



A new paludicolous species of *Malaxis* (Orchidaceae) from Argentina and Uruguay

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Abstract

Malaxis irmae, a new orchid species from the Paraná and Uruguay river basins in northeast Argentina and Uruguay, is described and illustrated. It is similar in size and overall floral morphology to *Malaxis cipoensis*, a species endemic to upland rocky fields on the Espinhaço range in Southeastern Brazil, which is its closest relative according to a cladistics analysis of nuclear (ITS) and plastid (*matK*) DNA sequences presented here. However, *M. irmae* is distinguished from *M. cipoensis* by inhabiting lowland marshy grasslands, possessing 3–5 long-petiolate leaves per shoot (vs. 2 shortly petiolate leaves), cylindrical raceme (vs. corymbose), pale green flowers (vs. green-orange flowers) and less prominent basal labellum lobules. *Malaxis irmae* is morphologically also similar to the Brazilian *M. warmingii*, which differs in its much larger plants and prominent basal labellum lobes.

Key words: ITS, *Malaxis irmae*, marshy grasslands, *matK*, phylogenetics

Introduction

As traditionally delimited, the genus *Malaxis* Solander ex Swartz (1788: 119) *s.l.* included about 300 species and had a worldwide distribution (Cribb 2005). However, a recent molecular phylogenetic analysis of tribe Malaxideae (Cameron 2005) showed that *Malaxis s.l.* is polyphyletic. Although much work on the phylogeny and taxonomy of the whole tribe Malaxideae remains to be done to clarify the generic limits, several morphology-based taxonomic studies have started to recognize less-inclusive segregated genera. Some of the current segregates of *Malaxis s.l.* include Old World tropical groups like *Crepidium* Blume (1825: 387), *Dienia* Lindley (1824: sub t. 825) and *Orestias* Ridley (1887: 197) (e.g. Szlachetko 1995, Clements & Jones 1996, Cribb 2005), whereas the New World segregated genera comprise *Crossoglossa* Dressler & Dodson (1993: 148), *Tamayorkis* Szlachetko (1995: 121) and *Crossoliparis* Margońska (2009: 298–299). Excluding such segregates, *Malaxis s.s.*, including *Microstylis* (Nuttall 1818: 196) Eaton (1822: 115), encompasses approximately 120 species restricted to the New World and temperate regions of Eurasia (G. A. Salazar, unpubl. data). Recently, Margońska *et al.* (2012) published a review of “Malaxidiinae” Bentham & Hooker (1883: 463, 465), a polyphyletic assemblage of taxa allegedly distinguishable from other Malaxideae by morphological traits such as column length relative to anther length, angle of the anther relative to the column, position of anther openings, degree of concavity of the stigma and structure of the nectary, but none of these traits is consistent in any of their purported subtribes, the limits of which grossly contradict the results of both, the molecular phylogenetic analysis of Cameron (2005) and Margońska *et al.*’s own cladogram based on ITS sequences (Margońska *et al.* 2012: Fig. 5). Likewise, the cumbersome infrageneric classification proposed in that work, which recognizes sections, subsections and series often based on unreliable characters (e.g. “Raceme apically dense and conical” vs. “Raceme dense and distinctly cylindrical all along its length”, a feature that often varies in the same plant depending on the stage of development of the inflorescence), results in grouping together disparate plants and segregating in different

supraspecific taxa species that hardly can be told apart, e.g. *M. excavata* (Lindley 1838: misc. 51) Kuntze (1891: 673) and *M. lepanthiflora* (Schlechter 1918: 200–201) Ames (1922: 84), which are ecologically and morphologically nearly indistinguishable but are placed in different sections by Margońska *et al.* (2012).

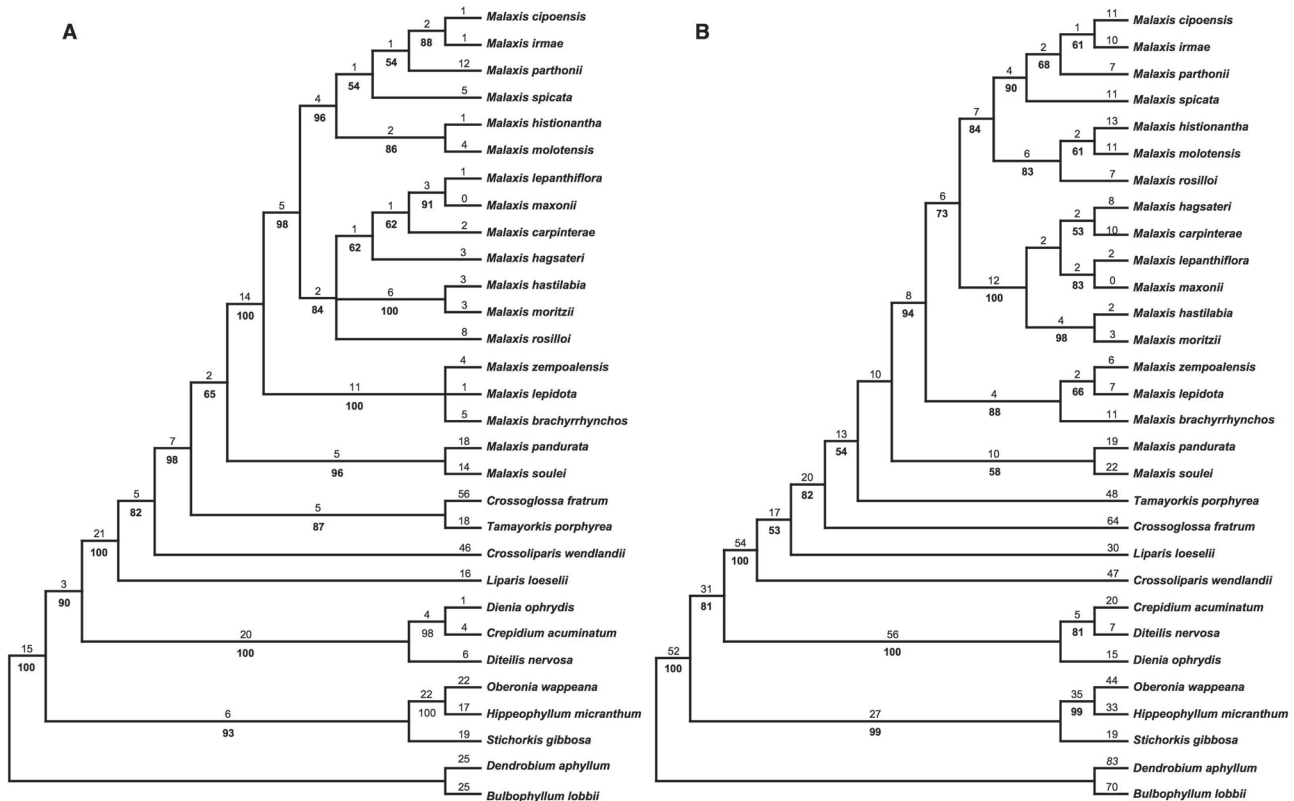


FIGURE 1. Phylogenetic relationships of selected Malaxideae inferred in the separate analyses of *matK* and ITS DNA sequences. A. The single most-parsimonious tree found by the analysis of *matK*. B. One of the three most-parsimonious trees recovered by the analysis of ITS. Numbers above the branches are branch lengths; numbers below the branches are bootstrap percentages.

Most published work on New World *Malaxis s.s.* consists of descriptions of new species, both as a result of access to previously unexplored areas and of the greater attention paid to these inconspicuous, little-studied orchids by local taxonomists (e.g. de Barros 1996, Salazar 1990, 1997, Salazar & de Santiago 2007, Dressler 2003, González *et al.* 2008, Carnevali & Noguera 2008). Here we describe an additional new species restricted to water-logged lowland vegetation in north-eastern Argentina and Uruguay. The phylogenetic position of the new species is assessed by means of a cladistics parsimony analysis of the same two molecular markers employed in Cameron’s (2005) molecular phylogenetic analysis of Malaxideae, namely the ITS region of nuclear ribosomal DNA (Baldwin *et al.* 1995) and the putative pseudogene *matK* of the plastid genome (Hilu & Liang 1997). The morphological and ecological peculiarities of the new species are discussed against the phylogenetic framework.

Materials and methods

Taxon sampling for the phylogenetic analysis:—We analysed samples of 28 species of Malaxideae, of which 18 belong to *Malaxis s.s.* and the others represent individual species of ten other genera of the tribe. One species each of *Dendrobium* Swartz (1799: 82) and *Bulbophyllum* Petit-Thouars (1822: table 3) were used as outgroups, following earlier works that indicate that Dendrobieae is the sister clade of Malaxideae (Cameron 2005, Cameron *et al.* 1999, Chase & Cribb 2005). Seventy five percent of the sequences were newly generated for this work and the rest downloaded from GenBank. A list of the taxa analysed, including voucher information and accession numbers in GenBank and the European Nucleotide Archive (<http://www.ebi.ac.uk/ena/data/view/HG970137-HG970159>) are provided in Table 1.

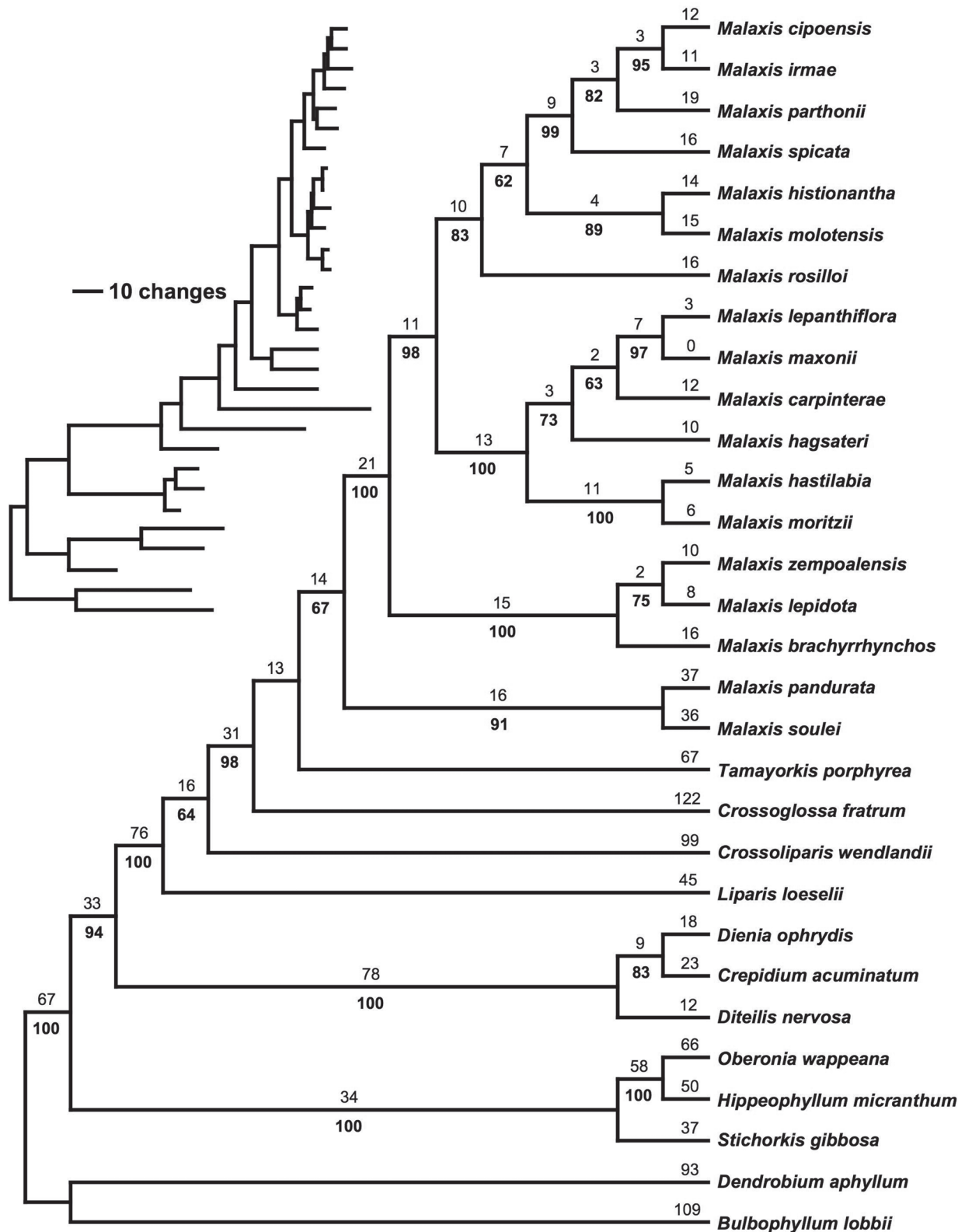


FIGURE 2. Phylogenetic relationships of selected Malaxideae inferred from combined *matK* and ITS DNA sequences. The main tree is the single most-parsimonious tree found; numbers above the branches are branch lengths; numbers below the branches are bootstrap percentages. The inset on the upper left hand is the same tree with branches drawn proportional to branch lengths.

TABLE 1. Taxa studied, voucher information or literature reference and GenBank/European Nucleotide Archive accessions.

Species	Voucher or reference	ITS	<i>matK</i>
Tribe Dendrobieae			
<i>Bulbophyllum lobbii</i> Lindl.	van den Berg <i>et al.</i> (2005)	AF521074	AY121740
<i>Dendrobium aphyllum</i> (Roxb.) C.E.C.Fisch.	Ding, X., Xu, L. & Wang, Z. (unpubl.) Teng, Y.-F., Wu, X.-J., Wang, Z.-T. & Yu, G.-D. (unpubl.)	AF355573 --	-- AF447068
Tribe Malaxideae			
<i>Crepidium acuminatum</i> (D.Don) Szlach.	Ohi-Toma <i>et al.</i> (2007)	AB290884	AB290892
<i>Crossoglossa fratrum</i> (Schltr.) Dressler ex Dodson	Costa Rica, <i>Dressler s.n.</i> (USJ)	HG970119	HG970141
<i>Crossoliparis wendlandii</i> (Rchb.f.) Marg.	Mexico, <i>Salazar et al. 6425</i> (MEXU)	HG970118	HG970140
<i>Dienia ophrydis</i> (J.Koenig) Seidenf.	Cameron (2005)	AY907114	AY907181
<i>Diteilis nervosa</i> (Thunb.) M.A.Clem. & D.L.Jones	Cameron (2005)	AY907092	AY907158
<i>Hippeophyllum micranthum</i> Schltr.	Philippines, <i>Salazar 7637</i> (MEXU)	HG970115	HG970137
<i>Liparis loeselii</i> (L.) Rich.	U.K., <i>Chase 7238</i> (K)	HG970117	HG970139
<i>Malaxis brachyrrhynchos</i> (Rchb.f.) Ames	Mexico, <i>Salazar et al. 7484</i> (MEXU)	HG970121	HG970143
<i>Malaxis carpintera</i> (Schltr.) Ames	Costa Rica, <i>Salazar s.n.</i> (MEXU, spirit)	HG970125	HG970147
<i>Malaxis cipoensis</i> F.Barros	Brazil, <i>Batista 2328</i> (BHCB)	HG970126	HG970148
<i>Malaxis hagsateri</i> Salazar	Mexico, <i>Salazar 6773</i> (MEXU)	HG970123	HG970145
<i>Malaxis hastilabia</i> (Rchb.f.) Kuntze	Costa Rica, <i>Salazar s.n.</i> (MEXU, spirit)	HG970128	HG970150
<i>Malaxis histionantha</i> (Link, Klotzsch & Otto) Garay & Dunst.	Mexico, <i>Soto 8958</i> (AMO)	HG970124	HG970146
<i>Malaxis irmae</i> Radins & Salazar	Argentina, <i>Radins 105</i> (CTES)	HG970127	HG970149
<i>Malaxis lepanthiflora</i> (Schltr.) Ames	Mexico, <i>Reyes 5469</i> (MEXU)	HG970129	HG970151
<i>Malaxis lepidota</i> (Finet) Ames	Mexico, <i>Soto 9733</i> (AMO)	HG970122	HG970144
<i>Malaxis maxonii</i> Ames	El Salvador, <i>Salazar & Linares 7519</i> (MEXU)	HG970130	HG970152
<i>Malaxis molotensis</i> Salazar & de Santiago	Mexico, <i>de Santiago 1320</i> (MEXU)	HG970131	HG970153
<i>Malaxis moritzii</i> (Ridl.) Kuntze	Venezuela, <i>Jardín Botánico Universidad de Mérida 27-07</i> (MEXU, spirit)	HG970132	HG970154
<i>Malaxis pandurata</i> (Schltr.) Ames	Mexico, <i>Rojas 54</i> (MEXU)	HG970135	HG970158
<i>Malaxis parthonii</i> C.Morren	Argentina, <i>Radins s.n.</i> (MEXU, photograph)	HG970133	HG970155
<i>Malaxis rosilloi</i> R.González & E.W.Greenw.	Mexico, <i>Salazar & Carnevali 6078</i> (MEXU)	HG970134	HG970156
<i>Malaxis soulei</i> L.O.Williams	Mexico, <i>Soto 9741</i> (AMO)	HG970136	HG970159
<i>Malaxis spicata</i> Sw.	Without locality, <i>Chase 377</i> (K)	AF521068	HG970157
<i>Malaxis zempoalensis</i> López-Ferr. & Espejo	Mexico, <i>Espejo et al. 5714</i> (AMO)	HG970120	HG970142
<i>Oberonia wappeana</i> J.J.Sm.	Cameron (2005)	AY907138	AY907206
<i>Stichorkis gibbosa</i> (Finet) J.J.Wood	South East Asia, <i>Heidelberg Botanical Garden s.n.</i> (HEID)	HG970116	HG970138
<i>Tamayorkis porphyrea</i> (Ridl.) Salazar & Soto Arenas	Cameron (2005)	AY907115	AY907182

DNA extraction, amplification and sequencing:—Genomic DNA was extracted from fresh, silica gel-dried or herbarium material using a 2× cetyltrimethylammonium bromide (CTAB) protocol based on Doyle & Doyle (1987), modified by the addition of 2% of polyvinylpyrrolidone (PVP) to the extraction buffer. PCR was carried out in 25 µL reactions using a commercial kit (*Taq* PCR Core Kit, Qiagen, Hilden, Germany), adding to the reaction mix 0.25 µL of each primer at a concentration of 100 ng/µL and 0.5 µL of a 0.4% aqueous solution of bovine serum albumin (BSA) to neutralize potential inhibitors (Kreader 1996). In the case of the ITS region, 0.5 µL of dimethylsulfoxide (DMSO) were

added to the reaction tube to reduce problems associated with DNA secondary structure. The PCR profile for *matK* consisted of a 2 min 30 s initial premelt at 94°C, 28–30 cycles with 1 min denaturation at 94°C, 1 min annealing at 52°C, a first 2 min 30 s extension at 72°C, increased by 8 s on each consecutive cycle, and final extension of 7 min at 72°C. The PCR profile for the ITS region consisted of an initial 2 min premelt at 94°C, 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 50°C, and 2 min extension at 72°C, with final extension of 7 min at 72°C. The primers used for PCR and sequencing are listed in Table 2.

TABLE 2. Primers used for PCR and sequencing.

Primer name	DNA region	Primer sequence	Reference
17SE	ITS	5'-ACG AAT TCA TGG TCC GGT GAA GTG TTC-3'	Sun <i>et al.</i> (1994)
26SE	ITS	5'-TAG AAT TCC CCG GTT CGC TCG CCG TTA-3'	Sun <i>et al.</i> (1994)
<i>matK</i> -19F	<i>matK</i>	5'-CGT TCT GAC CAT ATT GCA CTA TG-3'	Molvray <i>et al.</i> (2000)
<i>matK</i> 458F	<i>matK</i>	5'-CTA CTA ATA CCC YAT CCC ATC-3'	Molvray <i>et al.</i> (2000)
<i>matK</i> 556R	<i>matK</i>	5'-GAA GRA ACA TCT TTK ATC CA-3'	Molvray <i>et al.</i> (2000)
<i>matK</i> 731F	<i>matK</i>	5'-TCT GGA GTC TTT CTT GAG CGA-3'	Molvray <i>et al.</i> (2000)
<i>matK</i> 1326R	<i>matK</i>	5'-TCT AGC ACA CGA AAG TCG AAG T-3'	Cuénoud <i>et al.</i> (2002)
trnK 2R	<i>matK</i>	5'-AAC TAG TCG GAT GGA GTA G-3'	Steele & Vilgalys (1994)

PCR products were purified using QIAquick silica columns (Qiagen) and used in cycle sequencing reactions with the ABI Prism Big Dye® Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq® DNA polymerase version 3.1 (Applied Biosystems Inc., Foster City, California, USA). Cycle sequencing reactions included 2 µL terminator mix, 0.25 µL primer at the same concentrations as for PCR and 3 µL PCR product. Cycle sequencing products were purified with Centri-Sep sephadex columns (Princeton Separations, Inc., Adelphia, New Jersey, USA) and analysed in a 3100 Genetic Analyzer (Applied Biosystems Inc.).

Phylogenetic analysis:—The chromatograms were edited and assembled with Sequencher (GeneCodes Corp., Ann Arbor, Michigan, USA), and the resulting sequences were aligned by eye, trying to maximize sequence similarity (Simmons 2004). Individual gap positions were treated as missing data. The aligned matrix is available from TreeBase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S15688>). We analysed the *matK* and ITS data separately and in combination under the parsimony optimality criterion using the software PAUP* v. 4.02b (Swofford 2002). Each analysis consisted of a heuristic search with 1,000 replicates of random order of taxa for calculating the starting trees, tree-bisection-reconnection (TBR) branch-swapping and the “Multrees” option activated, saving all the most-parsimonious trees (MPTs) found. Clade support was assessed by means of 1,000 bootstrap replicates (Felsenstein 1985), each consisting of 20 heuristic searches conducted as above but saving up to 20 MPTs per heuristic replicate.

Morphological observations:—The description of the new species was based mainly on study and measurements from living, pressed and ethanol-preserved plants from Argentina under a stereomicroscope. Complementary information on the Uruguayan record was obtained from literature (Izaguirre 2010).

Results of the phylogenetic analysis

The *matK* matrix included 1,411 characters, of which 160 were parsimony-informative. The heuristic search found a single MPT (Fig. 1A) with a length of 509 steps, Consistency Index (CI, excluding uninformative characters) = 0.69 and Retention Index (RI) = 0.87. The ITS matrix consisted of 705 characters, of which 299 were parsimony-informative, and the search recovered 3 MPTs with a length of 1,034 steps, CI = 0.56 and RI = 0.71. One of the 3 MPTs is shown in Fig. 1B. As in the study of Cameron (2005), *matK* and ITS recovered similar relationships. The combined matrix consisted of 2,116 characters, of which 459 were parsimony-informative, and the analysis found a single MPT with a length of 1,453 steps, CI = 0.59 and RI = 0.75. The single MPT, with clade support from the bootstrap analysis (Bootstrap Percentages, BP), is shown in Fig. 2. In the following, we will refer only to this tree, which we consider as our best estimate of the phylogenetic relationships among the taxa analysed.

The earliest groups to diverge in Malaxideae consist of a grade of two strongly supported, successively diverging clades containing Old World taxa (Fig. 2). The first such clade encompasses *Stichorchis* Petit-Thouars (1809: 318) as sister to [*Hippeophyllum* Schlechter (1905: 107)-*Oberonia* Lindley (1830–1840: 15)], whereas the second clade includes *Diteilis* Rafinesque (1833: 73–74) and [*Crepidium-Dienia*] and is in turn sister to a strongly supported group (BP 100) in which Northern Temperate *Liparis loeselii* (Linnaeus 1753: 947) Richard (1817: 38) is the sister of a New World clade encompassing the remaining taxa. Within the latter, *Crossoliparis*, *Crossoglossa* and *Tamayorkis*

are successive sisters to weakly supported (BP 67) *Malaxis s.s.* The latter consists, in ascending branching order, of a group formed by *M. soulei* Williams (1934: 343) and *M. pandurata* (Schlechter 1906: 77–78) Ames (1922: 84) (BP 91), the clade *M. brachyrrhynchos* (Reichenbach filius 1888: 152–153) Ames (1922: 84)–[*M. lepidota* (Finet 1907: 531–532) Ames (1922: 84)–*M. zempoalensis* López-Ferrari & Espejo (2009: 45)] (BP 100) and a further clade (BP 100) that, except for *M. cipoensis* Barros (1996: 31), consists of species having above-ground, ovoid pseudobulbs separated by conspicuous rhizomes (cf. Salazar 1990), in contrast with the subterranean, globose corms without rhizomes found in other species of the genus. That last major clade consists in turn of two subclades, i.e. *M. moritzii* (Ridley 1888: 330) Kuntze (1891: 673) through *M. lepanthiflora*, and *M. rosilloi* González & Greenwood (1984: 387) through *M. cipoensis*. The latter includes the lectotype species of *Malaxis* (*M. spicata* Solander ex Swartz 1788: 119). The new species, hereafter referred to as *Malaxis irmae* Radins & Salazar (see Taxonomy, later) occupies a derived position in this clade as the sister of *M. cipoensis* (BP 95).

Taxonomy

Malaxis irmae Radins & Salazar, *sp. nov.* (Figs. 3, 4).

Similar to *Malaxis cipoensis* F.Barros, differing in inhabiting marshy lowland vegetation, 3–5 petiolate leaves per shoot, pale green flowers with somewhat darker green labellum and smaller basal lobules of the labellum.

Holotype:—ARGENTINA. Misiones: Garupá, 11 July 2008, *J. A. Radins 105* (CTES!).

Paludicolous herb 6–14 cm in height including the inflorescence. Roots cylindrical, sparsely pilose, up to 3 cm long, 0.5–1.0 mm in diameter. Rhizome whitish, up to several cm long, 3–5 mm in diameter. Pseudobulbs inconspicuous, ovoid, up to 10 mm long and 6 mm in diameter, when young concealed by the sheathing bases of the petioles. Leaves 3–5 per shoot, ascending, petiolate; petiole white at base but becoming green towards the apex, channelled, distinctly elongate, 12–55 mm long, 3–4 mm wide; blade deep green, broadly ovate to elliptic, base widely cuneate to rounded, apex obtuse-rounded, 14–50 mm long, 10–35 mm wide. Inflorescence provided with a laterally compressed peduncle 50–80 mm long, which is concealed at base by the petiole of the upper leaf; raceme 16–60 mm long, at first condensed and thus appearing somewhat umbellate but the rachis elongates as the flowers open successively and the raceme is cylindrical; rachis slightly angled. Floral bracts slightly concave, incurved, triangular, acute, 1–2 mm long. Flowers non-resupinate; sepals and petals pale green, labellum deep green. Sepals convex, with revolute lateral margins, 3-veined; dorsal sepal adpressed to the ovary, ovate, subacute, 2–2.5 mm long, ca. 1.5 mm wide; lateral sepals diverging, obliquely ovate-elliptic, obtuse, 1.8–2.3 mm long, ca. 1.8 mm wide. Petals strongly recurved, linear, subacute, 1-veined, 2.1–2.2 mm long, ca. 0.2 mm wide. Labellum fleshy, broadly cordate-sagittate, 1.4–1.7 mm long, 1.9–2.8 mm wide; proximal half provided with two rounded excavations; base provided at each side with a slightly retrorse, rounded lobule 0.5–0.6 mm long; apex apiculate, the apicule somewhat incurved in natural position. Column slightly compressed dorsiventrally, ca. 7 mm long and wide; anther dorsal, emarginate; rostellum broadly obtuse; stigma apical, concave, distinctly wider than long. Pollinaria 2, each formed by 2 fused pollinia, ca. 0.5 mm long (fide Izaguirre 2010; not seen). Ovary erect, straight, slightly twisted and somewhat thinner on the proximal one-third, above the middle slightly 6-angled, 6–8 mm long, ca. 1 mm in diameter near the apex. Capsule obovoid-ellipsoid, ca. 6 mm long (plus the pedicel of about the same length), to 5 mm in diameter.

Distribution and ecology:—*Malaxis irmae* is known only from the Río Paraná and Río Uruguay basins in Uruguay and north-eastern Argentina (Fig. 5), but it is expected also from southern Paraguay and the state of Rio Grande do Sul, Brazil. It inhabits in water-logged terrain dominated by grasses, and in neighbouring forest edges, between 60 and 100 m above sea level.

Conservation status:—Only three populations of this species have been recorded, but the Argentinian and Uruguayan populations are over 700 km apart in a straight line (Fig. 5) and it is likely that other populations exist, since suitable habitats (lowland wet grasslands) are widespread over the extensive Río de la Plata basin (which, among others, encompasses the Paraná and Uruguay rivers). At the type locality, urban expansion of the town of Garupá, on the outskirts of the city of Posadas, represents a short-term threat to that population; besides, the wet grasslands and associated water-logged forests that constitute the habitat of this species, are one of the most endangered vegetation types within the Atlantic rain forest biome in south-eastern South America (e.g. Bitetti *et al.* 2003, Krauczuk 2005, Overbeck *et al.* 2007). According to the IUCN Red List Categories and Criteria (IUCN 2012), *M. irmae* would qualify as Critically Endangered CR (criteria B2a, B2b and C2a(i)). However, both plants and flowers of this species are inconspicuous and can easily go unnoticed, even to trained botanical collectors. Therefore, further field studies are required to attain an objective assessment of its conservation status.

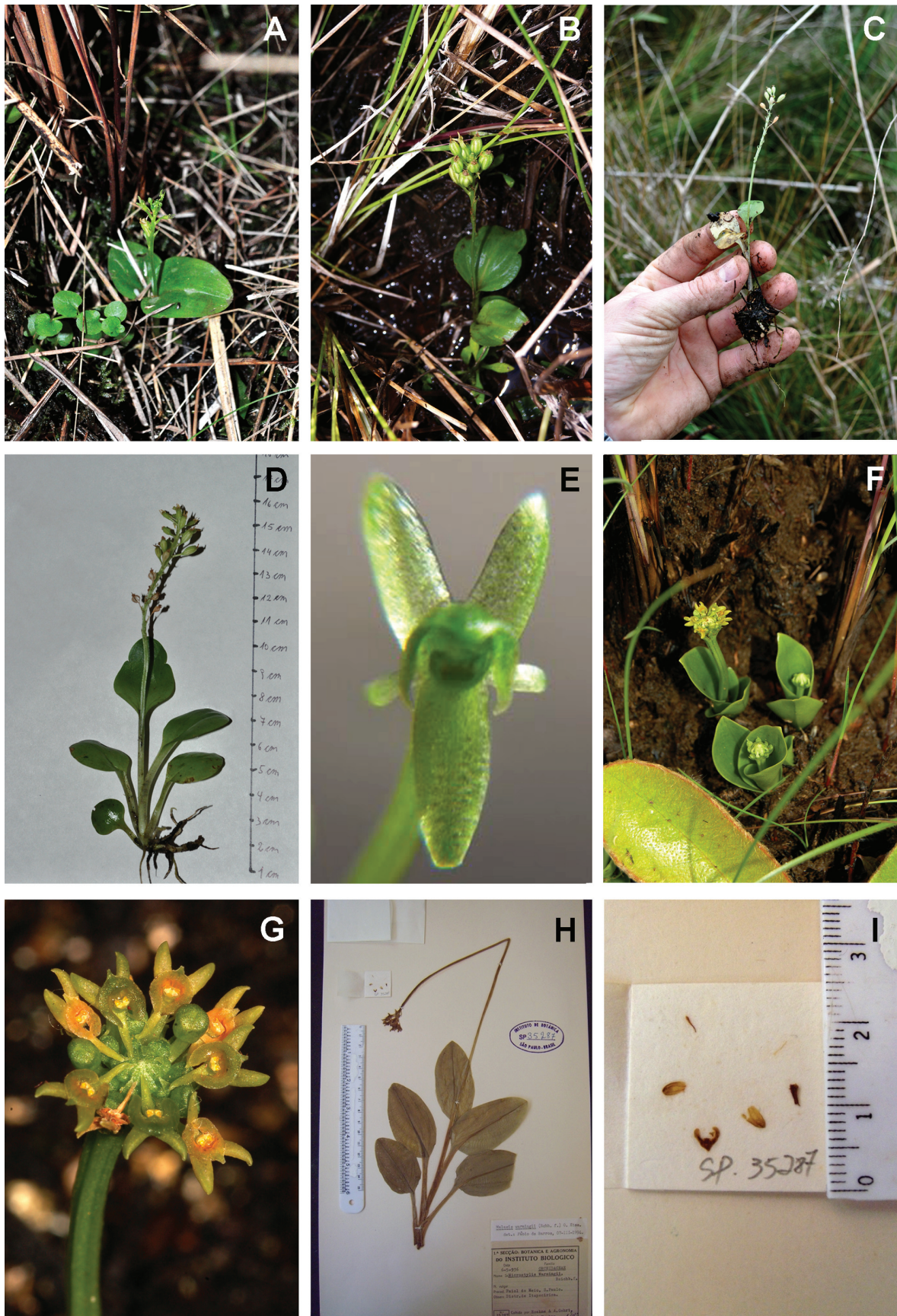


FIGURE 3. A–E, *Malaxis irmae* (from the type locality in Garupá, Misiones, Argentina). A. Flowering plant *in situ*. B. Fruiting plant *in situ*. C. Plant removed from substrate. D. Fruiting plant compared to a scale (in cm). E. Close-up of a flower. F–G. *M. cipoensis* (Brazil, Batista 2328, BHCB). F. Plants *in situ*. G. Inflorescence. H–I. *M. warmingii* (Brazil, Hoehne & Gehrt 35287, SP). H. Overview of the herbarium sheet with a 15 cm ruler for scale. I. Close-up of dissected flower. Photographers: A–E, J.A. Radins; F–G, J.A.N. Batista; H–I, F. de Barros.

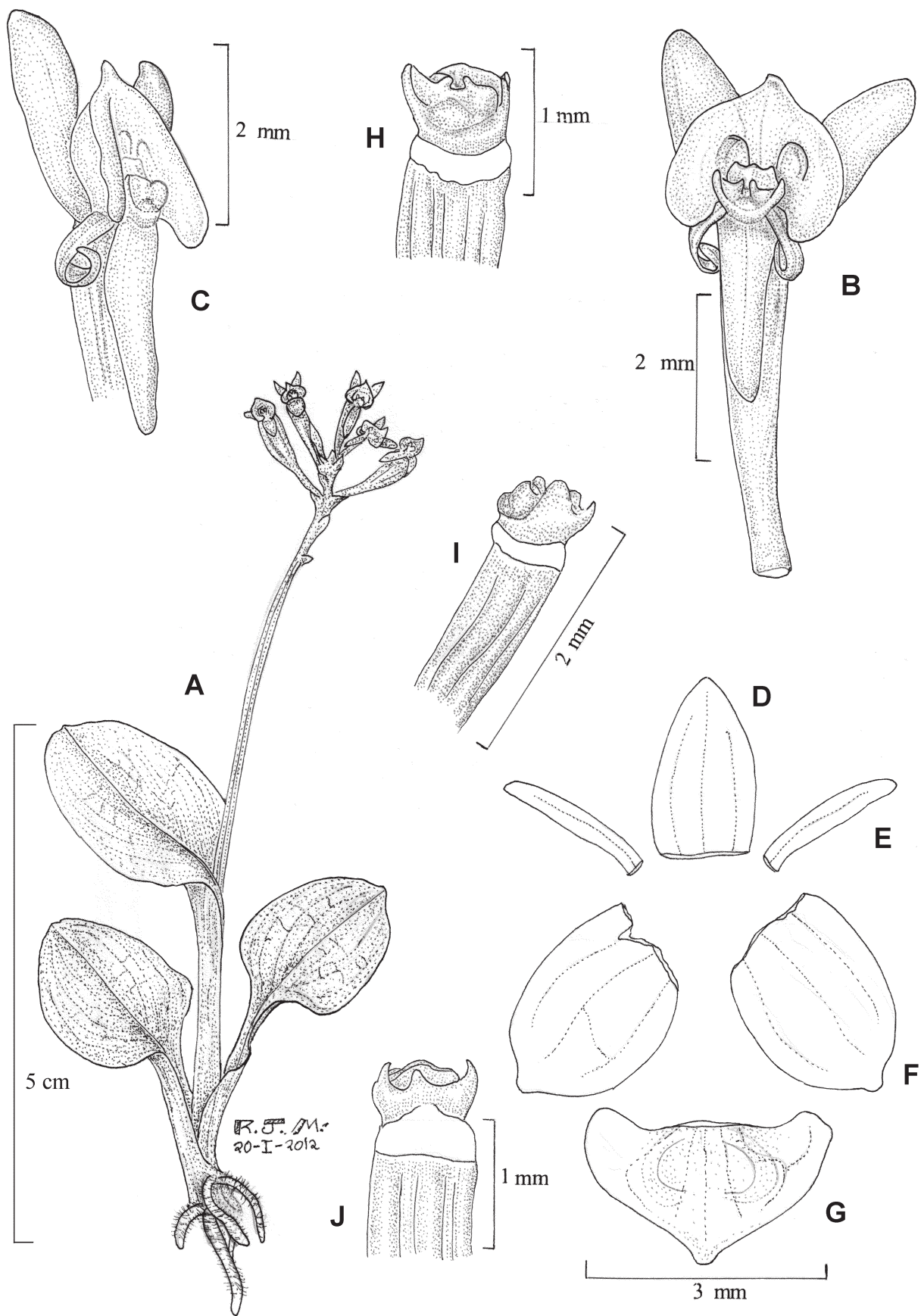


FIGURE 4. *Malaxis irmae*. A. Habit. B. Flower frontal view. C. Flower side view. D. Dorsal sepal. E. Petal. F. Lateral sepal. G. Labellum. H. Column from above. I. Column side view. J. Column from below. Drawn with camera lucida by Rolando Jiménez-Machorro from *Radins 105*.

Phenology:—Flowering from March to June. Capsules in advanced stage of development, including some already dehiscent, have been observed from May to August.

Etymology:—The specific epithet honours Ms. Irma Stella Insaurralde, long-term student of the orchids, and the flora in general, of the province of Misiones, Argentina.

Additional specimen examined:—ARGENTINA. Corrientes: Colonia Liebig, *D. Boicho s.n.* (CTES!).

Other records:—URUGUAY. Florida: precise locality not indicated, *E. Marchesi s.n.* (see Izaguirre 2010).

Discussion:—Our phylogenetic analysis strongly supports *M. irmae* as the sister of *M. cipoensis* (Fig. 1, 2), in agreement with their similar plant and flower size and overall floral morphology. However, they differ strikingly in habitat preferences, since *M. cipoensis* inhabits in well-drained soil on rocky field (*campo rupestre*) areas at 1,000 to 1,340 m elevation, having been found so far only on the Serra do Cipó and Serra da Moeda, both of which form part of the Espinhaço Range in the state of Minas Gerais, Brazil (Barros 1996, J.A.N. Batista, pers. obs.; Fig. 3F–G). *Malaxis irmae* thus differs from *M. cipoensis* in its lowland, water-logged habitat (Fig. 4 A–B), and morphologically it can also be distinguished from the latter by possessing 3–5, distinctly petioled leaves per shoot (vs. 2 shortly petiolate leaves), pale green flowers with a darker green labellum (vs. orangish-green flowers) and smaller basal labellum lobes (Fig. 4C–E; a good illustration of *M. cipoensis* for comparison is found in Barros 1996). *Malaxis warmingii* (Reichenbach 1881: 64) Kuntze (1891: 673), a relatively widespread, south-eastern Brazilian species that was not sampled for our molecular analysis, shares with *M. irmae* a preference for water-logged habitats, the 5–6-leaved shoots and a similar overall floral morphology, but it has much larger plants (40–60 cm in height) and prominent basal labellum lobes (Fig. 4H–I, Cogniaux 1893–1896). *Malaxis hieronymi* (Cogniaux 1893–1896: 279–280) Williams (1939: 363) inhabits marshes at high elevations (2,000–3,500 m) in Bolivia and the Argentinian provinces of Salta, Jujuy and Tucumán, being further distinguished from *M. irmae* by having pseudobulbs completely covered by fibrous leaf sheaths, two subsessile leaves and labellum lacking basal lobules. The Uruguayan material assigned here to *M. irmae* was identified by Izaguirre (2010) as *M. spicata*, a 2-leaved species with prominently lobed, brownish to orange-vermillion labellum provided with a deep triangular cavity, which is limited by a V-shaped, thickened rim. *Malaxis spicata* is restricted to the Antilles and the southeastern U.S.A. (Luer 1972).

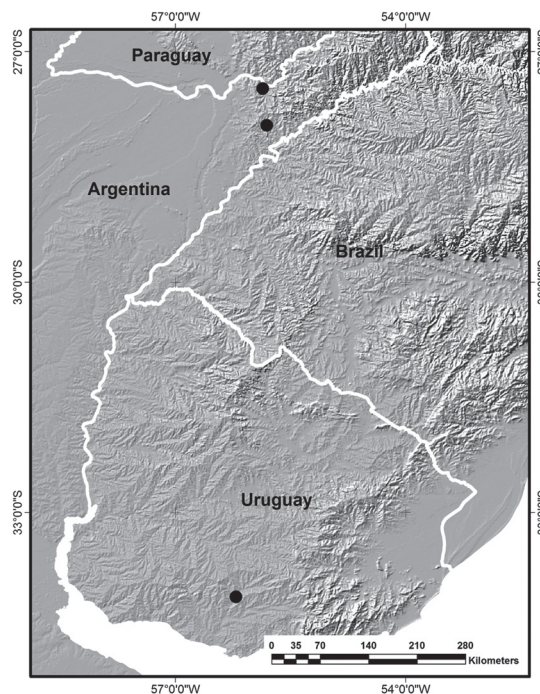


FIGURE 5. Known distribution of *Malaxis irmae* (dots).

The overall phylogenetic relationships in Malaxideae are beyond the focus of this paper and will be discussed in detail elsewhere (G. A. Salazar *et al.*, unpublished manuscript). The phylogenetic analysis conducted here was aimed mainly at setting up a context to determine the relationships of the new species, since a morphological comparison with other species of the genus indicated similarities to both *M. cipoensis* and *M. warmingii* (see earlier), and its preference

for water-logged habitats is shared, besides *M. warmingii*, by several other species, including South American *M. hieronymi* (as noted above) and Mexican *M. zempoalensis*. From our analysis, it is clear that the preference for wet places has evolved more than once in this genus. Although our sample of species of the genus is too sparse to draw conclusions at this time, the relationships recovered suggest that habitat divergence/specialisation may have played a role in promoting speciation in *Malaxis s.s.*, which is exemplified by the contrasting habitat preferences of *M. irmae* and *M. warmingii* with respect to *M. cipoensis* (the closest relative of *M. irmae* among the taxa we sampled). This and other interesting evolutionary questions, however, will have to be revisited when a more thorough sample of the genus is available for molecular phylogenetic study.

Barros (1996) placed *M. cipoensis* in *Malaxis* section *Umbellulatae* (Ridley 1888: 315) Barros (1996: 33) and *M. warmingii* in *M.* section *Spicatae* Ridley (1888: 315). The latter is obviously superfluous as it includes the (lecto-) type species of *Malaxis* (*M. spicata*), whereas our phylogenetic analysis shows that *M. cipoensis* belongs in the same clade as *M. spicata*, thus demonstrating that these infrageneric taxa are of little use. These and other infrageneric groups (e.g. those in Margońska *et al.* 2012), will have to be thoroughly assessed when the phylogenetic relationships in the genus are better understood.

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