



Phylogeny of the Andean genus *Deprea* (Physalideae, Solanaceae): testing the generic circumscription

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Abstract

Deprea (Solanaceae) is a small South American genus of 10 species occurring in Andean areas from Venezuela to Bolivia. The circumscription of *Deprea* has been repeatedly modified in recent years, with new species being described and others transferred into or out of the genus. The relationships of *Deprea* to other genera of Solanaceae are still poorly understood, although it seems to be closely related to *Larnax*. A phylogenetic analysis was performed to test the monophyly of *Deprea*. Sequences from three molecular markers (nuclear ITS and *waxy* and chloroplast *psbA-trnH*) were analyzed by parsimony and Bayesian methods. All the species of *Deprea* and *Larnax* sampled were intermixed in a strongly supported clade in the consensus trees, and therefore the currently recognized *Deprea* species do not form a monophyletic group. At least four strongly supported clades could be recovered within the *Deprea* + *Larnax* assemblage, but the affinities of several species of both genera remained unresolved. Additional sampling including the majority of the *Deprea* + *Larnax* species and more representatives of genera in the Physalideae should be done to clarify relationships within the clade and to pinpoint the closest relatives of *Deprea* + *Larnax*.

Key words: *Larnax*, ITS, *psbA-trnH*, *waxy*, South America

Introduction

Deprea Rafinesque (1838: 57) (Solanaceae) is a small South American genus of 10 species occurring in Andean areas from Venezuela to Bolivia. Two species, *D. orinocensis* (Kunth in Humboldt *et al.* 1818: 12) Rafinesque (1838: 57) and *D. bitteriana* (Werdermann 1937: 130) Sawyer & Benítez de Rojas (1998: 527), have broad distributions from Venezuela to Ecuador, while the remaining species have more restricted ranges. For instance, *D. zamorae* Barboza & Leiva González (2013: 42) is found only in southern Ecuador (Loja and Zamora–Chinchipec Provinces; Barboza *et al.* 2013) and *D. nubicola* Sawyer (2007: 54) is confined to the Sierra Nevada de Santa Marta in Colombia (Sawyer 2007).

The circumscription of *Deprea* has been repeatedly modified in recent years, with new species being described (Garzón-Venegas & Orozco 2007, Sawyer 2007, Cueva & Treviño 2012, Barboza *et al.* 2013) and others transferred into or out of the genus (Sawyer 2001, Leiva González *et al.* 2005). The relationships of *Deprea* to other genera of Solanaceae are still poorly understood. Hunziker (2001) placed the genus in tribe Solaneae subtribe Witheringiinae Reveal (2012: 220), while Sawyer (2005) considered *Deprea* to be a member of tribe Physalideae Miers (1849a: 179). Morphologically, *Deprea* is similar to *Larnax* Miers (1849b: 37) (Barboza & Hunziker 1994, Sawyer 1998, 2005, Garzón-Venegas & Orozco 2007), and some authors (D'Arcy 1979, 1993) have combined the two genera under *Deprea*, the earlier published name. Sawyer (2005) redefined *Deprea* as a monophyletic group excluding *D. glabra* (Standley 1935: 32) Hunziker (1977: 25) and *D. sylvarum* (Standley & Morton 1938: 1036) Hunziker (1977: 25), which he transferred to *Larnax* (Sawyer 2001). However, neither Hunziker (2001) nor Sawyer (2005) considered *Deprea* and *Larnax* to be sister taxa, but instead proposed that each was more closely related to different genera in the

Physalideae. Olmstead *et al.* (2008) did not include any *Deprea* species in their Solanaceae molecular phylogeny but they discussed its affinities with *Larnax* and other genera; *Larnax* was included by the authors in tribe Physalideae (as the Physaleae clade), although it was not assigned to any of the subtribes proposed and not closely related to other genera in the clade. Särkinen *et al.* (2013) and Ng & Smith (2016) included four species of *Deprea* and 11 species of *Larnax* in their molecular phylogenies. In the Särkinen *et al.* (2013) phylogeny these species formed a clade sister to the subtribe Withaninae Bohs & Olmstead (2008: 1171). Neither *Deprea* nor *Larnax* were supported as monophyletic genera in these analyses, but resolution and support were low in this portion of the tree.

A number of morphological characters, particularly corolla shape and stamen morphology, have been used to characterize *Deprea* and distinguish it from *Larnax* (Table 1), although some characters overlap in the two genera. *Deprea* and *Larnax* species are mostly shrubs with typically colorful and showy flowers (Fig. 1) and accrescent fruiting calyces tightly or loosely enveloping the berry. In *Deprea*, the corolla is funnel-shaped in the majority of the species, with lobes shorter than or rarely as long as the tube (Fig. 1a, d, g). The stamens are equal, with the basal part of the filament slightly expanded or not, not thickened, and fused to the corolla tube. In *Larnax*, which comprises 36 species (Deanna *et al.* 2014), the corolla is campanulate, stellate, or rotate, with lobes longer than or sometimes equal to the tube (Fig. 1b, c, e, f), the stamens are equal or unequal, and the basal part of the filament is thickened and usually bears two prominent tooth-like appendages.

TABLE 1. Diagnostic characters used to distinguish *Deprea* and *Larnax* (based on published literature).

Characters	<i>Deprea</i>	<i>Larnax</i>
Venation of calyx lobes		
Barboza & Hunziker 1994, Hunziker 2001	Lobes always with 3 nerves (main and secondary nerves)	Lobes with a single main nerve, sometimes also with 1 or 2 secondary or tertiary nerves
Corolla shape		
Barboza & Hunziker 1994	Funnel-shaped	Rotate
Sawyer 2001, 2005, 2007	Funnel-shaped	Rotate-campanulate
Hunziker 2001	Funnel-shaped or urceolate	Campanulate-rotate
Leiva González <i>et al.</i> 2005	Tubular to funnel-shaped	Shortly tubular, rotate or stellate
Barboza <i>et al.</i> 2013	Narrowly campanulate, funnel-shaped, urceolate	-----
Ratio corolla lobes/corolla tube		
Barboza & Hunziker 1994, Garzón-Venegas & Orozco 2007	Lobes always shorter than the tube	Lobes 2–4 times longer than the tube, rarely as long as the tube
Sawyer 2001	Lobes shorter than or equal to the tube	Lobes always longer than the tube
Hunziker 2001	Lobes 1.5–3 times shorter than the tube	Lobes usually 2–3 times longer than the tube, sometimes as long as the tube
Pubescence in the inner corolla		
Leiva González <i>et al.</i> 2005	Usually with a ring of hairs	Sometimes with a ring of hairs
Filament length		
Leiva González <i>et al.</i> 2005, Sawyer 2005	Usually equal	Usually unequal
Filament base expansion		
Barboza & Hunziker 1994	Expanded or not expanded	Expanded
Sawyer 2001, 2005, 2007	Never thickened and gradually expanding basipetally	Thickened and abruptly expanded
Leiva González <i>et al.</i> 2005	Absent	Present
Filament adnation		
Sawyer 2005	≥ 2.5 mm	≤ 1.8 mm

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TABLE 1. (Continued)

Characters	<i>Deprea</i>	<i>Larnax</i>
Tooth-like appendages on the filament base (= auricles)		
Barboza & Hunziker 1994	Always absent	Present or absent
Ratio filament length/anther length		
Barboza & Hunziker 1994, Hunziker 2001	Filaments always longer than the anthers (exception: <i>D. paneroi</i>)	Filaments longer, shorter, or as long as the anthers
Leiva González <i>et al.</i> 2005	Filaments > 140% the length of the anthers Filaments always longer than the anthers, rarely equal in length	Filaments ≤ 55% the length of the anthers Filaments usually shorter than the anthers, sometimes equal in length
Heteranthery		
Sawyer 2001, 2005, 2007 Hunziker 2001	Anthers of equal size Anthers equal	Anthers of 2 or 3 different sizes Anthers sometimes unequal
Mucronate anthers		
Leiva González <i>et al.</i> 2005	Anthers usually not mucronate	Anthers usually mucronate
Berry with stone cells		
Leiva González <i>et al.</i> 2005	Stone cells usually present	Stone cells absent
Pollen surface texture		
Sawyer 2001	Rugulate	Echinate
Sawyer 2005	Rugulate	Scabrate or psilate

Molecular data have led to new insights into phylogenetic relationships at all taxonomic levels, and Solanaceae has not been an exception to this trend. However, even though significant progress has been made in understanding evolutionary relationships in the family, the sole attempt to reconstruct phylogenetic relationships in *Deprea* was based on morphological characters and resulted in a weakly supported hypothesis (Sawyer 2005). Therefore, a molecular phylogenetic analysis using three markers (two nuclear and one chloroplast) was carried out in order to test the monophyly of *Deprea* and to infer interspecific relationships.

Methods

Species sampling

Eight out of 10 *Deprea* species (Table 2) were sampled in the field (2011–2012). The two missing species are *Deprea cardenasiana* Hunziker (1977: 10), which has not been collected since the 1940's (it was not found on two collecting trips to Bolivia by Barboza in 2012 and 2013), and *Deprea oxapampensis* Cueva & Treviño (2012: 144), a recently described species collected just once in 2007 (Cueva & Treviño 2012). A group of eight *Larnax* species was also sampled; three of these have been placed in *Deprea* in the past (Table 2). Based on Olmstead *et al.* (2008), five species from different clades of tribe Physalideae were sampled (Table 2) and *Solanum tuberosum* L. (1753: 185) was used as the outgroup.

Molecular analysis

Total genomic DNA was extracted from silica gel-dried leaves using the DNeasy Plant Mini Kit (Qiagen). Three molecular markers were analyzed: ITS and GBSSI (*waxy*) from the nuclear genome, and *psbA-trnH* from the chloroplast genome. PCR amplification followed the protocols of White *et al.* (1990) for ITS, Levin *et al.* (2005) for *waxy*, and Sang *et al.* (1997) for *psbA-trnH*. Universal primers were used for ITS (forward ITS5, reverse ITS4; White *et al.* 1990) and *psbA-trnH* (Sang *et al.* 1997). Two pairs of primers, namely 181F and 1171R (Walsh & Hoot, 2001), and 1058 (Levin *et al.* 2006) and 3'N (Peralta & Spooner 2001), forward and reverse respectively in each pair, were used to amplify a *waxy* fragment from introns 2 to 8 in two overlapping pieces. The internal primers Ex4F and Ex4R (forward

and reverse respectively; Stern *et al.* 2010) were used for amplification and sequencing of *waxy* in three pieces for difficult materials.

TABLE 2. List of species included in this study, with voucher and geographic origin or GenBank accessions* (^Dspecies formerly under *Deprea*; ¹species formerly under *Larnax*).

Species	Voucher	GenBank numbers	Geographic origin
<i>Deprea bitteriana</i> (Werderm.) Sawyer & Benítez	Orozco <i>et al.</i> 3871 (COL)	<i>psbA-trnH</i> - KP267779 ITS- KP267794 <i>waxy</i> - KP267808	Colombia
<i>Deprea cuyacensis</i> (Sawyer & S. Leiva) S. Leiva & P. Lezama ¹	Barboza <i>et al.</i> 3367 (CORD)	<i>psbA-trnH</i> - KP267786 ITS- KP267793 <i>waxy</i> - KP267807	Peru
<i>Deprea cyanocarpa</i> J. Garzón & C. I. Orozco	Muñoz 002 (COL)	<i>psbA-trnH</i> - KP267780 ITS- KP267797 <i>waxy</i> - KP267811	Colombia
<i>Deprea ecuatoriana</i> Hunz. & Barboza	Orozco <i>et al.</i> 3952 (COL)	<i>psbA-trnH</i> - KP267784 ITS- KP267795 <i>waxy</i> - KP267809	Ecuador
<i>Deprea nubicola</i> Sawyer	Orejuela 215 (COL)	<i>psbA-trnH</i> - KP267783 ITS- KP267796 <i>waxy</i> - KP267810	Colombia
<i>Deprea orinocensis</i> (Kunth) Raf.	Benítez & Mancilla 7460 (MY)	<i>psbA-trnH</i> - KP267774 ITS- KP267767 <i>waxy</i> - KP267762	Venezuela
<i>Deprea paneroi</i> Benítez & M. Martínez	Benítez <i>et al.</i> 7454 (MY)	<i>psbA-trnH</i> - KP267773 ITS- KP267768 <i>waxy</i> - KP267761	Venezuela
<i>Deprea zamorae</i> Barboza & S. Leiva	Orozco <i>et al.</i> 3926 (COL)	<i>psbA-trnH</i> - KP267785 ITS- KP267792 <i>waxy</i> - KP267806	Ecuador
<i>Larnax glabra</i> (Standley) Sawyer ^D	Orozco <i>et al.</i> 3812 (COL)	<i>psbA-trnH</i> - KP267782 ITS- KP267799 <i>waxy</i> - KP267813	Colombia
<i>Larnax hawkesii</i> Hunz.	Orozco <i>et al.</i> 3834 (COL)	<i>psbA-trnH</i> - KP267822 ITS- KP267821 <i>waxy</i> - KP267820	Colombia
<i>Larnax nieva</i> S. Leiva & N. W. Sawyer	Deanna & Leiva 46 (CORD)	<i>psbA-trnH</i> - KP267775 ITS- KP267769 <i>waxy</i> - KP267763	Peru
<i>Larnax psilophyta</i> Sawyer	Sawyer 770 (CONN)	<i>psbA-trnH</i> - KP267778 ITS- KP267772 <i>waxy</i> - KP267766	Ecuador
<i>Larnax purpurea</i> S. Leiva	Deanna & Leiva 21 (CORD)	<i>psbA-trnH</i> - KP267777 ITS- KP267771 <i>waxy</i> - KP267765	Peru
<i>Larnax sachapapa</i> Hunz.	Orozco <i>et al.</i> 3813 (COL)	<i>psbA-trnH</i> - KP267781 ITS- KP267798 <i>waxy</i> - KP267812	Colombia
<i>Larnax subtriflora</i> (Ruiz & Pav.) Miers. ^D	Barboza & Leiva 3663 (CORD)	<i>psbA-trnH</i> - KP267776 ITS- KP267770 <i>waxy</i> - KP267764	Bolivia
<i>Larnax sylvarum</i> (Standley & C. V. Morton) Sawyer ^D	Bohs 2504 (UT)	<i>psbA-trnH</i> - KP267819 ITS- KP267800 <i>waxy</i> - KP267814	Costa Rica

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TABLE 2. (Continued)

Species	Voucher	GenBank numbers	Geographic origin
<i>Cuatresia harlingiana</i> Hunz.	Smith 204 (WIS)	ITS- DQ314165.1* waxy- DQ309475.1*	Ecuador
<i>Dunalia brachyacantha</i> Miers	Barboza et al. 3626 (CORD)	<i>psbA-trnH</i> - KP267791 ITS- KP267804 waxy- KP267818	Bolivia
<i>Eriolarynx iochromoides</i> (Hunz.) Hunz.	Barboza et al. 1966 (CORD)	<i>psbA-trnH</i> - KP267789 ITS- KP267802 waxy- KP267816	Argentina
<i>Iochroma squamosum</i> S. Leiva & Quip.	Barboza & Leiva 3369 (CORD)	<i>psbA-trnH</i> - KP267790 ITS- KP267803 waxy- KP267817	Peru
<i>Vassobia dichotoma</i> (Rusby) Bitter	Nee et al. 51797 (UT)	<i>psbA-trnH</i> - KP267788 ITS- KP267801 waxy- KP267815	Bolivia
<i>Solanum tuberosum</i> L.		<i>psbA-trnH</i> - FN675829.1* ITS- AY875827.1* waxy- X83220*	

PCR products were cleaned with the Promega Wizard SV Gel and PCR Clean-up system (Promega) or using the enzyme combination of exonuclease I (Exo I, Thermo Scientific) and thermosensitive alkaline phosphatase (FastAP, Thermo Scientific), following Werle *et al.* (1994). PCR products were sequenced on an ABI automated DNA sequencer (University of Utah Core Facilities, USA, and CERELA-CONICET, Argentina). Sequence editing and alignment were done using MEGA5 (Tamura *et al.* 2011), with final manual adjustments.

Phylogenetic analysis

Maximum parsimony (MP) analysis was done using PAUP 4.0b 10 (Swofford 2002) with heuristic searches and tree bisection-reconnection (TBR) branch swapping (1000 replicates, saving 10 trees per replicate). All characters were weighted equally and gaps were treated as missing data. Bootstrap analyses in PAUP were performed to assess internal support (BS) for clades (1000 replicates, each with 10 replicates using TBR branch swapping and saving 10 trees per replicate).

Bayesian inference (BI) was performed using MrBayes 3.2.2 (Ronquist *et al.* 2012). The analysis was carried out for 5 million generations using the Markov chain Monte Carlo (MCMC) search. The initial 25% of trees were discarded as burn-in and the remaining trees were used to build a majority-rule consensus tree with posterior probability (PP) values. The nucleotide substitution model was selected prior to the analysis using the Akaike information criterion as implemented in JModelTest version 2.1.3 (Darriba *et al.* 2012). The best-fitting model was the GTR+I for the combined datasets.

Results

Complete sequences were obtained of all three markers for all the species analyzed. ITS sequences were 700–850 bp long, *waxy* sequences had ca. 1300 bp, and the chloroplast *psbA-trnH* spacer was ca. 560 bp long. Parsimony-informative (PI) characters were 11% for ITS, 6.5% for *waxy*, and 3% for *psbA-trnH*.

Parsimony analysis of the combined data set resulted in three most parsimonious trees of 583 steps (CI: 0.775; RI: 0.771; Fig. 1), with the number of PI characters 8.1% of the total. The *Deprea* + *Larnax* clade received 100% BS support, but neither genus was resolved as monophyletic. A similar result was obtained using BI (Fig. 1). Parsimony analyses performed on the individual markers separately resulted in 1277 most parsimonious (MP) trees with *psbA-trnH*, 22 with ITS, and a single tree with *waxy*. The separate analyses of the three regions all resolved a clade containing *Deprea* + *Larnax* species (76% and 100% BS support for ITS and *waxy*, respectively, 44% BS support for *psbA-trnH*; Fig. 2); none resolved *Deprea* or *Larnax* as monophyletic within this clade.

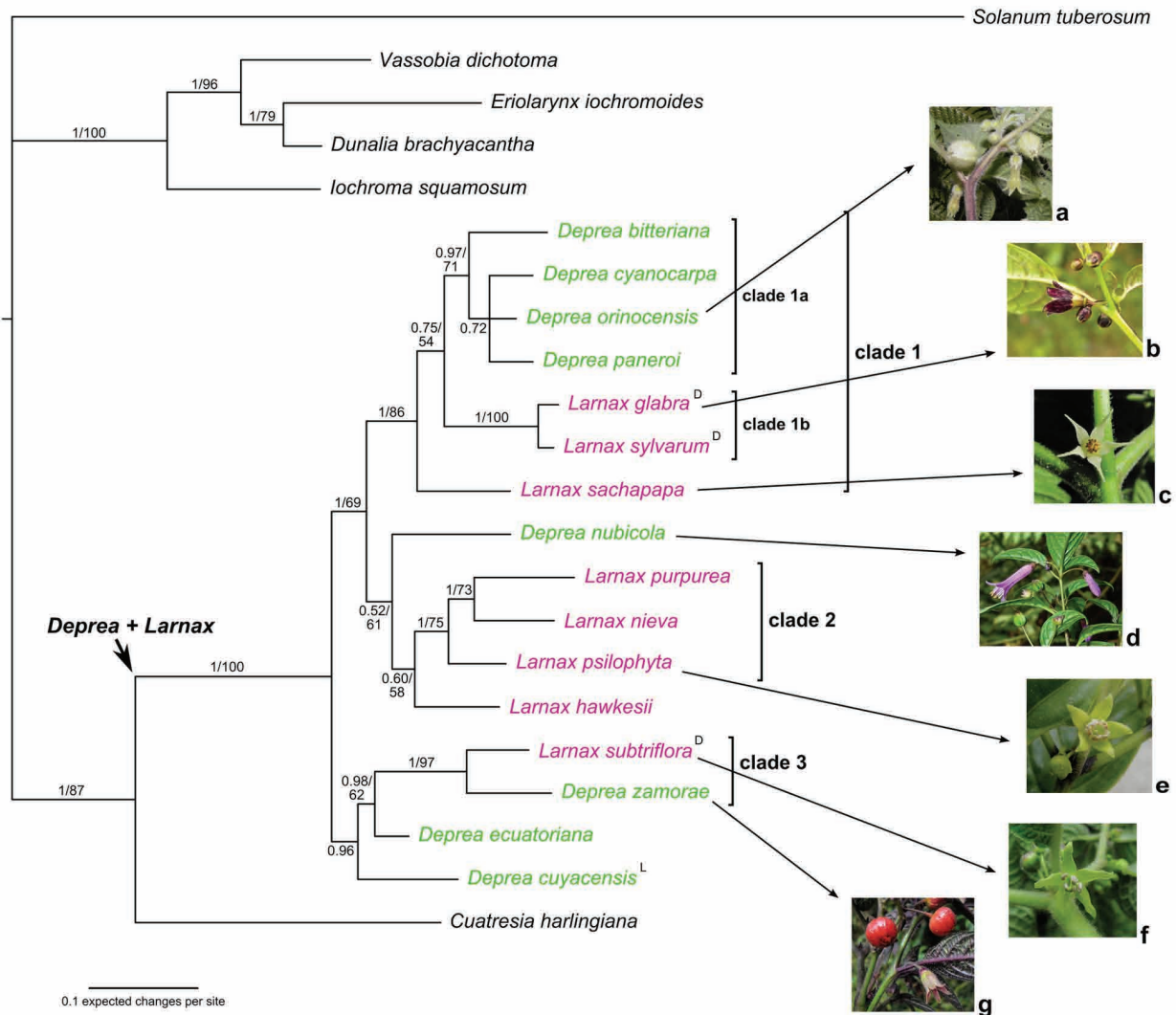


FIGURE 1. Phylogeny of *Deprea* and *Larnax*. Bayesian 50% majority-rule consensus tree obtained from the combined data set (chloroplast *psbA-trnH*, nuclear ITS and *waxy*). Posterior probabilities and Bootstrap support (1000 replicates) values above 50% are indicated on each branch. The topology of the parsimony strict consensus tree (from three most parsimonious trees of 583 steps, CI: 0.775, RI: 0.771) matches the Bayesian 50% majority-rule consensus tree except the branches with < 50% bootstrap support within clade 1 are collapsed in the parsimony tree. The species of each genus are identified by different colors: *Deprea* in green, *Larnax* in pink. ^D species formerly under *Deprea*; ^L species formerly under *Larnax*. (Photographs: a by J. C. Murillo; b by G. E. Barboza; c, f–g by S. Leiva González; d by A. Orejuela; e by R. Deanna).

The sister group to the *Deprea* + *Larnax* clade in the combined analyses is *Cuatresia harlingiana* Hunziker (1987: 92) (Fig. 1). *Cuatresia* Hunziker (1977: 15) is a genus unassigned to any Physalideae subtribe by Olmstead *et al.* (2008), as is the case in *Larnax*. The remaining Physalideae species studied form a clade in a polytomy with the remaining sampled taxa (Fig. 1).

Species relationships within the *Deprea* + *Larnax* clade are highly resolved in the combined analyses, although the BS for several branches is low (54–60%; Fig. 1). Three well-supported groups (i.e., those with BS >75% and PP > 0.8) are distinguished within the *Deprea* + *Larnax* clade (Fig. 1). Clade 1 includes *D. bitteriana*, *D. cyanocarpa* Garzón & Orozco (2007: 220), *D. orinocensis*, *D. paneroi* Benítez de Rojas & Martínez (1992: 270), *L. glabra* (Standley) Sawyer (2001: 460), *L. sylvarum* (Standley & Morton) Sawyer, and *L. sachapapa* Hunziker (1977: 13); the first four species form a clade (BS 71%, PP 0.97), designated as clade 1a on Fig. 1, and *L. glabra* and *L. sylvarum* are well-supported as sister taxa (BS 100%, PP 1.0; designated as clade 1b). *Larnax purpurea* Leiva González, *L. nieva* Leiva González & Sawyer in Leiva González & Lezama Asencio (2003: 106), and *L. psilophyta* Sawyer comprise clade 2, and *L. subtriflora* (Ruiz López & Pavón 1799: 42) Miers (1849b: 38) and *D. zamorae* form clade 3. In the BI tree, *D. ecuatoriana* Hunziker & Barboza (1996: 109) was strongly supported as sister to clade 3 (PP 0.98) and

D. cuyacensis was sister to this group (PP 0.96; Fig. 1). Trees from the individual markers resolved and supported different combinations of these clades, with the *waxy* tree being the best resolved (Fig. 2). Clade 1 was resolved in the *waxy* tree only, whereas clade 1b was resolved and well-supported in the *waxy* and ITS trees. Clade 2 was resolved and well-supported only in the ITS tree. Clade 3 was resolved in the trees from *waxy* and *psbA-trnH*, and the *waxy* tree also resolved *D. ecuatoriana* as sister to Clade 3 with moderate support (BS 79%).

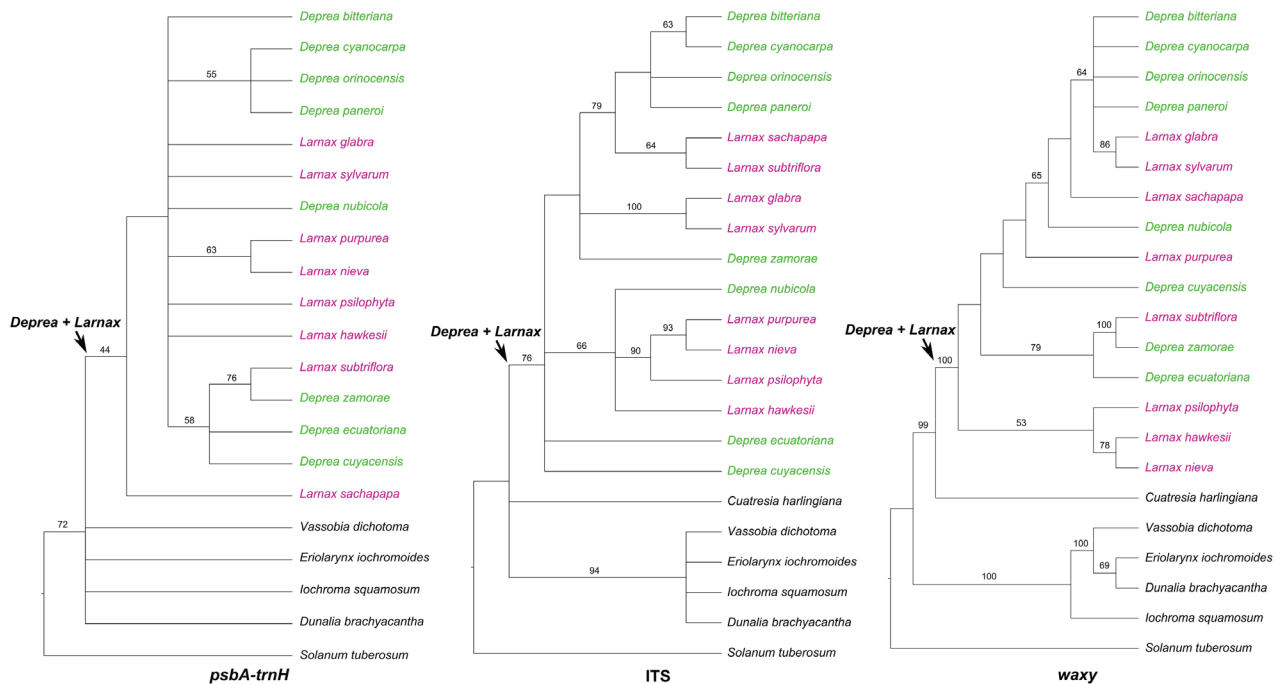


FIGURE 2. Parsimony analyses of single markers (chloroplast *psbA-trnH*, nuclear ITS and *waxy*); strict consensus trees for *psbA-trnH* (from 1277 most parsimonious trees of 50 steps; CI: 0.800, RI: 0.796) and ITS (from 22 most parsimonious trees of 257 steps; CI: 0.704, RI: 0.717), single most parsimonious tree for *waxy* (251 steps; CI: 0.920, RI: 0.921). Bootstrap values (1000 replicates) above 50% are indicated on each branch. The species of each genus are identified by different colors: *Deprea* in green, *Larnax* in pink.

Discussion

Because *Deprea* and *Larnax* do not emerge as monophyletic groups in the molecular trees, it is inferred that the diagnostic characters typically used to distinguish each of the genera (i.e., corolla shape, length of corolla lobes relative to the tube, filament base expansion, ratio of filament length to anther length, and equal vs. unequal stamens) are highly homoplastic and thus inadequate to define them. These results suggest that *Larnax* should be subsumed within *Deprea*, which has priority, and a revised suite of characters should be sought to define the *Deprea + Larnax* clade (Deanna *et al.* 2015).

The species of clade 1 inhabit the northernmost area of both genera, from Ecuador to Central America (Barboza *et al.* 2013, Deanna *et al.* 2014). All these species have non-mucronate anthers and equal filaments, with the filament base gradually expanded (except *D. paneroi*) and without appendages (Table 3). *Larnax sachapapa* is unique in having corolla lobes markedly longer than the tube and glabrous inner corolla surfaces (Fig. 1c), while *L. glabra* and *L. sylvarum* (clade 1b) are the only species with corolla lobes as long as or scarcely longer than the tube (Fig. 1b; Table 3). The *Deprea* species of clade 1a share funnel-shaped to narrowly campanulate corollas (Fig. 1a), with *D. paneroi* the most distinctive species; it is dioecious, with the filament bases not expanded (Sawyer & Benítez 1998, Sawyer & Anderson 2000) (Table 3). Within clade 1, the *Deprea* species form a monophyletic group (clade 1a; BS 71%, PP 0.97), but *Larnax* is paraphyletic.

The three *Larnax* species of clade 2 are glabrous to glabrescent plants with fleshy leaves, stellate or campanulate corollas (Fig. 1e), unequal filaments and subequal anthers, filament bases abruptly expanded with conspicuous appendages, and microechinate pollen surfaces (Table 3). *Larnax psilophyta* differs from the other two species in the presence of an annular ring of hairs on the inner corolla surface (Table 3) and in its orange berries. This species is restricted to the north of a biogeographic barrier, the Huancabamba Deflection in southern Ecuador and northern Peru (Sawyer 2005), while *L. purpurea* and *L. nieva* are found only to the south of this barrier (Sawyer 2005, Deanna pers. comm.).

TABLE 3. States of the diagnostic characters used to distinguish *Deprea* and *Larnax* in the species studied (based on the literature cited in Table 1 and our observations). Species are arranged according to the proposed phylogenetic groupings.

Characters /Species	Clade 1			Clade 1b			Clade 2			Clade 3							
	Clade 1a		<i>D. pamei</i>	<i>L. glabra</i>	<i>L. sylvanum</i>	<i>L. sachapapa</i>	<i>D. mibicola</i>	<i>L. hawkesii</i>	<i>L. nieva</i>	<i>L. psilophylla</i>	<i>L. purpurea</i>	<i>L. subinflora</i>	<i>D. zamorae</i>	<i>D. cenaloriana</i>	<i>D. cypacensis</i>		
Corolla shape	<i>D. bitteriana</i>	<i>D. cyanocarpa</i>	<i>D. orinocensis</i>	Funnel-shaped	<i>L. glabra</i>	<i>L. sylvanum</i>	<i>L. sachapapa</i>	<i>D. mibicola</i>	<i>L. hawkesii</i>	<i>L. nieva</i>	<i>L. psilophylla</i>	<i>L. purpurea</i>	<i>L. subinflora</i>	<i>D. zamorae</i>	<i>D. cenaloriana</i>	<i>D. cypacensis</i>	
Ratio corolla lobes/corolla tube	Funnel-shaped	Narrowly campanulate	Funnel-shaped	0.4–0.5	0.5	0.4–0.9	0.9–1.3	1–1.5	1–1.2	1–1.2	1.4–1.8	2–3.7	2–2.3	2–2.3	1–1.2	1.4–1.8	2–3.7
Annular ring of hair in the inner corolla	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Present	Absent	Present	Present	Absent	Present	Present
Filament length	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Unequal	Unequal	Unequal	Equal	Equal or slightly unequal	Equal	Equal	Equal
Filament base expansion	Gradually expanded	Gradually expanded	Gradually expanded	Not expanded	Gradually expanded	Gradually expanded	Gradually expanded	Gradually expanded	Abruptly expanded	Abruptly expanded	Abruptly expanded	Abruptly expanded	Gradually expanded	Gradually expanded	Not expanded	Gradually expanded	Gradually expanded
Filament adnation	3.2 mm ± 0.7	3.5 mm ± 0.5	4.1 mm ± 0.7	1.6 mm	1.1 mm ± 0.1	1.7 mm ± 0.3	1.1 mm ± 0.1	ca. 3 mm	1.1 mm ± 0.2	ca. 1 mm	1 mm ± 0.3	0.9 mm ± 0.1	1.3 mm ± 0.3	1.7 mm ± 0.2	ca. 3 mm	1.3 mm ± 0.3	1.3 mm ± 0.3
Appendage on the filament base expansion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Conspicuous	Very conspicuous	Conspicuous	Conspicuous	Absent	Inconspicuous	Absent	Absent	Absent
Ratio filament length/anther length	4.4 ± 0.7	7.5 ± 1.5	ca. 5.5	2.7 ± 7.1	1.2 ± 0.1	3.5 ± 0.5	1 ± 0.2	9.4 ± 0.6	0.8 ± 0.5	0.5 ± 0.1	1.10 ± 0.4	0.4 ± 0.1	1.2 ± 0.2	1.6 ± 0.1	2.1 ± 0.4	2.8 ± 0.6	2.8 ± 0.6
Anther length	Equal	Equal	Equal	Equal	Subequal	Subequal	Subequal	Equal	Subequal	Subequal	Subequal	Subequal	Subequal	Equal	Equal	Unequal	Unequal
Micro in anthers	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent
Stone cells in the berry	Present	Unknown	Present	Unknown	Present	Present	Present	Unknown	Present	Unknown	Unknown	Unknown	Unknown	Present	Absent	Absent	Present
Pollen surface	Psilate	Rugulate	Unknown	Rugulate	Dense, coarsely microechinate	Sparse, minutely microechinate	Dense, coarsely microechinate	Rugulate	Dense, coarsely microechinate	Sparse, minutely microechinate	Dense, coarsely microechinate	Sparse, minutely microechinate	Dense, minutely microechinate	Widely sparse, minutely microechinate	Unknown	Unknown	Granulate

The strongly supported clade 3 includes two species with different distributional ranges. *Deprea zamorae* is a southern Ecuadorian endemic (Barboza *et al.* 2013), whereas *L. subtriflora* is found from northern Peru to northwestern Bolivia (Leiva *et al.* 2013). Both species have glandular pubescence, orange or reddish-orange globose berries tightly enveloped by the calyx (Fig. 1g), an annular ring of hairs on the inner corolla surface, non-mucronate anthers, and microechinate pollen surfaces (Table 3). *Deprea ecuatoriana* is strongly supported as the sister species to clade 3 only in the BI analysis. It is morphologically and ecologically very different from both the species of clade 3 and the other species of *Deprea*. It is a low shrub from the paramos of southern Ecuador and northern Peru with urceolate orange corollas and ellipsoidal fruits.

Three species, *D. nubicola*, *D. cuyacensis*, and *L. hawkesii*, were not included in any of the delimited clades because their positions were weakly supported. Despite this, some morphological similarities support their relationships on the tree. *Larnax hawkesii* is similar to the species of clade 2 in its filament bases abruptly expanded with conspicuous appendages, stellate corollas, and microechinate pollen surfaces. On the contrary, *D. nubicola* does not share morphological characters with the species of clade 2, but is more similar to the species of clade 1 due to its funnel-shaped corolla and equal filaments gradually expanded at the base and lacking appendages. Finally, *D. cuyacensis* shares several morphological characters with the species of clade 3, including non-mucronate anthers and the absence of filament appendages. These three species have several traits that are unique among the *Deprea* + *Larnax* species analyzed here. *Larnax hawkesii* has elliptical-fusiform berries, *D. nubicola* has a funnel-shaped corolla without an inner ring of trichomes, and *D. cuyacensis* has granulate pollen grains.

Additional molecular studies including the majority of the *Deprea* + *Larnax* species and more representatives of genera in the Physalideae are in progress to pinpoint the closest relatives of *Deprea* + *Larnax*. Further taxonomic sampling with additional genes should increase support for species-level relationships within the clades, and character mapping on a well-resolved and supported phylogeny will elucidate the evolutionary patterns of floral characters such as corolla shape and androecium morphology that vary widely among species.

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