

SOTOA, A NEW GENUS OF SPIRANTHINAE (ORCHIDACEAE) FROM MEXICO AND THE SOUTHERN UNITED STATES

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ABSTRACT. Generic placement of “*Deiregyne*” *confusa* and “*D.*” *durangensis* has been inconsistent among several recent classifications of subtribe Spiranthinae based mainly on floral characters. In this work, we assessed the systematic position of these two species by means of cladistic parsimony analyses of nuclear (nrITS) and plastid (*trnL-trnF*) DNA sequences of 36 species/21 genera of Spiranthinae. Additionally, perceived differences in habitat preference between the two species were evaluated using geographic information system and niche modeling tools. Our results show that, in spite of their striking similarity in overall flower morphology, “*D.*” *confusa* and “*D.*” *durangensis* are only distantly related to one another. Instead, the former species is strongly supported as sister to *Svenkoeltzia*, whereas the latter groups with *Schiedeella*. Niche modeling revealed noticeable differences in the two species’ ecological preferences; no overlap of their potential distribution areas (as inferred using the Maxent modeling method) was predicted. A new monotypic genus, *Sotoa*, is proposed to accommodate “*Deiregyne*” *confusa* on the basis of genetic, morphological and (inferred) reproductive differences from other genera of the subtribe. The main morphological feature distinguishing *Sotoa* from other Spiranthinae is the folding of the bottom surface of the nectary, which is deeply concave from outside, resulting in an internally convex surface that is covered by dense pubescence.

KEY WORDS: Molecular phylogenetics, niche modeling, Orchidaceae, *Sotoa*, Spiranthinae.

In the early 1980s, Harvard orchidologist Leslie A. Garay described *Deiregyne confusa* Garay as a new species of Spiranthinae from Mexico and the southern United States. According to Garay (1982), all the specimens he assigned to *D. confusa* had previously been misidentified as *Spiranthes durangensis* Ames & C.Schweinf. (= *Deiregyne durangensis* [Ames & C.Schweinf.] Garay). Garay distinguished *D. confusa* from *D. durangensis* by its glandular-pubescent sepals, a differently proportioned labellum with a different callus at its base, and the shape of the rostellum [remnant] (Fig. 1). Balogh (1982; also as Burns-Balogh 1986) placed *D. durangensis* in *Schiedeella* Schltr., as did Schlechter (1920) previously with its synonym, *Schiedeella saltensis* (Ames) Schltr. (based on the illegitimate *Spiranthes saltensis* Ames, non Grisebach 1879). Subsequently Szlachetko (1991, 1993) included both *D. confusa* and *D. durangensis* in his newly created section

Lueretta Szlach. within the genus *Funkiella* Schltr. He treated *D. confusa* as a subspecies of *Funkiella durangensis* (Ames & C.Schweinf.) Szlach. because the distinguishing features noted by Garay (1982) vary substantially. More recently, however, Szlachetko *et al.* (2005) raised *Funkiella durangensis* subsp. *confusa* (Garay) Szlach. to species rank, as *Funkiella confusa* (Garay) Szlach., Rutk. & Mytnik, without a discussion of their rationale.

In the course of phylogenetic studies within subtribe Spiranthinae and other research focused on Mexican orchid diversity, the authors have had the opportunity to examine a number of specimens of both *D. confusa* and *D. durangensis*. Superficially plants and flowers of both species look very alike, which may explain the long-standing confusion reported by Garay (1982). However, under closer scrutiny, noticeable differences in floral indumentum and in the morphology of the

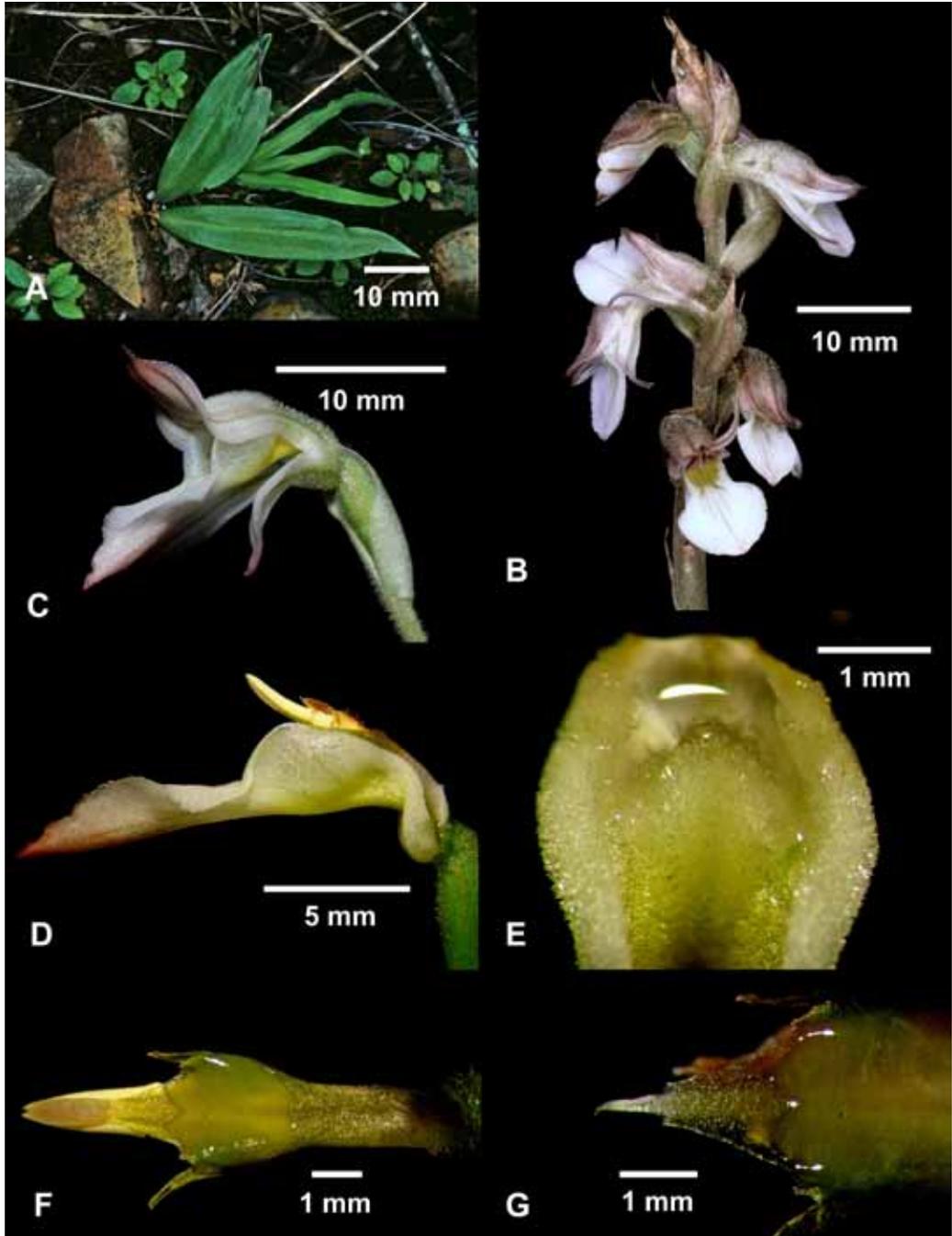


FIGURE 1. Morphology of *Sotoa confusa*. A. Plant *in situ* in Oaxaca during the season of vegetative growth, showing three oblong leaves forming a rosette. B. Inflorescence of a plant from Oaxaca (*Salazar 6575*). C-F. Flower and floral details of a plant from Guanajuato (*Reyes 6885-bis*). C. Flower from the side. D. Labellum and column from the side, with the other perianth segments excised. E. Inside of the nectary, showing the pubescent convexity and some nectar toward the base above it. F. Column with pollinarium in place, from below. G. Column apex after removal of the pollinarium, from below. Photo by G. A. Salazar.

nectary are evident. In *D. durangensis* the ovary bears a dense covering of opaque, curly, intermingling septate trichomes appressed against its surface; such trichomes barely reach the bases of the sepals and most of their outer surface is glabrous (or more precisely, cellular-papillose). In contrast, in *D. confusa* both the ovary and the proximal one half of the outer surface of the sepals bear translucent, sparse erect trichomes (instead of appressed) with a distinct apical swelling, hence the glandular pubescence described by Garay (1982). At the same time, in *D. durangensis* the bottom surface of the nectary at the base of the labellum is flat but bears a fleshy, lunate callus covered by short papillae, whereas in *D. confusa* the bottom of the nectary lacks a distinct callus. Instead, the bottom surface of the nectary is deeply concave from outside, resulting in an internally convex surface covered by dense pubescence (Fig. 1D). In rehydrated flowers from pressed specimens, the internal convexity of the nectary of *D. confusa* often looks like a longitudinal pubescent ridge. Therefore, the aforementioned characters allow for the distinction of the two species, as stated previously by Garay (1982), although the other features mentioned by him, namely lip shape and proportions, and rostellum remnant structure, seem to vary within each species and do not appear to provide clear-cut distinguishing attributes.

Besides their structural dissimilarities, there seem to be differences as well in their geographical ranges and ecological preferences. *Deiregyne durangensis* has a relatively restricted distribution, being known from the surroundings of the town of El Salto, state of Durango, in the Sierra Madre Occidental (including the type locality), plus a few of additional locations in the Estado de México (Luer 1975) and Michoacán (McVaugh 1985). On the other hand, *D. confusa* is a widespread species in eastern Mexico, barely reaching Texas (USA) and spreading through the Chihuahuan Desert to south of the Mexican Plateau, in the states of Coahuila, Estado de México, Guanajuato, Hidalgo, Jalisco, Nuevo León, Puebla, Oaxaca, San Luis Potosí, and the Distrito Federal (Salazar 2009; Salazar *et al.* 2006; Peinado & Riojas 2008) (Fig. 2A). As for their habitat preferences, *D. durangensis* inhabits in grassy open areas in pine-oak forest, and at least some of its populations occur in seasonally flooding terrain (Luer 1975; Hágsater *et al.* 2005). On the other hand,

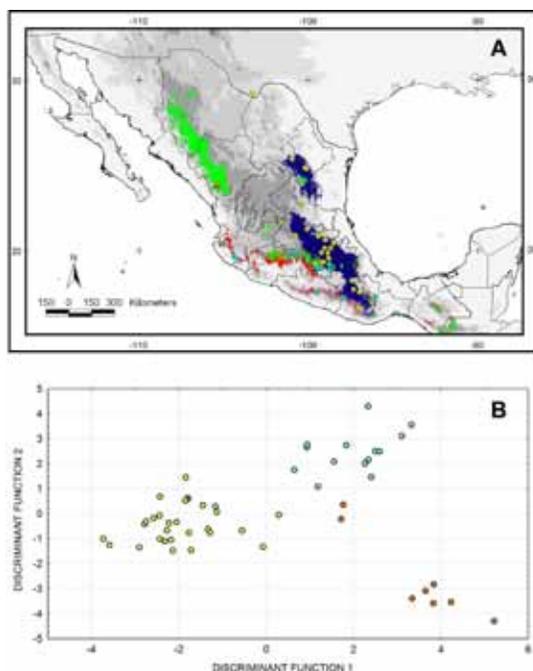


FIGURE 2. A. Potential distributions of *Sotoa* (“*Deiregyne*”) *confusa* (dark blue), *Schiedeella* (“*Deiregyne*”) *durangensis* (green), and *Svenkoeltzia* spp. (red) as inferred with Maxent; colored dots represent actual records of *Sotoa confusa* (yellow), *Schiedeella durangensis* (orange), and *Svenkoeltzia* spp. (pale blue). B. Plot of the discriminant function analysis; colored dots represent individual records of *Sotoa confusa* (yellow), *Schiedeella durangensis* (orange), and *Svenkoeltzia* spp. (pale blue) (see text).

D. confusa is found in a variety of habitats chiefly in semi-arid regions, including seasonally dry pine-oak and juniper-oak forest, tropical deciduous forest, various types of xerophilous scrub, and wastelands and induced pastures resulting from alteration of these plant associations (*e.g.*, Hágsater *et al.* 2005; Salazar *et al.* 2006; Salazar 2009; Peinado & Riojas 2008).

In all, the fact that *D. durangensis* and *D. confusa* represent two distinct species is now well established and they have been recognized as such in recent accounts of Mexican orchids (*e.g.*, Hágsater *et al.* 2005; Szlachetko *et al.* 2005; Soto *et al.* 2007). Nevertheless, their generic placement is a different matter. Taxonomists have included these two species either in *Deiregyne* (Garay 1982), *Schiedeella* (Schlechter 1920; Balogh 1982; Burns-Balogh 1986), or *Funkiella* (Szlachetko 1991, 1993; Szlachetko *et*

al. 2005), in all instances based on floral similarity. However, recent molecular phylogenetic studies have shown that floral morphology alone may not be a good indicator of phylogenetic relationship in some groups of Spiranthinae (Salazar *et al.* 2003; Górnaiak *et al.* 2006; Salazar & Dressler, submitted). In this work, the systematic position of *Deiregyne confusa* and *D. durangensis* is assessed by means of cladistic analyses of nucleotide sequences of two highly variable DNA markers, namely the region of the internal transcribed spacers of nuclear ribosomal DNA (nrITS; Baldwin *et al.* 1995; Álvarez & Wendel 2003) and plastid *trnL-trnF* region, which includes the intron of *trnL*, the intergenic spacer between *trnL* and *trnF*, and short exon portions (Taberlet *et al.* 1991; Kelchner 2000). Both these regions have been used previously, alone or in combination with each other and/or other DNA regions, for phylogeny reconstruction in Spiranthinae (Salazar *et al.* 2003; Górnaiak *et al.* 2006; Figueroa *et al.* 2008) and other orchidoid lineages (*e.g.*, Bellstedt *et al.* 2001; Clements *et al.* 2002; Bateman *et al.* 2003, 2009; van der Niet *et al.* 2005; Salazar *et al.* 2009; Álvarez-Molina & Cameron 2009).

We also evaluated the perceived differences in habitat preferences of *Deiregyne confusa*, *D. durangensis*, and, for reasons that will become evident later, *Svenkoeltzia congestiflora* and its kin, using geographic information systems (GIS) and niche modeling.

Materials and methods

Material studied. — Thirty-six species belonging to 21 genera of subtribe Spiranthinae *sensu* Salazar (2003) were analyzed, and representative species of subtribes Cranichidinae *s.l.*, Galeottiellinae, Goodyerinae, and Manniellinae were included as outgroups following previous phylogenetic studies (Salazar *et al.* 2003, 2009; Figueroa *et al.* 2008; Álvarez-Molina & Cameron 2009). A list of the taxa studied with voucher information and GenBank accessions is provided in Table 1.

Molecular methods. — Extraction, amplification, and sequencing of the DNA regions of interest were carried out using standard methods and primers described in Salazar *et al.* (2003). In all instances, bi-directional sequencing was performed and the chromatograms were edited and assembled with the program Sequencher (GeneCodes Corp.).

Phylogenetic analyses. — Alignment of the data matrices was done by eye and individual gap positions were treated as missing data.

All characters were treated as unordered and equally weighted. Parsimony analyses were conducted with the program PAUP* version 4.02b for Macintosh (Swofford 2002) for the nrITS region, the *trnL-trnF* region, and both regions in combination. Each analysis consisted of a heuristic search with 1000 replicates of random addition of sequences for the starting trees, branch-swapping using the “tree bisection-reconnection” (TBR) algorithm, and the option “MULTREES” was activated (to save multiple trees). All most-parsimonious trees (MPTs) were saved. Internal support for clades was assessed by nonparametric bootstrapping (Felsenstein 1985), in all cases consisting of 300 bootstrap replicates with heuristic searches, each including 20 random sequence additions for the starting trees and TBR branch-swapping. Up to 20 trees per bootstrap replicate were saved.

GIS and niche modeling. — Recently, several approaches to predictive modeling of the geographic distribution of species have been developed in a geographic information system (GIS) environment. Such modeling tools have been applied to problems in biogeography, conservation, evolutionary ecology, and ecological niche divergence among closely related species (*e.g.*, Ferrier 2002; Rice *et al.*, 2003; Kumar & Stohlgren 2009). In general, the procedure focuses on modeling ecological niches (the conjunction of ecological conditions within which a species is able to maintain populations without immigration; Grinnell 1917). Niche modeling uses environmental data and localities of occurrence of a species to produce a model of its requirements in those environmental/ecological dimensions (Stockwell & Peters 1999; Phillips *et al.* 2004), which is then projected on geographic space to create a map of the species’ potential distribution.

We assembled a database of 54 georeferenced herbarium records of *Deiregyne confusa*, *D. durangensis*, and *Svenkoeltzia* spp. based on the databases of two major collections of specimens of Mexican Orchidaceae, namely herbaria AMO and MEXU. In the last instance, records are publicly available through the portal of the Unidad de

Informática de la Biodiversidad (UNIBIO) of the Instituto de Biología, Universidad Nacional Autónoma de México (<http://unibio.ibiologia.unam.mx/>). We also incorporated records gathered in other herbaria, including AMES, K, ENCB, F, IEB, MEXU, MO, NY, SEL and US, as well as information from the literature. Subsequently, we used the Maximum Entropy modeling method (Phillips *et al.* 2004, 2006), as implemented in the software Maxent version 3.3.1 (freely available at <http://www.cs.princeton.edu/~schapire/maxent/>), to develop models of habitat suitability for the taxa. Maxent is a maximum entropy-based machine learning program that estimates the probability distribution for a species' occurrence based on environmental constraints (Phillips *et al.* 2006). Besides data on species presence, distribution models require environmental variable layers; we included four topographic data (U.S. Geological Survey; <http://edcdaac.usgs.gov/gtopo30/hydro/>) and 19 bioclimatic parameters (including precipitation and temperature variables) with spatial resolution of 30 arc sec (~1 km²) (WorldClim dataset; Hijmans *et al.* 2005). To assess model performance, we used Receiver Operating Characteristic (ROC) curves. The main advantage of ROC analysis is that the area under the ROC curve (AUC) provides a single measure of model performance, independent of the choice of threshold (Phillips *et al.* 2006).

We performed a discriminant function analysis (DFA) using the software package STATISTICA 6.0 (Statsoft, Inc.) to elucidate the differentiation between the niches of the species. This multivariate analysis works in the space defined by the environmental predictors and compares the distribution of the species to one another. It computes the factor that maximizes the inter-species variance while minimizing intra-species variance and therefore represents the direction along which the species are most differentially distributed. Then we calculated the distance measures between the centroids of each species to determine the similarity/dissimilarity between them based on the Mahalanobis distance.

Since species' limits in *Svenkoeltzia* are unclear (see Salazar 2003; Soto *et al.* 2007), we pooled all the records of *Svenkoeltzia* spp. available to us as a single taxonomic unit for comparison with the habitat preferences of *Deiregyne confusa* and *D. durangensis*.

Results

Phylogenetic analyses. — The nrITS data set consisted of 771 characters, of which 241 (31%) were potentially informative to parsimony. The analysis of this region yielded six equally most parsimonious trees (MPTs) with a length of 977 steps, consistency index excluding uninformative characters (CI) = 0.46, and retention index (RI) = 0.65. The strict consensus of the six MPTs is shown in Fig. 3A. On the other hand, the *trnL-trnF* matrix encompassed 1653 characters, 216 (13%) of which were potentially parsimony-informative, and again six MPTs were found, these being 804 steps long, with CI = 0.53 and RI = 0.71. The strict consensus calculated from these is depicted in Fig. 3B. Both analyses recovered similar overall patterns of supported relationships, and there were no instances of contradictory clades with bootstrap percentages (BP) > 50.

The combined dataset of the nrITS and *trnL-trnF* regions consisted of 2424 characters, 457 (19%) of which were potentially informative to parsimony. The heuristic search found a single MPT with a length of 1790 steps, CI = 0.48, and RI = 0.67. The single tree recovered is depicted in Fig. 4. Spiranthinae (a-d) form a strongly supported monophyletic group (BP 100) and within them three major clades were recovered, which match the groups referred to as the *Stenorhynchos* (a; BP 95), *Pelexia* (b; BP 53), and *Spiranthes* clades (d; BP 100) by Salazar *et al.* (2003), plus an additional clade consisting of *Eurystyles* and *Lankesterella* (c; BP 100). The latter obtained high support (BP 90) as sister to the *Spiranthes* clade, but the *Pelexia* clade did not obtain a BP > 50 as the sister of [[*Lankesterella-Eurystyles*]-*Spiranthes* clade]]. The internal relationships of the *Stenorhynchos* and *Pelexia* clades mirror closely the results of Salazar *et al.* (2003) and will not be dealt with further here. In the case of the *Spiranthes* clade, our taxonomic sampling was more comprehensive than in that study. This clade consists of two weakly supported subclades, the first of which (BP 62) includes two strongly supported groups; in the first of them, *Schiedeella faucisanguinea* is sister to [*Microthelys minutiflora-Funkiella hyemalis*], whereas the second encompasses, on the one hand, [*Deiregyne confusa-Svenkoeltzia congestiflora*] (BP 98), and on the other hand *Beloglottis costaricensis* as the sister of monophyletic (and strongly supported) *Aulosepalum*. The other major subclade of the *Spiranthes* clade comprises *Spiranthes*

TABLE 1. Voucher information and GenBank accessions for DNA sequences.

Taxon	Voucher specimen	nrITS	<i>trnL-trnF</i>
Subtribe Cranichidinae			
<i>Ponthieva racemosa</i> (Walt.) Mohr	Mexico, <i>Salazar 6049</i> (MEXU)	AJ539508	AJ544490
<i>Prescottia plantaginea</i> Lindl.	Brazil, <i>Salazar 6350</i> (K)	AJ539511	AJ544493
Subtribe Galeottiellinae			
<i>Galeottiella sarcoglossa</i> (A.Rich. & Galeotti) Schltr.	Mexico, <i>Jiménez 2334</i> (AMO)	AJ539518	AJ544500
Subtribe Goodyerinae			
<i>Goodyera pubescens</i> (Willd.) R.Br.	USA, <i>Chase 212</i> (NCU)	AJ539519	AM419815
Subtribe Manniellinae			
<i>Manniella cyrpedioides</i> Salazar, T.Franke, Zapfack & Benkeen	Cameroon, <i>Salazar 6323</i> (YA)	AJ539516	AJ544498
Subtribe Spiranthinae			
<i>Autosepalum hemichreum</i> (Lindl.) Garay	Mexico, <i>Salazar 6044</i> (MEXU)	–	FN641878
	Mexico, <i>Soto 8336</i> (MEXU)	FN641866	–
<i>Autosepalum pyramidale</i> (Lindl.) M.A.Dix & M.W.Dix	Mexico, <i>Salazar 6061</i> (MEXU)	AM884872	FN641877
<i>Autosepalum tenuiflorum</i> (Greenm.) Garay	Mexico, <i>Salazar 6150</i> (MEXU)	AJ539591	AJ544474
<i>Beloglottis costaricensis</i> (Rehb.f.) Schltr.	Mexico, <i>Soto 8129</i> (MEXU)	AJ539492	AJ544475
<i>Coccineorchis cernua</i> (Lindl.) Garay	Panama, <i>Salazar 6249</i> (MEXU, spirit)	AJ539502	AJ544485
<i>Cyclopogon epiphyticum</i> (Dodson) Dodson	Ecuador, <i>Salazar 6345</i> (K)	AJ539499	AJ544482
<i>Deirygyne albobaginata</i> (C.Schweinf.) Garay	Mexico, <i>Jiménez 2164</i> (AMO)	FN641870	FN641882
<i>Deirygyne (Dithyridanthus) densiflora</i> (C.Schweinf.) Salazar & Soto Arenas	Mexico, <i>Salazar 6125</i> (MEXU)	FN641874	FN641886
<i>Deirygyne diaphana</i> (Lindl.) Garay	Mexico, <i>Salazar 6172</i> (MEXU)	AJ539484	AJ544467
<i>Deirygyne eriophora</i> (B.L.Rob. & Greenm.) Garay	Mexico, <i>Salazar 6104</i> (MEXU)	FN641873	FN641885
<i>Deirygyne falcata</i> (L.O.Williams) Garay	Mexico, <i>Salazar 6112</i> (MEXU)	FN641871	FN641883
<i>Deirygyne pseudopyramidalis</i> (L.O.Williams) Garay	Mexico, <i>Salazar 6126A</i> (MEXU)	FN641872	FN641884
<i>Deirygyne rhombilabia</i> Garay	Mexico, <i>Salazar 6138</i> (MEXU)	FN641869	FN641881
<i>Dichromanthus aurantiacus</i> (La Llave & Lex.) Salazar & Soto Arenas	Mexico, <i>Salazar 6351</i> (K)	AJ539485	AJ544468
<i>Dichromanthus cinnabarinus</i> (La Llave & Lex.) Garay	Mexico, <i>Linares 4469</i> (MEXU)	AJ539486	AJ544469
<i>Eltroplectris calcarata</i> (Sw.) Garay & H.R.Sweet	Brazil, <i>Soares s.n.</i> (MEXU, photograph)	AJ519448	AJ519452

<i>Eitroplectris triloba</i> (Lindl.) Pabst	Argentina, <i>Munich Bot. Gard.</i> 96/4474 (M)	FN641864	FN641875
<i>Eurystyles borealis</i> A.H.Heller	Mexico, <i>Soto 9149</i> (AMO)	AJ539497	AJ544480
<i>Funktiella hyemalis</i> (A.Rich. & Galeotti) Schltr.	Mexico, <i>Salazar 6128</i> (MEXU)	AJ539495	AJ544478
<i>Lankesterella gnoma</i> (Kraenzl.) Hoehne	Brazil, <i>Warren s.n.</i> (K)	FN556163	FN556168
<i>Mesadenella petenensis</i> (L.O. Williams) Garay	Mexico, <i>Salazar 6069</i> (MEXU)	AJ539503	AJ544486
<i>Mesadenus lucayanus</i> (Britt.) Schltr.	Mexico, <i>Salazar 6043</i> (MEXU)	AJ539488	AJ544471
<i>Microthelys minutiflora</i> (A.Rich. & Galeotti) Garay	Mexico, <i>Salazar 6129</i> (MEXU)	AJ539494	AJ544477
<i>Odontorrhynchus variabilis</i> Garay	Chile, <i>Wallace 130/85</i> (CANB)	AJ539498	AJ544481
<i>Pelexia adnata</i> (Sw.) Poit. ex Spreng.	Mexico, <i>Salazar 6012</i> (MEXU)	AJ539501	AJ544484
<i>Sacoila lanceolata</i> (Aubl.) Garay	Guatemala, <i>Förther 2545</i> (M)	AJ539504	–
	Brazil, <i>da Silva 874</i> (MG)	–	AJ544529
<i>Sarcoglotis acaulis</i> (J.E.Sm.) Schltr.	Trinidad, <i>Salazar 6346</i> (K)	AJ544483	AJ539500
<i>Schiedeella crenulata</i> (L.O. Williams) Espejo & López-Ferrari	Mexico, <i>Goldman 902</i> (BH)	FN641868	FN641880
<i>Schiedeella (Deiregyne) durangensis</i> (Ames & C.Schweinf.) Burns-Bal.	Mexico, <i>Soto 10673</i> (AMO)	FN641867	FN641879
<i>Schiedeella faucisanguinea</i> (Dod) Burns-Bal.	Mexico, Jiménez s.n. (AMO)	AJ539496	AJ544479
<i>Schiedeella llaveana</i> (Lindl.) Schltr.	Mexico, <i>Salazar 6105</i> (MEXU)	AJ539487	–
	Mexico, <i>Salazar 6073</i> (MEXU)	–	AJ544470
<i>Sotoa (Deiregyne) confusa</i> (Garay) Salazar	Mexico, <i>Hernández 3320</i> (MEXU)	FN641865	FN641876
<i>Spiranthes cernua</i> (L.) Rich.	USA, <i>Nickrent 4188</i> (MEXU)	AJ539489	AJ544472
<i>Spiranthes spiralis</i> (L.) Cheval.	UK, <i>Rudall & Bateman s.n.</i> (K)	AJ539490	AJ544473
<i>Stenorhynchos glitsteinii</i> E.A.Christ. ^a	Mexico, <i>Salazar 6090</i> (MEXU)	AJ539505	AJ544487
<i>Svenkoeltzia congestiflora</i> (L.O. Williams) Burns-Bal.	Mexico, <i>Salazar 6143</i> (MEXU)	AJ539493	AJ544476

^a Originally submitted to GenBank as "*Stenorhynchos speciosum*" and thus referred in Salazar *et al.* (2003).

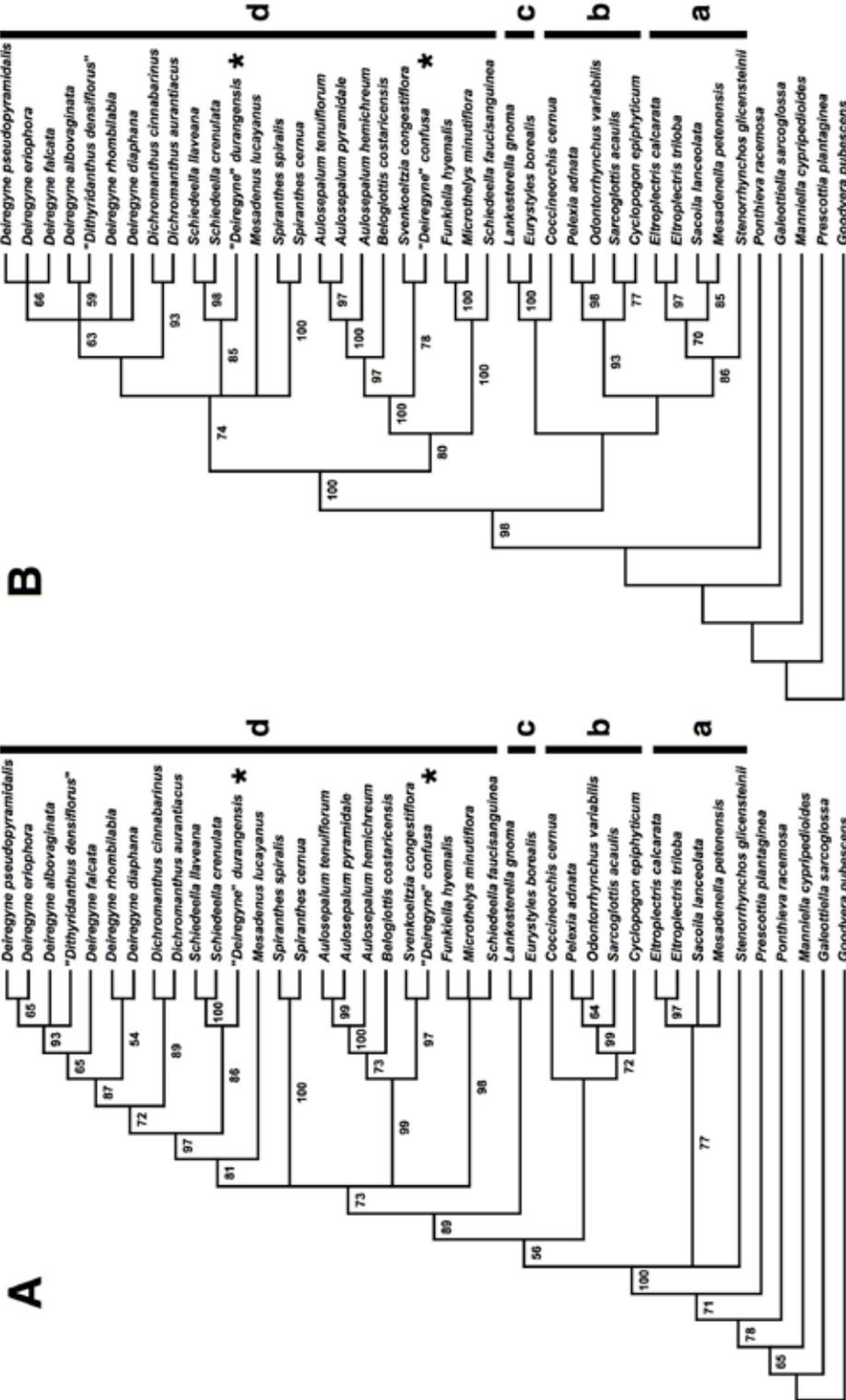


FIGURE 3. A. Strict consensus of the six MPTs found in the analysis of the nrITS region. B. Strict consensus of the six MPTs found in the analysis of the *trnL-trnF* region. Numbers below branches are bootstrap percentages. Bars marked with letters (a-d) refer to clades or groups discussed in the text. Asterisks mark the position of *Sotoa confusa* and *Schiedeella durangensis* in the trees.

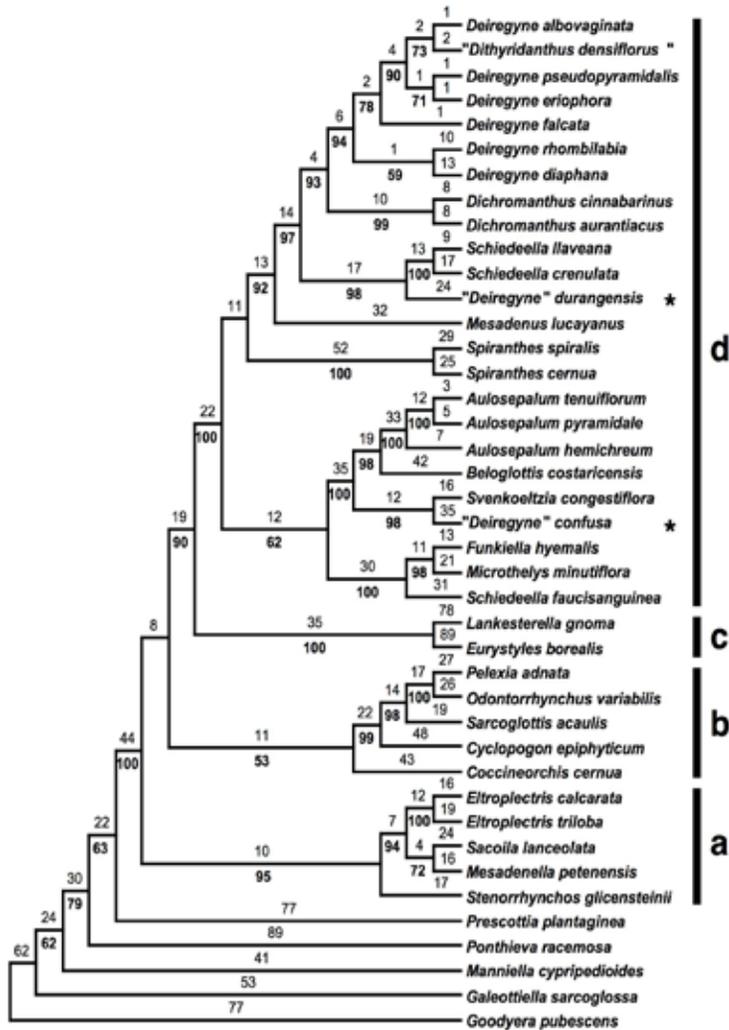


FIGURE 4. Single tree recovered in the analysis of combined nrITS and *trnL-trnF* DNA sequences. Numbers above branches are branch lengths, numbers below branches are bootstrap percentages. Bars marked with letters (a-d) refer to clades or groups discussed in the text. Asterisks mark the position of *Sotoa confusa* and *Schiedeella durangensis* in the trees.

(BP 100) as the sister of the rest (BP < 50), whereas *Mesadenus lucayanus* is strongly supported (BP 92) as sister to a clade encompassing [[*Deiregyne durangensis*-*Schiedeella crenulata*-*S. llaveana*]] in turn as the sister of *Dichromanthus* plus most species of *Deiregyne* (among which *Dithyridanthus densiflorus* is nested). All the internal relationships of these two groups are strongly supported. From Figs. 3-4 it is clear that neither *Schiedeella* nor *Deiregyne* is monophyletic.

Niche-modeling. — Distributional data are represented by 30, 15 and 9 unique localities for *Deiregyne*

confusa, *D. durangensis*, and *Svenkoeltzia* spp., respectively. Ecological niche models and associated distributional predictions developed for each species were all reasonably accurate; AUC values for all models are > 0.9, implying a potentially significant result. The potential ranges for the three taxa predicted by the models are shown in Fig. 2A. There is marginal overlap of the potential areas of *Svenkoeltzia* with those of *D. confusa* and *D. durangensis*, but they have never been found living sympatrically. These differences among the species are reflected also in the ecological distance measures that we calculated. According to the

Mahalanobis statistic (D2), the most similar taxa with respect to the environmental variables are *D. confusa* and *Svenkoeltzia* spp. (D2 = 18.78), whereas the most distinct are *D. confusa* and *D. durangensis* (D2 = 50.96). A plot constructed using individual scores on the two discriminant functions provides a picture of the pattern of segregation of the ecological parameters among the species investigated (Fig. 2B).

In the DFA, the first two discriminant functions are significant ($\lambda = 1$, $p < 0.0001$). The first and second discriminant functions explain 61.3% and 38.7% of the total variation of the sample, respectively. The most important environmental variables for group discrimination in the first function are precipitation of the rainiest period and precipitation of the warmest period, whereas for the second function they are basically temperature of the coldest month and temperature seasonality. The plot of the values of these functions recovers each taxon as a distinct cluster that can be characterized as follows: locations of *D. confusa* have low precipitation both during the rainiest period and the warmest period, intermediate temperature values for the coldest period, and intermediate seasonal temperature variation. *Deiregyne durangensis* occurs in locations where precipitation of the warmest period and temperature seasonality are higher, whereas *Svenkoeltzia* spp. are found in areas with higher precipitation during the rainiest period and with the lowest temperatures of the coldest period.

Discussion

The results of the present phylogenetic analyses show that "*Deiregyne*" *confusa* is only distantly related both to "*D.*" *durangensis* and to genuine members of *Deiregyne* as typified by *D. diaphana* (Lindl.) Garay (= *D. chloraeformis* (A. Rich. & Galeotti) Schltr.; see Garay 1982; Catling 1989; Salazar 2003; *contra* Balogh 1982; Burns-Balogh 1986, 1988; Szlachetko 1995). "*Deiregyne*" *durangensis* is strongly supported as member of a clade that includes the type species of *Schiedeella* (*S. llaveana* [Lindl.] Schltr. = *S. transversalis* Schltr.), and therefore could reasonably be included in *Schiedeella*, as in Hågsater *et al.* (2005) and Soto *et al.* (2007). We will refer to it as *Schiedeella durangensis* from here forth and the inclusion of this species does not significantly changes the circumscription of *Schiedeella* as interpreted by Salazar

(2003). The overall flower morphology of *Schiedeella* is similar to that of both *Deiregyne* and *Funkiella* but the former can be distinguished from these two genera by its herbaceous floral bracts that upon drying are scarious and opaque (vs. papery and translucent with contrasting dark veins) and the lack of orange-red to rust-red areas on the labellum, respectively. Our results also point to the polyphyly of *Schiedeella* as currently delimited because "*S.*" *faucisanguinea* (Dod) Burns-Bal. consistently groups with species of *Funkiella* and *Microthelys* (see below), sharing with them an affinity for high-montane habitats and the possession of red thickenings on the labellum (see Salazar 2003; Salazar *et al.* 2003). However, our present sampling of this clade is too sparse to sustain taxonomic changes at this time and this issue will be dealt with elsewhere (G.A. Salazar *et al.*, unpubl. data).

On the other hand, *Deiregyne* as interpreted here (following Garay 1982; Catling 1989; Salazar 2003) is the strongly supported sister of *Dichromanthus s.l.* (Salazar 2003; Salazar *et al.* 2002, 2003; Salazar & García-Mendoza 2009). The *Dichromanthus-Deiregyne* clade is sister to *Schiedeella*, in agreement with previous results of Salazar *et al.* (2003). Likewise, the nrITS analysis of Górnai *et al.* (2006) recovered *Deiregyne diaphana* (as its synonym, *Burnsbaloghia diaphana* [Lindl.] Szlach.) in a strongly supported clade that also included *Dichromanthus* ("*Stenorrhynchos*") *aurantiacus* and *Schiedeella llaveana*. Although the present study included only seven of the twelve species we currently recognize in *Deiregyne* (Salazar 2003; cf. Soto *et al.* 2007), the species analyzed here represent a significant portion of the structural variation and the geographic distribution displayed by the genus and few future changes in its limits are anticipated.

"*Deiregyne*" *confusa*, henceforth referred to as *Sotoa confusa* (Garay) Salazar (see Nomenclature, below), did not group either with *Schiedeella* or with *Deiregyne*, being instead strongly associated with *Svenkoeltzia congestiflora* within a robust clade that also includes *Beloglottis* and *Aulosepalum*. The last whole group is in turn sister to a strongly supported *Funkiella* subclade encompassing *F. hyemalis*, *Microthelys minutiflora*, and, as noted earlier, *Schiedeella faucisanguinea*. These relationships might appear surprising at first sight, given the noticeable likeness in overall flower appearance of *Schiedeella*

durangensis and the species here referred to as *Sotoa confusa*. Nevertheless, as mentioned earlier, there are substantial differences in nectary structure and floral pubescence between these species and their similar outward appearance likely is an indication of similar pollination mechanisms. Both *Schiedeella durangensis* and *Sotoa confusa* possess a generalized suite of floral morphological traits likely related to pollination by nectar-foraging bees (e.g. *Bombus* spp.); these traits include white flowers with darker veins on tepals, a contrastingly colored area on the throat of the labellum, and diurnal floral scents (cf. Catling 1983; Salazar 2003). Indeed, pollination of *Schiedeella durangensis* by *Bombus steidachneri* Handrilsch, 1988 was recorded by Luer (1975) in the Estado de México. This pollination syndrome is also displayed by members of “true” *Deiregyne* (i.e. *D. diaphana* and its kin), *Schiedeella*, and *Funkiella*, and it might represent the plesiomorphic condition in the whole *Spiranthes* clade. In contrast, *Svenkoeltzia* encompasses one to four ill-defined species with bright yellow, tubular flowers on a more or less congested inflorescence (Burns-Balogh 1989; González 2000; Salazar 2003; Szlachetko *et al.* 2005), which are most likely pollinated by hummingbirds (Salazar 2003).

Ecological niche modeling substantiates the existence of noticeable differences in the ecological preferences of *Schiedeella durangensis* and *Sotoa confusa*, and their potential distributions inferred with Maxent do not overlap (Fig. 2A, B). In the case of *Svenkoeltzia*, there is marginal overlap with the distributions predicted for both *Sotoa confusa* and *Schiedeella durangensis*, but the potential overlap may be an artifact of the scale of the underlying cartography, since there are profound differences in their particular habitats. For instance, plants of *Svenkoeltzia* live epiphytically or on rocks in forests, whereas both *Schiedeella durangensis* and *Sotoa confusa* are geophytes occurring in open areas; as far as we now, none of them has ever been found occurring sympatrically with any other.

Garay (1982) envisioned monotypic genera as “[...] the inevitable, peripheral products of anagenesis, i.e., the evolutionary refinements within a main phylogenetic branch of the family [...]”. This logic applies to some extent in the case of *Sotoa confusa*, which, in spite of its close relationship to *Svenkoeltzia*,

differs from it in habitat preferences, flower structure, and (likely) pollination biology. Given their divergent natural histories, it seems to us less confusing to create a new genus for “*D.*” *confusa* than lumping it in an undiagnosable broader concept of *Svenkoeltzia*. The inclusion of *Sotoa confusa* and *Svenkoeltzia congestiflora* in *Funkiella*, as in Szlachetko (1991, 1993; also Garay 1982 in the case of *S. congestiflora*) is untenable on phylogenetic grounds, unless one is willing also to sink into *Funkiella* the morphologically distinctive genera *Aulosepalum* and *Beloglottis* (see Figs. 3, 4). There is no obvious advantage in lumping these ecologically, structurally and genetically distinctive clades, and therefore we opt here for erecting *Sotoa* as a distinct genus from *Svenkoeltzia*.

Nomenclature

***Sotoa* Salazar, gen. nov.**

Type species: *Sotoa confusa* (Garay) Salazar.

Morphologia tota floris et rostellis Deiregynae, Funkiellae et Schiedeellae similis; differt a tribus generibus fundo nectarii valde concavo-convexo, convexitate interna dense pubescenti, saepe apparenti ut crista longitudinali pubescenti in floribus siccis rursus madefactis; etiam differt a Deiregynae bracteae floralibus neque albidis translucidis neque venatione atrata; etiam differt a Funkiellae labello sine areis aurantiis vel ferrugineo-rubrescentibus.

This genus is named in honor of Miguel Angel Soto Arenas (1963-2009), outstanding contemporary botanist and leading expert on the Orchidaceae of Mexico. So far, *Sotoa* includes a single widespread species from semiarid regions of Mexico and southern USA:

***Sotoa confusa* (Garay) Salazar, comb. nov.**

Basionym: *Deiregyne confusa* Garay, Bot. Mus. Leaflet 28: 238. 1982. Holotype: Mexico. Hidalgo: lagoon of Metztlán, 1600 m, 27 March 1933, J. González [sic] & O. Nagel (sub E. Östlund) 2194 (AMES!).

Other synonyms: *Spiranthes confusa* (Garay) Kartesz & Ghandi, Phytologia 73: 128. 1992. *Schiedeella confusa* (Garay) Espejo & López-Ferrari, Phytologia 82: 80. 1997. *Funkiella durangensis* (Ames & C.Schweinf.) subsp. *confusa* (Garay) Szlach., Fragm. Flor. Geobot. 36: 20. 1991.

Funkiella confusa (Garay) Szlach., Rutkowski & Mytnik, Polish Bot. Stud. 20: 227. 2005.

As noted earlier, "*Dithyridanthus*" *densiflorus* is nested in *Deiregyne* (Figs. 3, 4) and is most closely related (and morphologically similar) to *Deiregyne albovaginata*. Thus the following new combination in *Deiregyne* is required to make classification consistent with its phylogenetic position.

Deiregyne densiflora (C.Schweinf.) Salazar & Soto Arenas, *comb. nov.*

Basionym: *Spiranthes densiflora* C.Schweinf., Bot. Mus. Leafl. 4: 104. 1937. Holotype: Mexico. Morelos: Tepeyte, 2300 m, flowered at Cuernavaca, 10 Oct. 1932, P. Carbonero (sub E. Östlund) 1513 (AMES!).

Other synonyms: *Schiedeella densiflora* (C.Schweinf.) Burns-Bal., Orquidea (Mexico City), n.s., 8: 39. 1981. *Dithyridanthus densiflorus* (C.Schweinf.) Garay, Bot. Mus. Leafl. 28: 316. 1982. *Stenorrhynchos densiflorus* (C.Schweinf.) Szlach., Fragm. Flor. Geobot. 37: 200. 1992 (as "*densiflora*").

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