Metaxya lanosa, a Second Species in the Genus and Fern Family Metaxyaceae

ALAN R. SMITH

University Herbarium, 1001 Valley Life Sciences Building #2465, University of California,
Berkeley, California 94720-2465

HANNA TUOMISTO

Department of Biology, University of Turku, FIN-20014 Turku, Finland

KATHLEEN M. PRYER and JEFFERY S. HUNT

Department of Botany, The Field Museum of Natural History, 1400 S. Lake Shore Drive,
Chicago, Illinois 60605-2496

PAUL G. WOLF

Department of Biology, Utah State University, Logan, Utah 84322-5305

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ABSTRACT. We describe and illustrate **Metaxya lanosa,** the second known species in the genus and the fern family Metaxyaceae (Pteridophyta). It is currently known from four different watersheds in Amazonian Peru and Venezuela. It can be distinguished readily from *M. rostrata* by the noticeably woolly-hairy stipes and rachises (hairs red-brown or orange-brown and easily abraded), broader, more elliptic pinnae, cartilaginous and whitish pinna margins, more distinct veins abaxially, and longer pinna stalks, especially on the distal pinnae. *rbcL* data from a very limited sampling are ambiguous but do not reject support for the recognition of at least two species within *Metaxya*.

The genus *Metaxya* (Metaxyaceae) heretofore has been considered a monotypic genus in a monotypic family (Tryon 1970; Tryon and Tryon 1982; Kramer in Kubitzki 1990). Metaxyaceae is a member of the tree-fern assemblage of families (Smith 1995), which together are a well-supported monophyletic group (Hasebe et al. 1995; Pryer et al. 1995). Although relationships among the major lineages of tree ferns are still unclear (Wolf et al. 1999), *Metaxya* is likely the sister group of a clade comprising *Dicksonia* and *Calochlaena* (Dicksoniaceae), *Lophosoria* (Lophosoriaceae), *Cyathea* (Cyatheaceae), and *Hymenophyllopsis* (Hymenophyllopsidaceae), or at least intimately related to these four families (Hasebe et al. 1995; Pryer et al. 1995; Stevenson and Loconte 1996; Pryer et al. 1999; Wolf et al. 1999).

The finding of a second species of *Metaxya* in South America arouses considerable interest. The well-known *Metaxya rostrata* (Kunth) C. Presl is widespread and common in lowland Amazonian forests and also outside this area, occurring from southern Mexico (Chiapas) to Panama, Guadeloupe, Trinidad, and Colombia to the Guianas, Ecuador,

Peru, Bolivia, and northern Brazil. The new species is known from Amazonian Venezuela and Peru, from four different watersheds, and is likely to be found in white-sand areas of Colombia and Brazil as well. We name it for the densely woolly hairs of the stipe and rachis, because this is one of the most striking characteristics of the species, even though the hairs are easily abraded and may be lost through careless or rough handling of specimens.

Metaxya lanosa A. R. Sm. & H. Tuomisto, sp. nov. (Fig. 1).—TYPE: PERU. Loreto: Distr. Maynas, Experimental station ''El Dorado'' of INIA, Km 25 along the road Iquitos-Nauta, low-statured forest (varillal bajo húmedo) on white-sand soil, 100–200 m, 3°57′05″S, 73°24′31″W, 24 Sep 1998, *Tuomisto & Ruokolainen 13054* (holotype USM!; isotypes AMAZ!, TUR!, UC!). *Paratypes*: **Peru.** Loreto: Distr. Iquitos, El Dorado-INIA, Varillal Alto Húmedo, 03° 57′ 07″ S, 73° 24′ 36″ W, *Vásquez et al. 23785* (MO, UC). **Venezuela:** Amazonas: Dpto. Atures, río Coro-Coro, W of Serrania de Yutaje, 8 km N of settlement of Yutaje, 05° 41′ 45″ N, 66°
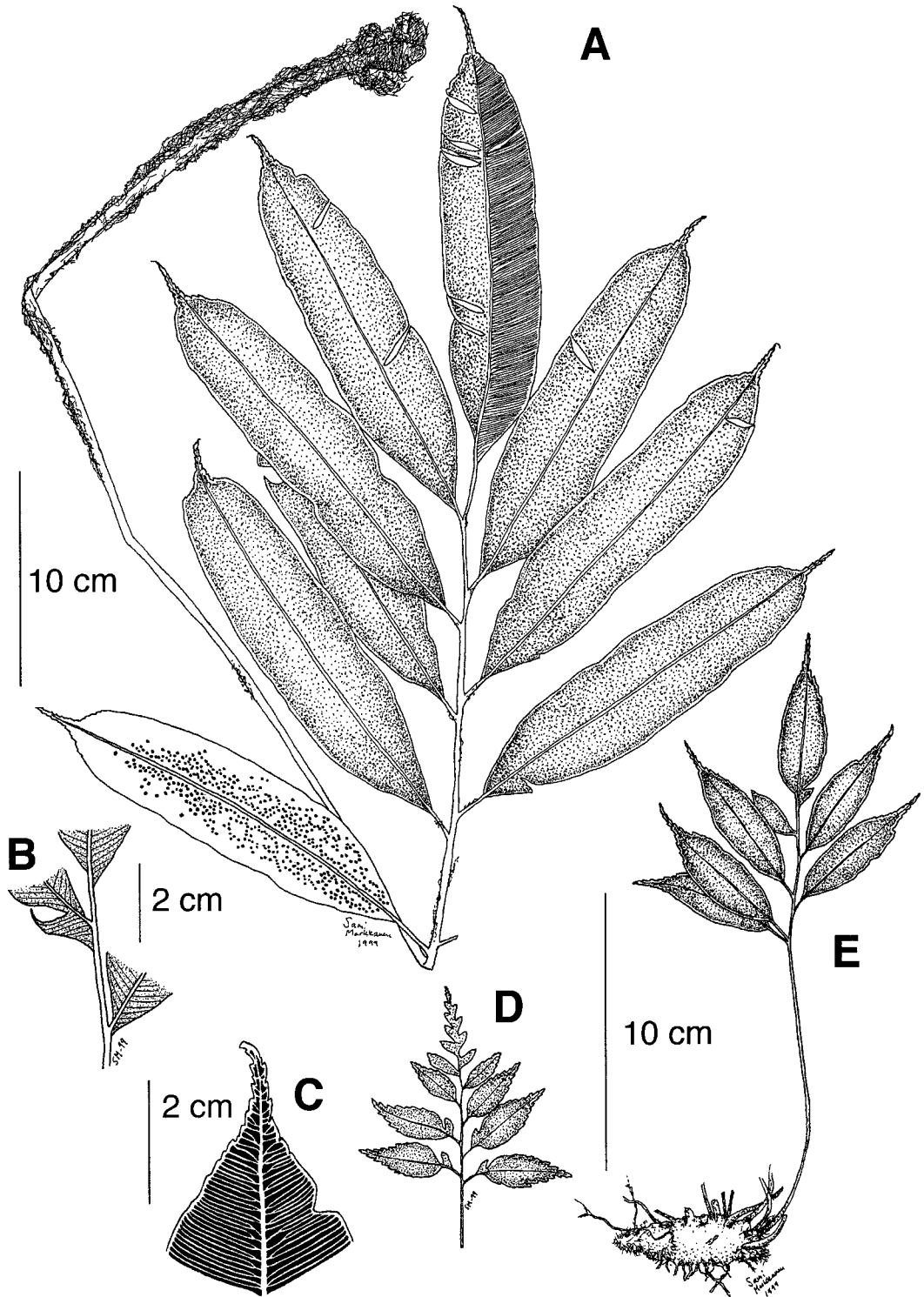
FIG. 1. A. *Metaxya lanosa*. Adult leaf, basal pinna shown with sori. B. *Metaxya rostrata*. Distal part of rachis with the bases of some pinnae shown. Note the decurrent base especially in the distalmost lateral pinna. C. *Metaxya lanosa*. Pinna apex showing details of venation and the cartilaginous margin. D–E. *Metaxya lanosa* juveniles. In small juveniles (D), the pinnae are dissected; in larger juveniles (E) they become entire and remain that way.

08′ W, 450 m, *Holst & Liesner 3150* (MO, UC-2 sheets); Dpto. Río Negro, Cerro de la Neblina, río Mawarinuma, afluente del río Baria, 0° 50′ N, 66° 10′ W, 110–130 m, *Steyermark & Luteyn 129730* (MO, UC-2 sheets, VEN); vicinity of Cerro Neblina base camp on río Mawarinuma, 0° 50′ N, 66° 10′ W, 140 m, *Croat 59331* (MO, UC); same locality, *Liesner 15643* (MO, UC); same locality, *Liesner 17315* (F, MO, UC); same locality, *Stein & Gentry 1733* (MO, UC); same general locality, *Plowman & Thomas 13569* (F, UC); same general locality, 0° 49′ 50″ N, 66° 09′ 40″ W, *Beitel 85026* (NY, UC); 3–5 km NE of Cerro de La Neblina base camp on río Mawarinuma, 150–200 m, *Liesner 16332* (MO, UC-2 sheets); Cerro Duida, southeastern facing slopes along Caño Negro, 305–1095 m, *Steyermark 57957* (F-2 sheets).

See note added in proof (p. 486) for additional paratypes.

Differt a *M. rostrata* (Kunth) C. Presl stipitibus rhachidibusque pilis rubiginosis vel aurantiacis persistentibus dense ferentibus, laminis coriaceis, crassis, pinnis utroque rhachidis latere 5–10, ellipticis, 18–31 × 4.0–6.5 cm, longistipitatis (stipitibus 10–20 mm proximaliter, 1–9 mm distaliter), marginibus cartilagineis, venis abaxialiter albidis prominentibus.

Stems stout, prostrate, with densely disposed, long, reddish brown to orange-brown hairs, especially toward the apex. Fronds ca. 0.9–1.5(–2.5) m long; stipes woolly with densely disposed, long (up to about 15 mm) reddish brown to orange-brown curly hairs throughout, these easily abraded with age or handling; similar hairs extending to some degree along the rachises. Blades coriaceous, 1-pinnate; pinnae 5–10 on each side of rachis, plus a conform terminal pinna, simple, elliptic, largest ones 18–31 × 4.0–6.5 cm, stalks 10–20 mm in proximal pinnae, (1–)3–9 mm in distal pinnae; margins cartilaginous, pronouncedly whitish, entire except at the tip, abruptly reduced to an attenuate-serrate tip. Veins free, readily visible on both sides of blades, raised and whitish abaxially. Sori round, exindusiate, scattered relatively evenly on the blades between the costae and 2/3 the distance toward the margin.

Morphology.   *Metaxya lanosa* differs from *M. rostrata* in having densely woolly-hairy stipes and rachises (the hairs ferrugineous, long and curly, easily abraded); lamina with a thicker, coriaceous texture; broader, more elliptic pinnae; cartilaginous and whitish pinna margins; more distinct veins abaxi-

ally; and longer-stalked pinnae, especially in the distal part of the leaf (Fig. 1). In *M. rostrata,* the distal pinnae are partially adnate and decurrent onto the rachis. *Metaxya rostrata* also has hairs on the rhizome and the basal part of the stipe, but the hairs are shorter (usually 1–3 mm), straight, and not nearly as densely disposed and woolly as in *M. lanosa.* On the croziers of *M. rostrata,* the hairs are longer (may exceed 5 mm) but are shed well before the frond is fully expanded, except at the very base of the petiole. The hairs of *M. rostrata* tend to be yellowish on the rhizome and brown on the petiole. *Metaxya lanosa* is relatively easy to spot among collections of *M. rostrata* in herbaria, using the aforementioned characters.

In western Amazonia, *Metaxya rostrata* comes in at least two forms, one with a few (about 10) relatively wide (up to 6 cm) pinnae, and the other with many (about 20) much narrower (less than 4 cm) pinnae. Both forms of *M. rostrata* are common in the field, but the differences between them are not clear-cut, and hence it is not known whether they merely represent wide variability in the morphology of the species, or whether they are constant enough to merit taxonomic recognition. Both forms of *M. rostrata,* however, share the same leaf texture, have non-cartilaginous (or only slightly so) pinna margins, and lack the ferruginous woolly hairs on the stipe, and are in these ways clearly distinct from *M. lanosa.* In addition, the pinnae in the narrower form of *M. rostrata* are typically much longer in relation to their width than the pinnae in *M. lanosa.* In the wider form of *M. rostrata,* where the pinna shape is more similar to that of *M. lanosa,* the decurrent bases of the distalmost pinnae tend to be especially wide (more than 1 cm in some cases).

Metaxya lanosa was first brought to our attention by the ornithologist José Alvarez in Iquitos, who had been struck by its strong smell of anise and brought some juvenile leaves to HT to ask the identity of the plant. Since *M. rostrata* normally has no such smell, and the leaves did not look like typical *M. rostrata* juveniles, HT followed JA to the locality and collected the specimen that in the present paper is designated the type of *M. lanosa.* Juveniles (e.g., isotype at TUR) show the same differences from typical *M. rostrata* as adult specimens do (more coriaceous blades, more elliptic, cartilaginous-margined pinnae, lanose stipes), and there is no difficulty in assigning juveniles to one or the other species. Juveniles of *M. rostrata* were described and illustrated by Tuomisto and Groot (1995).

Ecology and Distribution. This new species is apparently confined to forests on white-sand soils, or on sandstone boulders. It is terrestrial or occasionally on rocks and logs at 100–450 m. At the type locality, *M. lanosa* was very abundant in a patch of extremely nutrient-poor white-sand soil. The forest prevailing at the site is locally called Varillal bajo húmedo; it is characterized by low (about 15 m), sparse canopy and a relatively simple physiognomic structure. The other fern species encountered at the site are also most commonly (or even exclusively) found in such white-sand forests, including *Lindsaea tetraptera* K. U. Kramer, *L. hemiptera* K. U. Kramer, *L. jævitensis* Humb. & Bonpl. ex Willd., *Elaphoglossum glabellum* J. Sm., and *E. plumosum* (Fée) T. Moore. *Metaxya rostrata* occurs in the same general area, but has not been found in this particular forest type. In general, *M. rostrata* has a remarkably wide ecological range, and consequently it has been collected from a wide variety of different forests, the substrates of which range from relatively nutrient-rich clay soils to nutrient-poor loamy or even sandy soils. However, it does not seem to thrive on the most extreme white-sand soils, these being occupied by *M. lanosa* instead.

rbcL *Evidence for Species Delimitation in* **Metaxya.** Molecular data can serve as an independent test of phylogenetic hypotheses that are based on morphology (e.g., Pryer et al. 1995). In addition, it has been suggested that the extent of infra- and interspecific DNA sequence variation observed in ferns might assist in evaluating species limits for taxa that are often recognized by very few, and rather cryptic, morphological characters (Haufler and Ranker 1995; Hauk 1995; Gastony and Ungerer 1997; Yatabe et al. 1998).

The preferred target for recent molecular phylogeny studies in plants has been the chloroplast gene *rbcL* (Soltis and Soltis 1998). In *rbcL* studies on phanerogams, authors report sequence percent-divergence values as low as 0.6%–0.9% between *genera* (e.g., Gadek and Quinn 1993). By comparison, fern species have been shown on average to be more divergent than are seed plant species. Wolf et al. (1994), for example, reported *rbcL* divergence values for congeneric *species* that ranged from 0.2% (within *Odontosoria*) to 1.8% (within *Polypodium*). Hauk (1995) reported a 0.6% average sequence divergence between species of *Botrychium* subg. *Botrychium*. Although the average sequence divergence among species of *Polypodium* was reported as 1.87% by Haufler and Ranker (1995), two pairs of morphologically distinct sister-species, *P. amorphum*

Suksd. + *P. appalachianum* Haufler & Windham and *P. australe* Fée + *P. macaronesicum* A. E. Bobrov, were both shown to differ by only five out of 1323 *rbcL* nucleotide bases (0.38% sequence divergence). Similarly, Gastony and Ungerer (1997) calculated a 0.4% sequence divergence (five nucleotides) between *Cheilanthes aemula* Maxon and *C. alabamensis* (Buckley) Kunze, congeneric cheilanthoid species that are well differentiated by traditional morphological characters. Sequence divergence values of 0.3–0.5% (four to seven nucleotides) between two morphologically distinct varieties of *Onoclea sensibilis* L. prompted a change of status and a new combination to accommodate their recognition at the species level (Gastony and Ungerer 1997).

Yatabe et al. (1998), in a study that examined intrasubspecific variation for 1246 bp of the *rbcL* gene among 63 Japanese individuals (37 populations) of *Stegnogramma pozoi* (Lag.) K. Iwats. subsp. *mollissima* (Fisch. ex Kunze) K. Iwats., found three sequence "types" in the subspecies. The Atami-1 and Atami-2 types differed by a single base, whereas the geographically distinct Tateyama type differed from Atami-1 and Atami-2 by four and five fixed nucleotide differences (0.32% and 0.40% sequence divergence values, respectively). Given that there are also morphological features differentiating the Tateyama from the Atami sequence types, Yatabe et al. (1998) suggested that these two types might represent distinct species, a statement they reinforced by pointing out that two cryptic species in the Japanese *Ceratopteris thalictroides* (L.) Brongn. complex, which are not interfertile and that can be differentiated by their allozyme patterns, differ by only a single nucleotide in *rbcL*.

We considered it worthwhile to investigate whether any variation existed in *rbcL* sequence data among populations of *Metaxya,* including the new species described here. However, only a very limited sample of *Metaxya* material was available for DNA analysis. We present the data nevertheless, fully appreciating that the small sample size constrains us to be cautious, at best, with our interpretations. DNA extraction, *rbcL* amplification, and sequencing procedures were accomplished exactly as described in Pryer et al. (2001). Material was available for *M. rostrata* from Costa Rica (*Conant s.n.,* LSC) and Ecuador (*Tuomisto 11734,* UC), and for *M. lanosa* from Peru (*Tuomisto 13054,* UC). The three *rbcL* sequences obtained in this study have been assigned GenBank accession numbers AF317699–AF317701, respectively.
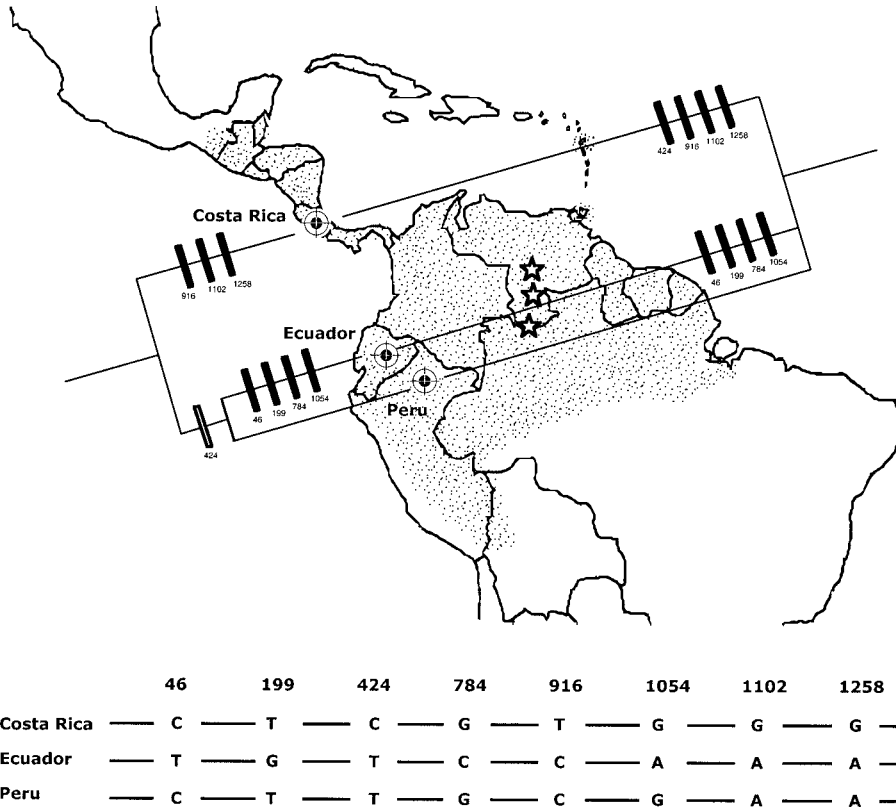
A single *rbcL* sequence for *Metaxya rostrata* was

	46	199	424	784	916	1054	1102	1258
Costa Rica	C	T	C	G	T	G	G	G
Ecuador	T	G	T	C	C	A	A	A
Peru	C	T	T	G	C	G	A	A

FIG. 2. Map of the Neotropics with the geographical range of *Metaxia rostrata* stippled (based on Tryon and Tryon 1982). The localities of the samples available for *rbcL* sequencing are indicated by a target symbol (*M. rostrata*: Costa Rica and Ecuador; *M. lanosa*: Peru). Additional known general localities for *M. lanosa* are indicated by open stars. An exhaustive parsimony search of *rbcL* for 11 tree ferns and allies resulted in the two equally most parsimonious topologies for *Metaxya* shown here. Nucelotide site differences among the three samples are numbered according to alignment position and indicated below the map. These character state changes are plotted onto the two topologies using Acctran optimization. Black boxes = unambiguous character state changes. White box = ambiguous character state change.

already available in GenBank (U05635, incorrectly spelled as *Metaxia*). However, we resequenced *rbcL* from the same Costa Rican voucher on which the GenBank sequence was based (*Conant s.n.*, LSC), and found nine nucleotide differences between our sequence (AF317699) and the published sequence (U05635). These nine discrepancies appear to be in error in U05635 because they are clustered in three regions that are conserved in their sequence among all three samples sequenced here. Therefore, U05635 was rejected from further comparisons.

Given the morphological differences we observed in *Metaxya,* we expected to find little or no *rbcL* sequence divergence between the two samples of *M. rostrata,* and greater divergence between them and *M. lanosa*. To our surprise, out of 1266 nucleotides we found eight differences (0.63% sequence

divergence) between our Costa Rican and Ecuadorian samples of *M. rostrata* and four differences (0.32% sequence divergence) between each of the *M. rostrata* samples and *M. lanosa*. All of these nucleotide differences are synonymous substitutions in the third codon position. As mentioned above, 0.30–0.60% is consistent with the level of *rbcL* sequence divergence typically reported between closely related fern species. The divergence observed among our *Metaxya* samples does not reject *M. lanosa* as a new species, but it provides too few data to definitively support it, given the greater divergence between the two *M. rostrata* accessions.

A data matrix of eleven published *rbcL* sequences for tree ferns and allies was assembled from GenBank, including the three *Metaxya* sequences presented here (data not shown). An exhaustive

parsimony search using PAUP* (version 4.0b2a; Swofford 1999) of 1266 equally weighted characters revealed two equally most parsimonious trees, which differed only in the relationships of the three *Metaxya* samples. In one topology, the three *Metaxya* samples were arranged in a polytomy, whereas in the other, a single ambiguous character state change supported a closer relationship between *M. lanosa* and *M. rostrata* from Ecuador (Fig. 2). The eight nucleotide sites that differed between the two *M. rostrata* samples are shown in Fig. 2 and these changes are compared to *M. lanosa* and plotted onto the two topologies using Acctran optimization. The *M. lanosa rbcL* sequence is more similar to each of the *M. rostrata* sequences than the latter are to each other, because *M. lanosa* retained plesiomorphic states found in the common ancestor of all three sequences, while the *M. rostrata* accessions evolved independently.

We suspect there may be more than one cryptic species masquerading under the name *M. rostrata,* even after the segregation of *M. lanosa.* We recommend a broad survey of genetic variation among populations of *Metaxya* using adequate sampling and a more appropriate and variable gene (e.g., chloroplast *trnL-trnF* spacer region, nuclear ITS) and/or technique (e.g., isozymes, cytogenetics). This would confirm whether there is molecular support for recognizing two (or more) species within *Metaxya.* We certainly do not advocate describing a new species on the basis of molecular character differences alone. However, given the number of easily observed morphological and ecological differences between *M. rostrata* and *M. lanosa,* we feel confident in their recognition as distinct species, despite the ambiguity observed in *rbcL* for our small sample.

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Note added in proof: Three more paratypes of *Metaxya lanosa,* from two additional countries, have been found recently by HT. The Colombian collection is from a white-sand area, and the Guyanan ones are from the Roraima sandstone: **Colombia**: Vaupes: Rio Cananari, Cerro Isibukuri, 250–700 m, *Schultes & Cabrera 13333* (U). **Guyana**: Pakaraima Mts., Mt. Membaru, 5° 57′ N, 60° 33–34′ W, 550–600 m. *Maas & Westra 4338* (U); Cuyuni-Mazaruni, Maipuri Falls, Karowrieng River, 5° 41′ N, 60° 14′ W, 650–770 m, *Gillespie & Smart 2747* (U).