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Rediscovery and Phylogenetic Placement of *Philcoxia minensis* (Plantaginaceae), with a Test of Carnivory

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The recently described genus *Philcoxia* comprises three rare species endemic to seasonally dry areas of deep white sand among cerrado vegetation in Brazil. One of these, *P. minensis*, was described from a single fragmentary specimen collected in the Serra do Cabral in Minas Gerais, Brazil, the detailed locality of which was unspecified. We report the rediscovery of *P. minensis* in this mountain range and provide an augmented description, detailed illustrations, and locality and habitat information. On the basis of morphology, *Philcoxia* has been considered to be a member of either the tribe Scrophularieae or tribe Gratioleae, in the latter case close to *Gratiola* or members of the informally named subtribe "Dopatriinae." We tested the classification of *Philcoxia* with a phylogenetic analysis of *P. minensis* and other samples of Gratioleae based on molecular sequence data from the internal transcribed spacer region of nuclear DNA and *rbcL*, *3′-ndhF*, *matK*/*3′-trnK*, and *trnL-trnF* regions of chloroplast DNA. Results demonstrate solid support for the inclusion of *P. minensis* within the Gratioleae, but relatively distant from both *Gratiola* and "Dopatriinae." Instead, it forms the second-divergent lineage among the samples tested in separate and combined-gene analyses. Previous workers have noted that the peltate leaves with stalked capitate glands on the upper surface and what they considered to be circinnate vernation in *Philcoxia* are similar to those found in some carnivorous plant families. Our additional observation of nematode worms on the surfaces of most leaves of all species of *Philcoxia* prompted us to conduct a test of carnivory in *P. minensis*. Negative results for protease activity suggest that *Philcoxia* is not carnivorous. Because of various potential sources of error, however, the possibility of carnivory in *Philcoxia* should not be entirely ruled out.

The recently described genus *Philcoxia* P. Taylor and V.C. Souza consists of three rare species endemic to Brazil (Taylor et al. 2000). The genus is characterized by subterranean stems, orbicular to reniform usually peltate leaves situated on or below the soil surface, flowers on a leafless scape, a deeply 5-lobed calyx with subequal lobes, two adaxial and included stamens, monothecous glabrous anthers that are oriented transversely to the filament, lack of staminodes, and a 4-valved capsule. The peltate leaves and unusual subterranean stems of *Philcoxia* are extraordinary features within Plantaginaceae (*sensu* Angiosperm Phylogeny Group 2003; Taylor et al. 2000). All species occur in areas of white sand surrounded by cerrado vegetation between 800 and 1450 m ele-

vation. Each of the species is named for the state of Brazil to which it is endemic: *P. bahiensis* V.C. Souza and Harley, *P. goiasensis* P. Taylor, and *P. minensis* V.C. Souza and Giulietti.

The last of these was until now only documented from the type, collected in the Serra do Cabral in 1981 with the precise locality not indicated. Some of the authors who described the genus conducted a field trip to the Serra do Cabral but could not relocate *Philcoxia minensis* (Taylor et al. 2000). During a field trip to the Serra do Cabral in October 2001 to study Melastomataceae and members of Ericales, the first three authors of the present paper by chance encountered *P. minensis* growing in a flat undisturbed area of very dry deep white sand among cerrado vegetation. The presence of *Discocactus placentiformis* (Lehm.) K. Schum. in the immediate vicinity indicated the well drained habitat in which *P. minensis* occurs.

The presumably highly specialized vegetative characters of *Philcoxia* have obscured the relationships of this genus to other members of Scrophulariaceae *sensu lato*. Souza (1996) placed it in tribe Scrophularieae *sensu* Thieret (1967) on the basis of the shared features of its posterior corolla lobes overlapping the lateral lobes and monotelic (cymose) inflorescence. In contrast, Taylor et al. (2000), in interpreting the inflorescence as polytelic (racemose) and citing a general, although unspecified, resemblance, suggested affinity with *Gratiola* L. and *Dopatrium* Buch.-Ham. ex Benth., predominantly aquatic genera in the tribe Gratioleae *sensu* Wettstein (1891). Fischer (2004) placed *Philcoxia* within an informally recognized subtribe "Dopatriinae" of tribe Gratioleae also containing the mostly aquatic genera *Deinostema* Yamazaki, *Dopatrium*, *Hydrotriche* Zucc., and *Limnophila* R. Br.

In their original paper describing *Philcoxia*, Taylor et al. (2000) noted the general convergent similarity to members of Lentibulariaceae, especially in the peltate leaves with reportedly circinnate vernation and abundant stalked capitate glands on the adaxial surfaces. They stated that field observations did not support the view that the glands had any insectivorous function, although the detailed basis for this conclusion was not mentioned. One piece of evidence for carnivory would be the presence of dead organisms on the leaf surfaces. We carefully examined all leaf surfaces from both our recent collections and the isotypes of the other two species, and did not observe any insects. Upon magnification to 60×, however, sparse to rather dense brown threads on the upper surfaces on most of the upper leaf surfaces of each species were apparent (Fig. 1A). Increasing the magnification to 1000× confirmed that these were nematode worms (Fig. 2). The leafless scapose inflorescence and open, nutrient-poor, fire-prone habitat in which *Philcoxia* species occur are consistent with the form and habitat of many carnivorous plants (Lloyd 1942; Givnish 1989). The habitat of white sand

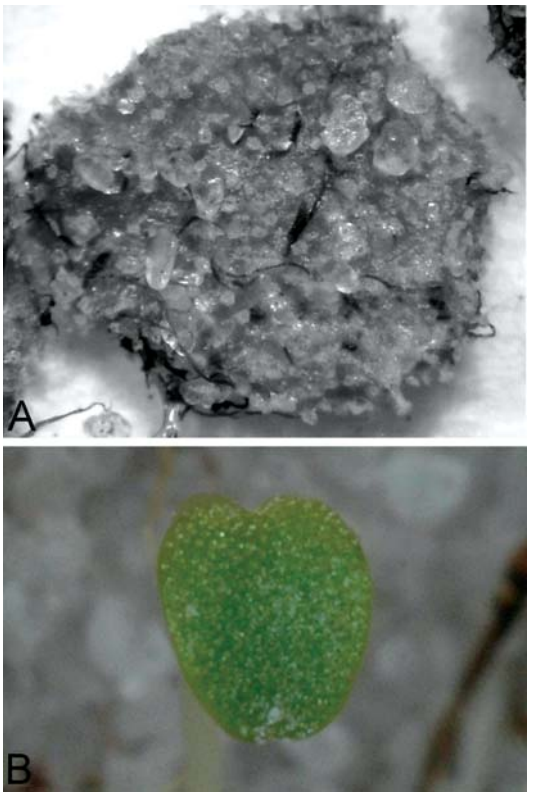


FIGURE 1. Leaf blades of *Philcoxia*. A. Nematode worms (the dark threads) attached to the upper surface of a leaf blade of *Philcoxia goiasensis*. All *Philcoxia* species exhibit leaves with such nematodes. B. Living leaf blade of *P. minensis*. B photo, J. L. M. Aranha Filho.

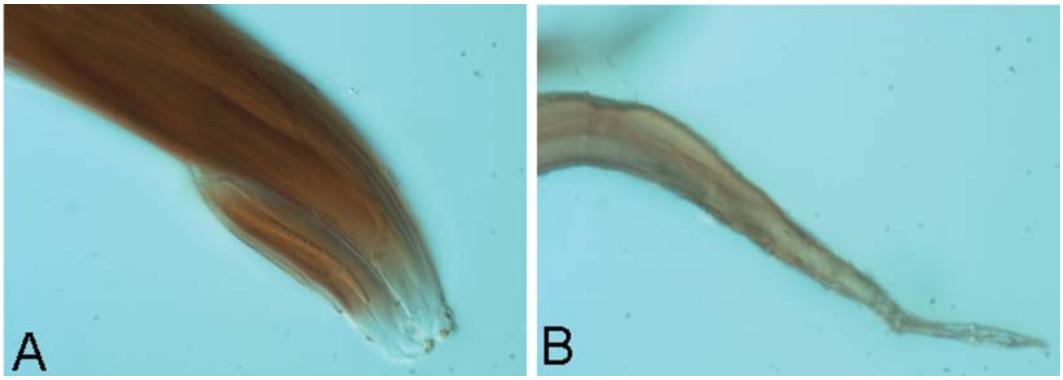


FIGURE 2. High magnification (1000x) of nematode worm found on the leaves of *Philcoxia minensis* showing head (A) and tail (B).

resembles particularly that of *Genlisea* Benth. and Hook. f. (Lentibulariaceae) in Brazil, a genus that traps and digests ciliate protozoa (Barthlott et al. 1998). We therefore hypothesized that *Philcoxia* was carnivorous in the broad sense, using nematodes and possibly other soil organisms as a source of nutrition.

Here we report the rediscovery of *Philcoxia minensis* in Serra do Cabral and provide an augmented description, detailed illustrations, and locality and habitat information for the species. We also infer the phylogenetic placement of *Philcoxia* using DNA sequence data from the internal transcribed spacer region of nuclear ribosomal DNA (ITS), and the *trnL-trnF* intergenic spacer, the *rbcL* gene, the 3′ end of the *ndhF* gene, and the *matK* gene/3′-*trnK* intron of chloroplast DNA with analyses that comprise both newly published sequence data from other members of Gratioleae and sequences from GenBank. Finally, we tested the hypothesis of carnivory in *Philcoxia* by conducting a simple test for protease activity in *P. minensis* with live field-collected plants from the Serra do Cabral.

MATERIALS AND METHODS

TAXONOMIC TREATMENT.—The description of *Philcoxia minensis* is based on field observations and collections made in October 2001 and September and October 2005 by the first three authors. Collected material consists of dried herbarium specimens and flowering and fruiting plants preserved in 95% ethanol.

PHYLOGENETICS.—Taxa of the tribes Scrophularieae and Gratioleae, the two groups considered likely to contain the closest relatives of *Philcoxia*, form two rather distantly related clades within Lamiales in analyses based on DNA sequence data, with other members of the former Scrophulariaceae interspersed among various clades of Lamiales (Olmstead et al. 2001; Bremer et al. 2002; Rahmanzadeh et al. 2004; Albach et al. 2005; Oxelman et al. 2005). We therefore assessed the general placement of *Philcoxia* among the Lamiales by constructing a data set that included one or more representatives of most well supported major clades of Lamiales recovered in the global analyses of Bremer et al. (2002). Sequence data from the chloroplast gene *rbcL*, chloroplast intergenic spacer region *trnL-trnF*, and the 5.8S region of nrDNA were employed for this analysis because taxa of Lamiales representative of the major clades have been sequenced for these three genic regions and are available from GenBank (Table 1). We particularly emphasized sampling taxa that have been placed in the tribes Gratioleae and Scrophularieae (*sensu* Fischer 2004). On occasion, different species in the same genus were sequenced for different genic regions and combined into a single terminal (Table 1). Because the combined terminals only occurred in clades that have

TABLE 1. GenBank accession numbers of taxa of Lamiales *trnL-trnF, rbcL*, and nuclear ribosomal 5.8S sequences used in this study. Herbarium voucher information is provided for the newly reported nr 5.8S sequence of *Angelonia*.

Taxon	*trnL-trnF*	*rbcL*	nr 5.8S
Acanthaceae: *Ruellia*	AF482604	L12595	AY530731
Bignoniaceae: *Jacaranda*	AJ430914	AF102647	—
Byblidaceae: *Byblis*	AF482605	L01891	—
Calceolariaceae: *Calceolaria*	AJ60861	AF123669	AJ579467
Gesneriaceae: *Columnea*	AF482612	AF170228	AF543251
Lamiaceae: *Lamium*	AJ608588	Z37403	AY443449
Lentibulariaceae: *Pinguicula*	AF482619	L01942	AB198348
Martyniaceae: *Proboscidea*	AJ608573	L01946	AY178642
Oleaceae: *Olea*	AF231867	AJ001766	AJ585193
Orobanchaceae: *Melampyrum*	AF482608	AF026834	—
Pedaliaceae: *Sesamum*	AF479010	L14408	AF478946
Pedaliaceae: *Uncarina*	AF482610	—	AY178650
Plantaginaceae: *Angelonia pratensis* Gardn. ex Benth.; *Almeda et al. 8960*, CAS, UEC)	AJ608618	AF123672	EU074164
Plantaginaceae: *Capraria*	AJ608608	—	—
Plantaginaceae: *Galvezia*	AY492177	—	AY492104
Plantaginaceae: *Lindenbergia*	AJ608586	AF123664	—
Plantaginaceae: *Melosperma*	AY492185	—	AY492112
Plantaginaceae: *Monttea*	AY492187	—	AY492114
Plantaginaceae: *Ourisia*	AY492189	—	AY492116
Plantaginaceae: *Plantago*	AY101952	L36454	AJ548984
Plantaginaceae: *Stemodiopsis*	AJ608565	—	—
Plantaginaceae: *Veronica*	AF513338	L36453	AY540868
Plocospermataceae: *Plocosperma*	AJ430903	Z68829	—
Schlegeliaceae: *Schlegelia*	AJ43093	L36448	—
Scrophulariaceae: *Diascia*	AJ608595	—	AJ616319
Scrophulariaceae: *Limosella*	AJ608587	—	AJ550588
Scrophulariaceae: *Myoporum*	AJ430934	L36445	—
Scrophulariaceae: *Nemesia*	AF380874	AF123663	AJ616325
Stilbaceae: *Stilbe*	AJ608629	Z68827	AJ616331
Tetrachondraceae: *Tetrachondra*	AJ430939	AF254787	—
Verbenaceae: *Verbena*	AF231885	Z37473	AF47779

previously been demonstrated to have strong statistical support, we assume that the use of such combinations did not affect the placement of *Philcoxia*. Based on the results of Bremer et al. (2002), we used *Plocosperma* (Plocospermataceae) as outgroup for the rest of Lamiales. Thirty-five terminals were included in this analysis.

After the general placement of *Philcoxia* was assessed, a second main data set was constructed to more specifically address the placement of *Philcoxia* among an expanded set of other species of "core" Gratioleae (Table 2). The genic regions employed for the analysis were ITS (including the ITS 1 and ITS 2 spacers and the 5.8S region), *trnL-trnF*, *rbcL*, *matK*/3′-*trnK*, and 3′-*ndhF*. These five regions were employed because of their demonstrated utility in resolving relationships of other groups within the former Scrophulariaceae (Olmstead et al. 2001; Rahmanzadeh et al. 2004; Albach et al. 2005; Oxelman et al. 2005) and the extensive number of GenBank sequences available for

TABLE 2. GenBank accession numbers of core Gratioleae ITS, *trnL-trnF*, 3′-*ndhF*, *rbcL*, and *matK*/3′-*trnK* sequences used in this study. Asterisks indicate newly reported sequences. Plus signs indicate that the taxon with the sign and the one below it have been combined into a single terminal in the analysis. Table cells with horizontal lines have no sequence data. Voucher and locality information is provided for newly reported sequences, with herbarium acronym in parentheses. CAS = California Academy of Sciences; UEC = Universidade Estadual de Campinas.

Taxon	Collection #	Locality	ITS	*trnL-trnF*	3′-*ndhF*	*rbcL*	*matK*/3′-*trnK*
Achetaria scutellarioides Wettst.	*D. Estes*		—	—	EF527469	—	—
Amphianthus pusillus Torr.			—	—	AF123674	AF123673	—
Bacopa eisenii (Kellogg) Pennell	*Fritsch & Cruz 1789* (CAS)	Butte Co., California, U.S.A.	EF467894*	EF467888*	EF467911*	EF467906*	EF467900*
Bacopa monnieri (L.) Pennell			AY492095	AY492170	EF527447	—	AY667458
Bacopa repens (Sw.) Wettst.	*Fritsch & Cruz 1788* (CAS)	Butte Co., California, U.S.A.	EF467893*	EF467887*	EF467910*	EF467905*	EF467899*
Dopatrium junceum (Roxb.) Buch.-Ham.	*Fritsch & Cruz 1787* (CAS)	Butte Co., California, U.S.A.	EF467891*	EF467885*	EF467908*	EF467903*	EF467897*
Gratiola neglecta Torr.[1]			—	AJ608591	AF188183	AF026827	—
Hydrotriche hottoniiflora Zucc.	*Fritsch 1791* (CAS)	Cultivated, Univ. of Wisconsin, U.S.A.	EF467892*	EF467886*	EF467909*	EF467904*	EF467898*
Leucospora multifida Nutt.			—	AJ608597	EF527453	—	—
Limnophila × *ludoviciana* Thieret	*Fritsch & Cruz 1790* (CAS)	Butte Co., California, U.S.A	EF467896*	EF467890*	EF467913*	—	EF467902*
Limnophila aromatica (Lam.) Merrill	*D. Estes*		—	—	EF527457	—	—
Mecardonia acuminata (Walter) + Small	*D. Estes*		—	—	EF527449	—	—
Mecardonia procumbens Small			AY492111	AY492184	—	—	AY492152
Otacanthus azureus (Linden) A. Ronse+			—	—	EF527468	—	—
Otacanthus caeruleus Lindl. +			—	—	—	—	AY667459
Otacanthus sp.			AY492115	AY492188	—	—	—
Philcoxia minensis V.C. Souza & Giulietti	*Almeda et al. 8544* (CAS, UEC)	Minas Gerais, Brazil	EF467895*	EF467889*	EF467912*	EF467907*	EF467901*
Scoparia dulcis L.			AY492119	AY492191	EF527450	—	AY492162
Scoparia 'Melongolly Blue'	*D. Estes*		—	—	EF527451	—	—
Scoparia plebeja Cham. & Schltdl.	*D. Estes*		—	—	EF527452	—	—
Sophronanthe pilosa (Michx.) Small	*D. Estes*		—	—	EF527459	—	—
Stemodia durantifolia (L.) Sw.			AY492120	—	—	—	AY492164
Stemodia glabra Spreng.			—	AJ608566	AJ617584	—	—
Stemodia schottii Holz.	*D. Estes*		—	—	EF527470	—	—
Stemodia suffruticosa HBK.	*D. Estes*		—	—	EF527455	—	—
Stemodia verticillata (Mill.) Hassler	*D. Estes*		—	—	EF527454	—	—

[1] As *Gratiola pilosa* in GenBank but probably *G. neglecta* based on comparative sequence data of D. Estes (unpubl. data).

these regions from members of core Gratioleae. Based on the results of Albach et al. (2005) and our Lamiales-wide analysis, we used *Mecardonia* as outgroup. Of the 64 sequences of core Gratioleae used in the study, 30 are here published for the first time, from seven taxa (Table 2). We conducted separate ITS and cpDNA analyses to detect any discordance between nuclear and chloroplast data partitions as determined from an incongruence length difference (ILD) test (Farris et al. 1994), and a combined 5-gene analysis to provide a total-evidence phylogenetic estimate.

Total genomic DNA was extracted from fresh, silica-gel dried, or herbarium leaf samples with DNeasy Plant Mini DNA extraction kits (Qiagen, Inc.). Extraction, PCR amplification, PCR prod-

uct purification, cycle sequencing, and sequence generation followed the protocols in Wang et al. (2004). Sequences were edited with the computer program Sequencher 4.7 (Gene Codes Corp.). All sequences have been deposited in GenBank (Tables 1 and 2). The gene *rbcL* was amplified and sequenced as in Fritsch et al. (2001) with primers from Olmstead et al. (1992), the 3′-*ndhF* region as in Fritsch et al. (2004), as modified from Clausing and Renner (2001), with primers from Olmstead and Sweere (1994), and the ITS, *trnL-trnF*, and *matK*/3′-*trnK* regions as in Wang et al. (2004) with primers from Swensen et al. (1998), Taberlet et al. (1991), and Sang et al. (1997), respectively. Target sequences unsuccessfully amplified with the external primers were often successfully amplified in two fragments with an external and one of the internal primers.

Sequence alignment was manual. The aligned sequence matrices are available from the authors upon request. Phylogenetic analyses employed maximum parsimony (MP) for the analysis of Lamiales, and MP, maximum likelihood (ML), and Bayesian inference (BI) for the placement of *Philcoxia* within core Gratioleae. MP heuristic searches and parsimony bootstrapping (bt; Felsenstein 1985) were conducted with the computer program PAUP* version 4.0b10 (Swofford 2002) by following the procedure of Wang et al. (2004). Gaps were treated as missing data (the default option in PAUP*). The ML analyses were performed with the PAUP* version 4.0b10 for UNIX (Swofford 2002) under the GTR + I + Γ model, in accordance with the recommendations of Huelsenbeck and Rannala (2004). One hundred ML bootstrap replicates were performed on the Gratioleae data set. Four iterations were run, with parameters for the initial iteration estimated from a neighbor-joining tree and those for subsequent iterations estimated from the previous iteration. The BI analysis was conducted with MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) by using uniform prior probabilities and estimating base frequencies and the parameters for the GTR + I + Γ model. Four chains of the Markov chain Monte Carlo were run by beginning with a random tree and sampling one tree every 100 generations for 3,000,000 generations. The phylogenetic estimate was based on trees sampled after the first 30,000 generations of the chain, which were used as "burn in" after stationarity was reached. To estimate the posterior probability (pP) of recovered branches, 50% majority-rule consensus trees were created.

Test for carnivory.—The protease test of Hartmeyer (1997) as modified by Meyers-Rice (1999) was performed to check for carnivory in *Philcoxia*. Due to the harsh environmental conditions (i.e., extreme heat, sand blown by wind) in which *Philcoxia* grows, the test could not be performed in the field. Thus, six whole plants were transported in their sand substrate to the laboratory where the test could be conducted more easily. A 10% solution of baker's yeast was pipetted onto the upper surface of the leaves (still attached to the plant) and the leaf was placed between two pieces of Ilford XP2 ASA 400 black and white film with the emulsion side of the film toward the leaf. The film was made flat with a herbarium paper backing that fit each piece of film, and the paper-film-leaf sandwich was clipped together so that the leaf pressed against the film. After 48 hours the film was examined; any clearing of the originally opaque surface would indicate digestion of the gelatin layer of the film and thus protease activity by the plant. All tests were conducted under ambient room conditions in indirect sunlight. Prior to field work in Brazil, a preliminary test was performed in the laboratory on a species of cultivated *Drosera* that produced a vigorous positive reaction. As a result, the reagents and equipment used were brought to Brazil for the test.

Because the leaves of *Philcoxia* were often found covered with sand grains adhering to the glands, care was taken to first remove as many grains as possible with forceps. Two of the plants were tested while remaining in their native soil. Because it was technically difficult to set up the test as such, the other four were tested by placing them in Petri dishes under a moist paper towel under natural indirect light. Some tests were also conducted with leaves clipped from the stems. Pieces of freshly cut pineapple were employed as a positive control. Three types of negative con-

trols were used: film only, film plus yeast extract, and film plus yeast extract on the presumably non-carnivorous plant *Ixora coccinea* L. (Rubiaceae). We performed the same test in the U.S. on *Hydrotriche hottoniiflora* and *Limnophila* × *ludoviciana* plants collected from the same locality as the material used for molecular analysis.

Results

Taxonomic Treatment

Philcoxia minensis V.C. Souza & Giulietti, Kew Bull. 55:161. 2000. Type.— Brazil. Minas Gerais: Joaquim Felício (município), Serra do Cabral, 17 April 1981, *Rossi et al. CFCR 1089* (holotype: SPF not seen). Figures 1B, 3–8.

Terrestrial, probably perennial delicate and wiry herbs 10–26 cm tall. Root unbranched or sparsely branched, knobby, dark orange, not fibrous. Rhizomes horizontal, arising from upright stems or rarely the root, unbranched, 0.5–5 cm long or more, mostly <0.25 cm thick, glabrous; old rhizomes dark orange, stiff-wiry, young rhizomes white, capillary and delicate. Upright stems produced at root apex and along rhizomes, subterranean, swollen or tuber-like, 2–5 mm long. Leaves 5–10, irregularly arranged on upright stems, or frequently borne on young rhizomes, then 1–6 and alternately arranged. Petiolar tissue not clearly differentiated from that of the rhizomes; petioles from upright stems 0.5–3 cm long or more, radiating in all directions, those from young rhizomes 0.1–2 cm long; young laminae conduplicate; lamina subterranean or (when mature) at soil surface, oriented at a ± 90° angle from petiole, green in living stage and when dry, suborbicular to subreniform, subpeltate to peltate, convex-hemispherical adaxially, slightly concave abaxially, 0.5–1.5 × 0.5–1.5 mm, vaguely palmately 3-nerved abaxially with each of two lateral nerves bifurcating distally, adaxially covered with ± sessile and stalked glands with pluricellular heads, abaxially glabrous, base rounded (when peltate) to cordate, margin entire, apex ± emarginate. Inflorescences usually several from each upright stem, aerial except at base, paniculate or occasionally unbranched, zigzag-racemose distally, erect, 10–26 cm long, peduncle green, terete, 0.5 mm thick, glabrous; bracts basifixed, inconspicuous, ± appressed to peduncle or pedicels at least basally, deltoid, 0.5–1.5 × ca. 0.25 mm at base, glabrous, margin entire, apex acute. Flowers often partly to completely resupinate, ebracteolate, unscented. Pedicels green, ascending or upcurved, terete, 1–2.7 cm long at anthesis, minutely glandular-puberulent, more densely so distally just below calyx, trichomes to ca. 0.07 mm long with unicellular stipe and pluricellular head, commonly elongating in fruit. Calyx ± equally 5-lobed; lobes distinct nearly to base, elliptic, 0.7–1 × 0.4–0.6 mm, glabrous or very sparsely glandular-puberulent at base abaxially, erect and persisting after fruits have fallen. Corolla sympetalous, salverform, bilabiate, upper (adaxial) lip (1-) 2-lobed, lower (abaxial) lip 3-lobed, upper lobes covering lower lobes in bud (antirrhinoid aestivation); tube 3–4 mm long, slightly incurved toward the adaxial side, slightly gibbous adaxially at base, lacking a palate at throat, flaring into the lobes, externally glabrous, internally very pale lavender, with a lighter square color pattern at floral orifice, white-clavate-puberulent abaxially, white-pilose adaxially on distal half and densely so just below filaments; lobes (4)5, pale lavender with darker unbranched or dichotomous venation, spreading, ± obovate, 2–3 × 1.75–3 mm, apically undulate, shallowly emarginate, or subtruncate. Stamens 2, adaxial, inserted on corolla ± midway up tube, included in the corolla; filaments straight, flattened, 0.5–0.7 mm long, sparsely puberulent on proximal half and bearing a callose knobby thickening (possibly a rudimentary theca) distally that is disjunct from and just below anther, connective flared toward the theca above the thickening; anthers positioned just below stigma in tube, oriented transverse to filament, dorsifixed, monothecous, dehiscing by a longitudinal

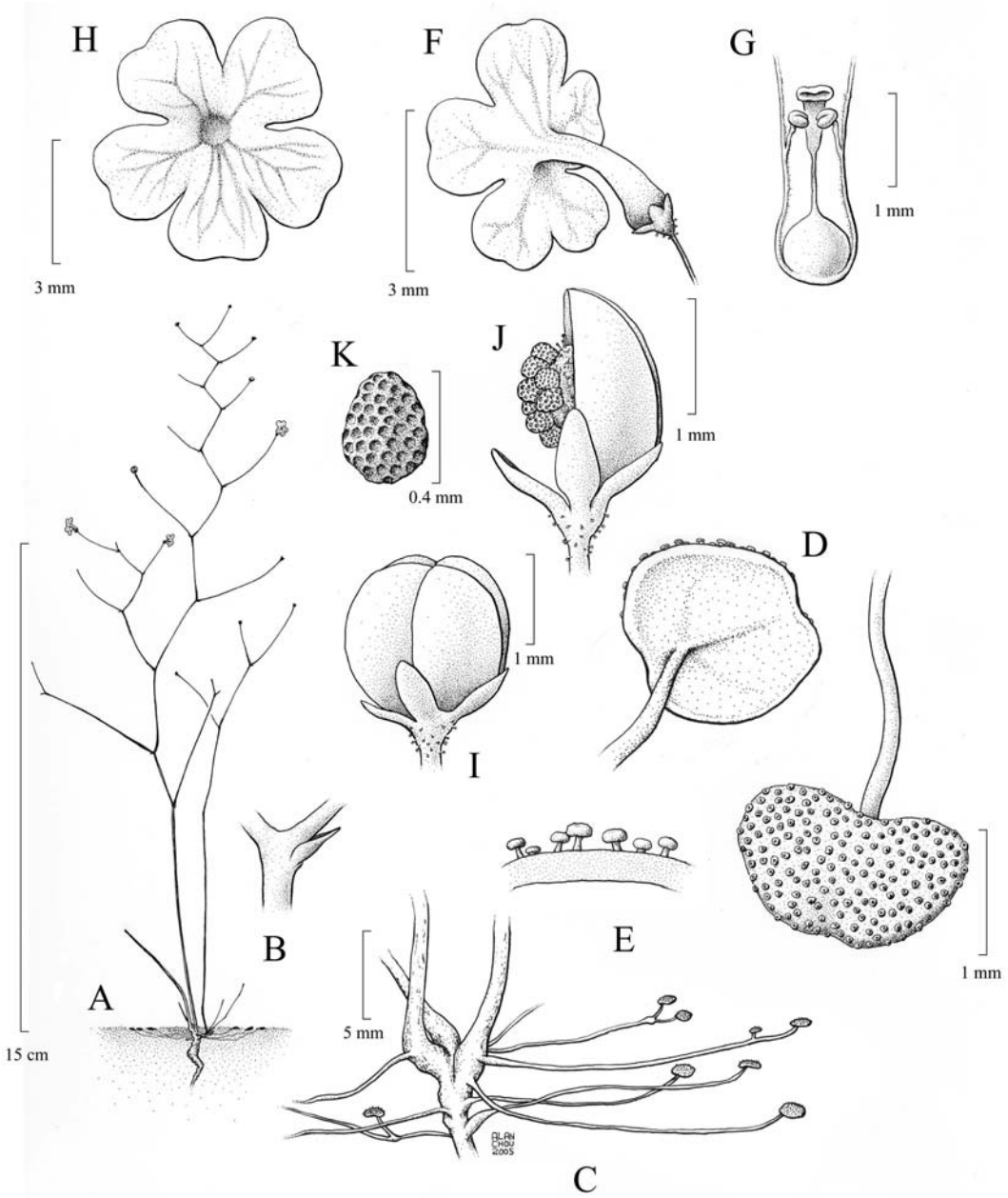


FIGURE 3. *Philcoxia minensis* V. C. Souza & Giulietti. A. Habit showing subterranean root, horizontal rhizomes, leaves, and aerial inflorescence. B. Inflorescence bract. C. Upright stem, rhizomes, leaves, and basal portion of inflorescence. D. Petiole and leaf blade, abaxial (left) and adaxial (right) surface. E. stalked glands on adaxial surface of leaf blade. F. Flower in lateral-rear view. Note gibbous portion at base of corolla tube. G. Corolla tube in longitudinal section showing arrangement of the androecium and gynoecium. H. Corolla, face view. I. Capsule. J. Capsule, half-view exposing the seeds. K. Seed. From *Almeda et al. 8544* (CAS).

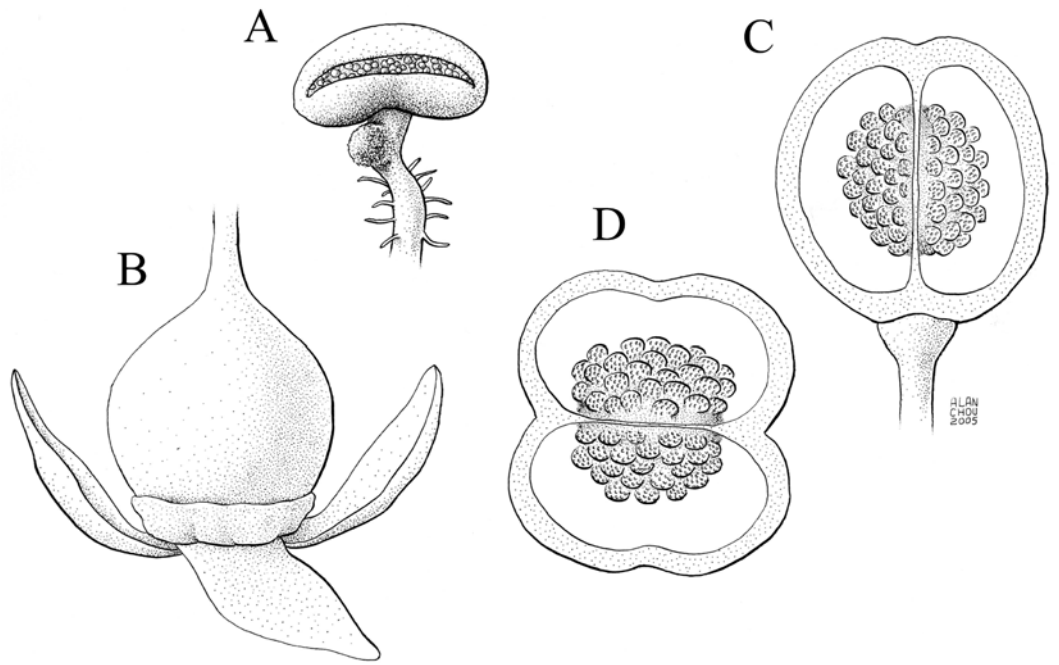


FIGURE 4. *Philcoxia minensis* V. C. Souza & Giulietti. A. Monothecous anther with callose thickening beneath it, the latter possibly a rudimentary theca and the flared portion above it thus part of the connective. B. Calyx and ovary with annular disk at ovary base. C, D. Ovary in longitudinal section (C) and cross-section (D) showing axile placentation and numerous ovules. From *Almeda et al. 8544* (CAS).

slit, ellipsoid or subreniform, 0.5 × 0.5 mm, rounded at the ends, glabrous; staminodia lacking. Gynoecium syncarpous, 2-carpellate; ovary superior, 2-locular, globose or ovoid, 0.7–0.8 mm long, with an annular nectary disk surrounding base; style terminal, solitary, filiform for basal 1–1.5 mm, abruptly expanded and laterally compressed-claviform or obconic for distal 0.8 mm, caducous; stigma positioned just above anthers in floral tube and bent toward abaxial side of corolla, bilabiate; lobes ± appressed to one another, similar in size and shape; ovules borne on two axile placentae, numerous. Fruit a dry capsule 2 × 2 mm, dehiscing septicidally and then loculicidally from apex along 4 valves; capsule valves entire, glabrous. Seeds black, ovoid, 0.4 × 0.25 mm long, estipitate; testa reticulate-foveolate.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. Minas Gerais: Município Joaquim Felício. Serra do Cabral, 16 km S of Armazém de Laje and 8 km N of Joaquim Felício, 17°42′S, 44°11′W, cerrado vegetation on white sand at 1067 m, 18 Oct 2001, *F. Almeda, A. B. Martins, P. W. Fritsch, and R. Belinello 8544* (BHCB, CAS, MO, UEC, USP); 24 Sep 2004, *F. Almeda*, *A. B. Martins, and R. Belinello 9137* (CAS, MO, NY, UEC).

PHYLOGENETICS.—MP analysis of the three-gene Lamiales data set resulted in 19 equally parsimonious trees of 1274 steps (CI = 0.47; RI = 0.82; Fig. 9). Although the strict consensus of these trees is highly unresolved, the placement of *Philcoxia* is recovered within a strongly supported clade comprising core Gratioleae (bt = 99), as sister to *Gratiola* (bt = 65). *Mecardonia* forms the first-diverging lineage of the clade (bt = 94).

The MP analysis of the expanded core Gratioleae clade with ITS (12 terminals) resulted in a single optimal tree of 406 steps (CI = 0.67; RI = 0.63; Fig. 10). The strict consensus recovered a clade of *Bacopa* species (bt = 100; pP = 1.00) as the first-diverging lineage (bt = 63; pP = 1.00). In the sister clade to *Bacopa*, the following successive sister lineages were recovered: *Dopatrium* +

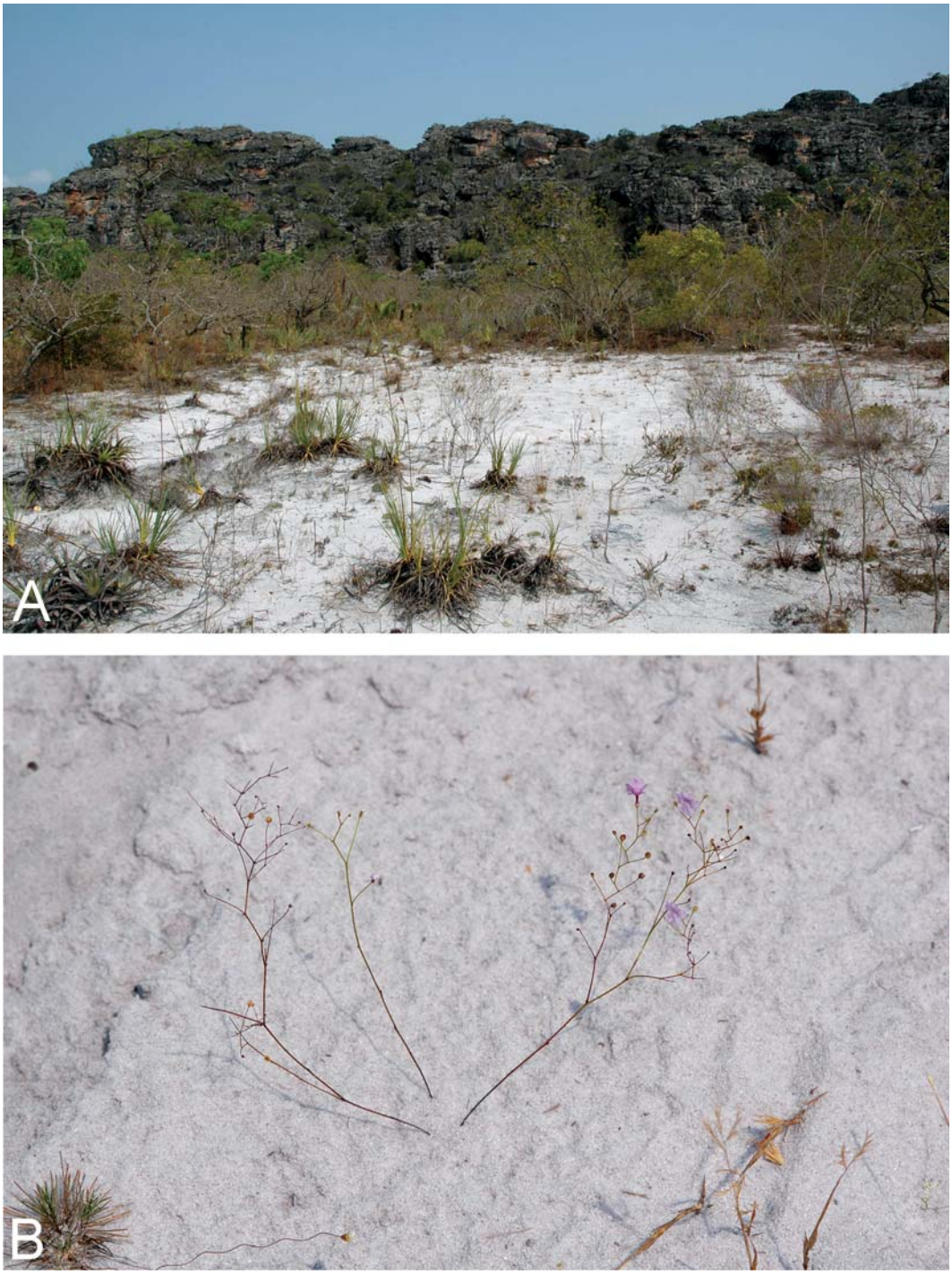


FIGURE 5. Habitat and habit of *Philcoxia minensis* at Serra do Cabral, Minas Gerais, Brazil. A. Habitat. B. Habit in white sand substrate. Photos by F. Almeda.

Hydrotriche (bt = 100; pP = 1.00), *Limnophila* (bt = 95; pP = 1.00), *Gratiola* (bt = 92; pP = 1.00), *Otacanthus* (bt = 72; pP = 1.00), *Stemodia durantifolia* (bt = 82; pP = 1.00), and *Philcoxia* (bt ≤ 50; pP = 1.00). The MP, ML, and BI analyses all recovered identical topologies.

The MP analysis of the expanded core Gratioleae clade with the chloroplast DNA genic regions (24 terminals) recovered seven equally optimal trees of 987 steps (CI = 0.73; RI = 0.76; Fig. 11). In the strict consensus, the species of *Bacopa* form the first-diverging lineage (bt = 100; pP = 1.00). In the sister clade to *Bacopa*, one clade consists of the species of *Scoparia* plus *Leucospora*, *Stemodia suffruticosa*, and *St. verticillata* (bt = 63; pP = 1.00). In the other clade, *Philcoxia* forms the first-diverging lineage (bt ≤ 0.50) whose sister (bt = 80; pP = 1.00) comprises a clade of *Achetaria*, *Otacanthus*, and the other species of *Stemodia* (bt = 75; pP = 1.00), and another of *Amphianthus*, *Dopatrium*, *Gratiola*, *Hydrotriche*, *Limnophila*, and *Sophronanthe* (bt = 80; pP = 1.00). The ML analysis resolved the placement of *Philcoxia* in the same way as did MP, whereas the BI analysis resolved it as sister to the clade of *Leucospora*, *Scoparia*, *St. suffruticosa*, and *St. verticillata* (pP ≤ 0.5). The only difference in the three analyses otherwise is the placement in the BI analysis of *St. durantifolia* as sister to the clade comprising *Achetaria*, *Otacanthus*, *St. glabra*, and *St. schottii* (pP ≤ 0.5) versus as sister to the clade of *Achetaria* and *Otacanthus* (bt ≤ 0.5).

FIGURE 6. Vegetative parts of *Philcoxia minensis* plants. A. Leaf blades on the surface of the white sand substrate. The two intersecting lines in the right half of the image are the basal portions of two peduncles of *Philcoxia*. B. Two upright stems of *P. minensis* with rhizomes, leaves, and (at right) an inflorescence base. Photos by (A) P. Fritsch; (B) F. Almeda.

The ITS and cpDNA data sets were not significantly incongruent as determined from the ILD test ($P = 0.94$). The MP analysis of the combined five-gene expanded core Gratioleae data set (24 terminals) resulted in a single optimal tree of 1394 steps (CI = 0.71; RI = 0.73; Fig. 12). The strict consensus is identical to that recovered from the cpDNA analysis and has higher levels of support. All clades were supported by pP = 1.00. The placement of *Philcoxia* was supported by bt = 52.

TEST FOR CARNIVORY.—The positive control exhibited a strong clear zone where the pineapple touched the film. In all other tests of *Philcoxia* protease activity, no clearing was observed after 12 and 24 hours. After this time some of the plants in the Petri dishes started to die and some clearing occurred but this was likely due to the plants rotting, because the area where the rhizomes/peti-

FIGURE 7. *Philcoxia minensis*, whole plant and inflorescence. A. Single individual removed from substrate. The coin is 2 cm in diam. B. Inflorescences in situ. The flower in the upper right is partly resupinate whereas the two on the lower half of the image are fully so. Photos by (A) F. Almeda; (B) P. Fritsch.

oles contacted the film were also cleared, including that of the negative control. All tests with *Hydrotriche hottoniiflora* and *Limnophila* × *ludoviciana* also returned a negative result.

DISCUSSION

NOTES ON HABITAT AND MORPHOLOGY OF *PHILCOXIA MINENSIS*.—Taylor et al. (2000) stated that species of *Philcoxia* might prefer habitats associated with mining disturbance. The area in

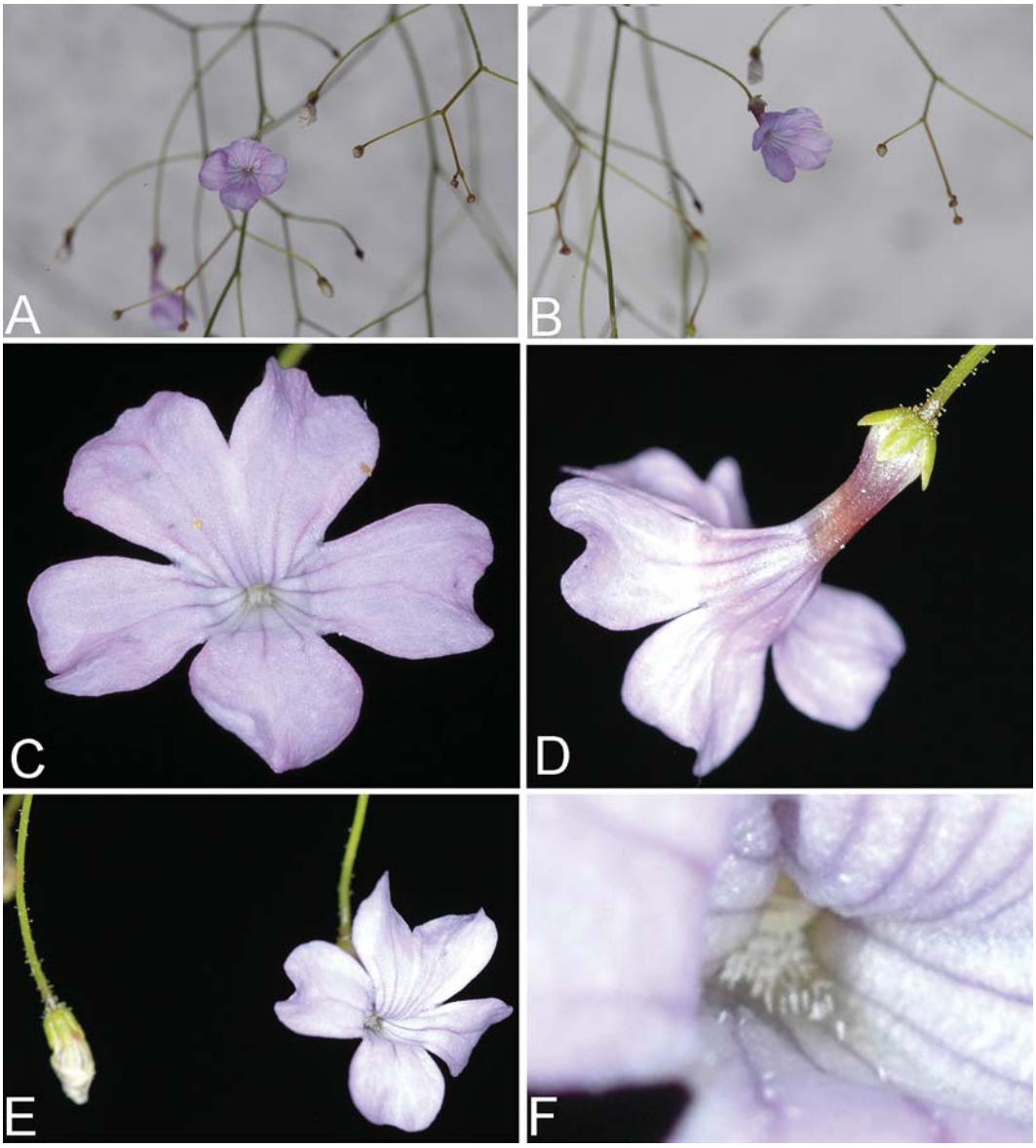


FIGURE 8. Flowers of *Philcoxia minensis*. All flowers are shown with the adaxial half oriented toward the top of the figure. A. Face view. A four-lobed flower. The top edges of the lateral lobes can be seen to be forward of that of the upper lobe, showing that the upper (adaxial) lobe covered the laterals in bud. B. Face-lateral view, showing the adaxial gibbous portion. C. Face view showing three corolla lobes abaxially and two adaxially. D. Upper part of pedicel and flower, rear-lateral view showing adaxial gibbous portion. E. Face-lateral view. F. Close-up of abaxial clavate pubescence. Photos by F. Almeda.

which we rediscovered the species appeared to us to be an undisturbed white sand island surrounded by cerrado. The approximately 50 to100 plants of *P. minensis* we observed occur in a single population across an area of approximately 10 m^2, with several more plants located approximately 30 m distant. Several hours of searching in the surrounding area revealed no additional individuals.

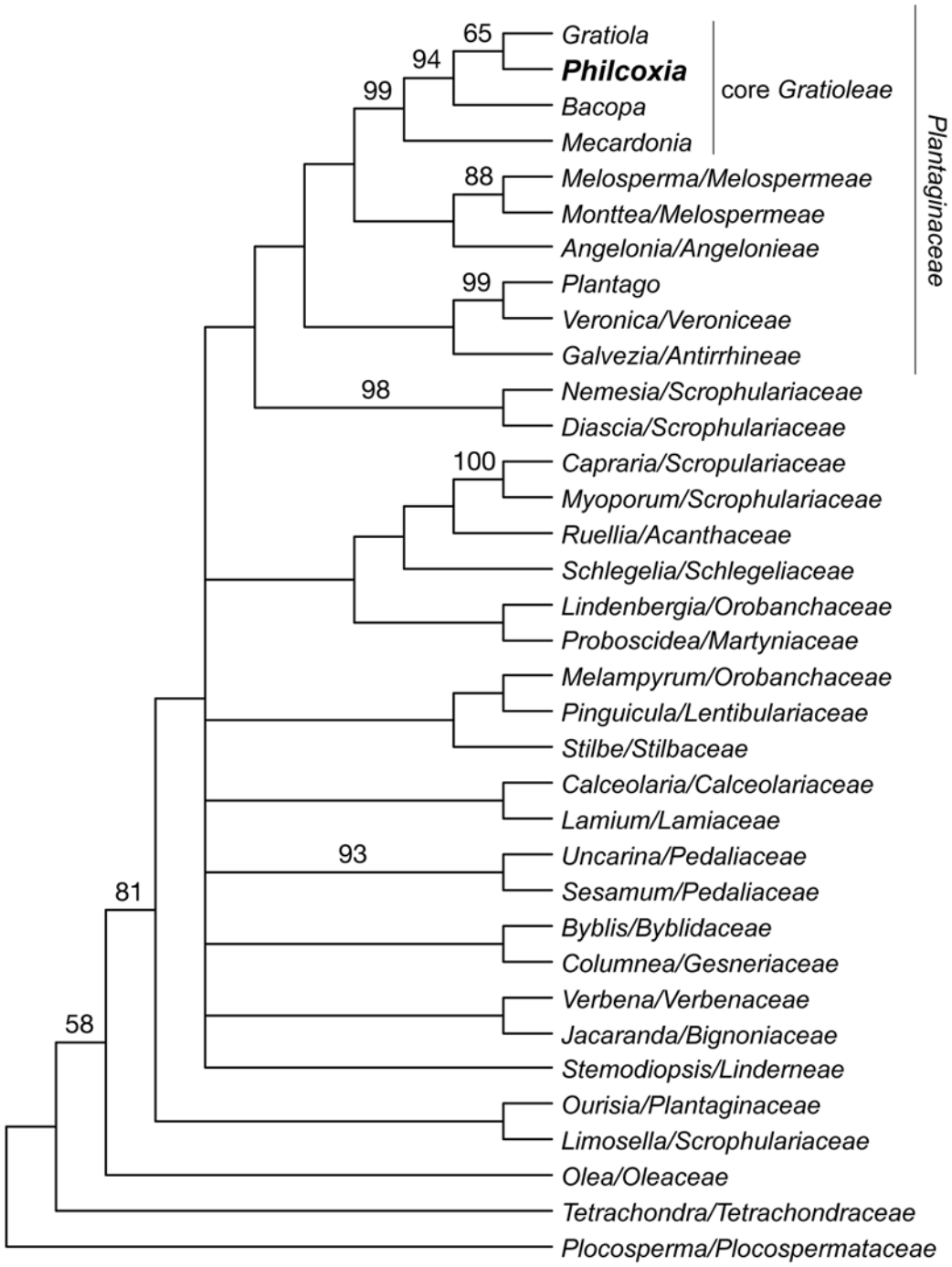


FIGURE 9. Strict consensus of 19 equally shortest trees from a maximum parsimony analysis of Lamiales with 5.8 S nrDNA, *rbcL*, and *trnL-trnF* sequences. Bootstrap values are shown above branches. *Philcoxia* groups strongly within a clade comprising other members of core Gratioleae. Taxon names from Bremer et al. (2002), Fischer (2004), Albach et al. (2005), Oxelman et al. (2005) and R. Olmstead (unpubl. data, available on line at *http://depts.washington.edu/phylo/classifications/Lamiales.html*.).

The species must thus be considered local and rare in the Serra do Cabral region until this area is better known floristically.

Cerrado, the second largest of Brazil's major biomes, is a mosaic of various vegetation types influenced by soil conditions. It is one of the richest of all tropical savanna regions in the world with high levels of endemism (da Fonseca et al. 2004). Estimates indicate that cerrado originally covered from 20 to 25% of the Brazilian territory (Gottsberger and Gottsberger 2006). Despite its geographic extent, cerrado is poorly represented in Brazil's system of protected areas. Current estimates put the size of all protected cerrado areas in Brazil at about 5.5% (da Fonseca et al. 2004). Serra do Cabral currently has no official protected status but it has been identified by the Brazilian government as a priority area of extreme biological importance in the cerrado biome because of its high incidence of endemic plants (Cavalcante and Joly 2002; Costa et al. 1998).

We compared our specimens of *Philcoxia minensis* directly with type material of *P. bahiensis* (*W. Ganev 918*, isotypes: K [96840 and 96841]) and *P. goiasensis* (*H. S. Irwin et al. 14397*, isotypes: K [96839], NY). Taylor et al. (2000) used petiole length, bract length, sepal length, and corolla lobe shape as key characters to distinguish between *P. goiasensis* and *P. bahiensis*/*P. minensis*, and lamina diameter, inflorescence structure, pedicel glandular trichome density, corolla color, and style shape to distinguish between *P. bahiensis* and *P. minensis*. Although most of these character differences are supported by our observations, several are not (Table 3). We consider petiole length in *P. minensis* to vary from 1–30 mm or more, thus overlapping the range of lengths of the other two species. The similarity in the stated corolla color difference (lilac in *P. bahiensis* versus pale

TABLE 3. Morphological character comparison of *Philcoxia bahiensis*, *P. goiasensis*, and *P. minensis* as modified and expanded from the key and descriptions in Taylor et al. (2000).

	P. bahiensis	*P. goiasensis*	*P. minensis*
Root thickness	2–3 mm	ca. 0.2 mm	ca. 1 mm
Rhizome structure	not leaf-bearing	not leaf-bearing	sometimes leaf-bearing
Upright stem structure	branched	unbranched	unbranched
Upright stem thickness	1.5–3.5	0.4–0.6	0.5–1.5
Leaf number per upright stem	numerous (>>20)	6–20	5–10
Petiole length	10–17 mm	2–7 mm	1–30 mm or more
Lamina diameter	1.2–2.5 mm	1.3–2.6 mm	0.5–1.5 mm
Inflorescence structure	simple	simple or branched	simple or branched
Inflorescence length	14–25 cm	9–15 cm	10–26 cm
Inflorescence bract length	0.5–0.8 mm	0.2–0.5 mm	0.5–1.5 mm
Pedicel length	9–16 mm	12–27 mm	10–25 mm
Glandular trichome length on pedicels	to ca. 0.2 mm	to ca. 0.2 mm	to ca. 0.07 mm
Glandular trichome stipe structure on pedicels	uniseriate	uniseriate	simple
Sepal length	1.5–2 mm	ca. 0.7 mm	1–1.5 mm
Corolla tube color[1]	lilac	yellow	pale lavender
Corolla limb width (adaxial to abaxial edges)	8–9 mm	4–5 mm	4–5 mm
Corolla lobe apex shape	emarginate or rounded	all bilobed	undulate, shallowly emarginate, or subtruncate

[1] *P. bahiensis* and *P. goiasensis* only determined from dried material.

lavender in *P. minensis*) renders this character of uncertain utility, although these colors clearly contrast with the yellow tube of *P. goiasensis*. The styles of *P. bahiensis* and *P. minensis*, stated as narrow at the base and widening abruptly towards the apex versus obconic respectively, appear to us to be indistinguishable. Both of them are filiform for the proximal half and flare distally into the stigma.

Irrespective of these and more minor differences in size estimates for various characters, we were able to confirm the distinctness of *P. minensis* as proposed by Taylor et al. (2000). At least five character state differences occur between *P. minensis* and the other two species (Table 3), including the thickness of the root (ca. 1 mm versus ca. 0.2 mm or 2–3 mm), structure of the rhizome (sometimes leaf-bearing versus not leaf-bearing), diameter of the lamina (0.5–1.5 mm versus 1.2–2.6 mm), length of the glandular trichomes on the pedicels (to ca. 0.07 mm versus to ca. 0.2 mm), and the structure of the stipe on the glandular trichomes of the pedicel (simple versus uniseriate).

In contrast to the observations of Taylor et al. (2000) as repeated by Fischer (2004), we did not observe evidence of circinnate vernation either in *Philcoxia minensis* or on the material available to us of the other two species. Instead, the growing tip of the delicate rhizome appears straight or upcurved (Figs. 3C, 6B). Young leaf blades are infolded lengthwise but are not inwardly inclined or coiled. On this basis, circinnate vernation should be removed from any enumeration of features in *Philcoxia* that resemble Lentibulariaceae or other carnivorous plants.

In Gratioleae, the adaxial corolla lobes cover the lateral lobes in bud (antirrhinoid aestivation; Fischer 2004). In *Philcoxia minensis*, the adaxial side is two-lobed or occasionally unlobed, white-pilose internally, and slightly gibbous at the base. The abaxial side has three lobes and clavate puberulence internally, and is the side toward which the stigma is curved. The flowers of *P. minensis* are often resupinate, i.e., with the three-lobed side sky-ward and the two-lobed side ground-ward, through torsion of the pedicel. Sometimes they are positioned at various angles between resupinate and nonresupinate within the same inflorescence (Fig. 7B).

The anther filaments of the two stamens are straight, as in the adaxial stamens of other members of Gratioleae (Fischer 2004). Only a few genera of Gratioleae have pubescent filaments. The filament pubescence in *Philcoxia* appears to be similar to that of *Dopatrium*. Most species of *Dopatrium* have pubescent anthers, in contrast to the glabrous anthers of *Philcoxia*. The knobby thickening just below the anther is here interpreted to be a rudimentary theca. In *Dopatrium* and other members of Gratioleae, the two fertile thecae are disjunct and are attached to the filament by a connective with two arms, each extending to one of the thecae (Fischer 2004). In *Philcoxia*, the flared portion above the sterile theca can therefore be interpreted as an arm of the connective, the other arm of which is absent by reduction if the monothecous condition is derived within the tribe (see below). The transverse orientation of the thecae to the filament in *Philcoxia* is similar to that of species of *Gratiola* excluding *G. hispida* and *G. pilosa*, which belong to *Sophronanthe* (D. Estes, unpublished data).

Although we designate the filamentous structure subtending the lamina as petiolar tissue, this structure and the delicate rhizomes are indistinguishable, at least at 60× magnification. This and the highly variable length of such structures in *P. minensis* lead to the question of whether the structures that are called "petioles" in *Philcoxia* are instead rhizomes, terminated by a sessile leaf blade. In young leaves, the abaxial tissue of the blade appears to be identical to the tissue of the so-called petiole and continuous with it, with the same white color and smooth texture and distinct in color and texture from the young adaxial blade surfaces. The leaves of *Philcoxia* are so unusual that it is possible they are not developmentally or positionally homologous with the leaves of the other members of Gratioleae.

Phylogenetic placement of *Philcoxia*.—Our results strongly support the general place-

ment of *Philcoxia* within tribe Gratioleae, as hypothesized by Taylor et al. (2000) and Fischer (2004). Other members of Gratioleae *sensu* Fischer (2004: at the subfamily level) have a combination of the following characters: glandular trichomes pluricellular-headed; inflorescences racemose; corollas often two-lipped, unspurred; adaxial corolla lip not galeate, covering the lateral lobes in bud; stamens two to four (rarely five in *Bacopa*), the abaxial pair often reduced to staminodes or lacking; anther thecae rounded at base; and ovary bilocular. The morphology of *Philcoxia* agrees well with these characters, with its two-lipped, unspurred corolla; non-galeate adaxial corolla lip that covers the lateral lobes in bud; two stamens, the abaxial pair lacking; rounded anther thecae; and bilocular ovary. The inflorescence of *Philcoxia* is unique in the tribe in its single bract per node (versus two per node) and zig-zag pattern of branching. This has made the basic structure of the inflorescence (racemose versus cymose) difficult to interpret from morphology alone. The unequivocal placement of *Philcoxia* in the Gratioleae demonstrated here supports the interpretation of the inflorescence as racemose as in other members of the tribe, rather than cymose as suggested by Souza (1996).

The results do not support the specific hypothesis put forward by Taylor et al. (2000) and Fischer (2004) of a close relationship of *Philcoxia* to *Dopatrium*, *Hydrotriche*, or *Limnophila*. In our analyses, these three genera form a clade that is sister to *Amphianthus*, *Gratiola*, and *Sophronanthe*, whereas *Philcoxia* is placed as sister to this clade plus *Achetaria*, *Otacanthus*, and *Stemodia* in part. This specific placement of *Philcoxia* received BI support of 1.00 but bt support of only 52 in the combined analysis, probably resulting from the very long branch of *Philcoxia* in both ITS and the cpDNA results (Figs. 10–12), thus leaving the specific placement of *Philcoxia* somewhat in question.

The characters defining the informally named subtribe "Dopatriinae" by Fischer (2004; i.e., the three genera above plus *Deinostema*) are plants mostly aquatic; bracteoles absent (except some species of *Limnophila*); flowers with two stamens, the abaxial pair usually reduced to staminodes or lacking; anthers with two separate thecae held together by a connective with two short arms; and seeds reticulate (smooth in some *Limnophila*). From our observations can be added the presence of chambered stems, and opposite or verticillate bracts and leaves. Of these characters, *Philcoxia* agrees only in the lack of bracteoles and presence of two stamens with abaxial staminodes lacking. Otherwise, it is terrestrial, the stems are solid, the bracts and leaves (and rhizome branches) are alternate, the anthers are monothecous, and the seeds are foveolate-reticulate. *Philcoxia* is the only genus in the tribe *sensu* Fisher (2004) with monothecous anthers.

Even with the limited sampling conducted here of the core members of tribe Gratioleae, results indicate that *Philcoxia* forms a distinct lineage relative to other members and this accords well with the unusual morphological features of the genus. The available data are unable to place *Philcoxia* with high confidence, but results are resolved enough to clearly establish that *Philcoxia* groups somewhere above *Bacopa* and *Mecardonia* as opposed to highly nested within the tribe. Although additional sampling may affect the interpretation of character state evolution in *Philcoxia*, the available data establish that the subterranean stems and petioles, peltate leaves, zigzag inflorescence, solitary inflorescence bracts, and monothecous anthers all represent uniquely derived character states within core Gratioleae. The addition of other genic regions and other taxa will be required to determine the precise placement of *Philcoxia* and provide more comprehensive statements of character state evolution. For now it is clear that because of the relatively basal placement of *Philcoxia* demonstrated here, it will be critical to include this genus in any tribe-wide assessments of character state evolution.

Negative evidence of carnivory.—Givnish (1989) has listed two requirements for a plant to be classified as carnivorous: it must be able to absorb nutrients from dead animals next to its sur-

faces, and it must have some morphological, physiological, or behavioral feature whose primary effect is the active attraction, capture, and/or digestion of prey. Thus, the inducement of proteases on the surface of leaves would go far toward demonstrating carnivory in particular plant species and the protease test employed here has been used to help distinguish between carnivorous plants, or those likely to be so (*Dionaea*, *Drosera*, *Drosophyllum*, *Pinguicula*, *Stylidium*), versus noncarnivorous plants (*Byblis*, *Ibicella*, *Proboscidea*, *Roridula*; Hartmeyer 1997; Meyers-Rice 1999; Darnowski et al. 2006), especially *in lieu* of detailed nutrient uptake experiments. The negative results for protease activity obtained for *Philcoxia* suggest that it is not carnivorous.

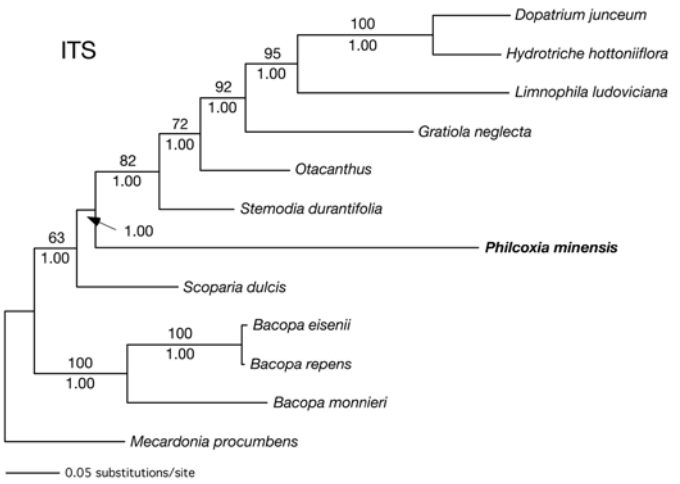
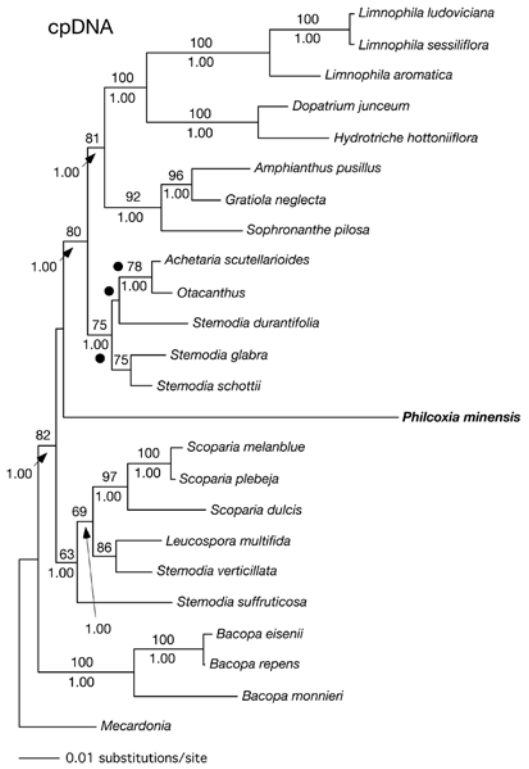


FIGURE 10. The single best maximum likelihood tree (= the single maximum parsimony and 50% majority-rule Bayesian inference trees) from analysis of core Gratioleae with ITS sequences. Bootstrap values >50% are shown above branches; posterior probabilities >50% are shown below branches.

There are several potential sources of error, however, that might have affected our ability to detect a positive test result for carnivory in *Philcoxia minensis*. First, the small leaf blades of *P. minensis* (0.5–1.5 mm diam.) on delicate petioles were difficult to manipulate, and the hemispherical shape of the blades with glands on the convex side restricted the area of leaf surface in contact with the surface of the film; flattening the blade to obtain more contact risked crushing the leaf tissue. A more reliable test would likely come from using one of the other two species of *Philcoxia*, because their leaves are substantially larger than those of *P. minensis*. *Philcoxia bahiensis* might be best suited for the test because it appears to have the most glands per unit area of leaf of any of the three species. Further, a known population is extant in Bahia, whereas *P. goiasensis* has not been rediscovered (Taylor et al. 2000). Second, the test might not have been sensitive enough to detect protease activity. Our trial tests with a species of *Pinguicula* failed to produce clearing on the film, whereas species of *Drosera* produced a dramatic area of clearing. In the case of *P. minensis*, the leaves are so small that they might not have been able to digest enough of the film for any clearing to be observed. Third, it is possible that *P. minensis* is carnivorous for only part of the year or under certain environmental conditions. Several of the known carnivorous plants show seasonality in carnivory (e.g., *Sarracenia*, *Stylidium*; Givnish 1989; Darnowski et al. 2006). A major environmental factor that the habitat of *Philcoxia* does not seem to share with that of known carnivorous plants is high water availability. When we sampled *Philcoxia* (late September and October), no water was detected in the white sand substrate, but this probably changes during the rainy season. If *Philcoxia* is actively digesting soil organisms only at times of adequate soil moisture, it would be critical to sample protease activity during these times. This becomes more likely when one considers that because many soil nematodes are drought-tolerant through anhydrobiosis (Demeure et al. 1979), carnivory could be timed to the rainy season when nematodes are active.

It would be desirable to be able to grow plants of *Philcoxia* under controlled conditions to be



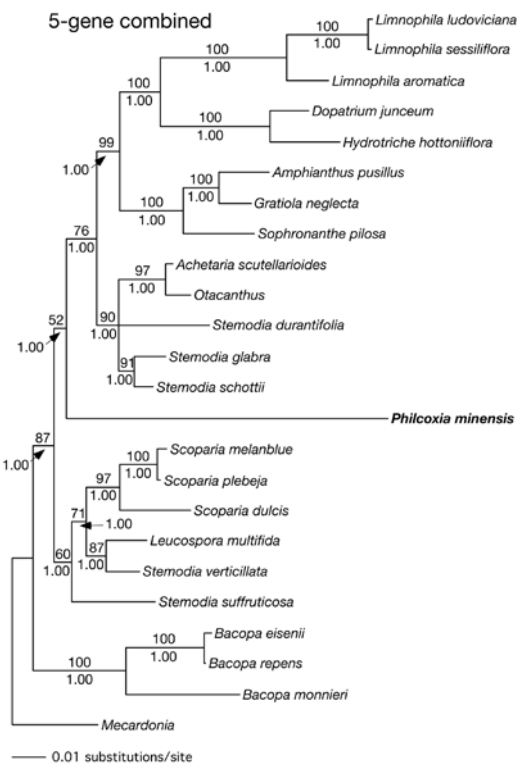


FIGURE 11 (left). The single best maximum likelihood (ML) tree from analysis of core Gratioleae with cpDNA sequences (*trnL-trnF*, *rbcL*, *matK/3′-trnk*, and 3′-*ndhF*). Dots indicate clades that collapse in the strict consensus of seven equally optimal trees in the maximum parsimony (MP) analysis; other clades in the MP analysis are identical to those recovered from ML. The tree from Bayesian inference is identical to that from the ML analysis except that the placement of *Philcoxia* is as sister to the clade comprising *Leucospora*, *Scoparia*, *Stemodia suffruticosa*, and *St. verticilla*ta (pP ≤ 0.5) and the placement of *St. durantifolia* is as sister to the clade comprising *Achetaria*, *Otacanthus*, *St. glabra*, and *St. schottii* (pP ≤ 0.5). Bootstrap values >50% are shown above branches; posterior probabilities >50% are shown below branches.

FIGURE 12 (right). The single best maximum likelihood (ML) tree (= the single maximum parsimony and 50% majority-rule Bayesian inference trees) from analysis of core Gratioleae with combined ITS, *rbcL, trnL-trnF, matK/3′-trnk*, and 3'-*ndhF* sequences. Bootstrap values >50% are shown above branches; posterior probabilities >50% are shown below branches.

able to conduct additional tests for carnivory, but our attempts to maintain the plants collected in the field or to grow them from seed have been unsuccessful. Until then, on the basis of our tests we assume that *Philcoxia* is not carnivorous and an alternative explanation must be sought for the unusual growth form, leaf shape, and abundance of glands on its leaf surfaces. One possible explanation for the habit of the species is that the plants could merely be adapted to the hot and dry environment in which they occur in keeping most of their parts underground, with only the mature leaf surfaces and inflorescences above the surface of the soil. The glandular hairs could thus serve as a defense against herbivory by small animals crawling on the surface of the soil. The glands also could provide a physical protective function against sharp sand grains, which could otherwise cut and injure the leaves. More study of *Philcoxia* in this context is clearly needed to understand the evolution of this highly unusual plant.

Acknowledgments

We thank the curators of K and NY for loaned herbarium material; Fabienne Audebert and Gary Williams for positive identification of nematodes on the leaves of *Philcoxia* and help in high-magnification imaging; João Aranha Filho for field collaboration, help with the protease test, and locating the NY material of *P. goiasensis* for loan to CAS; Renato Belinello for field collaboration; Kent McKenzie of the California Rice Experiment Station for permission to collect samples of Gratioleae; John Glaeser for the sample of *Hydrotriche*; Alan Chou for illustrations; Dominique Jackson for editing the photographic plates; and an anonymous reviewer for helpful comments on the manuscript. This research was supported in part by National Science Foundation grant DEB-0106631 to the first two authors.

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