Parnassia asarifolia: TX: Rusk Co., *Banks 3957* [ASTC]; Nacogdoches Co., *Nixon 15086* [ASTC], *Lacey 789* [BRIT], *MacRoberts & MacRoberts 3344* [TEX], *MacRoberts & MacRoberts 3343* [LSU].

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The plants were found while the senior authors were engaged in a Challenge Cost-Share Agreement with the Kisatchie National Forest. R. Dale Thomas aided with distribution information. Steve Leonard, Clifton Eakes, and Ken Gordon provided information on Mississippi populations. Bob O'Kennon, Lindsay Woodruff, Dorinda Scott, Geraldine Watson, and Tom Wendt provided information on Texas *Parnassia*. Robert E. Evans and Michael Dehnisch showed us *Parnassia asarifolia* populations in Nacogdoches County, Texas. Connie Taylor provided information on the Oklahoma plants. Alfred Schotz provided information on *Parnassia* in Alabama. Anne S.

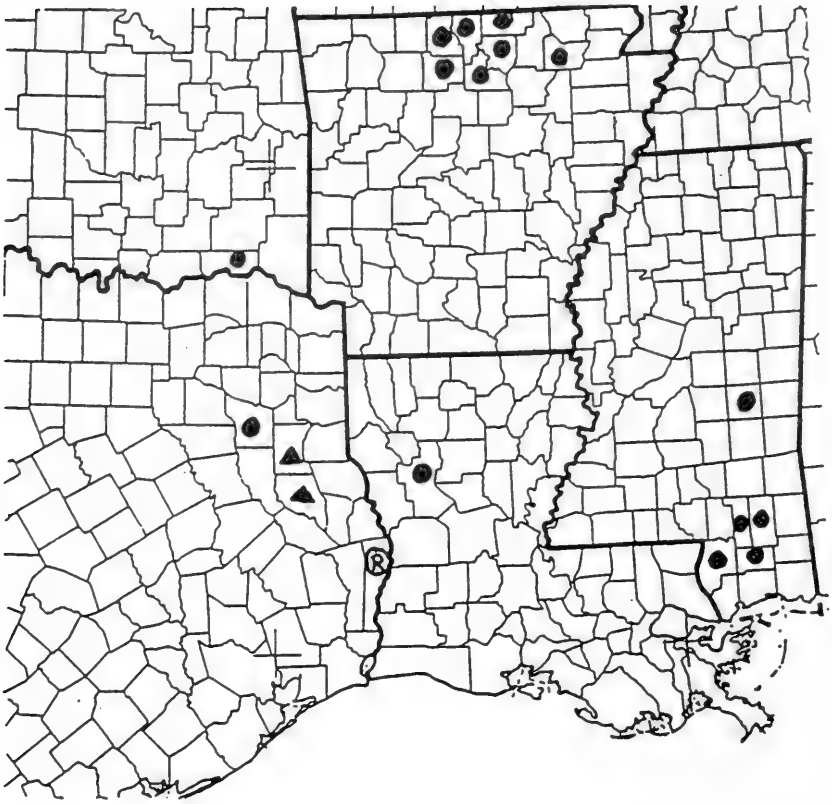

Figure 1. Distribution of Parnassia grandifolia (circles and R) and P. asarifolia (triangles) in Arkansas, Louisiana, Mississippi, Oklahoma, and Texas.

Bradburn and Susan L. Richardson provided information about J.L. Riddell and *Parnassia*. Connie Taylor and R. Dale Thomas reviewed an earlier version of the paper.

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ESTUDIO PALINOLOGICO DE LAS BURSERACEAE DEL ESTADO DE QUERETARO, MEXICO*

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RESUMEN

Se estudia e ilustra la morfología de los granos de polen de la familia Burseraceae del Estado de Querétaro. Los géneros y las especies que comprende son: *Bursera fagaroides* (HBK.) Engl. var. *fagaroides, B. galeottiana* Engl., *B. lancifolia* (Schlecht.) Engl., *B. morelensis* Ramírez, *B. palmeri* S. Wats., *B. schlechtendalii* Engl., *B. simaruba* (L.) Sarg., y *Protium copal* (Schlecht. & Cham.) Engl. var. *copal.*

El polen de las especies de *Bursera* resulto ser aspidado, tricolporado o triporado, esferoidal y con la ornamentación estriada reticulada. En *Protium copal* var. *copal* existen diferencias notables con el del género anterior no es aspidado, es tricolporado con colpos bien definidos y la ornamentación es lisa o psilada. Ambos géneros pudieron ser separados por medios palinológicos.

PALABRAS CLAVE: Burseraceae, morfología polen, sistematica

ABSTRACT

Pollen grains of genera and species belonging to Burseraceae from Querétaro state, México, are studied and illustrated. The taxa are: *Bursera fagaroides* (HBK.) Engl. var. *fagaroides, B. galeottiana* Engl., *B. lancifolia* (Schlecht.) Engl., *B. morelensis* Ramírez, *B. palmeri* S. Wats., *B. schlechtendalii* Engl., *B. simaruba* (L.) Sarg., and *Protium copal* (Schlecht. & Cham.) var. *copal.*

*Trabajo subsidiado por la Dirección de Posgrado e Investigación del IPN.
**Becarios de COFAA.

Pollen of *Bursera* species usually is tricolporate or triporate, spheroidal and with ornamentation striate reticulate and pollen of *Protium* lacks aspides, is tricolporate and prolate, and usually with ornamentation smooth or psilate. These differences facilitate the separation of genera by palynology, according to the key included.

KEY WORDS: Burseraceae, pollen morphology, systematics

INTRODUCCION

La familia Burseraceae la constituyen cerca de 20 géneros y 680 especies que se encuentran distribuídas en las partes tropicales de ambos hemisferios, (Engler 1913). En México según Rzedowski y Guevara (1992) existen dos taxa, *Bursera* y *Protium.*

El primero agrupa más de 100 especies que progresan en los bosques tropicales caducifolios y matorrales xerófilos, se encuentra distribuido desde el sur de Estados Unidos hasta sudamérica, principalmente en la vertiente del Pacífico en México.

A *Protium* lo constituyen cerca de 75 especies que progresan en las zonas tropicales de Asia, Madagascar, Mascareñas, y América (Rzedowski y Guevara 1992).

En el estado de Querétaro se encuentran las siguientes especies: *Bursera fagaroides* (HBK.) Engl. var *fagaroides* (HBK.) Engl., *B. galeottiana* Engl., *B. lancifolia* (Schlecht.) Engl., *B. morelensis* Ramírez, *B. palmeri* S. Wats., *B. schlechtendalii* Engl., *B. simaruba* (L.) Sarg., y *Protium copal* (Schlecht. & Cham.) Engl. var. *copal.*

Los estudios palinológicos, realizados en México de la familia Burseraceae generalmente versan sobre el género *Bursera* y hasta ahora no ha sido descrito, el polen de *Protium* tal vez por ser menos frecuente y contener un número menor de especies en el páis.

El polen de *Bursera* es de gran importancia en otros estudios como en las floras fósiles, donde es considerado como un indicador de clima caliente. Se encuentra en forma abundante en las lluvias de polen actual y como fósil ha sido encontrado por Graham (1976) en los sedimentos del Mioceno Superior en la formación de Paraje Solo de Coatzacoalcos, Veracruz.

Se ha encontrado también en los sedimentos del Mioceno Inferior del norte de Chiapas (Palacios y Rzedowski 1993), cabe agregar que en dichos depósitos también fue encontrado polen fósil de *Protium.*

Sobre la morfología del polen actual de *Bursera* existen varios trabajos regionales de México. Palacios Chávez (1966) describe el polen de siete especies del estado de Morelos; González (1969) estudia el de dos taxa perteneciente a la flora del Valle del Mezquital; Palacios Chávez (1984) en un trabajo más amplio describe el de 49 especies. Rzedowski y Palacios (1985) mediante el análisis de los granos de polen de

Bursera encuentran que dos de las especies pertenecen a *Commiphora*. Palacios Chávez *et al.* (1986) describen el polen de dos especies de la flora del Valle de México, y Palacios Chávez *et al.* (1991) incluyen la morfología de una de las especies que se encuentran en la flora de Sian Ka´an, Quintana Roo. Algunos otros investigadores también han estudiado la palinología de la familia Burseraceae; entre ellos Erdtman (1966), Huang (1972), La Asociación de los palinólogos de la lengua francesa (1974), Lobreau *et al.* (1975), Mitra *et al.* (1977), Roubik & Moreno (1991), y Harley & Daily (1995) quienes describen las Burseraceae de la tribu Protieae.

MATERIALES Y METODOS

Todas las muestras florales donde se obtuvieron los granos de polen fueron tomadas del Herbario (ENCB) del Departamento de Botánica de la Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional, se procuro que todas las colectas fueran de diferentes localidades del estado de Querétaro o de otros lugares cercanos; principalmente cuando no se encontraron ejemplares pertencecientes al estado o las muestras carecian de polen, como es el caso de *Bursera galeottiana*. Los granos de polen fueron procesados con la técnica de la acetolisis de Erdtman levemente modificada y las observaciones se hicieron con el microscopio de luz. En las descripciones se utiliza la terminología de Ertdman (1966), la de Faegri & Iversen (1975), y la de Punt *et al.* (1994). Las medidas de los ejes y diámetros se expresan en micras.

DESCRIPCION DE LOS GRANOS DE POLEN

Bursera fagaroides (HBK.) Engl. var. *fagaroides* 6 km. al NNW de Querétaro, Municipio de Querétaro, *J. Rzedowski 16240*. Lám. I, Figuras 1 a 4.

Polen triporado, aspidado, semitectado, esferoidal, de 30(34)38 × 28(32)35 µm. P/E=1.06. Vista polar circular de 30(32)35 µm de diámetro. Exina de 2.4 µm de grosor, con la sexina de mayor espesor que la nexina, engrosada a la altura de los poros hasta 4.5 µm, superficialmente estriada reticulada. Poros más o menos circulares de 5(6)7 µm de diámetro.

Bursera galeottiana Engl. Ixtla, Municipio el Grande, Estado de Guanajuato, *J. Rzedowski 37545*. Lám. I, Figuras 5 a 7.

Polen tricolporado, aspidado, semitectado, esferoidal, de 30(31)33 × 28(30)34 µm. P/E= 1.03. Vista polar de 30(32)34 µm de diámetro. Exina de 3 µm de grosor, con la sexina y la sexina de igual espesor, engrosándose a la altura de los poros hasta 5

µm, superficialmente estriada reticulada, colpos cubiertos con membranas lisas. Poros lalongados de 5(6)8 µm de largo por 4(5)6 µm de ancho.

Bursera lancifolia (Schlecht.) Engl. 2 km al S de Ayutla, Municipio de Arroyo Seco, Querétaro, *R. Fernández 2461*. Lám. I, Figuras 8 a 11.

Polen triporado, aspidado, semitectado, esférico de 30(32)34 µm de diámetro. Vista polar circular de 30(32)36 µm de diámetro. Exina de 3 µm de grosor, con la sexina y la nexina de igual espesor, engrosándose hasta 6 µm a la altura de los poros, superficialmente estriada reticulada. Poros lalongados de 5(6)7 µm de largo por 2(3)4 µm de ancho.

Bursera morelensis Ramírez Vista hermosa, Municipio de Cadereyta, Querétaro, *J. Rzedowski 43102*. Lám. I, Figuras 12 a 15.

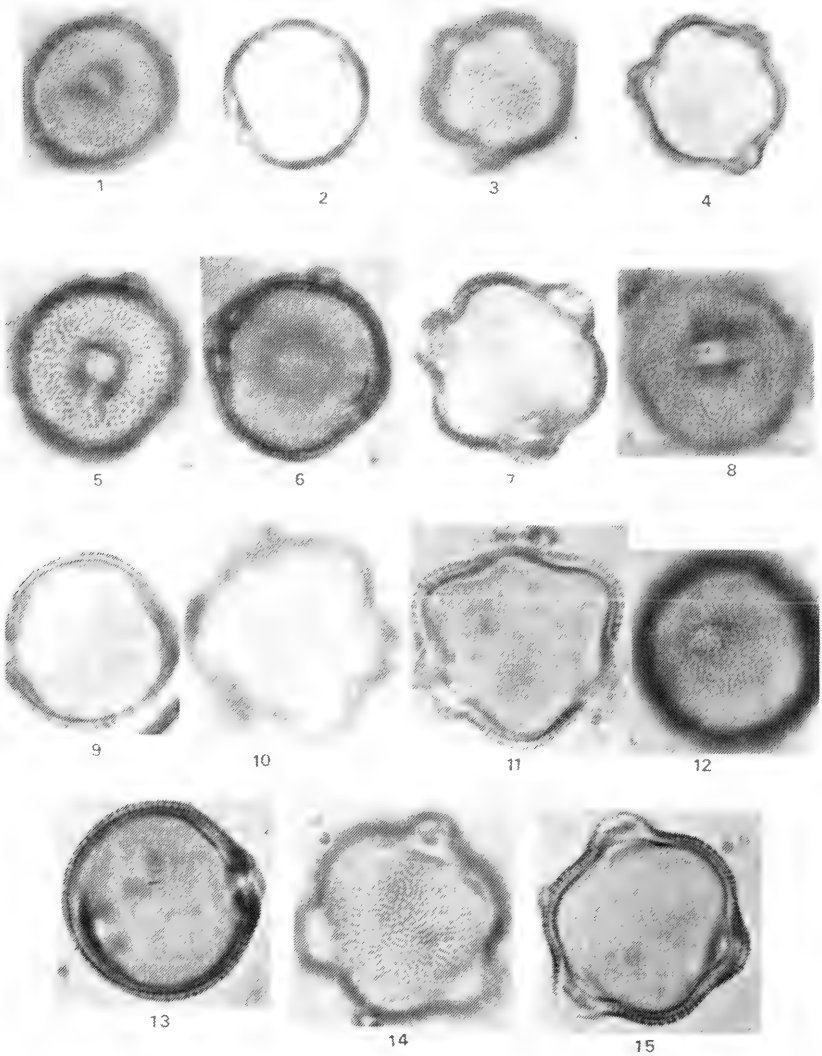
Polen tricolporado, aspidado, semitectado, esferoidal de 30(33)36 m × 28(31)34 µm. P/E=1.01. Vista polar circular de 30(31)34 µm de diámetro. Exina de 2.4 µm de grosor, con la sexina y la nexina de igual espesor, engrosándose a la altura de los poros hasta 4 µm, superficialmente estriada reticulada. Colpos cortos, difusos y mal definidos. Poros lalongados de 6(7)8 µm de largo por 4(5)6 µm de ancho.

Bursera palmeri S. Wats. 5 km al NNW de Querétaro, sobre la carretera a San Luis Potosí, Querétaro, *J. Rzedowski 16242*. Lám. II, Figuras 17 a 20.

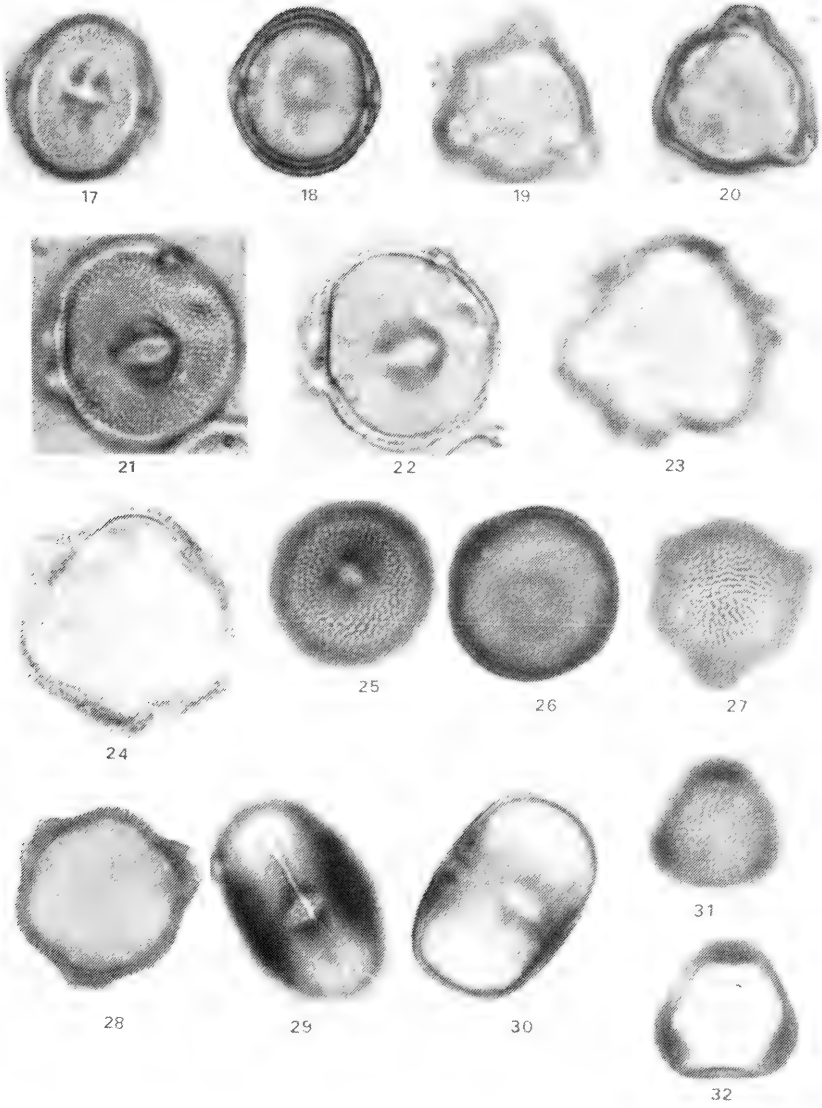
Polen tricolporado, aspidado, semitectado, esferoidal de 26(28)32 × 22(26)30 µm. P/E=1.1. Vista polar circular de 25(28)31 µm de diámetro. Exina de 2.4 µm de grosor, con la sexina y la nexina de igual espesor, engrosándose a la altura de los poros hasta 4 µm, superficialmente estriada reticulada. Colpos cortos y someros. Poros lalongados de 5(6)8 µm de largo por 3(4)5 µm de ancho.

Bursera schlechtendalii Engl. 11 km. al S de Camargo, Municipio de Peñamiller, Querétaro, *R. Fernández 2876*. Lám. II, Figuras 21 a 24.

Polen triporado, aspidado, semitectado, esferoidal de 25(27)29 × 24(26)29 µm. P/E=1.1. Vista polar circular de 25(27)29 µm de diámetro. Exina de 3 µm de grosor, con la sexina y la nexina de igual espesor, engrosándose a la altura de los poros hasta 4 µm, superficialmente estriada reticulada. Poros lalongados de 3(4)5 µm de largo por 2(3)4 µm de ancho.



Lám. I. *Bursera fagaroides* var. *fagaroides*. 1. Vista ecuatorial superficial; 2. Sección transversal; 3. Vista polar superficial; 4. Corte óptico de la vista polar. *B. galeottiana*. 5. Vista ecuatorial superficial, mostrando ornamentación y aberturas; 6. Corte óptico; 7. Corte óptico de la vista polar. *B. lancifolia*. 8. Vista ecuatorial superficial; 9. Corte óptico de la vista ecuatorial; 10. Vista polar superficial; 11. Sección óptica. *B. morelensis*. 12. Vista ecuatorial superficial en óptica; 13. Vista ecuatorial sección óptica; 14. Vista polar superficial; 15. Vista polar en sección óptica.



Lám. II. *Bursera palmeri*. 17. Vista ecuatorial superficial; 18. Vista ecuatorial, corte óptico; 19. Vista polar superficial; 20. Corte óptico de la vista polar. *B. schlechtendalii*. 21. Vista ecuatorial superficial; 22. Corte óptico; 23. Vista polar superficial; 24. Corte óptico de la vista superficial. *B. simaruba*. 25. Vista ecuatorial; 26. Corte óptico de la vista ecuatorial; 27. Vista polar superficial; 28. Vista polar en corte óptico. *Protium copal* var. *copal*. 29. Vista ecuatorial superficial; 30. Vista ecuatorial en corte óptico; 31. Vista polar superficial; 32. Corte óptico de la vista polar.

Bursera simaruba (L.) Sarg. 2 km al NE de Ayutla, sobre el Cañón del río Sta. María, Municipio de Arroyo Seco, Querétaro, *J. Rzedowski 43192*. Lám. II, Figuras 25 a 28.

Polen tricolporado, aspidado, semitectado, esferoidal de 28(33)38 × 27(32)36 µm. P/E=1.03. Vista polar circular de 25(27)29 µm de diámetro. Exina de 2.5 µm de grosor, con la sexina de mayor espesor que la nexina. Colpos cortos y cubiertos con membranas lisas. Poros lalongados de 8(9)11 µm de largo por 3(5)6 µm de ancho.

Protium copal (Schlecht. & Cham.) Engl. var. *copal* La Isla, 6 km al N de Carrizal, Municipio de Jalpan, Querétaro, *R. Fernández 4474*. Lám. II, Figuras 29 a 32.

Polen tricolporado, tectado, prolato de 28(32)37 × 20(23)27 µm. P/E=1.39. Vista polar angular de 20(23)27 µm de diámetro. Exina de 2.4 µm de grosor, con la sexina ligeramente de mayor espesor que la nexina, superficialmente psilada, ligeramente reticulada en los polos. Colpos delgados y bien definidos, cubiertos con membranas lisas. Poros lalongados de 8(9)12 µm de largo por 2.0(3.5)4.0 µm de ancho, cubiertos con membranas granulosas. Indice del área polar 0.4 grande.

CLAVE PARA LA SEPARACION DE GENEROS

1.- Polen aspidado, generalmente esferoidal o esférico con la ornamentación estriada reticulada. ..... *Bursera*
1.- Polen sin áspides, prolato, con la ornamentación lisa o psilada. ..... *Protium*

CONCLUSIONES

Las especies del género *Bursera* del estado de Querétaro presentaron granos de polen con características muy homogéneas. Las aberturas se encuentran en las partes altas de los áspides y pueden ser tricolporadas o triporadas, pudiéndos apreciar polen triporado en *B. fagaroides* var. *fagaroides, B. lancifolia, B. schlechtendalii,* y tricolporado en *B. galeottiana, B. morelensis, B. palmeri,* y *B. simaruba*, la ornamentación en todos los casos es estriada reticulada y la forma generalmente es esferoidal. Los colpos son difusos y mal definidos, pero la abertura central se aprecia visible, generalmente en forma transversal elíptica y siempre se encuentran en la parte alta de los aspides, rara vez circular.

El polen de *Protium* resulto ser muy diferente con las siguientes características palinológicas, carece de aspides, la forma es prolata, tricolporado, con colpos muy

bien definidos y con la ornamentación lisa o psilada, no existen levantamientos sexinosos en los lugares donde se encuentran las aberturas y la vista polar es angular (angulotriaperturada). Las diferencias palinológicas entre ambos géneros permiten la separación de cada taxon.

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MORFOLOGIA DE LAS ESPORAS DEL GENERO *PTERIS* PARA MEXICO

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RESUMEN

Se estudia la morfología de las esporas de catorce especies del género *Pteris* presentes en México: *Pteris altissima* Poir., *P. biaurita* L., *P. cretica* L., *P. erosa* Mickel & Beitel, *P. grandifolia* L., *P. longifolia* L., *P. muricata* Hook., *P. muricella* Fée, *P. orizabae* Mart. & Gal., *P. paucinervata* Fée, *P. podophylla* Sw., *P. pulchra* Schlecht. & Cham., *P. pungens* Willd., y *P. quadriaurita* Retzius. Se elaboro una clave palinológica para separar las especies de acuerdo con la morfología de las esporas, se tomo en consideración la presencia o ausencia de collar ecuatorial, grosor de este y ornamentación. Se dan algunas interpretaciones tomando en consideración la morfología de las esporas y se discute la posición taxonómica de algunas especies con base a los caracteres morfológicos encontrados y a los datos bibliográficos.

PALABRAS CLAVE: *Pteris*, Pteridaceae, Pteridophyta, morfología espora, México

ABSTRACT

This paper deals with the spore morphology of fourteen species of *Pteris* found in México: *Pteris altissima* Poir., *P. biaurita* L., *P. cretica* L., *P. erosa* Mickel & Beitel, *P. grandifolia* L., *P. longifolia* L., *P. muricata* Hook., *P. muricella* Fée, *P. orizabae* Mart. & Gal., *P. paucinervata* Fée, *P. podophylla* Sw., *P. pulchra* Schlecht. & Cham., *P. pungens* Willd., and *P. quadriaurita* Retzius. A key to taxa based on spore characters is included. The main characters useful for identification are presence or absence of equatorial collar, and its thickness and ornamentation. Some possible interpretations of the spore morphology are given, and taxonomic position of some taxa is discussed with regard to spore morphology and bibliographic data.

*Becarios de COFAA del IPN

KEY WORDS: *Pteris*, Pteridaceae, Pteridophyta, spore morphology, México

INTRODUCCION

Pteris es un género de aproximadamente 300 especies, 60 de ellas son americanas (Mickel & Beitel 1988) y en México, a la fecha solo prosperan quince. El propósito del trabajo es el de conocer la morfología de las esporas de *Pteris* y relacionarlas con los estudios taxonómicos del género.

ANTECEDENTES

Entre los trabajos que aportan datos sobre la morfología de las esporas del género *Pteris* tenemos la de Lugardon (1963) en cuyo estudio sobre las Pteridaceae de Francia, describe e ilustra las esporas de *Pteris cretica* L. y *Pteris longifolia* L. Nayar (1964), menciona algunos caracteres de las especies de *Pteris* e ilustra a *Pteris wallichiana* var. *magna*. Lugardon (1974) estudia la infraestructura de la pared de *Pteris longifolia*. Tryon & Tryon (1982), incluyen fotomicrografías de ocho especies americanas del género con breves comentarios sobre la ornamentación. Tryon & Lugardon (1991) mencionan los caracteres generales de 70 especies que estudiaron, de los cuales cuatro prosperan en México, además se incluyen fotomicrografías de diez y ocho taxones. Arreguín-Sánchez *et al.* (1996) estudian las esporas de cinco especies mexicanas del género *Pteris* para el estado de Querétaro.

METODOLOGIA

Se tomaron muestras de las esporas de catorce especies de *Pteris* depositadas en los herbarios de la Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional (ENCB) y del Instituto de Biología de la Universidad Nacional Autónoma de México (MEXU). Las esporas fueron tratadas con la técnica de Plá Dalmau (1961). Las esporas de *Pteris chiapensis* no fueron estudiadas por no encontrarse material de esta especie en los herbarios mexicanos, al parecer es una especie escasa y endémica de Chiapas cuyos ejemplares están depositados en herbarios del extranjero.

RESULTADOS

DESCRIPCION DE LAS ESPORAS

Pteris altissima Poir. 3-4 Km al E de Santa María Chimalpa, Oaxaca, H. Hernández 831 (ENCB). Lámina I, Figuras 1 a 3.

Espora trilete, vista proximal triangular de 30.4(32.7)35.5 por 32.0(34.0)35.5 µm. Vista lateral o ecuatorial ovada, de 30.4(33.1)34.6 por 20.3(23.4)26.2 µm. Exina de 6 µm de grosor. Nexina de 1 µm, sexina de 1 µm y perina de 4µm de espesor, rugulada, vista ecuatorial con collar de 10 µm de grosor. Brazos de la laesura de 10 a 13 µm de largo por 1 µm de ancho.

Pteris biaurita L. Pochutla, Oaxaca, *J. Mickel & Leonard 5166* (ENCB). Lámina I, Figuras 4 a 6.

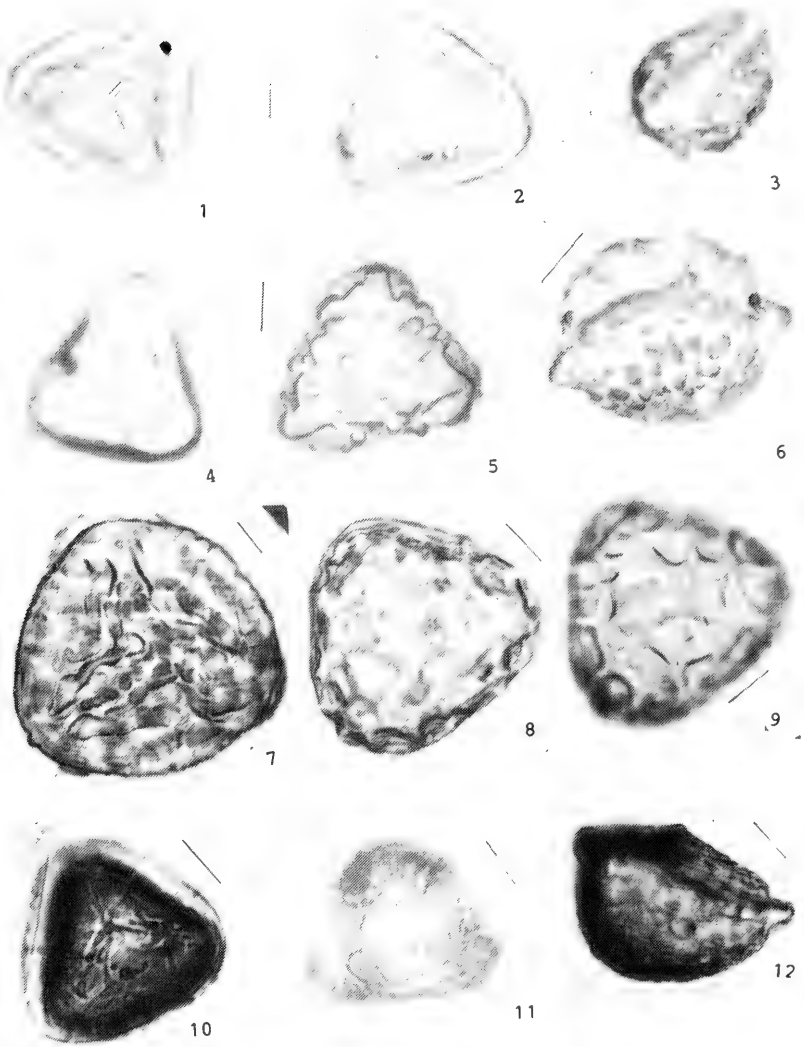
Espora trilete, vista proximal triangular de 39.0(40.7)42.3 por 43.0(45.0)46.5 µm. Vista lateral o ecuatorial ovada de 40.6(41.7)44.0 por 26.0(28.3)30.5 µm. Exina de 5 µm de grosor. Nexina de 1 µm, sexina de 2 µm de espesor, tuberculada y perina de 2 µm de espesor, psilada, vista ecuatorial con collar de 10 µm de grosor. Brazos de la laesura de 13 a 17 µm de largo por menos de 1 µm de ancho.

Pteris cretica L. Cerro Frío, Tlazapotla, Municipio Puente de Ixtla, Morelos, *A. Bonfil 271* (ENCB). Lámina I, Figuras 7 a 9.

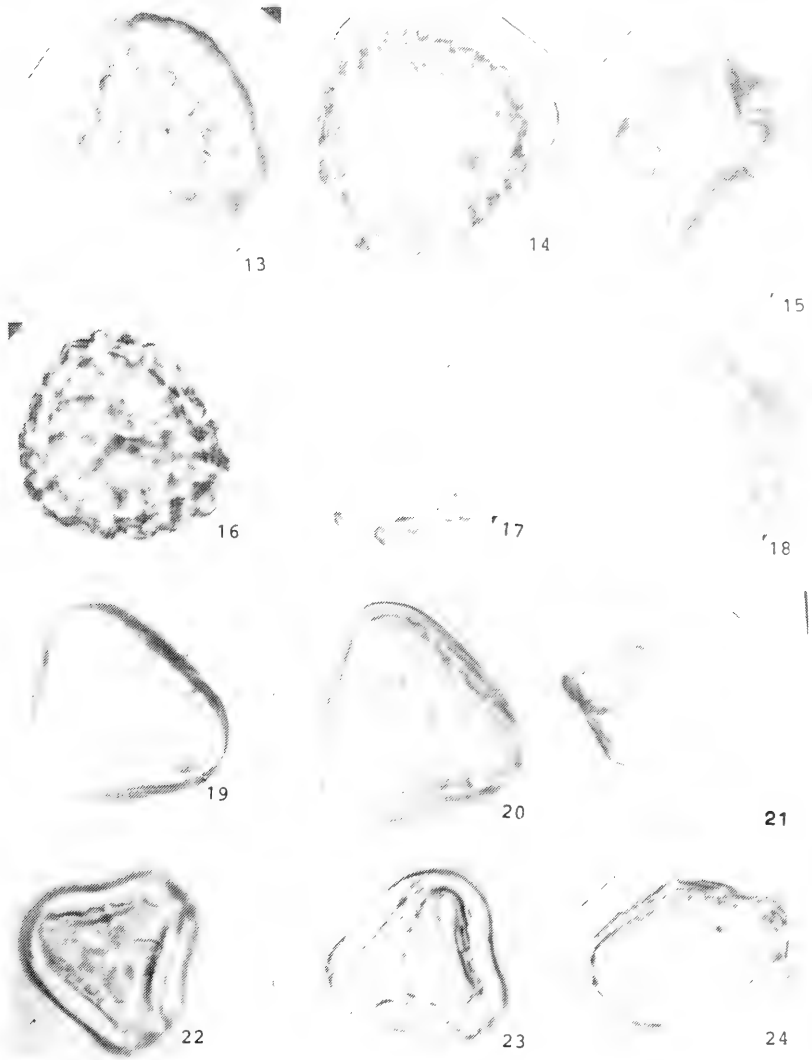
Espora trilete, vista proximal triangular de 48.0(45.0)51.5 por 45(42)48 µm. Vista lateral o ecuatorial ovada de 45.5(41.0)54.0 por 30(33)40 µm. Exina de 6 µm de grosor. Nexina de 1 µm, sexina de 1 µm y perina de 4 µm de espesor, rugulada en vista proximal y reticulada en vista distal, sin collar ecuatorial. Brazos de la laesura de 15 a 19 µm de largo por 2 a 3 µm de ancho.

Pteris erosa Mickel & Beitel. La Cuesta, Talpa de Allende, Jalisco, *R. McVaugh 23376* (MEXU). Lámina I, Figuras 10 a 12.

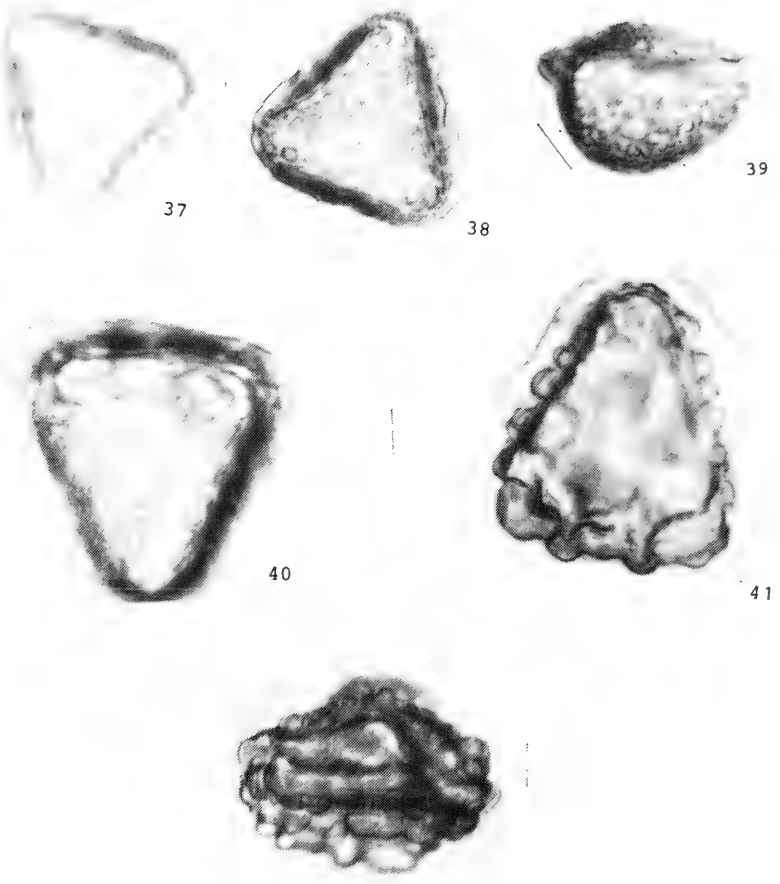
Espora trilete, vista proximal triangular de 33.0(33.5)40.0 por 37.0(39.0)42.3 µm. Vista lateral o ecuatorial ovada de 30.4(32.2)33.8 por 32.1(35.7)39.7 µm. Exina de 3.3 µm de grosor. Nexina menor de 1 µm, sexina de 1 µm y perina de 1.5 µm de espesor, rugulada, vista ecuatorial con collar de 8 µm de grosor. Brazos de la laesura de 13.5 a 16.0 µm de largo por mas o menos de 1 µm de ancho.



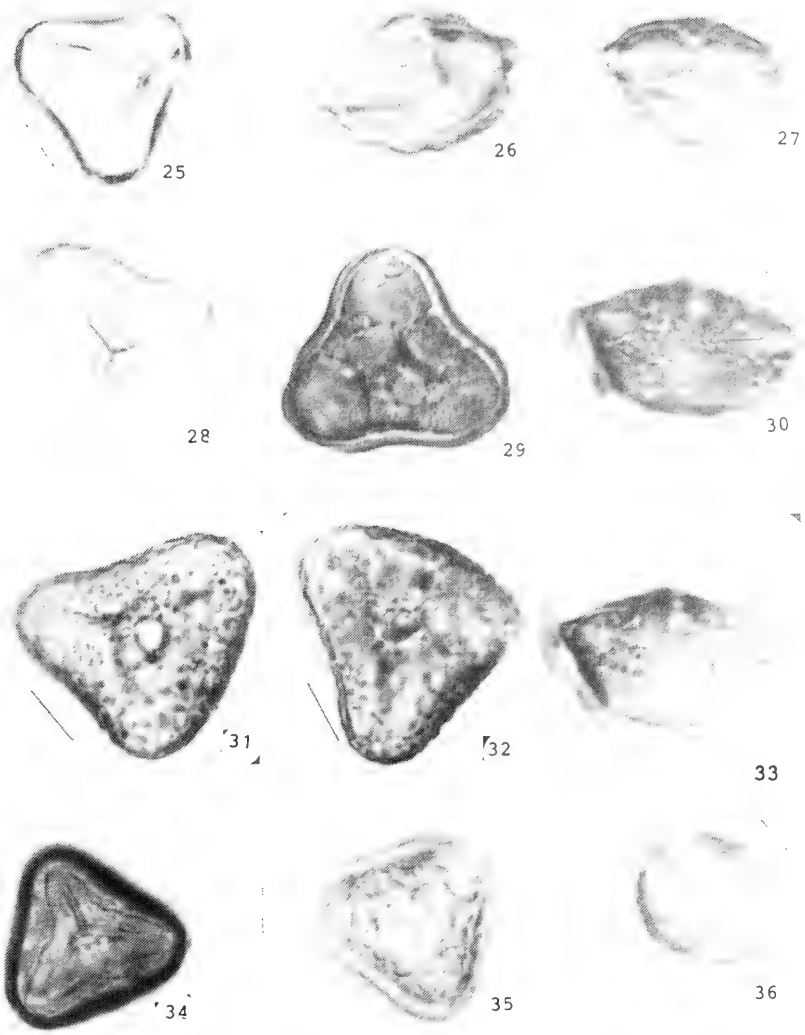
LAMINA I. ***Pteris altissima***. 1.- Vista proximal mostrando la cicatriz. 2.- Ornamentación de la espora. 3.- Vista ecuatorial mostando el collar. ***Pteris biaurita***. 4.- Vista proximal mostrando la cicatriz. 5.- Ormanentación de la espora. 6.- Vista ecuatorial mostrando el collar. ***Pteris cretica***. 7.- Vista proximal mostrando cicatriz. 8.- Vista distal mostrando grosor de la exina. 9.- Ornamentación de la vista distal. ***Pteris erosa***. 10.- Vista proximal mostrando cicatriz. 11.- Ornamentación de la espora. 12.- Vista ecuatorial mostrando el collar. La línea negra que se encuentra al lado de las fotomicrografías corresponde a 10 μ.



LAMINA II. ***Pteris grandifolia***. 13.- Vista proximal mostrando cicatriz. 14.- Ornamentación de la espora. 15.- Vista ecuatorial mostrando el collar. ***Pteris longifolia***. 16.- Vista proximal mostrando cicatriz. 17.- Vista distal mostrando grosor de la exina. 18.- Ornamentación de la vista distal. ***Pteris muricata***. 19.- Vista proximal mostrando cicatriz. 20.- Ornamentación de la espora. 21.- Vista ecuatorial mostrando el collar. ***Pteris muricella***. 22.- Vista proximal mostrando cicatriz. 23.- Ornamentación de la espora. 24.- Vista ecuatorial mostrando el collar. La línea negra que se encuentra al lado de las fotomicrografías corresponde a 10 μ.



LAMINA IV.- ***Pteris pungens*** 37.- Vista proximal mostrando cicatriz. 38.- Ornamentación de la espora. 39.- Vista ecuatorial mostrando el collar. ***Pteris quadriaurita*** 40.-Vista proximal mostrando cicatriz. 41.- Ornamentación de la espora. 42.- Vista ecuatorial mostrando el collar. La línea negra que se encuentra al lado de las fotomicrografías corresponde a 10 μ



LAMINA III. ***Pteris orizabae***. 25.- Vista proximal mostrando cicatriz. 26.- Vista ecuatorial mostrando grosor de la exina. 27.- Vista ecuatorial mostrando el collar. ***Pteris paucinervata*** 28.- Vista proximal mostrando cicatriz. 29.- Ornamentación de la espora. 30.- Vista ecuatorial mostrando el collar. ***Pteris podophylla*** 31.- Vista proximal mostrando cicatriz. 32.- Ornamentación de la espora. 33.- Vista ecuatorial mostrando el collar. ***Pteris pulchra*** 34.- Vista proximal mostrando cicatriz. 35.- Ornamentación de la espora. 36.- Vista ecuatorial mostrando el collar. La línea negra que se encuentra al lado de las fotomicrografías corresponde a 10 μ.

Pteris grandifolia L. 9 Km al sur de Palenque, Chiapas, *R. Fernández 2233* (ENCB). Lámina II, Figuras 13 a 15.

Espora trilete, vista proximal triangular de 39.5(43.5)47.5 por 43.0(46.5)51.5 µm. Vista lateral o ecuatorial ovada de 38.0(39.0)40.5 por 45.5(47.0)50.0 µm. Exina de 7 µm de grosor. Nexina de 1 µm, sexina de 3 µm, tuberculada y perina de 3 µm de espesor, psilada, vista ecuatorial con collar de 12 µm de grosor. Brazos de la laesura de 28 a 32 µm de largo por 2 a 3 µm de ancho.

Pteris longifolia L. Minatitlán, Municipio de Minatitlán, Colima, *López-Ferrari, Espejo, & A. Flores 627* (ENCB). Lámina II, Figuras 16 a 18.

Espora trilete, vista proximal triangular de 42(47)51 pór 40(45)50 µm. Vista lateral o ecuatorial circular de 41.5(44.5)47.5 µm de diámetro. Exina de 6 µm de grosor. Nexina de 1 µm, sexina de 1 µm y perina de 4 µm de espesor, vista proximal rugulada y distal reticulada, en vista ecuatorial sin collar. Brazos de la laesura de 11 a 15 µm de largo por mas o menos 1 µm de ancho.

Pteris muricata Hook. Talquián, Municipio de Unión Juárez, Chiapas, *E. Ventura & E. López 3315* (ENCB). Lámina II, Figuras 19 a 21.

Espora trilete, vista proximal triangular de 33.0(35.3)37.2 por 35.5(37.3)39.0 µm. Vista lateral o ecuatorial ovada de 28.0(29.7)31.3 µm de largo por 37.2(39.2)41.4 µm de ancho. Exina de 3.3 µm de grosor. Nexina menor de 1 µm, sexina de 1 µm y perina de 1.3 µm de espesor, psilada, vista ecuatorial con collar de 11 µm de grosor. Brazos de la laesura de 10 a 13 µm de largo por 1.5 a 2.0 µm de ancho.

Pteris muricella Fée. Laguna Ocotal, Municipio Ocotsingo, Chiapas, *D.E. Breedlove 33076* (ENCB). Lámina II, Figuras 22 a 24.

Espora trilete, vista proximal triangular de 28.7(30.4)32.0 por 33.0(34.5)36.3 µm. Vista lateral o ecuatorial ovada de 23.6(25.0)26.2 µm de largo por 34.6(37.0)39.0 µm de ancho. Exina de 3.3 µm de grosor. Nexina menor de 1 µm y sexina de 1.5 µm de espesor, tuberculada, perina de 1 µm, vista ecuatorial con collar de 10 µm de grosor. Brazos de la laesura de 8 a 12 µm de largo por 1.0 a 1.5 µm de ancho.

Pteris orizabae Mart. & Gal. Chipoca, cerca de Otongo, Municipio Tlalchinol, Hidalgo, *J. Rzedowski 32479* (ENCB). Lámina III, Figuras 25 a 27.

Espora trilete, vista proximal triangular de 27(29)36 por 31(38)36 µm. Vista lateral o ecuatorial ovada de 28(30)31 µm de largo por 28(35)43 µm de ancho. Exina de 3.5 µm de grosor. Nexina de 1 µm, sexina de 1 µm y perina de 1.5 µm de espesor, rugulada, vista ecuatorial con collar de 18 µm de grosor. Brazos de la laesura de 8 a 11 µm de largo por 1.5 a 2.0 µm de ancho.

Pteris paucinervata Fée. Km 10 camino Omiltemi, Guerrero, *F.G. Lorea 1171* (ENCB). Lámina III, Figuras 28 a 30.

Espora trilete, vista proximal triangular de 37.0(44.4)50.7 por 43.0(48.4)54.0 µm. Vista lateral o ecuatorial ovada de 41.4(42.3)43.0 µm de largo por 28.0(31.8)34.6 µm de ancho. Exina de 2.5 µm de grosor. Nexina menor de 1 µm y sexina de 1.7 µm de espesor, escabrosa, parecen unirse las escabras en forma irregular unas con otras, con collar ecuatorial de 9 µm de grosor. Brazos de la laesura de 15 a 17 µm de largo por 1 µm de ancho.

Pteris podophylla Sw. Dos Lagos, Municipio de la Trinitaria, Chiapas, *D.E. Breedlove 56487* (ENCB). Lámina III, Figuras 31 a 33.

Espora trilete, vista proximal triangular de 31(33)38 por 33.0(36.3)40.6 µm. Vista lateral o ecuatorial de 24.5(26.4)29.6 µm de largo por 36.3(39.0)42.3 µm de ancho. Exina de 2.5 µm de grosor. Nexina menor de 1 µm y sexina de 1.6 µm de espesor, psilada, vista ecuatorial con collar de 9 µm de grosor. Brazos de la laesura de 11 a 13 µm de largo por 1.0 a 1.5 µm de ancho.

Pteris pulchra Schlecht. & Cham. Santiago Atzalán, Municipio Atzalán, Veracruz, *F. Ventura 19494* (ENCB). Lámina III, Figuras 34 a 36.

Espora trilete, vista proximal triangular de 31.3(33.6)36.3 por 33.0(35.4)38.0 µm. Vista lateral o ecuatorial ovada de 35.5(37.0)39.7 µm de largo por 22.8(24.7)27.0 µm de ancho. Exina de 3.3 µm de grosor. Nexina menor de 1 µm y sexina de 1.0 µm de espesor, rugulada, perina de 1.5 µm, psilada, vista ecuatorial con collar de 13 µm de grosor. Brazos de la laesura de 8.5 a 13.0 µm de largo por 1.5 a 2.0 µm de ancho.

Pteris pungens Willd. 7 Km al N de Santa María Chimalapa, Oaxaca, *H. Hernández 686* (ENCB). Lámina IV, Figuras 37 a 39.

Espora trilete, vista proximal triangular de 39.0(41.5)44.8 por 37.2(40.0)43.0 µm. Vista lateral o ecuatorial ovada de 37.0(39.6)42.3 µm de largo por 25.3(28.0)30.4 µm de ancho. Exina de 6 µm de grosor. Nexina de 1 µm y sexina de 2.5 µm de espesor, tuberculada, perina de 2.5 µm de grosor, psilada, vista ecuatorial con collar de 11 µm de grosor. Brazos de la laesura de 15 a 18 µm de largo por 2 µm de ancho.

Pteris quadriaurita Retzius. La Barranca, Municipio de Teocelo, Veracruz, *F. Ventura 18217* (ENCB). Lámina IV, Figuras 40 a 42.

Espora trilete, vista proximal triangular de 52.4(54.3)55.8 por 56.6(58.6)61.0 µm. Vista lateral o ecuatorial ovada de 52.4(56.7)61.0 µm de largo por 30.4(32.4)33.8 µm de ancho. Exina de 8 µm de grosor. Nexina de 1 µm, sexina de 3.5 µm de espesor, tuberculada, perina de 3.5 µm de grosor, psilada, vista ecuatorial con collar de 11 µm de grosor. Brazos de la laesura de 17 a 20 µm de largo por 2.5 a 3.0 µm de ancho.

Con base a la morfología de las esporas podemos separar las especies de *Pteris* en los siguentes grupos tomando en consideración la presencia o ausencia de collar equatorial, ornamentación y grosor del collar ecuatorial.

1.- Ausencia de collar ecuatorial; vista proximal rugulada, distal reticulada..... *P. cretica*; *P. longifolia*
1.- Presencia de collar ecuatorial..... 2
2.- Exina tuberculada..... *P. biaurita*; *P. grandifolia*; *P. muricella*; *P. pungens*; *P. quadriaurita*
2.- Exina rugulada, psilada, o escabrosa..... 3
3.- Exina rugulada..... 4
4.- Grosor del collar ecuatorial menor de 15 µm..... *P. altissima*; *P. erosa*
4.- Grosor del collar ecuatorial 18 µm..... *P. orizabae*
3.- Exina psilada o escabrosa..... 5
5.- Exina psilada..... *P. muricata*; *P. podophylla*; *P. pulchra*
5.- Exina escabrosa..... *P. paucinervata*

CONCLUSIONES

Pteris presenta esporas triletes, generalmente con collar ecuatorial, vista proximal triangular, y distal ovada, en algunas especies la vista proximal y distal con diferente

ornamentación como *P. cretica* y *P. longifolia*; con exina rugulada como *P. altissima*, *P. erosa*, y *P. orizabae*; exina psilada como *P. podophylla*, *P. muricata*, y *P. pulchra*; escabrosa como *P. paucinervata*; y tuberculada como *P. biaurita*, *P. grandifolia*, y *P. muricella*.

Autores como Tryon & Tryon (1982) dividen al género en cinco grupos: Grupo *Pteris deflexa* donde se incluyen *P. altissima, P. muricata, P. muricella*, y *P. podophylla*; Grupo *Pteris quadriaurita* con *P. biaurita, P. pungens*, y *P. quadriaurita*; Grupo *Pteris longifolia* con *P. longifolia*; Grupo *Pteris cretica* con *P. cretica*; y grupo *Pteris haenkeana* con *P. grandifolia, P. orizabae, P. paucinervata, P. pulchra, P. pungens*, y *P. erosa* no son considerados en ninguno de estos grupos, citados por Tryon & Tryon (1982).

Al comparar las divisiones taxonómicas con las observaciones palinológicas observamos que en el Grupo de *Pteris deflexa, P. altissima* presenta exina rugulada, *P. muricata* y *P. podophylla* psilada y *P. muricella* tuberculada. Del grupo *Pteris quadriaurita, P. biaurita, P. pungens*, y *P. quadriaurita* presentan esporas muy semejantes con ornamentación tuberculada. Los grupos *Pteris cretica* y *Pteris longifolia* las especies que se estudiaron en este trabajo presentan esporas muy semejantes con ausencia de collar ecuatorial, vista proximal rugulada y distal reticulada, por lo que palinológicamente no se justifica la separación en dos grupos taxonómicos diferentes y por último, el grupo de *Pteris haenkeana* presentan ornamentación muy diversa, por ejemplo, *P. grandifolia* y *P. pungens* con ornamentación tuberculada, *P. orizabae* y *P. erosa* rugulada, *P. paucinervata* escabrosa, y *P. pulchra* psilada. Por lo anterior, se considera que la separación taxonómica no corresponde con la morfología de las esporas.

Tryon & Tryon (1982) indican que una característica de las esporas de *Pteris* es la presencia de un collar equatorial, sin embargo, Tryon & Lugardon (1991) indican que generalmente las esporas de *Pteris* presentan este collar, quizás este cambio de apreciación se deba a que nueve años después se han estudiado más especies de este género y se ha observado que pueden existir especies en las cuales no este presente este collar.

Mickel & Beital (1988) señalan que *Pteris erosa* y *P. orizabae* son especies muy cercanas y a nivel palinológico se encontró similitud entre ambos taxa, ambos con ornamentación rugulada, solo que en la primera la ornamentación de la exina mucho más conspícua que la segunda. También indican que varios ejemplares de *P. pungens* son confundidos con *P. paucinervata*, sin embargo, estas dos especies se separan fácilmente por sus esporas, la primera con esporas con ornamentación tuberculada y la segunda con ornamentación escabrosa.

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