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Polyphyly of *Mesadenus* (Orchidaceae, Spiranthinae) and a New Genus from the Espinhaço Range, Southeastern Brazil

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Abstract—*Mesadenus* displays an odd disjunction, with most species found in the southeastern USA, the Caribbean, and Mexico south to El Salvador, and two outliers occurring in southeastern Brazil. A recent molecular phylogenetic study showed that, as currently delimited, *Mesadenus* is polyphyletic, and further study of this little-known genus is required. Here we assessed relationships in *Mesadenus* and to other genera conducting a cladistic parsimony analysis of nucleotide sequences of nuclear (ITS) and plastid (*matK-trnK*, *trnL-trnF*) DNA for an enlarged sample of taxa, including five out of six currently accepted species of *Mesadenus* and representatives of nearly all (36 out of 41) genera of Spiranthinae, plus suitable outgroups. A detailed comparison of vegetative and floral attributes of relevant taxa was also conducted. Our results confirm the polyphyly of *Mesadenus* and support the creation of a new genus, ***Espinhassoa***, to include Brazilian '*Mesadenus*' *glaziovii* and '*M.*' *rhombiglossus* (as ***Espinhassoa glaziovii*** and ***E. rhombiglossa***, respectively). An identification key, descriptions, and illustrations of the two species herein included in ***Espinhassoa*** are provided.

Keywords—Endemism, *Espinhassoa*, floral morphology, leaf morphology, molecular phylogenetics.

Orchidaceae subtribe Spiranthinae Lindl. comprises about 41 genera and 520 species of primarily geophytic, Neotropical orchids, except for the widespread, chiefly North American genus *Spiranthes* Rich. Recent molecular phylogenetic analyses have shown that, with a few adjustments such as the exclusion of *Galeottiella* Schltr. and *Discyphus* Schltr., Spiranthinae are a strongly supported monophyletic group (see Salazar et al. 2018 and references therein). There are two main centers of diversity of Spiranthinae, one in Mexico and the other in southeastern Brazil (Balogh 1982; Salazar 2003b; Salazar et al. 2018). About 24 genera have been recorded in each of these countries; 11 such genera are shared by Mexico and Brazil, whereas 11 Brazilian genera are absent from Mexico, and 11 Mexican genera are in turn absent from Brazil (data based on Soto et al. 2007 for Mexico and De Barros et al. 2013 for Brazil). Nearly all of the shared genera, such as *Brachystele* Schltr., *Cyclopogon* C.Presl, *Pelexia* Poit. ex Lindl., *Sacoila* Raf., and *Sarcoglottis* C.Presl, are widespread in the Neotropics and show a more or less continuous distribution from Mexico to southeastern South America (see Salazar 2003b).

A notable exception is *Mesadenus* Schltr., a genus of six species displaying an odd geographical disjunction: four of them are found in the southeastern USA (Florida), the Caribbean (Bahamas, Puerto Rico, Hispaniola), and northern Mesoamerica (Mexico south to El Salvador), and the two remaining species occur in southeastern Brazil (Salazar 2003b). Such a disjunction, which spans over 6000 km in a straight line, is not an artifact of undercollecting, as comprehensive orchid inventories have been conducted in intermediary countries and regions (e.g. Peru: Dodson 1993; Ecuador: Dodson 1999; Costa Rica: Pupulin 2002; Central America: Szlachetko et al. 2005; Colombia: Dueñas and Fernández-Alonso 2009; Panamá: Bogarín et al. 2014). No other genus of Spiranthinae shows a similar disjunction, and the only two species of this subtribe shared by the two areas, namely *Sacoila lanceolata* (Aubl.) Garay and *Eltroplectris calcarata* (Sw.) Garay & H.R.Sweet, are widespread in the Neotropics.

Morphologically, *Mesadenus* is characterized by its small flowers with a narrow floral tube, flared floral segment apices, undivided labellum hardly distinct from other perianth parts, clavate column, and, especially, oval viscidium located near the middle of the ventral surface of the pollinarium (Schlechter 1920; Garay 1982; Szlachetko and Rutkowski 2000; Salazar 2003b; Figs. 1A–F, 2B–M, 3F, G, J). However, a recent phylogenetic analysis of nuclear and plastid DNA sequences that included nearly all the genera and over one-third of the species of Spiranthinae (Salazar et al. 2018) showed that, as currently delimited, *Mesadenus* is polyphyletic. The two Mesoamerican/Caribbean species included in that analysis, *M. lucayanus* (Britton) Schltr. and *M. polyanthus* (Rchb.f.) Schltr., are nested in the mainly North American '*Spiranthes* Rich. clade' with strong bootstrap support, whereas southeastern Brazilian *M. glaziovii* (Cogn.) Schltr. is in turn strongly supported as the earliest-diverging branch of the chiefly South American '*Stenorrhynchos* Rich. ex Spreng. clade' (Salazar et al. 2018: Figs. 3, 4).

A centrally placed viscidium is also found in the distantly related *Funkiella minutiflora* (A.Rich. & Galeotti) Salazar & Soto Arenas, *Schiedeella affinis* (C.Schweinf.) Salazar (both Spiranthinae), and *Galeottiella sarcoglossa* (A.Rich. & Galeotti) Schltr. (Galeottiellinae; Salazar 2003a, 2003b; Salazar et al. 2002, 2018), which suggests that similarities in floral morphology among these taxa, and between the northern and

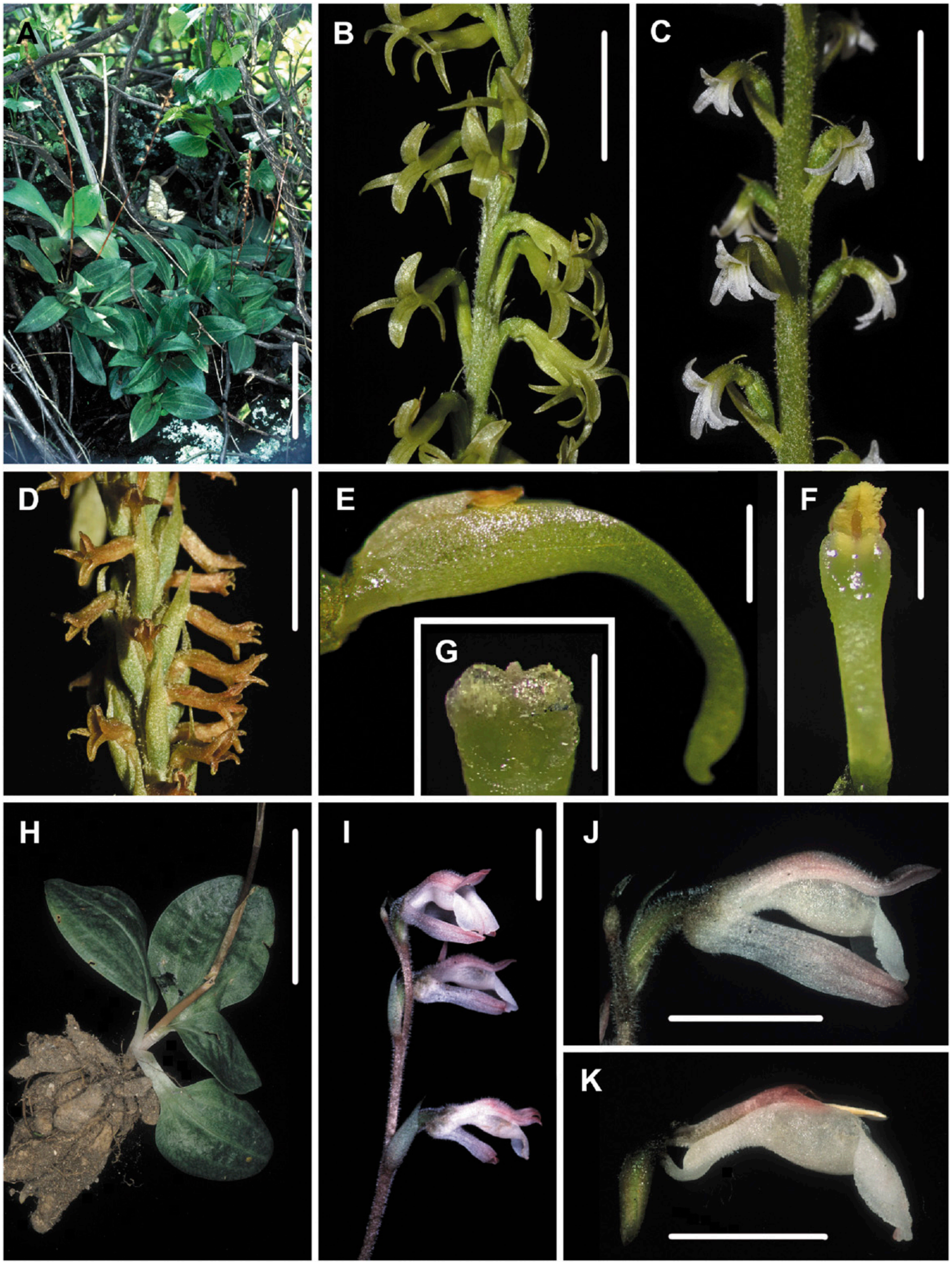

FIG. 1. *Mesadenus* species (various plants from Mexico). A. Clump of plants of *M. polyanthus*, some of them in fruit, living in a soil pocket on basaltic rock in Mexico City (*Salazar 6370*). B. Close-up of inflorescence of *M. lucayanus* (*Salazar 9518*). C. Close-up of inflorescence of *M. tenuissimus* (*Salazar 9855*). D. Close-up of inflorescence of *M. chiangii* (*Hernández-López et al. 416*). E. Flower of *M. lucayanus* with the sepals and petals excised to show the natural position of the labellum and column (*Salazar et al. 6593*). F. Column of previous flower. G. Column apex from below after removal of the pollinarium, showing the small, apicule-like rostellum remnant. H. Plant of *Schiedeella nagelii* removed from soil (*Salazar 6870*). I. Inflorescence of another plant (*Salazar et al. 6071*). J. Close-up of flower from side. K. Flower with the sepals and petals excised to show the natural position of the labellum and column. Scale bars: A = 10 cm; B–D, I–K = 1 cm; E, F = 1 mm; G = 500 µm; H = 5 cm. Photographer: Gerardo A. Salazar.

southern *Mesadenus* species, may represent convergence. Recurrent homoplasy of floral characters has been shown to occur in other groups of Spiranthinae that display similar floral morphology but are distantly related according to molecular data and other sources of evidence (Salazar et al. 2011). Moreover, molecular phylogenetic analysis and morphological comparisons revealed a close relationship between Mesoamerican/Caribbean *Mesadenus* and species

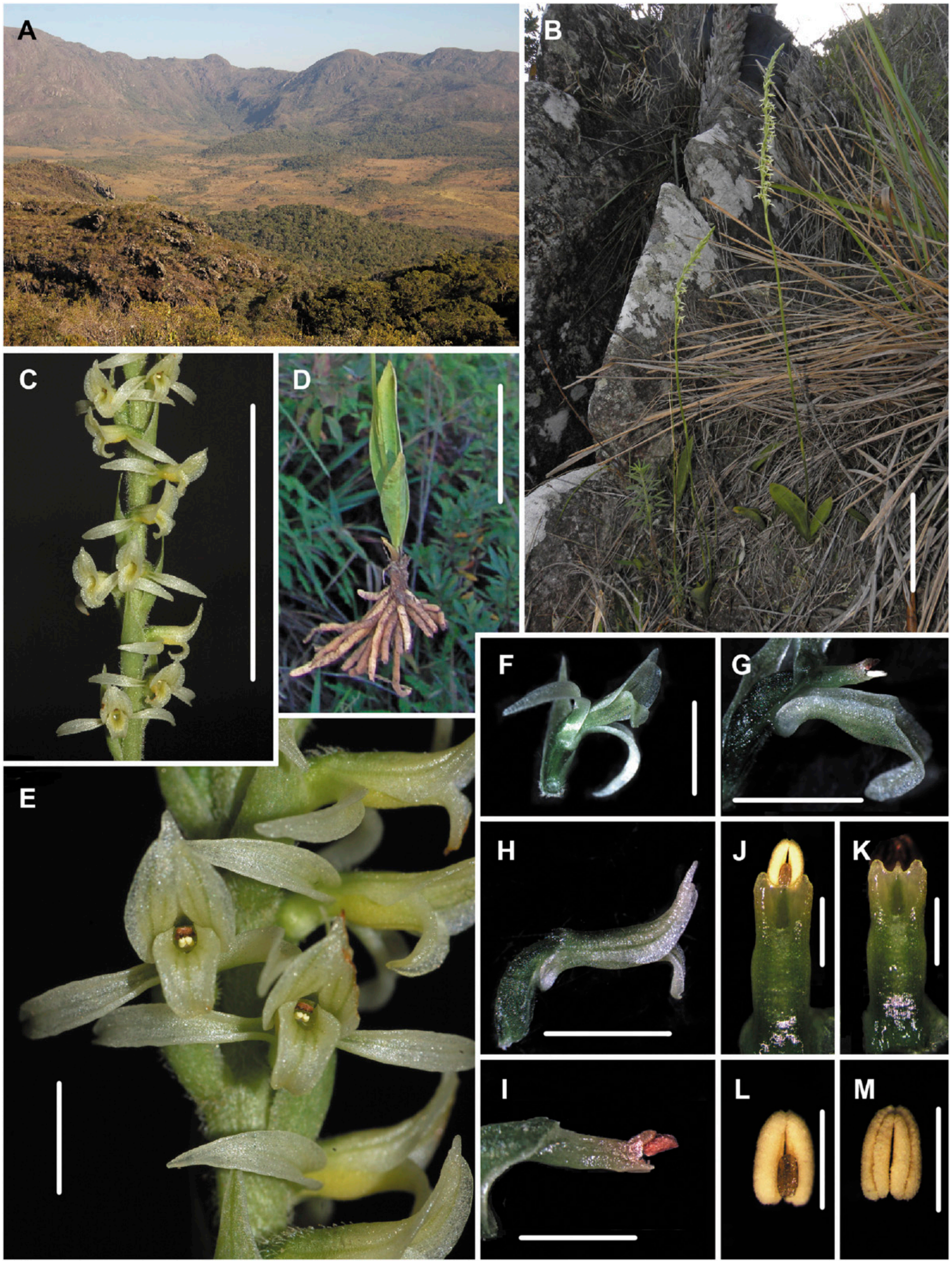

FIG. 2. *Espinhassoa glaziovii* (*Batista et al. 3158*). A. Habitat of *E. glaziovii* in the campo rupestre of the Serra do Caraça, Minas Gerais, Brazil. B. Two flowering plants in situ. C. Inflorescence. D. Plant removed from soil showing the ascending leaves and the fascicle of roots. E. Close-up of flowers. F. Flower, oblique view. G. Flower with sepals and petals excised to show the labellum and column. H. Flower from side with the lateral sepals excised to show the basal mentum. I. Column from side showing the apical anther and the prominent column foot. J. Column from below prior to removal of the pollinarium. K. Column from below after removal of the pollinarium. L. Pollinarium from below. M. Pollinarium from above. Scale bars: B = 10 cm; C, D = 5 cm; E = 3 mm; F–I = 5 mm; J–M = 1 mm. Photographer: J. A. N. Batista.

previously included in Mesoamerican/Caribbean *Schiedeella* Schltr. (Salazar et al. 2016, 2018). All the above indicates the need of a thorough assessment of relationships in these groups.

In this study, we assess the phylogenetic relationships of *Mesadenus* analyzing the same DNA regions and extensive generic sampling of Salazar et al. (2018), but including additional accessions of *M. lucayanus* and *M. glaziovii*, two

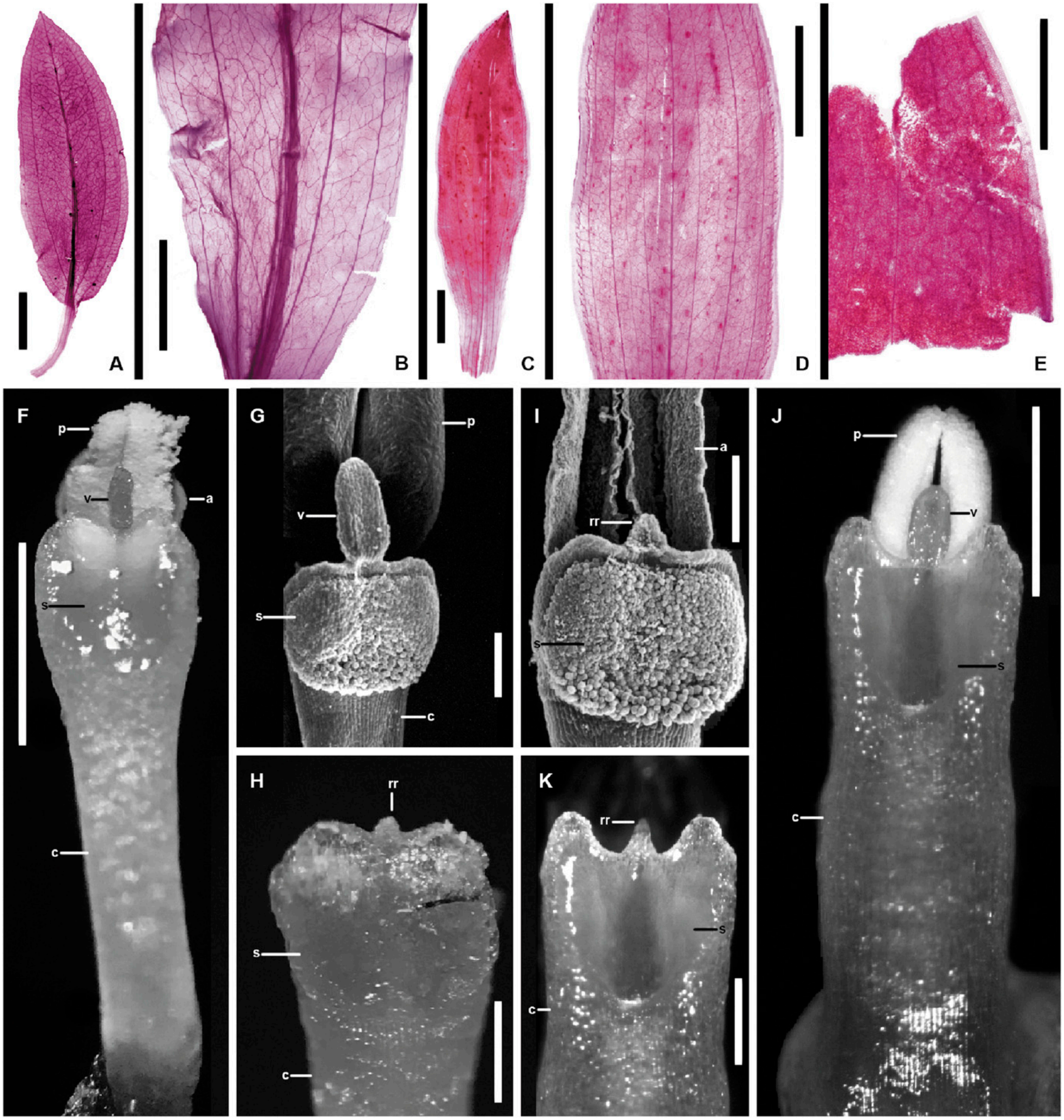

FIG. 3. Morphology of selected Spiranthinae. A. Diaphanized leaf of *Mesadenus polyanthus*. B. Detail of previous leaf. C. Diaphanized leaf of *Sacoila lanceolata*. D. Detail of previous leaf. E. Diaphanized leaf fragment of *Espinhassoa glaziovii*. F. Ventral view of column of *M. lucayanus* prior to removal of the pollinarium. G. SEM micrograph of column apex of *M. lucayanus* prior to removal of the pollinarium. H. Column apex of *M. lucayanus* after removal of the pollinarium. I. SEM micrograph of column apex of *M. lucayanus* after removal of the pollinarium. J. Ventral view of column of *E. glaziovii* prior to removal of the pollinarium. K. Column apex of *E. glaziovii* after removal of the pollinarium. Scale bars: A–E = 1 cm; F, J = 1 mm; G–I, K = 500 μm. Abbreviations: a = anther; c = column; p = pollinium; rr = rostellum remnant; s = stigma; v = viscidium. Photographers: A–E. Laura Calvillo-Canadell; F, H. Gerardo A. Salazar; G, I. Coyolxauhqui Figueroa; J–K. João A. N. Batista. (A, B from *Salazar 6370*; C–D from *Batista et al. 3158*; E, J, K from *Batista et al. 3158*; F, H from *Salazar 9518*; G, I from *Salazar et al. 6714*).

additional species of Mexican *Mesadenus* not analyzed previously, *M. chiangii* (M.C.Johnt.) Garay and *M. tenuissimus* (L.O.Williams) Garay, and three previously unsampled species of *Schiedeella*. We also conduct a comparison of the vegetative and floral morphology of the taxa concerned. Our goals are to determine the limits and species composition of *Mesadenus* as a basis for a revised taxonomy, with an emphasis on the little-known Brazilian species, and achieve a better understanding of the evolution of vegetative and floral morphology in the concerned taxa.

Materials and Methods

Taxon Sampling for Molecular Analysis—We analyzed exemplars of 83 species of Spiranthinae, which represent 37 out of the 41 genera accepted by Chase et al. (2015). Two accessions each of *Mesadenus glaziovii*, *M. lucayanus*, and *M. tenuissimus*, and one each of *M. chiangii* and *M. polyanthus*, were included. Our sampling was designed to maximize generic representation, especially in the *Spiranthes* and *Stenorrhynchos* clades, in which the species previously assigned to *Mesadenus* are nested. We also included various species and genera of subtribes Cranichidinae Lindl., Discyphinae Salazar & van den Berg, Galeottiellinae Salazar & M.W.Chase, Goodyerinae Klotzsch, and Manniellinae Schltr. as outgroups, following previous phylogenetic studies (e.g. Salazar et al. 2003, 2009, 2014, 2018; Álvarez-Molina and Cameron 2009). A list of the taxa analyzed with voucher information and GenBank accessions for the DNA sequences is given in Appendix 1. Those specimens also vouch for the morphological observations, in addition to those listed under the respective species in the Taxonomic Treatment section (see later). The aligned DNA data matrix in Nexus format was deposited in the Dryad repository (Salazar et al. 2019).

Molecular Methods—Genomic DNA was extracted from silica gel-dried leaf tissue or from small leaf fragments or flower buds from herbarium specimens, in all instances using a 2 × cetyltrimethylammonium bromide (CTAB) procedure based on Doyle and Doyle (1987), with the addition of 2% polyvinylpyrrolidone (PVP) to the extraction buffer. DNA extracts were purified by precipitation with absolute ethanol at -20°C.

The PCR was performed in 12.5 μL reaction volumes using the Taq PCR core kit (QIAGEN, Hilden, Germany), adding 0.125 mL of each primer (100 ng/μL), 0.0625 μL of polymerase, 0.5 μL of a 0.4% aqueous solution of bovine serum albumin (BSA) to neutralize potential inhibitors (Kreader 1996), and 0.25 μL of dimethyl sulfoxide (DMSO) to reduce problems associated with secondary structure of the DNA (Jensen et al. 2010). The ITS region was amplified and sequenced with primers ITS4 and ITS5 (White et al. 1990). The thermal cycler profile consisted of an initial denaturation at 94°C for 2 min, followed by 28–35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 7 min. The *trnL–trnF* region, including the intron of *trnL* and the *trnL-trnF* intergenic spacer, was amplified as a single fragment with primers c and f or as two non-overlapping fragments with primers c–d and e–f (all from Taberlet et al. 1991); the PCR profile for *trnL-trnF* was the same as for ITS and all four primers were used for sequencing. The *matK-trnK* region, including the *matK* gene and the 3′ portion of the *trnK* intron (downstream *matK*), was usually amplified as a single fragment with primers -19F (Molvray et al. 2000) and 2R (Steele and Vilgalys 1994). However, degraded DNA from herbarium specimens was amplified in smaller fragments with -19F, 2R, and internal primers 458F, 556R, and 731F (Molvray et al. 2000). The PCR profile for the *trnK–matK* region was as for ITS and *trnL-trnF*, except for increasing the extension time by 8 s on each cycle. The PCR products were purified with CentriSep columns with Sephadex (Princeton Separations, Inc., Adelphia, New Jersey) and sent for Sanger sequencing to the Laboratorio Nacional de Biodiversidad, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City.

Bidirectional sequence reads were assembled and edited with Sequencher v. 5 (GeneCodes Corp., Ann Arbor, Michigan) or with Geneious v. 8.1.9 (Biomatters, Ltd., Auckland, New Zealand). Each DNA region (*matK-trnK*, *trnL-trnF*, whole ITS region) was aligned separately using the default settings of the online version of the software package MAFFT v. 6 (Katoh and Standley 2013), with minor subsequent manual adjustment using Mesquite (Maddison and Maddison 2016).

Phylogenetic Analysis—We conducted a maximum parsimony analysis of concatenated plastid (*matK* gene, *trnK* intron downstream *matK*, *trnL* intron, and *trnL-trnF* spacer) and nuclear (ITS) DNA sequences. This approach is supported by the results of our previous phylogenetic analyses of Spiranthinae and other Cranichideae, in which both resolution and overall clade support improved in the combined analyses relative to the separate analyses, and groups that differed between datasets were not supported by high bootstrap percentages (e.g. Salazar et al. 2003, 2011, 2018; Cisternas et al. 2012). The analysis was conducted with the software package PAUP* v. 4.0a159 (Swofford 2018) or 32-bit Microsoft Windows and consisted of 1000 replicates of random addition of taxa for the starting trees, the option MulTrees activated to allow for storage of multiple trees in memory, and branch-swapping using the tree bisection-reconnection (TBR) algorithm, saving all the shortest trees found. All characters were treated as unordered and equally weighted and the individual gap positions were treated as missing data. Clade support was evaluated by 1000 bootstrap replicates (Felsenstein 1985), each consisting of a heuristic search with 20 starting trees with random addition of taxa and TBR branch-swapping, saving up to 20 trees per search. The phylogenetic trees were edited with FigTree v. 1.4.0 (Rambaut 2012) and Photoshop® CC (Adobe Systems Inc., San Jose, California). Bootstrap percentages of 51–74, 75–89 and 90–100 were considered as weak, moderate, and strong support, respectively.

Morphological Observations—We studied live, liquid-preserved, and herbarium specimens of all the species historically assigned to *Mesadenus* and other members of Spiranthinae that were sampled for molecular study. Selected species were examined in further detail to compare leaf and floral attributes, as follows.

For comparing leaf veining patterns and other leaf features, fresh leaves of *Mesadenus polyanthus*, *M. tenuissimus*, and *Schiedeella nagelii*, and leaves from herbarium specimens of *Mesadenus glaziovii* and *Sacoila lanceolata*, were diaphanized according to Payne (1969), with some modifications. Fresh leaves were fixed in FAA (5% formalin, 5% acetic acid, 50% ethanol; Sass 1958) for at least 4 d and rinsed with running water for 1–2 h to remove the fixative. Leaves from herbarium specimens were soaked in lukewarm water overnight prior to further treatment. Leaves were placed in a lukewarm 10–15% NaOH solution for 3–5 d, rinsed with water and transferred to a 10% solution of NaOCl (commercial bleach) for 2–3 h. Subsequently they were dyed with safranin O, dehydrated in an ethanol series (50%, 70%, 96%, 2–3 d each), placed in eugenol for 10 min to remove the excess of dye, transferred to xylene and photographed under a stereomicroscope. Structural leaf character terminology followed Ellis et al. (2009).

For comparing the structure of the perianth and the column, including the rostellum and pollinarium/viscidium, fresh flowers of *Mesadenus glaziovii*, *M. lucayanus*, *M. polyanthus*, and *M. tenuissimus* were dissected and photographed under a stereomicroscope. In the case of Brazilian *Mesadenus rhombiglossus*, only softened flowers from the holotype and an additional sample from the same region could be examined (Fig. 4).

Flowers and flower buds of *M. lucayanus* and *M. polyanthus* were also examined under a scanning electron microscope (SEM), for which they were fixed in FAA for at least four days, rinsed with running water for 1–2 h, dehydrated in an ethanol series (50%, 70%, 80%, 90%, and three changes at 100%, 48 h each), critical point-dried, covered with gold, and observed under a Hitachi S-2460 N SEM (Hitachi High-Technologies, Krefeld, Germany) operating at 15 kv. Micrographs were taken with a Pentax Z10 camera using 35 mm Kodak 100 TMAX film. Negatives were digitized with a Nikon Super Coolscan 5000 scanner (Nikon, Tokyo, Japan).

Conservation Status—Estimation of extent of occurrence and area of occupancy of the Brazilian species was performed using a beta version of GeoCAT (Bachman et al. 2011) as implemented in the Royal Botanic Gardens, Kew website (https://www.kew.org/science/projects/geocat-%E2%80%93-geospatial-conservation-assessment-tool), with the default setting of 2 km width cell. Preliminary conservation status was inferred using the World Conservation Union red list categories and criteria (IUCN 2012) and the guidelines for using the IUCN red list categories and criteria (IUCN 2016).

Results

Phylogenetic Analysis—The aligned matrix consisted of 101 terminals (98 spp., 51 genera; Appendix 1) and 4724 characters, of which 1204 were parsimony-informative. The analysis found eight most-parsimonious trees with a length of 5640 steps, consistency index (CI) excluding uninformative characters of 0.40 and retention index (RI) of 0.70. The strict consensus of the eight trees, on which bootstrap percentages (BP) from the bootstrap analysis are shown, is depicted in Fig. 5 (for ease of visualization, the outgroups were trimmed in this figure; the full consensus tree with bootstrap percentages is available as Supplemental Fig. S1, Salazar et al. 2019).

Spiranthinae are strongly supported (BP 100), and the five main clades recovered in our previous analyses (Salazar et al. 2018 and references therein) were also recovered here (marked by numbered black circles 1–5 in Fig. 5). The two accessions of Brazilian *Mesadenus glaziovii* analyzed are sister to one another (BP 100), and collectively are the strongly supported sisters of the reminder of the *Stenorrhynchos* clade (BP 100; Fig. 5, clade 3). On the other hand, all accessions of Mesoamerican/Caribbean *Mesadenus* form a

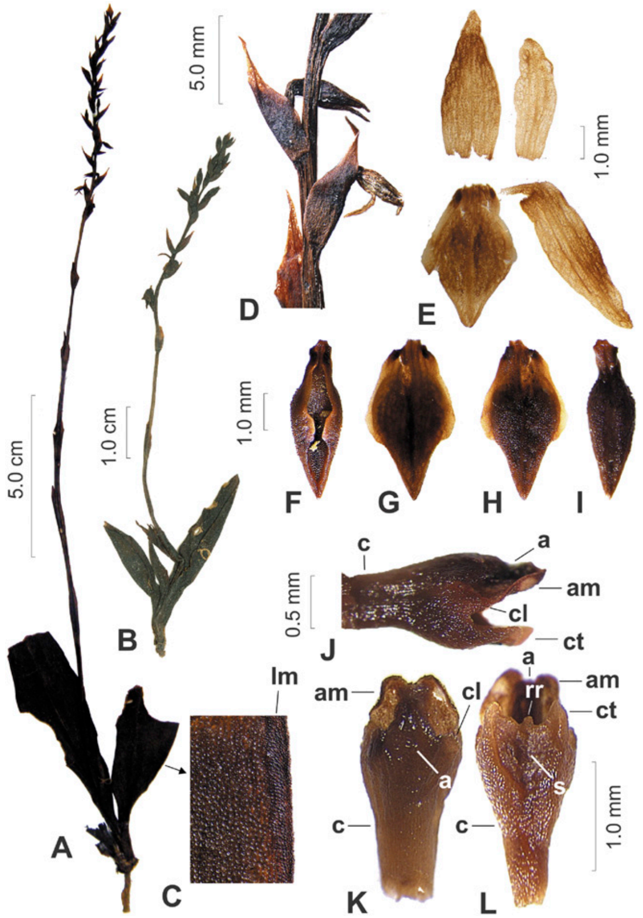

FIG. 4. *Espinhassoa rhombiglossa*. A. Habit. B. Habit from one of the specimens of the holotype sheet at RB. C. Section of the leaf showing the distinct margin. D. Side view of flowers. E. Dissected perianth; the margin on the left of the labellum is torn. F. Labellum, ventral view. G. Spread out labellum, ventral view. H. Spread out labellum, dorsal view. I. Labellum, dorsal view. J. Column, side view after removal of the pollinarium. K. Column, dorsal view. L. Column, ventral view after removal of the pollinarium. Abbreviations: a = anther; am = anther margin; c = column; cl = clinandrium; ct = column teeth; lm = leaf margin; rr = rostellum remnant; s = stigma; (A, C–L from *Ferreira Jr et al. 404*, BHZB); B from *Mello Barreto 524 and Brade 14877*, RB).

strongly supported group (BP 100) deeply nested in the *Spiranthes* clade, and their strongly supported sister is *Schiedeella nagelii* (BP 98; Fig. 5, clade 5). Other relationships match previous results of Salazar et al. (2016, 2018) and will not be dealt with in detail here, except when directly relevant to the relationships of *Mesadenus*.

Structural Characters—All species examined share a number of foliar features, including the palmate-parallelodromous primary vein pattern, presence of interior secondary veins, inconsistent intercostal tertiary vein angle, mixed epimedial tertiary fabric, and freely ramifying quaternary vein fabric. However, there are several consistent differences between the Mesoamerican *Mesadenus*/*Schiedeella nagelii* clade and Brazilian *Mesadenus glaziovii*, and the latter is most similar in these respects to *Sacoila lanceolata*. In *Mesadenus polyanthus*, *M. tenuissimus*, and *S. nagelii* the leaves are distinctly (although sometimes shortly) petiolate, spreading to horizontal, there are five primary veins and well-developed areolas, the margins are indistinct from the rest of the blade, and the ultimate marginal veins are recurved, forming pronounced loops that touch the margin (Figs. 1A, H, 3A, B). In contrast, in *Sacoila lanceolata* and *M. glaziovii* the leaves are sessile and attenuate at base, gently to rigidly ascending, they have seven primary veins and moderate development of areolas, the blade margins are translucent, and the ultimate marginal veins are barely recurved, do not form loops, and do not reach the margin (Figs. 2B, D, 3C–E).

Overall, floral organization is similar between Mesoamerican/Caribbean *Mesadenus* spp. and both *M. glaziovii* and *M. rhombiglossus*, but close examination reveals several differences, summarized in Table 1. In particular, the column, traditionally considered of prime importance for generic distinction in Spiranthinae, differs in several respects between *Mesadenus* s. s. and *M. glaziovii*/*M. rhombiglossus*. In the former group, the column is slightly arcuate when seen from one side, its ventral surface is flat, and the apex is essentially truncate to slightly emarginate when seen from below (Figs. 1E, F, 3I, H), whereas in the latter the column is straight, ventrally channeled, and bears a prominent lateral tooth at each side of the apex (Figs. 2I, K, 3K, 6L).

DISCUSSION

Our phylogenetic analysis corroborates the results of Salazar et al. (2016, 2018) regarding polyphyly not only of *Mesadenus*, but also of *Schiedeella* as delimited by most previous authors (e.g. Balogh 1981, 1982; Garay 1982; Szlachetko 1992; Salazar 2003b; Szlachetko et al. 2005). *Schiedeella tenella* (L.O. Williams) Burns-Bal. and *S. williamsiana* Szlach. are strongly supported as belonging in *Schiedeella* s. s., i.e. the clade that includes its type species, *S. transversalis* (A.Rich. & Galeotti) Schltr. However, *Schiedeella nagelii* is strongly supported as the sister of *Mesadenus* s. s. (Fig. 5). This association may appear surprising since the flowers of *S. nagelii* show obvious differences in size and form from those of *Mesadenus* s. s. (Fig. 1I–K), but vegetatively the former is very similar to *M. polyanthus* and other typical *Mesadenus* (Fig. 1H) and amply different from *Schiedeella* s. s. (cf. Salazar et al. 2016: Fig. 7D–F). Both typical *Mesadenus* s. s. and *S. nagelii* are nested in the *Spiranthes* clade, in which mellitophily seems to be the rule. This pollination syndrome includes predominantly white flowers, sometimes with the throat of the labellum tinged yellow or orange and the perianth bearing green or brownish veins (nectar guides), agreeable diurnal fragrance, and labellum apex somewhat expanded, forming a sort of landing or standing platform (cf. Salazar et al. 2018 and references therein). Bee pollination has been confirmed for several species of *Aulosepalum* Schltr., *Spiranthes*, *Schiedeella*, *Deiregyne* Schltr., and one species of *Dichromanthus* Garay, and it represents the plesiomorphic condition of the whole *Spiranthes* clade (Salazar and Ballesteros-Barrera 2010; Salazar et al. 2011, 2016, 2018). The large, showy flowers of *S. nagelii* (for *Mesadenus* standards) producing a faint diurnal odor (G. A. Salazar, pers. obs.) fit the mellitophilous floral pattern, whereas the combination of tiny, usually greenish flowers, narrow floral tube, and nocturnal floral odor of *Mesadenus* s. s., at least in some instances (*M. lucayanus*, *M. polyanthus*) reminiscent of lemongrass, all suggest pollination by small nocturnal insects, perhaps dipterans or lepidopterans (Salazar et al. 2016). Hence, the large, mellitophilous flowers of '*S.*' *nagelii* would represent an ancestral feature relative to the specialized, derived floral morphology of *Mesadenus* s. s.

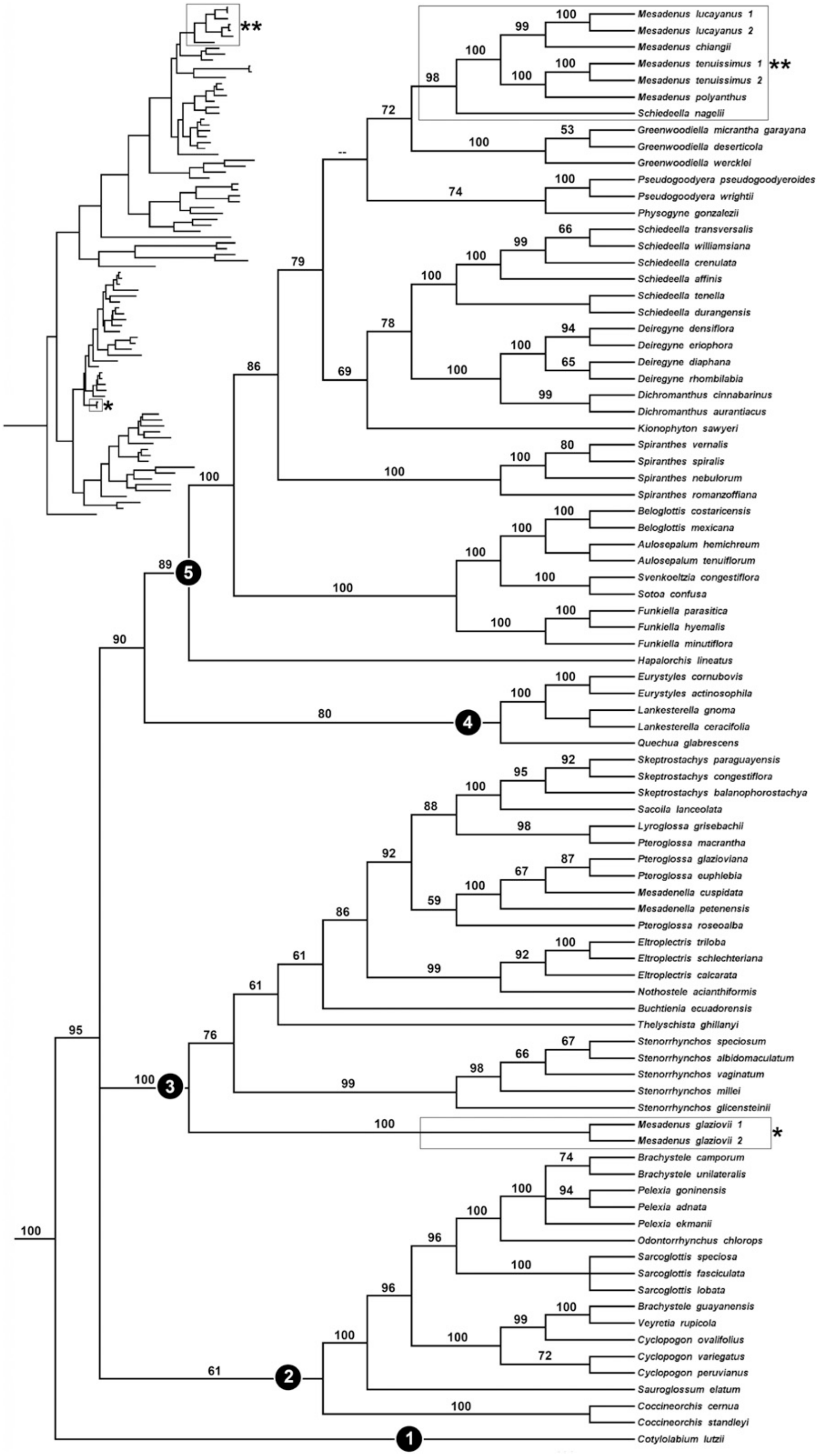

FIG. 5. Phylogenetic relationships in Spiranthinae inferred by maximum parsimony analysis of combined nuclear (ITS) and plastid (*trnK-matK*, *trnL-trnF*) DNA sequences (outgroups trimmed; see text). The main tree is the strict consensus of the eight shortest trees found by the analysis; the inset is one of the eight trees drawn with branch lengths proportional to the number of changes. Numbers above branches are bootstrap percentages (> 50). One asterisk (*) indicates the position of *Mesadenus* (*Espinhassoa*) *glaziovii* and two asterisks (**) that of *Mesadenus* s. s. as redelimited here, plus its sister *Schiedeella nagelii*. Numbers 1–5 in black circles refer to clades discussed in the text.

Mesadenus glaziovii has white flowers and the indication on the label of one herbarium sheet of a faint nocturnal odor (*Viana and Mota 1841*) suggests that it might be pollinated by small nocturnal lepidopterans, but this requires confirmation in the field. Salazar et al. (2018) noted the presence of various pollination syndromes in the *Stenorrhynchos* clade, including halictid bee pollination in *Mesadenella* Pabst & Garay (Singer 2002), hummingbird pollination in *Stenorrhynchos* and *Sacoila*

TABLE 1. Distinguishing features between *Espinhassoa* and *Mesadenus* as redelimited here.

Feature	*Espinhassoa*	*Mesadenus*
Root shape	Cylindrical	Fusiform
Root diameter	1.3–5.5 mm	5.0–11.0 mm
Root indumentum	Glabrous	Pilose
Leaf position	Ascending	Spreading to horizontal (often curved downwards)
Leaf number	1–3(4)	4–6(8)
Leaf base	Sessile, attenuate	Abruptly narrowed into a distinct petiole
Leaf surface (live condition)	Opaque, smooth (unknown in *E. rhombiglossa*)	Iridescent, smooth to fossulate
Leaf margin	Distinct, translucent	Indistinct, opaque
Flower color	White with proximal 2/3 of labellum cream-colored (pending confirmation in *E. rhombiglossa*)	Homogeneously pale green, bronzy green, brownish pink or white, with green or rosy-brown perianth base
Lateral sepal orientation (live condition)	Recurved or spreading from near the base	Proximally erect and parallel to dorsal sepal and petals, recurved (flared) above
Labellum indumentum	Base provided internally with retrorse, simple trichomes beneath the nectar glands and most of the external surface irregularly papillose, the papillae forming a distinct, ellipsoid, cream-colored area in fresh condition, which turns rusty red-brown in alcohol-preserved and herbarium material (*E. glaziovii*), or external surface mostly covered by rounded papillae, otherwise glabrous (*E. rhombiglossa*)	Whole surface glabrous, except for papillae on apical labellum lobe in in *M. tenuissimus*
Column shape when seem from one side	Straight	Arcuate
Ventral surface of column	Channeled	Flat
Stigma surface	Concave	Flat

(Singer and Sazima 2000; Siegel 2011), and butterfly pollination in *Pteroglossa* Schltr. (Pansarin and Ferreira 2015). The "basal" position of *Mesadenus glaziovii* in the *Stenorrhynchos* clade, close to such florally disparate taxa as *Stenorrhynchos* s. s., *Thelyschista* Garay, and *Buchtienia* Schltr. (Fig. 5), is suggestive of a period of "experimentation" with various pollination syndromes at the onset of the diversification of the *Stenorrhynchos* clade, which resulted in the array of floral morphologies displayed by this major clade.

As noted earlier, the main character that traditionally has defined *Mesadenus*, i.e. viscidium centrally placed on the ventral surface of the pollinarium, has evolved independently several times in Cranichideae: once in Galeottiellinae (*Galeottiella*; Salazar et al. 2002; Salazar 2003a) and four in Spiranthinae. Within the last subtribe, there is a single known instance of this condition in the *Stenorrhynchos* clade (*Mesadenus glaziovii*; Fig. 2J, L) but three independent ones within the *Spiranthes* clade: *Funkiella minutiflora* (see Salazar et al. 2018: Fig. 2L), *Schiedeella affinis* (formerly placed in *Brachystele* or *Mesadenus*), and *Mesadenus* s. s. (Figs. 1F, 3F–H). To understand how this sort of structure could evolve repeatedly, it is instructive to revisit column development in Spiranthinae. Floral developmental studies by Rasmussen (1982), Kurzweil (1988, 1998), Figueroa (2014), and Figueroa et al. (2012) have shown that, in Spiranthinae, anther initiation is followed by the emergence of the median carpel apex, which arises as a rounded bulge on the ventral side of the anther primordium and quickly elongates until it equals the length of the anther, the elongated portion constituting the rostellum (e.g. Figueroa et al. 2012: Fig. 2E–H). *Mesadenus* (and likely other taxa with similar column/pollinarium morphology) is the exception in that the median carpel apex stops elongating when it has reached about one half of the total length of the anther, resulting in the median positioning of the viscidium on the ventral side of the pollinarium (Fig. 3G). Hence, this condition could easily be derived from the regular pattern by suppressing or interrupting full elongation of the rostellum. Nevertheless, only a few species of Spiranthinae have been studied developmentally, and this hypothesis will have to be evaluated when floral ontogeny of more species displaying a centrally placed viscidium can be studied.

Regarding leaf morphology, *M. glaziovii* is very similar to *Sacoila lanceolata* in the number and position of the leaves, as well as in possession of seven main veins, lack of marginal vein loops, and a distinctly translucent or hyaline leaf margin. Leaves of *M. rhombiglossus* are similar in number and position to those of *M. glaziovii* and *Sacoila lanceolata* and appear to have also a translucent margin, although this and other features are somewhat obscured by the dark coloration acquired by the leaves upon drying (Fig. 4C). A translucent leaf margin is present in several other members of the *Stenorrhynchos* clade, including *Lyroglossa* Schltr., *Pteroglossa*, and *Skeptrostachys* Garay (Salazar 2003b), and some species of *Odontorrhynchus* M.N.Correa, *Pelexia* Poit. ex Lindl., *Sarcoglottis* C.Presl, and *Sauroglossum* Lindl. of the *Pelexia* clade (Salazar 2003b; Cruz-Lustre 2015), but is unknown in the *Spiranthes* clade (G. A. Salazar, pers. obs.). Cruz-Lustre (2015) showed that, in species of the *Pelexia* clade with a hyaline or translucent leaf margin, the marginal portion of the leaf lacks parenchyma and both epidermis (adaxial and abaxial) are in contact with each other, whereas, in species with indistinct margin, parenchyma virtually reaches the leaf edge. This sort of structural characters of leaves has rarely, if ever, been considered in taxonomy of Spiranthinae and deserves further study.

Judging from the protologue, *Mesadenus rhombiglossus* (as *Hapalorchis rhombiglossa* Pabst) appeared to differ from *Mesadenus glaziovii*, and all other Spiranthinae, in its unique combination of broad and short, rhombic labellum definitely three-lobed above the middle, and the winged column with bifurcate rostellum remnant (Pabst 1974: Table 1). Our repeated attempts to obtain material suitable both for DNA analysis and for a detailed study and comparison of its structural features have failed so far. However, examination of an additional specimen of *M. rhombiglossus* (*Ferreira Jr. et al. 404*, BHZB), recently collected in the same region as the type (Serra do Cipó), allowed us to attain a better characterization

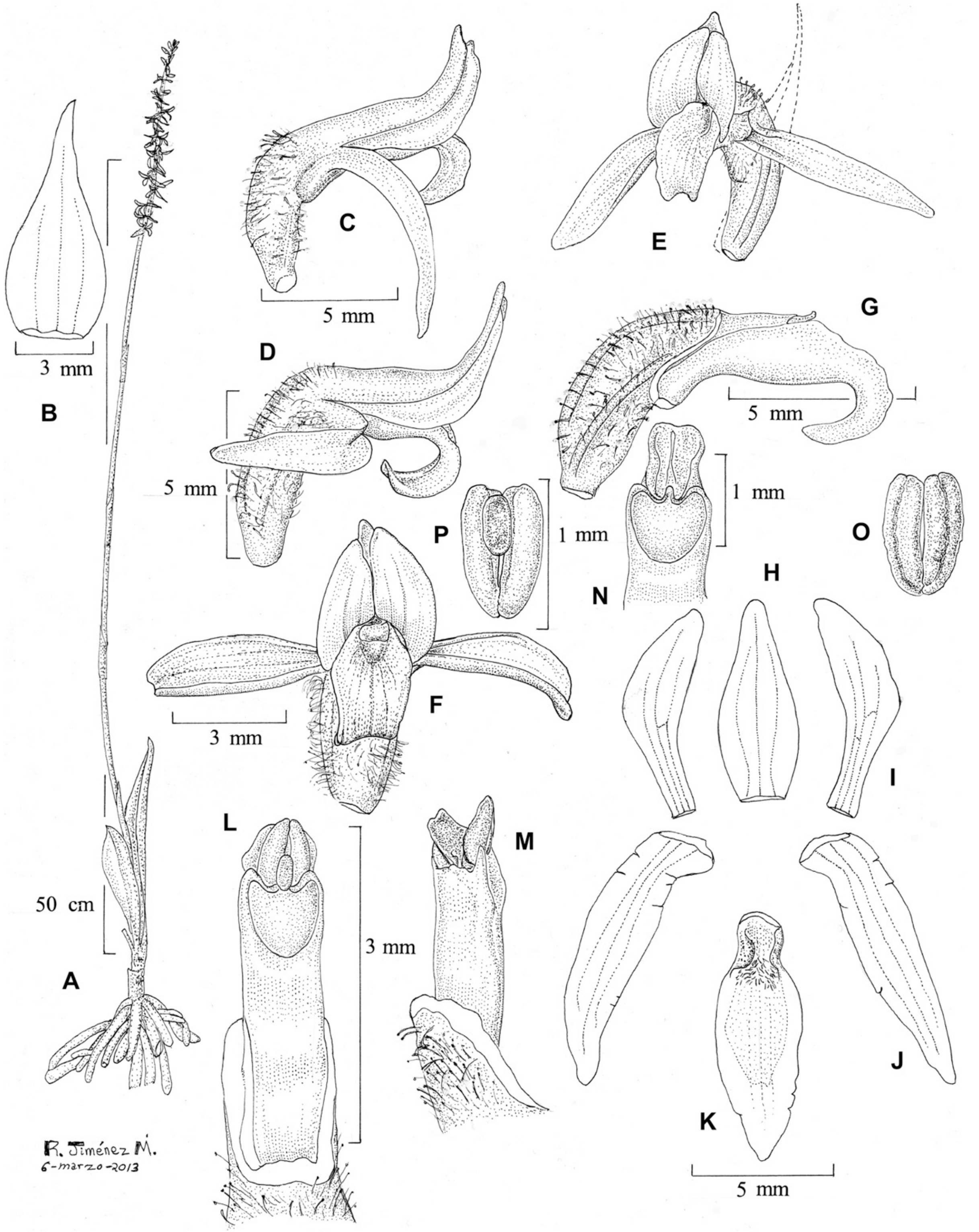

FIG. 6. *Espinhassoa glaziovii*. A. Habit. B. Floral bract. C, D. Two side views of flower. E. Oblique view of flower. F. Front view of flower. G. Labellum and column with the sepals and petals excised to show the position of labellum and column. H. Dorsal sepal. I. Petal. J. Lateral sepal. K. Labellum. L. Column from below with the pollinarium in place. M. Column from side. N. Column apex from below after removal of the pollinarium. O. Pollinarium from above. P. Pollinarium from below. Drawn with camera lucida by Rolando Jiménez-Machorro from *Batista et al. 3158*.

of the species and clarify some inaccuracies in Pabst's original description and illustration. As shown in our Fig. 4J–L, the column is clavate, i.e. somewhat expanded above from a narrower base, but lacks wings of any sort. Moreover, the rostellum is not deeply bipartite ("rostello profunde fisso," according to Pabst 1974), but entire and the rostellum remnant (i.e. what remains of the rostellum after removal of the pollinarium/viscidium) is a minute apicule about 0.1 mm long (Fig. 4L). In fact, the column apex of *M. rhombiglossus* is three-toothed as in *M. glaziovii* (Figs. 2K, 3K), the only difference being that the median tooth (i.e. the rostellum remnant) is proportionately smaller in *M. rhombiglossus*. It is worth noting that Pabst's original drawing, attached to the holotype (RB 162460!), clearly shows the minute rostellum remnant, but this detail somehow was lost in the figure reproduced in the published protologue (Pabst 1974: Table 1). Moreover, the labellum of *Mesadenus rhombiglossus* is fleshy with membranaceous margins, sharply channeled, and, when fully spread out, only obscurely three-lobed (Fig. 4G, H). Thus, *Mesadenus rhombiglossus* is similar in many respects to *M. glaziovii* but differs from it in several morphological aspects: the whole plant dries black, the inflorescence and flowers are completely glabrous, the filament is completely adnate to the column and hence the anther is solidly held in place (i.e. non-articulate), the perianth segments are fleshy, especially the labellum, and the rostellum remnant is proportionately smaller.

All the above shows that *M. glaziovii* and *M. rhombiglossus* are clearly out of place in *Mesadenus* s. s., typified by *M. galeottianus* (A.Rich.) Schltr. (a synonym of *M. polyanthus*). On the other hand, the morphological and eco-geographical features shared by the two Brazilian species, *M. glaziovii* and *M. rhombiglossus*, support a close relationship to each other, which could easily be tested when material suitable for DNA analysis of *M. rhombiglossus* is available. On that basis, a new genus is herein proposed to accommodate the Brazilian species. The systematic position of *S. nagelii* will be dealt with in another work focused on a thorough phylogenetic reassessment of the genus *Schiedeella* (G. A. Salazar unpubl. data).

Taxonomic Treatment

Espinhassoa Salazar & J.A.N.Bat., gen. nov. Type Species: *Espinhassoa glaziovii* (Cogn.) Salazar & J.A.N.Bat.

Similar to *Mesadenus* Schltr. in overall floral morphology but differing in the sessile, basally attenuate, ascending leaves with seven primary veins, lacking marginal vein loops, and with translucent margins; lateral sepals spreading from the base, i.e. not parallel and forming a distinct floral tube; column straight when seen from one side, ventrally channeled, with concave stigma and a tooth at each side of the apex, these more prominent than the central, apiculate to shortly triangular rostellum remnant.

A genus of two species restricted to the Espinhaço range in the state of Minas Gerais, Brazil (see below).

Etymology—The generic name refers to the Espinhaço range (Serra do Espinhaço), a predominantly quartzitic and sandstone mountain chain running about 1100 km north to south in the states of Minas Gerais and Bahia, Brazil, and renowned for its exceptionally high plant diversity and endemism (Giulietti et al. 1987, 1997; Rapini et al. 2008).

Key to the Species of *Espinhassoa*

1. Flowering from the beginning until the end of the dry season (May to November); plants 26–84 cm in height including the inflorescence, turning pale brownish when dried; rachis, ovary, and base of sepals pubescent; flowers (6–)18–48(–66); dorsal sepal 5.7–8.1 × 1.7–2.8 mm; labellum mostly membranaceous, 6.6–9.4 × 2.3–4.2 mm; filament not fully adnate to the column (thus the anther is articulate); rostellum remnant about the same size as the lateral teeth of the column, ca. 0.2 mm long . . . . *E. glaziovii*

1. Flowering at the end of the rainy season (April); plants 10–27 cm in height including the inflorescence, turning completely black when dried; rachis, ovary and perianth glabrous; flowers 10–18; dorsal sepal 3.5–4.6 × 1.2–1.9 mm; labellum fleshy with membranaceous margins, 3.5–4.4 × 2.0–2.6 mm; filament completely fused to the column (hence anther rigidly kept in place, nonarticulate); rostellum remnant distinctly shorter than the lateral teeth of the column, ca. 0.1 mm long . . . . *E. rhombiglossa*

Espinhassoa glaziovii (Cogn.) Salazar & J.A.N.Bat., comb. nov. *Stenorrhynchus glaziovii* Cogn., Fl. Bras. (Martius) 3(4): 171. 1895. *Mesadenus glaziovii* (Cogn.) Schltr., Beih. Bot. Centralbl. 37(2): 368. 1920. Type: Brazil. Rio de Janeiro, *Glaziou s.n.* (holotype: B, destroyed; lectotype, here designated: BR 13533793!).

Acaulescent, terrestrial **herb** 26–84 cm in height including the inflorescence. **Roots** 3–18, fasciculate, fleshy, cylindrical, glabrous, 5.4–17.0 cm long, 2.0–5.5 mm in diam. **Leaves** basal, 1–2(4), ascending, sessile, base attenuate, blade narrowly elliptic to oblanceolate, 3.3–18.0 × 1.1–4.3 cm, with seven primary veins, acute to obtuse, glabrous, subcoriaceous, smooth, uniformly light green with translucent margins. **Inflorescence** erect, racemose; scape glabrous, provided with 6–9 spaced, sheathing, glabrous, acuminate bracts 1.2–4.0 cm long; rachis densely to sparsely pubescent, trichomes septate, up to 8-celled, glandular, white in fresh condition, turning brown when dried; raceme spiral, 3.5–24.0 cm long, few- to many-flowered; floral bracts ascending, shortly and sparsely pubescent near the base, glabrous otherwise, much longer than the ovaries, lanceolate, long-acuminate, 6–12 × 2.7–3.4 mm, light green, with hyaline margins. **Flowers** (6)18–48(66), most of them opened simultaneously, horizontal, resupinate, white overall, slightly fragrant during nighttime; ovary ascending, densely pubescent, fusiform, slightly 3-costate, slightly twisted, arcuate above the middle, 5–6 × 1.3–1.6 mm. **Sepals** shortly and sparsely pubescent near the base, glabrous otherwise, three-veined, mostly white, base pale green; dorsal sepal erect below the middle, recurved above, lanceolate, apex acute, 5.7–8.1 × 1.7–2.8 mm; lateral sepals spreading, recurved from near the base, obliquely linear-lanceolate, acute, bases connate for about 1 mm and decurrent to the ovary apex for about 2.5 mm to form a prominent mentum, 7.8–11.5 × 1.7–2.7 mm. **Petals** erect below the middle, recurved above, adherent to the dorsal sepal above the middle, glabrous, obliquely oblanceolate, falcate, apex obliquely acute to obtuse, base cuneate, 4.8–8.3 × 1.1–2.1 mm, 2-veined, the innermost vein bifurcate above the middle, white with creamy veins. **Labellum** erect, base arcuate, distal 1/3 strongly recurved, sessile, entire, concave-channeled, adnate at base to the sepaline mentum, embracing the column without adhering to it, trullate when spread out, apex acute to obtuse, pale yellow on the proximal two-thirds, white with creamy veins above, 6.6–9.4 × 2.3–4.2 mm, base rounded, internally with an inconspicuous, lunate submarginal thickening at each side and retrorse, simple trichomes below it; external surface with short marginal trichomes outside the lunate thickenings and most of the surface covered by irregular papillae forming a distinctly colored area (pale yellow in fresh condition, brown in alcohol-preserved material). **Column** semi-terete, straight, nearly perpendicular to the ovary, glabrous, ventrally channeled, apex tridentate, 2.7–3.5 × 0.6–0.8 mm long; column foot adnate to the ovary apex, 1.6–1.9 mm long; clinandrium with short, membranaceous, irregularly dentate margins that do not extend to the apex of the lateral column teeth. **Anther** dorsal, articulate, prominent at the column apex, ovate, emarginate, margins recurved, 0.6–1.0 × 0.7–0.9 mm, filament not completely adnate, fleshy. **Pollinarium** aprox. 0.8 mm long; pollinia 2, deeply cleft, brittle, elliptic, pale yellow, viscidium oblong, channeled, translucid gray, placed on the proximal half on the ventral surface of the pollinarium. **Stigma** ventral, obcordate, slightly concave. **Rostellum** abbreviated, terminal; rostellum remnant a minute, triangular membranaceous apicule after removal of the viscidium, about 0.2 mm long, forming the central tooth of the column apex. **Capsule** not seen. Figures 2, 3E, J, K, 6.

***Distribution and Habitat*—**The species is an endemic of the Espinhaço range in the State of Minas Gerais, ranging from the Serra do Cabral and Grão Mogol in the north, to the Quadrilatero Ferrífero in the south. It is terrestrial in sandy, shallow soil on stony ground with rocky field vegetation ("campo rupestre"), from (700–)1000 to 1900 m elevation.

***Phenology*—**Flowering occurs from the beginning to the end of the dry season, from May to November, but mainly from July to October. That is the height of the dry period and plants usually flower after shedding the leaves, although specimens that bloom at the beginning of the dry season or that grow in shade or in protected places may have senescent leaves or leaf remnants.

***Conservation Status*—**According to the IUCN red list categories and criteria, and guidelines (IUCN 2012, 2016), this species could be classified as endangered (EN) due to its small area of occupancy, estimated at 136 km^2. However, the species is known from about 18 localities, occasionally it forms large populations, many of which occur within federal or state protected areas, and its extent of occurrence is estimated as 43,290 km^2, and we tentatively suggest that it is near threatened (NT) due to its restricted and specific habitats. At least one of the known populations of the species, that in the Serra da Piedade in Caeté, Minas Gerais, has disappeared. The species was collected there by Gardner in 1840, but the area has since suffered great impact from human occupation. Although numerous botanists later visited the region, the species has never been collected in that area again.

***Notes*—**Szlachetko and Rutkowski (in Rutkowski et al. 2008) intended to lectotypify *Stenorrhynchos glaziovii* Cogn., but instead of choosing a single specimen they cited two, the one originally located at B and destroyed during allied bombing raids in World War II, and another housed at BR. The latter, actually a fragment of the holotype consisting of a few flowers and one of Cogniaux's handwritten labels, was indicated by them as "isolectotype." A perusal of their nomenclatural synopsis of Spiranthinae shows that Szlachetko and Rutkowski (in Rutkowski et al. 2008) consistently committed this sort of error. In order to avoid future confusion, we here unequivocally designate the specimen housed at BR as the lectotype.

The protologue of *Stenorrhynchos glaziovii* stated "Rio de Janeiro" as the type locality, but all specimens that we have been able to verify come from the Espinhaço range in the state of Minas Gerais. The original label of the lectotype only states "Coll. Glaziou," without any indication of the geographic origin of the plant. Because the state of Rio de Janeiro is far outside the known distribution range of the species, and taking into account that many of the Glaziou collections have inaccurate or equivocal locality details (Wurdack 1970), we consider the presence of this species in Rio de Janeiro as doubtful. *Espinhassoa glaziovii* has also been reported from the Espinhaço range in the state of Bahia (Smidt 2003; Toscano de Brito and Cribb 2005), based on specimen *E. Pereira 9959 & A.P. Duarte 8945* (HB 41042!), from Morro do Chapéu, Bahia, with no collection date. However, a duplicate of the same collection, with inverted collectors (*A. P. Duarte 8945 & E. Pereira 9959*, RB 129394!), bears the following data: "Diamantina, Minas Gerais, 18 September 1965." Considering that the HB specimen is the only known record of the species for Bahia, the uncertainty as to its true origin, and the fact that a recent account of the orchids of the municipality of Morro do Chapéu (Bastos and van den Berg 2012) did not record or comment this species, we exclude its occurrence in Bahia until material unequivocally collected in that state is available.

Specimens *Hatschbach 16210* (MBM, US!), from Paraná, and *Menini Neto et al. 364* (CESJ!), from Rio Preto, southern Minas Gerais, both bear identifications as *Mesadenus* sp. but actually represent *Mesadenella cuspidata* (Lindl.) Garay.

***Additional Specimens Examined*—Brazil.**—MINAS GERAIS: Curimatahy [Buenópolis, Serra do Cabral], 1816–1821, *A. Saint-Hilaire B-2011* (P!); [Caeté], Serra da Piedade, Sept 1840, *G. Gardner s.n.* (BM 000032723!); Catas Altas, R.P.P.N. Serra do Caraça, caminho entre a Capelina e a Gruta de Lurdes, 8 Sept 2011, *J.A.N. Batista et al. 3158* (BHCB!, MEXU!); [Catas Altas], Serra do Caraça, Carapuça, 5 Sept 1977, *A. Ghillány s.n.* (HB 63889!); Catas Altas, Serra do Caraça, Campo de Fora, 27 Jun 2000, *R.C. Mota & J.R. Stehmann 230* (BHCB!); Catas Altas, Serra do Caraça, 4 Jul 2002, *R.C. Mota 2429* (BHCB!); Catas Altas, Serra do Caraça, 28 May 2002, *R.C. Mota 2433* (BHCB!); Catas Altas, Serra do Caraça, próximo do Pico do Sol, 7 Jul 2005, *R.C. Mota 2935* (BHCB!); Catas Altas, R.P.P.N. Santuário do Caraça, Pico Conceição, 26 Aug 2008, *C.T. Oliveira & L.L. Giacomin 21* (BHCB!); Catas Altas, R.P.P.N. Santuário do Caraça, Pico Cangerena, 27 Aug 2008, *C.T. Oliveira & L.L. Giacomin 61* (BHCB); Catas Altas, R.P.P.N. Santuário do Caraça, subida ao Pico do Sol, 1853 m, 29 Jun 2009, *C.T. Oliveira et al. 510* (BHCB!); Conceição do Mato Dentro, Parque Natural Municipal do Ribeirão do Campo, 9 Oct 2002, *R.C. Mota et al. 2537* (BHCB!); Diamantina, APE Manancial Pau de Fruta, área da COPASA, 29 Aug 2017, *J.A.N. Batista & G.M.C. Lustre 3449* (BHCB!); Diamantina, cerca de 20 km de Diamantina, na direção de Gouveia, 21 Sept 1998, *M.M.N. Braga et al. s.n.* (BHCB 47154!); Diamantina, Campus II da FAFEID, próximo à Fazenda Samambaia, 3 Sept 2004, *F.N. Costa & L.G. Lessa 807* (DIA, RB!); Diamantina, 18 Sept 1965, *A.P. Duarte 8945 & E. Pereira 9959* (RB!); Diamantina, Serra do Espinhaço, planalto, próximo de Diamantina, ca. 1200 m, 6 Oct 1972, *A.P. Duarte 14043* (AMES!, HB, K! drawing of dissected flower, M!, MBM!, NY!); Diamantina, estrada Diamantina-Conselheiro Mata, km 190, 30 Oct 1981, *A.M. Giulietti et al. in CFCR 2395* (BHCB!, SPF!); Diamantina, estrada para Salto da Divisa, 7 Sept 1971, *G. Hatschbach 27420* (MBM!, NY!); Diamantina, estrada para Conselheiro Mata, km 189, 2 Aug 1985, *J.R. Pirani et al. in CFCR 7921* (BHCB!, SP!, SPF!); Grão Mogol, 5–15 km ao norte da cidade, 16 Oct 1988, *R.M. Harley et al. 25114* (HUEFS!); Grão Mogol, ca. 5–15 km ao norte da cidade, 16 Oct 1988, *R.M. Harley et al. 25119* (K!); Grão Mogol, Córrego do Pasto, Alto da Serra Geral, 21 Oct 1978, *G. Hatschbach & A. Kasper 41630* (MBM!, MO, NY!); Grão Mogol, Trilha da Tropa, que vai da cidade até Periperi passando pelo alto da serra, nas encostas à esquerda do Ribeirão do Inferno, 27 Sept 1997, *R. Mello-Silva et al. 1434* (SP!); Itabira, Serra dos Alves, 22 Jun 2017, *P.B. Meyer 3085* (BHCB!); Itabirito, 28 Jun 1993, *W.A. Teixeira s.n.* (BHCB 26115!, SP 330923!); Itabirito, Pico do Moeda, Marinho da Serra/C1, 2 Jul 2007, *F.F. Carmo 672* (BHCB!); Itamarandiba, Parque Estadual da Serra Negra, Padre João Afonso, Serra Negra, 13 Sept 2006, *A.P. Fontana et al. 2417* (RB!); Joaquim Felício, Serra do Cabral, área da SCAI, cerca de 2,3 km da portaria, em direção a sede da empresa, 3 Nov 2009, *J.A.N Batista & E.K.O Hattori 2914* (BHCB!); Mariana, Mina de Conta História, Jun 2007, *M.S. Mendes et al. 82* (BHCB!); Mariana, R.P.P.N. Horto Alegria, 25 Jun 2015, *J.A. Paiva et al. 532* (BHCB!); Mariana, R.P.P.N. Horto Alegria, 26 Jun 2015, *M.O. Pivari et al. 2518* (BHCB!); Ouro Preto, Serra de Capanema/C2, 7 Jul 2008, *F.F. Carmo 3396* (BHCB!); Ouro Preto, Serra de Capanema/C2, 7 Jul 2008, *F.F. Carmo 5108* (BHCB!); Presidente Kubitschek, rodovia BR-259, próximo ao trevo para Presidente Kubitschek, 25 Jul 1998, *G. Hatschbach et al. 68231* (MBM!); Santa Barbara, Serra da Gandarela/C2, 1

Jul 2008, *F.F. Carmo 3161* (BHCB!); [Santa Barbara], Gandarela, 1570 m, 13 Jul 1972, *L. Emygdio 3281* (R!); Santana do Riacho, Serra do Cipó, R.P.P.N.s Brumas do Espinhaço e Ermos dos Gerais, próximo a sede da R.P.P.N., 11 Apr 2014, *J.A.N. Batista et al. 3326* (BHCB!); Santana do Riacho, R.P.P.N. Brumas do Espinhaço e Ermo do Gerais, 13 Sept 2012, *C.A. Ferreira Jr et al. 651* (BHCB!, BHZB!); Santana do Riacho, Serra do Cipó, bifurcação para Morro do Pilar, 9 Oct 1987, *F.R. Salimena-Pires et al. in CFSC 10746* (SPF!); Santana do Riacho, Serra do Cipó, rodovia MG 010, km 117, BH - Conceição do Mato Dentro, 20 Oct 1997, *P.T. Sano et al. 525* (BHCB!, SPF!); São Gonçalo do Rio Preto, P.E. do Rio Preto, próxima do Pico do Sol, 10 Oct 2007, *C.A.N. Martins 11* (BHCB!); São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, subida para o Pico Dois Irmãos, 23 May 2007, *N.F.O. Mota et al. 753* (BHCB!); São Gonçalo do Rio Preto, P.E. do Rio Preto, Chapada do Couto, base do Pico Dois Irmãos, 31 Aug 2007, *N.F.O. Mota et al. 869* (BHCB!); São Gonçalo do Rio Preto, P.E. do Rio Preto, 15 Sept 2006, *R.C. Mota et al. 3134* (BHCB!); São Gonçalo do Rio Preto, P.E. do Rio Preto, 10 May 2004, *P.L. Viana et al. 1764* (BHCB!); São Gonçalo do Rio Preto, P.E. do Rio Preto, 10 Aug 2004, *P.L. Viana & N.F.O. Mota 1841* (BHCB!); without locality, *Libon s.n.* (W-R 17337!).

Espinhassoa rhombiglossa (Pabst) Salazar & J.A.N.Bat., comb. nov. *Hapalorchis rhombiglossa* Pabst, Bradea 1: 468. 1974. *Mesadenus rhombiglossus* (Pabst) Garay, Bot. Mus. Leafl. 28: 336. 1982. TYPE: BRAZIL. Minas Gerais: Serra do Cipó, 15 April 1935, *H. L. Mello Barreto 524 & A. C Brade 14877* (holotype: RB 162460!, isotypes: AMES [00083999 fragment with flower from RB,] HB 52521!, HUEFS! [photo from HB] K! [drawing of dissected flower from HB].

Acaulescent, terrestrial **herb** 10–27 cm in height including the inflorescence. **Roots** few, fleshy, cylindrical, glabrous, 1.3–2.4 mm in diameter. **Leaves** basal, 2–3, ascending, sessile, base attenuate, blade elliptic, 2.3–8.9 × 0.8–2.1 cm, with seven primary veins, acute to obtuse, glabrous, smooth, margins paler than the rest of the blade, somewhat translucent. **Inflorescence** erect, racemose; scape glabrous, provided with 3–5 spaced sheathing, glabrous, acuminate, bracts 1.0–1.4 × 0.35–0.4 cm; rachis glabrous; raceme spiral, 2.8–7.0 cm long, few-flowered; floral bracts ascending, glabrous, longer than the ovaries, lanceolate, long-acuminate, 7.0–9.0 × 2.5–3.2 mm. **Flowers** 10–18, opening in succession towards the apex, horizontal, resupinate, green; ovary ascending, glabrous, fusiform, slightly twisted, arcuate above the middle, 3.4–5.0 × 0.8–1.0 mm. **Sepals** glabrous, three-veined; dorsal sepal lanceolate-ovate, apex acute, 3.5–4.6 × 1.2–1.9 mm; lateral sepals slightly spreading, obliquely lanceolate, acute, bases not connate, decurrent to the ovary apex for about 1.2–1.5 mm to form an obscure mentum, 4.0–5.3 × 1.0–1.6 mm. **Petals** adherent to the dorsal sepal, glabrous, obliquely lanceolate, 0–3.7 × 1.0–1.4 mm, 3-veined. **Labellum** erect, sessile, lightly trilobed, concave-channeled, adnate at base to the sepaline mentum, embracing the column without adhering to it, broadly rhombic when spread out, apex acute, 3.5–4.4 × 2.0–2.6 mm, fleshy with membranaceous margins, base truncate, internally with elliptic, submarginal thickenings at each side, glabrous, external surface mostly covered by rounded papillae. **Column** semi-terete, straight, perpendicular to the ovary, glabrous, ventrally channeled, 1.2–2.0 × 1.0 mm; column foot adnate to the ovary apex, 1.5–1.6 mm long, apex tridentate, lateral teeth about 0.3 mm long; clinandrium small, with short, irregularly dentate margins that mostly extend to the base of the lateral column teeth. **Anther** dorsal, poorly articulate, prominent at the column apex, ovate, about 1.1 × 0.9 mm, slightly exceeding the apex of the lateral column teeth, emarginate, margins prominent, membranaceous, recurved, filament completely adnate. **Pollinarium** not examined. **Stigma** ventral, obcordate, slightly concave. **Rostellum** abbreviated, terminal; rostellum remnant a minute, triangular membranaceous apicule after removal of the viscidium, about 0.1 mm long, forming the central tooth of the column apex. **Capsule** not seen. Figure 4.

Distribution and Habitat—Endemic to the Serra do Cipó in the Espinhaço range of the State of Minas Gerais, the species is terrestrial on rocky field vegetation ("campo rupestre") around 1300 m elevation.

Phenology—Flowering occurs at the very end of the rainy season in April.

Conservation Status—*Espinhassoa rhombiglossa* is an extremely rare species, currently known from only two collections from Serra do Cipó, in the Espinhaço range of Minas Gerais. The plants are small and slender, grow among grasses, and are difficult to find in the field. Several attempts to recollect the species have failed so far. The known collections are composed of few specimens, suggesting that the number of individuals found was small. According to the IUCN red list categories and criteria, and guidelines (IUCN 2012, 2016), the species can tentatively be considered Critically Endangered (CR) due to its limited geographic range, small area of occupancy estimated in 8 km^2, and two known populations [B1ab(iii) + 2ab(iii); C2a(i)].

Notes—To our knowledge, this rare species has only been collected twice, both times at the Serra do Cipó, and is known from only four specimens. The type specimen was merely labelled as "Serra de Cipó," but the type of *Habenaria mello-barretoi* Brade & Pabst, gathered by the same collectors (*Mello Barreto 521* and *Brade 14876*) on the same day as the type of *Hapalorchis rhombiglossa*, bears precise locality data ("km 134"). In 2008, one of us (JANB) collected *Habenaria mello-barretoi* at its type locality and conducted a search for *E. rhombiglossa*, which was unsuccessful. Additional searches in 2011, 2013, and 2016 in the area were also unsuccessful in locating the species. The second collection of *E. rhombiglossa* (*Ferreira Jr* et al. *404*, BHZB!), also from Serra do Cipó, has precise locality information (Santana do Riacho, RPPN Brumas do Espinhaço e Ermo dos Gerais), but a search in the area in April 2014 (JANB accompanied by the original collector) failed to reveal plants of this species. Hence, no material suitable for DNA extraction nor detailed morphological comparison has been available to us. However, from the careful examination of the collection *Ferreira Jr* et al. *404* (BHZB!), it is clear that the species is morphologically similar to *E. glaziovii*, but still unique in several aspects justifying both its transfer to *Espinhassoa* and its maintenance as a distinct species.

In general, plants of *E. rhombiglossa* are smaller and their inflorescences shorter with fewer flowers, but the habit of *E. glaziovii* is quite variable and, although larger specimens are easy to differentiate, smaller specimens overlap in meristic characters (size of plants and flowers, length of inflorescence, and number of flowers) with *E. rhombiglossa*. The column of *E. rhombiglossa* is proportionally larger and stouter than that of *E. glaziovii*, in spite of the usually larger flowers of *E. glaziovii*. Other distinctive features of *E. rhombiglossa* are the glabrous inflorescence and flowers, the filament completely adnate to the column resulting in a poorly articulated anther, the fleshy floral segments, especially the labellum, and the small rostellum remnant about 0.1 mm long, which is distinctly shorter than the column lateral teeth (Fig. 4). Moreover, the whole plants of *E. rhombiglossa*, including leaves, scape, bracts, and flowers, dry black, whereas in *E. glaziovii* plants and flowers dry pale brownish. The habitat of the two species seems to be similar, but *E. rhombiglossa* blooms at the end of the rainy season, in April, whereas *E. glaziovii* flowers from the beginning until the end of the rainy season, from May to November. Nevertheless, specimens of *E. rhombiglossa* are extremely sparse and therefore the characterization of the species presented here is provisional.

***Additional Specimen Examined*—Brazil.**—MINAS GERAIS: Santana do Riacho, R.P.P.N. Brumas do Espinhaço e Ermo dos Gerais, 19 Apr 2012, *C.A. Ferreira Jr. et al. 404* (BHZB!).

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AUTHOR CONTRIBUTIONS

GAS and JANB designed the study and led the discussion and taxonomic decisions; TECM contributed descriptive and taxonomic data of Brazilian taxa; LIC conducted molecular lab work; CF performed SEM study of flowers; LC-C carried out leaf anatomical work; AAdV participated in field- and descriptive work in Brazil; RJ-M contributed descriptive data of Mexican taxa and prepared the line drawing reproduced as Figure 6.

LITERATURE CITED

Álvarez-Molina, A. and K. M. Cameron. 2009. Molecular phylogenetics of Prescottiinae s.l. and their close allies (Orchidaceae, Cranichideae) inferred from plastid and nuclear ribosomal DNA sequences. *American Journal of Botany* 96: 1020–1040.

Bachman, S., J. Moat, A. W. Hill, J. de la Torre, and B. Scott. 2011. Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* 150: 117–126.

Balogh, P. 1981. Notas sobre nomenclatura del género *Schiedeella* Schlechter (Orchidaceae). *Orquídea (Mexico City), n.s.* 8: 37–40.

Balogh, P. 1982. Generic redefinition in subtribe Spiranthinae (Orchidaceae). *American Journal of Botany* 69: 1119–1132.

Bastos, C. A. and C. van den Berg. 2012. A família Orchidaceae no município de Morro do Chapéu, Bahia, Brasil. *Rodriguésia* 63: 883–927.

Bogarín, D., Z. Serracín, Z. Samudio, R. Rincón, and F. Pupulin. 2014. An updated checklist of the Orchidaceae of Panama. *Lankesteriana* 14: 135–364.

Chase, M. W., K. M. Cameron, J. V. Freudenstein, A. M. Pridgeon, G. A. Salazar, C. van den Berg, and A. Schuiteman. 2015. An updated classification of Orchidaceae. *Botanical Journal of the Linnean Society* 177: 151–174.

Cisternas, M., G. A. Salazar, G. Verdugo, P. Novoa, X. Calderón, and M. Negritto. 2012. Phylogenetic analysis of Chloraeinae (Orchidaceae) based on plastid and nuclear DNA sequences. *Botanical Journal of the Linnean Society* 168: 258–277.

Cruz-Lustre, G. M. 2015. *Evolución Estructural Vegetativa en las Especies Mexicanas del Género* Sarcoglottis *(Orchidaceae)*. M.S. thesis. Mexico City: Universidad Nacional Autónoma de México.

De Barros, F., F. Vinhos, V. T. Rodrigues, F. F. V. A. Barberena, C. N. Fraga, E. M. Pessoa, W. Forster, L. Menini Neto, S. G. Furtado, C. Nardy, C. O. Azevedo, and L. R. S. Guimarães. 2013. *Orchidaceae in Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB179.

Dodson, C. H. 1993. Orchidaceae. Pp. 763–867 in *Catalogue of the Flowering Plants and Gymnosperms of Peru*, eds. L. Brako and J. L Zaruchi. *Monographs in Systematic Botany of the Missouri Botanical Garden*. St. Louis: Missouri Botanical Garden Press.

Dodson, C. H. 1999. Orchidaceae. Pp. 630–775 in *Catalogue of the vascular plants of Ecuador*, eds. P. M. Jørgensen and S. León-Yánez. *Monographs in Systematic Botany of the Missouri Botanical Garden* 75. St. Louis: Missouri Botanical Garden Press.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Dueñas, H. del C. and J. L. Fernández-Alonso. 2009. Sinopsis de la subfamilia Spiranthoideae (Orchidaceae) en Colombia, parte II. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 33: 157–181.

Ellis, B., D. C. Daly, L. J. Hickey, K. R. Johnson, J. D. Mitchell, P. Wilf, and S. L. Wing. 2009. *Manual of Leaf Architecture*. Ithaca: Cornell University Press.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.

Figueroa, C. 2014. *Filogenia y Evolución Floral en Spiranthinae*. Ph.D. thesis. Mexico City: Universidad Nacional Autónoma de México.

Figueroa, C., G. A. Salazar, T. Terrazas, and P. Dávila. 2012. Estructura y desarrollo del ginostemio en *Dichromanthus michuacanus* (Orchidaceae, Spiranthinae). *Revista Mexicana de Biodiversidad* 83: 73–82.

Garay, L. A. 1982. A generic revision of the Spiranthinae. *Botanical Museum Leaflets, Harvard University* 28: 277–425.

Giulietti, A. M., N. L. Menezes, J. R. Pirani, M. Meguro, and M. G. L. Wanderley. 1987. Flora da Serra do Cipó, Minas Gerais: Caracterização e lista de espécies. *Boletim de Botânica da Universidade de São Paulo* 9: 1–152.

Giulietti, A. M., J. R. Pirani, and R. M. Harley. 1997. Espinhaço range region. Eastern Brazil. Pp. 397–404 in *Centres of Plant Diversity. A Guide and Strategies for the Conservation*, vol. 3, *The Americas*, eds. S. D. Davis, V. H. Heywood, O. Herrera-MacBryde, J. Villa-Lobos, and A. C. Hamilton. Cambridge: WWF/IUCN.

IUCN. 2012. IUCN red list categories and criteria. Version 3.1. Second edition. Gland and Cambridge: International Union for Conservation of Nature and Natural Resources. http://www.iucnredlist.org/technical-documents/categories-and-criteria.

IUCN. 2016. Guidelines for using the IUCN red list categories and criteria. Version 12. Gland, Switzerland and Cambridge, UK: IUCN. http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Jensen, M. A., M. Fukushima, and R. W. Davis. 2010. DMSO and betaine greatly improve amplification of GC-rich constructs in de novo synthesis. *PLoS One* 5: e11024, doi: 10.1371/journal.pone.0011024.

Katoh, K. and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Kreader, C. A. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology* 62: 1102–1106.

Kurzweil, H. 1988. Developmental studies in orchid flowers III: Neottioid species. *Lindleyana* 4: 1–5.

Kurzweil, H. 1998. Floral ontogeny of orchids: A review. *Beiträge zur Biologie der Pflanzen* 71: 45–100.

Maddison, W. P. and D. R. Maddison. 2016. Mesquite: A modular system for evolutionary analysis. Version 3.11. http://mesquiteproject.org.

Molvray, M. P., P. J. Kores, and M. W. Chase. 2000. Polyphyly of mycoheterotrophic orchids and functional influences on floral and molecular characters. Pp. 441–448 in *Monocots: Systematics and Evolution*, eds. K. L. Wilson and D. A. Morrison. Collingwood: CSIRO.

Pabst, G. F. J. 1974. Additamenta ad orchidologiam brasiliensem—XVI. Estudos em Spiranthinae. *Bradea* 1: 465–471.

Pansarin, E. R. and A. W. C. Ferreira. 2015. Butterfly pollination in *Pteroglossa* (Orchidaceae, Orchidoideae): A comparative study on the reproductive biology of two species of a Neotropical genus of Spiranthinae. *Journal of Plant Research* 128: 459–468.

Payne, W. W. 1969. A quick method for clearing leaves. *Ward's Bulletin New Series* 8: 4–5.

Pupulin, F. 2002. Catálogo revisado y anotado de las Orchidaceae de Costa Rica. *Lankesteriana* 4: 1–88.

Rambaut, A. 2012. FigTree, version 1.4.0. Available at: http://tree.bio.ed.ac.uk/software/figtree/.

Rapini, A., P. L. Ribeiro, S. Lambert, and J. R. Pirani. 2008. A flora dos campos rupestres da Cadeia do Espinhaço. *Megadiversidade* 4: 15–23.

Rasmussen, F. N. 1982. The gynostemium of the neottioid orchids. *Opera Botanica* 65: 1–96.

Rutkowski, P., D. L. Szlachetko, and M. Górniak. 2008. Phylogeny and Taxonomy of the Subtribes Spiranthinae, Stenorrhynchidinae and Cyclopogoninae (Spirantheae, Orchidaceae) in Central and South America. Gdańsk: Wydawnictwo Uniwerytetu Gdańskiego.

Salazar, G. A. 2003a. Galeottiellinae. Pp. 59–63 in Genera Orchidacearum vol. 3, Orchidoideae part 2, Vanilloideae, eds. A. M. Pridgeon, P. J. Cribb, M. W. Chase, and F. N. Rasmussen. Oxford: Oxford University Press.

Salazar, G. A. 2003b. Spiranthinae. Pp. 164–278 in Genera Orchidacearum vol. 3: Orchidoideae part 2, Vanilloideae, eds. A. M. Pridgeon, P. J. Cribb, M. W. Chase, and F. N. Rasmussen. Oxford: Oxford University Press.

Salazar, G. A. and C. Ballesteros-Barrera. 2010. *Sotoa*, a new genus of Spiranthinae (Orchidaceae) from Mexico and the southern United States. *Lankesteriana* 9: 491–504.

Salazar, G. A., M. W. Chase, and M. A. Soto Arenas. 2002. Galeottiellinae, a new subtribe and other nomenclatural changes in Spiranthinae (Orchidaceae, Cranichideae). *Lindleyana* 17: 172–176.

Salazar, G. A., M. W. Chase, M. A. Soto-Arenas, and M. Ingrouille. 2003. Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae, Orchidoideae): Evidence from plastid and nuclear DNA sequences. *American Journal of Botany* 90: 777–795.

Salazar, G. A., L. I. Cabrera, S. Madriñán, and M. W. Chase. 2009. Phylogenetic relationships of Cranichidinae and Prescottiinae (Orchidaceae, Cranichideae) inferred from plastid and nuclear DNA sequences. *Annals of Botany* 104: 403–416.

Salazar, G. A., L. I. Cabrera, and C. Figueroa. 2011. Molecular phylogenetics, floral convergence and systematics of *Dichromanthus* and *Stenorrhynchos* (Orchidaceae, Spiranthinae). *Botanical Journal of the Linnean Society* 167: 1–18.

Salazar, G. A., C. van den Berg, and A. Popovkin. 2014. Phylogenetic relationships of *Discyphus scopulariae* (Orchidaceae, Cranichideae) inferred from plastid and nuclear DNA sequences: Evidence supporting recognition of a new subtribe, Discyphinae. *Phytotaxa* 173: 127–139.

Salazar, G. A., T. J. Hernández-López, J. Sharma, R. Jiménez-Machorro, L. I. Cabrera, and J. Treviño-Carreón. 2016. *Greenwoodiella*, a new genus of Spiranthinae (Orchidaceae) from North- and Central America and the Greater Antilles, with a new species from the Chihuahuan Desert. *Systematic Botany* 41: 823–838.

Salazar, G. A., J. A. N. Batista, L. I. Cabrera, C. van den Berg, W. M. Whitten, E. C. Smidt, C. R. Buzatto, R. B. Singer, G. Gerlach, R. Jiménez-Machorro, J. A. Radins, I. S. Insaurralde, L. R. S. Guimarães, F. de Barros, F. Tobar, J. L. Linares, E. Mújica, R. L. Dressler, M. A. Blanco, E. Hágsater, and M. W. Chase. 2018. Phylogenetic systematics of subtribe Spiranthinae (Orchidaceae, Orchidoideae, Cranichideae) based on nuclear and plastid DNA sequences of a nearly complete generic sample. *Botanical Journal of the Linnean Society* 186: 273–303.

Salazar, G. A., J. A. N. Batista, T. E. C. Meneguzzo, L. I. Cabrera, C. Figueroa, L. Calvillo-Canadell, A. A. do Vale, and R. Jiménez-Machorro. 2019. Data from: Polyphyly of *Mesadenus* (Orchidaceae, Spiranthinae) and a new genus from the Espinhaço Range, Southeastern Brazil. Dryad Digital Repository. https://doi.org/10.5061/dryad.c163km7.

Sass, J. E. 1958. *Botanical Microtechnique*, ed. 3. Ames: Iowa State College Press.

Schlechter, R. 1920. Versuch einer systematischen Neuordnung der Spiranthinae. *Beihefte zum Botanischen Centralblatt* 37: 317–454.

Siegel, C. 2011. Orchids and hummingbirds: Sex in the fast lane. *The Orchid Digest* 75: 8–17.

Singer, R. B. 2002. The pollination biology of *Sauroglossum elatum* Lindl. (Orchidaceae: Spiranthinae): Moth-pollination and protandry in Neotropical Spiranthinae. *Botanical Journal of the Linnean Society* 138: 9–16.

Singer, R. B. and M. Sazima. 2000. The pollination of *Stenorrhynchos lanceolatus* (Aublet) L. C. Rich. (Orchidaceae: Spiranthinae) by hummingbirds in southeastern Brazil. *Plant Systematics and Evolution* 223: 221–227.

Smidt, E. C. 2003. A subtribo Spiranthinae Lindl. (Orchidaceae: Orchidoideae) na Chapada Diamantina, Bahia, Brasil. M.S. thesis. Feira de Santana: Universidade Estadual de Feira de Santana.

Soto, M. A., E. Hágsater, R. Jiménez-Machorro, G. A. Salazar, R. Solano-Gómez, R. Flores, and I. Ruiz. 2007. *The Orchids of Mexico: Digital Catalogue*. Mexico City: Instituto Chinoin.

Steele, K. P. and R. Vilgalys. 1994. Phylogenetic analysis of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. *Systematic Botany* 19: 126–142.

Swofford, D. L. 2018. PAUP* Phylogenetic analysis using parsimony (*and other methods), v. 4.0a159. Sunderland: Sinauer Associates.

Szlachetko, D. L. 1992. Genera and species of the subtribe Spiranthinae (Orchidaceae). 2. A revision of *Schiedeella*. *Fragmenta Floristica et Geobotanica* 37: 157–204.

Szlachetko, D. L. and P. Rutkowski. 2000. *Gynostemia orchidalium* I. Apostasiaceae, Cypripediaceae, Orchidaceae (Thelymitroideae, Orchidoideae, Tropidioideae, Spiranthoideae, Neottioideae, Vanilloideae). *Acta Botanica Fennica* 169: 1–379.

Szlachetko, D. L., P. Rutkowski, and J. Mytnik. 2005. Contributions to the taxonomic revision of the subtribes Spiranthinae, Stenorrhynchidinae and Cyclopogoninae (Orchidaceae) in Mesoamerica and the Antilles. *Polish Botanical Studies* 20: 3–387.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Toscano de Brito, A. L. V. and P. J. Cribb. 2005. *Orquídeas da Chapada Diamantina*. Rio de Janeiro: Nova Fronteira.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in *PCR Protocols: A Guide to Methods and Applications*, eds. M. Innis, D. Gelfand, J. Sninsky, and T. J. White. San Diego: Academic Press.

Wurdack, J. J. 1970. Erroneous data in Glaziou collections of Melastomataceae. *Taxon* 19: 911–913.

APPENDIX 1. Voucher information is listed as follows: taxon name, collector name and number (herbarium), country, GenBank accessions (*matK-trnK*, *trnL-trnF*, ITS).

Aa colombiana Schltr., *Aldana 2* (ANDES), Colombia (AM900802, AM412731, AM419766); *Altensteinia fimbriata* Kunth, *Salazar 6789* (MEXU), Ecuador (AM900801, AM412737, AM419765); *Aulosepalum hemichreum* (Lindl.) Garay, *Salazar 6044* (MEXU), Mexico (HE575491, FN641878, AM884879); *Aulosepalum tenuiflorum* (Greenm.) Garay, *Salazar et al. 6150* (MEXU), Mexico (AM884249, AJ544474, AJ539591); *Beloglottis costaricensis* (Rchb.f.) Schltr., *Soto 8129* (MEXU), Mexico (AJ543920, AJ544475, AJ539492); *Beloglottis mexicana* Garay & Hamer, *Hágsater 12018* (AMO), Mexico (LT600869, LT600887, LT600852); *Brachystele camporum* (Lindl.) Schltr., *Insaurralde s.n.* (MEXU, spirit), Argentina (HG425366, HG425369, HG425362); *Brachystele guayanensis* (Lindl.) Schltr., *Batista & Meneguzzo 2927* (BHCB), Brazil (MG460434, MG460399, MG460367); *Brachystele unilateralis* (Poir.) Schltr., *Lehnebach s.n.* (MEXU, spirit), Chile (MG755106, MG582315, MF464956); *Buchtienia ecuadorensis* Garay, *Simpson s.n.* (FLAS), Peru (MG755107, MG582297, MF464938); *Coccineorchis cernua* (Lindl.) Garay, *Salazar et al. 6249* (MEXU, spirit), Panama (AJ543930, AJ544485, AJ539502); *Coccineorchis standleyi* (Ames) Garay, *Salazar 6248* (MEXU, spirit), Panama (LT600868, FN996961, FN996949); *Cotylolabium lutzii* (Pabst) Garay, *Batista et al. 3216* (BHCB), Brazil (HG425363, HG425367, HG425360); *Cranichis engelii* Rchb.f., *Schott s.n.* (K, spirit), Ecuador (AM900814, AM412721, AM419779); *Cyclopogon ovalifolius* C.Presl, *Morón s.n.* (MEXU, spirit), Peru (MG755118, MG582356, MF464999); *Cyclopogon peruvianus* (C.Presl) Schltr., *Bernal s.n.* (ANDES), Colombia (HE575496, HE575523, HE575513); *Cyclopogon variegatus* Barb.Rodr., *Radins s.n.* (MEXU, photograph), Argentina (MG755121, MG582346, MF464988); *Deiregyne densiflora* (C.Schweinf.) Salazar & Soto Arenas, *Salazar et al. 6125* (MEXU), Mexico (HE575498, FN641886, FN641874); *Deiregyne diaphana* (Lindl.) Garay, *Salazar et al. 6172* (MEXU), Mexico (AJ543912, AJ544467, AJ539484); *Deiregyne eriophora* (B.L.Rob. & Greenm.) Garay, *Salazar et al. 6104* (MEXU), Mexico (HE575499, FN641885, FN641873); *Deiregyne rhombilabia* Garay, *Salazar et al. 6138* (MEXU), Mexico (HE575500, FN641881, FN641869); *Dichromanthus aurantiacus* (Lex.) Salazar & Soto Arenas, *Salazar 6351* (K, spirit), Mexico (AJ543913, AJ544468, AJ539485); *Dichromanthus cinnabarinus* (Lex.) Garay, *Linares 4469* (MEXU), Mexico (AJ543914, AJ544469, AJ539486); *Discyphus scopulariae* (Rchb.f.) Schltr., *Popovkin 338A* (UEFS), Brazil (LK391735, LK391734, LK391732); *Eltroplectris calcarata* (Sw.) Garay & H.R.Sweet, *Soares s.n.* (MEXU, photograph), Brazil (AJ519450, AJ519452, AJ519448); *Eltroplectris schlechteriana* (Porto & Brade) Pabst, *Insaurralde s.n.* (MEXU, photograph), Argentina (MG755124, MG582301, MF464942); *Eltroplectris triloba* (Lindl.) Pabst, *Munich Bot. Gard. 96/4474* (M), Argentina (FN868835, FN641875, FN641864); *Espinhassoa glaziovii* (Cogn.) Salazar & J.A.N.Bat. 1, *Batista et al. 3158* (BHCB), Brazil (MG460450, MG460417, MG460385); *Espinhassoa glaziovii* (Cogn.) Salazar & J.A.N.Bat. 2, *Harley et al. 25119* (K), Brazil (MK310237, —, MK309832); *Eurystyles actinosophila* (Barb.Rodr.) Schltr *Franke s.n.* (MEXU, spirit), Argentina (MG755125, MG582363, MF465007); *Eurystyles cornu-bovis* Szlach., *Maduro 230* (FLAS), Panama (FN556172, FN556167, FN556162); *Funkiella hyemalis* (A.Rich. & Galeotti) Schltr., *Salazar et al. 6128* (MEXU), Mexico (AJ543923, AJ544478, AJ539495); *Funkiella minutiflora* (A.Rich. & Galeotti) Schltr., *Salazar et al. 6129* (MEXU), Mexico (AJ543922, AJ544477, AJ539494); *Funkiella parasitica* (A.Rich. & Galeotti) Salazar & Soto Arenas, *Jiménez s.n.* (AMO), Mexico (AJ543924, AJ544479, AJ539496); *Galeoglossum thysanochilum* (B.L.Rob. & Greenm.) Salazar, *Tenorio 17900* (MEXU), Mexico (AM900810, AM412725, AM419775); *Galeottiella sarcoglossa* (A.Rich. & Galeotti) Schltr., *Jiménez 2334* (AMO), Mexico (AJ543945, AJ544500, AJ539518); *Gomphichis caucana* Schltr., *Díaz 159* (ANDES), Colombia (AM900805, AM412736, AM419770); *Greenwoodiella deserticola* Salazar, Hernández-López & J.Sharma 1, *Sharma JS0001* (AMES), USA (LT600880, LT600897, LT600862); *Greenwoodiella micrantha* (Lex.) Salazar & R.Jiménez var. *garayana* (R.González) Salazar & R.Jiménez 3, *Salazar et al. 9225* (MEXU), Mexico (LT600876, LT600893, LT600858); *Greenwoodiella wercklei* (Schltr.) Salazar & R.Jiménez 3, *Pérez-Bravo 100* (MEXU), Mexico (LT600884, LT600901, LT600866); *Hapalorchis lineatus* (Lindl.) Schltr., *Ackerman 3648* (UPRRP), Puerto Rico (FN556175, FN556170, FN556165); *Kionophyton sawyeri* (Standl. & L.O.Williams) Garay, *Salazar 7252* (MEXU), Mexico (LT600873, LT600891, LT600856); *Lankesterella ceracifolia* (Barb.Rodr.) Ames, *Salazar 7535* (MEXU, spirit), Argentina (FN556174, FN556169, FN556164); *Lankesterella gnoma* (Kraenzl.) Hoehne, *Warren s.n.* (K, spirit), Brazil (FN556173, FN556168, FN556163); *Ludisia discolor* (Ker-Gawl.) A.Rich., *Salazar 6344* (MEXU), Tropical Asia (AJ543911, AJ544466, AJ539483); *Lyroglossa grisebachii* (Cogn.) Schltr., *Batista & Bianchetti 3108* (BHCB), Brazil (KF548547, KF548549, KF548545); *Manniella cypripedioides* Salazar, T.Franke, Zapfack & Benkeen, *Salazar et al. 6323* (YA), Cameroon (AJ543943, AJ544498, AJ539516); *Mesadenella cuspidata* (Lindl.) Garay, *Penz s.n.* (MEXU, photograph), Argentina (MF179019, MG582306, MF464946); *Mesadenella petenensis* (Standl. & L.O.Williams) Garay, *Salazar 6069* (MEXU), Mexico (AJ543931, AJ544486, AJ539503); *Mesadenus chiangii* (M.C.Johnst.) Garay, *Hernández-López et al. 416* (MEXU), Mexico (MK310238, MK310230, MK309833); *Mesadenus lucayanus* (Britt.) Schltr. 1, *Salazar 6043* (MEXU), Mexico (AJ543916, AJ544471, AJ539488); *Mesadenus lucayanus* (Britt.) Schltr. 2, *Salazar 6007* (MEXU), Honduras (MK310239, MK310231, MK309834); *Mesadenus polyanthus* (Rchb.f.) Schltr. 1, *Salazar 6370* (MEXU), Mexico (AM902109, LT600902, AM778175); *Mesadenus tenuissimus* (L.O.Williams) Garay 1, *Salazar 6033* (MEXU), Mexico (MK310240, MK310232, MK309835); *Mesadenus tenuissimus* (L.O.Williams) Garay 2, *Salazar et al. 6102* (MEXU), Mexico (MK310241, MK310233, MK309836); *Nothostele acianthiformis* (Rchb.f. & Warm.) Garay, *Viana 767* (BHCB), Brazil (FN868833, FN868836, FN868838); *Odontorrhynchus chlorops* (Rchb.f.) Garay, *Rodríguez s.n.* (MEXU, spirit), Argentina (MG755131, MG582313, MF464954); *Pelexia adnata* (Sw.) Poit. ex Spreng., *Salazar 6012* (MEXU), Mexico (AJ543929, AJ544484, AJ539501); *Pelexia ekmanii* (Kraenzl.) Schltr., *Radins s.n.* (MEXU, photograph), Argentina (MG755134, MG582317, MF464959); *Pelexia goninensis* (Pulle) Schltr., *Smidt 681* (HUEFS), Brazil (MG755136, MG582321, MF464964); *Physogyne gonzalezii* (L.O.Williams) Garay, *Soto & Salazar 10557* (AMO), Mexico (LT600872, LT600890, LT600855); *Ponthieva racemosa* (Walt.) C.Mohr, *Salazar 6049* (MEXU), Mexico (AJ543936, AJ544490, AJ539508); *Porphyrostachys pilifera* Rchb.f., *Whalley s.n.* (K, photograph), Peru (AJ543942, AJ544496, AJ539514); *Prescottia plantaginea* Lindl., *Salazar 6350* (K, spirit), Brazil (AJ543939, AJ544493, AJ539511); *Pseudogoodyera pseudogoodyeroides* (L.O.Williams) R.González & Szlach., *Salazar et al. 6545* (MEXU), Mexico (LT600871, LT600889, LT600854); *Pseudogoodyera wrightii* (Rchb.f.) Schltr., *Mújica 4* (MEXU, photograph), Cuba (MG755148, MG582376, MF465021); *Pterichis habenarioides* Schltr., *Aldana 12* (COL), Colombia (AJ543937, AJ544491, AJ539509); *Pteroglossa euphlebia* (Oliv. ex Rchb. f.) Garay, *Guimarães 191* (SP), Brazil (MG755149, MG582305, MF464945); *Pteroglossa glazioviana* (Cogn.) Garay, *Radins s.n.* (MEXU, spirit), Argentina (MF179017, MG582304, MF464944); *Pteroglossa macrantha* (Rchb.f.) Schltr., *Flachsland s.n.* (MEXU, photograph), Argentina (MF179016, MG582303, MF464943); *Pteroglossa roseoalba* (Rchb.f.) Salazar & M.W.Chase, *Salazar 6023* (MEXU), El Salvador (FN868834, FN868837, FN868839); *Quechua glabrescens* (T.Hashim.) Salazar & Jost, *Jost 7916* (QCNE), Ecuador (HE575503, HE575526, HE575516); *Sacoila lanceolata* (Aubl.) Garay, *Salazar et al. 9917* (MEXU, spirit), Argentina (MG755150, MG582307, MF464947); *Sarcoglottis fasciculata* (Vell.) Schltr., *Salazar 6369* (K, spirit), Argentina (MG755156, MG582335, MF464978); *Sarcoglottis lobata* (Lindl.) P.N.Don, *Huerta 88* (AMO), Mexico (MG755158, MG582338, MF464981); *Sarcoglottis speciosa* C.Presl, *Hermans & Hermans 5276* (K, spirit), Peru (MG755166, MG582333, MF464976); *Sauroglossum elatum* Lindl., *Cabassi s.n.* (MEXU, spirit), Argentina (HG425365, HG425368, HG425361); *Schiedeella affinis* (C.Schewinf.) Salazar, *López s.n.* (MEXU), Mexico (LT600885, LT600903, LT600867); *Schiedeella crenulata* (L.O.Williams) Espejo & López-Ferrari, *Goldman 902* (BH), Mexico (HE575504, FN641880, FN641868); *Schiedeella durangensis* (Ames & C.Schweinf.) Burns-Bal., *Soto 10673* (AMO), Mexico (HE575505, FN641879, FN641867); *Schiedeella nagelii* (L.O.Williams) Garay, *Salazar et al. 6071* (MEXU), Mexico (MK310242, MK310234, MK309837); *Schiedeella tenella* (L.O. Williams) Burns-Bal., *Soto 10659* (AMO), Mexico (MK310243, MK310235, MK309838); *Schiedeella transversalis* (A.Rich. & Galeotti) Schltr., *Salazar 6073* (MEXU), Mexico (—, AJ544470, —); *Schiedeella transversalis* (A.Rich. & Galeotti) Schltr., *Salazar 6105 et al.* (MEXU), Mexico (AJ543915, —, AJ539487); *Schiedeella williamsiana* Szlach., *Salazar et al. 6148* (MEXU), Mexico (MK310244, MK310236, MK309839); *Skeptrostachys balanophorostachya* (Rchb.f. & Warm.) Garay, *Baptista s.n.* (BHCB), Brazil (KF548546, KF548548, KF548544); *Skeptrostachys congestiflora* (Cogn.) Garay, *van den Berg 1425* (HUEFS), Brazil (MG755169, MG582310, MF464950); *Skeptrostachys paraguayensis* (Rchb.f.) Garay, *Radins s.n.* (MEXU, photograph), Argentina (MG755170, MG582309, MF464949); *Sotoa confusa* (Garay) Salazar, *Hernández 3320* (MEXU), Mexico (HE575506, FN641876, FN641865); *Spiranthes nebulorum* Catling & V.R.Catling, *Soto 10057* (AMO), Mexico (HE575507, HE575527, HE575517); *Spiranthes romanzoffiana* Cham., *Chase 4746* (K), USA (MG755179, MG582368, MF465012); *Spiranthes spiralis* (L.) Cheval., *Bateman s.n.* (K, spirit), United Kingdom (AJ543918, AJ544473, AJ539490); *Spiranthes vernalis* Engelm. & Gray, *Nickrent 4189* (MEXU), USA (MG755180, MG582370, MF465014); *Stenoptera ecuadorana* Dodson & C.Vargas, *Salazar 6357* (K, spirit), Ecuador (AJ543940, AJ544494, AJ539512); *Stenorrhynchos albidomaculatum* E.A.Christ., *Salazar 7662*, (MEXU, spirit), Costa Rica (MF179014, FN996960, FN996948); *Stenorrhynchos glicensteinii* Christenson, *Salazar 6090* (MEXU), Mexico (AJ543532, AJ544487, AJ539505); *Stenorrhynchos millei* Schltr., *Portilla s.n.* (K, spirit), Ecuador (MF179013, FN996958, FN996946); *Stenorrhynchos speciosum* (Jacq.) Rich. ex Spreng., *Salazar 7661* (MEXU, spirit), Virgin Islands (HG425364, FN996959, FN996947); *Stenorrhynchos vaginatum* (Kunth) Spreng., *Giraldo s.n.* (COL), Colombia (MF179015, MG582297, MF464939); *Svenkoeltzia congestiflora* (L.O.Williams) Burns-Bal., *Salazar et al. 6143* (MEXU), Mexico (AJ543921, AJ544476, AJ539493); *Thelyschista ghillanyi* (Pabst) Garay, *van den Berg 1435* (HUEFS), Brazil (MG755181, MG582296, MF464937); *Veyretia rupicola* (Garay) F.Barros, *van den Berg 1477* (UEFS), Brazil (MG755182, MG582359, MF465003).