**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**

**POSGRADO EN CIENCIAS BIOLÓGICAS**

FACULTAD DE ESTUDIOS SUPERIORES ZARAGOZA

SISTEMATICA

# SISTEMATICA FILOGENETICA DEL COMPLEJO ELAPHOGLOSSUM PETIOLATUM (DRYOPTERIDACEAE)

## TESIS

QUE PARA OPTAR POR EL GRADO DE:

**DOCTOR(A) EN CIENCIAS**

PRESENTA:

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**Presente**

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **19 de septiembre de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **MARTÍNEZ BECERRIL ANA GABRIELA** con número de cuenta **303286383** con la tesis titulada **“SISTEMÁTICA FILOGENÉTICA DEL COMPLEJO ELAPHOGLOSSUM PETIOLATUM (DRYOPTERIDACEAE)”,** realizada bajo la dirección del **DR. ELOY SOLANO CAMACHO,** quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

**ATENTAMENTE**
**“POR MI RAZA HABLARÁ EL ESPÍRITU”**
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**COORDINADOR DEL PROGRAMA**

**DR. ADOLFO GERARDO NAVARRO SIGÜENZA**

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*a todas las MUJERES.*

*a quienes aman la vida y además la estudian.*

*a quienes por causa o destino están leyendo esto.*

# ÍNDICE

# RESUMEN

*Elaphoglossum* (Sw.) Urb. es un género de helechos considerado megadiverso debido a su riqueza calculada en 600 especies aproximadamente. El género se distingue fácilmente de otros helechos por sus láminas simples, rara vez divididas, con dimorfismo entre hojas estériles y fértiles, venas libres y soros acrosticoides.

La clasificación infragenérica de *Elaphoglossum* reconoce siete clados principales o secciones (sec.), de las cuales una de las que presenta mayor riqueza es la sec. *Lepidoglossa* con cerca de 200 especies. Dentro de esta sección, se ubica un grupo de 11 especies con morfología similar, denominado complejo de *Elaphoglossum petiolatum*. Las especies de este grupo comparten características como escamas del rizoma obscuras con márgenes enteros, escamas de la porción proximal de los pecíolos con ápice oscuro, escamas de la lámina planas o estrelladas y puntos resinosos en la parte inferior de las láminas. La taxonomía y delimitación de las especies del complejo no está bien definida debido a que existen pocos caracteres diagnósticos, hay una gran variación en los caracteres morfológicos y no existen trabajos filogenéticos moleculares sobre el grupo.

Algunas filogenias moleculares previas que no incluyeron todas las especies del complejo sugieren que éste no es un grupo monofilético, sin embargo, las especies mexicanas que hacen parte de él se recuperan en el mismo clado. No obstante, la representación de especies mexicanas en la filogenia es escasa.

El presente estudio tuvo como objetivos principales: I) probar la monofilia del complejo de *Elaphoglossum petiolatum* incrementando el muestreo de sus especies y de los representantes mexicanos en la filogenia general de *Elaphoglossum,* II) conocer las relaciones entre las especies del complejo y III) elaborar la monografía del grupo monofilético que resultará de los análisis moleculares. Para ello, se realizaron dos análisis

filogenéticos utilizando secuencias de marcadores de cloroplasto, *atpβ-rbcL, rps4-trnS y trnL-trnF*, y se analizó la morfología de las especies involucradas, incluida la de las esporas. Los resultados de esta investigación están divididos en cuatro capítulos:

**Capítulo I.** Systematics of the *Elaphoglossum petiolatum* complex (Dryopteridaceae). Este capítulo se enfocó en poner a prueba la monofilia del complejo de *E. petiolatum* utilizando las secuencias de ADN del cloroplasto e incrementando la representación tanto de las especies del complejo como de los representantes mexicanos del género en la filogenia general. Los resultados confirman que el complejo no es un grupo natural, pero se recuperó un grupo monofilético que incluye las especies mexicanas. Este clado, nombrado aquí "clado de *E. petiolatum",* representa el objeto de estudio en los siguientes capítulos.

**Capítulo II.** Spore morphology of the Mexican species of the *Elaphoglossum petiolatum* complex (Dryopteridaceae). En este capítulo se analizó la morfología de las esporas de las especies que integran el "clado de *E. petiolatum".* Se encontraron caracteres morfológicos con valor taxonómico que ayudan a reconocer algunas especies del clado.

**Capítulo III.** Taxonomic revision of the *Elaphoglossum petiolatum* clade (Dryopteridaceae). Este capítulo corresponde con el tratamiento taxonómico de las especies que integran clado de *E. petiolatum* definido con base en la evidencia molecular del primer capítulo. Este trabajo provee una clave dicotómica, descripciones ilustradas, sinonimia, mapas de distribución y notas nomenclaturales para todas las especies del clado.

**Capitulo IV.** *Elaphoglossum auralolitae,* a new species of *Elaphoglossum* sect. *Lepidoglossa* (Dryopteridaceae) from Guatemala. Se describe una nueva especie de *Elaphoglossum* endémica de Guatemala que pertenece al "clado de *E. petiolatum".*

# ABSTRACT

*Elaphoglossum* (Sw.) Urb. is a fern genus considered megadiverse due to its richness estimated at ca. 600 species. The genus is easily distinguished from other ferns by its simple, rarely divided laminae, with dimorphism between sterile and fertile leaves, free veins, and acrostichoid sori.

The infrageneric classification of *Elaphoglossum* recognizes seven main clades or sections (sect.), one of those with more richness is sect. *Lepidoglossa* with about 200 species. Within this section, there is a group of 11 species with similar morphology, called the *Elaphoglossum petiolatum* complex. Species belonging to this group share characteristics such as dark rhizome scales with entire margins, proximal petiole scales with dark apex, flat or stellate blade scales, and resinous dots on the abaxial laminar surface. The taxonomy and delimitation of the species that are part of the complex are not well defined due to there being few diagnostic characters, there is a great variation in the morphological characters and there are no molecular phylogenetic works on this complex.

Previous molecular phylogenies that do not include all the species of this complex suggest it is not a monophyletic group but, the Mexican species that belong to the complex were recovered in the same clade. Nevertheless, Mexican species are not well represented in the phylogenies of the genus.

The main aims of this study were: I) to test the monophyly of the *Elaphoglossum petiolatum* complex by increasing the sample of its species and the Mexican representants in the global phylogeny of *Elaphoglossu*m, II) to know the phylogenetic relationships among the species of the *E. petiolatum* complex and III) to carry out the monograph of the monophyletic group resulting of the molecular analyses. To reach all these aims, we carried out two phylogenetic analyses using sequences of chloroplast markers, *atpβ-rbcL, rps4-trnS*

and *trnL-trnF*, and we analyzed the morphology of the species involved and its spores. The results of this research are divided into four chapters:

**Chapter I.** Systematics of the *Elaphoglossum petiolatum* complex (Dryopteridaceae). This chapter focused on testing the monophyly of the *E. petiolatum* complex using chloroplast DNA sequences and increasing the representants of both the complex species and the Mexican species of the genus in the overall phylogeny. The results show that the complex is not a natural group, but a monophyletic group that includes the Mexican species was recovered. This clade named here "*E. petiolatum* clade", is the object of study in the following chapters.

**Chapter II.** Spore morphology of the Mexican species of the *Elaphoglossum petiolatum* complex (Dryopteridaceae). In this chapter, we analyze the morphology of the spores of the species belonging to the "*E. petiolatum* clade". We found some morphological characters with a taxonomic value that helped distinguish some species of the clade.

**Chapter III.** Taxonomic revision of the *Elaphoglossum petiolatum* clade (Dryopteridaceae). This chapter corresponds to the taxonomic treatment of the species belonging to the "*E.* petiolatum clade", the monophyletic group defined based on the molecular evidence of the first chapter. This work provides a dichotomous key, illustrated descriptions, synonymy, distribution maps, and nomenclatural notes for all the species of the clade.

**Chapter IV.** *Elaphoglossum auralolitae*, a new species of *Elaphoglossum* sect. *Lepidoglossa* (Dryopteridaceae) from Guatemala. We describe a new species of *Elaphoglossum* endemic to Guatemala that belongs to the "*E. petiolatum* clade".

# INTRODUCCIÓN GENERAL

Los helechos son el segundo grupo de plantas vasculares con mayor riqueza en el mundo tan solo detrás las plantas con semilla, las cuales también representan su grupo hermano (Pryer et al., 2001; Barker y Wolf, 2010; Moran, 2018). Se conocen aproximadamente 12,000 especies de helechos distribuidas en todos los ecosistemas del mundo, pero con mayor representación en los trópicos.

Entre los géneros de helechos con mayor riqueza se encuentra *Elaphoglossum* (Sw.) Urb. con al menos 600 especies aceptadas, pero con más de 1000 descritas (Moran, 2018; PPG I, 2016; Rouhan, 2020). Las especies de este género se distribuyen en las zonas tropicales de todo el mundo, pero al menos ¾ del total de especies conocidas se encuentra en los trópicos de América (Fig.1) (Mickel, 1995; Moran, 2018; Moran et al., 2010). *Elaphoglossum* se distingue fácilmente de otros helechos por sus láminas simples, rara vez divididas (sólo seis especies presentan láminas divididas), con dimorfismo entre hojas estériles y fértiles, venas libres y soros acrosticoides (Fig. 2). La mayoría son epífitas, pero también las hay terrestres y rupícolas (Mickel y Atehortúa, 1980; Moran 2018; Moran et al., 2007; Moran et al., 2010; Vasco et al., 2009a; Vasco et al., 2013; Rouhan et al., 2020).

Las primeras especies de *Elaphoglossum* se describieron como parte de *Acrostichum* L., un género que agrupaba especies con soros acrosticoides. En1834 Heinrich Wilhelm Schott reconoció por primera vez el género *Elaphoglossum,* sin embargo, no elaboró una descripción lo que dificultó que el nombre fuera fácilmente aceptado. Por lo anterior, en muchas especies y en los primeros tratamientos taxonómicos que se elaboraron para el género se siguió utilizando el nombre de *Acrostichum*. (Mickel y Atehortúa, 1980).

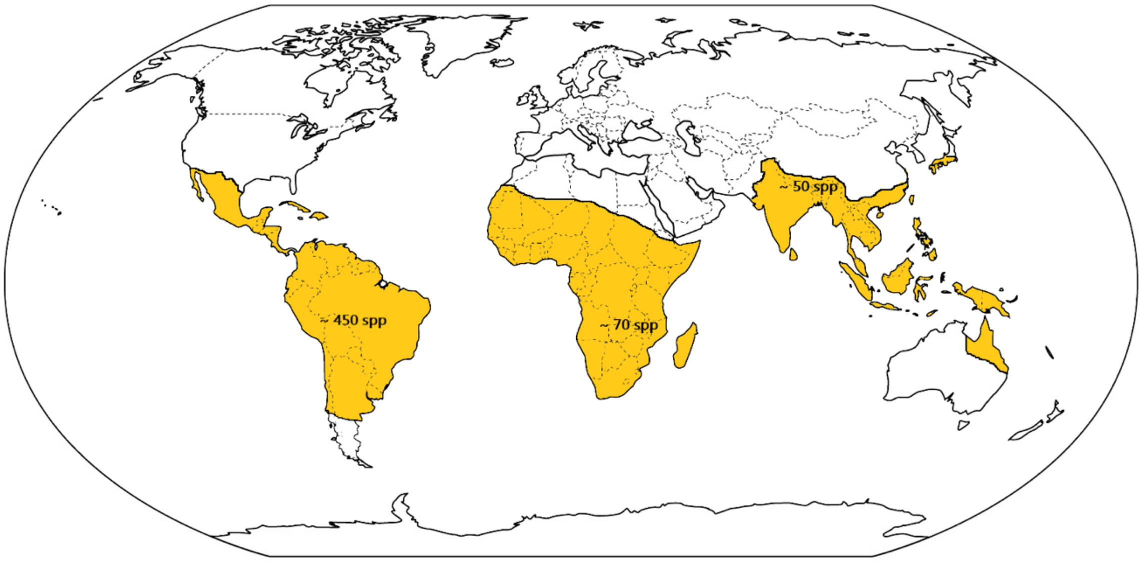


*Figura 1.* Distribución general de las especies de *Elaphoglossum.*

La descripción formal de *Elaphoglossum* la realizó John Smith en 1841, quien transfirió a este género seis especies de *Acrostichum* que diferían morfológicamente del tipo (*Acrostichum aureum* L.) (Mickel y Atehortúa, 1980). El primer tratamiento taxonómico conocido para *Elaphoglossum* fue preparado por Fée (1845) aunque este se realizó aún bajo el nombre de *Acrostichum*. En este trabajo, Fée reconoció dos grupos infragenéricos principales: *Oligolepideae* y *Polylepideae*, mismos que subdividió con base en el tamaño de la hoja. Posteriormente, Fée (1852) decidió reordenar al género y utilizar cuatro grupos sin subdivisiones: *Oligolepideae, Polylepideae, Pilosellae* y *Chromatolepideae* (Mickel y Atehortúa, 1980).

Las combinaciones posteriores las realizó Moore (1857 a,b,c; 1862) quien separó al género en dos grupos, *Oligolepidum* y *Polylepidum,* de acuerdo con la cantidad de escamas de la lámina. A esta clasificación le sucedieron la de Sodiro (1897) y la de Diels (1899). Sodiro (1897) reconoció ocho subgrupos dentro del género, pero también siguió tratándolo como *Acrostichum*, y Diels (1899) nombró dos secciones: *Eu-Elaphoglossum* y

*Hymenodium* en las que acomodó la mayoría de los grupos propuestos por Sodiro, a los que asignó la categoría de subsecciones.

*Figura 2.* Representación de la morfología de las especies de *Elaphoglossum.* A. *E. lanceum* Mickel (Sundue 3472). B. *E. macrophyllum* (Mett. ex Kuhn) Christ (Nathan Smith 255_0296). C. *E. potosianum* Christ (Martínez-Becerril 399). A y B tomadas de www.fernsoftheworld.org.

En 1899 el abogado y botánico alemán Hermann Christ llevó a cabo el único trabajo monográfico para *Elaphoglossum* que abarcó todas las especies descritas hasta entonces. El trabajo incluyó el estudio minucioso de las 142 especies conocidas en ese momento y su clasificación en 32 grupos de acuerdo con características morfológicas como el tipo de venación, tipo de escamas de la lámina, tamaño de la hoja y el hábito del rizoma. Aunque estos grupos eran diagnosticables y fácilmente reconocibles, el trabajo no incluyó una clave de identificación para cada uno.

Posterior a la monografía de Christ, los trabajos sobre el género se limitaron a la descripción de nuevas especies, hasta que en 1980 Mickel y Atehortúa publicaron una nueva clasificación infragenérica. En esta clasificación, dividieron a *Elaphoglossum* en grupos con base en características morfológicas como el hábito del rizoma, el tipo y color de las escamas del rizoma y la lámina, la textura y forma de la hoja, la presencia o ausencia de hidátodos y filopodios y la micromorfología de las esporas. De esta manera, los autores reconocieron nueve secciones y 21 subsecciones dentro del género, algunas de las cuales ya habían sido propuestas previamente por Christ (1899).

En las últimas dos décadas nuestra comprensión sobre la diversidad y las relaciones evolutivas dentro *Elaphoglossum* ha cambiado debido a la evidencia que han aportado los datos moleculares (Rouhan et al., 2004; Skog et al., 2004; Liu et al., 2007; Vasco et al., 2009b; Lóriga et al., 2014). Es así como la monofilia de *Elaphoglossum* y su posición como grupo hermano del género *Mickelia* R. C. Moran, Labiak & Sundue y dentro el clado bolbitidoide, subfamilia *Elaphoglossoideae* (Pic. Serm.) Crabbe, familia Dryopteridaceae están fuertemente respaldadas y dentro de su división infragenérica se reconocen siete secciones (sec.) monofiléticas: 1) *Elaphoglossum* sec. *Amygdalifolia* (Christ) Mickel y Atehortúa; 2) sec. *Wrightiana* J. Lóriga, A. Vasco, L. Regalado, Heinrichs y R.C. Moran; 3) sec. *Elaphoglossum*, 4) sec. *Squamipedia* Mickel y Atehortúa; 5) sec. *Polytrichia* Christ; 6) sec. *Setosa* (Christ) Mickel y Atehortúa; y 7) sec. *Lepidoglossa* Christ (Fig. 3) (Skog et al., 2004; Rouhan et al., 2004; Lóriga et al., 2014).

Seis de estos clados se corresponden con las secciones propuestas previamente por Mickel y Atehortúa (1980) y el séptimo fue descrito en 2014 como una sección monotípica (Rouhan et al., 2004; Skog et al., 2004; Moran et al., 2010; Lóriga et al., 2014; Liu et al., 2016; PPGI, 2016).

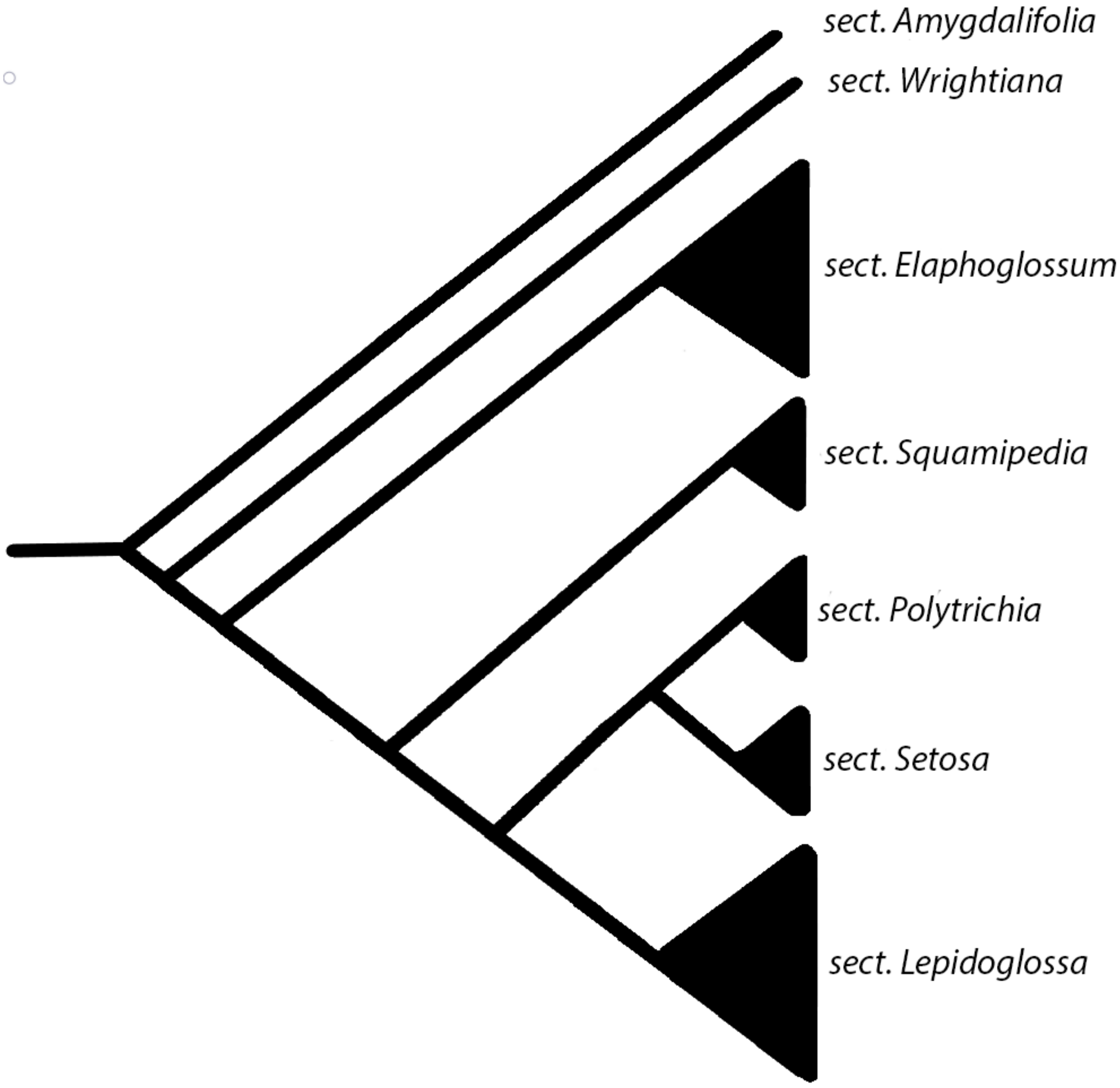


*Figura 3.* Representación gráfica de la clasificación infragenérica del género *Elaphoglossum* en siete secciones. El tamaño de los triángulos es proporcional a la riqueza de especies dentro de cada sección.

El conocimiento geográfico y taxonómico sobre el género se ha incrementado en los últimos 30 años debido a revisiones para floras locales, por ejemplo, en Sudáfrica (Roux, 1982), Perú (Mickel, 1991), Mesoamérica (Mickel, 1995a), Venezuela (Mickel, 1985, 1995b), México (Mickel y Smith, 2004), África Oriental Tropical (Mickel, 2002), Hawái

(Palmer, 2003), Islas Mascareñas (Lorence y Rouhan, 2004), Polinesia Francesa (Rouhan et al., 2008), África, Macronesia, y las Islas del Atlántico medio y el sureste del océano Índico (Roux, 2011) y Bolivia (Kessler et al., 2018); y a revisiones taxonómicas que se han centrado en pequeños grupos monofiléticos o algunas secciones (Matos y Mickel, 2014, 2018, 2019; Vasco, 2011; Vasco, et al., 2009a; Vasco, et al., 2013). No obstante, estas monografías han tratado menos del 10% de las especies descritas para *Elaphoglossum,* por lo que el trabajo taxonómico del género aún está lejos de ser completado.

Una de las secciones con mayor número de especies dentro del género es la sec. *Lepidoglossa*, con aproximadamente 200 especies (Vasco, 2011). Las especies de esta sección se caracterizan por tener escamas del rizoma y la lámina con dientes marginales aciculares, por presentar escamas planas y/o estrelladas (no subuladas) y en ocasiones, puntos resinosos en las láminas y pecíolos (Kessler et al., 2018). Esta subsección fue dividida por Mickel y Atehortúa (1980) en seis subsecciones (subsec.): subsec. *Muscosa* Mickel y Atehortúa, subsec. *Polylepidea* Christ, subsec. *Microlepidea* Christ, subsec. *Petiolosa* Christ, subsec. *Pilosa* Christ y subsec. *Huacsaro* Mickel y Atehortúa, las cuales se diferencian principalmente, por la forma y la densidad de las escamas de las hojas y los rizomas, el ápice de las láminas y la micromorfología de las esporas.

Dentro de la subsec. *Pilosa,* Mickel y Atehortúa (1980) agruparon especies con rizomas rastreros compactos, generalmente con escamas oscuras con márgenes enteros o con largas proyecciones, láminas con puntos resinosos, escamosas a subglabras y esporas con surcos y crestas (Mickel y Atehortúa 1980). Las escamas de la lámina pueden ser lanceoladas con márgenes con proyecciones cortas, estrelladas, subestrelladas o, a veces, tan reducidas que parecen pelos estrellados.

Como parte del trabajo taxonómico sobre el género *Elaphoglossum* en México, y con base en caracteres morfológicos, Mickel (2001) describió un grupo de especies dentro de la subsec. *Pilosa*, al que denominó el complejo de *Elaphoglossum petiolatum*. Este grupo incluyó 11 especies distribuidas en los trópicos, tres de ellas endémicas de México, (Cuadro 1), las cuales se caracterizan por presentar escamas del rizoma obscuras con márgenes enteros, escamas de la porción proximal de los pecíolos con ápice oscuro, escamas de la lámina planas o estrelladas y puntos resinosos en la parte inferior de las láminas (Fig. 4) (Mickel 2001, Mickel y Smith 2004). No obstante, la delimitación de las especies de este complejo no es clara debido a la gran variación de los caracteres morfológicos, a los pocos caracteres diagnósticos que existen para diferenciar a las especies -principalmente se utilizan la presencia, tamaño, tipo y densidad de las escamas que se encuentran mezcladas con los puntos resinosos- y a las diferencias en la densidad y el tamaño de los puntos resinosos (Mickel, 2001).

Si bien hasta ahora no existen trabajos sobre la sistemática del complejo de *Elaphoglossum petiolatum*, análisis filogenéticos moleculares previos, que incluyeron algunas de las especies de este complejo, sugirieron que este grupo no era monofilético (Rouhan et al. 2004, Vasco et al. 2009b, Lóriga et al. 2014). Los taxones pertenecientes al complejo incluidos en estos análisis fueron recuperados en tres clados diferentes que a su vez incluían otras especies fuera del complejo: el “grupo *Elaphoglossum ciliatum*”, el “clado mexicano de *E. petiolatum*” – el cual incluye las especies mexicanas del clado-, y un tercer clado sin nombre que incluía dos especímenes de Ecuador determinados como *E. aff. petiolatum* (Vasco et al., 2009b).

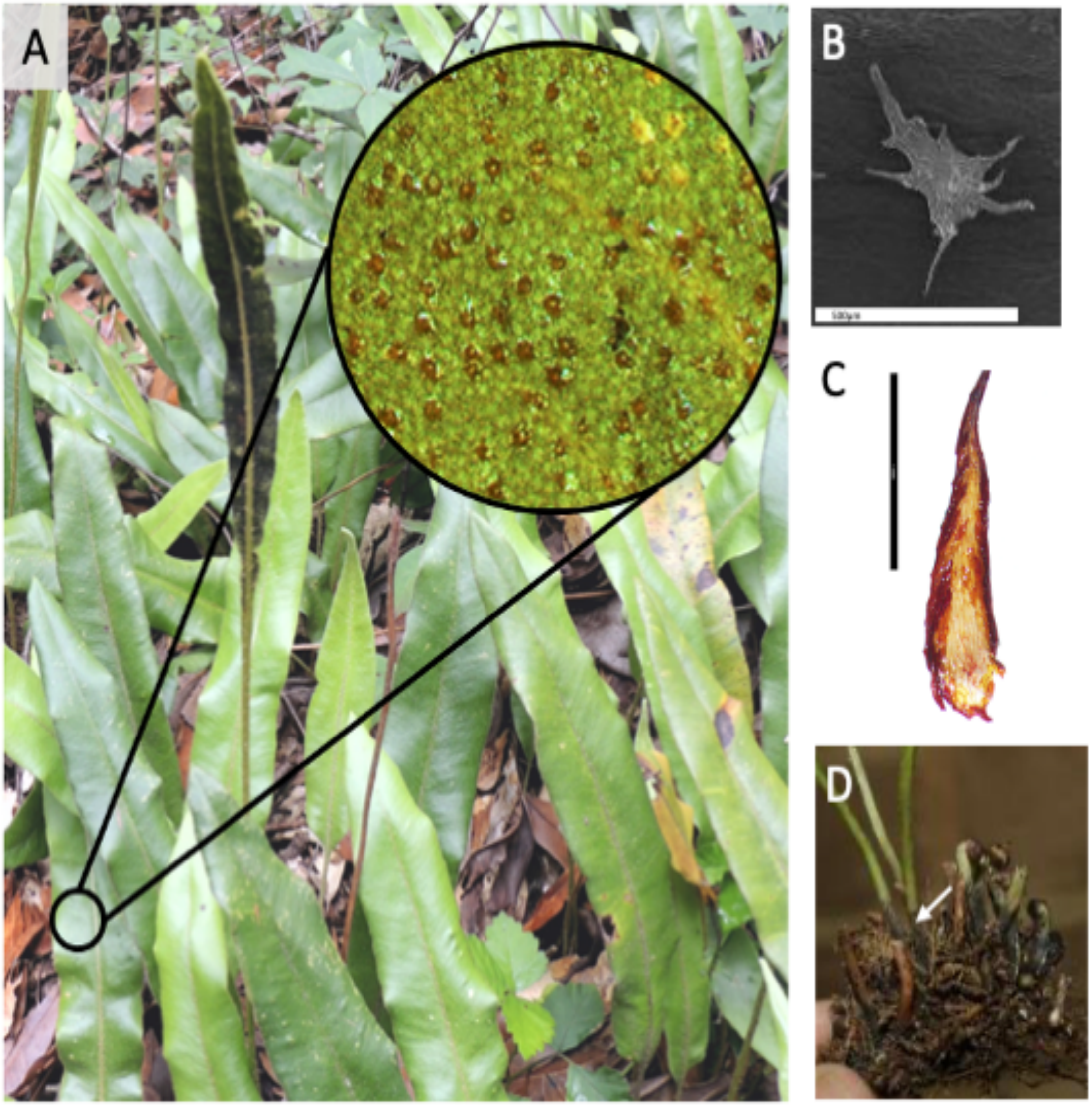


*Figura 4.* Morfología general de las especies que integran el complejo de *Elaphoglossum petiolatum.* A. Hábito y acercamiento a los puntos resionos de las láminas. B. Escama plana de la lámina (escala= 500μm). C Escama del rizoma (escala= 1μm). D. porción proximal de los pecíolos con ápice oscuro. Fotos A y C por Ana Gabriela Martínez; B y D por A. Vasco.

**Cuadro 1.** Especies que integran el complejo de *Elaphoglossum petiolatum* sensu Mickel (2001).

| Especies | Distribución |
|---|---|
| *Elaphoglossum petiolatum* (Sw.) Urban | De México a Perú y Las Antillas |
| *E. salicifolium* (Willd. ex Kaulf.) Alston (ahora *E. lancifolium* (Desv.) C.V. Morton) | Bioko, Camerún, Comoros, Congo, Gaba, Guinea, La Reunión, Liberia, Islas Mauricio, Madagascar, Malawi, Mozambique, Ruanda, Sierra Leona, Seychelles, Tanzania, Zambia, Zimbabwe |
| *E. nervosum* (Bory) H. Christ | Isla de Santa Helena (Reino Unido) |
| *E. dimorphum* (Hook. & Grev.) T. Moore | Isla de Santa Helena (Reino Unido) |
| *E. bifurcatum* (Jacq.) Mickel | Isla de Santa Helena (Reino Unido) |
| *E. stelligerum* (Wall. ex Baker) T. Moore ex Alston & Bonner | India |
| *E. yunnanense* (Baker) C. Chr. | China, India, Malasia, Vietnam |
| *E. blumeanum* (Fee) J. Smith | Archipielago de Bismark, Borneo, China, Indonesia, Filipinas, Malasia, Islas Salomón, Sumatra. |
| *E. rzedowskii Mickel* | México |
| *E. pringlei* (Davenp.) C. Chr. | México |
| *E. trichomidiatum* Mickel (ahora *E. potosianum* Christ) | México |

Por lo anterior, el presente estudio tuvo como objetivos principales: I) ampliar la representación de las especies del complejo y de las especies mexicanas de *Elaphoglossum* en la filogenia global del género para poner a prueba la hipótesis de monofilia del complejo de *E. petiolatum,* II) conocer las relaciones entre las especies del complejo y III) elaborar la monografía del grupo monofilético que resulte de los análisis moleculares. Las preguntas específicas que se buscaron responder fueron (1) ¿El complejo de *E. petiolatum* es monofilético cuando se incluye una muestra más amplia de sus especies? De ser así, ¿cuántas y cuáles son las especies que pertenecen al complejo? Si no lo es, ¿dónde se recuperan las especies del complejo? (2) ¿Existe evidencia morfológica que sustente la posición filogenética de las especies pertenecientes al complejo? y (3) ¿Se sigue recuperando el "clado mexicano" cuando se incluyen otras especies distribuidas en México, especialmente aquellas especies que son morfológicamente similares a las recuperadas en este clado?

Para responder a estas preguntas, se realizaron dos análisis filogenéticos utilizando secuencias de tres marcadores de cloroplasto, *atpβ-rbcL, rps4-trnS y trnL-trnF*, y se analizó la morfología de las especies involucradas, incluida la de las esporas, en busca de caracteres morfológicos que aporten información taxonómica que ayuden a la diferenciación de las especies. Los resultados completos de esta investigación están divididos en los siguientes capítulos:

**Capítulo I.** Systematics of the *Elaphoglossum petiolatum* complex (Dryopteridaceae). Este capítulo se enfocó en poner a prueba la monofilia del complejo de *E. petiolatum* utilizando secuencias de ADN de los marcadores del cloroplasto *atpβ-rbcL, rps4-trnS* y *trnL-trnF*. En este trabajo se incrementó la representación de las especies del complejo y de los representantes mexicanos del género en la filogenia general de

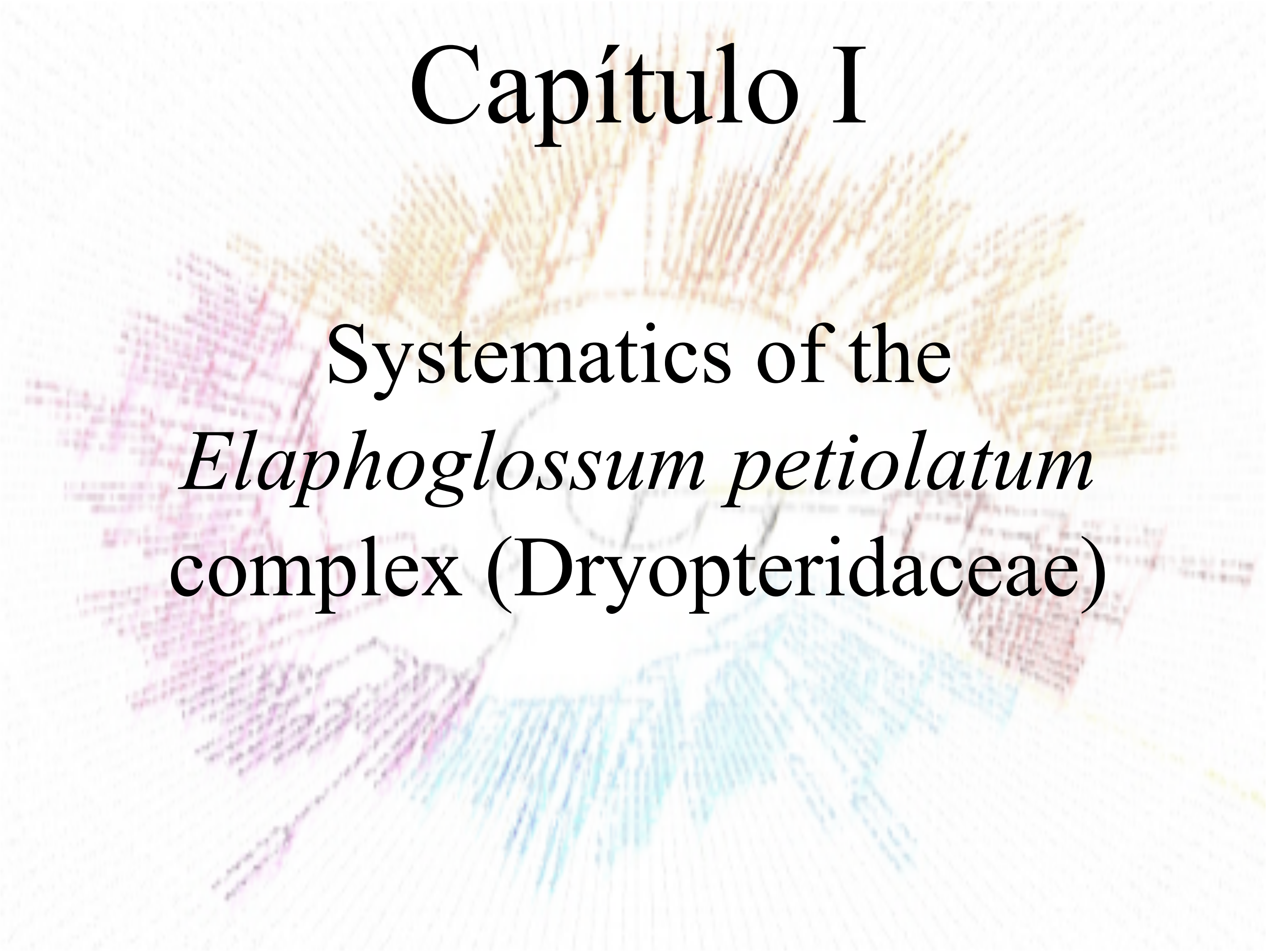
*Elaphoglossum*. Los resultados comprueban que el complejo de *E. petiolatum* no es un grupo monofilético pues las especies que lo integran se recuperan en tres clados que no son hermanos, denominados aquí como "el clado de *E. ciliatum", "*el clado de *E. lancifolium"* y "el clado de *E. petiolatum".* Este último clado recuperó el representante de *E. petiolatum* de Jamaica, localidad tipo de este taxón, las especies mexicanas consideradas previamente parte del complejo original, más *E. schiedei* y *E. schmitzii,* especies que con base en la evidencia de este trabajo, fueron sacadas de la sinonimia de *E. petiolatum*.

Las especies que pertenecen al "clado de *Elaphoglossum petiolatum"* se distribuyen exclusivamente en el continente americano, de México a Costa Rica incluyendo Las Antillas, y al menos cuatro de ellas son endémicas de México (Mickel y Smith, 2004; Vasco et al., 2009; Martínez-Becerril et al., 2021). Su morfología se caracteriza por la presencia de escamas del rizoma con ápices agudos a filiformes con el margen generalmente más obscuro que el resto de la escama (excepto en *E. petiolatum*, que presenta escamas del rizoma concoloras), láminas escamosas en ambas superficies (en diferentes grados) con escamas planas, en ocasiones estrelladas, y con puntos resinosos pegajosos y/o secos (en diferente densidad), y esporas con perisporas espinosas, con espinas que miden menos de 1µm (excepto *E. petiolatum* que presenta depósitos irregulares en lugar de espinas).

**Capítulo II.** Spore morphology of the Mexican species of the *Elaphoglossum petiolatum* complex (Dryopteridaceae). El capítulo corresponde al análisis morfológico de las esporas de las especies que integran el "clado de *E. petiolatum".* Las esporas se observaron, fotografiaron y midieron utilizando microscopía electrónica de barrido y los resultados mostraron diferencias en la ornamentación y en el tamaño de las esporas. Estas diferencias son útiles para distinguir algunas especies de este grupo. La variación encontrada en el tamaño de las esporas sugiere la posibilidad de que existan especies poliploides dentro de este clado.

**Capítulo III.** Taxonomic revision of the *Elaphoglossum petiolatum* clade (Dryopteridaceae). Este capítulo corresponde con el tratamiento taxonómico de las especies que integran el grupo monofilético definido con base en la evidencia molecular del primer capítulo, donde se integra el representante de Jamaica de *E. petiolatum* y varias especies endémicas de México. Este trabajo constituye una herramienta para la delimitación taxonómica de la especie *E. petiolatum* y para la identificación de todas las especies del "clado de *E. petiolatum"* ya que provee una clave dicotómica, descripciones ilustradas, sinonimia, mapas de distribución y notas nomenclaturales para cada especie.

**Capitulo IV.** *Elaphoglossum auralolitae,* a new species of *Elaphoglossum* sect. *Lepidoglossa* (Dryopteridaceae) from Guatemala. Se describe una nueva especie de *Elaphoglossum,* endémica de Guatemala que presenta como características distintivas la abundante densidad de escamas blanquecinas, con margen ciliado en la superficie adaxial de la lámina; la presencia de puntos resinosos en la cara abaxial de la lámina y esporas con una perispora espinosa. Aunque esta especie no se incluyó en los análisis filogenéticos, la morfología sugiere que pertenece al "clado de *E. petiolatum"*.



# Capítulo I

## Systematics of the *Elaphoglossum petiolatum* complex (Dryopteridaceae)

Este artículo se someterá a *Systematic Botany*

# SYSTEMATICS OF THE ELAPHOGLOSSUM PETIOLATUM COMPLEX (DRYOPTERIDACEAE)

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***Abstract***– This work is an approach to the study of a group of 11 species informally called the *Elaphoglossum petiolatum* complex. The species belonging to this complex are characterized by the presence of resinous dots on the abaxial lamina surface, black rhizome scales and spiny perispores. Previous molecular phylogenetic studies suggested that the complex was polyphyletic, but the Mexican species considered as a part of this complex were recovered in the same clade. However, these studies did not include all species in the complex or samples of *E. petiolatum* from Jamaica, the type locality for this species. The objectives of our study were to test the monophyly of this complex including sequences of *E. petiolatum* from Jamaica and a better representation of the species of the complex as well as the species reported for Mexico. Our analyses included sequences of three chloroplast markers from 194 species, including 9 of the 11 species in the complex. Our topologies support the non-monophyly of the *E. petiolatum* complex recovering its species in three separate clades: the "clade of *E. ciliatum"*, the "clade of *E. lancifolium*" and the "clade of *E. petiolatum"*, however, they do not show good resolution of the relationships among the species.

**Keywords**– *Elaphoglossum*, endemic, ferns, Mexico, monophyletic, taxonomy, tropics.

*Elaphoglossum* (Sw.) Urb. is one of the largest fern genera in the world, as it includes at least 600 taxonomically accepted species (Moran 2018, PPG I 2016, Rouhan 2020). According to molecular and morphological evidence, *Elaphoglossum* belongs to the bolbitidoid clade of the subfamily Elaphoglossoideae (Pic. Serm.) Crabbe, of the family Dryopteridaceae Herter, and represents the sister group of the genus *Mickelia* R. C. Moran, Labiak & Sundue (Liu et al. 2016, Moran et al. 2010, Moran 2018 Rouhan et al. 2004, Skog et al. 2004, PPGI 2016).

*Elaphoglossum* species are easily distinguished from other ferns by their simple, rarely divided laminae (only four species have divided laminae), dimorphic sterile and fertile leaves, free veins, and acrostichoid sori (Mickel and Atehortúa 1980, Moran et al. 2007, Vasco et al. 2009a, Rouhan et al. 2020). At least 60% of the species are epiphytes, but they can also be terrestrial or epipetric (Moran 2018, Moran et al. 2010, Zots et al. 2021). The genus is present in tropical areas around the world with its greatest diversity in the American tropics, where approximately 450 species have been recorded (Mickel 1995, Moran 2018, Moran et al. 2010).

The most recent infrageneric classification of *Elaphoglossum* was based on morphological characters and represents the most solid base in the taxonomic study of this very challenging genus, mainly because it provides clear hypotheses about important characters for *Elaphoglossum* taxonomy and taxonomic keys to the proposed sections and subsections (Mickel and Atehortúa 1980). This classification focuses on spore and sporophyte characters, such as spore ornamentation, rhizome shape and color, petiole and lamina scales shape, size and density, and laminae shape, size, and texture (Mickel and Atehortúa 1980).

Since the publication of the infrageneric classification (Mickel and Atehortúa 1980), knowledge about *Elaphoglossum* has increased substantially. In the last four decades several local floras for the genus have been published, for example, in South Africa (Roux 1982), Peru (Mickel 1991), Mesoamerica (Mickel 1995a), Venezuela (Mickel 1985, 1995b), Mexico (Mickel and Smith 2004), Tropical East Africa (Mickel 2002), Hawaii (Palmer 2003), Mascarene Islands (Lorence and Rouhan 2004, 2008), French Polynesia (Rouhan et al. 2008), in Africa, Macaronesia, the mid-Atlantic, and southern Indian Ocean Islands (Roux 2011), Bolivia (Kessler et al. 2018), Brazil (Matos and Mickel 2014, 2018, 2019), and Madagascar (Rouhan 2020). In addition, molecular phylogenetic studies have provided a robust phylogenetic framework (Rouhan et al. 2004, Skog et al. 2004, Liu et al. 2007, Vasco et al. 2009b, Lóriga et al. 2014), and have promoted the elaboration of taxonomic treatments for small clades within the genus (Matos et al. 2019, Vasco 2011, Vasco, et al. 2009a, Vasco et al. 2013).

Phylogenetic studies with chloroplast markers have recovered seven major clades within *Elaphoglossum*, six of which largely correspond to the sections (sect.) recognized by Mickel and Atehortúa (1980) based on morphology (Rouhan et al. 2004, Skog et al. 2004, Liu et al. 2007, Schuettpelz and Pryer 2007), and a seventh one recently described (Lóriga et al. 2014). That is how currently seven sections are recognized within the genus: sect. *Amygdalifolia* (Christ) Mickel & Atehortúa, sect.

*Lepidoglossa* Christ., sect. *Elaphoglossum*, sect. *Polytrichia* Christ, sect. *Setosa* (Christ) Mickel & Atehortúa, sect. *Squamipedia* Mickel & Atehortúa, and sect. *Wrightiana* J. Lóriga, A. Vasco, L. Regalado, Heinrichs & R.C. Moran (Mickel and Atehortúa 1980, Rouhan et al. 2004, Skog et al. 2004, Liu et al. 2007, Schuettpelz and Pryer 2007, Lóriga et al. 2014).

With more than 200 species, sec. *Lepidoglossa* is the most specious section within *Elaphoglossum* (Vasco 2011). Species in the section are characterized by laminae generally and notoriously scaly, rhizome and blade scales typically stellate or flat (not subulate) and with unicellular acicular marginal projections (these marginal processes in other sections end in a slightly swollen bulbous cell); sometimes the laminae and petioles have resinous dots (Mickel and Atehortúa 1980, Vasco 2009a, Kessler et al. 2018). The section was subdivided into six subsections (subsect.) by Mickel and Atehortúa (1980), differing mainly by leaf and rhizome scale shape and density, laminae apex, and spore micromorphology. These were named subsec. *Muscosa* Mickel and Atehortúa, subsec. *Polylepidea* Christ, subsec. *Microlepidea* Christ, subsec. *Petiolosa* Christ, subsec. *Pilosa* Christ, and *subsec*. Huacsaro Mickel and Atehortúa.

Within subsec. *Pilosa*, Mickel and Atehortúa (1980) grouped species with short creeping rhizomes, usually with dark scales with entire margins or with long projections, lamina with resinous dots, scaly to subglabrous, and spores with grooves and ridges (Mickel and Atehortúa 1980). The lamina scales can be lanceolate with margins with short projections, substellate, or sometimes so reduced that they look like stellate hairs.

As part of his taxonomic work on the genus *Elaphoglossum* in Mexico, and based on morphological characters, Mickel (2001) described a group within subsec. *Pilosa*, the *Elaphoglossum petiolatum* complex. Species within this complex are characterized by black rhizome scales with entire margins, scales of the proximal portion of the petioles with a dark apex, and resinous dots on the lower side of the laminae (Mickel 2001, Mickel and Smith 2004). Within this complex Mickel grouped 11 species distributed across the tropics, three of them endemic to Mexico (Table 1) (Mickel, 2001). Previous molecular phylogenetic analyses with three chloroplast markers (*atpβ-rbcL, rps4-trnS, and trnL-*

*trnF*), which did not include all the species in the *Elaphoglossum petiolatum* complex, suggested that the complex was not monophyletic (Rouhan et al. 2004, Vasco et al. 2009b, Lóriga et al. 2014). The taxa belonging to the complex and that were included in these analyses were recovered in three different clades that in turn included other species outside the complex: the "*Elaphoglossum ciliatum* group", the "Mexican clade of *E. petiolatum*", and a third unnamed clade that included two specimens from Ecuador determined as *E.* aff. *petiolatum* (Vasco et al., 2009b).

The present study focused on the *E. petiolatum* complex and aimed to test its monophyly and the relationships among its species. We wanted to answer three questions (1) Is the *E. petiolatum* complex monophyletic when a broader sampling of its species is included? If so, how many and which are the species that belong to the complex? If not, where are the species of the complex recovered? (2) Is there morphological evidence that supports the phylogenetic position of the species belonging to the complex?, and (3) Which species belong to the "Mexican clade" when other species distributed in Mexico are included, particularly those that are morphologically similar to the species already recovered in this clade.

To answer these questions, we performed phylogenetic analyses using sequences of three chloroplast markers, *atpβ-rbcL*, *rps4-trnS*, and *trnL-trnF*. We assembled a matrix that included the most comprehensive sampling of the species belonging to the *E. petiolatum* complex to date –including a sequence of *E. petiolatum* from Jamaica, the country where the nomenclatural type of this species comes from (Swartz,1778, Proctor 1985), species morphologically similar to those of the complex, and various species of *Elaphoglossum* from Mexico. Our sampling includes many new sequences of Mexican *Elaphoglossum.*

Our results corroborate the previous hypothesis that the *Elaphoglossum petiolatum* complex is not a monophyletic group and that the species originally described as part of the complex are recovered in three non-sister clades, all of them including other species of *Elaphoglossum* besides those of the complex. Here we informally name these clades as "*E. ciliatum* clade", "*E. lancifolium* clade", and "*E. petiolatum* clade". The *E. petiolatum* clade recovers the endemic Mexican species originally described as part of the complex, as well as a sequence of a specimen of *E. petiolatum* from Jamaica, and sequences of other two species endemic to Mexico –*E. schiedei* and *E. schmitzii*. *Elaphoglossum gratum*, a species distributed in Mexico that is morphologically similar to some of the species of the *E. petiolatum* complex, does not belong to any of the three clades. A description and discussion of each clade and a dichotomous key to differentiate them are presented.

**Table 1.** Species belonging to the *Elaphoglossum petiolatum* complex sensu Mickel (2001). (*) indicates the species included in this analysis.

| Species | Distribution |
|---|---|
| **Elaphoglossum petiolatum* (Sw.) Urban | From Mexico to Peru and the West Indies |
| **E. salicifolium* (Willd. ex Kaulf.) Alston (ahora *E. lancifolium* (Desv.) C.V. Morton) | Bioko, Cameroon, Comoros, Congo, Gaba, Guinea, La Reunion, Liberia, Mauritius Island, Madagascar, Malawi, Mozambique, Rwanda, Sierra Leone, Seychelles, Tanzania, Zambia, Zimbabwe |
| **E. nervosum* (Bory) H. Christ | St. Helena Island |
| **E. dimorphum* (Hook. & Grev.) T. Moore | St. Helena Island |
| **E. bifurcatum* (Jacq.) Mickel | St. Helena Island |
| *E. stelligerum* (Wall. ex Baker) T. Moore ex Alston & Bonner | India |
| *E. yunnanense* (Baker) C. Chr. | China, India, Malaysia, Vietnam |
| **E. blumeanum* (Fee) J. Smith | Bismark Archipielago, Borneo, China, Indonesia, Philippines, Malasyia, Salomon Islands, Sumatra. |
| **E. rzedowskii Mickel* | Mexico |
| **E. pringlei* (Davenp.) C. Chr. | Mexico |
| **E. trichomidiatum* Mickel (ahora *E. potosianum* Christ) | Mexico |

## METHODS

*Sampling*. – The matrix analyzed included 187 new sequences here generated, and sequences generated in previous works available in GenBank (Skog et al. 2004, Rouhan et al. 2004, Vasco et al. 2009b, Moran et al. 2010, Lóriga et al. 2014, https://www.ncbi.nlm.nih.gov/genbank/). Information on the samples used in the analyses is in Appendix I. All new sequences will be submitted to GenBank.

We used DNA sequences of three chloroplast markers, *atpβ-rbcL*, *rps4-trnS*, and *trnL-trnF*, from 213 individuals. We included several individuals for the species belonging to the *E. petiolatum* complex when it was possible. Sequences of two genera closely related to *Elaphoglossum* were selected as outgroups: *Bolbitis serratifolia* (Mertens) Schott, and *Mickelia nicotianifolia* (Sw.) R.C. Moran, Labiak & Sundue (Rouhan et al. 2004, Moran et al. 2010).

*DNA extraction, amplification and alignment.* – Chloroplast DNA was extracted from leaf tissue collected in the field and dried in silica gel or from herbarium specimens. Extraction was performed at the Molecular Systematics Laboratory of the Institute of Biology, UNAM, using the DNeasy Plant Mini Kit (Qiagen®, Hilden, Germany) following the manufacturer's protocol. To obtain better quality DNA from herbarium samples, we added 30 μL of β-mercaptoethanol to each sample, in addition to the extraction buffer (step two of the original protocol), and incubated for 24 h at 42°C (Vasco et al. al. 2009b). The quality of the DNA obtained was evaluated visually with electrophoresis in a 1% agarose gel.

The non-coding regions of chloroplast DNA: *atpβ-rbcL* (which includes a fragment of the *atpβ* gene), *rps4-trnS,* and *trnL-trnF,* were amplified by means of the polymerase chain reaction (PCR), using the same primers as in Rouhan et al. (2004, 2007). The details of each PCR run are detailed in Table 2. For each sample, the success of the reaction was evaluated by electrophoresis in a 1% agarose gel. Successful amplifications were sent to the Laboratory of Molecular Biology of Biodiversity and Health of the UNAM Institute of Biology or to the High-Throughput Genomics Unit, Department of Genome Sciences, University of Washington, for purification and bidirectional sequencing. New forward and reverse sequences were assembled and edited manually with the Geneious® program, version 6.01 (https://www.geneious.com). We generated a single matrix for each marker.

**Table 2.** Details of the number of cycles, temperature, time, and primers used per marker for each step in the polymerase chain reaction (PCR). °C= Celsius degrees; ‘= minutes; “= seconds. The direction of primers’ sequences is 5’-3’

| | ***atpB-rbcl*** | ***rps4-trnS*** | ***trnL-trnF*** |
|---|---|---|---|
| Cycles | 35 | 30 | 30 |
| Step 1 | 97°C / 50“ | 94°C / 5’ | 94°C / 5’ |
| Step 2 | 97°C / 50“ | 94°C / 30” | 94°C / 30” |
| Step 3 | 53°C / 45“ | 50°C / 1’ | 50°C / 1’ |
| Step 4 | 72°C / 1:15‘ | 72°C / 1’ | 72°C / 1’ |
| Step 5 | 72°C / 7’ | 72°C / 7’ | 72°C / 7’ |
| Primer F | ACACTWAGAGGRGCTCCCGTATCAA | AGTTGTTAGTTGTTGAGTAT | GGTTCAAGTCCCTCTATCCC |
| Primer R | CACCAGCTTTGAATCCAACACTTGC | TACCGAGGGTTCGAATC | ATTTGAACTGGTGACACGAG |

Each matrix was individually aligned using the MAFFT online version with the preset parameters (https://www.ebi.ac.uk/Tools/msa/mafft/) and then adjusted manually using Mesquite version 3.51 (Maddison and Maddison 2019). For the alignment of the *atpβ* gene fragment, the first, second, and third position of the nucleotides within each amino acid were considered.

*Phylogenetic analyses.* – Matrices of each marker were analyzed independently with maximum parsimony (MP) using the TNT software v.1.5 (Goloboff et al. 2008) with the “New Technology” search option with 1000 replicates, and maximum retention of 1200 trees (topologies not included here). The resulting topologies were compared to test the null hypothesis that the three markers were congruent in their phylogenetic information, and to identify potential lab errors or conflicts. Because no incongruence was found between the strict consensus trees from each of the three plastid regions, they were combined into a single alignment.

To study the phylogenetic relationships among the species and to evaluate the monophyly of the *Elaphoglossum petiolatum* complex, we performed two analyses with the concatenated matrix. Maximum likelihood (ML) analyses using IQ-Tree v.2.1.2 (Nguyen et al. 2015) and Bayesian inference (BI) analyses with Mr. Bayes 3.2.7 (Ronquist and Huelsenbeck 2003).

We carried out a maximum likelihood (ML) analysis with the dataset divided into six partitions previously estimated by the ModelFinder option implemented in IQ-Tree (Kalyaanamoorthy et al. 2017), on the digital platform from Cipres (https://www.phylo.org/portal2/home.action, Miller et al. 2010)1). These partitions were: *atpβ-rbcL* (K3P+F+G4), 2) the first position of the *atpβ* gene fragment (K2P+G4), 3) the second position of the *atpβ* gene fragment (TVMe+G4), 4) the third position of the *atpβ* gene fragment (K2P+G4), 5) *rps4-trnS* (TVM+F+G4), and 6) *trnL-trnF* (TPM2+F+G4). The search for the best-scoring ML tree was conducted in a single run using IQ-Tree in the Cipres platform, and we choose a fast bootstrap analysis (ultrafast bootstrap) with 1000 replicates (Nguyen et al. 2015, Hoang et al. 2018).

Bayesian inference analyses were conducted using MrBayes 3.2.7 (Ronquist and Huelsenbeck 2003), with the dataset partitioned into three parts as estimated by the Bayesian information criterion (BIC) implemented in PartitionFinder (Lanfear et al. 2012). The three partitions were: 1) *atpβ-rbcL,* the third position of the *atpβ* gene fragment, and *rps4-trnS* (GTR+G), 2) first and second position of the *atpβ* gene fragment (SYM+G), and 3) *trnL-trnF* (GTR+G). The analyses were run for 10 million generations with four Markov Chains (three heated and one cold) and sampling the posterior probability distribution every 1000th generation. Samples corresponding to the initial phase of the Markov chains (20%) were discarded as burn-in, after visualizing in Tracer v.1.7 (Rambaut et. al., 2018). Post burn-in trees were combined to obtain a single majority consensus tree and the respective node posterior probabilites. The topologies obtained from the ML and BI analyses were viewed and edited with FigTree v. 1.4.4 (Rambaut 2010) and Adobe Illustrator (Adobe Inc., 2019).

*Morphological analyses.* – To better understand the morphological characteristics of the species of the three clades in which the original species belonging to the *E. petiolatum* complex are recovered, we studied herbarium specimens (BRIT, MEXU, MO, NY, P, US, and VT), digital images available on the website pteridoprtal.org, taxonomic literature (Vasco et al. 2009a, Rouhan et al. 2020), and spores images made by us or available on plantsystematics.org. After that, a dichotomous taxonomic key for the three clades was completed.

## RESULTS

The final matrix comprised 171 species of *Elaphoglossum*, including nine of the 11 species of the *E. petiolatum* complex, and 42 species reported from Mexico, including two taxa we quit of the synonymy of *E. petiolatum* and considered as independent species, and two specimens we could not identify. This represents 81% of the species of the *E. petiolatum* complex, 59% of the species of the genus known for Mexico, and 28.5% of all the species recognized for *Elaphoglossum*.

The concatenated alignment included 1704 characters, of which 748 are constant, 393 autapomorphic, and 563 informative (Table 3). The topologies obtained from the ML and BI analyses of the concatenated matrix are very similar and recovered the seven major clades previously recognized for *Elaphoglossum* (Figs. 1 and 2). Although there are minor topological differences between the topologies, mainly in the arrangement of a few species, we decided to describe the species' relationships based on the ML topology (Fig. 1). The 42 *Elaphoglossum* species from Mexico are recovered within five of these large clades and, in general, their position is consistent with the morphological characteristics that define each section.

**Table 3.** Constant and informative sites for the matrices of the three chloroplast markers used in this study.

| Matrix/ marker | ***atpB-rbcL*** | ***rps4-trnS*** | ***trnL-trnF*** | **Total** |
|---|---|---|---|---|
| Number of bases (% of the total) | 876 (51.4) | 441 (25.8) | 387 (22.7) | 1704 |
| Constant (%) | 447 (59.7) | 174 (23.2) | 127 (16.9) | 748 |
| Single (%) | 195 (39.5) | 108 (21.9) | 90 (38.5) | 393 |
| Parsimony informatives characters (%) | 234 (41.5) | 159 (28.2) | 170 (30.1) | 563 |

The nine species originally described as part of the *Elaphoglossum petiolatum* complex included in this study (Table 1), are all recovered in three clades that are not sister to each other, within the sec.

*Lepidoglossa* (Figs. 1 and 2). The *" E. ciliatum* clade" (BS=81, PP=1) recovers three of these nine species: *E. bifurcatum*, *E. dimorphum*, and *E. nervosum*; the "*E. lancifolium* clade" (BS= 84, PP= 1), recovers two of the nine species: *E. blumeanum* and *E. lancifolium;* and the “*E. petiolatum* clade” (BS=100, PP= 1), recovers the sequences of the remaining four species: *E. petiolatum*, *E. potosianum*, *E. pringlei*, and *E. rzedowskii* (Figs. 1 and 2, Table 4).

This latter clade was previously called the “Mexican clade of *E. petiolatum*” by Vasco et al. (2009b) but we renamed it here because the *E. petiolatum* sequence from the type locality of this species was recovered here. None of the other Mexican species of *Elaphoglossum* included in this study is recovered in any of the three clades mentioned above, not even *E. gratum*, a species with very similar morphology to that of the species of the *E. petiolatum* complex. Next, we briefly describe the topologies of these three clades.

The “*Elaphoglossum ciliatum* clade”– was previously monographed by Vasco et al. (2009a) with nine species recognized. In this study, we found that *E. palmeri* Underw. & Maxon, species from Cuba and the Dominican Republic, was recovered as part of this clade (Table 4). The *E. ciliatum* clade has a polytomy that includes three branches, the first corresponds to a sequence of *E. huacsaro*, the second corresponds to a clade (BS=100, PP=1) recovering the sequences of *E. ciliatum* and *E. nigrescens*, and the third is a clade (BS=85, PP=1) with two subclades: one recovering sequences of *E. gramineum*, *E. dusii,* and *E. palmeri*, and the other recovers sequences of *E. bifurcatum*, *E. burchellii*, *E. dimorphum*, and *E. nervosum* (Figs. 1 and 2).

The "*Elaphoglossum lancifolium* clade" has a polytomy that includes three branches. The first is a clade (BS=90, PP=1) where sequences from *E. tenuiculum* and *E. viscidum* are recovered. The second corresponds to a sequence of *E. ipshookense* from Mexico. The third branch is a clade (BS=75 PP=--) that includes two subclades: the first (BS= 92, PP=--) groups two sequences, one from Colombia and another from Ecuador, of a specimen that has not been identified so far, plus *E. blumeanum* and *E. welwitschii,* both species from the paleotropics. The second subclade (BS=75, PP=--), recovers an *E. caricifolium* sequence as sister to a clade (BS=100, PP=--) that includes sequences from *E. lancifolium* from La

Reunion and Mauritius Islands*,* *E. yungense* from Bolivia, and a specimen from Brazil identified as *E. viscidum* (Figs. 1 and 2, Table 4).

**Table 4.** Species belonging to the three clades mentioned in this study. Species in bold correspond with those part of the original *Elaphoglossum petiolatum* complex sensu Mickel (2001).

| Species | Distribution | Clade |
|---|---|---|
| ***E. bifurcatum* (Jacq.) Mickel** | St. Helena Island, | *E. ciliatum* clade |
| *E. burchelli* (Baker) C. Chr. | Costa Rica, Panama, Dominican Republic, Colombia, Venezuela, Ecuador, Peru, Brazil, Bolivia, Paraguay | |
| *E. ciliatum* (C. Presl) T. Moore | Costa Rica, Panamá, Colombia, Ecuador, Perú | |
| ***E. dimorphum* (Hook. & Grev.) T. Moore** | St. Helena Island | |
| *E. dusii* Underw. & Maxon | Puerto Rico, Montserrat, San Cristóbal Island, Guadeloupe, Dominica, Martinica, St. Vincent, Grenada | |
| *E. gramineum* (Jenman) Urb. | Honduras, Jamaica, Cuba, Dominican Republic | |
| *E. huacsaro* (Ruiz) Christ. | Mexico, Guatemala, Costa Rica, Jamaica, Haiti, Dominican Republic Colombia, Venezuela, Ecuador, Peru, Brazil, Bolivia | |
| ***E. nervosum* (Bory) Christ** | St. Helena Island | |
| *E. nigrecsens* (Hook.) T. Moore ex Diels | Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Guyana, Ecuador, Peru, Brazil, Bolivia | |
| *E. palmeri* Underw. & Maxon | Cuba and Dominican Republic | |

| | | |
|---|---|---|
| ***E. petiolatum* (Sw.) Urban** | Jamaica, Dominican Republic, Costa Rica, Panama, Honduras, Nicaragua, Guatemala and Mexico. | *E. petiolatum* clade |
| ***E. pringlei* (Davenp.) C. Chr.** | Mexico | |
| ***E. potosianum* Chris** | Mexico | |
| ***E. rzedowskii Mickel*** | Mexico | |
| *E. schiedei* (Kunze) T. Moore. | Mexico and Guatemala | |
| *E. schmitzii* (Mett. ex Khun) Martínez-Becerril, A. Vasco. | Mexico | |
| ***E. blumeanum* (Fee) J. Smith** | China, Philippines, Indonesia, Java, Papua New Guinea | *E. lancifolium* clade |
| *E. caricifolium* Mickel | Costa Rica, Panama | |
| *E. ipshookense* Mickel | Mexico | |
| ***E. lancifolium* (Willd. ex Kaulf.) Alston** | Bioko, Cameroon, Comoros, Congo, Gaba, Guinea, La Reunion, Liberia, Mauritius Islands, Madagascar, Malawi, Mozambique, Ruanda, Sierra Leone, Seychelles, Tanzania, Zambia, Zimbawe | |
| *E. tenuiculum* (Fée) T. Moore ex C. Chr. | Mexico, Guatemala, El Salvador, Honduras, Colombia, Costa Rica, Venezuela, Ecuador, Peru, Brazil, Bolivia | |
| *E. viscidum* | Brazil | |
| *E. welwitschii* (Baker) C. Chr. | Angola, Malawi, Tanzania, Zambia, Zimbawe | |
| *E. yungense* de la Sota | Peru, Bolivia, NE of Argentina | |

The "*Elaphoglossum petiolatum* clade" (BS=100, PP=1) comprises two subclades. The first subclade (BS=82, PP=0.97) includes sequences of *E. pringlei*, *E. schiedei*, a sequence of *E. petiolatum* from Jamaica, and sequences of a Mexican specimen that has not been identified. The second subclade

(BS=82, PP=--) includes sequences of the Mexican endemics *E. rzedowskii*, *E. schmitzii*, and *E. potosianum* (Figs. 1 and 2, Table 4).

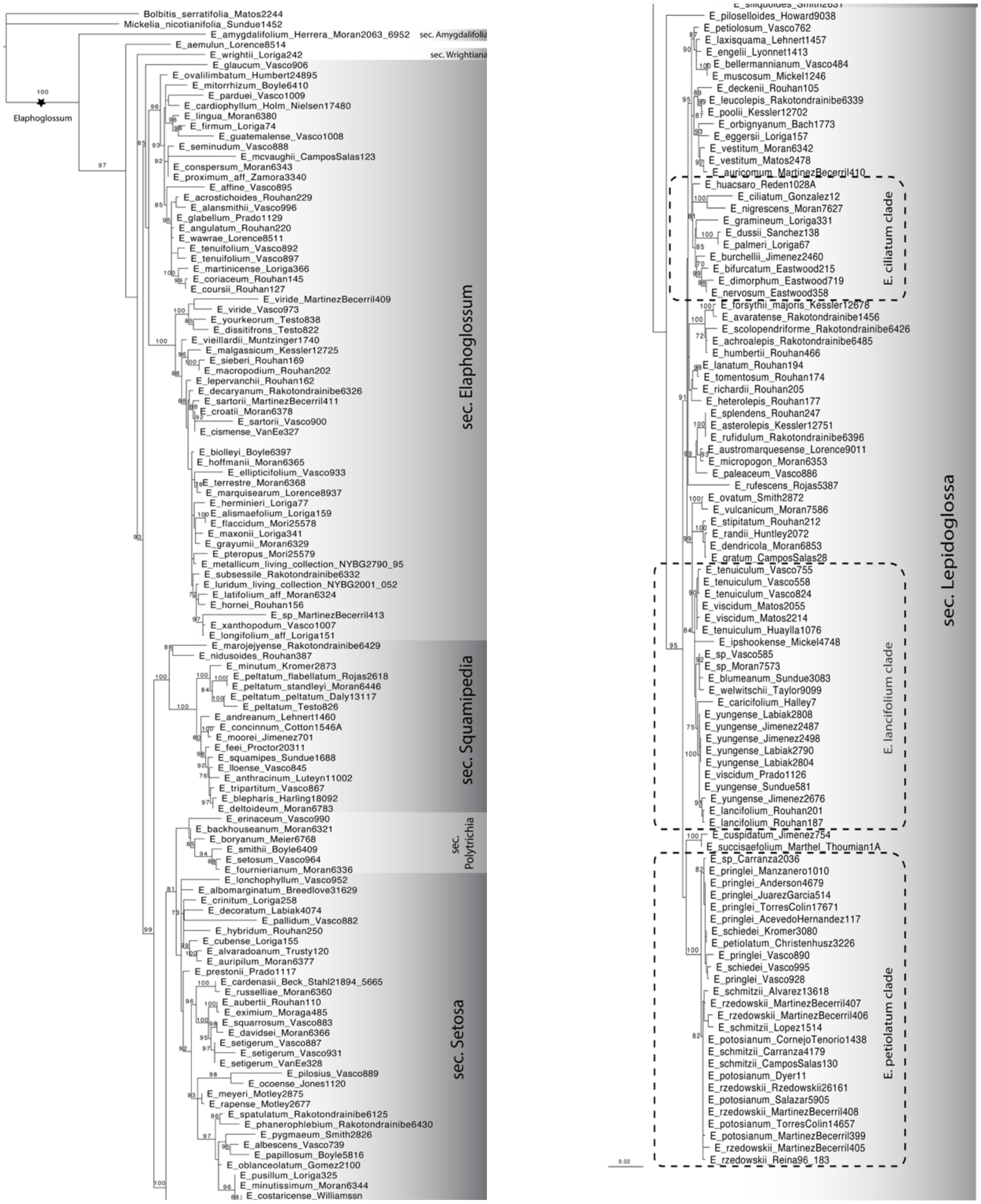

**Figure 1.** Maximum likelihood topology for the concatenated matrix with the three chloroplast markers *atpB-rbcL, rps4-trnS,* and *trnL-trnF*. Numbers on the branches indicate the bootstrap support (%). The names of the terminals including the collection numbers.

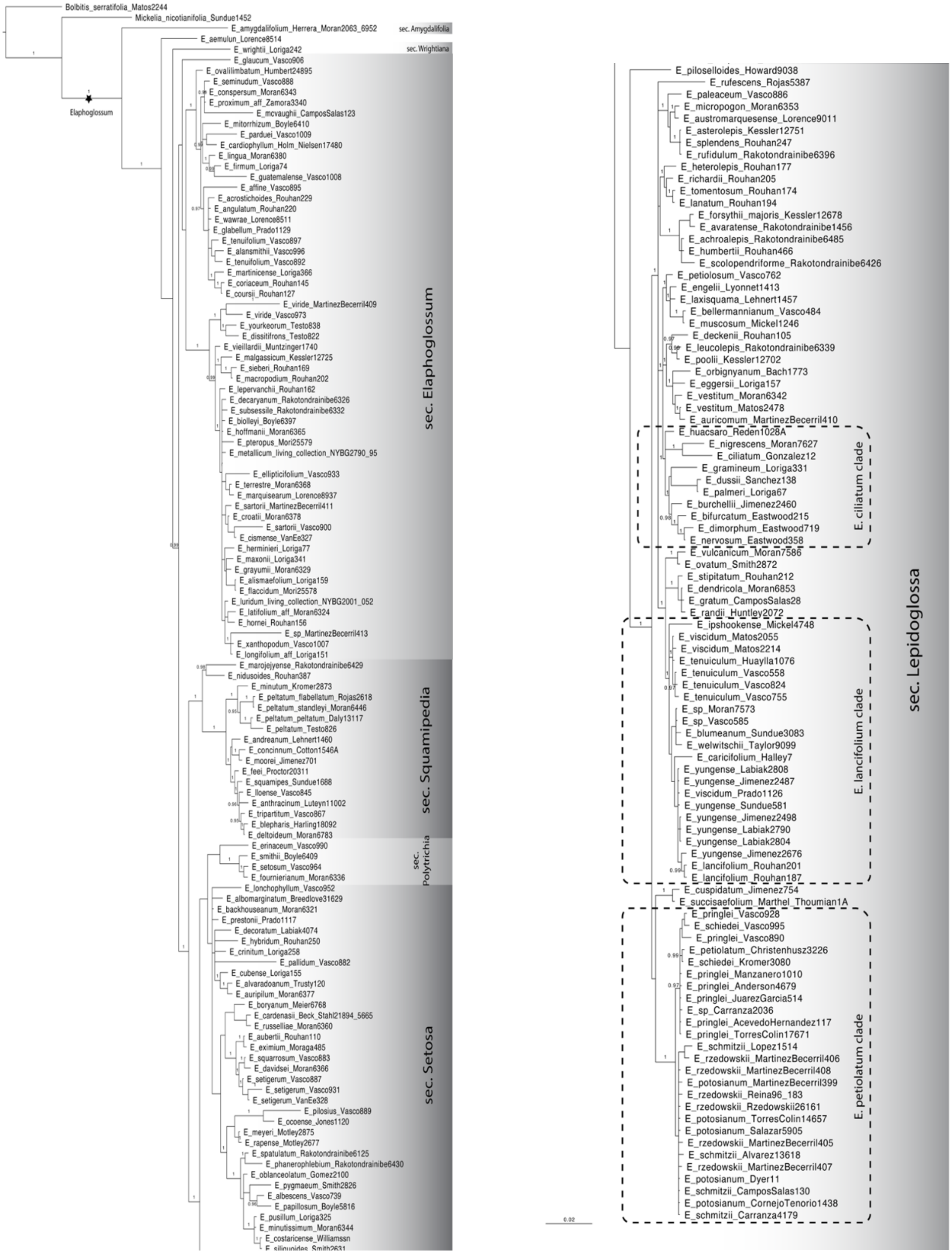

**Figure 2.** Bayesian Inference topology for the concatenated matrix with the three chloroplast markers *atpB-rbcL, rps4-trnS,* and *trnL-trnF*. Numbers on the branches indicate the posterior probability support (%). The names of the terminals including the collection numbers.

## DISCUSSION

The *Elaphoglossum petiolatum* complex was defined as a group within subsec. *Pilosa*, based on morphological characters (Mickel 2001). Previous molecular phylogenetic studies suggested that neither the subsection nor the complex were monophyletic (Rouhan et al. 2004; Vasco et al., 2009b). These studies did not include all the species of the complex, but of those included, only the Mexican species were recovered in a clade, called “the Mexican clade of *E. petiolatum*” by Vasco et al. (2009b). The phylogenetic analyses presented here, which included the best representation of the species of the complex so far, including half of the *Elaphoglossum* species recorded for Mexico, and other species of the genus, agree with the previous analyses recovering subsec. *Pilosa* and the *E. petiolatum* complex as polyphyletic, and, in addition, recovering a well-supported clade of mostly Mexican species, that coincides with the previously reported “Mexican clade of *E. petiolatum*”.

In our analyses, the species originally placed in the *Elaphoglossum petiolatum* complex (Mickel 2001) are recovered in three non-sister clades within sec. *Lepidoglossa*, referred to herein as the “*E. ciliatum* clade”, “*E. lancifolium* clade” and “*E. petiolatum* clade” (= "Mexican *E. petiolatum* clade"). These three clades include other species not previously considered as part of the *E. petiolatum* complex. Species of these three clades have flat and/or stellate scales on the laminae and spores with spiny perispores, some taxa also have resinous dots on the abaxial surface of the laminae (Fig. 4). Laminar flat and/or stellate scales are present in most species of sec. *Lepidoglossa* (Mickel 1985, Mickel and Atehortúa 1980, Kessler et al. 2018) and spiny perispores are an independently evolved character distinctive to at least five subclades in this section (Moran et al. 2007, Vasco et al., 2009a, Martínez-Becerril et al., 2021, pers. obs.). On the other hand, resinous dots were important in the definition of the *Huacsaro*, *Petiolosa*, and *Pilosa* subsections, and in the *E. petiolatum* complex (Mickel and Atehortúa 1980, Mickel 2001).

The presence of these characters in multiple clades suggests that the *E. petiolatum* complex was defined based on plesiomorphic characters, meaning characters that have evolved several times during the evolutionary history of the section.

In the genus *Elaphoglossum*, resinous dots have been interpreted as the bases of reduced scales that remain embedded in the epidermis of the laminae (Vasco et al. 2009a). Although these dots are not present in all the species of the three clades in which the species originally described as part of the *E. petiolatum* complex were recovered, all the *Elaphoglossum* species that have laminar resinous dots are part of sec. *Lepidoglossa* and were recovered in one of these three clades. There is one exception: *E. petiolosum* (Desv.) T. Moore, which has resinous dots on the laminae, but it is recovered in a different clade within sec. Lepidoglossa and was not considered part of the *E. petiolatum* complex. *Elaphoglossum petiolosum* has, in addition to resinous dots on its abaxial lamina, tiny glandular trichomes on the petiole and costa, an unusual feature in species of sec. *Lepidoglossa* (Mickel and Atehortúa 1980). Our results suggest that the presence of resinous dots evolved independently on at least four occasions during the evolutionary history of sec. *Lepidoglossa*.

Although spiny perispores are present in other subclades of sec. *Lepidoglossa* (Moran et al. 2007, Martínez-Becerril et al. 2021), in this work we detected a variation in the size and density of the spore spines that is useful to differentiate our three clades. The species of the *"E. ciliatum* clade" have dense spines that are generally ≥1μm (Fig. 4A-C), while in the species of the "*E. lancifolium*" and the "*E. petiolatum*" clades the spines are scattered and ≤1μm (Fig. 4D-L). To better understand the variation and evolution of the spines in sect. *Lepidoglossa,* comparative morphological and anatomic analyses are necessary to corroborate whether these structures are homologous between the clades.

In addition to the size and density of the spines in the spores, there are other characteristics that differentiate the clades in which the species of the *E. petiolatum* complex were recovered. For example, the presence and density of stem and lamina scales, the shape of lamina apices, and the geographic distribution of the taxa (see key at end of discussion). Next, we make a brief discussion of each of these clades.

*"Elaphoglossum ciliatum* Clade"– It has ten species, three of which, *E. bifurcatum, E. dimorphum* and *E. nervosum*, were part of the original complex of *E. petiolatum* (Mickel, 2001). Nine of

the species of this clade were previously treated in a monograph (Vasco et al. 2009a). This work confirmed that *E. palmeri*, a species present in Cuba and the Dominican Republic, is also part of this clade (Lóriga et al. 2014). *Elaphoglossum palmeri* has been commonly compared with *E. petiolatum*, but it differs from it mainly by the absence of resinous dots, by its stem scales with long projections, and by the presence and density of stellate scales on the lamina (Maxon 1908, Lóriga et al. 2014) (Table 4).

Besides having resinous dots, species in the "*E. ciliatum* clade" are distinguished by their blackish stems with a shiny exudate similar to lacquer when dry, and by spores that are mostly equinate, that is, with dense spines ≥1µm in high (Fig. 4A -C) (Vasco et al. 2009a). Some species such as *E. dimorphum, E. dusii, E. nigrescens*, and *E. palmeri* can have spines ≤1µm long (pers. obs.). The clade distribution includes the American tropics, from Mexico and the Antilles to southeastern Brazil and the volcanic island of Santa Helena in the Atlantic Ocean (Fig. 3, Table 4) (Vasco et al. 2009a). According to the age of diversification estimated for this clade, between 5.36–10.35 mya (Lóriga et al. 2014b), the presence of some of its species on the island of Santa Elena can be explained by long-distance dispersal, a successful and common phenomenon among ferns that has allowed them to colonize island ecosystems thanks to the ease with which the spores can travel long distances (Janssen et al. 2007, Ranker et al. 2004). For more information on the phylogenetic relationships, description, and distribution of this group, see the studies by Vasco et al. (2009a,b).

"*Elaphoglossum lancifolium* Clade"– It is perhaps the clade with the most species of the three discussed here and its taxonomy requires further study. We named it *E. lancifolium* because this is the oldest described species of the clade (Desvaux 1811). The clade contains at least eight species of which two, *E. blumenaum* and *E. lancifolium* (formerly *E. salicifolium*), were previously recognized as members of the *E. petiolatum* complex by Mickel (2001). In general, the species of this clade are characterized by resinous dots (except *E. tenuiculum* and *E. ipshookense*), attenuated to long attenuated lamina apices, whitish and abundant scales on the adaxial surface of the laminae, and spores with scattered spines ≤1µm in height (Fig. 4D-F) (Moran et al. 2007; www.plantsystematics.org).

The “*Elaphoglossum lancifolium* clade” is the only one of the three that has a pantropical distribution, with species distributed in the Paleotropics (Africa, Madagascar, and Southeast Asia, including India) and species distributed in the Neotropics (Mexico, Central, and South America) (Fig. 3, Table 4). Although not all the species included in this study were considered, the age of diversification of this clade was calculated between 2.96–7.47 mya (Lóriga et al. 2014b), which suggests that long-distance dispersal, and not vicariance, is the most likely explanation for understanding the distribution of species because when the diversification of the group began, the tropics of America, Africa, and Asia were already geographically isolated (Barrington 1993, Janssen et al. 2007, Lóriga et al 2014b, Moran and Smith 2001, Smith 1993, Tryon 1970, 1986, Perrie et al 2010).

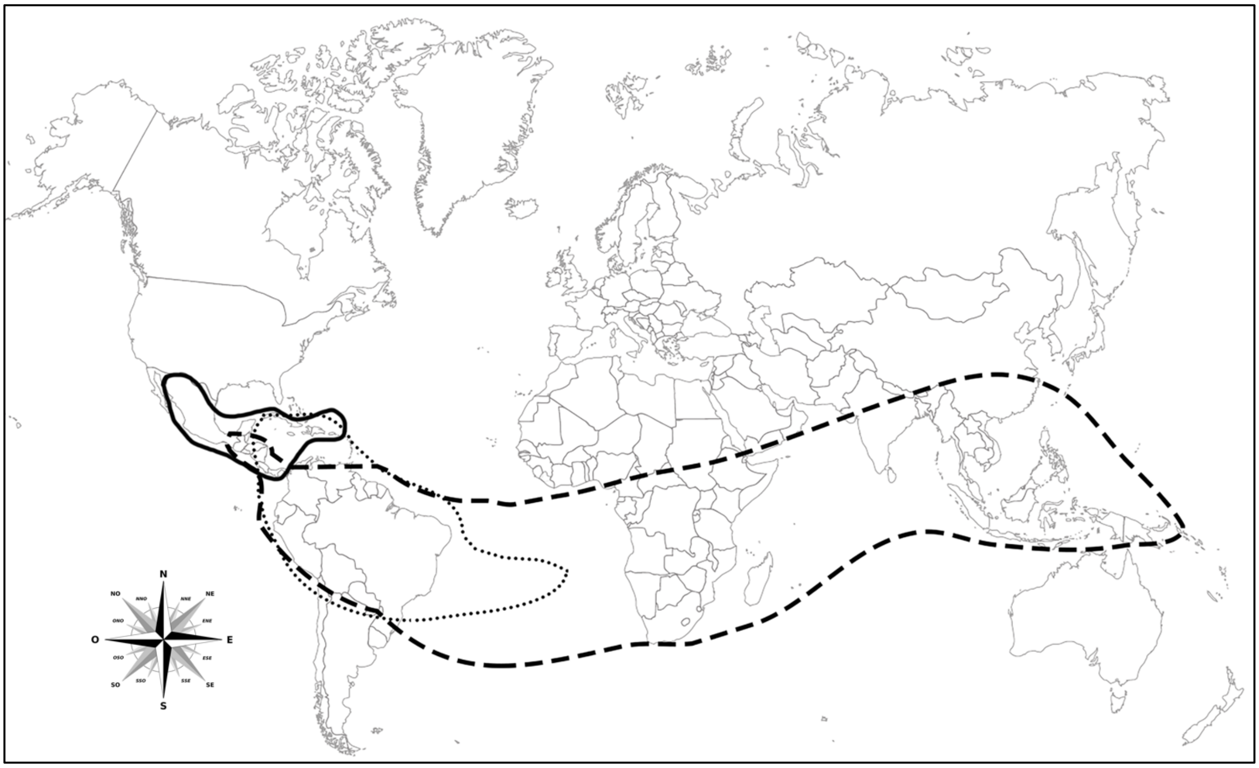

**Figure 3.** General distribution of the three clades that were described in this study. The dotted line indicates the distribution for the “*E. ciliatum* clade*”*, the interrupted line indicates the distribution for the “*E. lancifolium* clade*”,* and the continuous line indicates the distribution of the *“E. petiolatum* clade*”*.

It has been shown that the Africa-Madagascar region functions as a contact point for the floras of America, Africa, and Asia, and that could explain why tropical America and Africa-Madagascar share more than 10% of their fern species (Moran and Smith 2001, Janssen et al. 2007, Ranker et al. 2004). A list of "species pairs", where from two morphologically and phylogenetically related species, one occurs in the American tropics and the other in Africa or Madagascar (Moran and Smith 2001), indicates the pair of *E. lancifolium* (Africa) and *E. yungense* (South America). Our topologies show that these species are closely related, however, a sister group relationship between them cannot be established, because *E. viscidum* is recovered in the same subclade. Our study also suggests that there might be another species pair between *E. blumeanum*, from Southeast Asia, and *E. welwitschii* distributed in different African countries, and an unidentified species from Colombia and Ecuador (Figs. 1 and 2, Table 4).

In the molecular work of Rouhan et al. (2004), the phylogenetic closeness between *E. killipii* Mickel (South America), *E. lancifolium* (Africa), and *E. tectum* (Humb. & Bonpl. ex Willd.) T. Moore (from Mexico to South America) is mentioned. In this study, we corroborate the identifications of the specimens used in those analyses and find that the collections determined as *E. killipii* Mickel (M. Sundue 581, LPB, NY, USZ) and *E. tectum* (J. Prado 1126, NY) correspond to *E. yungense* and *E. viscidum*, respectively. Interestingly *E. viscidum* from *Prado 1126* is not recovered with the rest of the sequences of the same species in our topologies, so what we have called *E. viscidum* likely corresponds to several taxa.

Our study did not include sequences of *E. killipii* or *E. tectum*; however, based on their distribution and morphology, we think they could be part of the "*E. lancifolium* clade". *Elaphoglossum killipii* is endemic to Peru, it has abundant stellate scales and resinous dots on the abaxial surface of the lamina, and its spores have scattered spines ≤1µm high (Mickel 1991, Mickel and Smith 2004, plantsystematics.org). *Elaphoglossum tectum* lacks resinous dots, and the morphology of its spores is unknown, however, it occurs from Mexico to Brazil and has abundant whitish scales with long acuminate apices (Mickel and Smith 2004).

Besides these two species, there are others, of which there are no sequences, but their morphology (presence of resinous dots and/or laminae with attenuated to largely attenuated apices) suggests they might belong to the "*E. lancifolium* clade"; for example, *E. ballivianii* Rosenst. and *E. stenophyllum* (Sodiro) Diels from South America and *E. ambrense* Rouhan, *E. anjanaharibense* Rouhan, *E. gladiifolium* Rouhan, *E. patriceanum* Rouhan, *E. subglabricaule* Rouhan, *E. stelligerum* (Wall. ex Baker) T. Moore ex Alston & Bonner, and *E. yunnanense* from Madagascar (Baker) C. Chr., (Mickel 2001, Rouhan 2020).

The "*Elaphoglossum lancifolium* clade" is an ideal candidate for future phylogenetic studies and for an in-depth taxonomic revision because, although the group can be delimited morphologically (see key), many of its species are poorly defined and frequently confused.

"*Elaphoglossum petiolatum* clade"– This was the original "Mexican clade of *E. petiolatum*" proposed by Vasco et al. (2009b), but we rename it because we found that the sequence of the specimen of *E. petiolatum* from Jamaica (*Christenhusz 3226*, NY), the type locality of the species (Swartz 1778, Proctor 1985) is recovered here. Seven species were recovered as part of this clade, four of which –*E. petiolatum, E. potosianum, E. pringlei,* and *E. rzedowskii* were considered part of the complex described by Mickel (2001). The other three species are *E. schiedei, E. schmitzii,* and a third, from the Mexican state of Querétaro (Carranza 2036 MEXU, IEB) which was initially determined to be *E. potosianum*, but our molecular analyses and morphology suggests it is probably an undescribed species.

Most of the species of this clade are endemic to Mexico, except for *E. schiedei*, which is present in Mexico and Guatemala, and for *E. petiolatum*, which occurs from Mexico to Panama and in the Antilles (Table 4) (Mickel and Smith 2004, Chapter IV). The current distribution of the *E. petiolatum* clade and the diversification age of a similar clade, calculated between 0.42–3.49 million years ago (Lóriga et al. 2014b), suggests that the Mexican territory was its center of diversification.

In general, the species of the *Elaphoglossum petiolatum* clade are distinguished, by resinous dots on the lamina, bicolorous stem scales (with the margin generally darker than the rest of the scale) with acute to filiform apices, and spores with scattered spines ≤1µm high (except *E. petiolatum* which has concolorous scales and spores with irregular deposits) (Fig.4G-I) (Mickel and Smith 2004, Vasco et al. 2009a, Martínez-Becerril et al 2021).

Apart from the endemic species, from *E. schiedei*, and from the probable new species from Querétaro, no other species of *Elaphoglossum* from Mexico is recovered within the *E. petiolatum* clade, not even *E. gratum*, which has been misidentified as *E. petiolatum* because its stem scales can sometimes be entire; or as *E. pringlei* because of its scaly laminae (Mickel and Smith 2004) (Figs. 1 and 2). *Elaphoglossum gratum* differs from species in the *E. petiolatum* clade because its laminar scales are very large (1–1.5mm) compared to the lamina scales of *E. petiolatum* and *E. pringlei* (up to 1mm), the absence of laminar resinous dots, and the presence of perispores with dense spines ≥1µm in height (vs. spores with scattered spines ≤1µm in height in *E. pringlei* and spores without spines in *E. petiolatum*).

The original sampling of this study included sequences of *Elaphoglossum petiolatum* from Mexico and Central America and of *E. muelleri*, another species with similar morphology to the species of this clade. However, the herbarium work for the taxonomic revision of this clade allowed a better determination of the specimens and resulted in the non-inclusion of representatives of these two species. After analyzing the morphology of the plants and the spores, we conclude that it is very likely that the Mexican and Central American specimens of E. *petiolatum* and *E. muelleri* are part of this clade (Martínez-Becerril et al. 2021, Martínez-Becerril et al., in prep.). On one hand, the morphology of the Central American specimens of *E. petiolatum,* including that of spores with irregular deposits instead of spines coincides with the morphology of the Antillean specimens of this species (Fig.4L ). On the other hand, *Elaphoglossum muelleri* is similar to *E. potosianum* as it has resinous dots and trichomidia on the abaxial surface of the laminae and spores with scattered spines ≤ 1µm high (Fig. 4I). *Elaphoglossum muelleri* is distinguished from *E. potosianum* by its abundant stellate laminar scales and its orange stem scales.

The "clade of *E. petiolatum*" is divided into two moderately well-supported subclades within which interspecific relationships are not fully resolved. This low resolution has been reported in other works of the genus using the same molecular markers or a subset of them (Skog et al. 2004, Rouhan et al. 2004, Vasco 2009b, 2011, Lóriga 2014b), and is probably due to the exclusive use of plastid DNA as this type of data is not always suitable for work at the species level in groups of plants that have reticulate or recent evolutionary histories, such as ferns (Voegel et al. 1998; Stein and Barrington 1990; Guillon and Raquin 2000). In this sense, phylogenetic estimates from chloroplast genes can produce misleading inferences because plastid genomes are uniparentally inherited, so evolutionary events such as hybridization and polyploidy are often overlooked (Lovis 1978). Furthermore, unlike nuclear genomes, plastid genomes do not undergo extensive recombination, so inferences are also susceptible to events such as deep coalescence. To fully unravel the relationships in many fern groups requires the development of unlinked markers that clearly reflect hybridization events, e.g., nuclear markers or genomes (Roethfels et al 2013).

Despite the low resolution at the species level, all sequences from the same species were recovered only in one of the two subclades of the "*E. petiolatum* clade" and are morphologically diagnosable. For example, *E. pringlei* and *E. rzedowskii* had been considered variations of the same species (Mickel and Smith 2004), however in this study, they are recovered in different subclades and our morphological analyses show that they have different characteristics such as the size of their spores (larger in *E. pringlei* than in *E. rzedowskii*) (Martínez-Becerril et al. 2021), the apex of the stem scales (long attenuated to filiform-tortuous in *E. pringlei* vs. acuminate, sometimes attenuated in *E. rzedowskii*) and the density of the lamina scales (sparse to dense, non-imbricate in *E. pringlei* vs. dense, sometimes imbricate in *E. rzedowskii*). Something similar happens with *E. schiedei*, and *E. schmitzii*, which were considered synonyms of *E. petiolatum* (Mickel and Smith 2004), but which are recovered in different subclades within the "*E. petiolatum* clade" (*E. schmitzii* in one and *E. schiedei* plus *E. petiolatum* in another).

The distribution of *E. schmitzii, E. schiedei* and *E. petiolatum* overlaps in the state of Oaxaca while that of *E. schiedei* and *E. petiolatum* also overlaps in Chiapas and Guatemala. These species can be distinguished by many morphological characters, including spore ornamentation (spiny in *E. shiedei* and *E. schimitzii* and with irregular deposits in *E. petiolatum*) (Fig. 4J–L) (Martínez-Becerril in prep.). The review of herbarium specimens for this study and for the monographic study of this clade showed that many of the Mexican specimens determined as *E. petiolatum* correspond to *E. schiedei* or *E. schmitzii*.

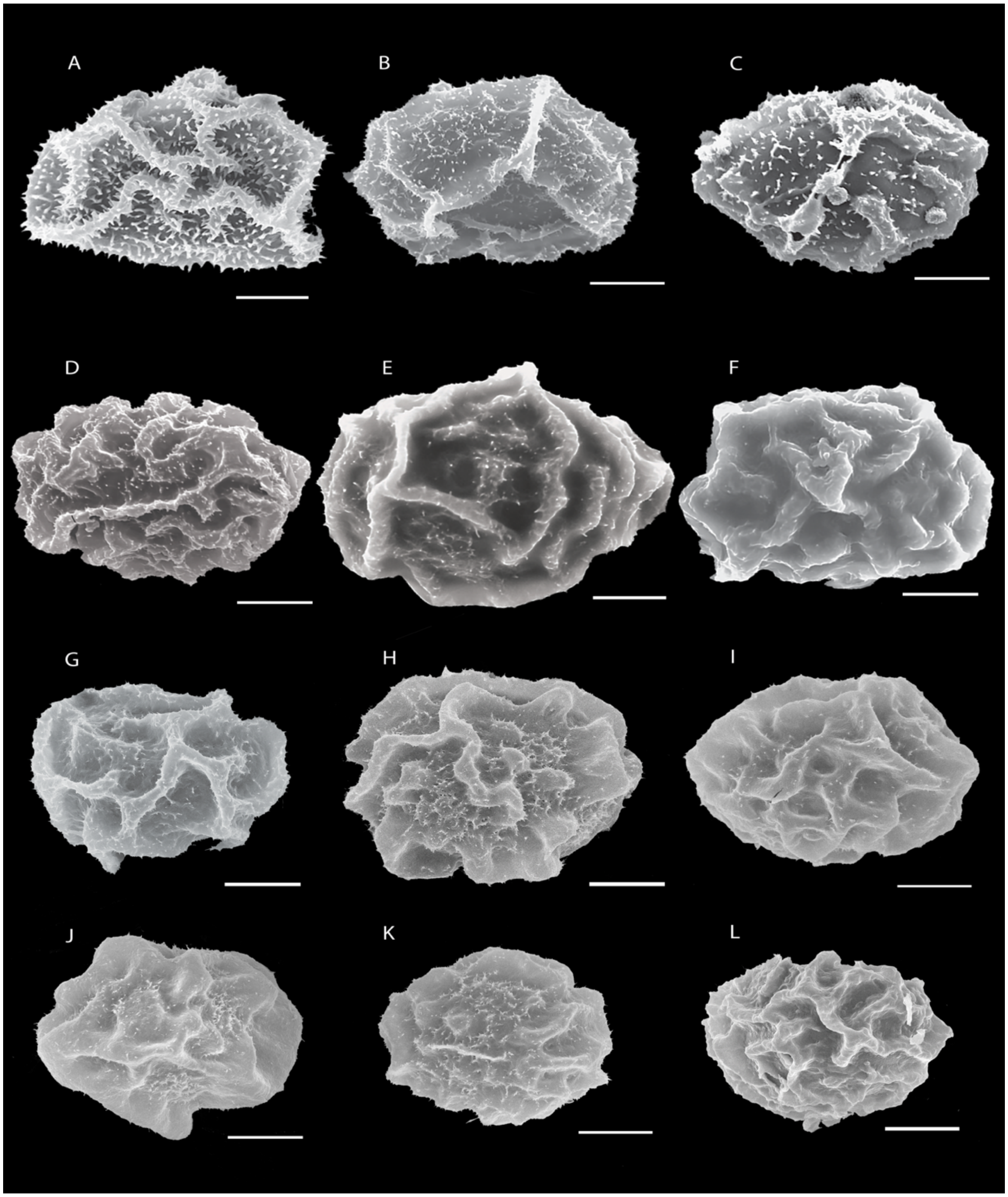

**Figure 4.** Spores of the species belonging to the three clades described in this work. Scale= 10μm. "*Elaphoglossum ciliatum* clade" (A–C), "*E. lancifolium* clade" (D–F), and "*E. petiolatum* clade" (G–L). A. *E. ciliatum* (*Neill 9648*, MO). B. *E. burchelli* (*Dusen 7225*, NY). C. *E. gramineum* (*Valeur 634*, NY). D. *E. killipii* (*Buchtein 4239*). E. *E. lancifolium* (*Rouhan & Grangaud 201*, P). F. *E. yungense* (*Kessler 6444*, NY). G. *E. pringlei* (*Anderson 4778*, MEXU). H. *E. rzedowskii* (*Martinez-Becerril 405*, MEXU). I.

*E. muelleri (Anderson 13211,* MEXU), J. *E. schmitzii (McVaugh 23885,* MEXU), K. *E. schiedei (Gallardo Hernández 836,* MEXU), L. *E. petiolatum* (*Proctor 5297*, MO).

This confusion in the identification explains the differences in the micromorphology of the spores indicated by Martínez-Becerril et. al. (2021) between the Mexican specimens of *E. petiolatum* with respect to the Central American and Antillean ones.

*Notes on the application of the name Elaphoglossum petiolatum*. –For a long time, the name *E. petiolatum* was used interchangeably to identify specimens of *Elaphoglossum* throughout the world with dark rhizome scales and resinous dots on the laminar abaxial surfaces (Mickel 2001). In an attempt to understand and clarify the morphological variation of this species, the name *E. petiolatum* was restricted to the specimens from the American tropics, from Mexico and the Antilles to Peru and Brazil, while to accommodate those from southeast Asia, Africa, and India, names such as *E. blumeanum*, *E. yunnanense, E. salicifolium*= *E. lancifolium*, and *E. stelligerum*, respectively, were used (Proctor 1985, Mickel 1995, 2001, Mickel and Smith 2004). In this work, we corroborate that *E. petiolatum* is not present in South America and that its distribution is restricted to Antilles, Mexico, and Central America. South American specimens identified as *E. petiolatum* belong to the "*E. lancifolium* clade" and need to be renamed. For this, there are at least five published names that could be applied: *E. killipii* (Peru), *E. ballivianii* (Bolivia), *E. stenophyllum* (Colombia and Venezuela), *E. viscidum* (Brazil), and *E. yungense* (from Peru to Argentina). These species are similar to *E. petiolatum* because of the presence of resinous dots on the laminae, however, they differ from it in several aspects, such as the density of the lamina scales (generally dense vs. glabrous or scattered to dense in *E. petiolatum*) and the shape of the laminae apex (attenuated to largely attenuated vs. acute to acuminate in *E. petiolatum*) (de la Sota 1973, Tryon 1991).

In addition to helping resolve the phylogenetic relationships of the *E. petiolatum complex*, the present study defines the distribution of *E. petiolatum* and allowed the recognition of two new monophyletic groups – the *E. lancifolium* and *E. petiolatum* clades, with a manageable number of species to carry out detailed taxonomic revisions.

## Key to identify the different clades in which the species of the Elaphoglossum petiolatum complex sensu Mickel (2001) were segregated

1. Stems black with a shiny, lacquer-like exudate when dried, spores with dense spines ≥1µm high …………………….........................................……………… clade of *E. ciliatum*

1. Stems brown, without shiny exudate, spores with scattered spines ≤1µm high or without spines

2. Laminae apices attenuate to largely attenuate, spores with spines ≤1µm high …………………. ........................................................................................... clade of *E. lancifolium*

2. Laminae apices acute or acuminate, spores with spines ≤1µm high or with irregular deposits… ……………………………………...………….……….…. clade of *E. petiolatum*

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**Appendix I.** Voucher information for sequences used in this study and their GenBank accession numbers. X= indicates the new sequences generated for this study). (*) indicates the species considered as a part of the *Elaphoglossum petiolatum* complex sensu Mickel (2001).

| **Genus** | **Species** | **Collection and herbarium** | **Section** | **Country** | ***atpB*** | ***rps4*** | ***trnL-F*** |
|---|---|---|---|---|---|---|---|
| *Bolbitis* | *B. serratifolia* (Mert. ex Kaulf.) Schott | Matos 2244, NY | Outgroup | Brazil | MG600314 | MG600344 | MG600372 |
| *Mickelia* | *M. nicotianifolia* (Sw.) R. C. Moran, Labiak & Sundue | Sundue 1452, NY | Outgroup | Costa Rica | | GU376666 | GU376519 |
| *Elaphoglossum* | *E. achroalepis* (Baker) C. Chr. | Rakotondrainibe 6485, P | *Lepidoglossa* | Madagascar | EF040636 | AY540225 | AY536288 |
| | *E. acrostichoides* (Hook. & Grev.) Schelpe | Rouhan 229, P | *Elaphoglossum* | La Reunion | EF040654 | EF040628 | EF040614 |
| | *E. aemulun* (Kaulf.) Brack | Lorence 8514, P | *Elaphoglossum* | Hawaii | | AY540227 | AY536290 |
| | *E. affine* (M. Martens & Galeotti) T. Moore | Vasco 895, MEXU | *Elaphoglossum* | Mexico | X | X | X |
| | *E. alansmithii* Mickel | Vasco 996, MEXU | *Elaphoglossum* | Mexico | X | X | X |
| | *E. albescens* (Sodiro) Christ | Vasco 739, HUA, NY | *Subulata* | Colombia | X | GU376678 | GU376532 |
| | *E. albomarginatum* A. R. Sm. | Breedlove 31629, CAS, MEXU, NY | *Subulata* | Mexico | MG600320 | MG600350 | MG600378 |
| | *E. alismaefolium* (Fée) T. Moore | Loriga 159, HAC | *Elaphoglossum* | Cuba | KF212374 | KF212425 | KF212399 |
| | *E. alvaradoanum* A. Rojas | Trusty 120, NY | *Subulata* | Costa Rica | EF040651 | EF040625 | EF040611 |
| | *E. amygdalifolium* (Mett. ex Kuhn) Christ | Herrera 2063, CR, INB, NY, USJ | *Amygdalifoila* | Costa Rica | | AY536173 | AY534845 |
| | | Moran 6952, NY | | | KJ528036 | | |
| | *E. andreanum* Christ | Lehnert 1460, GOET, QCA, NY, UC | *Squamipedia* | Ecuador | KJ528044 | KJ528131 | KJ528167 |
| | *E. angulatum* (Blume) T. Moore | Rouhan 220, NY, P | *Elaphoglossum* | La Reunion | EF040655 | AY540230 | AY536293 |
| | *E. anthracinum* A. Vasco, Mickel & R. C. Moran | Luteyn 11002, NY | *Squamipedia* | Ecuador | KJ528017 | KJ528116 | KJ528172 |
| | *E. asterolepis* (Baker) C. Chr. | Kessler 12751, P | *Lepidoglossa* | Madagascar | EF040642 | AY540231 | AY536294 |
| | *E. aubertii* (Desv.) T.Moore | Rouhan 110, P | *Subulata* | Comoros | EF040647 | EF040622 | EF040608 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *E. auricomum* (Kunze) T. Moore | MartinezBecerril 410, BRIT, FEZA, MEXU (aun no está) | *Lepidoglossa* | Mexico | X | X | X |
| | *E. auripilum* Christ | Moran 6377, NY | *Polytrichia* | Costa Rica | EF040652 | EF040626 | EF040612 |
| | *E. austromarquesense* Rouhan & Lorence | Lorence 9011, PTBG | *Lepidoglossa* | Marquesas Islands | | AY540292 | AY536355 |
| | *E. avaratense* Rakotondr. | Rakotondrainibe 1456, P | *Lepidoglossa* | Madagascar | EU907660 | AY540233 | AY536296 |
| | *E. backhouseanum* T. Moore | Moran 6321, NY | *Polytrichia* | Costa Rica | | AY540234 | AY536297 |
| | *E. bellermannianum* (Klotzsch) T. Moore | Vasco 484, HUA, NY | *Lepidoglossa* | Colombia | KJ528040 | KJ528161 | KJ528173 |
| | *E. bifurcatum* (Jacq.) Mickel | Eastwood 215, E | *Lepidoglossa* | Sta. Helena Island | EU907661 | EU907737 | AY194070 |
| | *E. biolleyi* Christ | Boyle 6397, NY | *Elaphoglossum* | Costa Rica | | AY540235 | AY536298 |
| | *E. blepharis* A. Vasco, Mickel & R. C. Moran | Harling 18092, AAU | *Squamipedia* | Ecuador | KJ528030 | KJ528146 | KJ528174 |
| | **E. blumeanum* (Fée) J. Sm. | Sundue 3083, NY | *Lepidoglossa* | Philippines | X | X | X |
| | *E. boryanum* (Fée) T. Moore | Meier 6768, NY | *Setosa* | Venezuela | | AY536133 | AY534804 |
| | *E. burchellii* (Baker) C. Chr. | Jimenez 2460, NY | *Lepidoglossa* | Bolivia | EU907663 | EU907738 | EU907803 |
| | *E. cardenasii* W. H. Wagner | Stahl 5665, NY | *Setosa* | Bolivia | EF040648 | | |
| | | Beck 21894, NY | | | | AY536131 | AY534802 |
| | *E. cardiophyllum* (Hook) T.Moore | Holm-Nielsen 17480, NY | *Elaphoglossum* | Ecuador | | AY536171 | AY534842 |
| | *E. caricifolium* Mickel | Halley 7, NY | *Lepidoglossa* | Costa Rica | | AY536143 | AY534814 |
| | *E. ciliatum* (C. Presl) T. Moore | Gonzalez 12, CR, INB, UCR | *Lepidoglossa* | Costa Rica | EU907665 | EU907740 | EU907805 |
| | *E. cismense* Rosenst. | VanEe 327, NY | *Elaphoglossum* | Costa Rica | | AY540237 | AY536300 |
| | *E. concinnum* Mickel | Cotton 1546A, NY | *Squamipedia* | Ecuador | KJ528019 | KJ528151 | KJ528179 |
| | *E. conspersum* Christ | Moran 6343, NY | *Elaphoglossum* | Costa Rica | | AY540238 | AY536301 |
| | *E. coriaceum* Bonap. | Rouhan 145, P | *Elaphoglossum* | Seychelles | EF040653 | EF040627 | EF040613 |
| | *E. costaricense* Christ | Williams sn, NY | *Setosa* | Costa Rica | | AY536128 | AY534799 |
| | *E. coursii* Tardieu | Rouhan 127, P | *Elaphoglossum* | Comoros | | AY540240 | AY536303 |
| | *E. crinitum* (L.) Christ | Loriga 258, HAC | *Polytrichia* | Cuba | KF212377 | KF212428 | KF212403 |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
|  | *E. croatii* Mickel | Moran 6378, NY | *Elaphoglossum* | Costa Rica |  | AY540241 | AY536304 |
|  | *E. cubense* (Mett. ex Kuhn) C. Chr. | Loriga 155, HAC | *Polytrichia* | Cuba | KF212378 | KF212429 | KF212404 |
|  | *E. cuspidatum* (Wild.) T. Moore | Jimenez 754 | *Lepidoglossa* | Bolivia | EU907675 | EU907750 | EU907815 |
|  | *E. davidsei* Mickel | Moran 6366, NY | *Setosa* | Costa Rica |  | AY540242 | AY536305 |
|  | *E. decaryanum* Tardieu | Rakotondrainibe 6326, P | *Elaphoglossum* | Madagascar | EF040658 | AY540243 | AY536306 |
|  | *E. deckenii* (Kuhn) C. Chr. | Rouhan 105, CNDRS, NY, P, PTBG | *Lepidoglossa* | Comoros |  | AY540244 | AY536307 |
|  | *E. decoratum* (Kunze) T. Moore | Labiak 4074, NY | *Subulata* | Brazil | KJ528071 | KJ528165 | KJ528181 |
|  | *E. deltoideum* (Sodiro) Christ | Moran 6783, NY | *Squamipedia* | Ecuador | KJ528031 | KJ528148 | KJ528240 |
|  | *E. dendricola* (Baker) Christ | Moran 6853 | *Lepidoglossa* | Ecuador | EU907676 | EU907751 | EU907816 |
|  | *E. dimorphum* (Hook. & Grev.) T. Moore | Eastwood 719 | *Lepidoglossa* | Sta. Helena Island | EU907678 | EU907753 | EU907817 |
|  | *E. dissitifrons* Mickel | Testo 822, MEXU | *Elaphoglossum* | Mexico |  | X | X |
|  | *E. dussii* Underw. ex Maxon | Sanchez 138, NY | *Lepidoglossa* | Puerto Rico | EU907681 | EU907755 | EU907819 |
|  | *E. eggersii* (Baker) Christ | Loriga 157, HAC | *Lepidoglossa* | Cuba | KF212381 | KF212431 | KF212406 |
|  | *E. ellipticifolium* A. Rojas | Vasco 933, MEXU | *Elaphoglossum* | Mexico | X | X | X |
|  | *E. engelii* (H. Karst.) Christ | Lyonnet 1413, US | *Lepidoglossa* | Mexico | X | X | X |
|  | *E. erinaceum* (Fée) T. Moore | Vasco 990A, MEXU | *Subulata* | Mexico | X | X | X |
|  | *E. eximium* (Mett.) Christ | Moraga 485, NY | *Setosa* | Costa Rica |  | AY536132 | AY534803 |
|  | *E. feei* (Bory ex Fée) T. Moore | Proctor 20311, US | *Squamipedia* | Guadeloupe | KJ528029 | KJ528123 | KJ528187 |
|  | *E. firmum* (Mett. ex. Kuhn) Urb. | Loriga 74, HAC | *Elaphoglossum* | Cuba | KF212382 | KF212433 | KF212408 |
|  | *E. flaccidum* (Fée) T. Moore | Mori 25578, NY | *Elaphoglossum* | French Guiana | EF040657 | AY540246 | AY536309 |
|  | *E. forsythii-majoris* Christ | Kessler 12678, P | *Lepidoglossa* | Madagascar | EF040644 | EF040620 | EF040606 |
|  | *E. fournierianum* L.D. Gómez | Moran 6336, NY | *Setosa* | Costa Rica |  | AY540248 | AY536311 |
|  | *E. glabellum* J. Sm. | Prado 1129, NY | *Elaphoglossum* | Brazil |  | AY536167 | AY534839 |
|  | *E. glaucum* T. Moore | Vasco 906, MEXU |  | Mexico |  | X | X |
|  | *E. gramineum* (Jenman) Urb. | Loriga 331, HAC | *Lepidoglossa* | Cuba | KF212383 | KF212434 | KF212409 |
|  | *E. gratum* (Fée) T. Moore | CamposSalas 28, MEXU | *Lepidoglossa* | Mexico | X | X | X |

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|---|---|---|---|---|---|---|---|
| | *E. grayumii* Mickel | Moran 6329, NY | *Elaphoglossum* | Costa Rica | | AY540250 | AY536313 |
| | *E. guatemalense* (Klotzsch) T. Moore | Vasco 1008, MEXU | *Elaphoglossum* | Mexico | X | X | X |
| | *E. herminieri* (Bory & Fée) T. Moore | Loriga 77, HAC | *Elaphoglossum* | Cuba | KF212384 | KF212435 | KF212410 |
| | *E. heterolepis* (Fée) T. Moore | Rouhan 177, P | *Lepidoglossa* | Mauritius | EU907683 | AY540251 | AY536314 |
| | *E. hoffmanii* (Mett. ex Kuhn) Christ | Moran 6365, NY | *Elaphoglossum* | Costa Rica | | AY540252 | AY536315 |
| | *E. hornei* C. Chr. | Rouhan 156, NY | *Elaphoglossum* | Seychelles | | AY540253 | AY536316 |
| | *E. huacsaro* (Ruiz) Christ | Reden 1028A, NY | *Lepidoglossa* | Costa Rica | EU907688 | EU907763 | EU907826 |
| | *E. humbertii* C. Chr. | Rouhan 466, P | *Lepidoglossa* | Madagascar | EU907696 | EU907771 | EU907834 |
| | *E. hybridum* (Bory) Brack. | Rouhan 250, P | *Subulata* | La Reunion | EU907697 | EU907772 | EU907835 |
| | *E. ipshookense* Mickel | Mickel 4748, NY | *Lepidoglossa* | Mexico | EU907698 | EU907773 | EU907836 |
| | *E. lanatum* (Bojer ex Baker) Lorence | Rouhan 194, MAU, NY, P, PTBG | *Lepidoglossa* | Mauritius | | AY540258 | AY536321 |
| | **E. lancifolium* (Desv.) C. V. Morton | Rouhan 201, P | *Lepidoglossa* | La Reunion | EU907699 | AY540259 | AY536322 |
| | | Rouhan 187, P | | Mauritius | EF040640 | EF040619 | EF040605 |
| | *E. latifolium*_aff | Moran 6324, NY | *Elaphoglossum* | Costa Rica | | AY540245 | AY536308 |
| | *E. laxisquama* Mickel | Lehnert 1457, NY | *Lepidoglossa* | Ecuador | X | X | X |
| | *E. lepervanchii* (Bory & Fée) T. Moore | Rouhan 162, P | *Elaphoglossum* | Seychelles | | AY540260 | AY536323 |
| | *E. leucolepis* (Baker) Krajina ex Tardieu | Rakotondrainibe 6339, P | *Lepidoglossa* | Madagascar | EF040638 | AY540261 | AY536324 |
| | *E. lingua* (C. Presl) Brack. | Moran 6380, NY | *Elaphoglossum* | Costa Rica | | AY540262 | AY536325 |
| | *E. lloense* (Hook.) T. Moore | Vasco 845, HUA, MER, NY, VEN | *Squamipedia* | Venezuela | KJ528066 | KJ528127 | KJ528201 |
| | *E. lonchophyllum* (Fée) T. Moore | Vasco 952, MEXU | *Setosa* | Mexico | X | X | X |
| | *E. longifolium*_aff | Loriga 151, HAC | *Elaphoglossum* | Cuba | KF212375 | KF212426 | KF212401 |
| | *E. luridum* (Fée) Christ | living_collection_NYBG 2001-052, NY | *Elaphoglossum* | Peru | | AY540263 | AY536326 |
| | *E. macropodium* (Fée) T. Moore | Rouhan 202, P | *Elaphoglossum* | La Reunion | | AY540264 | AY536327 |
| | *E. malgassicum* C. Chr. | Kessler 12725, NY | *Elaphoglossum* | Madagascar | EF040659 | AY540265 | AY536328 |

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|---|---|---|---|---|---|---|---|
| | *E. marojejyense* Tardieu | Rakotondrainibe 6429, P | *Squamipedia* | Madagascar | EF040630 | AY540266 | AY536329 |
| | *E. marquisearum* Bonap. | Lorence 8937, PTGB | *Lepidoglossa* | Marquesas Islands | | AY540267 | AY536330 |
| | *E. martinicense* (Desv.) T. Moore | Loriga 366, HAC | *Elaphoglossum* | Cuba | KF212387 | KF212437 | KF212412 |
| | *E. maxonii* Underw. ex C.V. Morton | Loriga 341, HAC | *Elaphoglossum* | Cuba | KF212388 | KF212438 | KF212413 |
| | *E. mcvaughii* Mickel | CamposSalas 123, MEXU | | Mexico | | X | |
| | *E. metallicum* Mickel | living_collection_NYBG 2790/95, NY | *Elaphoglossum* | Peru | | AY536160 | AY534832 |
| | *E. meyeri* Rouhan | Motley 2875, NY | *Setosa* | Rapa | | AY540287 | AY536350 |
| | *E. micropogon* Mickel | Moran 6353, NY | *Lepidoglossa* | Costa Rica | EF040643 | AY540268 | AY536331 |
| | *E. minutissimum* R.C. Moran & J.T. Mickel | Moran 6344, NY | *Setosa* | Costa Rica | | AY540293 | AY536356 |
| | *E. minutum* (Pohl ex Fée) T. Moore | Krömer 2873, NY | *Squamipedia* | Mexico | KJ528073 | KJ528099 | KJ528206 |
| | *E. mitorrhizum* Mickel | Boyle 6410, CR, INB, NY, USJ | *Elaphoglossum* | Costa Rica | EF040656 | AY540269 | AY536332 |
| | *E. moorei* (Britton) Christ | Jimenez 701, NY | *Squamipedia* | Bolivia | KJ528024 | KJ528156 | KJ528210 |
| | *E. muscosum* (Sw.) T. Moore | Mickel 1246, NY | *Lepidoglossa* | Mexico | X | X | X |
| | *E. nervosum* (Bory) Christ | Eastwood 358, E | *Lepidoglossa* | Sta. Helena Island | EU907700 | EU907774 | AY194069 |
| | *E. nidusoides* Rouhan & Rakotondr. | Rouhan 387, P | *Squamipedia* | Madagascar | EF040634 | EF040618 | EF040604 |
| | *E. nigrescens* (Hook.) T. Moore ex Diels | Moran 7627, NY | *Lepidoglossa* | Ecuador | EU907709 | EU907782 | EU907844 |
| | *E. oblanceolatum* C. Chr. | Gomez 2100, NY | *Setosa* | Costa Rica | | AY540271 | AY536334 |
| | *E. ocoense* C. Chr. | Jones 1120, NY | *Setosa* | Dominican Republic | | KF212441 | KF212414 |
| | *E. orbignyanum* (Fée) T. Moore | Bach 1773 | *Lepidoglossa* | Bolivia | EU907710 | EU907783 | EU907845 |
| | *E. ovalilimbatum* Bonap. | Humbert 24895, P | *Elaphoglossum* | Madagascar | | AY540272 | AY536335 |
| | *E. ovatum* (Hook. & Grev.) T. Moore | Smith 2872, UC | *Lepidoglossa* | Ecuador | EF040641 | AY540273 | AY536336 |

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|---|---|---|---|---|---|---|---|
| | *E. paleaceum* (Hook. & Grev.) Sledge | Vasco 886, MEXU | *Lepidoglossa* | Mexico | X | X | X |
| | *E. pallidum* (Baker ex Jenman) C. Chr. | Vasco 882, MEXU | *Subulata* | Mexico | X | X | X |
| | *E. palmeri* Underw. & Maxon | Loriga 67, HAC | *Lepidoglossa* | Cuba | KF212391 | KF212442 | KF212415 |
| | *E. papillosum* (Baker) Christ | Boyle 5816, NY | *Setosa* | Costa Rica | | AY536129 | AY534800 |
| | *E. parduei* Mickel | Vasco 1009, MEXU | *Elaphoglossum* | Mexico | X | X | X |
| | *E. peltatum_peltatum* (Sw.) Urb. | Testo 826, MEXU | *Squamipedia* | Mexico | X | X | X |
| | *E. peltatum_flabellatum* (Humb. & Bonpl. Ex Willd.) Mickel | Rojas 2618, NY | *Squamipedia* | Costa Rica | KJ528043 | KJ528110 | KJ528217 |
| | *E. peltatum_peltatum* (Sw.)Urb. | Daly 13117, NY | *Squamipedia* | Mexico | KJ528055 | KJ528100 | KJ528218 |
| | *E. peltatum_standleyi* (Maxon) Mickel | Moran 6446, NY | *Squamipedia* | Costa Rica | KJ528045 | KJ528111 | KJ528229 |
| | **E. petiolatum* (Sw.) Urb. | Christenhusz 3226, NY | *Lepidoglossa* | Jamaica | | | X |
| | *E. petiolosum* (Desv.) T.Moore | Vasco 762, HUA, NY | *Lepidoglossa* | Colombia | KJ528052 | KJ528160 | KJ528230 |
| | *E. phanerophlebium* C. Chr. | Rakotondrainibe 6430, P | *Setosa* | Madagascar | EF040646 | AY540276 | AY536339 |
| | *E. piloselloides* (C. Presl) T. Moore | Howard 9038, NY | *Setosa* | Dominican Republic | | KF212445 | KF212418 |
| | *E. pilosius* Mickel | Vasco 889, MEXU | | Mexico | X | X | X |
| | *E. poolii* (Baker) Christ | Kessler 12702, NY | *Lepidoglossa* | Madagascar | EF040639 | AY540278 | AY536341 |
| | **E. potosianum* Christ | MartinezBecerril 399, BRIT, FEZA, MEXU | *Lepidoglossa* | Mexico | | X | X |
| | | Dyer 11, MEXU | | | X | X | X |
| | | CornejoTenorio 61438, MEXU | | | X | X | X |
| | | Salazar 5905, MEXU | | | | X | X |
| | | TorresColin 14657, MEXU | | | | X | X |
| | *E. prestonii* J. Sm. | Prado 1117, NY | *Polytrichia* | Brazil | | AY536139 | AY534810 |
| | | Campos 2650, NY | | | EU907716 | EU907787 | EU907850 |

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|---|---|---|---|---|---|---|---|
| | **E. pringlei* (Davenp.) C. Chr. | Manzanero 1010, NY | *Lepidoglossa* | Mexico | | X | X |
| | | AcevedoHernandez 117, NY | | | X | X | X |
| | | Anderson 4679, MEXU | | | X | X | X |
| | | Vasco 890, MEXU | | | X | X | X |
| | | Vasco, 928, MEXU | | | X | X | X |
| | | JuarezGarcia 514, MEXU | | | X | X | X |
| | | TorresColin 17671, NY | | | X | X | X |
| | *E. proximum*_aff | Zamora 3340, INB | *Elaphoglossum* | Costa Rica | | AY540236 | AY536299 |
| | *E. pteropus* C. Chr. | Mori 25579, NY | *Elaphoglossum* | French Guiana | | AY540280 | AY536343 |
| | *E. pusillum* (Mett. ex Kuhn) C. Chr. | Loriga 325, NY | *Setosa* | Cuba | KF212394 | HG428762 | KF212420 |
| | *E. pygmaeum* (Mett. ex Kuhn) Christ | Smith 2826, UC | *Setosa* | Ecuador | | AY540281 | AY536344 |
| | *E. randii* Alston and Schelpe | Huntley 2072, P | *Lepidoglossa* | Mariana Islands | | AY540282 | AY536345 |
| | *E. rapense* Rapel. | Motley 2677 | *Setosa* | French Polynesia | | AY540283 | AY536346 |
| | *E. richardii* (Bory & Fée) Christ | Rouhan 205, P | *Lepidoglossa* | | EF040645 | EF040621 | EF040607 |
| | *E. rufescens* (Liebm.) T. Moore | Rojas 5387, MEXU | *Lepidoglossa* | Mexico | X | X | X |
| | *E. rufidulum* (Willd. ex Kuhn) C. Chr. | Rakotondrainibe 6396, P | *Lepidoglossa* | Madagascar | | AY540285 | AY536348 |
| | *E. russelliae* Mickel | Moran 6360, NY | *Setosa* | Costa Rica | | AY540286 | AY536349 |
| | **E. rzedowskii* Mickel | MartinezBecerril 406, BRIT, FEZA, MEXU | *Lepidoglossa* | Mexico | X | X | X |
| | | MartinezBecerril 407, BRIT, FEZA, MEXU | | | X | X | X |

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|---|---|---|---|---|---|---|---|
| | | MartinezBecerril 405, BRIT, FEZA, MEXU | | | X | X | X |
| | | Reina 96-183, MEXU | | | | X | X |
| | | Rzedowskii 26161, NY | | | | X | X |
| | | MartinezBecerril 408, BRIT, FEZA, MEXU | | | | X | X |
| | *E. sartorii* (Fée) T. Moore | MartinezBecerril 411, BRIT, FEZA, MEXU | *Elaphoglossum* | Mexico | X | X | X |
| | | Vasco 900, MEXU | | | X | X | X |
| | *E. schiedei* | Vasco 995, MEXU | *Lepidoglossa* | Mexico | X | X | X |
| | | Kromer 3080, MEXU | | | X | X | X |
| | *E. schmitzii* | Alvarez 13618, MO | *Lepidoglossa* | Mexico | X | X | X |
| | | Carranza 4179, MEXU | | | X | X | X |
| | | CamposSalas 130, MEXU | | | X | X | X |
| | | Lopez 1514, CHAPA | | | X | X | X |
| | *E. scolopendriforme* Tardieu | Rakotondrainibe 6426, P | *Lepidoglossa* | Madagascar | EU907719 | AY540288 | AY536351 |
| | *E. seminudum* Mickel | Vasco 888, MEXU | *Elaphoglosusm* | Mexico | X | X | X |
| | *E. setigerum* (Sodiro) Diels | Vasco 887, MEXU | *Setosa* | Mexico | X | X | X |
| | | Vasco 931, MEXU | | | X | X | X |
| | | VanEe 328, NY | | Costa Rica | | AY540289 | AY536352 |
| | *E. setosum* (Liebm.) T. Moore | Vasco 964, MEXU | *Setosa* | Mexico | X | X | X |
| | *E. sieberi* (Hook. & Grev.) T. Moore | Rouhan 169, P | *Elaphoglossum* | Mauritius | EU907720 | AY540290 | AY536353 |
| | *E. siliquoides* (Jenman) C. Chr. | Smith 2631, UC | *Setosa* | Costa Rica | | AY536127 | AY534798 |
| | *E. smithii* (Baker) Christ | Boyle 6409, NY | *Setosa* | Costa Rica | | AY540291 | AY536354 |

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|---|---|---|---|---|---|---|---|
| | *E. sp.* | MartinezBecerril 413, FEZA | *Elaphoglossum* | Mexico | | X | |
| | *E. sp.* | Vasco 585, HUA, MO, NY | *Lepidoglossa* | Colombia | EU907713 | | EU907847 |
| | *E. sp.* | Moran 7573, NY | *Lepidoglossa* | Ecuador | EU907714 | EU907785 | EU907848 |
| | *E. sp.* | Carranza 2036, NY | *Lepidoglossa* | Mexico | X | X | X |
| | *E. spatulatum* (Bory) T. Moore | Rakotondrainibe 6125, P | *Setosa* | Madagascar | EF040649 | EF040623 | EF040609 |
| | *E. splendens* (Bory ex Willd.) Brack. | Rouhan 247, P | *Lepidoglossa* | La Reunion | EU907721 | AY540296 | AY536359 |
| | *E. squamipes* (Hook.) T. Moore | Sundue 1688, INB, NY | *Squamipedia* | Costa Rica | KJ528067 | KJ528119 | KJ528235 |
| | *E. squarrosum* (Klotzsch) T. Moore | Vasco 883, MEXU | *Squamipedia* | Mexico | X | X | X |
| | *E. stipitatum* (Bory ex Fée) T. Moore | Rouhan 212, NY, P, PTBG | *Lepidoglossa* | La Reunion | | AY540297 | AY536360 |
| | *E. subsessile* (Baker) C. Chr. | Rakotondrainibe 6332, P | *Elaphoglossum* | Madagascar | EF040660 | AY540298 | AY536361 |
| | *E. succisaefolium* (Thouars) T. Moore | Marthel-Thoumian 1A, P | *Lepidoglossa* | Amsterdam Island | KJ528047 | AY540299 | AY536362 |
| | *E. tenuiculum* (Fée) T. Moore ex | Vasco 558, NY | *Lepidoglossa* | Colombia | EU907722 | | EU907852 |
| | | Vasco 755, HUA, NY | | Colombia | X | X | X |
| | | Vasco 824, HUA, MER, NY, VEN | | Venezuela | X | X | X |
| | | Huaylla 1076, HSB | | Bolivia | X | X | X |
| | *E. tenuifolium* (Liebm.) T. Moore | Vasco 897, MEXU | *Lepidoglossa* | Mexico | X | X | X |
| | | Vasco 892, MEXU | | | X | X | X |
| | *E. terrestre* A. Rojas | Moran 6368, NY | *Elaphoglossum* | Costa Rica | | AY540294 | AY536357 |
| | *E. tomentosum* (Bory ex Willd.) Christ | Rouhan 174, P | *Lepidoglossa* | Mauritius | EU907723 | AY540300 | AY536363 |
| | *E. tripartitum* (Hook ex Grev.) Mickel | Vasco 867, QCA, NY | *Squamipedia* | Ecuador | KJ528078 | KJ528140 | KJ528242 |
| | | Matos 2478, NY | *Lepidoglossa* | Mexico | X | X | X |

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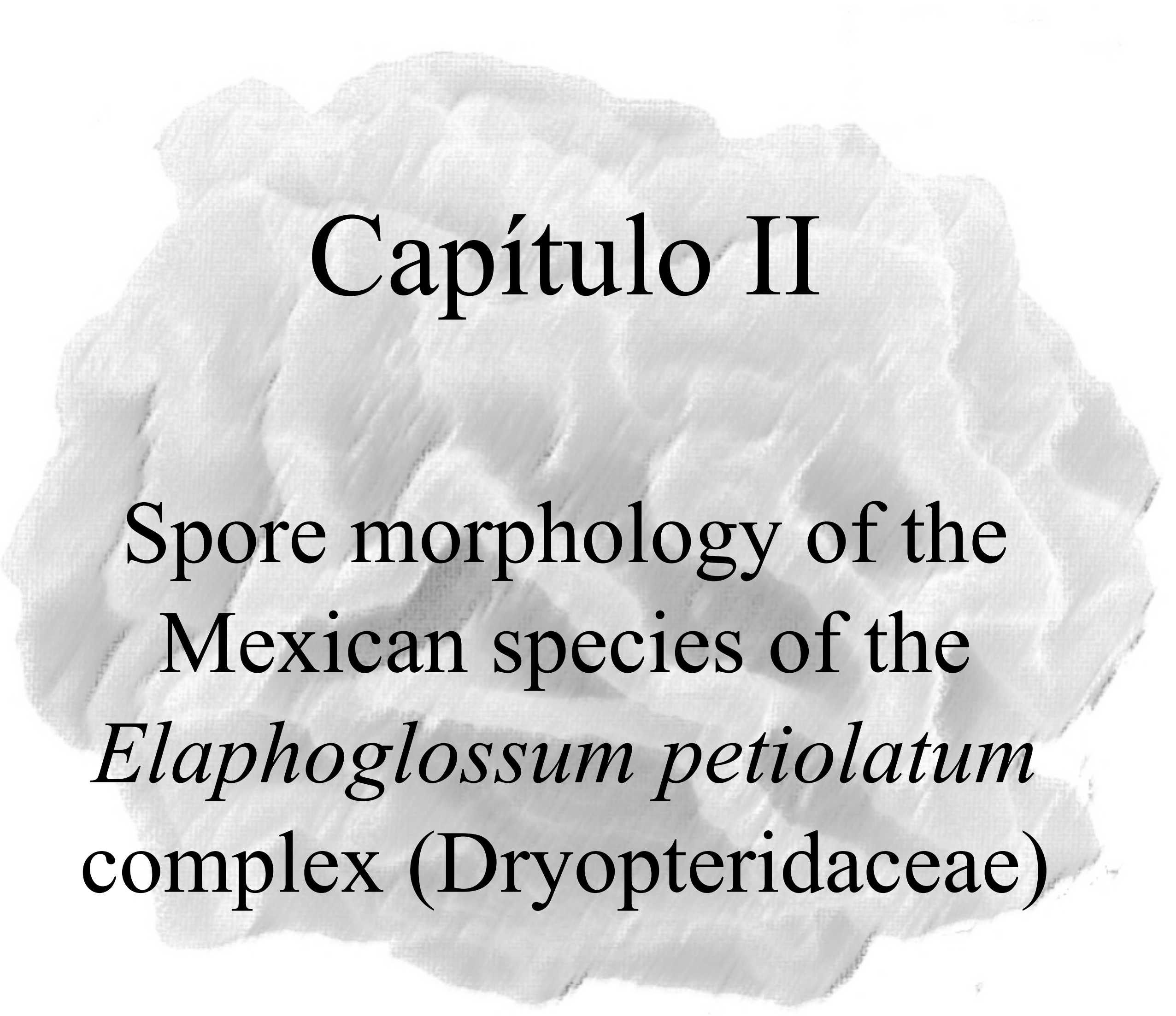
|---|---|---|---|---|---|---|---|
| | *E. vestitum* (Schltdl. & Cham.) T. Moore | Moran 6342, NY | *Lepidoglossa* | Costa Rica | EF040637 | AY540270 | AY536333 |
| | *E. vieillardii* (Mett.) T. Moore | Muntzinger 1740, P | *Elaphoglossum* | New Caledonia | | AY540301 | AY536364 |
| | *E. viride* Christ | MartinezBecerril 409, BRIT, FEZA, MEXU | *Lepidoglossa* | Mexico | X | X | X |
| | | Vasco 973, MEXU | | | X | X | X |
| | *E. viscidum* (Fée) Christ | Matos 2055, NY | *Lepidoglossa* | Brazil | X | X | X |
| | | Prado 1126, NY | | | | AY536142 | AY534813 |
| | | Matos, 2214, NY | | | X | X | X |
| | *E. vulcanicum* Christ | Moran 7586, NY | *Lepidoglossa* | Ecuador | EU907728 | EU907793 | EU907856 |
| | *E. wawrae* (Luerss.) C. Chr. | Lorence 8511, PTBG | *Elaphoglossum* | Hawaii | | AY540302 | AY536365 |
| | **E. welwitschii* (Baker) C. Chr. | Taylor 9099, P | *Lepidoglossa* | Tanzania | | AY540303 | AY536366 |
| | *E. wrightii* (Mett.) T. Moore | Loriga 242, NY | *Squamipedia* | Cuba | KF212396 | KF212446 | KF212422 |
| | *E. xanthopodum* Mickel | Vasco 1007, MEXU | | Mexico | X | X | X |
| | *E. yourkeorum* Mickel | Testo 838, MEXU | | Mexico | X | X | X |
| | **E. yungense* de la Sota | Jimenez 2487, NY | *Lepidoglossa* | Bolivia | EU907731 | EU907796 | EU907859 |
| | | Jimenez 2498, NY | | | EU907732 | EU907797 | EU907860 |
| | | Jimenez 2676, NY | | | EU907733 | EU907798 | EU907861 |
| | | Labiak 2808, | | | EU907736 | EU907801 | EU907864 |
| | | Labiak 2790 | | | EU907734 | EU907799 | EU907862 |
| | | Labiak 2804 | | | EU907735 | EU907800 | EU907863 |
| | | Sundue 581, LPB, NY, USZ | | | | AY540257 | AY536320 |



# Capítulo II

## Spore morphology of the Mexican species of the *Elaphoglossum petiolatum* complex (Dryopteridaceae)

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# Spore morphology of the Mexican species of the *Elaphoglossum petiolatum* complex (Dryopteridaceae)

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**Abstract**. *Elaphoglossum* is a taxonomically challenging fern genus with about 600 species. The present study concerns a group of five *Elaphoglossum* species mainly distributed in Mexico, four of which were recovered as monophyletic and are here called the Mexican-petiolatum clade (*E. petiolatum, E. potosianum, E. pringlei,* and *E. rzedowskii)*. The fifth species, *E. muelleri*, is distributed in Central America and Mexico and is morphologically similar to species of the Mexican-petiolatum clade. This study investigates the spores of these five species and has three objectives: first, to describe and compare their spore ornamentation; second, to analyze the variation in spore size among the species and within the widely distributed species *E. petiolatum*; and third, to assess whether perine ornamentation and exine size are useful to recognize species of the Mexican-petiolatum clade and/or for species delimitation. We studied 26 herbarium specimens of the five species and used scanning electron microscopy and statistical analyses to examine, measure, and analyze 133 spores. All the spores examined have uniform primary ornamentation (broad and discontinuous folds), but the secondary ornamentation is variable. Spores from Mexican specimens, regardless of their species, have small spines with narrow bases; spores of *E. petiolatum* from Central America have spines with wider bases; and spores of *E. petiolatum* from the West Indies have verrucae. Statistical analyses revealed that there are significant differences in spore size among all the studied species and within specimens identified as *E. petiolatum*. Within *E. petiolatum,* spores from the Dominican Republic are larger than those from the other countries. The results support our hypothesis that *E. muelleri* is part of the Mexican-petiolatum clade and suggest that *E. petiolatum* in Mexico, Central America, and the West Indies likely represent different taxa. Spore size differences help distinguish closely related species in the group such as *E. pringlei* and *E. rzedowskii* and indicate that there might be polyploid species within the Mexican-petiolatum clade.

**Keywords:** Comparative morphology, ferns, Mexico, polyploidy, species complex, spores, taxonomy.

**Resumen**. *Elaphoglossum* es un género de helechos taxonómicamente desafiante con alrededor de 600 especies. El presente estudio se enfocó en un grupo de cinco especies pertenecientes al género *Elaphoglossum*, distribuidas principalmente en México. De éstas, cuatro se recuperaron en un grupo monofilético denominado aquí como el clado mexicano de *E. petiolatum* (*E. petiolatum, E. potosianum, E. pringlei* y *E. rzedowskii*). La quinta especie, *E. muelleri*, se distribuye en América Central y México y es morfológicamente similar a las especies del clado mexicano de *E. petiolatum*. Este estudio investiga las esporas de estas cinco especies y tiene tres objetivos: primero, describir y comparar la ornamentación de las esporas; segundo, analizar la variación en el tamaño de las esporas entre las especies y dentro de la especie ampliamente distribuida *E. petiolatum*; y tercero, evaluar si la ornamentación de la

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perina y el tamaño de la exina son útiles para reconocer y/o delimitar estas especies. Examinamos, medimos y analizamos 133 esporas de 26 especímenes de herbario pertenecientes a las cinco especies, mediante microscopía electrónica de barrido y análisis estadísticos. Todas las esporas examinadas tienen ornamentación primaria uniforme (pliegues anchos y discontinuos), pero la ornamentación secundaria es variable. Las esporas de especímenes mexicanos, independientemente de su especie, tienen espinas pequeñas con bases estrechas; las esporas de *E. petiolatum* de América Central tienen espinas con bases más anchas; y las esporas de *E. petiolatum* de las Antillas tienen verrugas. Los análisis estadísticos revelaron que existen diferencias significativas en el tamaño de las esporas entre todas las especies estudiadas y dentro de los especímenes identificados como *E. petiolatum*. Dentro de *E. petiolatum*, las esporas de República Dominicana son más grandes que las de los demás países. Los resultados obtenidos apoyan nuestra hipótesis de que *E. muelleri* es parte del clado mexicano de *E. petiolatum* y sugieren que *E. petiolatum* de México, América Central y las Antillas, probablemente representan taxones diferentes. Las diferencias en el tamaño de las esporas ayudan a distinguir especies estrechamente relacionadas como *E. pringlei* y *E. rzedowskii*, e indican que puede haber especies poliploides dentro del clado mexicano de *E. petiolatum*.

*Elaphoglossum* Schott ex J. Sm. is a monophyletic fern genus belonging to the bolbitidoid clade of Dryopteridaceae (Skog et al., 2004; Rouhan et al., 2004; Vasco et al., 2009; Lóriga et al., 2014; Liu et al., 2015; PPG I, 2016). The genus has about 600 species and a pantropical distribution, with ca. 80% of the species occurring in the Neotropics (Moran et al., 2007). *Elaphoglossum* species are similar morphologically and usually characterized by simple laminae, entire margin, dimorphic leaves, acrostichoid sori, phyllopodia, free veins, and monolete spores (Moran et al., 2007, 2010). A large number of species and the similarity among them make the taxonomy of *Elaphoglossum* challenging.

Efforts to understand the taxonomy of *Elaphoglossum* include an infrageneric classification based on sporophyte morphology and spore ornamentation (Mickel & Atehortúa, 1980), and several molecular phylogenetic studies (Skog et al., 2004; Rouhan et al., 2004; Lóriga et al., 2014). Phylogenetic studies have largely recovered as monophyletic most of the large sections proposed in the morphological classification of Mickel and Atehortúa (1980). These are in seven well-supported clades: *Elaphoglossum* sect. *Wrigthiana* J. Lóriga, A. Vasco, L. Regalado, Heinrichs & R.C. Moran, sect. *Amygdalifolia* (Christ) Mickel & Atehortúa, sect. *Elaphoglossum,* sect. *Squamipedia* Mickel & Atehortúa, sect. *Polytrichia* Christ, sect. *Setosa* (Christ) Mickel & Atehortúa, and sect. *Lepidoglossa* Christ (Skog et al., 2004; Rouhan et al., 2004; Lóriga et al., 2014).

Spore morphology has been useful to define some of the unnamed large clades, proposed sections and subsections, and unnamed species groups (Mickel & Atehortúa, 1980; Skog et al., 2004; Rouhan et al., 2004; Lóriga et al., 2014). The main studies of *Elaphoglossum* spore morphology are that of Mickel and Atehortúa (1980), which included SEM spore images of 163 species, and that of Moran et al. (2007), which was the first study to assess perine morphology in a phylogenetic context and included spore SEM images of 221 species. Both studies showed that the perine morphology of *Elaphoglossum* displays considerable diversity and is mainly uniform within species. Moran et al. (2007) also showed that, although perine characters can show considerable homoplasy, they provide some synapomorphies for most of the large clades and for some smaller clades within *Elaphoglossum* (Moran et al., 2007). Other studies in *Elaphoglossum* have used perine morphology to define several small groups and distinguish closely related and morphologically similar species (Mickel, 1985; Rouhan et al., 2004; Vasco et al., 2009, 2013; Vasco, 2011; Matos et al., 2018).

The present study concerns a group of five species of *Elaphoglossum* sect. *Lepidoglossa*, four of which were recovered as monophyletic in a previous molecular phylogeny (Vasco et al., 2009). *Elaphoglossum* sect. *Lepidoglossa* Christ, a section of about 200 species, was originally based on morphology and has been subsequently recovered as a well-supported clade by molecular phylogenetic studies (Mickel & Atehortúa, 1980; Skog et al., 2004; Rouhan et al., 2004). Species of sect. *Lepidoglossa* are usually characterized by phyllopodia, scaly laminae, and scales with acicular marginal cells (Mickel & Atehortúa, 1980; Vasco et al., 2009). Spores of sect. *Lepidoglossa* species are generally characterized by broad and continuous folds, nonperforated perines, and absence of spines (Moran et al., 2007). Spines, however, appear to have originated several times and are a synapomorphy for smaller clades within the section (Moran et al., 2007).

The four species of sect. *Lepidoglossa* that were previously recovered as monophyletic are *Elaphoglossum petiolatum* (Sw.) Urb., *E. potosianum* Christ, *E. pringlei* (Davenp.) C. Chr., and *E. rzedowskii* Mickel (Vasco et al., 2009). These four species were traditionally considered part of the *E. petiolatum* complex, a worldwide group of about 12 species (Mickel, 2001). Species in the complex are distinguished by resinous dots on the abaxial lamina surface, black rhizome scales, and scales of the proximal portions of the petiole with dark apices and entire margins (Mickel, 2001; Mickel & Smith, 2004). Molecular phylogenetic studies suggested that, as traditionally defined, the *E. petiolatum* complex is not monophyletic and that only the Mexican species, including specimens of *E. petiolatum* from Mexico, form a monophyletic group named the Mexican-petiolatum clade (Vasco et al., 2009). The three species of the latter clade (*E. potosianum, E. pringlei,* and *E. rzedowskii*) are endemic to Mexico (Mickel & Smith, 2004). The fourth species, *E. petiolatum,* had been considered to be widely distributed from Mexico to South America, including the West Indies (Mickel & Smith, 2004). Previous molecular phylogenetic analyses, however, have shown that what has been called *E. petiolatum* in South America, belongs to a different clade than the Mexican-petiolatum clade (Vasco et al., 2009).

In the present study, we investigate perine morphology and exine size in the four species of the Mexican-petiolatum clade. We include specimens of *E. petiolatum* from its entire geographical distribution: Jamaica (where the type of *E. petiolatum* was collected), the Dominican Republic, Mexico, and Central America. Also, we studied the spores of *E. muelleri* (E. Fourn.) C. Chr., a species morphologically similar to those of the Mexican-petiolatum clade. We had three objectives: first, to describe and compare the ornamentation of species of the Mexican-petiolatum clade and *E. muelleri*; second, to analyze the variation in spore size among species of the Mexican-petiolatum clade and within *E. petiolatum*; and third, to assess if spore ornamentation and spore size are useful to recognize species that belong to the Mexican-petiolatum clade and/or for species delimitation.

## Material and methods

*Taxonomic sampling.*— We studied specimens representing the four species belonging to the Mexican-petiolatum clade (*Elaphoglossum petiolatum, E. pringlei, E. potosianum,* and *E. rzedowskii*), and *E. muelleri*, a species distributed in Mexico and Central America and morphologically similar to the other species of the Mexican-petiolatum clade. To evaluate spore ornamentation and size variation within *E. petiolatum*, we analyzed specimens from its entire distribution range (Costa Rica, the Dominican Republic, Jamaica, Mexico, and Panama). We identified the specimens using taxonomic keys (Proctor, 1985; Mickel, 1995; Mickel & Smith, 2004) and specimens or images of types.

*Spore ornamentation and size.*— We took spore samples from specimens at FEZA, MEXU, MO, and NY. The number of spores sampled per species varied depending on the number of fertile specimens found for each species. For each specimen, we transferred three closed sporangia to an aluminum stub covered with adhesive carbon tape. We opened the sporangia with a dissection needle and scattered the spores on the stub surface. Stubs were coated with a gold layer using a metallizer QUORUM (Q150R ES) for two minutes at 20 mA. We imaged the spores using a HITACHI SU1510 scanning electron microscope (SEM) with an accelerating voltage of 10 or 15 kV. Image background and brightness were edited with Corel PHOTO-PAINT X7 and Adobe Photoshop. Also, we used some spore microphotographs publicly available at Plantsystematics.org posted by R. C. Moran.

We examined the spore images to describe the laesura type and the primary and secondary ornamentation. A laesura is the scar formed on the proximal faces of a spore where it was in contact with the three other spores in the tetrad (Erdman, 1943; Punt et al., 2007). Primary ornamentation describes the overall sculpture of the spore surface (e.g., broad folds, crests, large spines), whereas secondary ornamentation describes microstructures smaller than 1 µm (Olejnik et al., 2018). For spore descriptions, we followed the terminology of Punt et al. (2007). To describe the secondary ornamentation, we use the terms spines and verrucae. In palynology, the definition of spines refers to long and tapering pointed elements (Erdman, 1952; Punt et al., 2007), whereas verrucae describe elements more than 1 µm wide, wider than tall, and not constricted at the base (Iversen & Troels-Smith, 1950; Punt et al., 2007).

Equatorial and polar diameters were measured using the software ImageJ (Schneider et al., 2012). The equatorial diameter (ED) refers to the line lying in the spore equatorial plane and passing through it (Erdman, 1943; Punt et al., 2007). The polar diameter (PD) is the straight line between the spore distal and proximal poles, and it is perpendicular to the equatorial axis (Wodehouse, 1935; Punt et al., 2007).

*Statistical analyses.*— We conducted all statistical analyses in R (R Core Team, 2014) using the "car" package (Weisberg, 2019). To assess if there are significant differences in spores ED and PD means among all the studied species, we conducted a Welch's test of variance for samples with unequal variance, because the number of spores measured per species is different (function "oneway.test" in R) (Table 1). To assess the differences between all possible pairs of species, we used the Fishers Least Significant Difference (LSD) test. To assess if there are significant differences in ED and PD in the spores of *E. petiolatum* among the different countries where this species is distributed, we conducted a traditional ANOVA (function "aov" in R) followed by a Tukey Honest Significant Differences (THSD) post-hoc test to assess the differences between all possible pairs of countries.

## Results

We measured 133 spores from 26 different collections belonging to the four species of the Mexican-petiolatum clade and *Elaphoglossum muelleri* (Table 1, Appendix 1). Most of the collections included in this study came from Mexico, but for *E. petiolatum,* we examined spores from Costa Rica, the Dominican Republic, Jamaica, Mexico, and Panama (Appendix 1). We found that spores were often collapsed, for the measurements we used only well-shaped spores. For the statistical analyses, the sample size was 131 spores because we excluded the atypical values (outliers). Spores of *E. muelleri, E. potosianum,* and *E. rzedowskii* are imaged and described here for the first time.

*Spore ornamentation.*— Spores of the five species studied are similar in shape and primary ornamentation. All the species have rounded and monolete spores, and nonperforated perines with broad discontinuous folds as primary ornamentation (Figs. 1 and 2). All specimens from Mexico, regardless of the species they belong to, have

**Table 1.** Number of spores sampled and spore size of all the *Elaphoglossum* species included in this study. *Elaphoglossum petiolatum* specimens are divided in three groups

| Species | Sample size | Equatorial diameter (µm) min[a]–(χ)[b]–max[c] | sd[d] | Polar diameter (µm) min–(χ)–max | sd | Fig. |
|---|---|---|---|---|---|---|
| *E. muelleri* | 11 | 29.5–**(37.5)**–44.1 | 5.9 | (23.9*–)28.1–**(30.5)**–33.7 (−36*) | 1.8 | 1A, B |
| *E. petiolatum "Mexican group"* | 7 | 29.4–**(34.4)**–38.6 | 3.4 | 24.8–**(27.4)**–30.1 | 2 | 1I, 2A |
| *E. petiolatum "Central American group"* | 15 | 29.3–**(33.5)**–38.4 | 3.1 | 22.5–**(25.2)**–28.9 | 2 | 2B–E |
| *E. petiolatum "West Indian group"* | 28 | 31.3–**(38.1)**–46.7 | 4.4 | (20.7*–)22.8– **(28.9)**–34.6 | 2.7 | 2F–I |
| *E. potosianum* | 20 | (33.1*–)35–**(39.4)**–43.1 (−69.5*) | 2.2 | 24.7– **(31.1)**–38.3(−43.1*) | 3.6 | 1C, D |
| *E. pringlei* | 28 | 30–**(40.4)**–64.3(−68.2*) | 8.3 | 20–**(31.6)**–46.3(−51.5*) | 6.5 | 1E, F |
| *E. rzedowskii* | 24 | 28.5– **(34.6)**–43.5 | 3.6 | 21.8–**(27.9)**–35.7 | 3.2 | 1G, H |

[a] Minimum size, [b] size mean, [c] maximum size, [d] standard deviation; *atypical value not considered in the mean calculation.

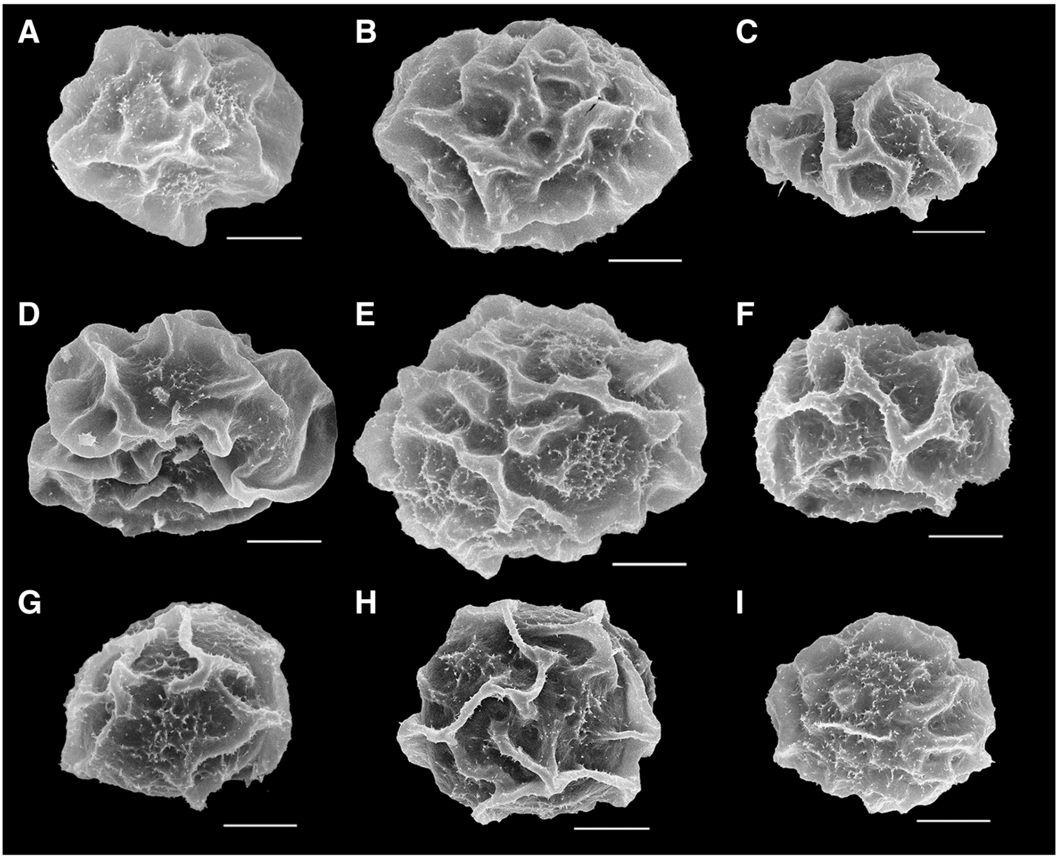

**Fig. 1.** Scanning electron microscopy images of spores from five species of *Elaphoglossum*. **A, B.** *E. muelleri*. **C, D.** *E. potosianum*. **E, F.** *E. pringlei*. **G, H.** *E. rzedowskii*. **I.** *E. petiolatum* "Mexican group". Scale bars = 10μm. (A–I from Mexico, A, *McVaugh 23,885*, MEXU; B, *Anderson 13,211*, MEXU; C, *Martínez-Becerril 399*, FEZA, MEXU; D, *Carranza 5140*, MEXU; E, *Martínez-Becerril 405*, FEZA, MEXU; F, *Anderson 4778*, MEXU; G, *Cornejo-Tenorio 1438*, MEXU; H, *Martínez-Becerril 408*, FEZA, MEXU; I, *Gallardo-Hernández 836*, MEXU.)

spines as secondary ornamentation (Figs. 1 and 2a). These spines are less than 1 μm tall and have a base less than 0.3 μm (Fig. 3a). Specimens of *Elaphoglossum petiolatum* from Central America also have small spines less than 1.2 μm tall, but these have a wider base (0.7–1.2 μm) than the spines found in Mexican specimens (Figs. 2b–e and 3b). Specimens of *E. petiolatum* from Jamaica and the Dominican Republic have verrucae instead of spines (Figs. 2f–i and 3c). Because of the consistent and considerable differences that we found in spore secondary ornamentation among Mexican, Central American (Costa Rica and Panama), and West Indian (Jamaica and the Dominican Republic) specimens of *E. petiolatum*, we described their spores and analyzed them as three different groups (Tables 1 and 2).

*Spore Size*. In the five species studied, ED varies between 28.5 and 69.5 μm and PD between 20 and 51.5 μm (Table 1, Appendix 1). Of all the species studied, *Elaphoglossum pringlei* and *E. potosianum* have the largest spores, whereas *E. rzedowskii* and the Central American group of *E. petiolatum* have the smallest spores (Figs. 4 and 5; Table 1). Within *E. petiolatum* (considering the specimens from all countries), ED varies between 29.3 and 46.7 μm and PD between 20.7 and 34.6 μm (Table 2, Appendix 1). In average, spores from the Dominican Republic are the biggest and the ones from Costa Rica the smallest (Figs. 6 and 7, Table 2).

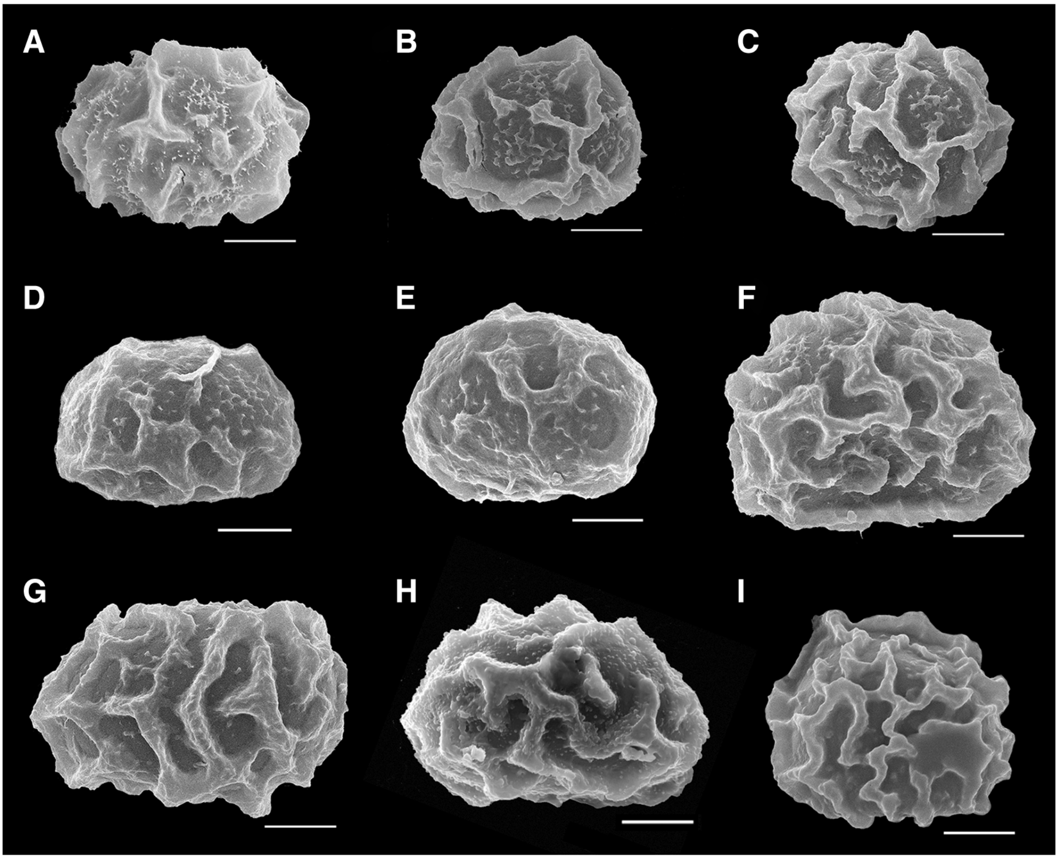

**Fig. 2.** Scanning electron microscopy images of *Elaphoglossum petiolatum* spores from five countries. **A.** Mexico. **B–E.** Central America. **F–I.** West Indies. Scale bars = 10μm. (A from Mexico, *Gallardo-Hernández 836*, MEXU; B, C from Costa Rica, *Lloyd 4134*, NY; D, E from Panama, *Croat 26,996*, NY; F, G from Dominican Republic, *García 958*, NY; H, I from Jamaica, H, *Underwood 3141*, NY; I, *Underwood 3214*, NY.)

The Welch's test to assess differences in spore size among the studied species, showed that there are significant differences in the means of the spores ED ($p$=0.000005, Fig. 4) and PD ($p$=0.000001, Fig. 5). The LSD test revealed there are differences in the spores ED and PD between many of the species pairs (Suppl. Material 1). The ANOVA to assess differences in the spore size of *Elaphoglossum petiolatum* among countries, showed that there are significant differences in

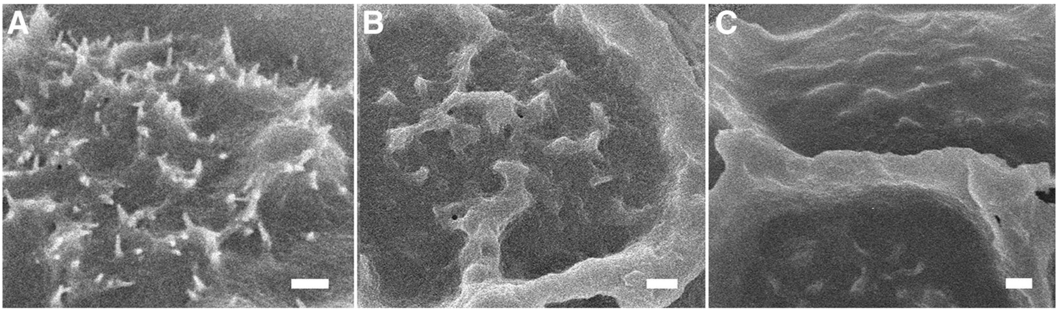

**Fig. 3.** Types of secondary ornamentation found in the perine of the five species of *Elaphoglossum* studied. **A.** Spines with narrow base, *Gallardo-Hernandez 836,* MEXU. **B.** Spines with wider base, *Lloyd 4134,* NY. **C.** Verrucae, *Underwood 545,* NY. Scale bars = 1μm.

**Table 2.** Number of spores sampled and spore size of *Elaphoglossum petiolatum* by country.

| Country | Sample size | Equatorial diameter (µm) min[a]–(χ)[b]–max[c] | sd[d] | Polar diameter (µm) min–(χ)–max | sd |
|---|---|---|---|---|---|
| Costa Rica | 8 | 29.3–**(32.5)**–38 | 3 | 22.5–**(25.1)**–28.7 | 2.1 |
| Dominican Republic | 8 | 36.5–**(42.5)**–46.7 | 3.2 | 28.1–**(30.6)**–34.6 | 2.2 |
| Jamaica | 20 | 31.3–**(36.3)**–43.5 | 3.4 | (20.7*–)22.8–**(28.1)**–33.1 | 2.6 |
| Mexico | 7 | 29.4–**(34.4)**–38.6 | 3.4 | 24.8–**(27.4)**–30.1 | 2 |
| Panama | 7 | 30.1–**(34.6)**–38.4 | 3.2 | 22.9–**(25.4)**–28.9 | 2 |

[a] Minimum size, [b] size mean, [c] maximum size, [d] standard deviation, * atypical value not considered in the mean calculation.

the mean spores ED (p=0.000005, Fig. 6) and PD (*p*=0.0006, Fig. 7). The posterior comparisons using the THDS test revealed there were significant differences in spore ED between the spores from the Dominican Republic and: Costa Rica, Jamaica, Mexico, and Panama (Fig. 6; Suppl. Material 1). Spore PD displayed significant differences between the Dominican Republic and: Costa Rica and Panama (Fig. 7; Suppl. Material 1).

## Discussion

All the spores we examined have uniform primary ornamentation consisting of nonperforated perines with discontinuous and broad folds. Although these results match previous reports for spores of species belonging to *Elaphoglossum* sect. *Lepidoglossa* (Mickel & Atehortúa, 1980; Tryon & Lugardon, 1991; Moran et al., 2007), nonperforated spores with discontinuous and broad folds have been shown to be plesiomorphic for *Elaphoglossum*, thus many species belonging to other sections of *Elaphoglossum*, such as sect. *Elaphoglossum* and sect. *Polytrichia*, can have similar spore primary ornamentation (Moran et al., 2007). On the other hand, the spore secondary ornamentation of our studied species presents differences (Fig. 3). All spores from Mexican specimens, regardless of their species, have small spines with narrow bases (Figs. 1, 2a and 3a). These include *E. muelleri* and all the species recovered as part of the Mexican-petiolatum clade (Vasco et al., 2009). Spores of *E. petiolatum* from Central America also have small spines but those have wider bases and thus are different from the spines found in Mexican specimens (Figs. 2b–e and 3b). Spores of *E. petiolatum* from the West Indies differ from the rest by the presence of verrucae (Fig. 2f–i, 3c). Although closely related species can have different spore ornamentation, the fact that all Mexican specimens studied have the same ornamentation, supports our hypothesis that *E. muelleri* is part of the Mexican-petiolatum clade.

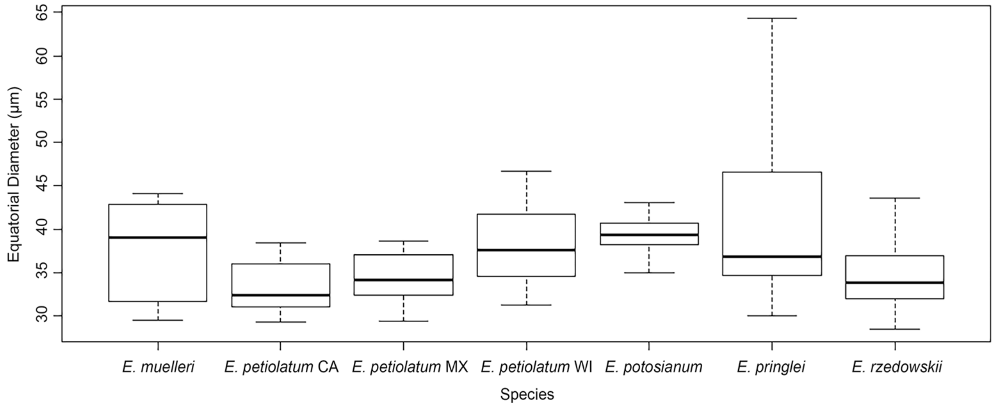

**Fig. 4.** Boxplot showing the variation of the spore equatorial diameter of the five species of *Elaphoglossum* included in this study. Lower and upper portions of boxes correspond with the first and third quartile respectively. The thick line inside boxes corresponds to the median. Lower and upper whiskers indicate the minimum and maximum values of spore equatorial diameter.

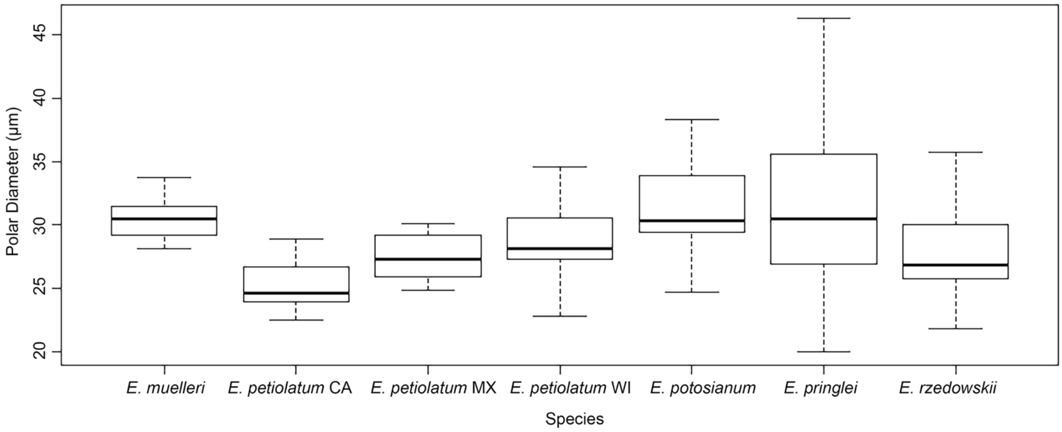

**Fig. 5.** Boxplot showing the variation of the spore polar diameter of the five species of *Elaphoglossum* included in this study. Lower and upper portions of boxes correspond with the first and third quartile respectively. The thick line inside boxes corresponds to the median. Lower and upper whiskers indicate the minimum and maximum values of spore polar diameter.

Previous studies on spore morphology of *Elaphoglossum* have suggested that spore ornamentation and size are constant within species (Mickel, 1980, 1985; Mickel & Atehortúa, 1980; Tryon & Tryon, 1982; Tryon & Lugardon, 1991; Moran et al., 2007; Rouhan et al., 2008; Vasco et al., 2015; Matos et al., 2018; Barrington et al., 2020). The differences in spore ornamentation and size within *E. petiolatum* (Figs. 2f–i and 3c, Table 2) suggest this species might consist of several different ones. *Elaphoglossum petiolatum* is the only species in the Mexican-petiolatum clade that has been reported to have a wide distribution, occurring from Mexico to South America, and in the West Indies (Mickel & Smith, 2004). A previous

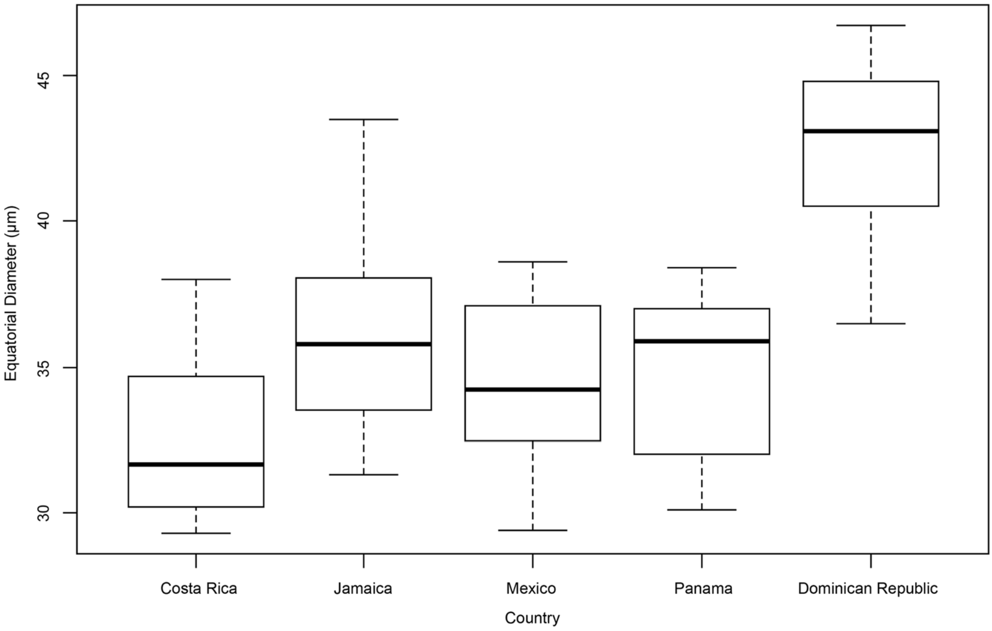

**Fig. 6.** Boxplot showing the variation of the spore equatorial diameter in five of the countries where *Elaphoglossum petiolatum* is distributed. Lower and upper portions of boxes correspond with the first and third quartile respectively. The thick line inside boxes corresponds to the median. Lower and upper whiskers indicate the minimum and maximum values of spore equatorial diameter.

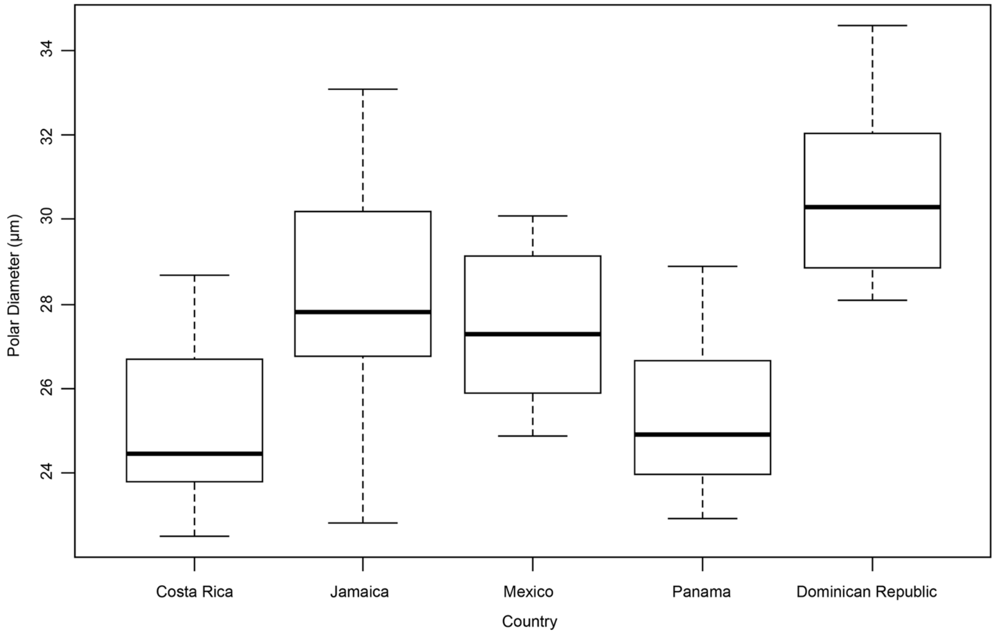

**Fig. 7.** Boxplot showing the variation of the spore polar diameter in five of the countries where *Elaphoglossum petiolatum* is distributed. Lower and upper portions of boxes correspond with the first and third quartile respectively. The thick line inside boxes corresponds to the median. Lower and upper whiskers indicate the minimum and maximum values of spore polar diameter.

molecular phylogeny has shown that what had been considered *E. petiolatum* in South America belongs to a different clade (Vasco et al., 2009). Central America and West Indies specimens of *E. petiolatum,* however, have not been included in molecular phylogenies; thus, it is not clear if specimens from those countries constitute part of the Mexican-petiolatum clade. Previous studies and ours show that there is considerable variation within *E. petiolatum* in the density of scales and of resinous dots, especially on the abaxial leaf surfaces (Mickel & Smith, 2004). This variation does not correlate with geographical distribution as does the variation in spore ornamentation and size reported here. Based on these differences, we suspect that *E. petiolatum* consists of at least three different species (Mexican, Central American, and West Indian), and that *E. petiolatum* from West Indies is probably not part of the Mexican-petiolatum clade. Further studies of the morphology and the size of rhizome and petiole scales, resinous dots, and molecular phylogenetic studies that include specimens from Central America and the West Indies, are necessary to confirm our hypothesis. If our hypothesis is corroborated, the name *E. petiolatum* would apply to specimens from the West Indies because the type of that name was collected in Jamaica (Swartz, 1788). For the Mexican specimens, two names are available: *E. schiedei* Kunze and *E. schmitzii* Mett. ex Kuhn. Both have been previously put in the synonymy of *E. petiolatum* (Mickel & Smith, 2004; Mickel & Velázquez-Montes, 2009).

We also found significant differences in spore size among the species studied (Suppl. Material 1). The most important taxonomically was between *Elaphoglossum pringlei* and *E. rzedowskii.* The former species had the biggest spores and *E. rzedowskii* had the smallest. Spores of *E. pringlei* are on average 5.8 µm (ED) and 3.7 µm (PD) larger than *E. rzedowskii* (Table 1). These two species are very similar morphologically. Both have linear-lanceolate, dark brown, lustrous rhizome scales, adaxial blade surfaces with lanceolate deeply toothed or stellate scales, and abaxial surfaces densely scaly with overlapping ciliolate or substellate scales. Mickel & Smith (2004) suggested that *E. pringlei* and *E. rzedowskii* could be conspecific and represent regional variations of a single taxon. Our results show that spore size is an important taxonomic character to separate them.

Size differences between closely related species may result from polyploidy, which is the condition of possessing more than two complete sets of chromosomes in a cell (Stebbins, 1950; Wagner, 1974; Barrington et al., 1986, 2020; Farrar, 2006). In this study, polyploidy could explain the larger size of *Elaphoglossum pringlei* spores, compared to those of the morphologically closely related species *E. rzedowskii*, or the larger size of *E. petiolatum* spores from the West Indies compared to those of other countries. Specimens of *E. petiolatum* from the West Indies have larger spores than those from Central America and Mexico (Figs. 6 and 7, Table 2). Particularly, spores from the Dominican Republic are significantly larger than those from the other countries, including Jamaica (ED=6.2–10 µm bigger on average, Fig. 6 and Table 2). Chromosome counts and isoenzymes have shown that polyploidy occurs in at least five species of *Elaphoglossum* (Walker, 1973, 1985; Ghatak, 1977; Tsai & Shieh, 1985; Manton et al., 1986; Bhavanandan & Ammal, 1992; Irudayaraj & Manickam, 1992; Eastwood et al., 2004; Tindale & Roy, 2002). This is less than 1% of the 600 *Elaphoglossum* species. Chromosome counts, flow cytometry data, and nuclear phylogenetic studies are necessary to further test the hypothesis that the larger spores in our studied species indicate polyploidy and to better understand how polyploids shape the diversity of the Mexican-petiolatum clade.

Adaptative mechanisms for dispersal and different environmental parameters have also been proposed to explain the larger size of some fern spores (Wagner, 1961; Carlquist, 1966; Cox & Hickey, 1984; Barrington et al., 1986, 2020). It has been proposed that spores of fern species from discontinuous, drier habitats or islands are larger when compared to their counterparts in continuous, wet, and mainland habitats, without necessarily being polyploids (Carlquist, 1966; Barrington et al., 2020). Species with larger spores from discontinuous habitats or islands could avoid dispersal to unfavorable surrounding habitats, for example the sea (Carlquist, 1966; Barrington et al., 2020). This could be an explanation for the significant differences in spore size we found between West Indian and continental *E. petiolatum* (Table 2).

## Conclusions

Here we report the ornamentation and size of spores of five species of *Elaphoglossum* that likely belong to the Mexican-petiolatum clade. Spore ornamentation and size within this clade was useful to distinguish closely related species. The differences in spore size between some of the species suggest that polyploidy occurs within the group. We demonstrate that careful studies of spore morphology are useful in *Elaphoglossum* taxonomy and systematics.

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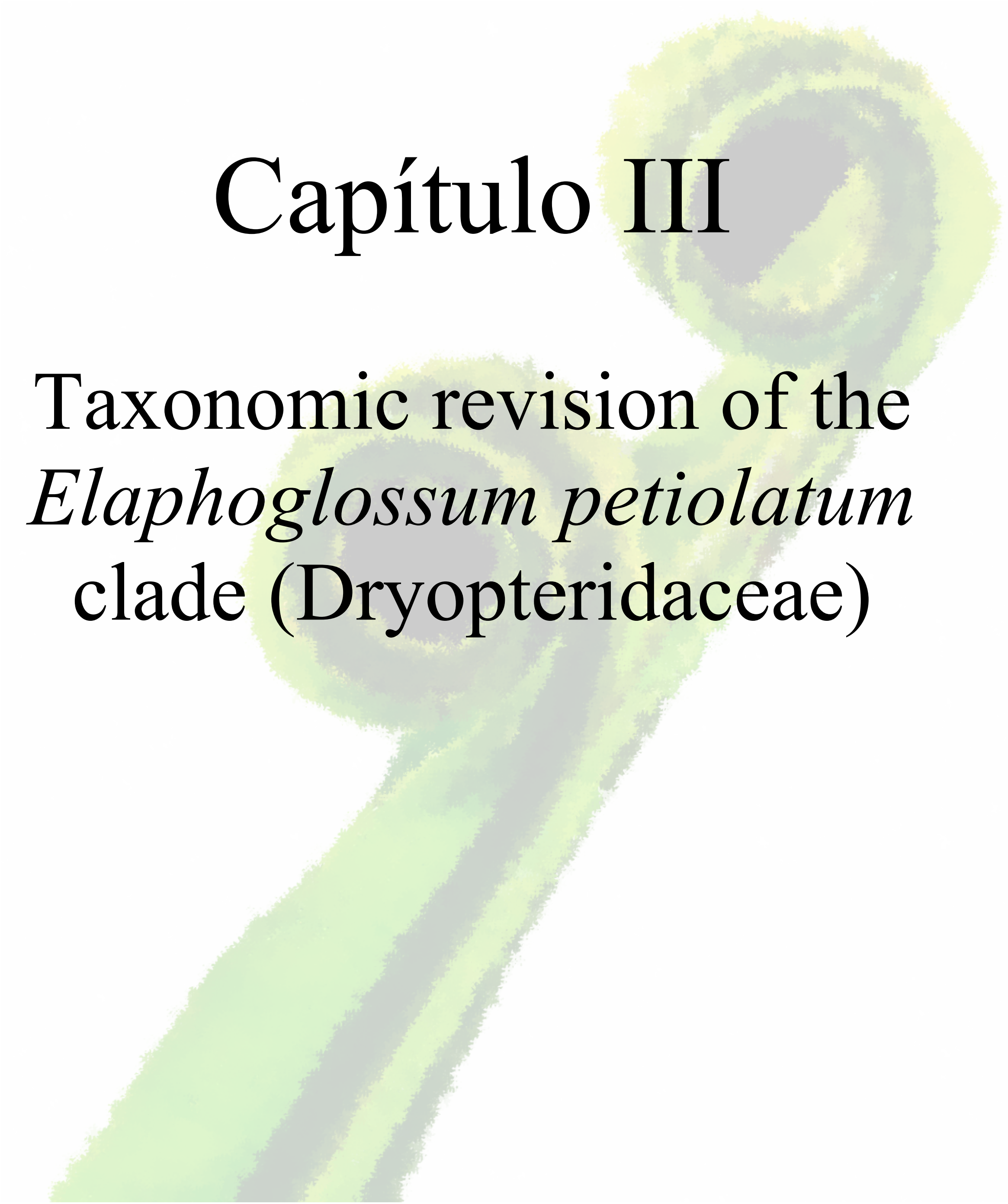
**Appendix 1.** Voucher information for all *Elaphoglossum* spores included in the analyses. Stars indicate spore images taken from Plantsystematics.org

***Elaphoglossum muelleri (E. Fourn.) C. Chr.*** **MEXICO.** *Anderson 13,211* (MEXU); *McVaugh 23,885* (MEXU). ***Elaphoglossum petiolatum*** **(Sw.) Urb. COSTA RICA.** *Lloyd 4134* (NY); *Moran 3079** (NY). **DOMINICAN REPUBLIC**. *Garcia 958* (NY); *Lloyd 753** (NY). **MEXICO.** *Gallardo-Hernandez 836* (MEXU); *Reveal & Atwood 3572** (NY). **JAMAICA.** *Proctor 5297* (MO); *Underwood 545, 3141*, 3214* (NY). **PANAMA**. *Croat 26,996* (NY). ***Elaphoglossum potosianum*** **Christ. MEXICO.** *Carranza 5140* (MEXU, NY*), *2036* (MEXU); *Martínez-Becerril 399* (FEZA, MEXU); *Parry & Palmer 1007** (NY). ***Elaphoglossum pringlei*** **(Davenp.) C. Chr. MEXICO.** *Anderson 4778* (MEXU); *Ernest 2371* (MEXU); *Martínez-Becerril 405, 407* (FEZA, MEXU); **Mickel 4463* (NY).** ***Elaphoglossum rzedowskii*** **Mickel. MEXICO.** *Cornejo-Tenorio 1438* **(MEXU);** *Martínez-Becerril 406, 408* (FEZA, MEXU); *Rzedowski 26032** (NY).

# FE DE ERRATAS

Capítulo II: “Spore morphology of the Mexican species of the *Elaphoglossum petiolatum* complex (Dryopteridaceae)”

- La ornamentación de las perisporas de *Elaphoglossum petiolatum* que en este Capítulo/artículo se describe como “con verrugas” equivale a lo que en los capítulos subsecuentes describimos como “con microestructuras dispersas e irregulares”. El cambio se da después de analizar mayor cantidad de esporas de diferentes ejemplares durante la elaboración de la monografía de las especies del clado de *E. petiolatum*.
- El país de origen de la colección *Lloyd 753* (NY) debería decir Dominica en lugar de República Dominicana.



# Capítulo III

## Taxonomic revision of the *Elaphoglossum petiolatum* clade (Dryopteridaceae)

Este artículo será sometido a *Annals of the Missouri Botanical Garden*

# TAXONOMIC REVISION OF THE NEOTROPICAL FERN CLADE ELAPHOGLOSSUM PETIOLATUM (DRYOPTERIDACEAE)

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***Resumen***–Se presenta una monografía del clado de *Elaphoglossum petiolatum* (sección *Lepidoglossa)*, un grupo recuperado a partir de análisis filogenéticos moleculares con ADN del cloroplasto. La monografía incluye una clave de identificación, sinonimia completa, descripciones detalladas, especímenes representativos examinados, índice de colectores y números de colección, ilustraciones, imágenes de esporas al microscopio electrónico y mapas de distribución para todas las especies. Morfológicamente, las especies de este clado se reconocen por escamas del rizoma con ápice agudo a filiforme-tortuoso, escamas de la porción proximal del peciolo con ápice obscuro, láminas con puntos resinosos y escamas planas y/o estrelladas, y perisporas con crestas y espinas pequeñas, menores a 1µm (excepto *E. petiolatum* que presenta microestructuras dispersas e irregulares). Se reconocen ochos especies sin subespecies o variedades. Todas las especies de este clado se distribuyen en el continente americano, desde México hasta Panamá, con una especie en Las Antillas Mayores. La mayoría ocurre entre los 1000–2500 msnm en bosques de pino-encino y bosques mesófilos de México. En este trabajo se lleva a cabo la combinación nomenclatural para *E. schmitzii*, se designan lectotipos para *E. potosianum* y *E. schmitzii* y un probable holotipo para *Acrostichum intermedium* y se realiza una lectotipificación en segundo grado para *E. pringlei*.

***Palabras clave*–** florística, helechos, México, morfología, nomenclatura, pteridofitas, puntos resinosos, taxonomía, trópicos.

***Abstract***–The present paper provides a monograph of the *Petiolatum* Clade of *Elaphoglossum*, section *Lepidoglossa*. This group was recovered as a monophyletic in molecular phylogenetic analyses with chloroplast DNA. Morphologically, this clade is recognized by rhizome scales with acute to filiform-tortuous apex, scales on the proximal portion of the petiole with a dark apex, blades with resinous dots and flat and/or stellate scales, and spores with ridges and spines less than 1µm (except *E. petiolatum,* which has sparsely irregular surface microstructures). Eight species are recognized without subspecies or varieties. All the species of this clade are distributed in the American continent, from Mexico to Panama, with one species in the Greater Antilles. Most of the species occur between 1000–2500 m in pine-oak forests and cloud forests of Mexico. Presented here are an identification key, full synonymy, detailed descriptions, representative specimens examined, an index to collectors and numbers, illustrations, spore microphotographs, and distribution maps for all species. A new nomenclatural combination is made for *E. schmitzii*, lectotypes are designated for *E. potosianum* and *E. schmitzii*, a probable holotype is designated for *Acrostichum intermedium* and a second step lectotypification is made for *E. pringlei.*

**Keywords**.— floristics, ferns, lectotype, Mexico, morphology, nomenclature, pteridophytes, resinous dots, taxonomy, tropics.

*Elaphoglossum* Schott ex J.Sm. (Dryopteridaceae) is a genus of tropical ferns with more than 600 generally accepted species out of the more than 1000 that have been described (Rouhan 2020). The genus is mainly distributed in wet forests and cloud forests throughout the tropics, but it is most diverse in the American tropics, from Mexico to Argentina and in the Antilles, where approximately 75% of the species occur (Mickel and Atehortúa 1980, Mickel 1991, Moran 2018, Moran et al. 2007, PPG I 2016, IPNI 2022, Rouhan 2020).

*Elaphoglossum* is distinguished from other ferns by laminae that are almost always simple (six of the 600 species have divided laminae), dimorphic sterile, and fertile leaves, free veins, and acrostichoid sori. Most species are epiphytes, but terrestrial, hemiepiphytic, and epipetric are also known (Lagomarsino et al. 2012, Mickel 1995, Moran et al. 2010, Rouhan et al. 2008, Vasco et al. 2013, Rouhan 2020, Zots et al. 2021).

Phylogenetic molecular analyses have recovered *Elaphoglossum* sister to *Mickelia* R.C. Moran, Labiak & Sundue. Both generabelong to the Bolbitidoid clade of subfamily Elaphoglossoideae (Pic.Serm.) Crabbe of Dryopteridaceae, one of the largest fern families in the world, harboring about 1700 species (Hasebe et al. 1995, Rouhan et al. 2004, Skog et al. 2004, Liu et al. 2007, 2016, Schuettpelz and Pryer 2008, Moran et al. 2010a, Vasco et al. 2009b, Lóriga et al. 2014, PPGI 2016, Moran 2018). Within *Elaphoglossum,* seven main and major clades, which have been ranked as sections, are currently recognized: 1) *Elaphoglossum* sect. *Amygdalifolia* (Christ) Mickel and Atehortúa, 2) sect. *Wrigthiana* J.Lóriga, A.Vasco, L.Regalado, J.Heinrichs and R.C.Moran, 3) sect. *Elaphoglossum*, 4) sect. *Squamipedia* Mickel and Atehortúa, 5) sect. *Polytrichia* Christ, 6) sect. *Setosa* (Christ) Mickel and Atehortúa, and 7) sect. *Lepidoglossa* Christ, (Skog et al.. 2004, Rouhan et al. 2004, Lóriga et al. 2014).

Of these sections, the sect. *Lepidoglossa* is by far the most species-rich, containing more than 200 species (Vasco 2011). The section is characterized by laminae obviously scaly, stem and blade scales typically flat (not subulate), and scale margins with unicellular, acicular, non-glandular marginal cells (Mickel and Atehortúa 1980, Vasco 2009, Kessler et al. 2018). Based on morphology, the section was subdivided into six subsections differing mainly by the shape and density of scales, the shape of the lamina apices, and spore morphology (Mickel and Atehortúa, 1980). The subsections (subsect.) of *Lepidoglossa* are subsect. *Huacsaro* Mickel and Atehortúa, subsect. *Muscosa* Mickel and Atehortúa, subsect. *Microlepidea* Christ, subsect. *Petiolosa* Christ, subsect. *Pilosa* Christ, and subsect. *Polylepidea* Christ.

As part of his floristic work on *Elaphoglossum* in Mexico, Mickel (2001) described a group of about 11 species called the "*Elaphoglossum petiolatum* complex". Due to the species which gives the name to the complex, *Elaphoglossum petiolatum* (Sw.) Urb., was assigned by Mickel and Atehortúa (1980) to subsect. *Pilosa* is considered the whole complex belonging to this subsection. Subsection *Pilosa* was defined as having short-creeping stems, usually dark, entire, or with long projections rhizome scales, laminae with resinous dots or scaly to subglabrous, and spores with low ridges (Christ 1899, Mickel and Atehortúa 1980, Moran et al., 2007).

Defined as such, the *Elaphoglossum petiolatum* complex was considered pantropical, with three of the species endemic to Mexico (Table 1; Mickel, 2001). Previous molecular phylogenetic analyses, which did not include all the species in the *Elaphoglossum petiolatum* complex, suggested that the complex was not monophyletic (Rouhan et al. 2004, Vasco et al. 2009, Lóriga et al. 2014). Nevertheless, the Mexican species of the complex were recovered in a single clade, called the "Mexican-*petiolatum* clade" by Vasco et al. (2009).

Our molecular phylogenetic work (Martínez-Becerril et al., in press) tested the monophyly of this complex including a better representation of taxa belonging to the complex and increasing the sampling of Mexican species of the genus. That study corroborated that the *E. petiolatum* complex is not monophyletic and that the species originally described as part of the complex belonged to three non-sister clades. Those three are the *E. ciliatum* clade, the *E. lancifolium* clade, and the *E. petiolatum* clade (Martínez-Becerril et al., in press).

These three clades include other species not previously considered part of the *E. petiolatum* complex, and surprisingly, all the species of *Elaphoglossum* with resinous dots in their laminae and petiole were recovered in any of these clades. Resinous dots have been interpreted as the bases of reduced scales that remain embedded in the epidermis of the laminae

(Vasco et al., 2009a). These resinous dots are atypical within the sect. *Lepidoglossa,* however, they evolved independently multiple times during the evolutionary history of this section (Martínez-Becerril et al., in press.).

**Table 1.** Species belonging to the *Elaphoglossum petiolatum* complex *sensu* Mickel (2001). (*) indicates the species included in this analysis.

| Species | Distribution |
|---|---|
| *Elaphoglossum petiolatum* (Sw.) Urban | From Mexico to Peru and the West Indies |
| **E. salicifolium* (Willd. ex Kaulf.) Alston (ahora *E. lancifolium* (Desv.) C.V. Morton) | Bioko, Cameroon, Comoros, Congo, Gaba, Guinea, La Reunion, Liberia, Mauritius Island, Madagascar, Malawi, Mozambique, Rwanda, Sierra Leone, Seychelles, Tanzania, Zambia, Zimbabwe |
| **E. nervosum* (Bory) H. Christ | St. Helena Island |
| **E. dimorphum* (Hook. & Grev.) T. Moore | St. Helena Island |
| **E. bifurcatum* (Jacq.) Mickel | St. Helena Island |
| *E. stelligerum* (Wall. ex Baker) T. Moore ex Alston & Bonner | India |
| *E. yunnanense* (Baker) C. Chr. | China, India, Malaysia, Vietnam |
| **E. blumeanum* (Fee) J. Smith | Bismark Archipielago, Borneo, China, Indonesia, Philippines, Malasyia, Salomon Islands, Sumatra. |
| **E. rzedowskii Mickel* | Mexico |
| **E. pringlei* (Davenp.) C. Chr. | Mexico |
| **E. trichomidiatum* Mickel (ahora *E. potosianum* Christ) | Mexico |

Here we present the monograph of the *Elaphoglossum petiolatum* clade (hereafter *Petiolatum* Clade), which was called earlier the "Mexican-*petiolatum* clade" (Vasco et al. 2009). We rename it here because our analyses (Martínez-Becerril et al., in press) show that some species outside of

Mexico are also part of this clade; and importantly the namesake species of the clade, *E. petiolatum* has its type from Jamaica (Swartz 1778, Proctor 1985). We recognize and monograph eight species within this clade. Six of these species are included in the clade based on cpDNA sequence evidence (Martínez-Becerril et al., in press) and the other two were considered as a part of this group based on the morphology of the sporophyte and the spores.

Morphologically the species of the *Petiolatum* Clade have, besides resinous dots, stem scales with acute to filiform apices and the margins generally darker than the rest of the scale; echinate perispores with short spines less than 1µm high (except *E. petiolatum* which has concolorous stem scales and perispores broadly folded with sparsely irregular surface microstructures); and sometimes, petioles seem to develop from the ventral side of the rhizome (Mickel and Smith 2004, Vasco et al. 2009, Martínez-Becerril et al. 2021). Defined as such, the clade is distributed from Mexico to Panama, and in the Greater Antilles. Seven out of the eight species in the clade occur in Mexico, and four of them are endemic to that country (Table 2).

**Table 2.** Species belonging to the *Elaphoglossum petiolatum* clade.

| Species | Distribution |
|---|---|
| *Elaphoglossum auralolitae* Martínez-Becerril & A. Vasco | Guatemala |
| *E. muelleri* (E. Fourn.) C. Chr. | From Mexico to Honduras |
| *E. petiolatum* (Sw.) Urban | Jamaica, Dominican Republic, Costa Rica, Panama, Honduras, Nicaragua, Guatemala and Mexico. |
| *E. pringlei* (Davenp.) C. Chr. | Mexico |
| *E. potosianum* Christ | Mexico |
| *E. rzedowskii* Mickel | Mexico |
| *E. schiedei* (Kunze) T. Moore | Mexico and Guatemala |
| *E. schmitzii* (Mett. ex Khun) Martínez-Becerril & A. Vasco | Mexico |

## MATERIALS AND METHODS

This study is based on examination of over 2000 herbarium sheets representing about 809 gatherings deposited in 13 herbaria (Table 3). Also, digital specimens from virtual herbaria were examined (Table 3). The protologues and types of all the species of this group and their synonyms were checked, and all relevant literature was consulted (e.g., Mickel 1981, Proctor 1985, Mickel and Smith 2004).

For the descriptions and the key, the terminology of Lellinger (2002) was mainly used. Measurements, colors, and characteristics described are based on the herbarium specimens that were examined. The scales located in the middle of the stems, petioles, and laminae were the ones measured and used in the descriptions and key. The measurements of the plants, including the length and width of the laminae and the proportion of the petiole, were taken from a representative sample of approximately 20 dry individuals per species that reflected the intraspecific morphological variation. Once selected, the largest leaf of each specimen was the one measured. The stem scales describe here are those located in the middle of the stem; those that are found at the apex can be larger, which could confuse the reader in determining the specimens. The term “small projection” used to describe the scale's margins, refers to projections that size less than half of the scale width whereas the “long projections” size more than half of the scale width. The term trichomidia used to describe the indument mixed with the abaxial laminar scales, refers to small, reduced hairs found mainly on the petiole and lamina (Lellinger, 2002). The term “short spines” used to describe the persipore morphology, refers to those long and tapering pointed elements that size ≥1µm.

For most of the specimens, geographic coordinates were estimated using Google Earth (https://www.google.com/earth/) because this information was often not provided on the labels.

These coordinates were used to make dot distribution maps for the species and are given in brackets for the herbarium specimens cited below. Distribution maps (Fig. 1) were made using the Rstudio program (Racine 2012) after converting the coordinates from degrees to decimals. To show the elevation intervals of the species, a box-and-whisker plot (Fig. 2) was produced in RStudio using the elevation of all collections recorded in meters.

Illustrations and spore microphotographs (Fig. 3). were prepared from herbarium specimens for all species. Illustrations consist of plates made using photographs taken with a camera coupled to a LEICAM165C® microscope. Spores were examined and photographed following the methodology described by Moran et al. (2007) and using scanning electron microscopes (HITACHI SU1510 and HITACHI SU3600) at the Microscopy and Biodiversity Photograph Laboratory of the Biology Institute, UNAM, and in the Microscopy Laboratory of the Botanical Research Institute of Texas (BRIT), respectively. Fieldwork was carried out in the Mexican states of Aguascalientes, Chihuahua, Jalisco, Puebla, and San Luis Potosí.

For the designation of the lectotypes, and according to the International Code of Nomenclature for algae, fungi, and plants, were given priority those specimens from the original material that were cited in the protologues or that were part of the herbarium of the species' author. In the cases where these were not found, the specimens considered original material and that were in the best condition were chosen, that is, specimens that preserved and showed all the structures, and where the specimen labels coincided with the data stated in the protologues, for example, collector, collection-number, locality, and date of collection. Collections with a single gathering were assigned as holotypes (Turland et al., 2018, Chapter II, Section 2, Article 9).

**Table 3.** Acronym, institution, and country of all herbaria that were consulted in this study. (*) indicates those from which we examined specimens.

| Acronym | Institution | Country |
|---|---|---|
| ASU | Arizona State University | U.S.A., Arizona |
| B | Botanischer Garten und Botanisches Museum Berlin, Zentraleinrichtung der Freien Univeristät Berlin | Germany, Berlin |
| BIGU | Universidad de San Carlos de Guatemala | Guatemala, Guatemala City |
| BRIT* | Botanical Research Institute of Texas | U.S.A., Texas |
| CHAPA* | Colegio de Postgraduados | Mexico, State of México |
| CR | Museo Nacional de Costa Rica | Costa Rica, San José |
| ENCB* | Escuela Nacional de Ciencias Biológicas, IPN | Mexico, Mexico City |
| F* | Field Museum of Natural History | U.S.A., Chicago |
| GH | Harvard University | U.S.A., Massachusetts |
| H | University of Helsinki | Finland, Helsinki |
| HAJB | Jardín Botánico Nacional, Universidad de La Habana | Cuba, La Habana. |
| HAL | Martin-Luther-Universität | Germany, Halle |
| HUMO* | Universidad Autónoma del Estado de Morelos | Mexico, Morelos |
| IEB* | Instituto de Ecología A.C. | Mexico, Pátzcuaro |
| IND | Indiana University | U.S.A., Indiana |
| K | Royal Botanic Gardens | UK, England |
| LE | Komarov Botanical Institute of RAS | Russia, St. Petersburg |
| LP | Museo de La Plata | Argentina, Buenos Aires |
| MEXU* | Herbario Nacional de México, UNAM | Mexico, Mexico City |
| MICH | University of Michigan | U.S.A., Michigan |
| MO* | Missouri Botanical Garden | U.S.A., Missouri, Saint Louis |
| NCU | University of North Carolina at Chapel Hill | U.S.A., North Carolina |
| NY* | The New York Botanical Garden | U.S.A., New York |
| P* | Muséum National d'Histoire Naturelle | France, Paris |
| PMA | Universidad de Panamá | Panama, Panama |
| RB | Jardim Botânico do Rio de Janeiro | Brazil, Rio de Janeiro |
| S | Swedish Museum of Natural History | Sweden, Stokholm |
| SERBO | Sociedad para el Estudio de los Recursos Bióticos de Oaxaca, A. C. | Mexico, Oaxaca |
| TEFH | Universidad Nacional Autónoma de Honduras | Honduras, Tegucigalpa |

| | | |
|---|---|---|
| UCR* | University of California, Riverside | U.S.A., California |
| US* | Smithsonian Institution | U.S.A., Columbia |
| VT* | University of Vermont | U.S.A., Vermont |
| WIS | University of Wisconsin | U.S.A., Wisconsin |
| WTU | University of Washington | U.S.A., Washington |
| YPM-YU | Yale University | U.S.A., Connecticut. New Haven |
| Y | Yale University | U.S.A., Connecticut. New Haven |

## RESULTS

Eight species from Mexico to Central America and the West Indies are recognized in this treatment as a part of the *Petiolatum* Clade (Fig. 1). Four of these are endemic to Mexico. Most of the species occur between 1000–2500 m in pine-oak forests and mesophilic forests of Mexico. (Fig. 2). Two lectotypes and a probable holotype are designated for species in the group or their synonyms. No infraspecific taxa are recognized. A synopsis of the species distributions is given in Table 2. A list of collectors and their numbers for all examined specimens is given in Appendix I.

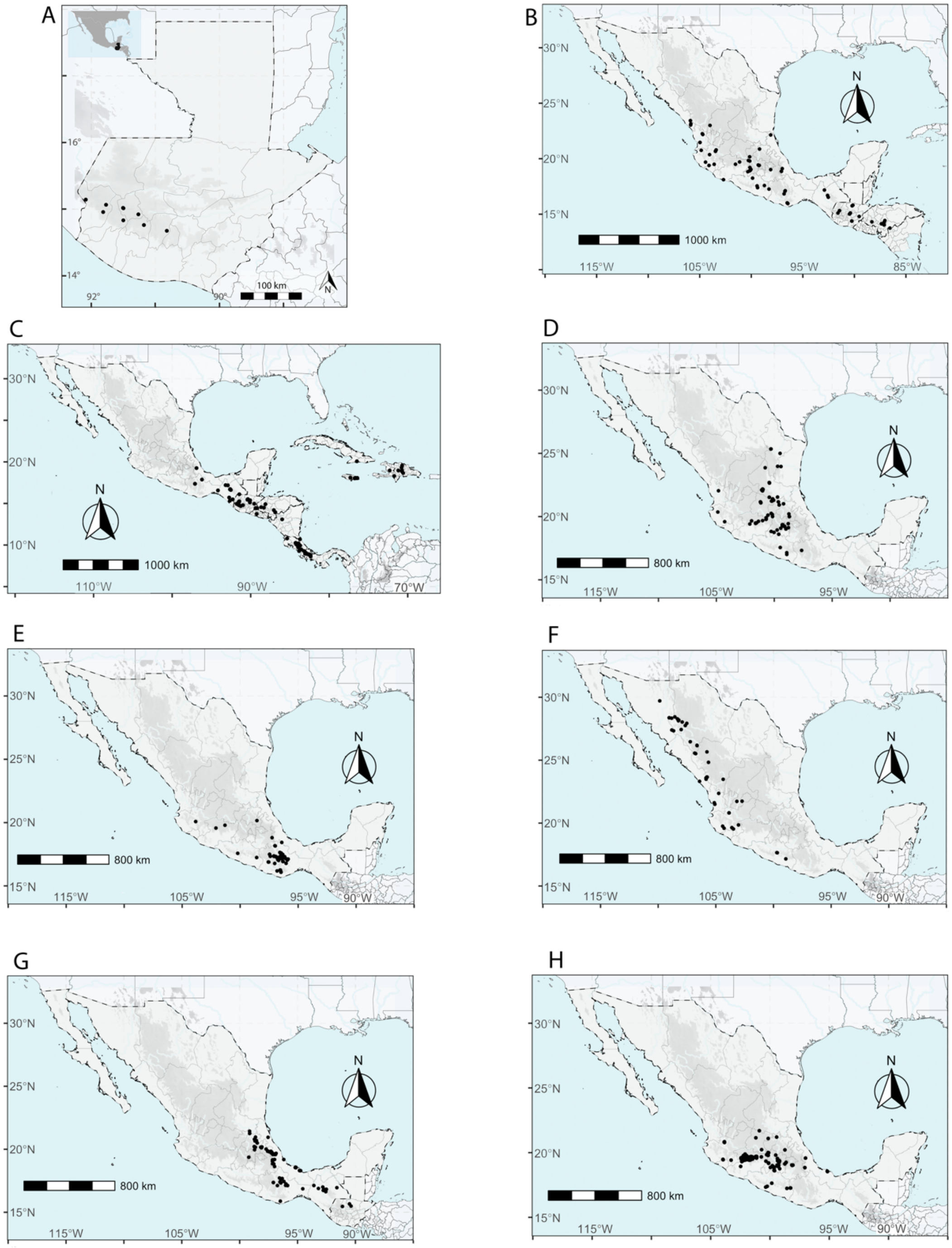

**Figure 1.** Distribution maps of all species in the *Elaphoglossum petiolatum* clade. A. *E. auralolitae,* B. *E. muelleri,* C. *E. petiolatum,* D. *E. potosianum,* E. *E. pringlei,* F. *E. rzedowskii,* G. *E. schiedei,* H. *E. schmitzii.*

## KEY TO THE SPECIES OF THE *PETIOLATUM* CLADE

1. Rhizome scales scattered, appressed, 0.7–1.2(–1.5) mm long, concolorous, oblanceolate, sclerotic, lustrous ……………………………….…………………………...… 7. *E. schiedei*

1. Rhizome scales dense, spreading, 1.4–5 mm long, bicolorous or concolorous, linear to lanceolate, no sclerotic, dull or lustrous.

2. Rhizome scales concolorous

3. Trichomidia absent on the abaxial laminar surface (use magnification)

4. Adaxial laminar scales absent or scattered to dense, not imbricate, when present ≤ 1mm long, with occasional short or long projections and attenuate apices; phyllopodia clearly visible; petiole scales scattered or dense mainly at the base; perispores broadly folded with sparsely irregular surface microstructures; plants epiphytic or terrestrial …………. ……………………………………………………………………………. 3. *E. petiolatum*

4. Adaxial laminar scales dense, usually imbricate, 0.5–1.4 mm long, with numerous long projections and long-attenuate to filiform apices; phyllopodia usually obscured by the rhizome scales; petiole scales dense throughout; echinate perispores; plants terrestrial …....…....……………………….....……………………………………..…. 1. *E. auralolitae*

3. Trichomidia present on the abaxial laminar surface (use magnification)

5. Abaxial laminar scales usually dense (only occasionally scattered), usually stellate or occasionally irregular; abaxial laminar trichomidia scattered (hard to see, even under a stereoscope); laminae narrowly elliptic …...…..………….……………..…. 2. *E. muelleri*

5. Abaxial laminar scales absent or scattered, irregular; abaxial laminar trichomidia dense (easy to see under a stereoscope); laminae linear-lanceolate to narrowly elliptic …….……… ……………………………………………………………………………4. *E. potosianum*

2. Rhizome scales bicolorous

6. Abaxial laminar scales dense, sometimes imbricate; phyllopodia usually obscured by the rhizome scales; rhizome scales with acuminate to long-attenuate apex; laminae linear to elliptic ……..………………………………………………..…...…. 6. *E. rzedowskii*

6. Abaxial laminar scales absent or scattered to dense, but never imbricate; phyllopodia clearly visible, not obscured by the rhizome scales; rhizome scales with attenuate to filiform-tortuouse apices (oftentimes broken); laminae narrowly elliptic or elliptic to lanceolate.

7. Laminae elliptic to lanceolate, the bases decurrent; abaxial laminar scales present, substellate, irregular or lanceolate; abaxial resinous dots absent or present ..................... 5.*E. pringlei*

7. Laminae narrowly elliptic, base cuneate; abaxial laminar scales absent or present, when present, linear-lanceolate; abaxial resinous dots present …..………..…… 8. *E. schmitzii*

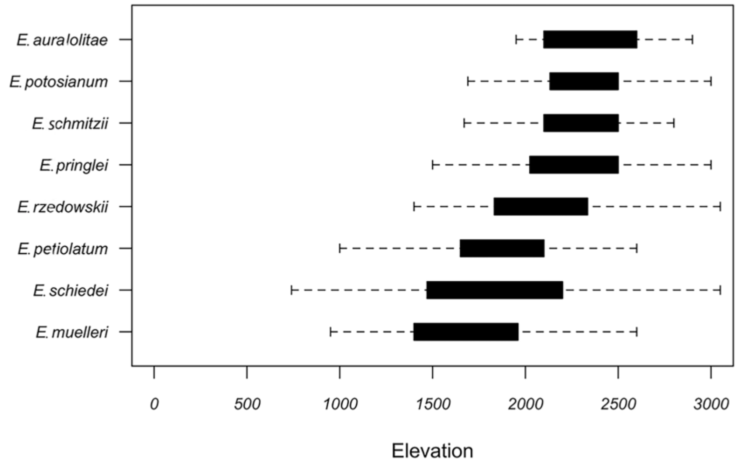

**Figure 2.** Boxplot depicting elevational ranges (in meters) of all species in the *Elaphoglossum petiolatum* clade.

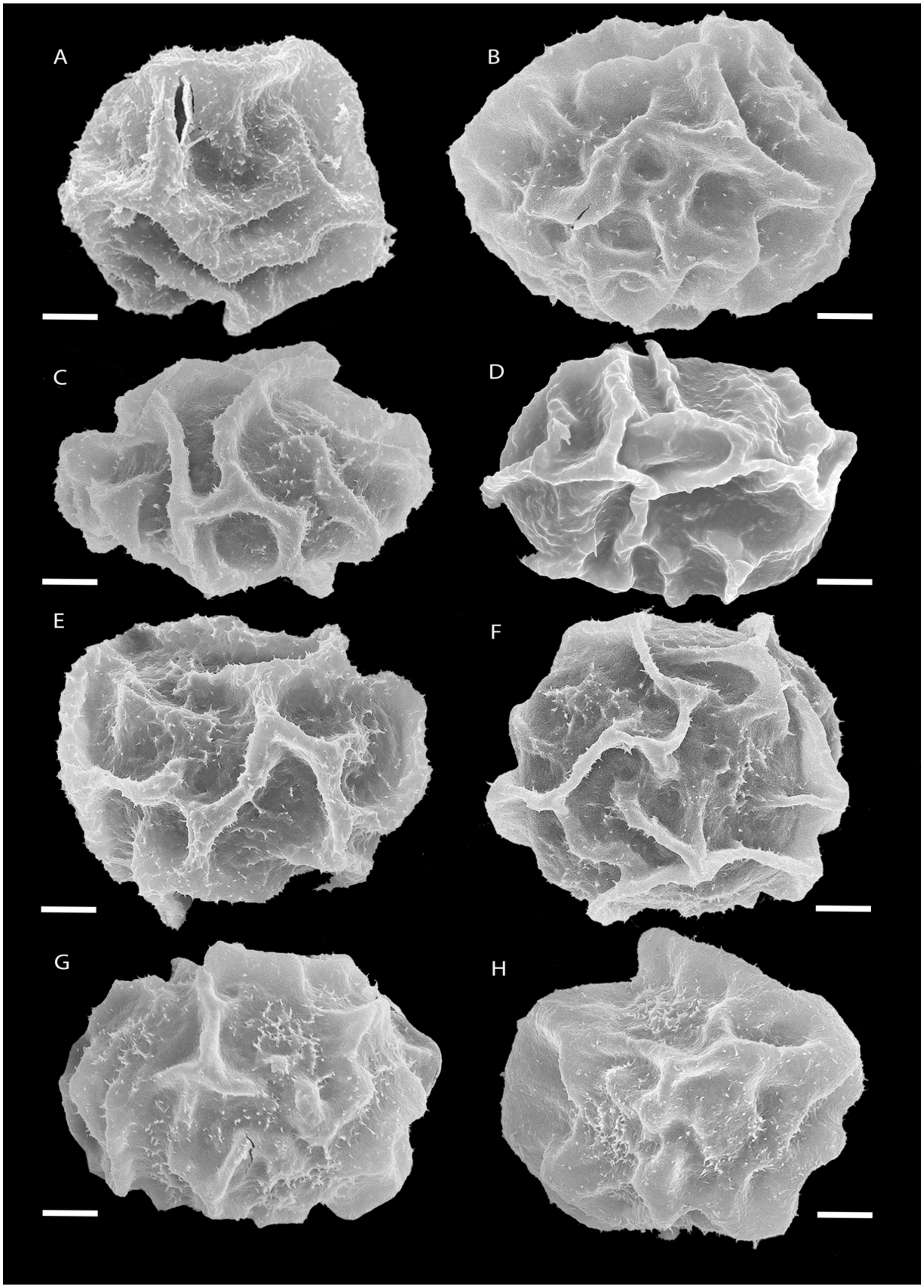

**Figure 3.** Spores of species of the *Elaphoglossum petiolatum* clade. A. *E. auralolitae* (*Standley 58933*, F). B. *E. muelleri* (*Anderson 13211*, MEXU), C. *E. potosianum* (*Martínez-Becerril 399*, FEZA), D. *E. petiolatum* (*Hellwig 440*, NY), E. *E. pringlei* (*Anderson 4778*, MEXU), F. *E. rzedowskii* (*Martínez-Becerril 408*, FEZA), G. *E. schiedei* (*Gallardo-Hernandez 836*, MEXU), H. *E. schmitzii* (*McVaugh 23885*, MEXU). Scale bar= 10µm.

## TAXONOMIC TREATMENT

**1.** ***Elaphoglossum auralolitae*** **Martínez-Becerril & A. Vasco. Here would be the publication cite.** Type: Guatemala. San Marcos: San Pedro Sac. [Sacatepéquez], 2300m, [14°57'22.22"N, 91°48'54.27"W], 19 Aug 1992, *M. Véliz 92.2319* (holotype: MEXU 903674, isotypes: BIGU 0030067).

**Plants** Terrestrial. **Rhizomes** 3–3.5 mm wide, short creeping; **rhizome scales** 2.5–4 mm long, dense, spreading, linear-lanceolate to lanceolate, entire or with occasional short projections (shorter than the width of the scale), concolorous, dark-reddish brown to dark brown, lustrous, base round, apex attenuate to filiform-tortuouse. **Sterile leaves** 13.5–39.5(–45) cm long, approximate; **phyllopodia** present, usually obscured by the rhizome scales, 5–10 mm long; **petiole** 5–15.5(–18) cm long, 1/3–2/5 the length of the sterile leaves; **larger petiole scales** to 3.5(–4) mm long, dense, usually imbricate, spreading to appressed, linear-lanceolate to ovate, with occasional short projections, stramineous to brown, usually darker at the apex, base round, apex attenuate to filiform-tortuouse; **smaller petiole scales** to 0.3 mm long (difficult to see), dense, appressed, irregular or lanceolate, with numerous long projections (as long or longer than the width of the scale) or with occasional short projections, stramineous, base auriculate, apex attenuate, usually with a long projection close to the apex; **laminae** 8.5–24(–27) × 1.2–2.4 cm, linear-elliptic to elliptic, chartaceous, base cuneate, apex acute to acuminate; **adaxial laminar scales** 0.5–1.4 mm long, dense, usually imbricate, lanceolate, with numerous long projections, stramineous to whitish, darker at the point of attachment, base auriculate, apex long-attenuate to filiform, **adaxial resinous dots** absent; **abaxial laminar scales** to 0.5 mm long, scattered to dense, not imbricated, irregular to lanceolate, when lanceolate, with numerous long projections, stramineous to light brown, base auriculate, apex attenuate; **abaxial resinous dots** present; **abaxial costal scales** 0.8–2(–3) mm long, dense, usually imbricate, lanceolate with occasional short projections, stramineous, base round with long projections, apex long-attenuate to filiform; **marginal scales** 0.5–0.8(–1) mm long, dense, not imbricate, lanceolate or irregular, when lanceolate, margin with numerous long projections, stramineous, base truncate with long projections, apex attenuate to filiform-tortuouse. **Fertile leaves** similar or sometimes longer, than the sterile; **petiole** 1/3–3/5 the length of the fertile leaves; **laminae** 28.9–36.2 × 0.9–1 cm, linear-elliptic to elliptic, base cuneate, apex acute, apiculate; **petiole scales** 2.0–3.1 mm long, scattered to dense, not imbricated, deciduous, lanceolate to oblanceolate, with numerous long projections or with occasional short projections, stramineous, base round with long projections or truncate, apex long-attenuate to filiform; **adaxial scales** 1–1.5 mm long, dense, usually imbricate, lanceolate with numerous long projections, stramineous, base auriculate, apex long-attenuate to filiform; **abaxial costal scales** 0.5–1.5 mm long, scattered to dense, not imbricate, deciduous, lanceolate to ovate-lanceolate or irregular, with numerous long projections, yellowish, base truncate with long projections, apex long-attenuate to filiform; **intersporangial scales** not visible. **Perispores** broadly folded, echinate with short spines. Figures 3A and 4.

*Distribution.*—Known only from the Sierra Madre in Guatemala, departments of Chimaltenago, Quetzaltenango, Quiché, San Marcos, Sacatepéquez, Sololá and Totonicapán. Figure 1A.

*Elevation*—1950–2900 m (Fig. 2)

*Etymology.*—This species is named for Aura Lolita Chávez Ixcaquic (1972– ), indigenous feminist from the K'iche' region and community in Guatemala, who is the leader of the "Council of K'iche's People for the Defense of Life, Nature, Land, and Territory of Guatemala". She was nominated for the "Sájarov for Freedom and Conscience Award" by the European Parliament and the winner of the "Ignacio Ellacuría Award" by the Vasque Agency for Development Cooperation (https://mujeresbacanas.com/aura-lolita-chavez-ixcaquic-1972/).

*Notes—Elaphoglossum auralolitae* resembles *E. petiolatum* by linear-lanceolate to lanceolate, concolorous, dark-reddish brown to dark brown rhizome scales with filiform-tortuose apices and resinous dots on the laminae abaxially; however, they differ in several important respects. The sterile leaves of *E. auralolitae* have adaxial laminar scales that are 0.5–1.4 mm long, dense, usually imbricate with many long projections along the margin, and with long-attenuate to filiform apices. The smallest scales on the costae abaxially are 0.8 mm long, dense, usually imbricate, and lanceolate. In contrast, in the sterile leaves of *E. petiolatum* the adaxial laminar scales are not always present, but when present, they are ≤1 mm long, scattered to dense, not imbricate, with occasional short or long projections along the margin, and attenuate apices. Also, its smallest scales on the costae abaxially are 0.5 mm long, scattered to dense, not imbricate, and lanceolate, substellate, or irregular. There are also differences among the fertile leaves of these two species: in *E. auralolitae,* the petiole scales are 2–3.3 mm long, lanceolate to oblanceolate, and with numerous long projections along the margin. The adaxial laminar scales are 1–1.5 mm long, dense, and usually imbricate; and the costal abaxial scales are up to 1.5 mm long with long attenuate to filiform apex. In contrast, the fertile leaves of *E. petiolatum* have petiole scales ≤1 mm long, substellate or linear to linear-lanceolate, and with occasional short projections along the margin when the scales are lanceolate; the adaxial laminar scales are ≤0.7 mm long, scattered to dense, and not imbricated; and the costal abaxial scales are up to 0.8 mm long with acuminate to attenuate apex. Finally, *E. auralolitae* has echinate perispores with spines

≤1 μm long, whereas *E. petiolatum* has perispores with broadly folded with sparsely irregular surface microstructures (Fig. 3A). Both species overlap in the distribution range of *E. auralolitae*, and some specimens of *E. petiolatum* from Guatemala are exceptionally scaly and could be easily confused with *E. auralolitae*. However, the characters above are sufficient to distinguished both species.

*Elaphoglossum auralolitae* also resembles *E. rubescens* Christ, a species that also occurs in Guatemala and has a scaly laminae but does not belong to the *Petiolatum* Clade. *Elaphoglossum auralolitae* differs by the presence of resinous dots in the abaxial laminar surface, by the dense, usually imbricated petiole scales, and by adaxial laminar scales that are dense and usually imbricate, lanceolate, and with numerous long projections. In contrast, *E. rubescens* lacks resinous dots on the abaxial laminar surface, has scattered petiole scales, and has adaxial laminar scales absent or dense, not imbricated, round to subdeltate, and with short projections. The shape and color of the abaxial laminar scales also differ between the two species, being irregular to lanceolate and stramineous to light brown in *E. auralolitae* and narrow to deltate and orange in *E. rubescens*.

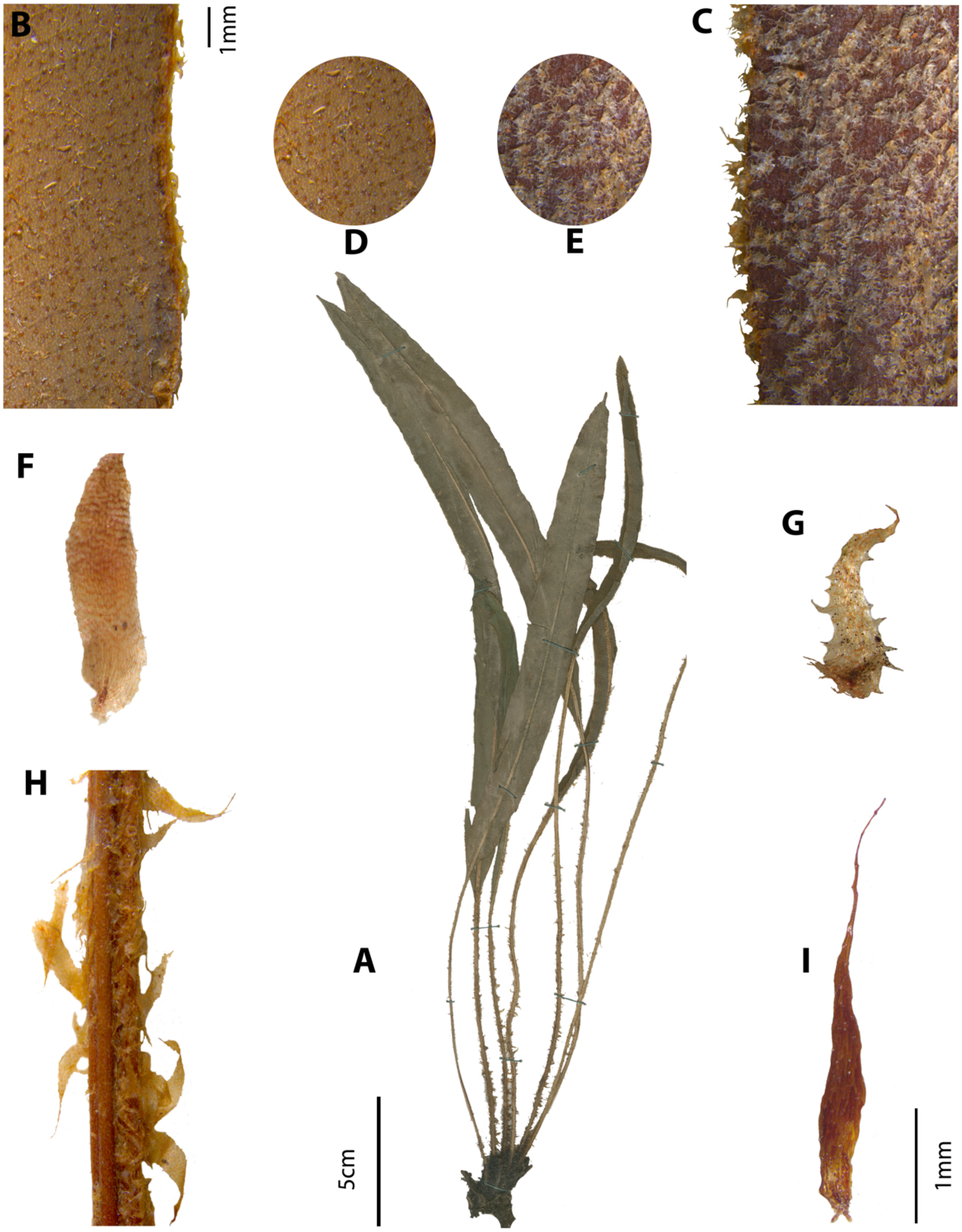

**Figure 4.** *Elaphoglossum auralolitae*. A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A from *Véliz 92.2319,* MEXU; B–J from *Molina 30311,* MO).

**2. Elaphoglossum muelleri (E. Fourn.) C. Chr.,** Index Filic. p. 311. 1905. *Acrostichum muelleri* E. Fourn., Mexic. Pl. 1: 68. 1872. Type: México. Veracruz: Mpio. Orizaba Uluapa, [1560 m], [18°51'44.62"N, 97° 5'12.70"W], 3 Aug 1853, *F. Müller 41 quinter* (holotype: P 00603618 [image!]).

*Acrostichum intermedium* Feé, Mém. Foug. 8: 69. 1857, *non Elaphoglossum intermedium* Brack., 1854. Type: México. Veracruz: prope Huatuzco, Cordova en terre [in protologue: sur la terre], [1370 m], [19° 6'18.97"N, 96°59'39.68"W], Jul 1854, *W. Schaffner 22* (probable holotype: RB 00543157 [image!]).

*Elaphoglossum araneosum* (D.C. Eaton) C. Chr. Index Filic. p. 303. 1905. *Acrostichum araneosum* D.C. Eaton, Proc. Amer. Acad. Arts 22: 461. 1887. Type: México. Jalisco: Río Blanco: in wet shady spots in ravines, Jun-Oct [in the protologue: Aug], [1530 m], [20°46'43.15"N, 103°23'46.16"W], 1886, *E. Palmer 333* (lectotype inadvertently designated by Mickel and Beitel, 1988: YU YU.000790 [image!], isolectotypes: GH 00102874 [image!], NY 00149579!, 00149581!, US 00067262!, 01100828!).

**Plants** terrestrial**. Rhizomes** 4–5(–10) mm wide, short creeping; **rhizome scales** (2–) 3–5 mm long, dense, spreading, lanceolate, with occasional short projections (shorter than the width of the scale), concolorous, orange to light brown, darker at the point of attachment, dull, base round, apex long-attenuate. **Sterile leaves** (7.5–) 14–31 (–40) cm long, approximate; **phyllopodia** present, usually obscured by the rhizome scales, ca. 5 (–15) mm long; **petioles** (2–) 4–10.5 (–15.5) cm long, 1/5–1/3 (–1/2) the length of the sterile leaves; **larger petiole scales** to 4 mm long, scattered to dense, not imbricate, spreading to appressed, linear-lanceolate to lanceolate, with numerous long projections (as long or longer than the width of the scale), stramineous, base truncate, apex attenuate to filiform; **smaller petiole scales** to 1.5 mm long, dense, not imbricate, mostly appressed, irregular, substellate or lanceolate, when lanceolate, margin with occasional long projections, stramineous, base auriculate, apex attenuate to filiform; **laminae** (3.5–) 6–21 x (0.7–) 1–2 cm; narrowly elliptic, papyraceous, base cuneate to decurrent, apex obtuse or acute, sometimes apiculate*;* **adaxial laminar scales** to 1mm long, dense, not imbricate, deciduous, linear-lanceolate, with numerous long projections, whitish to stramineous, darker at the point of attachment, base auriculate, apex long-filiform; **adaxial resinous dots** absent; **abaxial laminar scales** mixed with 2 (–3) armed reddish trichomidia ca. 0.1 mm long, the scales to 0.7 mm long, scattered to dense, not imbricate, deciduous, stellate to irregular, whitish to stramineous; **abaxial resinous dots** usually present; **abaxial costal scales** 0.5–1 mm long, dense, not imbricate, lanceolate with numerous long projections, stramineous, darker at the point of attachment, base auriculate, apex acuminate to filiform; **marginal scales** (0.5)–1 mm long, dense, usually imbricate, lanceolate to ovate-lanceolate, with numerous long projections, stramineous to yellowish, base truncate with long projections, apex long-attenuate to filiform, sometimes with a long projection close to the apex. **Fertile leaves** longer than the sterile; **petioles** 1/2– 2/3 the length of the fertile leaves; **laminae** (5–) 10–15.5 (–18) x 0.7–0.9 (–1.2) cm, linear-elliptic, papyraceous, base long-cuneate, apex round or acute, sometimes apiculate; **petiole scales** 0.5–2 mm long, scattered to dense, not imbricate, linear-lanceolate to lanceolate*,* with numerous long projections, stramineous, base truncate, apex attenuate to filiform; **adaxial indument** not seen; **abaxial costal scales** to 2 mm long, scattered to dense, not imbricate, deciduous, linear-lanceolate, with numerous long projections, whitish to stramineous, darker at the point of attachment, apex attenuate or filiform; **intersporangial scales** not visible. **Perispores** broadly folded, echinate with short spines and ridges. Figure 3B and 5.

*Distribution*—Mexico (Chis, CDMX, Col, Dgo, Edo. Mex., Gro, Jal, Mich, Nay, Oax, Pue, Sin, Ver), El Salvador, Guatemala, Honduras, and Nicaragua (Fig. 1B). Although *E. muelleri* was reported by Mickel and Smith (2004) for the Mexican states of Guanajuato and San Luis Potosí, as well as for Costa Rica and Panama, we did not find collections of this species from these areas. Mexican collections *Ventura & López 7446* (MEXU) and *Schaffner sn* (NY) from Guanajuato and San Luis Potosí, respectively, correspond to *E. potosianum.*

*Elevation*—(960–) 1300–2600 m (Fis. 2).

*Etymology*—This species was named in honor of Fritz Müller [Johan Friederich Theodor] (1822–1897). German zoologist and botanist who also studied medicine, and that was appointed as a "traveling naturalist" by the Brazilian government. His collections are at B, G, K, and NY (TL-2).

*Notes—Elaphoglossum muelleri* can be distinguished from all the other species in the *Petiolatum* Clade by the largest rhizome scales 3–5 mm long, scalier lamina margins (the marginal scales sometimes deciduous), and by the presence of trichomidia on the abaxial laminar surface.

*Elaphoglossum muelleri* can be confused with *E. potosianum* because both have trichomidia on the abaxial laminar surfaces and tend to have orange to light-brown lamina scales (some specimens of *E. potosianum* can have dark brown scales). These two species can be distinguished by the rhizome scales, the abaxial laminar scales, and trichomidia length. In *E. muelleri* the rhizome scales are (2–)3–5 mm long, and always orange to light brown; the abaxial laminar scales are scattered to dense, not imbricate, deciduous, stellate to irregular; and the trichomidia ca. 0.1 mm and inconspicuous even with a stereomicroscope. In contrast, in *E. potosianum* the rhizome scales are 2–3.5 mm long, orange or light brown to dark brown; the abaxial laminar scales are absent or scattered and irregular; and the trichomidia are 0.2–0.3 mm and easy to see with a magnifying glass or stereomicroscope.

Some collections were not considered for the description because they show small variations outside of our typical concept of *E. muelleri.* For example: *Goldsmith 41* (F, MO, NY, US) from Colima (Mexico) has brown rather than typical orange to light-brown rhizome scales; *Rzedowskii 24310* (NY) from the State of Mexico (Mexico) does not present stellate scales on the abaxial lamina surface, some leaves just present a few substellate scales; *Croat 46092* (MO) from Oaxaca (Mexico) has longer leaves than those typical of this species; *Seiler 31* (F) from El Salvador has rhizome scales with long projections instead of short projections; *Hernández 99* (MO) from Honduras is the only specimen collected below 1000 m (all the other specimens have been collected at 1000 m or above), *Martin 61* (MO) and *Berrios 97* (MO) also from Honduras, are smaller than typical *E. muelleri.* Both specimens are sterile specimens and thus probably not yet fully grown.

*Müller 41 quinter* is considered a holotype because there are no duplicates (Turland et al., 2018, Chapter II, Section 2, Article 9). *Müller 41* ex parte NY (003231439!) is not considered an isolectotype of *E. muelleri,* despite it has the same collector and collection number, because the locality and date on the label do not correspond to those in the protologue. We neither consider *Müller 41* ex parte, NY (03231440!) as a part of the original material because this specimen represents *E. schiedei*, and the specimen label suggests it was collected in 1855 and not in 1853 as the original material of *E. muelleri*.

*Pringle 2590* (B) has an anonymous note stating it is an isotype of *Acrostichum araneosum*, however, the collectors, collection date, and locality are different from those mentioned in the protologue. Therefore, it could not be an isotype or an isolectotype for this name (https://www.jacq.org/detail.php?ID=938981).

Lectotypification for *Acrostichum intermedium* was made inadvertently by Mickel and Beitel (1988) who cited *Schaffner 22* from P. However, we did not find this specimen among the material we examined, nor did Dr. Germinal Rouhan (pers. comm.) find it at P. There is, however, a specimen of *Schaffner 22* at P (P00255156) and it belongs to *Kyllinga pumila* Michx. (Cyperaceae). We also asked for the specimen *Schaffner 22* at STR thinking it could be there because Antonie Laurent Apollinaire Fée, the author of this species, deposited his types at BR, P, and STR (TL-II). Nevertheless, it is not there either. We have been able to find the only specimen from *Schaffner 22* in BR, for that reason, we cited this as the probable holotype.

There is no combination of *Acrostichum intermedium* Feé for *Elaphoglossum*, and we could not make one with the same epithet, because there is already a valid species from Brazil with that name –*Elaphoglossum intermedium* Brack (Brackenridge, 1854). Because we are considering *A. intermedium* a synonym of *E. muelleri* we are not making any combination for this name (Turland et al., 2018, Chapter II, Section 3, Article 11).

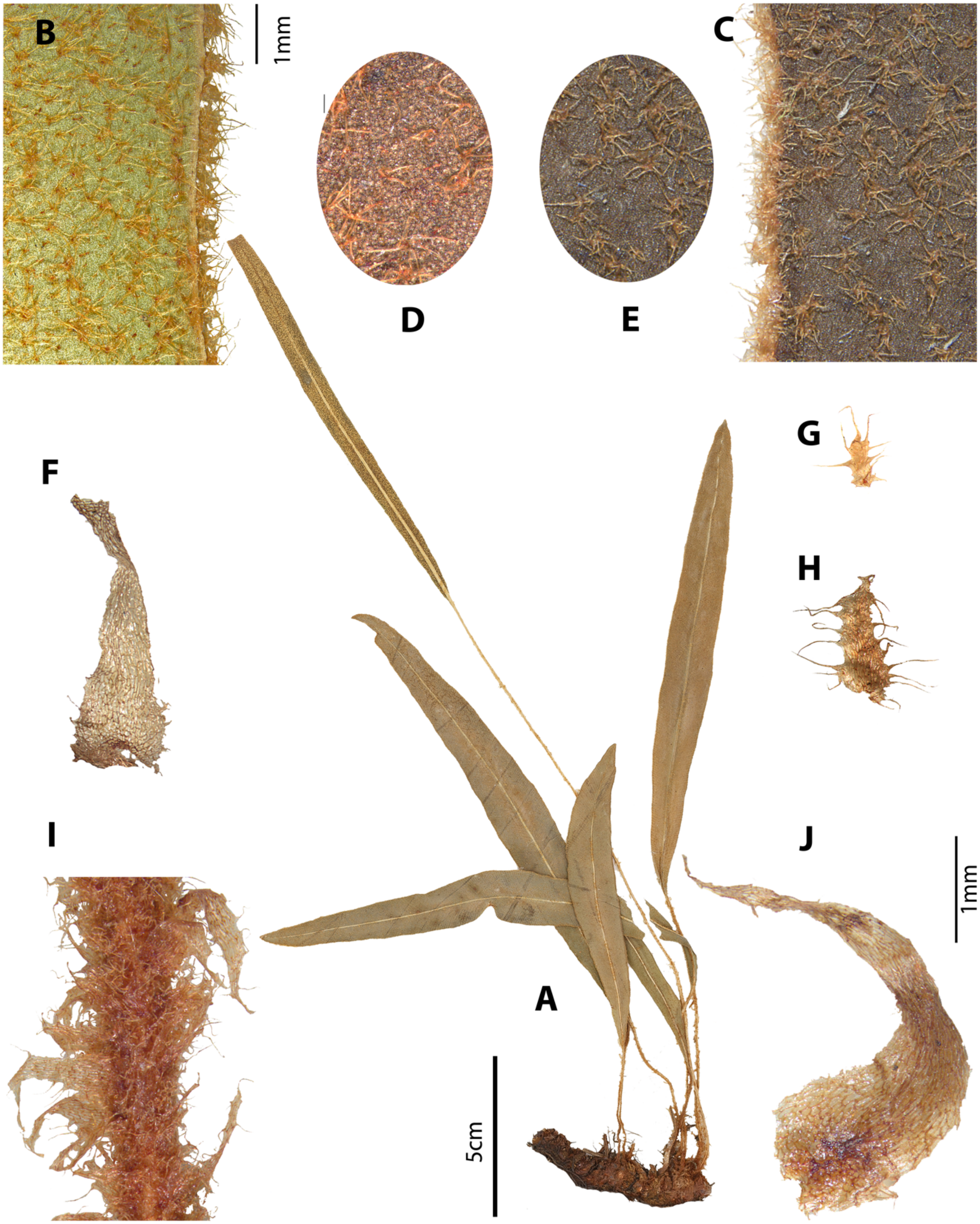

**Figure 5.** *Elaphoglossum muelleri.* A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A from *Mickel 1164*, NY; B, F–H from *Breedlove 17056*, NY; C–E, J from *Müller 41,* NY; I from *Pringle 2590,* NY).

**3. Elaphoglossum petiolatum (Sw.) Urb.,** Symb. Antill. 4: 61. 1903. *Acrostichum petiolatum* Sw. Prodr. 128. 1788. *Acrostichum viscosum* Sw., Syn. Fil. 10: 193. 1806. *Elaphoglossum viscosum* (Sw.) J. Sm., J. Bot. (Hooker) 4: 61. 1842 [1841]. Type: Jamaica. In 1778, *Swartz s.n.* (lectotype, inadvertently designated by Proctor, 1985: S, No. S-R-73 [image!], isolectotype: BM 000592488 [image!]).

**Plants** Terrestrial or epiphyte**. Rhizomes** 2–5 mm wide, short creeping; **rhizome scales** 1.5–3.7 (–5) mm long, dense, spreading, linear to lanceolate, with occasional short projections (shorter than the width of the scale), concolorous, dark-reddish brown to dark brown, dull, base truncate, apex filiform-tortuouse, oftentimes broken. **Sterile leaves** (9.5–) 10–36.5 (–43.5) cm long, approximate; **phyllopodia** present, clearly visible, 5–18 mm long; **petioles** 3–15 cm long, (1/6–) 1/4–1/3 (–2/5) the length of the sterile leaves; **larger petiole scales** to 3.5 mm long, scattered, denser at the base, not imbricate, spreading to appressed, linear to linear-lanceolate, entire or with occasional short projections, stramineous, sometimes darker at the apex, base round, apex long-attenuate to filiform-tortuouse, sometimes longer than the scale body; **smaller petiole scales** to 1 mm long, scattered to dense, usually imbricate, mostly appressed, irregular, substellate or lanceolate, when lanceolate, margin with occasional short or long projections (as long or longer than the width of the scale), stramineous, base round or auriculate, sometimes resinous at the point of attachment, apex acuminate; **laminae** 6.5–30 x (0.6–) 0.8–2 (–2.3) cm; elliptic, chartaceous, base cuneate, apex acute to acuminate, usually apiculate*;* **adaxial laminar scales** when present to 1mm long, absent or scattered to dense, not imbricate, lanceolate, with occasional short or long projections, whitish to yellowish, sometimes darker at the point of attachment, base auriculate, apex attenuate; **adaxial resinous dots** sometimes present; **abaxial laminar scales** when present to 1 mm long, usually absent, when present scattered, lanceolate, with numerous long projections, yellowish, base truncate with long projections, apex attenuate to long-attenuate; **abaxial resinous dots** present; **abaxial costal scales** 0.5–1.5 mm long, scattered to dense, not imbricate, lanceolate, substellate, or irregular when lanceolate, margin with occasional short and/or long projections, stramineous, base, when lanceolate, truncate or auriculate, apex acuminate to attenuate; **marginal scales** to 0.8 mm long, absent or scattered to dense, not imbricate, irregular or lanceolate, when lanceolate, margin with numerous long projections and occasionally also with short projections, whitish to yellowish, when lanceolate, base auriculate, apex long-attenuate, sometimes with a long projection close to the apex. **Fertile leaves** slightly shorter to longer than the sterile; **petioles** (2/5–) 3/5–1/2 the length of the fertile leaves; **laminae** (8.5–) 10–16.5 (–20.5) x 0.6–1 cm, elliptic, chartaceous, base cuneate to decurrent, apex acute or obtuse; **petiole scales** to 1mm long, scattered to dense, not imbricate, linear to linear-lanceolate or substellate*,* when linear to linear-lanceolate, margin with occasional short projections, yellowish to light brown, darker towards the apex, base round, apex long-attenuate to filiform-tortuouse; **adaxial scales** to 0.7 mm long, scattered to dense, not imbricate, linear-lanceolate to lanceolate or substellate when linear-lanceolate to lanceolate, margin with numerous long projections or with occasional short projections*,* whitish to light brown, base truncate, apex attenuate to long-attenuate; **abaxial costal scales** to 0.8 mm long, scattered, linear to lanceolate or substellate, when linear or lanceolate, margin with occasional short projections, yellowish, base auriculate, apex acuminate to attenuate; **intersporangial scales** not visible. **Perispores** broadly folded with sparsely irregular surface microstructures. Figures 3C and 6.

*Distribution*—Mexico (Chis, Gro, Oax, Ver), Costa Rica, Cuba, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Nicaragua, Panama, and Dominican Republic (Fig. 1C). Although *E. petiolatum* has been reported for Cuba (Eaton 1860, Mickel and Smith 2004), we were able to find only one collection of this species (*Sánchez C. y Morejón R. s.n.,* HAJB). Most of the other reports of *E. petiolatum* from Cuba are based on misidentified specimens that mostly correspond to *E. dussii* Underw. & Maxon. and *E. palmerii* Underw. & Maxon (Lóriga, com. pers). *Elaphoglossum petiolatum* was reported as occurring widely from Mexico to Venezuela (Mickel and Smith, 2004); however, most of the Mexican collections cited correspond to other species of the. *Petiolatum* Clade. As defined here, *E. petiolatum* is absent from South America, (Martínez-Becerril et al., in press, and see below).

*Elevation–* (847–) 900–2600 m (Fig. 2).

*Etymology*—The specific epithet apparently refers to the long and conspicuous petioles of the type.

*Notes*—*Elaphoglossum petiolatum* has been the most commonly used name specimens with resinous dots and dark rhizome scales in Mexico and Central America. This is the only species in the clade that can have both terrestrial or epiphytic habit and spores with perispores broadly folded with sparsely irregular surface microstructures. All other species of the *Petiolatum* Clade are always terrestrial and have echinate spores with small spines (Fig. 3). Another characteristic that distinguishes *E. petiolatum* from most species of the clade is the presence of non-shiny resinous dots on the abaxial laminar surface. In all other species with resinous dots, the dots are shiny. These non-shiny dots can sometimes be mixed with the typical shiny ones and are most common in specimens from Central America. The non-shiny resinous dots look dull in herbarium specimens and can easily be mistaken for peltate scales if not viewed under a stereoscope or light microscope. They tend to come off easily if touched with a needle, unlike shiny dots that remain attached.

*Elaphoglossum petiolatum* can be confused with *E. auralolitae, E. pringlei*, *E. schiedei*, and *E. schmitzii*, mainly because of their overlapping distributions and presence of resinous dots on the abaxial laminar surfaces, however, rhizome scales and the presence or absence of resinous dots on the adaxial laminar surface help distinguish them. *Elaphoglossum petiolatum* can be distinguished from *E. pringlei* by rhizome scales 1.5–3.7 (–5) mm long that are concolorous and dark-reddish brown to dark brown. In contrast, *E. pringlei* has rhizome scales 1.4–2.5 (–3.5) mm long that are bicolorous, light brown, and darker at the point of attachment. Also, *E. petiolatum* often has resinous dots on the adaxial laminar surface, whereas *E. pringlei* does not.

*Elaphoglossum petiolatum* can be distinguished from *E. schiedei*, by its longer rhizome scales 1.5–3.7 (–5) mm, which are dense, spreading, linear to lanceolate, dull, not sclerotic, and with a filiform-tortuouse apex. In contrast, *E. schiedei* has rhizome scales 0.7–1.2 (–1.5) mm, which are scattered, appressed, oblanceolate, reddish-brown to dark brown, lustrous, sclerotic, and with an acute to short-filiform apex. Also, *E. petiolatum* often has resinous dots on the adaxial laminar surface, whereas *E. schiedei* does not.

*Elaphoglossum petiolatum* and *E. schmitzii* are alike by the presence of resinous dots on the laminae adaxially, but they can be distinguished by their rhizome scales. In *E. petiolatum,* these are concolorous, dark-reddish brown to dark brown, and with a filiform-tortuouse apex. In contrast, *E. schmitzii* they are bicolorous, light brown only darker at the point of attachment and towards the apex, and with an attenuate to filiform apex.

See *Elaphoglossum auralolitae* for a comparison with that species.

*Elaphoglossum petiolatum* has the greatest intraspecific morphological variation of all the species of the *Petiolatum* Clade. It varies in habit, leaf size, shape and color of the rhizome scales, density of the laminar scales, and density and type (shiny and non-shiny) of the laminar resinous dots. For example, many collections from Guatemala have lighter or bicolorous rhizome scales with darker margins and apex, and their petioles, laminae, and costae tend to be more scaly on the adaxial surface than the typical *E. petiolatum*. Those can be confused with *E. auralolitae,* but see this species for comparison. Most of the Central American specimens are epiphytes with ascending rhizomes and, in general, are smaller compared to the Mexican and the Antillean representatives. Specimens from Jamaica and the Dominican Republic usually have resinous dots on both laminar surfaces.

Another variation is represented by *Mickel 9677* (NY) from Oaxaca. It has glabrescent rhizomes; however, unlike *E. schiedei* which also has glabrescent rhizomes, the few scales on the rhizome and petioles have the typical filiform-tortuous apex characteristic of *E. petiolatum*. Another variation is represented by *Molina 26627* (MO) from Guatemala, which has larger, ovate costal scales up to 2 mm long (*vs.* lanceolate, substellar, or irregular, 0.5–1.5 mm costal scales in most specimens). Further variation is found in *Seiler 698* (NY) from El Salvador has substellate and lanceolate scales with short projections on the abaxial laminar surface, in addition to the typical resinous dots. A specimen from Nicaragua (*Standley 10011*, F) varies by lighter rhizome scales that are quite unlike the typical dark-reddish brown to dark brown.

*Elaphoglossum petiolatum* is considered to have extensive morphological variation and be widely distributed in tropical America, Asia, and Africa (Mickel, 2001). A study of this variation (Mickel, 2001) concluded that the name *E. petiolatum* should be used only for specimens from the American tropics, and specimens from Southeast Asia, Africa, and India should be assigned to, respectively, *E. blumeanum* (Fée) J. Sm., *E. yunnanense* (Baker) C. Chr., *E. lancifolium* (Desv.) C.V. Morton (antes *E. salicifolium* (Willd. ex Kaulf.) Alston)*,* and *E. stelligerum* (Wall. ex Baker in Hook. & Baker) T. Moore ex Alston & Bonner (Mickel, 2001).

Two varietal names were described in part to represent the variation within *Elaphoglossum petiolatum: E. petiolatum* (Sw.) Urb. var. *dussii* (Underw. ex Maxon) Proctor and *E. petiolatum* var. *salicifolium* (Willd. ex Kaulf.) C. Chr., (Cristensen 1932, Proctor 1977). Morphological and phylogenetic analyses have shown that neither *E. petiolatum* var. *dussii* (= *E. dussii* Underw. & Maxon) nor *E. petiolatum* var. *salicifolium* (=*E. lancifolium*) are part of the *Petiolatum* Clade but they are part to the *E. ciliatum* and *E. lancifolium* clades respectively (Vasco et al 2009b, Martínez-Becerril et al., in press). The name of *E. petiolatum* only applies to specimens from Mexico (Oaxaca and Chiapas), Central America (from Guatemala to Panama), and the Greater Antilles (Cuba and Jamaica) (Martínez-Becerril et al., in press).

To reassign the South American specimens that have been misidentified as *Elaphoglossum petiolatum*, further morphological and taxonomic revisions are needed. For these specimens, there are several names available (e.g., *E. ballivianii* Rosenst., *E. killipii* Mickel, *E. stenophyllum* (Sodiro) Diels, *E. viscidum* (Feé) Christ, and *E. yungense* de la Sota) and some of the entities in the group might be undescribed.

The name *Acrostichum viscosum* and its combination with *Elaphoglossum* correspond to a nomen superflua because this species was described in 1806 using the same type used for *E. petiolatum* (*A. petiolatum*) in 1778, so it lacks taxonomic validity (Turland et al. 2018, Chapter VII, Article 52).

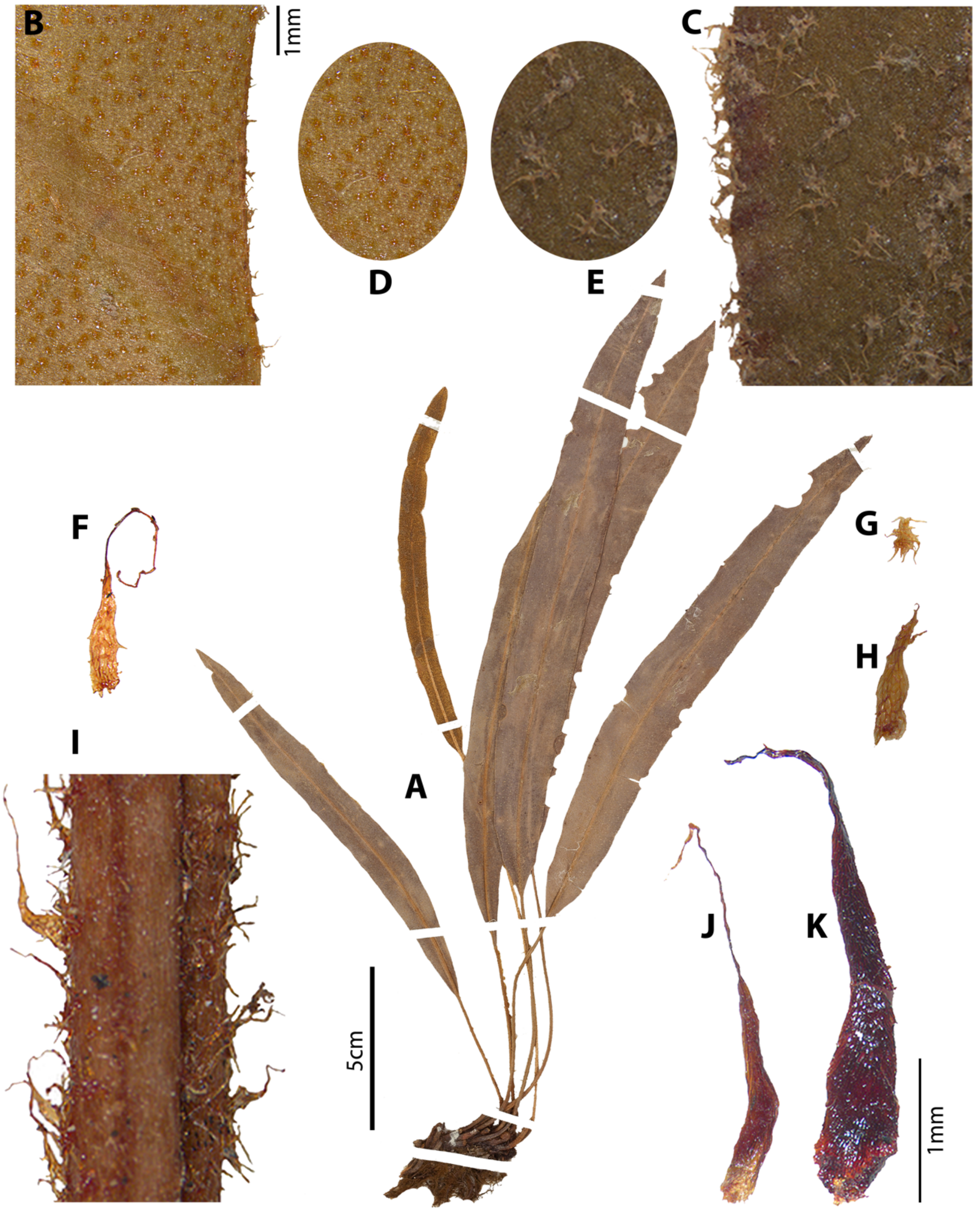

**Figure 6.** *Elaphoglossum petiolatum*. A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A from *Clute 312*, NY; B–E,H from *Maxon 2648,* NY; F,I from *Mickel 8447,* NY; G from *Evans 2702,* MO; J from *Stern 1112,* MO; K from *Moran 5646,* NY).

**4. Elaphoglossum potosianum Christ,** Neue Denkschr. Allg. Schweiz. Ges. Gesammten Naturwiss. 36: 119. 1899. Type: México. San Luis Potosí: San Migualito [Miguelito]: in montibus [according to the specimen at P, not in the protologue], [1890 m], [22°10'46.51"N, 101°0'26.09"W], 1877, *W. Schaffner 77* (lectotype, designated here: P 00249860 [image!], isolectotype: P 00249859 [image!]).

*Elaphoglossum trichomidiatum* Mickel, Brittonia 53: 488. 2001[2002]. Type: México. San Luis Potosí: in the region of San Luis Potosí, 6000–8000 feet [1828–2438 m], [22° 4'46.09"N, 100°59'58.72"W], 1878, *Parry y Palmer 1007* (holotype: NY 03231441!; isotypes: F C0620656F!, MO 1867264!, US 01564938!, VT UVMVT212789!).

**Plants** Terrestrial**. Rhizomes** 2–4 mm wide, short creeping; **rhizome scales** 2–3.5 mm long, dense, spreading, linear-lanceolate, with occasional short projections (shorter than the width of the scale), concolorous, orange or light brown to dark brown, darker at the point of attachment, dull, base truncate, apex attenuate to long-attenuate. **Sterile leaves** 12–26 (–46) cm long, approximate; **phyllopodia** present, sometimes obscured by the petiole scales, 2–4 (–40) mm long; **petioles** 2–12 (–29) cm long, (1/5–) 1/3–1/2 (–3/5) the length of the sterile leaves; **larger petiole scales** to 3 mm long, scattered to dense, not imbricate, spreading to appressed, linear-lanceolate to lanceolate, with occasional short projections and usually with occasional long projections (as long or longer than the width of the scale) located mostly towards the base, stramineous, sometimes darker at the apex, base truncate, apex attenuate to filiform; **smaller petiole scales** to 1mm long, dense, not imbricate, mostly appressed, lanceolate, with occasional long projections, stramineous, base truncate, apex acute; **laminae** 7.5–23 (–31.5) x 1.4–3.3 (–4) cm; linear-lanceolate to narrowly elliptic, chartaceous, base cuneate, apex acute, sometimes apiculate*;* **adaxial laminar scales** to 1mm long, scattered to dense, not imbricate, deciduous, irregular, substellate or lanceolate, when lanceolate, margin with numerous long projections, stramineous, darker at the point of attachment, base auriculate, apex acuminate; **adaxial resinous dots** absent; **abaxial laminar scales** mixed with (2–) 3 armed reddish trichomidia 0.2–0.3 mm long, the scales ca. 0.5 mm long, absent or scattered, irregular, with numerous long projections, stramineous, sometimes darker at the point of attachment; **abaxial resinous dots** present; **abaxial costal scales** 1–1.2 mm long, scattered to dense, not imbricate, lanceolate with occasional long projections, stramineous to yellowish, base truncate, apex acuminate; **marginal scales** to 0.5 mm long, dense, not imbricate, lanceolate, with occasional long projections, stramineous, darker at the point of attachment, base auriculate, apex long-acuminate. **Fertile leaves** longer than the sterile; **petiole** 3/5–2/3 the length of the fertile leaves; **laminae** (8.2–) 10–11.5 x 0.6–1 cm, linear, chartaceous, base attenuate to long-attenuate, apex acute or obtuse; **petiole scales** to 2.5 mm long, scattered to dense, not imbricate, linear-lanceolate to lanceolate*,* with occasional long projections, stramineous, sometimes with darker apex, base round or truncate, apex attenuate or filiform; **adaxial scales** lanceolate or substellate, when lanceolate, margin with numerous long projections*,* yellowish, apex acuminate to attenuate; **abaxial costal scales** to 1 mm long, scattered to dense, not imbricate, lanceolate, with occasional long projections, stramineous, base auriculate, apex acute; **intersporangial scales** not visible. **Perispores** broadly folded, echinate with short spines and ridges. Figures 3D and 7..

*Distribution.*—Endemic to Mexico. CDMX, Edo. de Mex., Gto, Gro, Hgo, Jal, Mich, Nay, Nvo. León, Oax, Qro, S.L.P, Sin, Tamps (Fig. 1D). Besides the states by Mickel y Smith (2004), *E. potosianum* has been collected in Estado de México, Guerrero, Jalisco, Michoacan, Nayarit, Oaxaca, and Queretaro.

*Elevation.*—(480–) 1500–2500 (–3000) m (Fig. 2).

*Etymology.*—The specific epithet *potosiaunum* refers to San Luis Potosí, the Mexican state where the holotype was collected.

*Notes—Elaphoglossum potosianum* differs from all other species of the *Petiolatum* Clade by its concolorous rhizome scales, which are orange or light brown to dark brown, darker at the point of attachment, and dull. Also distinctive is the presence of trichomidia mixed with resinous dots on the abaxial laminar surface. The larger specimens of *E. potosianum* have phylopodia up to 4 cm and highly conspicuous .

*Elaphoglossum potosianum* resembles *E. muelleri*, see *E, muelleri* for comparison.

Within *E. potosianum*, the density of the laminar scales, trichomidia, and resinous dots is variable. In some specimens, it is easier to observe these structures than in others. Usually, trichomidia can be most easily detected by observing the specimen in profile.

According to the protologue of *E. potosianum,* a specimen was at HB (currently the Berlin Herbarium B) (Christ, 1899). However, we were not able to locate it, and therefore we designate *Schaffner 77*, P (P00249860) as the lectotype.

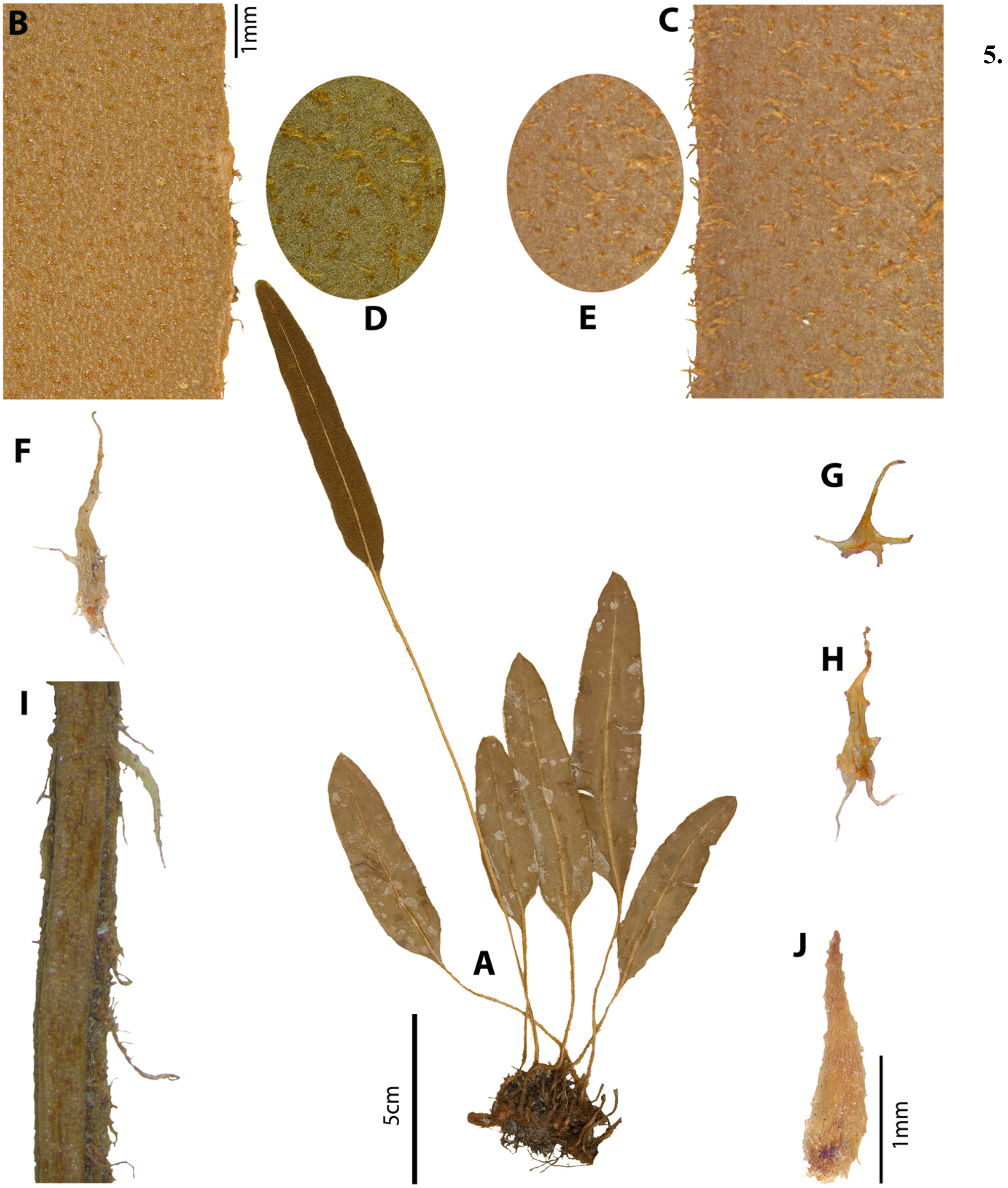

**Figure 7.** *Elaphoglossum potosianum*. A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A from *Parry & Palmer 1007,* VT; B–H, J from *Schaffner 92,* NY; I from *Schaffner 77,* P).

**Elaphoglossum pringlei (Davenp.) C. Chr.,** Index Filic. 313. 1905. *Acrostichum pringlei* Davenp. Bot. Gaz. 21 (5): 253. 1896. Type: México. Oaxaca: Sierra de San Felipe, 10,000 feet [3048 m], [17°25'46.28"N, 96°28'5.90"W], 25 Sep 1894, *C. G. Pringle 5605* (first-step lectotype inadvertently designated by Mickel & Beitel, 1988: GH; second-step lectotype designated here: GH 00020353 [image!]; isolectotypes F V0075926F!, GH 00102873 and 00102874 [images!], K 000994450 and 000994451 [image!], US 01564931!, VT UVMVT001435 [images!]).

**Plants** Terrestrial**. Rhizomes** 2–2.5 (– 4.5) mm wide, short creeping; **rhizome scales** 1.4–2.5 (–3.5) mm long, dense, spreading, linear to lanceolate, with occasional short projections (shorter than the width of the scale), bicolorous, light brown becoming dark brown with age, darker at the point of attachment, dull, base round, apex long-attenuate to filiform-tortuouse, oftentimes broken. **Sterile leaves** 7–38 cm long, approximate; **phyllopodia** present, clearly visible, 5–20 (–30) mm long; **petioles** (1.3–) 3.5–13 (–20) cm long, (1/4–) 3/8–3/7 (–3/5) the length of the sterile leaves; **larger petiole scales** to 2.5 mm long, scattered to dense, not imbricate, spreading to appressed, linear-lanceolate to lanceolate, with occasionally short projections or with long projections (as long or longer than the width of the scale), stramineous, sometimes darker at the apex, base round or auriculate, apex acuminate to long-attenuate; **smaller petiole scales** to 1 mm long, dense, not imbricate, deciduous, mostly appressed, irregular, substellate or lanceolate, when lanceolate, margin with occasional short or long projections, stramineous, sometimes darker at the apex, base round or auriculate, apex acuminate to long-attenuate; **laminae** 4–22.5 x (0.5–) 1–2.5 (–3) cm; elliptic to lanceolate, chartaceous, base decurrent, apex obtuse or acute, sometimes apiculate*;* **adaxial laminar indument** consisting of scales to 1 mm long, dense or sometimes scattered, not imbricate, irregular, substellate or lanceolate, when lanceolate, margin with numerous long projections, whitish to yellowish, darker at the point of attachment, base auriculate, apex long-attenuate; **adaxial resinous dots** absent; **abaxial laminar indument** consisting of scales to 1mm long, dense, not imbricate, substellate, irregular, or lanceolate, when lanceolate, with numerous long projections, stramineous to yellowish, sometimes darker at the point of attachment, base when lanceolate, auriculate, apex long-attenuate to filiform; **abaxial resinous dots** present or absent; **abaxial costal scales** 0.5–1 mm long, dense, not imbricate, lanceolate, substellate, or irregular, when lanceolate, margin with numerous long projections or with occasional short projections, stramineous, base, when lanceolate, auriculate, apex long-attenuate; **marginal scales** to 0.5 mm long, dense, not imbricate, lanceolate, with numerous long projections, stramineous to yellowish, base truncate, apex long-attenuate, sometimes with a long projection close to the apex. **Fertile leaves** longer than the sterile; **petioles** (2/3–) 3/5–5/7 the length of the fertile leaves; **laminae** 4–15.5 x 0.5–1.2 cm, elliptic to lanceolate, chartaceous, base long-cuneate, apex round or acute; **petiole scales** to 1 mm long, scattered, linear-lanceolate to lanceolate or irregular*,* when linear to linear-lanceolate, margin with occasional short projections, stramineous, sometimes with darker margin, base round or auriculate, apex acuminate to long-attenuate; **adaxial indument** consisting of scales lanceolate or substellate, when lanceolate, margin with numerous long projections*,* whitish to yellowish, apex attenuate to long-attenuate; **abaxial costal scales** to 1 mm long, scattered, linear-lanceolate to lanceolate or irregular, when linear-lanceolate or lanceolate, with numerous long projections, stramineous to yellowish, base auriculate, apex acuminate to long-attenuate; **intersporangial scales** not visible. **Perispores** broadly folded, echinate with short spines and ridges. Figures 3E and 8.

*Distribution*.—Endemic to Mexico. Chis, Gro, Hgo, Jal, Mich, Oax, Ver. Most of the collections of *Elaphoglossum pringlei* examined in this study, and those cited by Mickel and Smith (2004) are from Oaxaca (Fig. 1E).

*Elevation*.—(1280–)1500–3050 m (Fig. 2).

*Etymology.—*The specific epithet honors Cyrus Guernsey Pringle (1838–1911), an American botanist who collected between 1880 and 1909 in the Pacific Coast states of the United States, and in Mexico. In 1902, Pringle funded the herbarium of the University of Vermont, and currently, that herbarium carries out his name as The Pringle Herbarium (VT) (TL-2).

Notes.—*Elaphoglossum pringlei* has the largest spores of any species the *Petiolatum* Clade, suggesting it is polyploid (Martinez-Becerril et al., 2021). The spores average 40.4 μm in equatorial diameter and 31.6 μm in polar diameter. In contrast, the other species in the clade average 31.2 μm and 28.5 μm, respectively.

*Elaphoglossum pringlei* is often misidentified as *E. petiolatum* because of its similar linear to lanceolate rhizome scales and lamina shape . Mickel and Smith (2004) suggested that one of the main differences was that *E. pringlei* lacked resinous dots on the laminae, and that the scales were substellate abaxially. However, we found that at least half of the specimens of *E. pringlei* had resinous dots on the laminae abaxially, and some specimens of *E. petiolatum* had substellate scales on the abaxial surface of the laminae.

*Elaphoglossum pringlei* and *E. petiolatum* can be distinguished by rhizome-scale color, presence and kind of resinous dots on the laminae, spore morphology and geographic distribution. *Elaphoglossum pringlei* has bicolorous and light brown rhizome scales with a darker point of attachment, and long-attenuate apices ending in a thin hair; laminae without resinous dots on the adaxial surface and, when present, shiny resinous dots on the abaxial surface; and echinate perispores. In contrast, *E. petiolatum* has concolorous, dark-reddish brown to dark brown rhizome scales with a filiform to tortuouse apex (very long, but wider that a thin hair); laminae sometimes with resinous dots on the adaxial surface and always with shiny and/or non-shiny resinous dots on the abaxial surface; and perispores with broadly folded with sparsely irregular surface microstructures.

Mickel and Smith (2004) suggested that *Elaphoglossum pringlei* was related to *E. rzedowskii*, and that these two represented morphological variations of the same species. Our molecular phylogenetic analyses recover them in two separate clades (Martínez-Becerril et al., in press), and the differences found in their spore morphology (Martínez-Becerril et al., 2021) and the plants show they are two separate species.

*Elaphoglossum rzedowskii* differs from *E. pringlei* by rhizome scales with acuminate to long-attenuate apices, linear to elliptic laminae with dense scales, sometimes imbricated, deciduous, and resinous dots on the adaxial laminar surface. In contrast, *E. pringlei* has long-attenuate to filiform-tortuouse, oftentimes broken, elliptic to lanceolate laminae with dense scales, not imbricate, and absence of adaxial resinous dots. The average size of the spores is also a good character to distinguish these species; in *E. pringlei* they measure 40.4 µm and 31.6 µm, whereas in *E. rzedowskii* they measure 34.6 µm and 27.9 µm, in equatorial diameter and in polar diameter respectively.

Within *Elaphoglossum pringlei* there is a gradient of shapes and colors of the rhizome scales, from linear to lanceolate and different tones of brown, because they tend to darken with age. The laminar scales and resinous dots, some specimens have very scattered scales, while others can have dense scales, and some specimens do not have any resinous dots on the laminae.

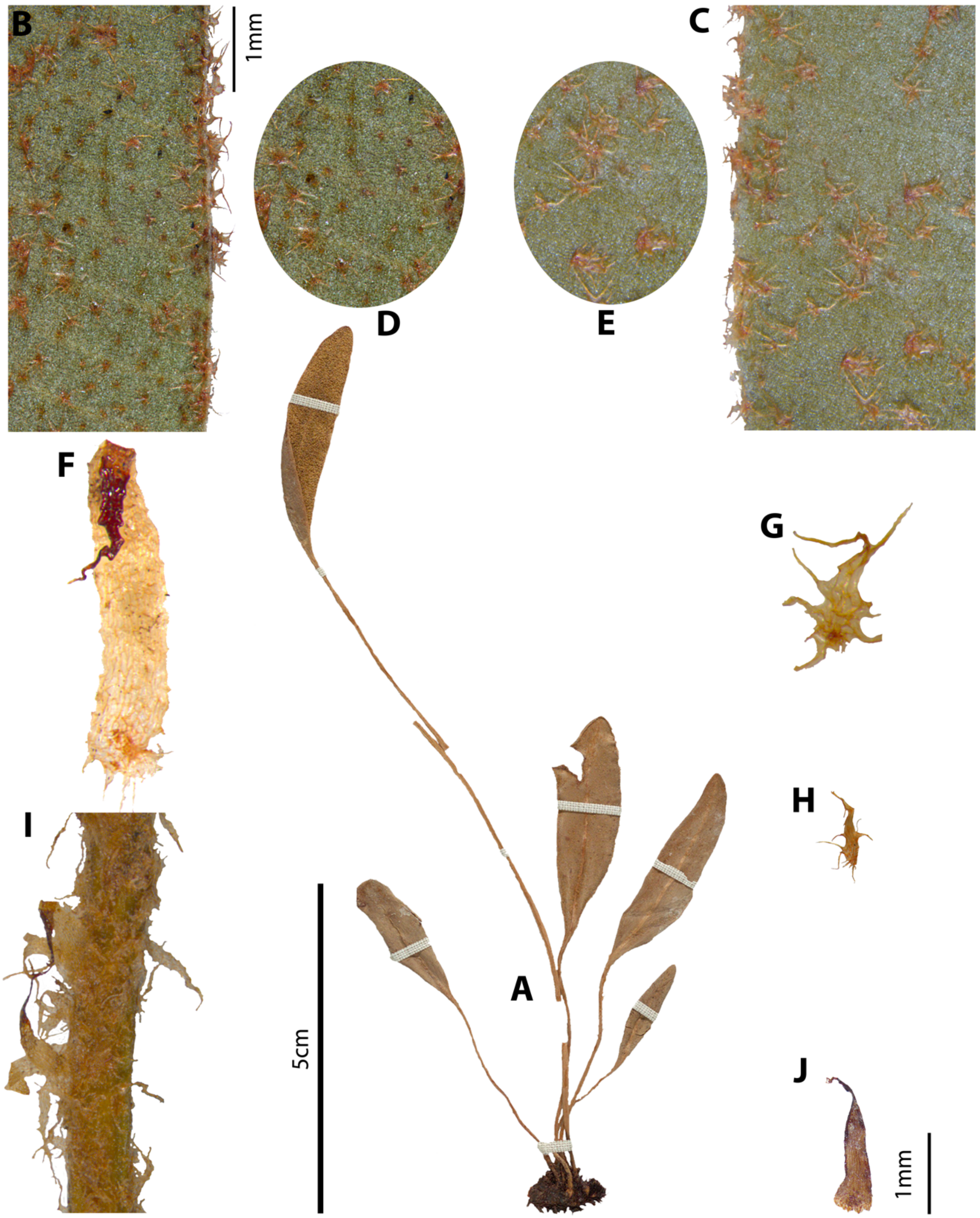

**Figure 8.** *Elaphoglossum pringlei*. A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A from *Pringle 5605,* US; B– E from *Mickel 4778,* NY; F from *Mickel 3866,* NY; G–H from *Pringle 5605,* F; I–J from *Mickel 3866,* NY).

**6. Elaphoglossum rzedowskii Mickel,** Brittonia 32: 337. 1980. Typo: México. Jalisco: Mpio. San Martín de Bolaños, Las Vidrieras, 10 km NW of El Platanar, 2450 m, [20°28'56.01"N, 103° 4'41.83"W], 1 Sep 1968, *J. Rzedowski 26161* (holotype: MICH 1287073! [image!]; isotype: NY 00149704!).

**Plants** Terrestrial**. Rhizomes** 2.5–4 mm wide, short creeping; **rhizome scales** (1–) 1.5–2 (–3) mm long, dense, spreading, linear-lanceolate, with occasional short projections (shorter than the width of the scale), bicolorous, stramineous to brown, darker at the point of attachment and towards the margin and the apex, dull, base round, apex acuminate to long-attenuate. **Sterile leaves** (8–) 16–25 cm long, approximate; **phyllopodia** present, usually obscured by the rhizome scales, ca. 5 mm long; **petiole** 3–6.5 (–11.5) cm long, 1/6–1/4 (–1/2) the length of the sterile leaves; **larger petiole scales** to 3 mm long, dense, usually imbricate, spreading to appressed, linear-lanceolate to lanceolate, with occasional short projections, stramineous, sometimes darker towards the apex, base truncate, apex attenuate to filiform; **smaller petiole scales** to 1mm long, dense, usually imbricated at the petiole base, mostly appressed, irregular, substellate or lanceolate, when lanceolate, margin with occasional long projections (as long or longer than the width of the scale), stramineous, darker at the point of attachment, base auriculate, apex acute ; **laminae** 9.5–18.5 x (0.7–) 1–1.5 (–2) cm; linear to elliptic, chartaceous, base decurrent, apex acute to acuminate, sometimes apiculate*;* **adaxial laminar indument** consisting of scales to 1mm long, dense, not imbricate, deciduous, linear-lanceolate, with numerous long projections (as long or longer than the width of the scale), stramineous, darker at the point of attachment, base auriculate, apex long-filiform, **adaxial resinous dots** present, sometimes not visible due to the density of the laminar scales; **abaxial laminar indument** consisting of scales, the scales to 0.7 mm long, dense, sometimes imbricate, lanceolate, with numerous long projections, stramineous, darker at the point of attachment, base auriculate, apex long-filiform; **abaxial resinous dots** present, sometimes not visible due to the laminar scale density; **abaxial costal scales** 1–2 mm long, dense, usually imbricate, linear-lanceolate with occasional short and/or long projections, stramineous, sometimes whitish , base auriculate, apex long-filiform; **marginal scales** to 1mm long, dense, usually imbricate, lanceolate, with numerous long projections, stramineous, base truncate with long projections, apex long-attenuate, sometimes with a long projection close to the apex. **Fertile leaves** similar or slightly longer than the sterile; **petiole** 3/7–1/2 the length of the fertile leaves; l**aminae** 11–13.5 x 0.9–1.1 cm, linear-elliptic, chartaceous, base long-cuneate, sometimes unequal, apex acute to acuminate; **petiole scales** (1.5–) 2.5 (–3) mm long, dense, usually imbricate, linear-lanceolate to lanceolate or irregular*,* when linear to linear-lanceolate, margin with occasional short or long projections, stramineous, sometimes with darker apex, base round or truncate, apex attenuate to filiform; **adaxial indument** consisting of scales linear-lanceolate to lanceolate, when linear-lanceolate to lanceolate, margin with numerous long projections*,* yellowish, apex long-attenuate to filiform; **abaxial costal scales** to 2 mm long, dense, usually imbricate, linear-lanceolate, with occasional short and/or long projections, stramineous, base auriculate, apex long-filiform; **intersporangial scales** not visible. **Perispores** broadly folded, echinate with short spines and ridges. Figures 3F and 9.

*Distribution–* Endemic to Mexico. Ags, Chih, Dgo, Gro, Jal, Nay, Sin, Son (Fig. 1F). This species was also reported by Mickel and Smith (2004) for the states of Guanajuato, Michoacán, and Zacatecas, however, we did not find specimens belonging to this species in these states.

*Elevation.*—(800–) 1400–2700 (–3050) m (Fig. 2).

*Etymology.—*The specific epithet honors Jerzy Rzedowski Rotter (1926- ), a Polish botanist, professor, and emeritus researcher who has dedicated his career to studying Mexican plants. (González 2009).

*Notes*.—Among the species of the *Petiolatum* Clade, *E. rzedowskii* has the northernmost distribution, occurring even in the Mexican states of Chihuahua and Sonora. Also, it is the only species of the clade with the scales of the laminae sometimes imbricated and so dense that they obscure the surface beneath Because of this, the resinous dots on the abaxial surface are difficult to observe; however, those on the adaxial surface are evident in most of the specimens.

*Elaphoglossum rzedowskii* and *E. pringlei* were hypothesized to represent regional morphological variations of the same species (Mickel and Smith 2004). See *E. pringlei* for a comparison and discussion of differences with this species.

Compared to other species in the *Petiolatum* Clade, *Elaphoglossum rzedowskii* has the fewest collections, and only a few are fertile. These were collected during August and September.

In general, specimens from Durango and Guerrero have fewer laminar scales than the rest, and the only collection recorded above 2700 m is *Gentry 18160* (NY) from Durango.
The holotype of *Elaphoglossum rzedowskii* is mixed. Two plants are mounted on the sheet that houses the holotype of this species *Rzedowski 26161* (MICH). In order to differentiate the two plants, someone marked them directly on the sheet with the letters a and b, and later they were assigned different barcodes: plant “a” (MICH 1287073) and plant “b” (MICH 1259599). In the original description of this species, Mickel (1980), explained that the holotype of *E. rzedowskii* is plant “a”, and plant “b” is *E. gratum* (Fée) T. Moore. The current annotation labels on the specimen at MICH indicates that in 1988 Mickel identified specimen “b” as *E. rzedowskii*

(determination corroborated in 2008 by R. K. Rabeler). We believe that the initial determination by Mickel (1980) is correct: the holotype of *E. rzedowskii* is plant “a,” and plant “b” is *E. gratum*.

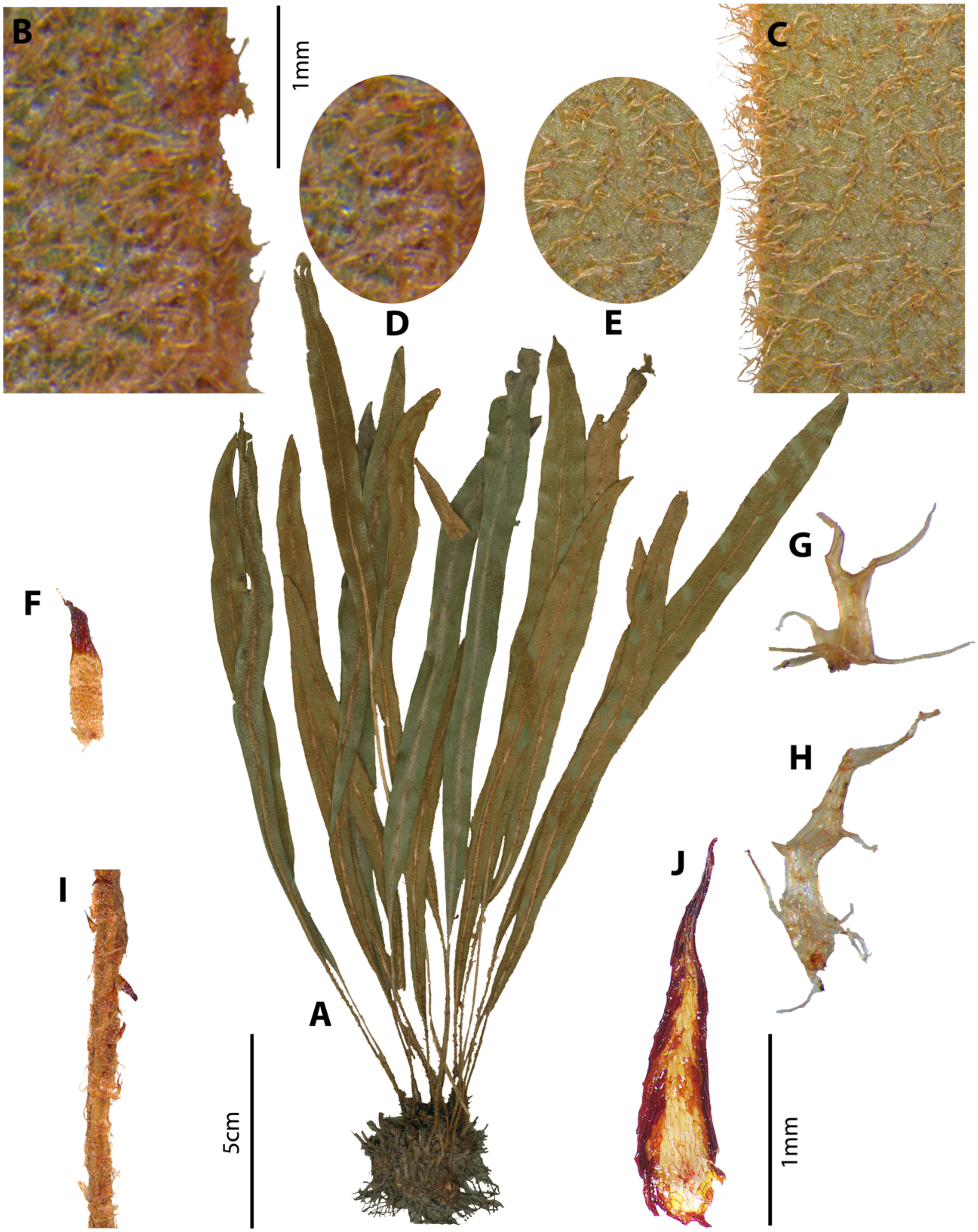

**Figure 9.** *Elaphoglossum rzedowskii*. A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A–B, D–F, I from *Rzedowski 26161,* NY; C, E, G–H, from *Correl & Gentry 22972,* MO; J from *Reina 96-183*, MO).

**7. *Elaphoglossum schiedei* (Kunze) T. Moore,** *Acrostichum schiedei* Kunze, Analecta Pteridogr. 10. 1837. Type: México. Veracruz: Mpio. Jalapa: ad ladera montimum prope El Molino de Pedreguera conf, [1430m], [19°33'7.02"N, 96°56'23.47"W], Junio 1829, *W. Schiede s.n.* (lectotype inadvertently designated by Mickel & Beitel, 1988: B (B 20 0071835) [image!], isolectotypes: BR 00000697192 [image!], HAL 0137800 [image!], LE 00008871 [image!], P 00603624 [image!]).

**Plants** Terrestrial**. Rhizomes** 2–10 mm wide, short creeping; **rhizome scales** 0.7–1.2 (–1.5) mm long, scattered, appressed, oblanceolate, with occasional short projections (shorter than the width of the scale), concolorous, reddish-brown to dark brown, sometimes lighter at the point of attachment, sclerotic, lustrous, base round or truncate, apex acute to short-filiform. **Sterile leaves** 17–45.5 (–75) cm long, approximate; **phyllopodia** present, clearly visible, 5–15 (–19) mm long; **petioles** 7–12 (–18) cm long, 1/3–3/5 (–3/4) the length of the sterile leaves; **larger petiole scales** to 2 mm long, sacttered to dense, not imbricate, spreading to appressed, linear-lanceolate to lanceolate, with occasional short projections, stramineous, sometimes reddish-brown towards the apex, base round or truncate, apex acuminate to filiform; **smaller petiole scales** to 1 mm long, sacttered to dense, not imbricate, mostly appressed, lanceolate, with short or long projections (as long or longer than the width of the scale), stramineous, base truncate or round, apex attenuate; **laminae** 10–25 (–57) x 1.5–2.8 (–3.2) cm; elliptic, papyraceous, base acute, apex acute to acuminate*;* **adaxial laminar indument** consisting of scales to 1 mm long, scattered to dense, not imbricate, deciduous, substellate or lanceolate, when lanceolate, margin with numerous long projections, whitish to yellowish, darker at the point of attachment, base auriculate, apex attenuate, **adaxial resinous dots** absent; **abaxial laminar indument** consisting of scales to 0.7 mm long, absent or scattered to dense, not imbricated, stellate to irregular, yellowish, sometimes darker at the point of attachment; **abaxial resinous dots** present; **abaxial costal scales** 0.7–1.2 (–1.5) mm long, sacttered to dense, not imbricate, ovate to lanceolate with occasional short projections, stramineous to light brown, base truncate, apex acute to attenuate; **marginal scales** to 0.6 mm long, scattered to dense, not imbricate, deciduous, linear-lanceolate or substellate, when linear-lanceolate, margin with numerous long projections, yellowish, base when linear-lanceolate, truncate, apex when lanceolate, long-attenuate. **Fertile leaves** longer than the sterile; **petiole** 1/3–2/3 (–3/4) the length of the fertile leaves; l**aminae** (7.5–) 10–18 x (0.3–) 0.7–1.3 cm, elliptic, papyraceous, base cuneate, apex acute or obtuse, sometimes apiculate; **petiole scales** to 2 mm long, scattered to dense, not imbricate, lanceolate or substellate*,* when lanceolate, margin with short projections, stramineous to light brown, with darker apex, base round or truncate, apex acuminate or filiform; **adaxial indument** consisting of scales to 0.5 mm long, dense, not imbricate, lanceolate or substellate, when lanceolate, margin with numerous long projections or with occasional short projections*,* yellowish, base truncate, apex attenuate; **abaxial costal scales** to 0.7 (–1.8) mm long, scattered to dense, not imbricate, lanceolate to oblanceolate or substellate, when lanceolate or oblanceolate, with occasional long projections, yellowish, base auriculate, apex acute to short-filiform; **intersporangial scales** not visible. **Perispores** broadly folded, echinate with short spines and ridges. Figures 3G and 10.

*Distribution*.—Mexico (Chis, CDMX, Hgo, Oax, Pue, Qro, Ver) and northwest Guatemala (Fig. 1G)

*Elevation*.—(274–) 740–3050 m (Fig. 2).

*Etymology*.— The specific epithet honors Christian Julius Wilhelm Schiede (1798–1836), German botanist, gardener, traveler, and plant collector who arrived in Mexico in 1828 and spent the rest of his life exploring the states of Veracruz (Jalapa and Orizaba) and Morelos (Cuautla and Cuernavaca) (https://www.sil.si.edu/DigitalCollections/tl-2/browse.cfm?vol=5#page/160).

*Notes*.—*Elaphoglossum schiedei* is the only species in the *Petiolatum* Clade with glabrescent rhizomes and sclerotic (hard) appressed rhizome scales. Furthermore, the rhizome scales are the smallest (0.7–1.2 (–1.5) mm long) and the only ones in the clade which are wider than long. Sometimes the bases of the petioles and their scales densely cover the rhizomes, making it difficult to find the rhizome scales.

*Elaphoglossum schiedei* was considered a synonym of *E. petiolatum* (Proctor 1985, Mickel and Beitel 1988, Mickel and Smith 2004), morphological and molecular evidence, however, supports its recognition as a different species. *Elaphoglossum schiedei* has 0.7–1.2(–1.5), scattered, appressed, oblanceolate rhizome scales with an acute to short filiform apex; lamina with a papyraceous consistency; stellate to irregular scales on the abaxial laminar surface; and echinate perispore ornamentation. In contrast, *E. petiolatum* has 1.5–3.7 (–5), dense, spreading, linear to lanceolate rhizome scales with a filiform-tortuouse apex, oftentimes broken; lamina with a chartaceous consistency; lanceolate scales on the abaxial laminar surface; and perispore ornamentation with broadly folded with sparsely irregular surface microstructures.

*Mickel 4706*, *4843*, and *4857* (all NY) from Oaxaca, Mexico, are unusual because they have rhizome scales longer than the other species (up to 1.5 mm), resinous dots non-shiny, and substellate scales on the laminae abaxially. However, they agree with our concept of *E. schiedei* by having glabrescent rhizomes, and rhizome scales with the shape and color typical of this species. *Standley 92203* (F), from Guatemala, is atypical in having been collected as an epiphyte.

According to our interpretation of the protologue, it is possible that the original material of this species was given "in goodwill" to Schlechtendal and represented the same species that

Karwinski previously reported for Mexico as *A. salicifolium*. Following our interpretation of the protologue, it is probable that Karwinski prepared a list of the material deposited in his herbarium where he clarified that what he called *A. salicifolium* was the same as *A. schiedei*. However, we currently know that *A. salicifolium* (=*E. lancifolium*) represents a different species, one that is distributed only in the Old World. This species belongs to the *E. lancifolium* clade, not the *Petiolatum* Clade (Martínez-Becerril et al., in press).

Mickel and Beitel (1988) inadvertently lectotypified *Elaphoglossum schiedei* when they cited a type specimen of it from B (B 20 0071835). We do not consider as original material the specimens from NY 00149705 and 00334944, both specimens are anonymously annotated as "cotypes" of *E. schiedei*, because, although they come from the same locality indicated in the protologue (Jalapa), the collection and collection year shown on the labels do not coincide with the one in the protologue (*Schiede s.n.*, in 1829). *Schlechtendal 736a* (NY 00149705) lacks a collection date, whereas a duplicate (NY 00334944) lacks both the collector and collection date. The term "cotype" was previously used to refer to what we now know as syntype and sometimes, and erroneously, to refer to an isotype and/or a paratype, however, this term is not used today (Hawksworth 2010).

BR 00000697171 is *Elaphoglossum schiedei*, but it cannot be determined whether it is a syntype because it does not indicate the date or the precise location. This specimen has a note by Gustav Kunze, the author of *A. schiedei,*mentioning that the specimen resembles *A. schiedei* but does not correspond to that species ("*Acrostichum schiedei* simili. Frondes stes. ad comparant. México Schiede" [similar to *Acrostichum schiedei*, fronds sterile, purchased from Mexico Schiede]). MO (MO-255729) marked as an isotype cannot be confirmed as original material due to lack of locality data and collector.

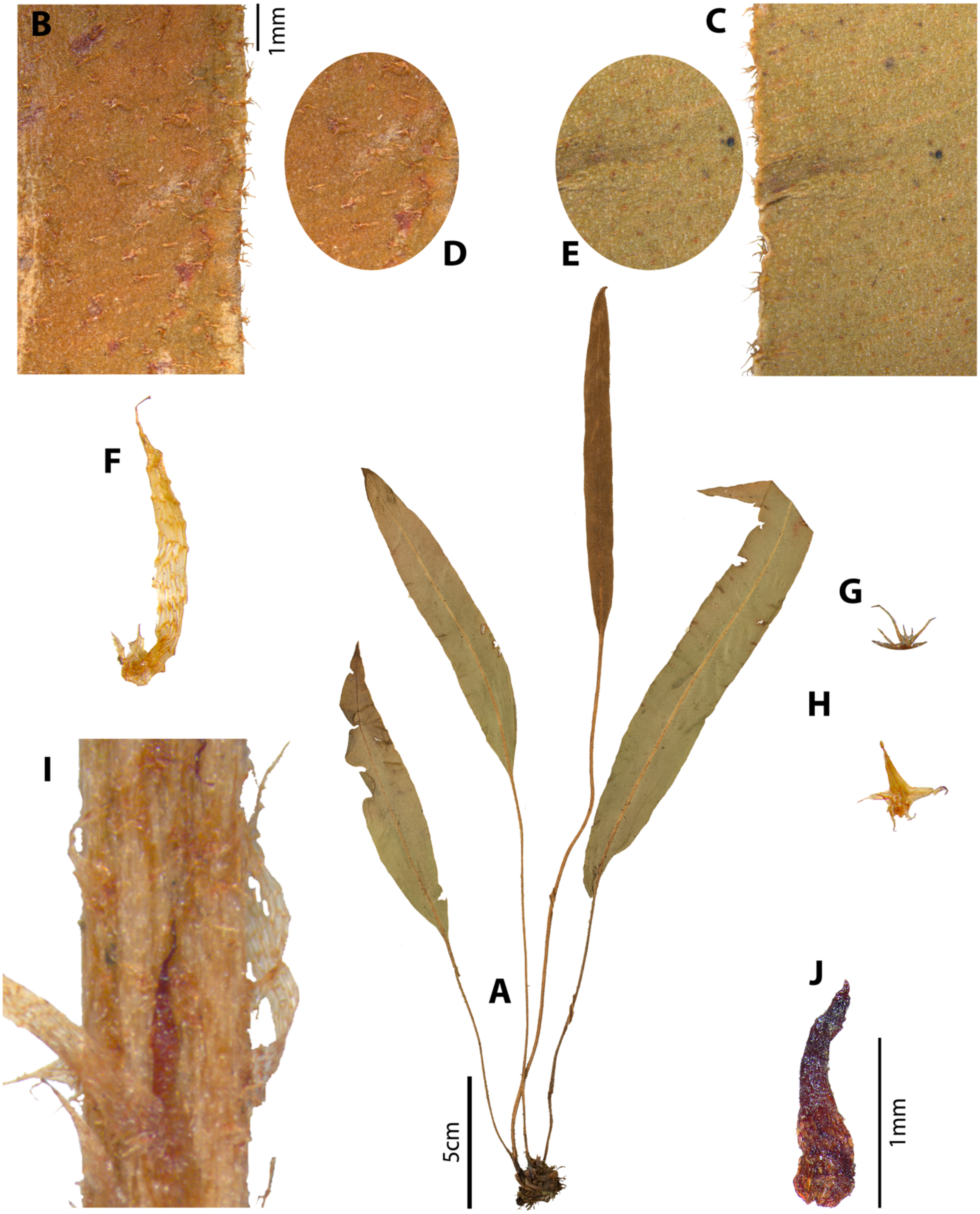

**Figure 10**. *Elaphoglossum schiedei*. A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A from *Pringle 13292*, VT; B–I *Castillo Campos 19132*, CHAPA; J from *Christ s.n.,* P).

**8. *Elaphoglossum schmitzii* (Mett. ex Kuhn) Martínez-Becerril & A. Vasco,** *Acrostichum schmitzii* Mett. ex Khun, Linnaea 36: 51. 1869. Syntypes: México. México: *Schmitz 3* (ex parte), *Karwinski s.n., Schaffner s.n.* (lectotype here designated: *Schaffner s.n.* B 20 0071856 [image!], isolectotypes: B 20 0071857 and, B 20 0071858 [images!]).

**Plants** Terrestrial**. Rhizomes** 1.5–3.5 mm wide, short creeping; **rhizome scales** 2–4 mm long, dense, spreading, linear to lanceolate, entire or with occasional short projections (shorter than the width of the scale), bicolorous, light brown becoming brown with age, darker at the point of attachment and towards the apex, dull, base round, apex attenuate to filiform. **Sterile leaves** (11–) 14–34 (–61) cm long, approximate; **phyllopodia** present, clearly visible, 5–10 mm long; **petioles** (3.5–) 4–17.5 cm long, (1/5–) 1/4–1/2 the length of the sterile leaves; **larger petiole scales** to 2.5 mm long, sacttered to dense, not imbricate, spreading, linear-lanceolate to lanceolate, with occasional short projections, stramineous, sometimes darker at the apex, base round with long projections (longer than the width of the scale), apex long-attenuate to filiform; **smaller petiole scales** to 1 mm long, scattered to dense, not imbricate, mostly appressed, substellate, stramineous; **laminae** (7.7–) 11–25.5 (–57) × 0.9–1.5 (–2) cm; narrowly elliptic, papyraceous or chartaceous, base cuneate, apex acute to acuminate; **adaxial laminar indument** consisting of scales to 0.8 mm long, dense, not imbricate, sometimes scattered, substellate, linear-lanceolate to lanceolate, when linear-lanceolate or lanceolate, margin with numerous long projections (as long or longer than the width of the scale), stramineous, darker at the point of attachment, base truncate with long projections, apex long-attenuate to filiform, **adaxial resinous dots** sometimes present; **abaxial laminar indument** consisting of scales to 1 mm long, absent or scattered to dense, not imbricated, linear-lanceolate, with numerous long projections, yellowish, sometimes darker at the point of attachment, base truncate with long projections, apex long-attenuate; **abaxial resinous dots** present; **abaxial costal scales** 0.7 (–1.5) mm long, scattered, lanceolate with occasional short projections, stramineous, base truncate, apex acuminate to filiform; **marginal scales** 0.7 (–1.2) mm long, usually dense, not imbricate, deciduous, oblanceolate or substellate, when oblanceolate, margin with numerous long projections, yellowish, base round or truncate, apex attenuate. **Fertile leaves** longer than the sterile; **petiole** 1/2- 2/3 (–5/7) the length of the fertile leaves; **laminae** (5.5–) 9–15 x (0.5–) 0.8–1.1 cm, narrowly elliptic, papyraceous or chartaceous, base cuneate to attenuate, apex acute or obtuse; **petiole scales** to 2.5 mm long, dense, not imbricate, deciduous, linear-laceolate to lanceolate or substellate*,* when linear to linear-lanceolate, margin with numerous long projections or with occasional short projections, stramineous, sometimes reddish-brown towards the apex, base round with long projections or truncate, apex long-attenuate to filiform; **adaxial indument** consisting of scales to 0.7 mm long, scattered to dense, not imbricate, lanceolate or substellate when lanceolate, margin with numerous long projections*,* yellowish, base truncate with long projections, apex long-attenuate to filiform; **abaxial costal scales** to 1 mm long, scattered to dense, not imbricate, deciduous, linear to lanceolate or stellate, when linear or lanceolate, with numerous long projections or with occasional short projections, yellowish, base truncate with long projections, apex attenuate; **intersporangial scales** not visible. **Perispores** broadly folded, echinate with short spines and ridges. Figures 3H and 11.

*Distribution.*—Endemic to Mexico (CDMX, Edo. de Mex., Gto, Gro, Hgo, Jal, Mich, Mor, Oax, Pue, Qro, Ver) (F1g. 1 H).

*Elevation*.—(609–) 1015–2800 m (Fig. 2).

*Etymology.—*The specific epithet honors Albert Schmitz (fl. 1870s) who collected in Mexico (Clifford and Bostock, 2007). We could not find any additional information about this collector.

*Notes*.—*Elaphoglossum schmitzii* is distinguished from the rest of the species of the clade by having lamina margins thinner and lighter than the rest of the lamina. Also the marginal scales of the laminae are 0.7 (–1.2) mm long, dense but not imbricate, substellate or oblanceolate, with long projections, and yellowish.

*Elaphoglossum schmitzii* was considered a synonym of *E. petiolatum* (Proctor 1985, Mickel and Beitel 1988, Mickel and Smith 2004); Molecular evidence also shows that these species belong to two different clades within the *E. petiolatum* group (Martínez-Becerril et al., in press.). Morphologically, *E. schmitzii* differs from *E. petiolatum* by the size, color, and apex shape of the rhizome scales and the ornamentation of the perispore. See *E. petiolatum* for comparison.

*Elaphoglossum schmitzii* can also be distinguished from *E. schiedei*, other species considered a synonym of *E. petiolatum* (Proctor 1985, Mickel and Beitel 1988, Mickel and Smith, 2004)*,* by rhizome scales 2–4 mm long, dense, spreading, linear to lanceolate, bicolorous, light brown, not sclerotic, and dull. In contrast, *E. schiedei* has rhizome scales 0.7–1.2(–1.5) mm long, scattered, appressed, oblanceolate, concolorous, reddish-brown to dark brown, sclerotic, and lustrous.

*Elaphoglossum schmitzii* varies greatly in the density of the laminar and petiole scales and resinous dots. Also, some specimens present petioles that seem to develop from the ventral side of the rhizome and thus, surround the rhizome; that is, the leaves are polystichously (radially) arranged. In other specimens, the rhizome scales are concolorous (apices and margins both brown), and the laminae have persistent bases of deciduous scales rather than resinous dots. Persistent deciduous scale bases fall off easily when touched with a dissecting needle. The non-shiny resinous dots do the same, but unlike the scales, their bases are flat.

Some other atypical specimens are the following: *Cochrine 12559* (NY) from Jalisco (Mexico) is a large specimen with leaves to 54 cm long, laminae 38 cm long, and petioles to 16 cm long. *Rose 7256* (US) from Morelos (Mexico) has the smallest sterile leaf recorded for this species: 4.4 cm long. Leaves of most *E. schmitzii* specimens are 14{34 cm long, and although the aforementioned collections were not incorporated in the descriptions, they were identified as *E. schmitzii* because the scales of the rhizome, petiole, and lamina (including those of the margin ) correspond to those described for this species. *Breedlove 18691* (NY) from Michoacan (Mexico), is the only registered collection below 1015 m.

The syntypes of this species, were probably part of Georg Heinrich Mettenius's personal collection of ferns, which was deposited  at LZ where he was director from 1851–1866 (https://www.sil.si.edu/DigitalCollections/tl-2/browse.cfm?vol=3#page/448). The LZ herbarium was destroyed in 1943 during World War II, and although many of its fern collections (exclusively) were acquired previously (between 1870–1875) by B and W, we were unable to find syntypes of *E. schmitzii* at these herbaria. We did, however, find three syntypes of *Schaffner s.n.* at B. We chose B 20 0071856 as the lectotype because a syntypes it was annotated by Mettenius as *A. schmitzii* and is in good condition.

We do not consider *Schmitz s.n.* (B 20 0071855), which was annotated as a type, is as a syntype because the location on its label differs from the one mentioned in the protologue. *Schmitz s.n.* (NY 00149707), *Schmitz 56a* (NY 00149706), and *Schaffner s.n.* P (P00249994) were annotated by someone previously as probable syntypes. We do not consider them to be syntypes. The first two specimens are incomplete specimens and therefore it is impossible to identify them. The second sheet has a collection number not cited in the protologue, and the third sheet comes from a locality not indicated in the protologue (Veracruz. Orizaba. Sierra de Christobal pr. Eugenio).

**Figure 11**. *Elaphoglossum schmitzii*. A. Habit. B. Abaxial lamina surface. C. Adaxial lamina surface. D. Abaxial lamina scales close-up. E. Adaxial lamina surface close-up. F. Petiole scale. G. Marginal scale. H. Midvein scale. I. Petiole. J. Rhizome scale. (A from *Brunch 2751*, NY; B–E, G–H from *Garcia 3984*, IEB; F,I from *Rzedowskii 46967*, IEB; J from *Arsene 8469,* P).

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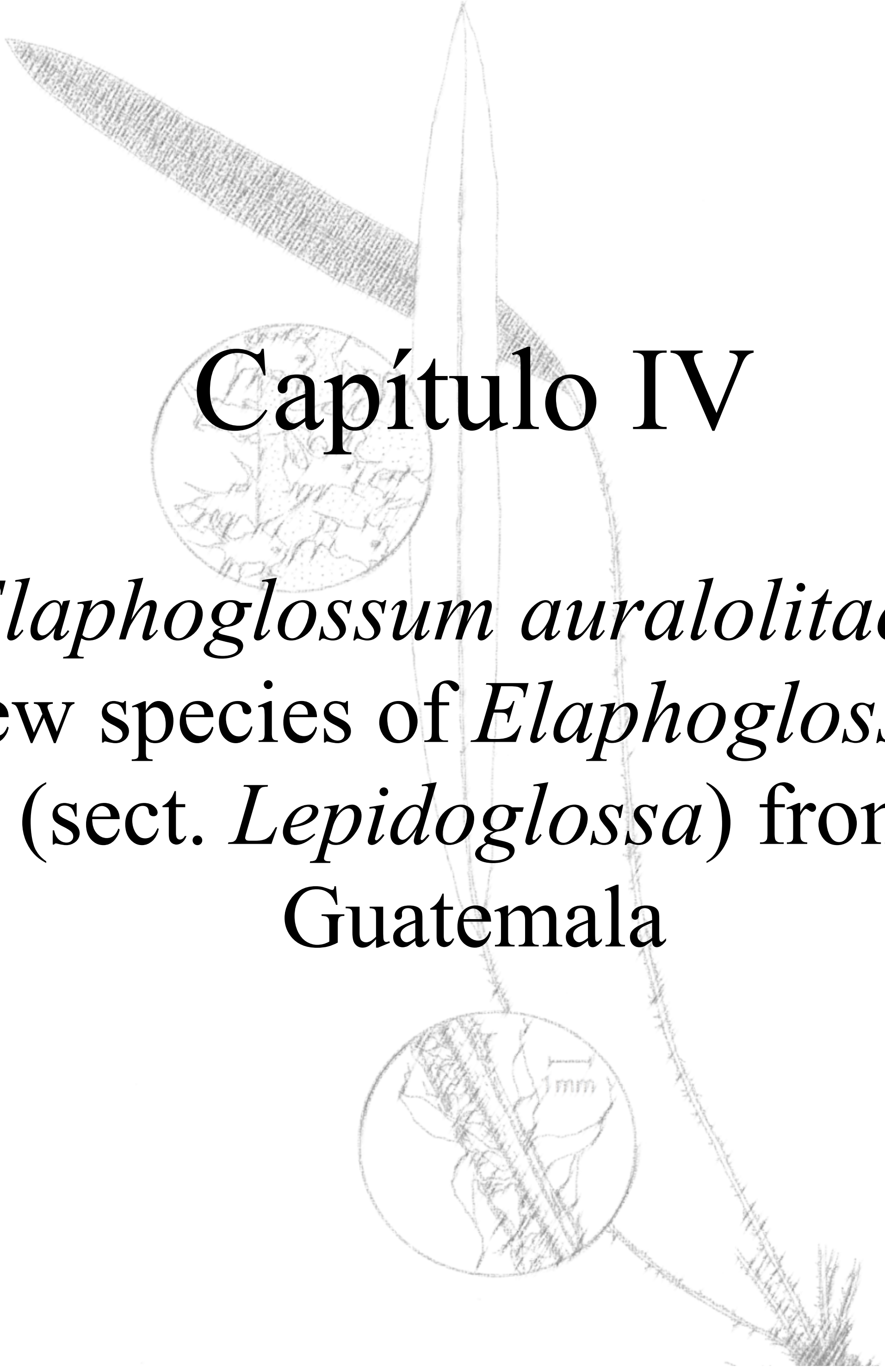

# Capítulo IV

## *Elaphoglossum auralolitae*, a new species of *Elaphoglossum* (sect. *Lepidoglossa*) from Guatemala

Artículo aceptado en *American Fern Journal*

# ELAPHOGLOSSUM AURALOLITAE, A NEW SPECIES OF *ELAPHOGLOSSUM* SECT. *LEPIDOGLOSSA* (DRYOPTERIDACEAE) FROM GUATEMALA

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ABSTRACT.— We describe *Elaphoglossum auralolitae*, a new species from Southwestern Guatemala. The new species belongs to the *Elaphoglossum petiolatum* complex, in the *Elaphoglossum* section *Lepidoglossa,* one of the largest sections within the genus. *Elaphoglossum auralolitae* is characterized by the presence of resinous dots, petioles, and laminae densely scaly with notoriously big scales (including those of costae and margins), and spores with spiny perispores. There are only a dozen collections known of this species all of them from the Sierra Madre mountains, in southwestern Guatemala. The first collection of this species was made in 1938 and the most recent was in 1992. We provide a description, taxonomic and ecological comments, geographic distribution, phenology, and illustrations for this new species.

KEY WORDS.— *Elaphoglossum petiolatum* complex, ferns, resinous dots, Sierra Madre, species-richness, spiny perispore, taxonomy.

During our ongoing research on the systematics of the *Elaphoglossum petiolatum* complex, a morphological group belonging to *Elaphoglossum* section *Lepidoglossa* (Mickel, 2001), we found some specimens from southwestern Guatemala that did not conform with any of the species in the complex described so far.

*Elaphoglossum* (Dryopteridaceae) is a very species-rich fern genus in the American tropics, where ca. 450 species occur (Mickel and Atehortúa, 1980; Mickel 1991,1995; Moran *et al.,* 2010; PPGI, 2016; Moran, 2018; IPNI, 2022). The genus can be easily distinguished from other fern genera by its simple leaves (only six species have divided leaves), free veins, dimorphic sterile and fertile leaves, and acrostichoid sori (Rouhan *et al*., 2004; Moran *et al.,* 2010; Vasco *et al.,* 2013). Within *Elaphoglossum,* seven main and major clades, which have been ranked as sections, are currently recognized: 1) *Elaphoglossum* sect. *Amygdalifolia* (Christ) Mickel and Atehortúa, 2) sect. *Wrigthiana* J.Lóriga, A.Vasco, L.Regalado, J.Heinrichs and R.C.Moran, 3) sect. *Elaphoglossum*, 4) sect. *Squamipedia* Mickel and Atehortúa, 5) sect. *Polytrichia* Christ, 6) sect. *Setosa* (Christ) Mickel and Atehortúa, and 7) sect. *Lepidoglossa* Christ, (Skog *et al*., 2004; Rouhan *et al.,* 2004: Lóriga *et al.,* 2014).

One of the most species-rich sections is the sect. *Lepidoglossa,* with ca. 350 species. This section is characterized by acicular, one-celled, non-glandular projections along the scale margins, flat scales, stellate scales, and/or resinous dots on blades and/or petioles (Mickel and Atehortúa, 1980; Moran, Hanks, and Rouhan*,* 2007; Vasco, Moran, and Rouhan, 2009a; Kessler *et al*., 2018; Matos and Vasco, 2022).

Mickel (2001) described a group of 11 worldwide distributed species belonging to the sect. *Lepidoglossa* and called them the *Elaphoglossum petiolatum* complex. Species within this complex are characterized by resinous dots in their laminae and/or petioles, black rhizome scales with entire margins, and scales of the proximal portion of the petioles with a dark apex (Mickel and Smith, 2004). Molecular phylogenetic analyses with chloroplast markers suggested that the species of the complex and most species of *Elaphoglossum* with resinous dots, were not monophyletic, because they were recovered in three different clades (Rouhan *et al*., 2004; Vasco, Moran, and Rouhan, 2009b; Lóriga *et al*., 2014; Martínez-Becerril *et al.,* in prep.). One of these three clades includes mainly Mexican species, mostly with spiny perispores, and was called the Mexican Clade of *E. petiolatum* (Mexican Clade, Vasco, Moran, and Rouhan*,* 2009b; Martinez-Becerril, Vasco, and Solano, 2021).

The morphology and geographical distribution of the new species here described, suggest it likely belongs to the Mexican Clade. What first surprised us about the dozen of gatherings of this new species was that compared to other specimens of the Mexican Clade with resinous dots, they had a higher density of scales on petioles and laminae and these scales were larger, in addition to the fact that all of them came from the Sierra Madre Mountains in southeastern Guatemala. We then decided to look at their spores under the scanning electron microscope (SEM) and found out they all have spiny perispores, a typical spore ornamentation for most species belonging to the Mexican Clade.

The specimens we cite here as belonging to the new species were previously identified with, at least, six names: *Elaphoglossum crinipes* C. Chr. (=*E. setigerum* (Sodiro) Diels), *E. gratum* (Fée) T. Moore*, E. petiolatum, E. pilosum* (Willd.) T.Moore*, E. rubescens* Christ, or *E. tectum* (Humb. & Bonpl. ex Willd.) T. Moore. From all these names *E. petiolatum* is the only species with resinous dots.

There are no recent treatments for *Elaphoglossum* from Guatemala and very few recent collections are available online. The published floras and the recent addition of new records in

Guatemala, report 41 species of *Elaphoglossum* for the country (Mickel, 1981, 1995a; Mickel and Smith, 2004; Jimenez and Rodas, 2013). Three of these species have resinous dots: *E. huacsaro* (Ruíz) Christ, *E. muelleri* (E. Fourn.) C. Chr., and *E. petiolatum* (Sw.) Urb. Our specimens do not belong to any of these three species, although they are very similar to *E. petiolatum*. To accommodate this morphotype , here we provide a description of this new species, along with a discussion of its morphological affinities within the genus, line drawings, spore micrographs, a distribution map, and other pertinent comments.

## MATERIALS AND METHODS

Before describing the new species, we searched the protologues, descriptions, type specimens, and the available spore images of the six species mistakenly associated with the new one (*Elaphoglossum crinipes* (=*E. setigerum)*, *E. gratum, E. petiolatum, E. pilosus, E. rubescens*, and *E. tectum*), and other similar species with resinous dots occurring in Central America and Mexico. Our description is based on the observation under a dissecting microscope of 23 specimens and three digital images, corresponding to 12 gatherings deposited at BIGU, F, MEXU, MO, NY, TEX, and US herbaria, and spore microphotographs.

For the spore examination, we selected six fertile specimens (*Jhonston J.R. 1346*, F; *Molina A. 30311*, F; *Standley 58933*, F; *Véliz M. 92.2319*, MEXU; *Williams O. 22688*, *22656*, US) and transferred three to five closed sporangia per specimen to an aluminum stub, previously covered with adhesive carbon tape, opened the sporangia, and scattered the spores on the stub surface with a dissection needle. Stubs were coated with a gold layer using a metallizer QUORUM (Q150R ES) for one minute, at 20 mA, with an accelerating voltage of 15.0kV. These samples were digitally imaged under the scanning electron microscope. The background and brightness of the images were edited with Adobe Photoshop (Fig. 1).

The line drawings were made based on *Veliiz 92.2319*, MEXU and *Molina 30311*, MO (Fig. 2). For the distribution map (Fig. 3) we convert the geocoordinates of all specimens examined into decimal degrees and generated the map with Rstudio (Racine, 2012). For those specimens lacking precise label data, geocoordinates were estimated using the Google Earth application and they are indicated in brackets.

## TAXONOMIC TREATMENT

***Elaphoglossum auralolitae*** Martínez-Becerril & A. Vasco, **sp. nov**. —HOLOTYPE: GUATEMALA. San Marcos: San Pedro Sac. [Sacatepéquez], 2300 m, [14°57'22.22"N, 91°48'54.27"W], 19 Aug 1992, *M. Véliz 92.2319* (holotype: MEXU 903674!; isotype: BIGU 0030067 [image seen]).

*Diagnosis.*—This species resembles *Elaphoglossum petiolatum* but differs by the longer and denser sterile and fertile laminar scales, especially those of the adaxial surface and abaxial costae, and those of the fertile petiole; and by the spores with spiny perispores.

**Plants** terrestrial. **Rhizomes** 3–3.5 mm wide, short creeping; **rhizome scales** 2.5–4 mm long, dense, spreading, linear-lanceolate to lanceolate, entire or with occasional short projections (shorter than the width of the scale), concolorous, dark reddish-brown to dark brown, lustrous, base round, apex attenuate to filiform-tortuous. **Sterile leaves** 13.5–39.5(–45) cm long, approximate; **phyllopodia** present, usually obscured by the rhizome scales, 5–10 mm long; **petiole** 5–15.5(–18) cm long, 1/3–2/5 the length of the sterile leaves; **larger petiole scales** to

3.5(–4) mm long, dense, usually imbricate, spreading to appressed, linear-lanceolate to ovate, with occasional short projections, stramineous to brown, usually darker at the apex, base round, apex attenuate to filiform-tortuous; **smaller petiole scales** to 0.3 mm long (difficult to see), dense, appressed, irregular or lanceolate, with numerous long projections (as long or longer than the width of the scale) or with occasional short projections, stramineous, base auriculate, apex attenuate, usually with a long projection close to the apex; **laminae** 8.5–24(–27) x 1.2–2.4 cm, linear-elliptic to elliptic, chartaceous, base cuneate, apex acute to acuminate; **adaxial laminar scales** 0.5–1.4 mm long, dense, usually imbricate, lanceolate, with numerous long projections, stramineous to whitish, darker at the point of attachment, base auriculate, apex long-attenuate to filiform, **adaxial resinous dots** absent; **abaxial laminar scales** to 0.5 mm long, scattered to dense, not imbricate, irregular to lanceolate, when lanceolate, with numerous long projections, stramineous to light brown, base auriculate, apex attenuate; **abaxial resinous dots** present; **abaxial costal scales** 0.8–2(–3) mm long, dense, usually imbricate, lanceolate with occasional short projections, stramineous, base round with long projections, apex long-attenuate to filiform; **marginal scales** 0.5–0.8(–1) mm long, dense, not imbricate, lanceolate or irregular, when lanceolate, margin with numerous long projections, stramineous, base truncate with long projections, apex attenuate to filiform-tortuous. **Fertile leaves** similar or sometimes longer than the sterile; **petiole** 1/3–3/5 the length of the fertile leaves; **laminae** 28.9–36.2 x 0.9–1 cm, linear-elliptic to elliptic, base cuneate, apex acute to acuminate; **petiole scales** 2–3.1 mm long, scattered to dense, not imbricate, deciduous, lanceolate to oblanceolate, with numerous long projections or with occasional short projections, stramineous, base round with long projections or truncate, apex long-attenuate to filiform; **adaxial scales** 1–1.5 mm long, dense, usually imbricate, lanceolate with numerous long projections, stramineous, base auriculate, apex long-attenuate to filiform; **adaxial costal scales** 0.5–1.5 mm long, scattered to dense, not imbricate, deciduous, lanceolate to ovate-lanceolate or irregular, with numerous long projections, yellowish, base truncate with long projections, apex long-attenuate to filiform; **intersporangial scales** not visible. **Spores** monolete, with nonperforated perines, broad discontinuous folds, and spiny perispore, spines ≤1µm. Figures 1 and 2.

*Distribution.*—Known only from the Sierra Madre Mountains in Guatemala, in the departments of Chimaltenango, Quetzaltenango, Quiché, San Marcos, Sacatepéquez, Sololá, and Totonicapán (Fig. 3).

*Ecology and Elevation.*—1950–2900 m in cloud forest.

*Etymology.*—This species is named in honor of Aura Lolita Chávez Ixcaquic (1972-), an indigenous feminist from the K'iche' region and community in Guatemala, who is the leader of the "Council of K'iche's People for the Defense of Life, Nature, Land, and Territory of Guatemala". She was nominated for the “Sakharov Prize for Freedom of Thought” (better known as “Sakharov Prize”) by the European Parliament and the winner of the “Ignacio Ellacuría Award” by the Basque Agency for Development Cooperation (https://mujeresbacanas.com/aura-lolita-chavez-ixcaquic-1972/).

*Notes.—Elaphoglossum auralolitae* most closely resembles *E. petiolatum,* because both species have resinous dots on the laminar surface abaxially and rhizome scales that are linear-lanceolate to lanceolate, dark-reddish brown to dark brown with filiform-tortuous apex. The two species can be distinguished by the adaxial laminae and abaxial costae scales of both sterile and fertile leaves, and by the spore ornamentation (see Table 1 for a comparison of both species). Both species occur in Guatemala and their distribution ranges overlap.

TABLE 1. Main morphological differences between *Elaphoglossum auralolitae* and *E. petiolatum*.

| | *Elaphoglossum auralolitae* | *Elaphoglossum petiolatum* |
|---|---|---|
| **Sterile leaves** | | |
| Adaxial laminar scales | Present, 0.5–1.4 mm long, dense, usually imbricate, margin with numerous long projections, apex long-attenuate to filiform | Sometimes present, when present ≤1mm long, scattered to dense, not imbricate, margin with occasional short or long projections, apex attenuate |
| Abaxial costal scales | 0.8–2(–3) mm long, dense, usually imbricate, lanceolate | 0.5–1.5 mm long, scattered to dense, not imbricate, lanceolate, substellate, or irregular |
| **Fertile leaves** | | |
| Petiole scales | 2–3.1 mm long, lanceolate to oblanceolate, margin with numerous long projections or with occasional short projections | ≤1 mm long, substellate or linear to linear-lanceolate, when linear or linear-lanceolate margin with occasional short projections |
| Adaxial laminar scales | 1–1.5 mm long, dense, usually imbricate | ≤0.7 mm long, scattered to dense, not imbricate |
| Abaxial longest costal scales | 1.5 mm long, apex long-attenuate to filiform | 0.8 mm long, apex acuminate to attenuate |
| Perispores | with spines (≤1μm) | with irregular deposits |
| **Distribution** | Western Guatemala | southern Mexico to Panama, and the West Indies |

There are five specimens of *Elaphoglossum petiolatum* from Guatemala, that are unusual for that species because they are scalier than the typical (*Steyermark 47116* F, US; *Standley 86178, 85402* F, US; *Kellerman 5592 US*; and *Williams 22175* F, US). These specimens were not considered in Table 1 and they are very hard to distinguish from *E. auralolitae*. We identified them as *E. petiolatum* because their adaxial laminar scales are less dense than what is typical for *E. auralolitae* and because the spores of the only fertile collection (*Williams 22175* F), have irregular deposits instead of small spines, agreeing with our concept of *E. petiolatum* (Martinez-Becerril, Vasco, and Solano, 2021). The distribution of these five specimens overlaps with that of *E. auralolitae*, this and their great morphological similarity  could be evidence of possible events of hybridization between these two species.

*Elaphoglossum auralolitae* can also be confused with *E. rufescens*, a Mexican species with abundant scales on the laminae and costae (Mickel and Smith, 2004), from which it differs by the presence of resinous dots (vs. absent in *E. rufescens*); the dense, usually imbricate petiole scales (vs. scattered, not imbricate); the lanceolate, dense, usually imbricate adaxial laminar scales (vs. round to subdeltate, scattered, not imbricate); and the irregular to lanceolate, stramineous to light brown abaxial laminar scales (vs. narrow to deltate, orange).

From the other two species with resinous dots in the flora of Guatemala, *Elaphoglossum auralolitae* differs from *E. huacsaro* because it has short-creeping rhizomes with dark-reddish brown to dark brown scales, instead of the erect or ascending rhizomes with black scales typical of *E. huacsaro* (Vasco, Moran, and Rouhan, 2009a). From *E. muelleri* the new species differs because of its dark-reddish brown to dark brown rhizome scales and irregular to lanceolate abaxial laminar scales, instead of the orange rhizome scales and stellate abaxial laminar scales of *E. muelleri* (Mickel and Smith, 2004).

Specimens of *Elaphoglossum auralolitae* have also been previously identified as *E. crinipes* (= *E. setigerum*), *E. gratum, E. pilosum,* and *E. tectum*. It is easy to distinguish all these species from *E. auralolitae* because they do not have resinous dots in their sterile laminae abaxially.

All known collections of this new species are from Guatemala in the departments of Chimaltenango, Quetzaltenango, Quiché, San Marcos, Sacatepéquez, Sololá, and Totonicapán. These departments are part of the Sierra Madre Mountains, also called Sierra Madre of Chiapas or the Cordillera Central. This cordillera crosses Guatemala from West to East and is considered the longest mountain range in Central America ranging from southern Mexico to Honduras along 600 km (Pérez-Irungaray *et al.,* 2018).

The first collection of the new species described here (*Johnston 1346,* F) was made in 1938 by the American botanist John Robert Johnston (1880–1953), who worked at Harvard and published the Catalog of Plants from Guatemala (1938). Besides this first collection, there are only eleven known gatherings of this species to date. Fertile specimens were collected mainly during the months of December and January. The most recent record was made in 1992 by Guatemalan botanist Mario Véliz (*Véliz 92.2319,* BIGU, MEXU) and because the MEXU specimen was the first specimen we detected as a probable new species, we decided to choose it as the holotype. This specimen shows all the plant structures, including a fertile lamina.

Although we have not included *Elaphoglossum auralolitae* in any molecular phylogenetic analyses, its distribution, overall morphology, and spore's ornamentation suggest that it belongs to the Mexican *petiolatum* Clade of Vasco, Moran, and Rouhan (2009b).

*Additional specimens examined.* —GUATEMALA. Chimaltenango: [14.6743667°, -90.8210861°], 21 Dec 1972, *Williams 41580* (F). Pacaya, [14.4028667°, -90.6071444°], 20 Aug 1938, *Johnston 1346* (F). Quetzaltenango: [14.8317528°, -91.5001972°], 15 Jan 1941, *Standley 83423* (F); Sierra Madre Mountains, about 4 km N of Olintepeque, [14.9579111°, -

91.5314889°], elev. 2600 m, 12 Dec 1962, *Williams 22861* (F, US, TEX [image seen]). Quiché: San Tomás Chichicastenango, on moist forest bank of El Molino, 4 km from Chichicastenango, [14.9218889°, -91.2650028°], elev. 2100 m, 14 Jan 1974, *Molina 30311* (F, MO). Sacatepéquez: Cuesta de las cañadas, above Antigua, [14.5881028°, -90.7383306°], elev. 1950 m, 06 Dec 1938, *Standley 58933* (F). San Marcos: Comitancillo, below Comitancillo and Santa Rosa junction, Sierra Madre Mountains, [15.0650528°, -91.7698472°], elev. 2900 m, 02 Jan 1965, *Williams 27065* (F, NY); San Pedro Sacatepéquez, [14.9561722°, -91.815075°], elev. 2300 m, 19 Aug 1992, *Véliz 92.2319* (MEXU, BIGU [image seen]); volcán Tacaná, dry white clay slopes on ridge leading towards Volcán Iacana, but on this side of sibinal, vicinity of Sibinal [interpreted], [15.1425333°, -92.085925°], 18 Feb 1940, *Steyermark 35963* (F, US). Sololá: Sololá, [14.7606694°, -91.1788194°], elev. 2400 m, 22 Apr 1974, *Copeland s.n.* (NY). Totonicapán: Ravine in cypress forest near Polagua, Sierra Madre Mountains, 20–25 km. north of Cristobal, [15.0133583°, -91.4976361°], 05 Dec 1962, *Williams 22688* (F, US, TEX [image seen]); ravine in cypress forest near Pologuá, Sierra Madre Mountains, 20–25 km. north of Cristobal, [15.0182806°, -91.5043611°], 05 Dec 1962, *Williams 22656* (F, US, TEX [image seen]).

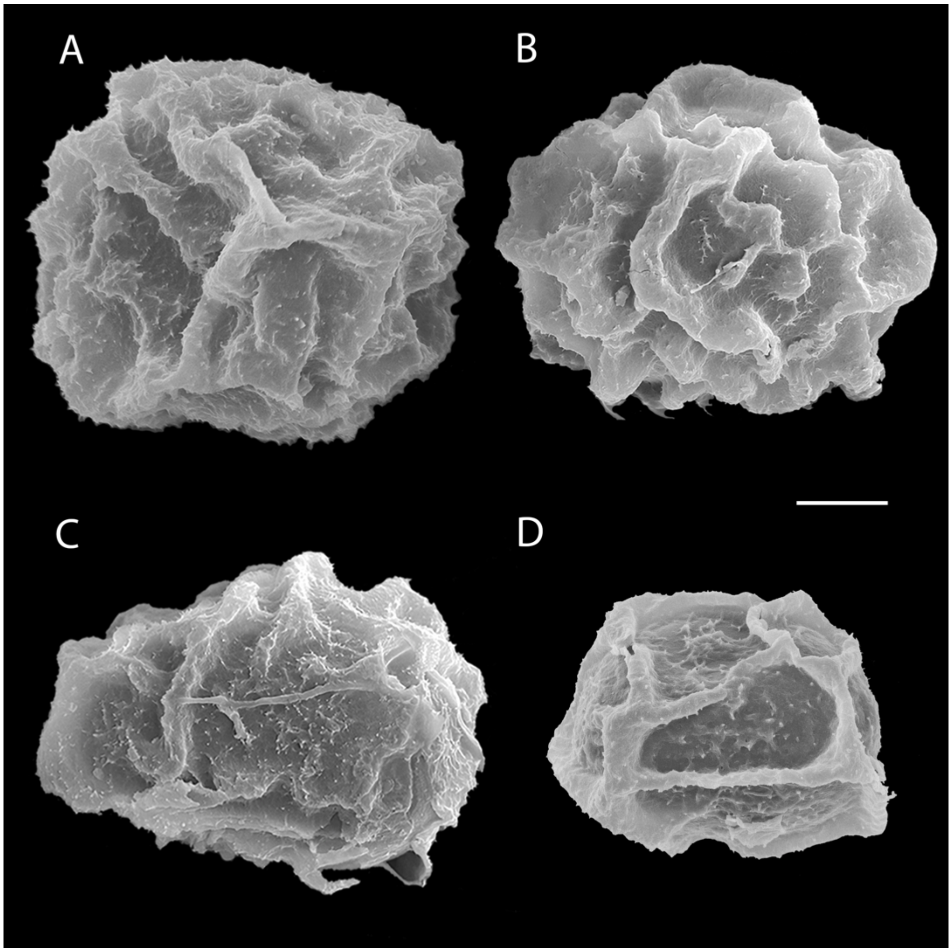

FIG. 1. Spores of *Elaphoglossum auralolitae.* **A.** *Molina 30311*, MO. **B.** *Williams 22656*, US. **C.** *Johnston 1346*, F. **D.** *Véliz 92.2319*, MEXU. Scale bar = 10µm.

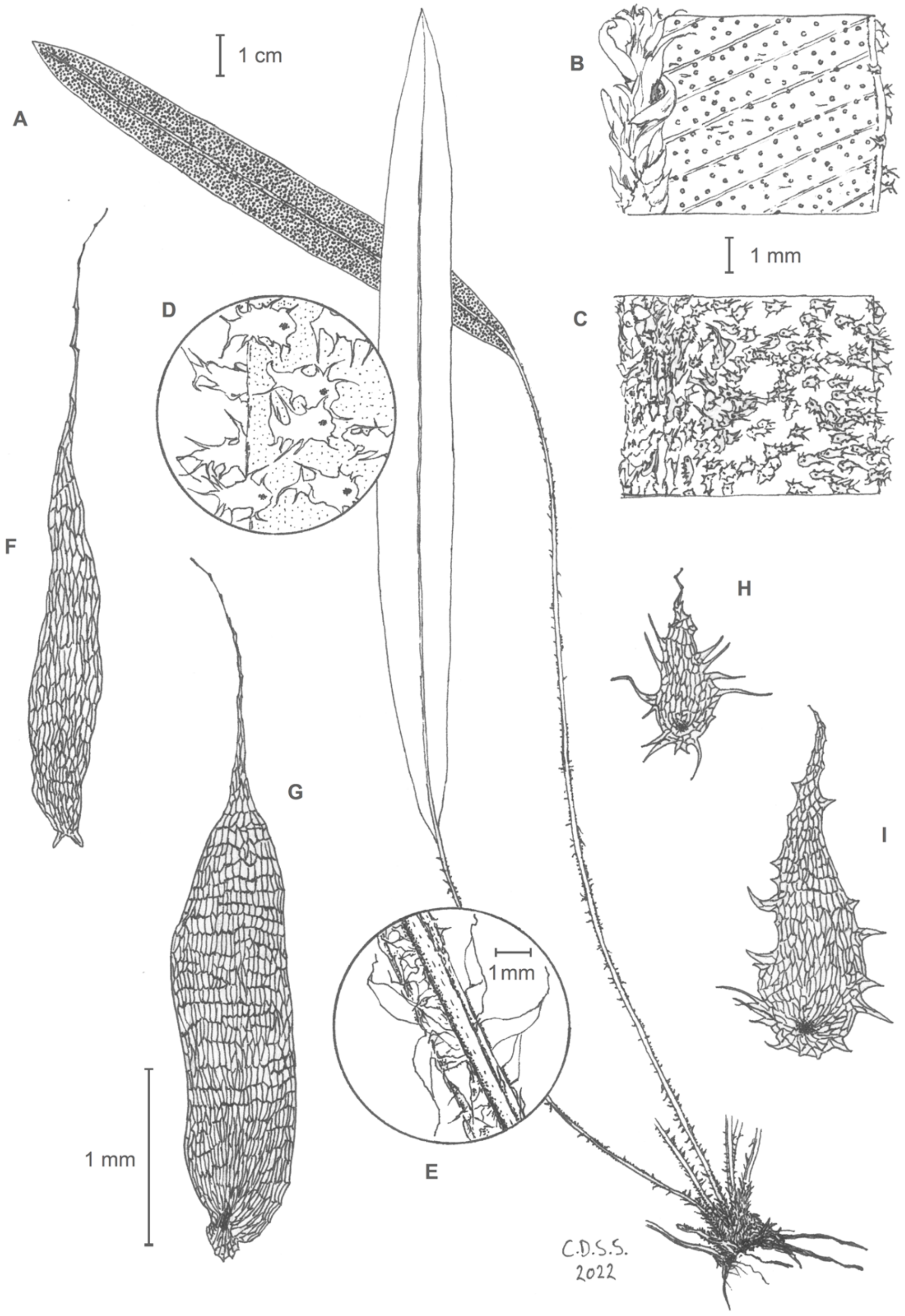

FIG. 2. *Elaphoglossum auralolitae.* **A.** Habit. **B.** Abaxial lamina detail. **C.** Adaxial lamina detail. **D.** Margin scales close-up. **E.** Petiole detail. **F.** Rhizome scale. **G.** Petiole scale. **H.** Adaxial laminar scale. **I.** Costal scale (**A–E** *Véliz 92.2319*, MEXU, **F–I** *Molina 30311*, MO).

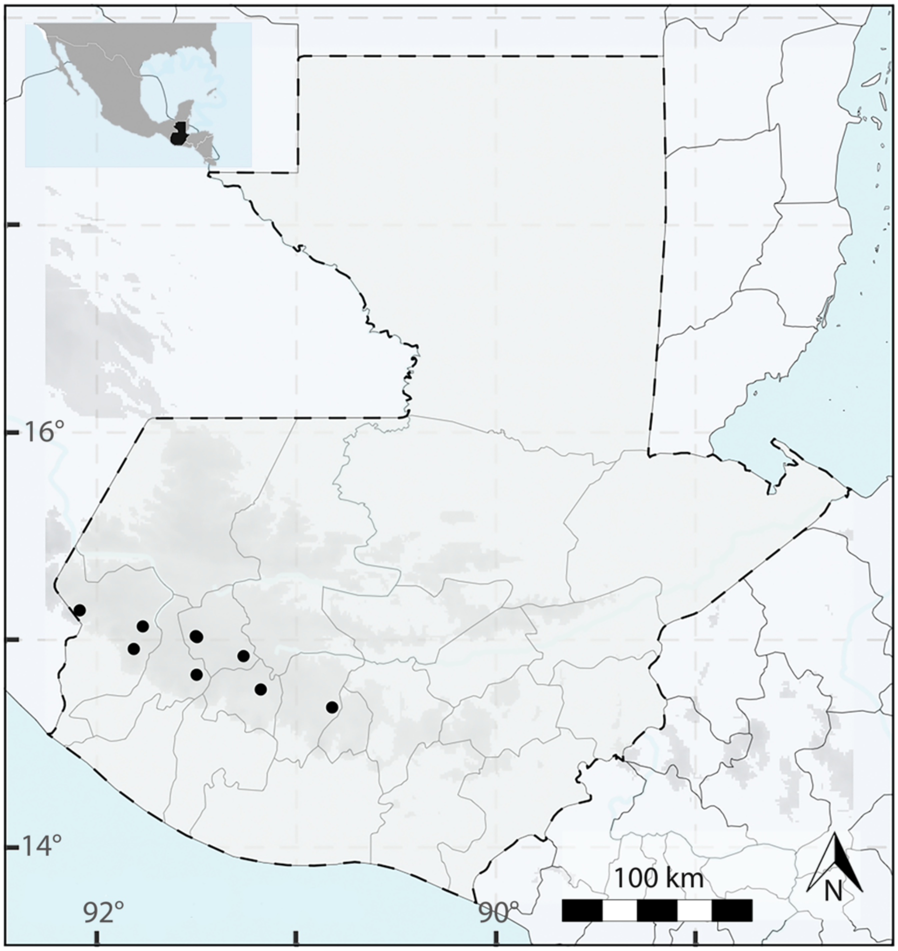

FIG. 3. Distribution map of *Elaphoglossum auralolitae.*

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# DISCUSIÓN GENERAL

La primera parte de esta investigación se centró en responder las siguientes preguntas: ¿el complejo de *Elaphoglossum petiolatum* es un grupo monofilético? de ser así, ¿cuántas y cuáles son las especies que pertenecen a él? y si no lo es, ¿dónde se recuperan las especies que lo integran? ¿Existe evidencia morfológica que sustente la posición filogenética de estas especies? ¿Se sigue recuperando el "clado mexicano" después de incrementar el muestreo de especies mexicanas en la filogenia? Para ello, se llevaron a cabo dos análisis filogenéticos moleculares que incluyeron 9 de las 11 especies del complejo, 59% de las especies reportadas para México y 28.5% del total de las especies reconocidas dentro del género, además de una secuencia de *E. petiolatum* (Sw.) Urb., proveniente de Jamaica, localidad tipo de la especie (Swartz, 1778; Proctor, 1985). Es así como nuestros análisis incluyen la mejor representación hasta el momento, de las especies del complejo y de las especies reportadas para México, en la filogenia general de *Elaphoglossum*.

Las topologías obtenidas recuperaron las siete secciones propuestas previamente para *Elaphoglossum* (Mickel y Atehortúa, 1980; Rouhan et al., 2004; Vasco et al., 2009; Lóriga et al., 2014) y mostraron que el complejo de *E. petiolatum* no es monofilético. Las especies que originalmente integraban este complejo se recuperaron, junto con otras especies, en tres clados no hermanos, denominados aquí como "clado de *E. ciliatum*", "clado de *E. lancifolium*" y "clado de *E. petiolatum*" (= "clado mexicano de *E. petiolatum*" por Vasco et al., 2009). Las secuencias de *E. petiolatum* de Jamaica se recuperaron como parte del clado de *E. petiolatum*, y ninguna otra de las especies mexicanas incluidas se recuperó en alguno de los tres clados aquí descritos.

Las especies que hacen parte de los clados de *E. ciliatum, E. lancifolium* y *E. petiolatum* presentan una morfología similar pues comparten características como las escamas planas y/o estrelladas en las láminas y esporas con perisporas espinosas. Además, algunos taxones presentan puntos resinosos en la superficie abaxial de las láminas. Las escamas planas y/o estrelladas en láminas son típicas en las especies de la sec. *Lepidoglossa* (Mickel, 1985; Mickel y Atehortúa, 1980; Kessler et al*.*, 2018) y las perisporas espinosas son una característica distintiva de algunos de los subclados de esta sección (Moran et al., 2007; Vasco et al., 2009; Martínez-Becerril et al., 2021). Los puntos resinosos no están presentes en todas las especies de estos clados, sin embargo, todas las especies de *Elaphoglossum* que los presentan, conocidas hasta el momento, se recuperaron en alguno de estos tres grupos. Lo anterior revela que caracteres morfológicos utilizados en la definición del complejo, como la presencia de puntos resinosos y perisporas con espinas*,* son homoplásicos (Capítulo I).

En este trabajo detectamos que algunas características, como el tamaño y la densidad de las espinas de las esporas, la presencia y densidad de las escamas del rizoma y de la lámina y la forma de los ápices de la lámina, así como la distribución geográfica, son útiles para diferenciar a los tres clados aquí nombrados, lo que los convierte en grupos morfológica y geográficamente diagnosticables. A continuación, se describe brevemente cada uno:

El clado de *Elaphoglossum ciliatum* integra diez especies que se distribuyen desde México y Las Antillas, hasta el sureste de Brasil y la isla volcánica de Santa Elena en el Océano Atlántico (Vasco et al., 2009). Tres de las diez especies, *E. bifurcatum* (Jacq.) Mickel*, E. dimorphum* (Hook. & Grev.) T. Moore y *E. nervosum* (Bory) Christ, formaban parte del complejo original de *E. petiolatum* (Mickel, 2001) y nueve de éstas fueron tratadas previamente en una monografía por Vasco et al. (2009a). La presente investigación confirmó que una décima especie hace parte de este clado: *E. palmeri* Underw. & Maxon, reportada para Cuba y República Dominicana (Lóriga et al., 2014).

Este grupo de especies, además de presentar los puntos resinosos en las láminas, comparten los rizomas negros con exudado brillante semejante a la laca cuando están secos y esporas con espinas ≥1µm de alto.

El clado de *Elaphoglossum lancifolium* contiene al menos ocho especies con distribución pantropical (México, Centro y Sudamérica, África y el sureste de Asia, incluyendo India). Dos de las ocho especies de este clado, *E. blumenaum* (Fée) J. Sm. y *E. lancifolium* (Desv.) C.V. Morton (anteriormente *E. salicifolium*), fueron previamente reconocidas como miembros del complejo *E. petiolatum* por Mickel (2001).

En general, las especies de este clado se caracterizan por la presencia de puntos resinosos (excepto *E. tenuiculum* (Fée) T. Moore ex C. Chr. y *E. ipshookense* Mickel), ápices de láminas atenuadas a largas atenuadas, escamas blanquecinas y abundantes en la superficie adaxial de las láminas, y esporas con espinas dispersas de1µm de altura (Moran et al., 2007; www.plantsystematics.org).

El clado de *Elaphoglossum petiolatum* es el grupo que Vasco et al. (2009b) denominaron como "clado mexicano de *E. petiolatum*" pero en este trabajo lo renombramos porque encontramos que la secuencia del espécimen de *E. petiolatum* de Jamaica, localidad tipo de esta especie (Christenhusz 3226, NY) se recupera en este grupo (Swartz, 1778; Proctor, 1985). El clado está integrado por siete especies que se distribuyen desde México hasta Panamá y en las Antillas, pero tres son endémicas de México (Mickel y Smith, 2004; Capítulo IV).

Las especies que fueron consideradas parte del complejo original descrito por Mickel (2001) son: *E. petiolatum, E. potosianum* Christ, *E. pringlei* (Davenp.) C. Chr. y *E. rzedowskii*

Mickel. Las otras tres son *E. schiedei, E. schmitzii*, y una tercera, que inicialmente se determinó como *E. potosianum* (*Carranza 2036,* MEXU, IEB) pero que posteriormente, con base en nuestros análisis moleculares y en la morfología del ejemplar se determinó que pertenece a una especie no descrita.

Las especies de este grupo presentan, además de puntos resinosos en las láminas, escamas del rizoma con ápices agudos a filiformes, y esporas con espinas ≤1µm (excepto *E. petiolatum* que presenta esporas con depósitos irregulares) (Martínez-Becerril et al., 2021; Capítulo I).

Los marcadores del cloroplasto empleados en esta investigación no resuelven por completo las relaciones interespecíficas dentro de los tres clados mencionados, principalmente dentro del clado de *E. petiolatum,* no obstante, permitieron comprobar que: 1) el complejo de *Elaphoglossum petiolatum* no es un grupo natural, 2) el clado de especies mexicanas (=clado de *E. petiolatum*) se mantiene como monofilético, 3) el representante de *E. petiolatum* de Jamaica pertenece al clado de *E. petiolatum* y 4) el clado de *E. petiolatum* está integrado por dos subclados, los cuales no comparten especies (Capítulo I).

Una vez comprobada la monofilia del clado de *Elaphoglossum petiolatum,* la segunda parte de esta investigación consistió en llevar a cabo la monografía de este grupo. Si bien la evidencia molecular empleada en este trabajo no permite delimitar las especies que integran este clado, nos permitió reconocer dos subclados como parte del grupo. Estos subclados no comparten especies pues las secuencias de un mismo taxon se recuperaron sólo en uno de los dos subclados. Lo anterior, junto con la evidencia morfológica recabada a partir de la revisión de ejemplares herborizados y esporas (Capitulo II), nos permitió diferenciar y delimitar ocho taxones dentro del clado de *E. petiolatum* en lugar de los siete sugeridos con base en la filogenia molecular. Estos taxones son: *E. auralolitae* Martínez-Becerril & Vasco sp. nov. (Capítulo IV)*,* *E. muelleri* (E. Fourn.) C. Chr.*, E. petiolatum, E. potosianum, E. pringlei* (Davenp.) C. Chr., *E. rzedowskii* Mickel*, E. schiedei* (Kunze) T. Moore y *E. schmitzii* (Mett. *ex* Kuhn) Martínez-Becerril & Vasco (Capítulo III).

*Elaphoglossum pringlei* y *E. rzedowskii* se consideraban variaciones de una misma especie (Mickel y Smith, 2004), sin embargo, sus secuencias se recuperaron en clados diferentes y el análisis de la morfología de las esporas encontró que las esporas de *E. pringlei* son significativamente más grandes (40.4 µm en el diámetro ecuatorial y 31.6 µm en el diámetro polar que en el resto de las especies del complejo (en promedio 31.2 µm y 28.5 µm en el diámetro ecuatorial y polar, respectivamente). Lo anterior sugiere que probablemente *E. pringlei* es una especie poliploide y respalda la hipótesis molecular que indica que *E. pringlei* y *E. rzedowskii* son taxones independientes (Martínez-Becerril, et al., 2021; Capítulos I y II).

*Elaphoglossum schmitzii* y *E. schiedei* estaban tratados como sinónimos de *E. petiolatum,* no obstante, las secuencias de *E. schmitzii,* se recuperaron en un subclado y las de *E. schiedei* +*E. petiolatum* en otro. Las diferencias entre *E. schiedei* y *E. petiolatum* son el tamaño, la densidad, la forma y el ápice de las escamas del rizoma: de 0.7–1.2(–1.5), dispersas, adpresas, oblanceoladas con ápices agudos a cortamente filiformes (vs. escamas del rizoma de 1.5–3.7 (–5), densas, no adpresas, linear a lanceoladas con ápices filiforme-tortuouso en *E. petiolatum*) y por la ornamentación de sus esporas (espinosa en *E. schiedei* y con depósitos irregulares en *E. petiolatum*) (Capítulo II).

*Elaphoglossum auralolitae* y *E. muelleri* no están incluidas en los análisis moleculares del Capítulo I, sin embargo, consideramos que también hacen parte del complejo de *E.*

*petiolatum* ya que la evidencia morfológica de las plantas y las esporas, y la distribución geográfica de los ejemplares respalda esta hipótesis.

La evidencia molecular y morfológica aportada en este trabajo, modifica la circunscripción del taxón *Elaphoglossum petiolatum,* pues, aunque el nombre era utilizado ampliamente para identificar ejemplares con escamas del rizoma oscuras y puntos resinosos en la superficie abaxial que habitaban los trópicos americanos, desde México y Las Antillas hasta Perú y Brasil (Mickel 2001), nuestros resultados señalan que *E. petiolatum* solo se distribuye de México a Centroamérica y en Las Antillas (Capítulos I y III). Los ejemplares sudamericanos identificados como *E. petiolatum*, además de pertenecer al clado de *E. lancifolium,* presentan esporas con espinas en lugar de depósitos irregulares, lo que sugiere que corresponden con otra especie y necesitan renombrarse.

La monografía resultante de esta investigación incluye una clave de identificación, sinonimia completa, descripciones detalladas, índice de colectores y números de colección, ilustraciones, imágenes al microscopio electrónico de esporas y mapas de distribución para todas las especies. Se designaron siete lectotipos y se llevó a cabo una combinación nomenclatural nueva.

Finalmente, la descripción de *E. auralolitae* como una especie nueva se llevó a cabo debido a que, durante la revisión de los ejemplares de herbario, identificamos 23 especímenes de Guatemala con puntos resinosos, que no se ajustaban a ninguna de las especies conocidas hasta el momento. De acuerdo con la riqueza de *Elaphoglossum* en Guatemala, calculada en 41 especies, solo tres presentan puntos resinosos en las láminas: *E. huacsaro* (Ruíz) H. Christ, *E. muelleri* y *E. petiolatum* (Mickel 1981, 1995a, Mickel y Smith 2004) y los ejemplares encontrados no corresponden con ninguna de éstas (Capítulo IV).

# CONCLUSIONES GENERALES

- El complejo de *Elaphoglossum petiolatum* es polifilético.
- Las especies del complejo de *E. petiolatum* se recuperan en tres clados morfológicamente diagnosticables pero que no son hermanos: clado de *E. ciliatum,* clado de *E. lancifolium* y clado de *E. petiolatum.*
- Todas las especies de *Elaphoglossum* que presentan puntos resinosos se recuperan en alguno de los tres clados aquí descritos.
- Las secuencias del taxón *E. petiolatum* provenientes de Jamaica, la localidad tipo de esta especie, se recuperan dentro del clado de *E. petiolatum*.
- Los marcadores moleculares del cloroplasto empleados en este estudio no resuelven las relaciones interespecíficas dentro del clado de *E. petiolatum.* No obstante, la morfología, (incluida la de las esporas) permite reconocer y separar ocho especies como parte de este clado.
- *Elaphoglossum petiolatum* se distribuye exclusivamente de México a Panamá y en Las Antillas.

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