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Cheilanthes ecuadorensis: A New Species of *Cheilanthes* s. s. (Pteridaceae) from Northern South America

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Abstract—Ongoing research on the taxonomically complex genus *Cheilanthes* (Pteridaceae; Cheilanthoideae) has resulted in the identification of a new species from Loja Province in Ecuador, ***Cheilanthes ecuadorensis***, described and illustrated herein. Originally collected in 1988 and identified as *C. cf. rufopunctata*, *C. ecuadorensis* is clearly distinct from that species in having pubescent adaxial blade surfaces and narrow, poorly-differentiated false indusia (rather than the glabrous adaxial surfaces and wide false indusia of *C. rufopunctata*). Among the South American species currently included in *Cheilanthes*, *C. ecuadorensis* is superficially most similar to *C. pilosa*. However, our molecular phylogenetic analyses indicate that *C. ecuadorensis* is sister to *C. micropteris*, the morphologically disparate generitype of *Cheilanthes*. Here we examine the phylogenetic relationships, morphology, cytogenetics, and geography of these four South American *Cheilanthes* species in a study that, once again, highlights the importance of herbaria in the process of new species discovery.

Resumen—Nuestra investigación en el complicado género *Cheilanthes* (Pteridaceae; Cheilanthoideae) ha resultado en la identificación de una nueva especie de la Provincia de Loja en Ecuador, ***Cheilanthes ecuadorensis***, descrita e ilustrada aquí. Colectada originalmente en 1988 e identificada como *C. cf. rufopunctata*, *C. ecuadorensis* se distingue claramente por tener pubesencia en las superficies adaxiales y falso indusio angosto y poco diferenciado (en comparación a las superficies glabras y el falso indusio ancho de *C. rufopunctata*). Entre las especies Sudamericanas que a la fecha se consideran *Cheilanthes*, *C. ecuadorensis* se parece superficialmente a *C. pilosa*. Sin embargo, nuestros análisis de filogenia molecular muestran que *C. ecuadorensis* es hermana a *C. micropteris*, el generitipo de *Cheilanthes* y una especie morfológicamente dispar. Aquí examinamos las relaciones filogenéticas, la morfología, la citogenética, y la geografía de estas cuatro especies Sudamericanas en un estudio que, una ves más, muestra la importancia de los herbarios en el descubrimiento de nuevas especies.

Keywords—Cheilanthoid ferns, cytogenetics, geographical distribution, spore number per sporangium.

While studying collections of South American *Cheilanthes* Sw. [Pteridaceae; Cheilanthoideae sensu PPG I (2016)] obtained on loan from various herbaria, we encountered three unusual specimens (representing two separate collections) from Ecuador originally identified as *Cheilanthes cf. rufopunctata* Rosenst. These collections looked quite different from that species and, instead, appeared most similar to *C. pilosa* Goldm. There were, however, several key differences between the Ecuadorian collections and typical *C. pilosa*, and these were sufficient to suggest that the former might represent an undescribed species. To investigate this possibility, we conducted molecular phylogenetic, morphological, and cytogenetic investigations of select species currently assigned to *Cheilanthes* or one of its segregate genera. The goals of this study were threefold: 1) explore the phylogenetic relationships of these anomalous Ecuadorian specimens, 2) ascertain whether they are sufficiently distinct from their closest relatives to warrant recognition as a new species, and 3) determine if this taxon is appropriately assigned to *Cheilanthes* s. s.

Materials and Methods

Taxon Sampling——Our sample of 26 cheilanthoids spans the hemionitid clade (Windham et al. 2009; Eiserhardt et al. 2011). It includes the conserved type species of *Cheilanthes* (*C. micropteris* Sw.; Appendix 1) as well as one of the specimens from the anomalous Ecuadorian collections (*Madsen and Sanchez 7940* (AAU); additional sampling was limited due to funding). Our choice of outgroup was informed by previous analyses (e.g. Link-Pérez et al. 2011; Yesilyurt et al. 2015), all of which indicate that the small genus *Pentagramma* Yatsk., Windham & E.Wollenw. is sister to the remainder of the hemionitid clade.

DNA Isolation, Amplification, and Sequencing—Genomic DNA was extracted from silica-dried or herbarium leaf material. DNA extraction, amplification, and sequencing followed protocols in Schuettpelz et al. (2007) and Nagalingum et al. (2007). Sequences were obtained for three plastid loci (*atpA*, *rbcL*, *trnGR*) for nearly all specimens (Appendix 1). Primers for amplification and sequencing for all three loci were those used in Schuettpelz et al. (2007) and Rothfels and Schuettpelz (2014). PCR purification and sequencing followed Schuettpelz et al. (2008). Sixty-four newly obtained DNA sequences were deposited in GenBank (Appendix 1).

DNA Sequence Alignment and Data Sets—DNA sequence data for each locus were individually aligned using MUSCLE (Edgar 2004) in AliView 1.19 (Larsson 2014). Alignments were then visually inspected. In *trnGR*, any ambiguously aligned regions with insertions or deletions were excluded from the analyses to ensure positional homology. Details on alignments and characters included are listed in Table 1. Alignments and phylogenetic trees are deposited in the Dryad Digital Repository (Sosa et al. 2021).

Phylogenetic Analyses—Sequence evolution models for each dataset (*aptA*, *rbcL*, and *trnGR*) were chosen using the automated model selection in PAUP* (Swofford 2003) under the Akaike information criterion (Table 1). Separate phylogenetic analyses were conducted for each of the three plastid regions using maximum likelihood (ML) in GARLI v. 2.0 (Zwickl 2006) with "genthreshfortopoterm" set to 1,000,000. Bootstrap support (BS) was calculated from 1000 replicates with "genthreshfortopoterm" set to 1,000,000. Once we confirmed there were no topological conflicts among loci (BS threshold > 70), all loci were combined into a single matrix. The final ML analysis was run with three partitions, with the best-fit model assigned to each locus, maintaining the same parameters as above.

Morphology of Sporophytes and Spores—The three known specimens of the anomalous cheilanthoid fern taxon from Ecuador were carefully examined for distinctive morphological traits. These were compared to representative collections of *C. micropteris*, *C. pilosa*, and *C. rufopunctata* listed in Appendix 2. A specimen of the rare species *C. andina* Hook. was also examined (Appendix 2). Specimens were observed using a Leica MZ 12.5 stereomicroscope and illustrated by the lead author. Spore number per sporangium (32 vs. 64) was determined for all taxa using the protocols described by George et al. (2019).

Cytogenetics——The chromosome number of the Ecuadorian taxon was determined by germinating spores collected from *Madsen and Sanchez 7940* (AAU) on Hevly's medium (Hevly 1963). Plates were kept at room temperature with a 12h–12h light–dark cycle under 39 μmol s^{-1} m^{-2} per μA of

TABLE 1. Details on the molecular datasets used in this study of select hemionitid ferns.

Dataset	Alignment length	Characters included	Variable characters	Best-fitting model
atpA	1846	1543	252	TrN+I+G
rbcL	1309	1309	213	GTR+I+G
trnGR	1308	941	373	TrN+I+G

fluorescent light, measured with a LI-COR LI-250A light meter. Prothalia were produced in 27 d, and sexually mature gametophytes were evident after nine weeks of growth. The mature gametophyte cultures were flooded with water to release spermatozoa, and an abundance of new sporophytes appeared 30 d later, most likely as a result of sporophytic selfing (Haufler et al. 2016). The young sporophytes were transferred to plastic pots with Biocomp BC-5 soil kept under the same light conditions and watered as needed when the soil dried out. Sporangia began to develop after about ten weeks. Chromosome counts were obtained using standard protocols for meiotic material (Windham and Yatskievych 2003) and results were documented using a Canon EOS Rebel T3i digital camera mounted on a Meiji MT5310L phase contrast microscope.

Species Distributions—All georeferenced specimen records for *C. micropteris* and *C. rufopunctata* were downloaded from GBIF (Flemons et al. 2007). Each record was inspected and cross-referenced with digitized herbarium specimens to ensure accuracy of locality and correct species identification. Records lacking precise locality data or whose identification could not be confirmed were excluded; the entire sampling is summarized in Appendix 3. The Ecuadorian taxon and *C. pilosa* are more difficult to distinguish based on digitized images, therefore specimen localities for these taxa were obtained only from herbarium specimens on loan from AAU, DUKE, F, GH, MO, NY, TEX/LL, and UC (Appendix 2). The map was prepared using Quantum GIS 2.18.2 (QGIS 2017) (geographical projection, decimal degrees, datum: EPSG:4326, coordinate system: WGS84). For visualization, Central and South American administrative boundaries were downloaded from GADM (Hijmans et al. 2010). Full specimen records are deposited in the Dryad Digital Repository (Sosa et al. 2021).

RESULTS

All evidence gathered during this study supports the hypothesis that the three Ecuadorian specimens represent a previously undescribed taxon assignable to *Cheilanthes* s. s. In the text that follows, we refer to this taxon as *C. ecuadorensis*, a

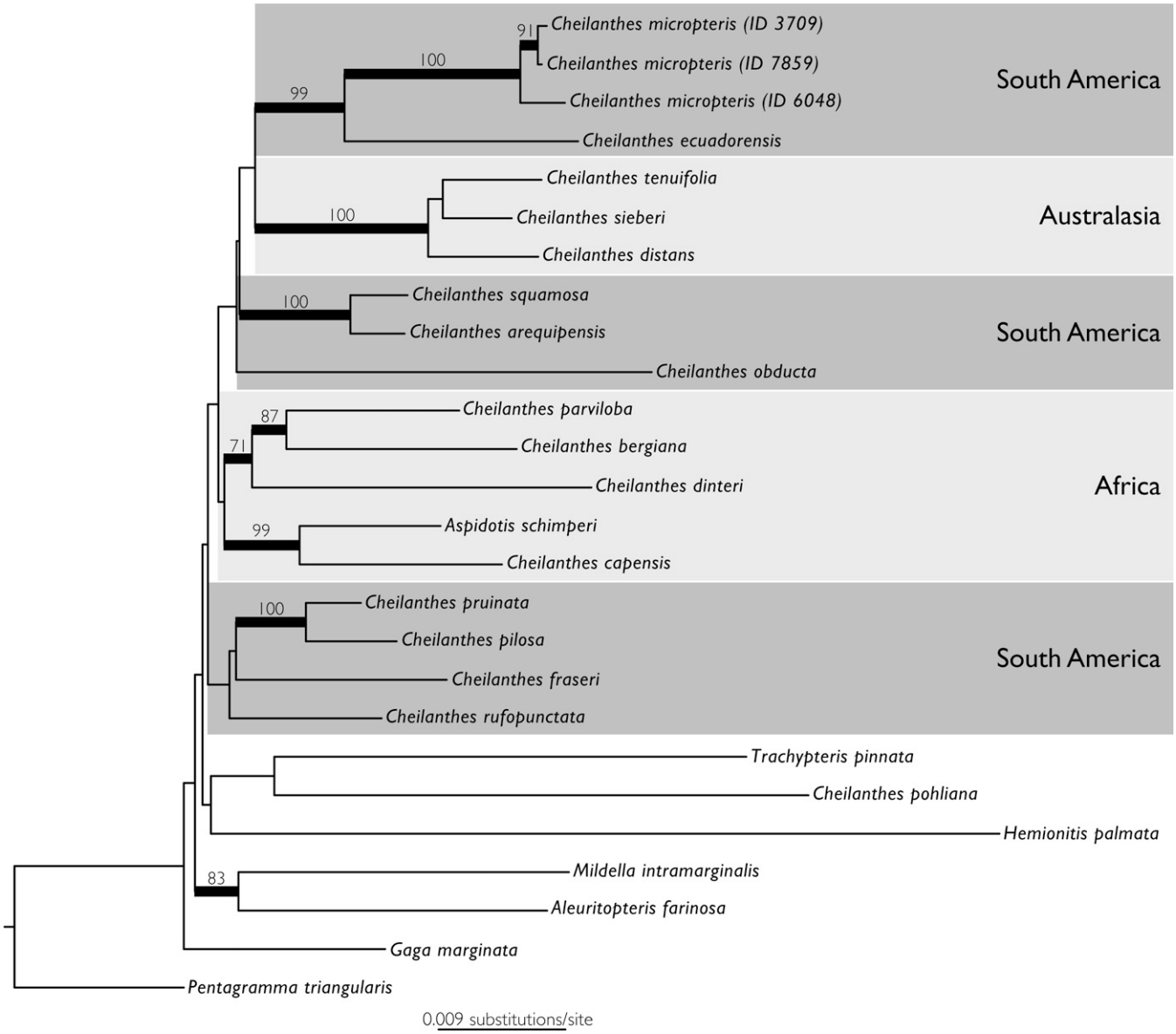

FIG. 1. Maximum likelihood (ML) phylogeny of select hemionitid ferns based on a combined plastid *atpA*, *rbcL*, and *trnG-R* dataset. Thickened branches received ML bootstrap support values ≥ 70 (actual values appear above those branches).

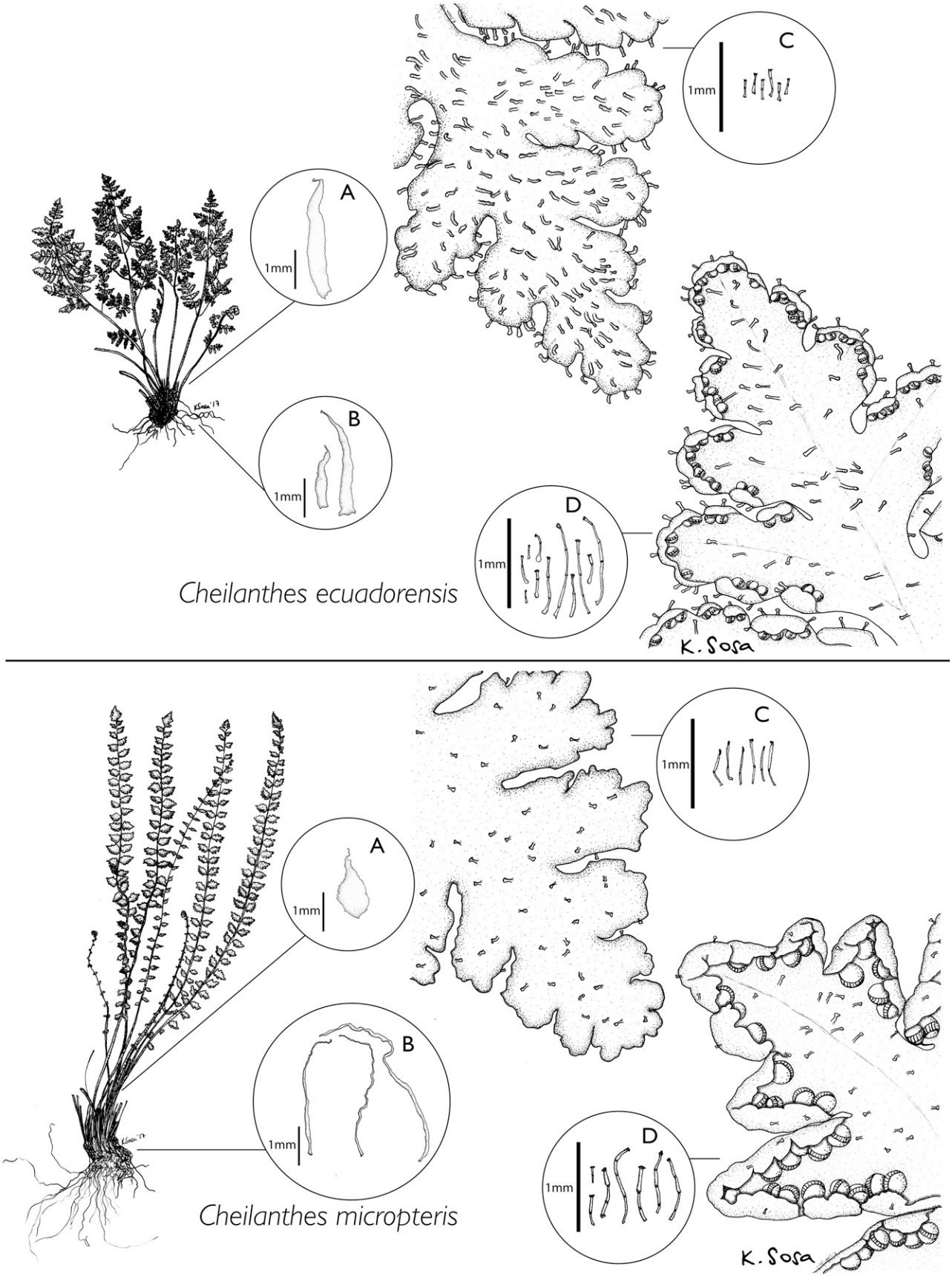

FIG. 2. Line drawings of *Cheilanthes ecuadorensis* and its sister species *C. micropteris*. Leftmost drawing shows whole plant, rightmost top depicts adaxial surfaces, and rightmost bottom depicts abaxial surfaces. Insets: A. Lower petiole scales; B. Rhizome scales; C. Adaxial blade trichomes; D. Abaxial blade trichomes. Drawings by first author, Karla Sosa.

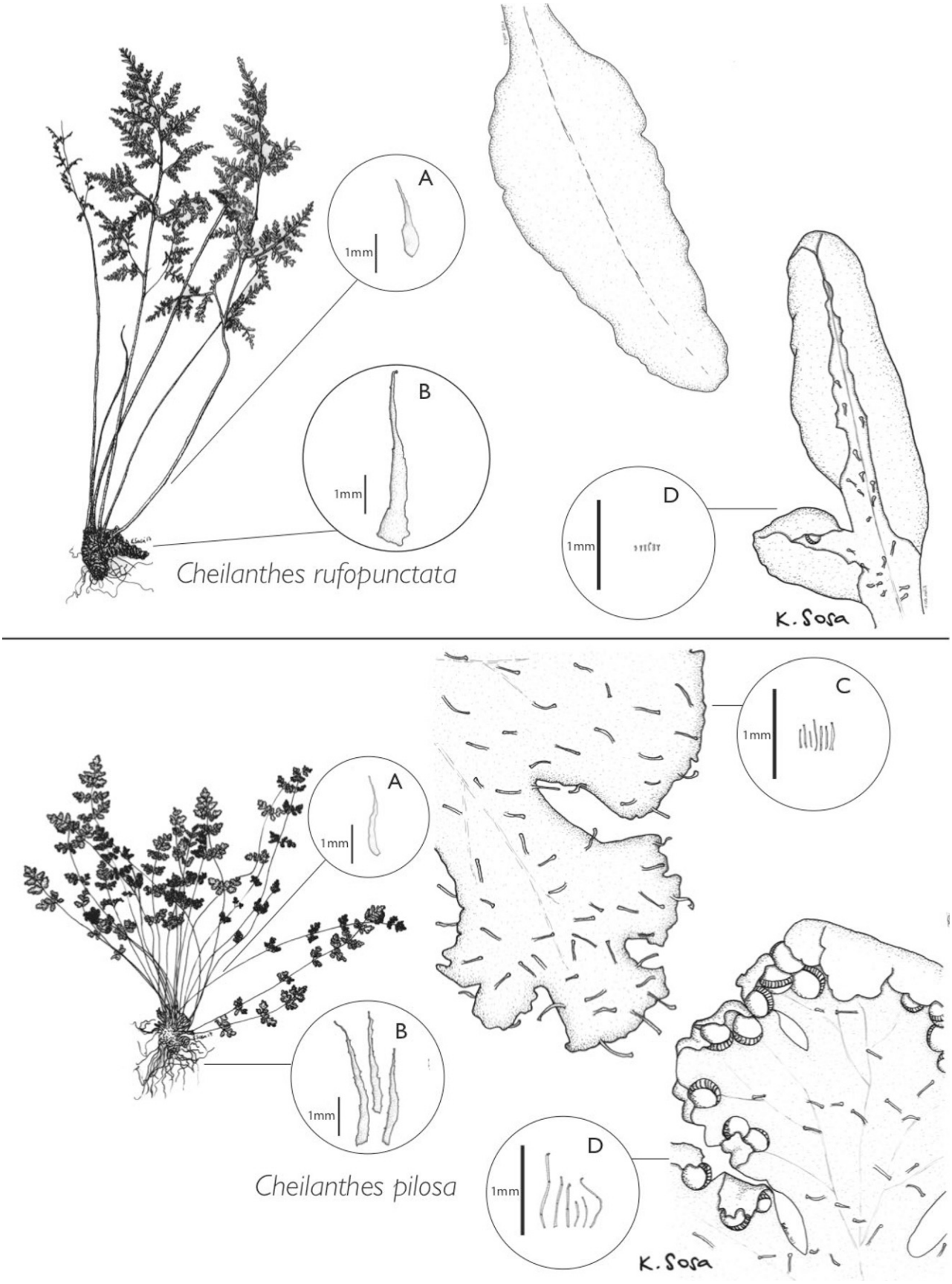

FIG. 3. Line drawings of *Cheilanthes rufopunctata* and *C. pilosa*, species historically confused with *C. ecuadorensis*. Leftmost drawing shows whole plant, rightmost top depicts adaxial surfaces and rightmost bottom depicts abaxial surfaces. Insets: A. Lower petiole scales; B. Rhizome scales; C. Adaxial blade trichomes; D. Abaxial blade trichomes. Drawings by first author, Karla Sosa.

TABLE 2. Morphological comparison of *Cheilanthes ecuadorensis* and the three species with which it has been aligned.

Taxon	Blade shape	Blade dissection	Number of pinnae per fertile leaf	Adaxial trichomes	Abaxial trichomes	False indusium (width)	Scale color	Spore number per sporangium
C. ecuadorensis	Lanceolate	Bipinnate-pinnatifid	8–12	Dense, 100–230 µm long	Sparse, 80–400 µm long	0–130 µm, sporangia visible	Brown	32
C. micropteris	Linear	Pinnate- pinnatifid	26–52	Dense, 70–150 µm long	Dense, 150–250 µm long	100–290 µm, sporangia visible	Brown	32
C. pilosa	Linear-lanceolate	Bipinnate- pinnatifid	8–17	Dense, 140–860 µm long	Dense, 220–900 µm long	340–790 µm, sporangia visible	Dark brown	64
C. rufopunctata	Lanceolate	Tripinnate	8–14	Absent	Sparse, 50–90 µm long	350–650 µm, sporangia not visible	Dark brown	64

new species formally recognized at the end of the discussion (see Taxonomic Treatment).

Phylogenetic Analyses—The alignment included 26 sequences each for *rbcL* and *atpA*, and 24 for *trnGR* (Appendix 1). Of the 4463 nucleotide positions, 3793 were included in the analyses, and a total of 401 were parsimony informative (Table 1). The maximum likelihood tree (Fig. 1) provides strong support (BS 99%) for a sister relationship between *C. ecuadorensis* and *C. micropteris*, the generitype of *Cheilanthes*. Although our analysis provides little support for the relationships of major hemionitid subclades to one another, *C. rufopunctata* (the species to which the collections of *C. ecuadorensis* were originally assigned) and *C. pilosa* (the species most easily confused with *C. ecuadorensis*) appear to represent a distinct South American clade. And, although the precise relationship of *C. rufopunctata* is unresolved, *C. pilosa* is strongly supported (BS 100%) as sister to *C. pruinata* Kaulf. (Fig. 1).

Morphology of Sporophytes and Spores—Morphological differences among the target taxa are illustrated in Figs. 2 and 3, with distinguishing features summarized in Table 2. A scan of the type specimen is shown in Fig. 4. *Cheilanthes ecuadorensis* is easily distinguished from its sister species *C. micropteris* by blade shape (lanceolate vs. linear), blade dissection (bipinnate pinnatifid vs. pinnate pinnatifid), and number of pinnae per fertile leaf (8–12 vs. 26–52). This newly recognized species differs from the superficially similar *C. pilosa* by its much narrower (0–130 vs. 340–790 µm) false indusia, its paler (brown vs. dark brown) rhizome scales, its reduced number of spores per sporangium (32 vs. 64), and its generally shorter leaf trichomes (Table 2). *Cheilanthes rufopunctata* shares the broader false indusia, dark brown rhizome scales, and 64-spored sporangia of *C. pilosa* and is further distinguished from *C. ecuadorensis* by its lack of adaxial leaf pubescence. We also examined a single specimen of *C. andina,* a rare species that has often been synonymized with *C. pilosa*. This specimen of *C. andina* resembles *C. pilosa,* and can be distinguished from *C. ecuadorensis* due to the lack of dense glandular trichomes adaxially, its wide, chartaceous false indusium, and the presence of 64 spores per sporangium.

Cytogenetics—Spores taken from the holotype of *C. ecuadorensis* were successfully germinated, yielding an abundance of gametophytes that were initially protogynous but eventually produced antheridia as well. These gametophyte populations were selfed to produce sporophytes that were subjected to meiotic chromosomal analyses upon reaching maturity. Spore mother cells undergoing meiosis consistently exhibited 30 bivalents at diakinesis (Fig. 5), indicating the plants are sexual diploids. The reduced number of spores per sporangium observed in *C. ecuadorensis* (32 instead of 64) is associated with the elimination of one of the four mitotic events just prior to meiosis, resulting in 8 spore mother cells per sporangium rather than the 16 typical of most sexually-reproducing leptosporangiate ferns.

Species Distributions—Specimen mapping revealed a very localized range for *C. ecuadorensis*, which is currently known from only two localities in eastern Loja Province in southern Ecuador (Fig. 6). Its sister species, *C. micropteris,* occurs in Bolivia, Paraguay, southern Brazil, and Argentina, but the closest known populations to *C. ecuadorensis* are disjunct by nearly 2000 km. *Cheilanthes pilosa*, an Andean species, is reported from Chile and Argentina (Zuloaga et al. 2008) north to Bolivia and Peru; it is geographically separated from *C. ecuadorensis* by more than 500 km. Our map does not include any *C. pilosa*

FIG. 4. Scan of the type specimen for *Cheilanthes ecuadorensis* (*J.E. Madsen and O.A. Sanchez 7940* (AAU)), reproduced with the permission of AAU.

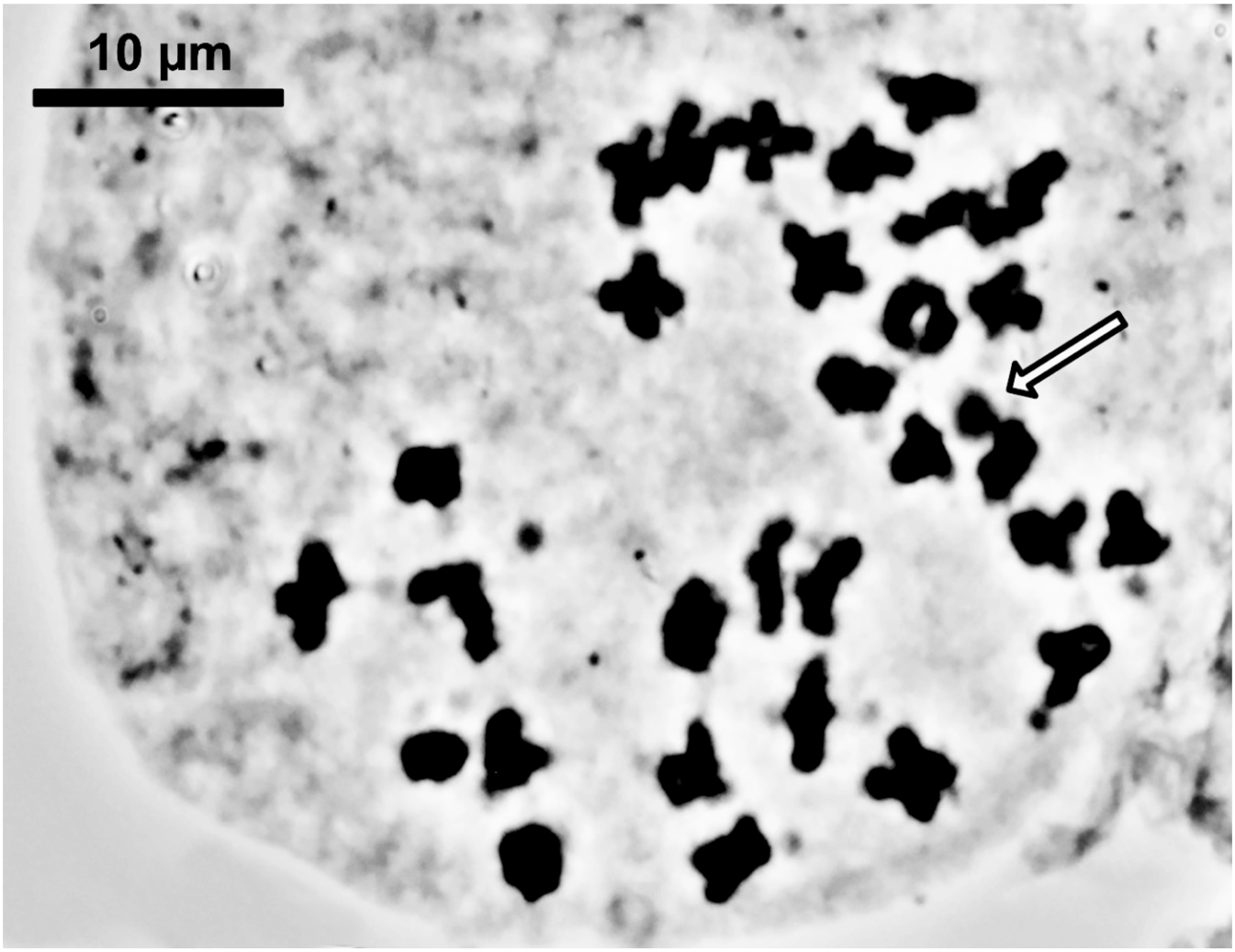

FIG. 5. Meiotic chromosome squash for *Cheilanthes ecuadorensis* showing 30 bivalents at late prophase I. Generated from sporophytes grown from *J.E. Madsen and O.A. Sanchez 7940* (AAU). Arrow points to nucleolus.

specimens from Chile nor Argentina as the loans we had did not include any samples from these countries. Finally, *C. rufopunctata*, known only from Bolivia and Peru, is isolated from *C. ecuadorensis* by nearly 800 km (Fig. 6).

DISCUSSION

The phylogenetic analyses reported here yield strong support (Fig. 1; BS 99%) for a sister relationship between *C. ecuadorensis* and *C. micropteris*. These two taxa are quite disparate morphologically (Fig. 2; Table 2), and it is clear that they are not conspecific. Although the backbone of the phylogenetic tree receives little or no bootstrap support, it is also clear that the superficially similar *C. ecuadorensis* and *C. pilosa* belong to different lineages (Fig. 1), further supporting recognition of the former as a new species. The only remaining question is whether this newly-discovered taxon should be assigned to *Cheilanthes* s. s. or to one of its segregate genera.

Cheilanthes and its relatives belong to a large group (400 + species) commonly known as cheilanthoid ferns [Pteridaceae; Cheilanthoideae sensu PPG I (2016)]. This group often occurs in xeric habitats, where strong selection pressures on various leaf traits have led to extensive morphological convergence (Gastony and Rollo 1995, 1998; Kirkpatrick 2007; Prado et al. 2007; Schuettpelz et al. 2007; Zhang et al. 2007; Rothfels et al. 2008; Eiserhardt et al. 2011; Yesilyurt et al. 2015). *Cheilanthes*, for example, is traditionally recognized by its highly dissected leaf blades with minute ultimate segments, leaf margins recurved to protect the abaxial surface, and abundant hairs that trap moisture and reflect light (Hevly 1963). However, molecular analyses indicate that this combination of traits appears in all major lineages of cheilanthoid ferns and that geography is more indicative of relationship than the morphological traits emphasized over the past two centuries (Eiserhardt et al. 2011). As a result, the genus *Cheilanthes*, as traditionally defined, spans the entire cheilanthoid phylogenetic tree, with all other genera (except *Bommeria* E.Fourn. and *Baja* Windham & L.O.George; George et al. 2019) nested within it.

Ongoing efforts to circumscribe a diagnosable, monophyletic group that can be called *Cheilanthes* s. s. have, so far, been more successful at identifying species groups that need to be excluded from it. This has led to the recircumscription of genera such as *Adiantopsis* Fée (Link-Pérez et al. 2011), *Myriopteris* Fée (Grusz and Windham 2013), and *Doryopteris* J.Sm. (Yesilyurt et al. 2015), as well as the naming of several new genera, including *Gaga* Pryer, F.W.Li & Windham (Li et al. 2012) and *Baja* (George et al. 2019). Ultimately, the key to redefining

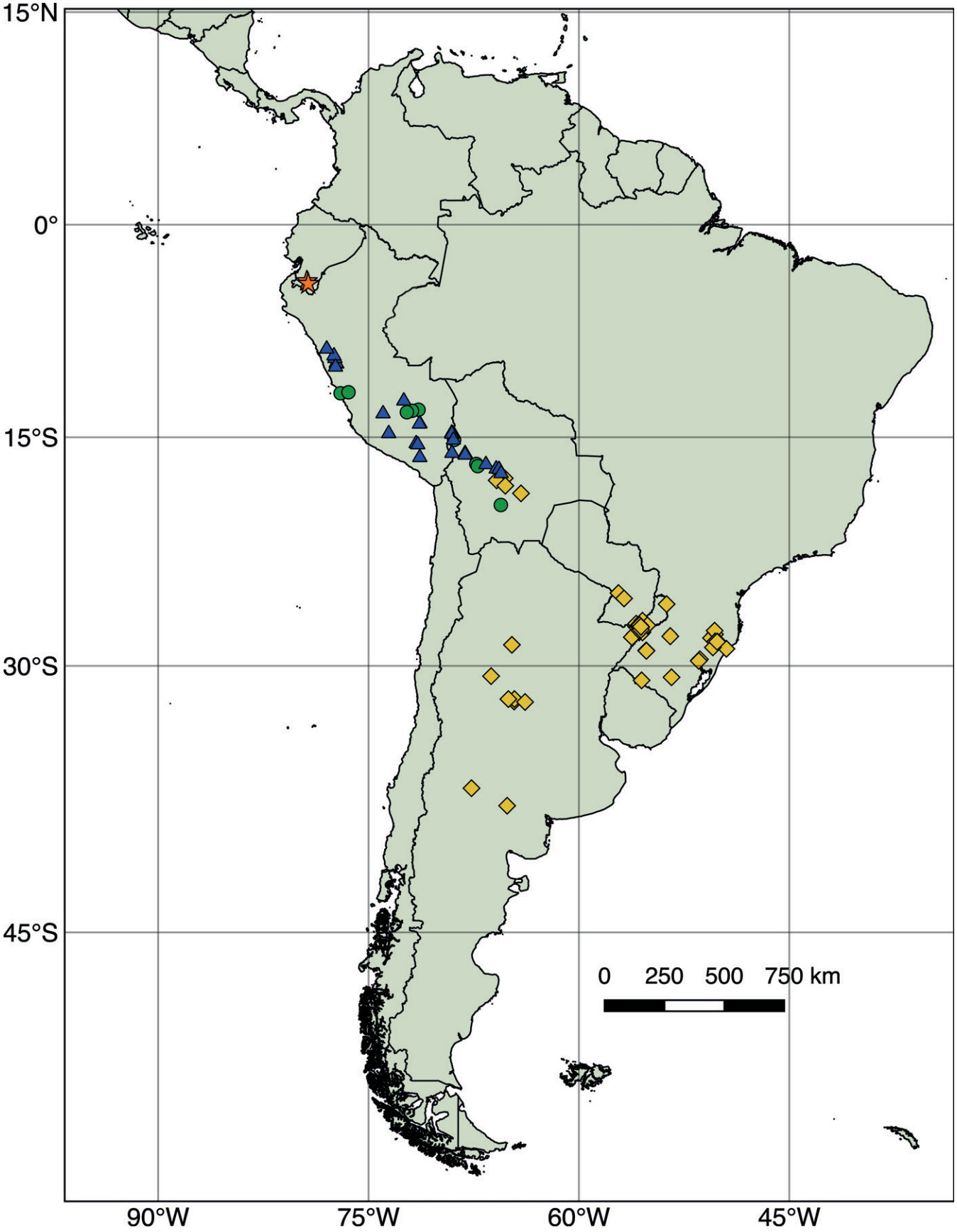

FIG. 6. Geographic distribution of *Cheilanthes ecuadorensis* (orange star), *C. micropteris* (yellow diamonds), *C. pilosa* (blue triangles), and *C. rufopunctata* (green circles); all taxa are restricted to South America.

Cheilanthes will be a relatively comprehensive sampling of Southern Hemisphere cheilanthoids that includes *C. micropteris,* the type species of the genus. This critical species has been included in few studies to date and has always been isolated on a long branch weakly associated with either core hemionitids (Link-Pérez et al. 2011; Li et al. 2012) or various Australasian *Cheilanthes* species (Bouma et al. 2010; Eiserhardt et al. 2011; Ponce and Scataglini 2018). Our study is the first to identify a close relative of *C. micropteris* in the form of our new species, *C. ecuadorensis*. Given the strongly supported sister relationship between the conserved type species of *Cheilanthes* (*C. micropteris*) and our new taxon, we believe *C. ecuadorensis* is correctly assigned as belonging to the genus *Cheilanthes*.

Taxonomic Treatment

Cheilanthes ecuadorensis Windham & K.Sosa, sp. nov. Type: Ecuador. Loja: 10–15 km W of Malacatos on gravel roads, surroundings of Rio Catamayo, 04°13'S, 79°18'W, 8 May 2001, *J.E. Madsen & O.A. Sanchez 7940* (holotype: AAU!; isotype: MO!).

Most similar to *C. pilosa* but differing in its reduced number of spores per sporangium (32 vs. 64), much narrower (0–0.13 vs. 0.3–0.8 mm) false indusia, brown (vs. dark brown) rhizome scales, and shorter (mostly less than 0.5 mm) blade surface trichomes.

Plants rupestral. **Stems** ascending, compact, to 10 mm in diameter including dense covering of persistent petiole bases, scaly. **Stem scales** linear-lanceolate, mostly ca. 1 × 0.2 mm, brown, thin, chartaceous, margins entire, apices attenuate, ending in dark glandular tip. **Leaves** ± monomorphic, the small sterile leaves of immature plants eventually completely replaced by fertile leaves 8–26 cm. **Petioles** ca. 1/4 of leaf length, with 1 vascular bundle, dark brown to dark reddish brown, terete to slightly flattened adaxially, non-abraded surfaces pubescent throughout with > 25 trichomes per mm^2, trichomes spreading 0.1–1.0 mm, with prominent 2–6-celled stalk and enlarged, spherical, glandular cell at apex, distinctly viscid when fresh, scaly proximally, the scales linear-lanceolate, to 3 × 0.7 mm, brown, thin, chartaceous, margins entire, apices long attenuate, ending in dark glandular tip. **Fertile blades** narrowly triangular to lanceolate, 2–3-pinnate-pinnatifid proximally, 3–5 cm at widest point. **Rachises** dark reddish brown proximally, green distally, flattened to very slightly grooved adaxially, with trichomes like those of the petioles throughout. **Pinnae** 8–12 per fertile leaf, alternate to subopposite, the proximal with dark reddish brown stalks 3–9 mm, broadly triangular to ovate, the largest 15–40 × 10–25 mm, pubescent with trichomes similar to those of petioles but often at lower density (> 10 per mm^2) and shorter (mostly 0.1–0.6 mm abaxially and 0.1–0.4 mm adaxially). **Ultimate segments** oblong-ovate, mostly 5–10 × 3–5 mm, usually broadly attached to subtending costae (rarely short stalked), margins shallowly to deeply 5–11-lobed, usually not recurved but occasionally slightly cupped around distal side of sori, with very narrow (< 0.1 mm), pale green to hyaline edges bearing glandular trichomes similar to those of adaxial pinna surfaces. **Veins** mostly obscure, segment midveins mostly with 5–11 alternately-branched secondary veins, these 1–4-forked, not anastomosing, terminating before reaching segment margins. **Sori** solitary on marginal lobes, consisting of 1–8 sporangia attached to enlarged vein tips in close proximity (ca. 0.1 mm) to apex of marginal lobes, discontinuous but occasionally appearing confluent with age, 10–50 per ultimate segment. **False indusia** 0–0.13 mm wide, scarcely differentiated, most sporangia exposed throughout development. **Sporangia** containing 32 spores, the stalk multicellular, < 0.1 mm, annulus 18–21-celled. **Spores** trilete, averaging 48–52 μm in diameter, dark brown, the perispore prominently cristate. **Chromosome number** $n = 30$. See Figs. 2 and 4 for illustrations and images of sporophyte features and Fig. 5 for image of meiotic chromosome squash.

Distribution and Habitat*—Cheilanthes ecuadorensis* is known from two localities, both in the eastern portion of Loja Province in southern Ecuador (Fig. 6). It occurs on dry or exposed slopes, usually in rock fissures, in association with *Acacia*, *Furcraea*, and cacti.

***Etymology*—**The specific epithet refers to this species' native range located, to the best of our knowledge, exclusively in Ecuador.

***Additional Specimens Examined*—Ecuador.** —Loja: Valley of Catamayo, S of the village, at Algarrobera, 1350–1450m, 19 May 1988, *B. Ollgaard, J.E. Madsen, & L. Christensen 74251* (AAU).

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Author Contributions

Conceptualization: MDW, KMP, and GY; Data Curation: KS and LH; Formal Analysis: KS; Funding Acquisition: KMP, MDW, and GY; Investigation: KS and MDW; Project Administration: KMP; Supervision: MDW; Visualization: KS; Writing – Original Draft Preparation: KS, KMP, and MDW; Writing – Review and Editing: KS, KMP, LH, GY, and MDW.

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Appendix 1. Vouchers and GenBank accession numbers for taxa used in the molecular phylogenetic analyses: Taxon, Collection locality, Voucher collector and collector number (Herbarium Acronym), FernLab database number (fernlab.biology.duke.edu), GenBank accession numbers for *atpA*, *rbcL*, *trnGR* (in that order). Loci missing from molecular analyses are indicated with a dash (—).

Ingroup: *Aleuritopteris farinosa* (Forssk.) Fée, TANZANIA, Iringa, *C.J. Kayombo 2700* (DUKE), 4406, EU268721.1, MH170409, EU268667.1. *Aspidotis schimperi* (Kunze) Pic.Serm., ZAMBIA, S. Gwembe, *B. Luwiika et al. 637* (MO), 5034, MH170450, MH170410, MH170460. *Cheilanthes arequipensis* (Maxon) R.M.Tryon & A.F.Tryon, PERU, Arequipa, *H. van der Werff et al. 20479* (MO), 6190, MH170444, MH170411, MH170468. *Cheilanthes bergiana* Schltdl., TANZANIA, Kagera, *L. Festo & W. Bayona 810* (DUKE), 4709, MH170429, MH170412, MH170456. *Cheilanthes capensis* (Thunb.) Sw., SOUTH AFRICA, Northern Cape, *M.S. Mothogoane 734* (US), 6175, MH170451, MH170413, MH170466. *Cheilanthes dinteri* Brause, NAMIBIA, Sargdeckelberg, *R. Seydel 3112* (US), 6174, MH170439, MH170414, MH170462. *Cheilanthes distans*, AUSTRALIA, New South Wales, *N.S. Nagalingum 23* (DUKE), 3894, MH170430, HM003029.1, EU268680.1. *Cheilanthes ecuadorensis*, ECUADOR, Loja, *J.E. Madsen & O.A. Sanchez 7940* (MO), 8991, MH170433, MH170415, —. *Cheilanthes fraseri* Mett. ex Kuhn, PERU, Cuzco, *W.L. Galiano et al. 5034* (MO), 4991, MH170447, MH170416, MH170461. *Cheilanthes micropteris*, ARGENTINA, Misiones, *N.B. Deginani et al. 1363* (MO), 3709, MH170434, EF452145.1, EU268683.1. *Cheilanthes micropteris*, BRAZIL, Rio Grande do Sul, *J. Prado & R.Y. Hirai 2132* (DUKE), 7859, MH170431, MH170417, MH170454. *Cheilanthes micropteris*, BOLIVIA, Chuquisaca, *H. Huaylla 69* (MO), 6045, MH170435, MH170408, —. *Cheilanthes obducta* Mett. ex Kuhn, BOLIVIA, Santa Cruz, *M.H. Nee 51199* (MO), 4994, MH170449, MH170418, MH170455. *Cheilanthes parviloba* Sw., NYBG living collection, 5324, MH170440, MH170419, MH170458. *Cheilanthes pilosa*, BOLIVIA, La Paz, *A.F. Fuentes et al. 10353* (UC), 5628, MH170437, MH170420, MH170463. *Cheilanthes pohliana* Mett., BRAZIL, Goias, *E. Schuettpelz et al. 1372* (SP), 8306, MH170432, MH170421, MH170457. *Cheilanthes pruinata*, PERU, Cuzco, *L. Valenzuela 2058* (MO), 4984, MH170445, MH170422, MH170465. *Cheilanthes rufopunctata*, BOLIVIA, Cochabamba, *M. Sundue et al. 613* (UC), 5629, MH170448, JX313528.1, JX313448.1. *Cheilanthes sieberi* Kunze, AUSTRALIA, New South Wales, *N.S. Nagalingum 20* (DUKE), 3891, MH170452, MH170423, MH170469. *Cheilanthes squamosa* Gilles & Hook. ex Grev., BOLIVIA, Tarija, *H. Huaylla et al. 1920* (MO), 4982, MH170441, MH170424, MH170459. *Cheilanthes tenuifolia* (Burm. f.) Sw., MALAYSIA, Kuala Lumpur, *A. Grusz et al. 1* (DUKE), 4345, MH170453, MH170425, MH170464. *Gaga marginata* (Kunth) F.W.Li & Windham, BOLIVIA, Tarija, *M. Serrano et al. 6089* (MO), 4986, MH170442, MH170426, MH170467. *Hemionitis palmata* L., COSTA RICA, Heredia, *C.J. Rothfels et al. 08-84* (DUKE), 5137, MH170436, KC984525.1, EU268690.1. *Mildella intramarginalis* (Kaulf. ex Link) Trevis., MEXICO, Oaxaca, *V.W. Steinmann et al. 2549* (MO), 4988, MH170438, MH170427, MH170470. *Trachypteris pinnata* (Hook. f.) C.Chr., ECUADOR, Galapagos, *I. Aldaz s.n.* (MO), 4978, MH170443, MH170428, MH170471.

Outgroup: *Pentagramma triangularis* (Kaulf.) Yatsk., Windham & E.Wollenw., UNITED STATES, California, *J. Metzgar et al. 179* (DUKE), 3832, MH170446, JX313531.1, KR132637.1.

Appendix 2. List of specimens examined microscopically for this project: Taxon, Collection locality, Voucher collector and collector number (Herbarium Acronym).

Cheilanthes andina, BOLIVIA, La Paz, *G.H. Tate 126* (NY). *Cheilanthes ecuadorensis*, ECUADOR, Loja, *Madsen & Sanchez 7940* (MO), *B. Ollgaard et al. 74251* (AAU). *Cheilanthes micropteris*, ARGENTINA, Misiones, *Múlgura de Romero et al. 2883* (MO), *Deginani et al. 1363* (MO); BOLIVIA, Oropeza, *Huaylla 71* (MO); BRAZIL, Rio Grande do Sul, *Wasum s.n.* (MO); Jaquirana, *Prado & Hirai 2132* (MO). *Cheilanthes pilosa*, BOLIVIA, La Paz, *Jimenez 1097* (UC); Franz Tamayo, *Fuentes et al. 10353* (UC); Murillo, *Fay & Fay 2844* (MO); PERU, Ancash, *Rothfels & Zylinski 3980* (DUKE). *Cheilanthes rufopunctata*, PERU, Chuquisaca, *Wood 14579* (UC); Cusco, *Valenzuela et al. 8350* (MO).

Appendix 3. GBIF record numbers for *Cheilanthes micropteris* and *C. rufopunctata* that are mapped in Fig. 6. As mentioned in the text, *C. ecuadorensis* and *C. pilosa* are difficult to distinguish in digitized images, so only specimens on loan were used to map their distributions.

Cheilanthes micropteris; 416878824, 416878829, 416878871, 416878872, 416878881, 416878892, 416878901, 416878902, 416878903, 416878947, 416878966, 416878981, 416878982, 684432105, 814356353, 814356357, 814356358, 814356359, 814356362, 1055479628, 1055614441, 1055774061,

1055809943, 1055909511, 1055948785, 1056017114, 1056188270, 1056477388, 1056618321, 1056671949, 1056902020, 1057170855, 1057549706, 1077299985, 1077414193, 1086676467, 1090550472, 1095631685, 1095637655, 1095638578, 1097637893, 1098095286, 1135825931, 1209423942, 1213528053, 1252588985, 1257640598, 1258000647, 1259526380, 1259526406, 1259588829, 1259668274, 1259668338, 1259715300, 1260639954, 1260639961, 1261066314, 1264700921, 1291016793, 1291017233.

Cheilanthes rufopunctata; 1260832280, 1260511806, 1259119872, 1261048427, 1056739392, 1055883370, 1261127205, 1261349799, 1057511088.