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Phylogenetic relationships of Cranichidinae and Prescottiinae (Orchidaceae, Cranichideae) inferred from plastid and nuclear DNA sequences

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- *Background and Aims* Phylogenetic relationships of subtribes Cranichidinae and Prescottiinae, two diverse groups of neotropical terrestrial orchids, are not satisfactorily understood. A previous molecular phylogenetic study supported monophyly for Cranichidinae, but Prescottiinae consisted of two clades not sister to one another. However, that analysis included only 11 species and eight genera of these subtribes. Here, plastid and nuclear DNA sequences are analysed for an enlarged sample of genera and species of Cranichidinae and Prescottiinae with the aim of clarifying their relationships, evaluating the phylogenetic position of the monospecific genera *Exalaria*, *Ocampoa* and *Pseudocranichis* and examining the value of various structural traits as taxonomic markers.
- *Methods* Approx. 6000 bp of nucleotide sequences from nuclear ribosomal (ITS) and plastid DNA (*rbcL*, *matK-trnK* and *trnL-trnF*) were analysed with cladistic parsimony and Bayesian inference for 45 species/14 genera of Cranichidinae and Prescottiinae (plus suitable outgroups). The utility of flower orientation, thickenings of velamen cell walls, hamular viscidium and pseudolabellum to mark clades recovered by the molecular analysis was assessed by tracing these characters on the molecular trees.
- *Key Results* Spiranthinae, Cranichidinae, paraphyletic *Prescottia* (with *Pseudocranichis* embedded), and a group of mainly Andean 'prescottioid' genera (the '*Stenoptera* clade') were strongly supported. Relationships among these clades were unresolved by parsimony but the Bayesian tree provided moderately strong support for the resolution (Spiranthinae–(*Stenoptera* clade-(*Prescottia*/*Pseudocranichis*–Cranichidinae))). Three of the four structural characters mark clades on the molecular trees, but the possession of a pseudolabellum is variable in the polyphyletic *Ponthieva*.
- *Conclusions* No evidence was found for monophyly of Prescottiinae and the reinstatement of Cranichidinae *s.l.* (including the genera of 'Prescottiinae') is favoured. Cranichidinae *s.l.* are diagnosed by non-resupinate flowers. Lack of support from parsimony for relationships among the major clades of core spiranthids is suggestive of a rapid morphological radiation or a slow rate of molecular evolution.

Key words: Cranichideae, Cranichidinae, *matK-trnK*, molecular phylogenetics, nrITS, Orchidaceae, Prescottiinae, resupination, *trnL-trnF*.

INTRODUCTION

Circumscription of subtribe Cranichidinae Lindl. has varied among the several orchid classifications published during the last century in whether or not some of its constituent genera are placed in a distinct subtribe, Prescottiinae Dressler (e.g. Schlechter 1911, 1926; Brieger, 1974–75; Dressler, 1974, 1981; Chase *et al.*, 2003; Pridgeon *et al.*, 2003; *contra* Dressler, 1990, 1993; Szlachetko, 1995). Dressler (1990, 1993) segregated the genera *Aa* Rchb.f., *Altensteinia* Kunth, *Gomphichis* Lindl., *Myrosmodes* Rchb.f., *Porphyrostachys* Rchb.f., *Prescottia* Lindl. ex Hook. and *Stenoptera* C.Presl in Prescottiinae, distinguishing them from Cranichidinae by the possession of velamen of the *Spiranthes* type (after Porembski and Barthlott, 1988), a laminar rostellum, soft pollinia and lack of a hamular viscidium (Rasmussen, 1982). In contrast, Cranichidinae *sensu stricto* (*s.s.*) have a velamen of the *Calanthe* type, a pointed rostellum, brittle pollinia and a hamular viscidium. However, Prescottiinae lack unique distinctive features, and those separating them from Cranichidinae are shared, in various combinations, with subtribes Galeottiellinae Salazar & M.W.Chase, Manniellinae Schltr. and Spiranthinae Lindl., probably representing symplesiomorphies of 'core spiranthids' *sensu* Salazar *et al.* (2003) and Chase (2003). On the other hand, Cranichidinae and Prescottiinae are unique in Cranichideae in having non-resupinate flowers (Fig. 1), and this feature was the reason to group their component genera in Cranichidinae *sensu lato* (*s.l.*) in the first place (e.g. Lindley, 1840, in part; Schlechter, 1911, 1926; Brieger, 1974–75; Dressler, 1981).

Salazar *et al.* (2003) carried out a phylogenetic assessment of tribe Cranichideae based on nucleotide sequences of plastid and nuclear ribosomal (nrITS) DNA. In their combined analysis, four main clades of 'core spiranthids' received moderate to strong internal support, namely Cranichidinae *s.s.*, Spiranthinae, *Prescottia* and a group encompassing predominantly high-Andean genera *Aa*, *Gomphichis*, *Porphyrostachys* and *Stenoptera*, assigned to Prescottiinae by Dressler (1990, 1993) and here referred to as the '*Stenoptera* clade'.

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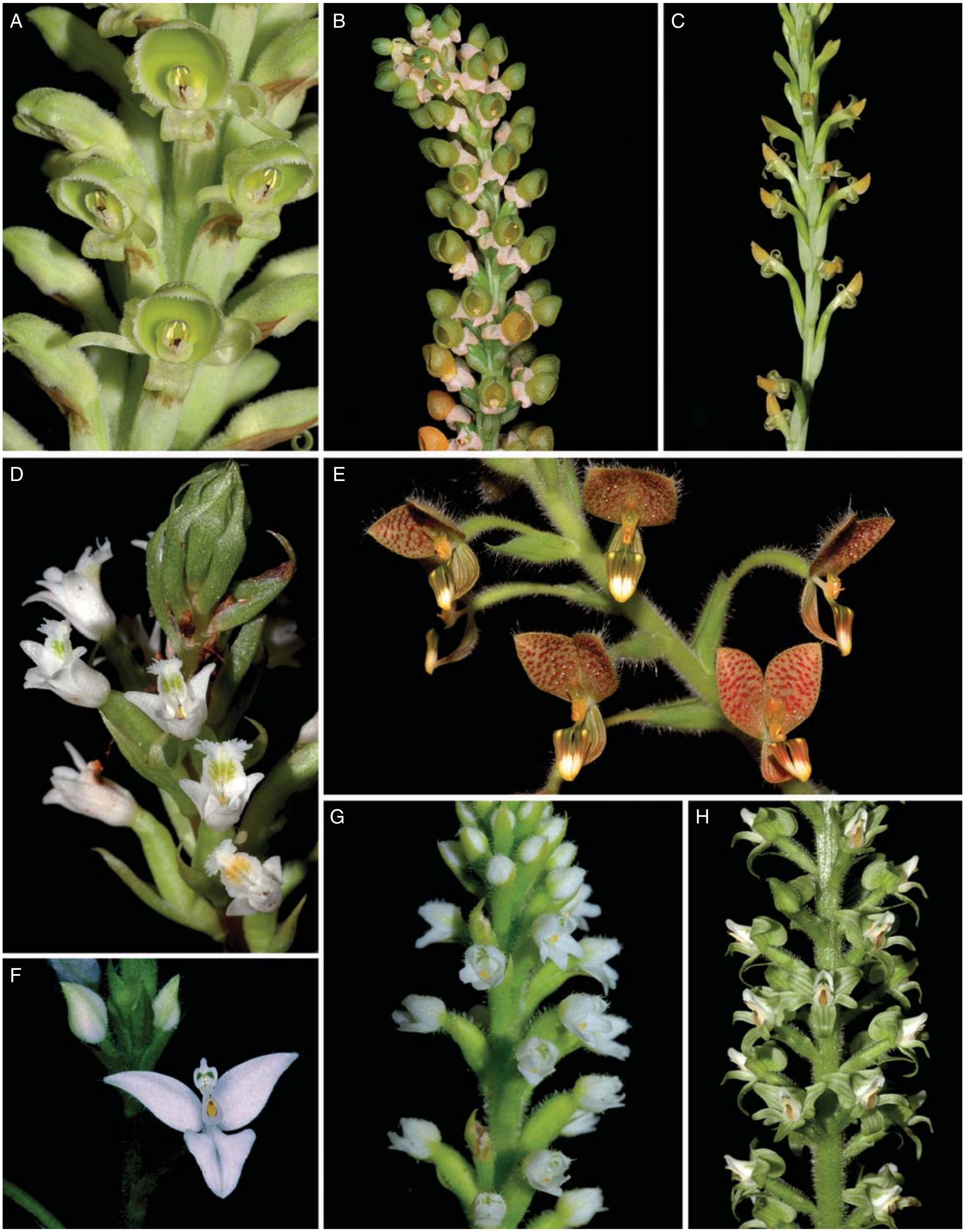

FIG. 1. Flowers of representative species previously assigned to Prescottiinae (A–D) and Cranichidinae (E–H): (A) *Altensteinia fimbriata* (Ecuador, *Salazar 6789*); (B) *Prescottia plantaginea* (Brazil, *Salazar 6350*); (C) *Prescottia tubulosa* (Mexico, *Reyes 5767*); (D) *Pseudocranichis thysanochila* (Mexico, *Reyes 5523*); (E) *Ponthieva formosa* (Mexico, *Salazar 6539*); (F) *Ponthieva ephippium* (Mexico, *Salazar 6440*); (G) *Ponthieva fertilis* (formerly *Exalaria parviflora*; Ecuador, *Salazar 7641*); (H) *Ponthieva* (*Ocampoa*) *mexicana* (Mexico, *Salazar 6474*).

However, the *Stenoptera* clade and *Prescottia* were not sisters; instead the former diverged first and *Prescottia* was weakly supported as sister to Cranichidinae. *Prescottia*/Cranichidinae were in turn weakly supported as collective sisters of Spiranthinae (Salazar *et al.*, 2003, fig. 6).

Recently, Figueroa *et al.* (2008) assessed the phylogenetic relationships of 26 species of Cranichideae with the aim of exploring the evolution and systematic value of several anatomical characters of the root, including some attributes used by previous authors to define so-called velamen types (Porembski and Barthlott, 1988). They did so by analysing cladistically three structural attributes in combination with nucleotide sequences of a nuclear (nrITS) and a plastid DNA region (*matK-trnK*). Their analysis recovered a single most-parsimonious tree (MPT) with the same four main clades of core spiranthids as in Salazar *et al.* (2003). Cranichidinae were sister to a clade in which paraphyletic *Prescottia* (with *Pseudocranichis* embedded) was in turn the sister of a group consisting of *Aa*/*Altensteinia* (representatives of the *Stenoptera* clade) and Spiranthinae. With the exception of *Prescottia*/*Pseudocranichis*, which received weak bootstrap support (BS), all these main clades were strongly supported. Relationships among the four main clades lacked BS >50 % (Figueroa *et al.*, 2008, fig. 4), but the three structural characters (thickenings of secondary walls of velamen cells, lamellate tilosomes and supraendodermal spaces) marked monophyletic groups recovered by the combined analysis.

Together, Cranichidinae *s.s.* and Prescottiinae include about 210 species in 17 genera (Pridgeon *et al.*, 2003), contributing significantly to the terrestrial orchid diversity of the neotropics. However, they are still one the least studied orchid groups. A better understanding of their phylogenetic relationships will provide a more objective basis for their classification and a background for addressing questions on various aspects of their evolution. One such question concerns the evolution of structural characters; for instance, as stated above, Cranichidinae *s.s.* and Prescottiinae differ from all other subtribes of Cranichideae in their non-resupinate flowers, but it is not clear whether this condition represents a uniquely derived, shared feature or a parallelism in these groups, given the lack of support for their relationships (Chase, 2003; Salazar *et al.*, 2003; Figueroa *et al.*, 2008).

Previous phylogenetic analyses of Cranichideae (Salazar *et al.*, 2003; Figueroa *et al.*, 2008) have included only a few representatives of Cranichidinae *s.s.* and Prescottiinae. In this study, the phylogenetic relationships of Cranichidinae and Prescottiinae are assessed by analysing a broader taxonomic sample of both groups with the same DNA regions used by Salazar *et al.* (2003), namely plastid genes *matK* and *rbcL*, plastid *trnK* intron, *trnL* intron and *trnL-trnF* intergenic spacer and the nuclear ribosomal (nr) ITS region. The aims were: (*a*) evaluate subtribal and generic limits and relationships of Cranichidinae *s.s.* and Prescottiinae; (*b*) clarify the systematic position of the monospecific genera *Exalaria* Garay & G.A.Romero-González, *Ocampoa* A.Rich. & Galeotti and *Pseudocranichis* Garay; and (*c*) gain insight into the value of various structural traits as taxonomic markers, including flower orientation and thickenings of the wall of velamen cells, hamular viscidia and the 'pseudolabellum' (a broad surface on the lower side of the flower formed by the expanded, approximate petals, whereas the true labellum is inconspicuous and stands in an upright position; Dressler, 1993).

MATERIALS AND METHODS

Taxonomic sample

Exemplars of 45 species/14 genera belonging to subtribes Cranichidinae and Prescottiinae were analysed, together with 23 species of Spiranthinae. Twelve additional species that represent all remaining subtribes of Cranichideae according to Chase (2003), namely Achlydosinae M.A.Clem. & D.L.Jones (formerly Megastylidinae Schltr., in part), Chloraeinae Rchb.f., Galeottiellinae, Goodyerinae, Manniellinae and Pterostylidinae Pfitz., were used as outgroups following previous phylogenetic studies (Kores *et al.*, 1997, 2001; Cameron *et al.*, 1999; Salazar *et al.*, 2003). A list of the taxa analysed with voucher information and GenBank accessions is provided in Appendix 1.

Molecular methods

Extraction, purification, amplification and sequencing of DNA were carried out following standard procedures explained in Salazar *et al.* (2003) and Figueroa *et al.* (2008). For all DNA regions analysed, both DNA strands were sequenced and then edited and assembled with Sequencher versions 3·1 to 4·6 (GeneCodes Corp.). Alignment of sequences was done by visual inspection, using as templates the alignments of Salazar *et al.* (2003) and trying to maximize sequence similarity (Simmons, 2004). No data were excluded from the analyses due to unambiguous alignment, and the individual gap positions were treated as missing data.

Phylogenetic analyses

A previous assessment of phylogenetic relationships of Cranichideae (Salazar *et al.*, 2003) showed that separate analyses of *rbcL* and the *trnL-trnF*, *matK-trnK* and nrITS regions recovered similar relationships, and no instances of conflicting resolution among different datasets obtaining strong internal support occurred. Furthermore, the combined analysis of all the datasets enhanced resolution and increased the proportion of clades that obtained strong support from the various measures of support applied. Therefore, in this study it was decided to analyse all datasets in combination to maximize resolution and support.

A parsimony analysis was conducted in PAUP* version 4·02b for Macintosh (Swofford, 2002) and consisted of a heuristic search with 1000 random sequences of taxon addition for the starting trees, tree–bisection–reconnection (TBR) branch swapping and the 'MULTREES' option on (storing multiple trees), saving all MPTs. All characters were treated as unordered and equally weighted. Internal support for clades was evaluated by 300 bootstrap replicates (Felsenstein, 1985), each with 20 random sequences of taxon addition and TBR branch swapping, saving up to 20 shortest trees from each addition replicate. Various alternative resolutions were examined by means of the 'Constraints' option in PAUP*, i.e.

constraining the analysis to enforce monophyly of specific groups to examine the effect on tree length and consistency and retention indices.

A model-based phylogenetic analysis of the combined matrix using Bayesian Markov Chain Monte Carlo inference was also carried out as implemented in MrBayes version 3·1·2 (Ronquist *et al.*, 2005). A six-parameter model of molecular evolution with gamma distribution and a proportion of invariant sites fit best the *rbcL*, *matK*, *trnL* intron, *trnL-trnF* intergenic spacer and nrITS data sets according to the Akaike information criterion (Akaike, 1974) in Modeltest version 3·7 (Posada and Crandall, 1998). In the case of the *trnK* intron, a six-parameter model with gamma distribution but with no invariant characters was selected. These models were accordingly assigned to two partitions in MrBayes. Two parallel analyses, each consisting of four Markov chains, were run for 1000 000 generations, sampling from the trees every 100 generations. In both runs, stationarity was reached around generation 70 000 and the first 150 000 generations were discarded as the 'burn-in'. A summary Bayesian tree was calculated from the remaining 8500 trees from each run. Both runs yielded topologically identical trees with most clades being supported by a high posterior probability (PP). The trees from both analyses (17 000 trees) were then pooled into a single summary tree, and the discussion will be based on that tree.

Four morphological characters (flower orientation, thickenings of velamen cell walls, hamular viscidium and pseudolabellum) were optimized on the molecular trees using the program MacClade version 4·02 (Maddison and Maddison, 2001).

RESULTS

Parsimony analysis

The combined dataset comprised 5944 aligned nucleotide positions, of which 2103 were variable and 1381 were potentially parsimony informative. The heuristic search found six MPTs with a length of 5841 steps, consistency index (CI) excluding uninformative characters = 0·43 and retention index (RI) = 0·74. In the strict consensus of the six trees (Fig. 2A), the core spiranthids as defined in Salazar *et al.* (2003) are strongly supported as monophyletic and consist, in successive branching order, of *Galeottiella* (Galeottiellinae), *Manniella* (Manniellinae) and a polytomy formed by Spiranthinae (BS 100), the *Stenoptera clade* (BS 100), paraphyletic *Prescottia* with *Pseudocranichis thysanochila* embedded (BS 92) and Cranichidinae (BS 99).

Strongly supported Spiranthinae encompass three major clades, identified by Salazar *et al.* (2003) as the *Stenorrhynchos*, *Pelexia* and *Spiranthes* clades. Relationships within Spiranthinae are unchanged with respect to previous analyses by Salazar *et al.* (2003, q.v.) and will not be dealt with further here. Within the *Stenoptera* clade, *S. ecuadorana* is sister of the rest, and *Altensteinia fimbriata* is sister (BS 76) to a strongly supported group formed by monophyletic *Gomphichis* sister to *Porphyrostachys pilifera*/*Aa*. In the *Prescottia*/*Pseudocranichis* clade, *Prescottia tubulosa* and *Pseudocranichis thysanochila* are strongly supported as sister to the remaining species of *Prescottia*. With the exclusion of *Pseudocranichis*, Cranichidinae *s.s.* are strongly supported as monophyletic, with *Pterichis* Lindl. being sister to the other members. These other members form two strongly supported clades: *Cranichis* Sw. and a group with *Baskervilla colombiana*, *Exalaria parviflora* and *Ocampoa mexicana* nested among species of *Ponthieva* R.Br. *Baskervilla colombiana* occupies a derived position in a subclade that also includes, in succession, *Ponthieva formosa*, *P. elata* and *P. tuerckheimii*. The other major subclade of *Ponthieva* includes *P. guatemalensis* as the sister of a trichotomy formed by *Exalaria parviflora*, *P. ephippium*/*Ocampoa mexicana*, and a clade comprising *P. triloba*, *P. schaffneri*, *P. trilobata*, *P. parvula* and *P. racemosa*/*P. brittoniae*.

Enforcing monophyly for the group with non-resupinate flowers (i.e. Cranichidinae *s.l.*) in a parsimony analysis by means of a constraint tree in PAUP* resulted in two MPTs only two steps longer (with the same CI and RI) than the six MPTs from the unconstrained analysis.

Bayesian analysis

Relationships recovered by the Bayesian analysis for the most part mirror those of the parsimony analysis, but the tree is fully resolved (Fig. 2B). Spiranthinae, the *Stenoptera* clade, *Prescottia*/*Pseudocranichis* and Cranichidinae are all strongly supported (PP 1·00). Spiranthinae are sister to a moderately supported group (PP 0·91) comprising the *Stenoptera* clade as the sister of a group that includes *Prescottia*/*Pseudocranichis*, which in turn is sister of Cranichidinae *s.s.* (PP 0·71). Internal relationships of these groups are similar to those recovered in the parsimony analysis. However, the topology of the Bayesian tree matched none of the six MPTs found by parsimony.

DISCUSSION

Relationships among the four major clades of 'core spiranthids'

The lack of supported resolution for the relationships among Spiranthinae, Cranichidinae, *Prescottia* and the *Stenoptera clade* noted by Salazar *et al.* (2003) was also observed in the parsimony analysis. In the consensus tree, these four clades form a polytomy (Fig. 2A). However, in the Bayesian tree, Spiranthinae are sister to the rest with a moderately high posterior probability (PP 0·91) and the *Stenoptera clade* diverges next, with paraphyletic *Prescottia* (including *Pseudocranichis*) as sister to Cranichidinae (PP 0·71; Fig. 2B). None of the MPTs of the parsimony analysis matches the topology of the Bayesian tree. Instead, each of the following resolutions was recovered by two of the six parsimony cladograms: (*a*) (*Stenoptera* clade–(*Prescottia*/*Pseudocranichis*–(Cranichidinae-Spiranthinae))); (*b*) (*Stenoptera* clade–(Cranichidinae–(*Prescottia*/*Pseudocranichis*–Spiranthinae))); and (*c*) (*Prescottia*/*Pseudocranichis*–(*Stenoptera* clade–(Cranichidinae-Spiranthinae))).

The parsimony analysis constrained to enforce monophyly of Cranichidinae *s.l.* resulted in two cladograms only two steps longer that the six MPTs of the unconstrained analysis.

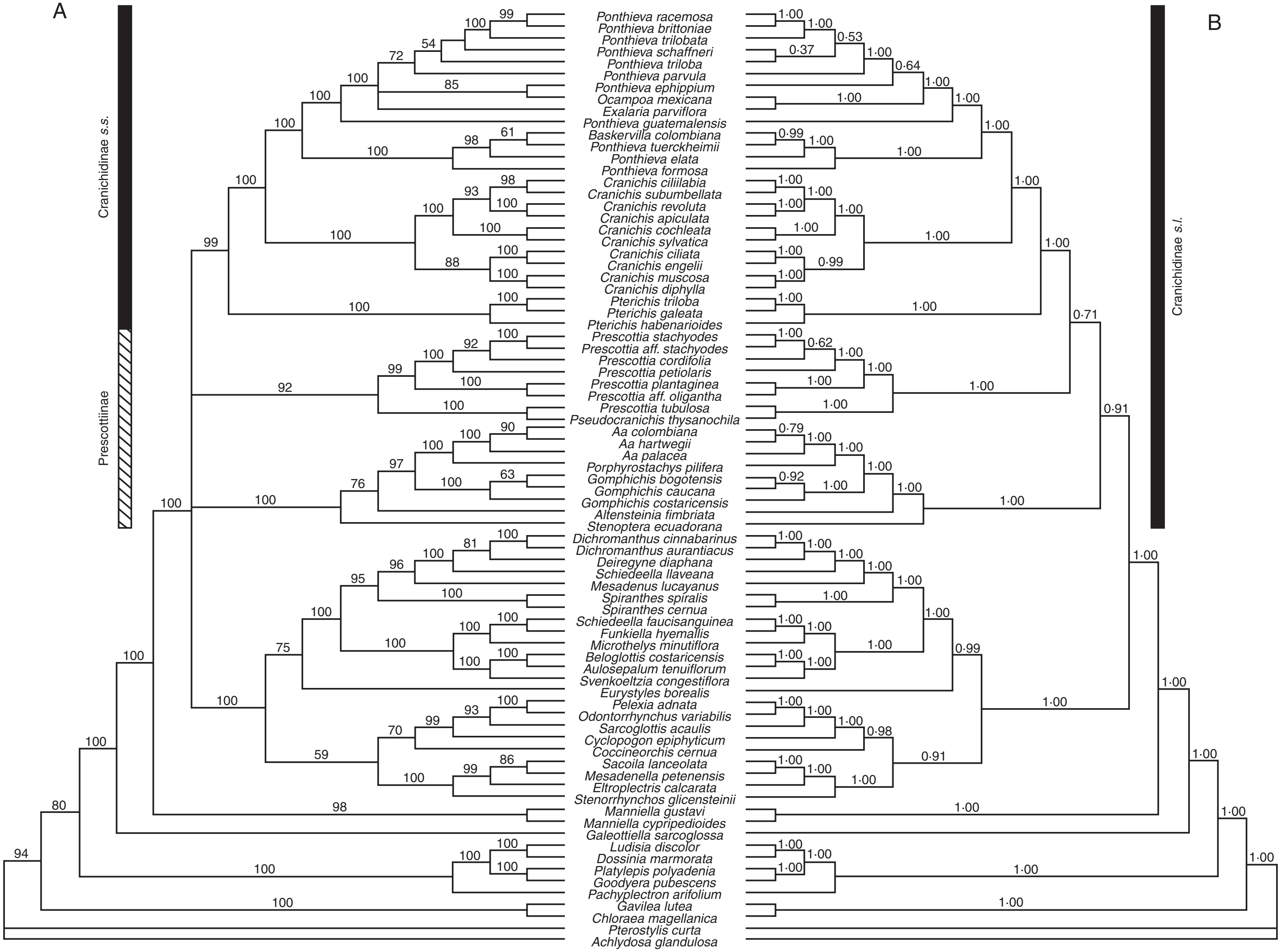

FIG. 2. Phylogenetic relationships of Cranichidinae and Prescottiinae inferred from combined analyses of *rbcL*, *matK-trnK*, *trnL-trnF* and nrITS. (A) Strict consensus of the six MPTs found by the parsimony analysis (numbers above branches are bootstrap proportions). (B) Bayesian summary tree (numbers above branches are posterior probabilities). Bars indicate taxonomic limits of Cranichidinae and Prescottiinae.

Therefore, the topology recovered by the Bayesian analysis is not substantially worse (in terms of parsimony steps) than the three (unsupported) resolutions recovered by the parsimony analysis. Cranichidinae *s.l.* can be unambiguously diagnosed by the non-resupinate flowers, and this requires a single transition from resupination to non-resupination in Cranichideae (Fig. 3A), since with the exception of a few species of Spiranthinae (e.g. *Aracamunia liesneri*, *Cyclopogon glabrescens*) and a few genera of Goodyerinae such as *Hetaeria* Bl. and *Macodes* Lindl., resupination is uniform in the tribe. Flower orientation is important for pollination (van der Pijl and Dodson, 1966), and transitions between resupination and non-resupination might have important evolutionary consequences, e.g. promoting divergence between lineages by adaptation to different types of pollinator. We believe that, in the absence of evidence on the contrary, a phylogenetic hypothesis that minimizes the number of transitions between these two conditions (such as that of Fig. 2B) is to be preferred.

The lack of support for relationships among Spiranthinae, Cranichidinae, *Prescottia* and the *Stenoptera* clade in the study of Salazar *et al.* (2003) led Chase (2003) to adopt a conservative approach and resurrect Cranichidinae in the broad sense, i.e. putting back the genera transferred to Prescottiinae by Dressler (1990, 1993). Chase (2003) stated that this was a compromise solution pending more data, which may be less misleading than recognizing more and more narrowly circumscribed subtribes. At least the results of the present Bayesian analysis support his approach, since Cranichidinae *s.l.* are recovered as monophyletic. We have considered the alternative option, i.e. creation of a new subtribe for the *Stenoptera clade*, thus restricting Prescottiinae to include only *Prescottia*/*Pseudocranichis*. However, we are unaware of any morphological attributes diagnostic for the *Stenoptera* clade, and it seems pointless to propose a new undiagnosable subtribe, which only complicates further the nomenclature of these groups. Therefore, we support the merging of ‘Prescottiinae’ with Cranichidinae *s.s.* proposed by Chase (2003) until compelling phylogenetic evidence clearly demonstrates otherwise.

In discussing relationships between Cranichidinae and Prescottiinae, Salazar *et al.* (2003) stated that a hamulus, a diagnostic feature of Cranichidinae *s.s.*, is also present in the prescottioid genus *Gomphichis*, but subsequent observations (A. Álvarez, Missouri Botanical Garden, Ecuador Program, Quito, Ecuador, pers. comm., 2007; see also Rasmussen, 1982) indicated that this may not be the case, and further study is required to determine the nature of the viscidium in that genus. On the other hand, the differences in velamen characteristics noted by Porembski and Barthlott (1988) between one prescottioid species, *Aa palacea* (as *Altensteinia palacea*) and two species of Cranichidinae *s.s.*, namely *Ponthieva schaffneri* (as *Cranichis schaffneri*) and *P. petiolata*, have been confirmed for various other taxa by Figueroa *et al.* (2008). Their study showed that in Spiranthinae and most representatives of ‘Prescottiinae’ analysed (except *Pseudocranichis thysanochila*) secondary walls of velamen cells bear conspicuous thickenings, which are absent in members of Cranichidinae *s.s.* (and *Pseudocranichis*) examined, as well as in the species of *Goodyera*, *Ludisia* (both Goodyerinae) and *Manniella* (Manniellinae) they used as outgroups. In their phylogenetic tree, Cranichidinae were sister to the two prescottioid clades plus Spiranthinae, and thus absence of thickenings was interpreted as the plesiomorphic condition, with their presence representing a synapomorphy of the Prescottiinae/Spiranthinae grade (Figueroa *et al.*, 2008, fig. 4, and 5A). Nevertheless, the relationships recovered by the present Bayesian analysis imply a different scenario, in which thickenings of velamen cell walls are synapomorphic for the whole Spiranthinae/Cranichidinae *s.l.* clade, with the absence of thickenings being best interpreted as a reversion (secondary loss) diagnostic of what might be termed ‘core Cranichidinae’ and evolving independently in *Pseudocranichis thysanochila* (Fig. 3B).

Lack of clear patterns of support for relationships among the four major clades of core spiranthids in the parsimony analysis discussed above contrasts with the otherwise strongly supported relationships at lower and higher hierarchical levels of the phylogenetic tree (Fig. 2A and B) and might be suggestive of a rapid morphological differentiation (i.e. rapid enough, in a geological timeframe, not to allow for the accumulation of nucleotide substitutions between successive divergences) or to a slower rate of molecular evolution. However, our studies have so far included only DNA sequence data, and it is necessary to conduct cladistic analyses of as many structural characters as possible to contrast results of the molecular trees. This would allow us to evaluate whether those portions of the evolutionary history of core spiranthids that have not been resolved clearly using only DNA sequences correspond to the appearance of structural changes that may have promoted rapid lineage divergence (cf. Bateman, 1999). One promising candidate for such a role as promoter of divergence is the change in flower orientation from resupinate to non-resupinate, which may have given these species access to previously unexploited types of pollinators. However, much more work is required on both assembling and analysing structural datasets for these orchids and investigating factors underlying such apparently radical changes as switching of flower orientation, to say nothing of a better understanding of pollination of these groups.

Internal relationships of the three major clades of Cranichidinae s.l.

Stenoptera *clade.* This group received strong support in the analyses of Salazar *et al.* (2003) and also in this study (Fig. 2). Figueroa *et al.* (2008) analysed only one species each of *Aa* and *Altensteinia*, which likewise formed a strongly supported group. No obvious features diagnosing this clade are known, but, as currently recognized, the genera it includes (*Aa*, *Altensteinia*, *Gomphichis*, *Myrosmodes*, *Porphyrostachys* and *Stenoptera*) are each clearly defined by floral characters. In both the present parsimony and Bayesian analyses, *Stenoptera ecuadorana* was sister to the rest, followed by *Altensteinia fimbriata*. Monophyletic *Gomphichis* was recovered as sister to a clade consisting of *Porphyrostachys pilifera* plus *Aa*. *Porphyrostachys pilifera* is distinctive among the group for its relatively large, bright red flowers with two white blotches on the labellum, which is funnel-shaped and adnate at its base to the prominent column foot (thus forming a deep floral tube). The remaining genera, as well as the other species of *Porphyrostachys* (*P. parviflora*) have

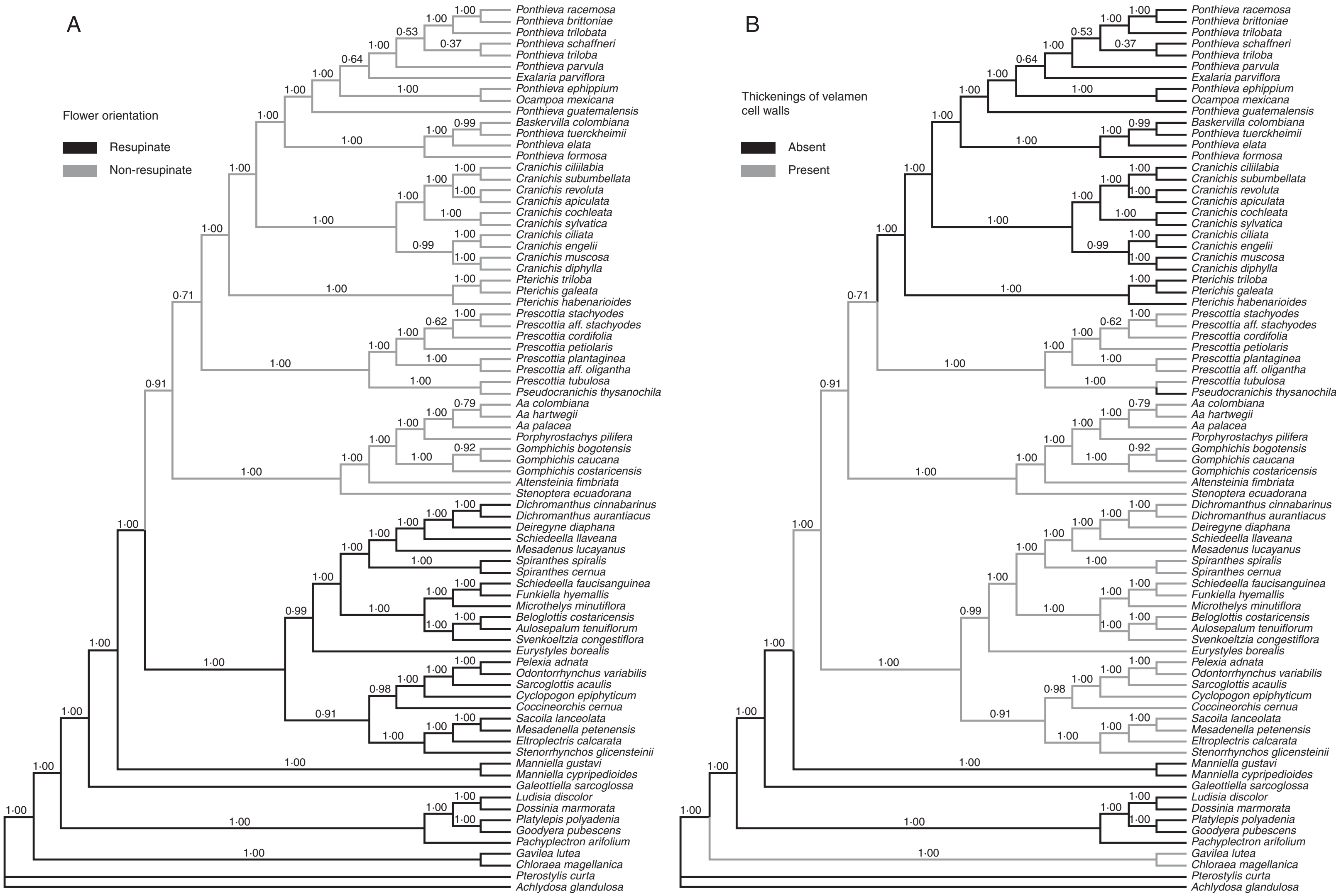

FIG. 3. Optimization of flower orientation and thickenings of velamen cell walls on the Bayesian tree of Fig. 2B (see text).

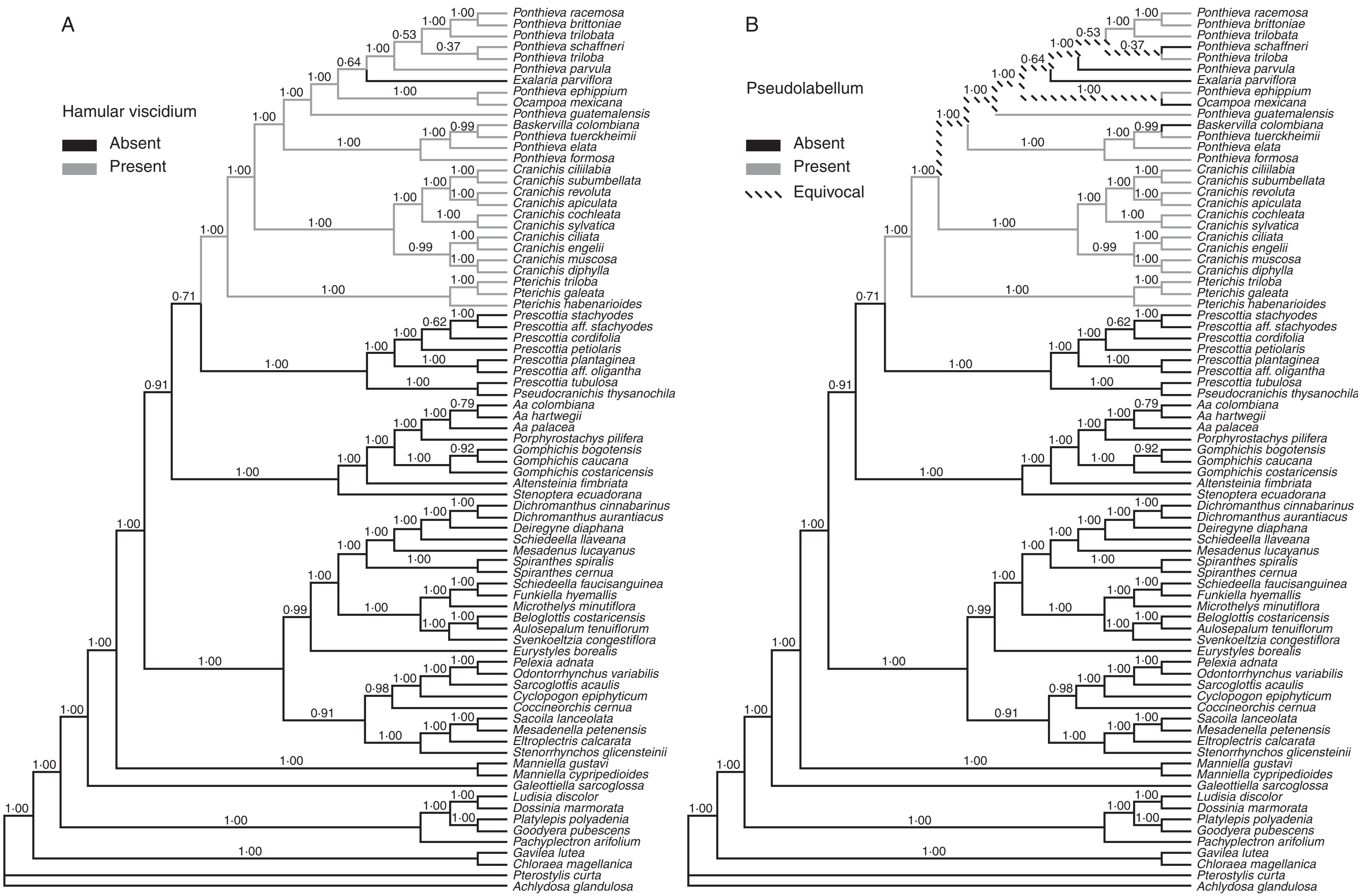

FIG. 4. Optimization of hamular viscidium and pseudolabellum on the Bayesian tree of Fig. 2B (see text).

flowers that vary in size and colour but are always much smaller and less showy than in *P. pilifera*, the only member of Cranichideae outside Spiranthinae that appears to be pollinated by hummingbirds (van der Pijl and Dodson, 1966).

Although no representatives of *Myrosmodes* were included in the combined analyses because it was not possible to sequence plastid DNA reliably from the degraded sample available to us, a preliminary heuristic search, in which an ITS sequence of *Myrosmodes cochleare* Garay (GenBank accession AM419768) was included, placed this species as the strongly supported sister of *Aa* (results no shown), in agreement with their shared possession of lateral inflorescences, scarious bracts and a lacerate-fimbriate labellum.

Prescottia *and* Galeoglossum *(including* Pseudocranichis*).* As noted by Vargas (1997) and Salazar *et al.* (2003), *Prescottia tubulosa* differs from the other members of the genus in various attributes, such as the absence of functional leaves at flowering time and the slightly concave labellum with incurved ('involute') lateral margins (instead of calceolate). Salazar *et al.* (2003) suggested a close relationship between *P. tubulosa* and *Pseudocranichis thysanochila* based on similarities in labellum and column morphology (*P. thysanochila* was not available for molecular study at that time). The present analyses corroborate a sister-group relationship between *P. tubulosa* and *P. thysanochila* that makes *Prescottia* paraphyletic (see also Figueroa *et al.*, 2008). Monophyly can be achieved either by sinking *Pseudocranichis* in *Prescottia* or by removing *Prescottia tubulosa* from the latter. Here we argue for the second approach, noting that the earliest generic name available for the clade that includes *P. tubulosa* and *P. thysanochila* is *Galeoglossum* A.Rich. & Galeotti (Salazar, 2009). Thus redelimited, *Galeoglossum* (including *Pseudocranichis*) is restricted to the floristically distinctive, seasonally dry/cool pine–oak forests occurring throughout the major mountain ranges of Mexico and Guatemala (Hágsater *et al.*, 2005; Salazar *et al.*, 2006). *Galeoglossum* is readily distinguished from *Prescottia* by a labellum with incurved lateral margins but open apically (not calceolate) and provided with a distinct apical lobule, the saddle-shaped stigma with two receptive areas separated by a central sterile area and the hairpin-shaped, slender pollinia. A review of the floral morphology and taxonomy of *Galeoglossum*, including the required new combinations, will be published elsewhere (Salazar, 2009).

The remaining species of *Prescottia* analysed here form a strongly supported clade with two subgroups, the first of which consists of *P. plantaginea* (the type species of the genus) and *P.* aff. *oligantha*. Both these species, as with most of the remaining 20-odd species of the genus, are restricted to Brazil. The second clade includes the long-petioled, broad-leaved species *P. petiolaris*, *P. cordifolia*, *P. stachyodes* and *P.* aff. *stachyodes*. All these species occur in continuously moist or wet tropical and cloud forests; the range of widespread *P. stachyodes* includes southern Mexico, but its habitat preferences are amply distinct from those of *Galeoglossum*. The six species of *Prescottia s.s.* sampled for this study encompass a good deal of the morphological variation recognized within the genus (cf. Hoehne, 1945; Vargas, 1997). All species of *Prescottia s.s.* have in common a calceolate labellum lacking apical lobulation, a single receptive stigmatic area located on the ventral surface of the column and ovate pollinia.

'Core' Cranichidinae. As stated earlier, Cranichidinae *sensu* Dressler (1993) are paraphyletic because *Pseudocranichis thysanochila* is strongly supported as sister of *Prescottia tubulosa*, but once the former species is excluded their monophyly is strongly supported by the present data and the unique possession of a hamular viscidium (except *Exalaria parviflora*; cf. Rasmussen, 1982; Szlachetko and Rutkowski, 2000) (Figs 1G and 4A). The position of *Pterichis* as sister to the rest in the analyses of Salazar *et al.* (2003) is confirmed here. The three species of *Pterichis* analysed in this study form a strongly supported clade in which *P. habenarioides* is sister to *P. triloba*/*P.galeata*. As currently delimited, *Pterichis* is a predominantly Andean genus encompassing about 20 species, one of which is found in Costa Rica and Panama and another in Jamaica. However, the single species of the genus *Fuertesiella* Schltr. (*F. pterichoides*), found in Cuba and Hispaniola, is morphologically similar to the species of *Pterichis*, and further study might demonstrate that *Fuertesiella* should be synonymized with *Pterichis*. No suitable material of *Fuertesiella* has so far been available for molecular analysis.

The strongly supported sister group of *Pterichis* consists of two clades. The first clade is *Cranichis* (BS 100, PP 1·00), which is fully resolved with all of its subclades receiving strong support. The group formed by *Cranichis engelii*/*C. ciliata* and *C. diphylla*/*C. muscosa* consists of species widespread in the neotropics (except for *C. engelii*, restricted to Andean Colombia, Ecuador and Venezuela), whereas its sister group includes only Mesoamerican taxa. Recently González (1996) proposed a new genus, *Nezahualcoyotlia*, for the Mexican endemic *Cranichis gracilis* on the basis of differences in lobulation of the clinandrium, projection of the lower margin of the stigma, size of the anther relative to the column, fusion of veins of the floral bracts and coloration of the leaves (among others). Many of these characters show gradual variation among the species, and some, such as the veining of the floral bracts, have not been adequately described for most species of the genus, thus making comparisons difficult. Although it was not possible to obtain suitable material for DNA analysis, in our view *C. gracilis* shows the basic floral structure of *Cranichis* and should be retained in this genus until there is convincing phylogenetic evidence to the contrary.

The second major clade of core Cranichidinae includes species of *Ponthieva* mingled with some species currently placed in other genera. Therefore, as currently delimited, *Ponthieva* is polyphyletic. There are two groups containing species of *Ponthieva*. The first one includes *Baskervilla colombiana* in a derived position within the grade formed by *Ponthieva formosa* (Fig. 1E), *P. elata* and *P. tuerckheimii*. The last three species represent a chiefly Andean group that differs from 'typical' members of *Ponthieva* in various morphological features, such as the possession of a fleshy rhizome, pollinia of two different sizes and a pair of basal labellum lobes of variable size but similar in position to the basal 'flaps' characteristic of the labellum of *Baskervilla*

Lindl. These structural features agree with the DNA sequences in the present study and suggest that all these species might be grouped under *Baskervilla*. However, the sampling in this clade is too sparse, and making nomenclatural changes seems inadvisable at this time.

The second group of *Ponthieva* species encompasses all other species of *Ponthieva* analysed, including the type species of the genus, *P. racemosa*, but has both *Exalaria parviflora* and *Ocampoa mexicana* embedded among them. *Ocampoa* was originally proposed to include the single species, *O. mexicana*, characterized by a long C-shaped labellum claw and strongly oblique lateral sepals. Schlechter (1918) sank *Ocampoa* in the synonymy of *Cranichis* without discussing his rationale, and most subsequent flora writers have followed Schlechter (e.g. Williams, 1951; McVaugh, 1985), but contemporary Mexican orchid students have resurrected *Ocampoa* on account of its unique suite of floral characters (González, 1995; Hágsater *et al.*, 2005; Soto, 2008) (Fig. 1H). Nevertheless, the present data firmly place *O. mexicana* in the clade that includes the type of the genus *Ponthieva*, with *O. mexicana* being sister to the 'typical' *P. ephippium* (BS 87, PP 1), in spite of its unusual labellum and lateral sepal morphology. On the other hand, Garay and Romero-González (1999) segregated the Andean species previously known as *Cranichis fertilis* into a monotypic new genus, *Exalaria*, combining the latter with the specific epithet of the earliest name of the species, [*Ophrys*] *parviflora* (which for priority reasons cannot be used in *Cranichis*). *Exalaria* was distinguished from *Cranichis* mainly by its short, broadly triangular, excised rostellum and wingless clinandrium in contrast to the pointed rostellum and a more or less conspicuous wing or flap on each side of the column of typical *Cranichis*. Garay and Romero-González (1999) also proposed that the New Caledonian endemic *Coilochilus neocaledonicus* is the closest relative of *Exalaria*. However, phylogenetic analyses of plastid DNA sequences (Kores *et al.*, 2000, 2001) have shown that *Coilochilus* is sister to *Cryptostylis* (subtribe Cryptostylidinae, tribe Diurideae). Alternatively, an extremely divergent (and probably paralogous) ITS sequence relates it to subfamily Epidendroideae (Clements *et al.*, 2002). Furthermore, *Coilochilus neocaledonicus* and *Exalaria parviflora* differ sharply in vegetative morphology, and the purported similarity between them is restricted to the overall appearance of the minute flowers (Fig. 1G). Such similarity likely resulted from extreme reduction of all floral parts undergone independently by these two distantly related, apparently self-pollinating species (Bower, 2001; G. A. Salazar, pers. obs.). As in the case of *Ocampoa mexicana*, the embedding of *Exalaria parviflora* in the clade that includes the type of *Ponthieva* sustains its inclusion in *Ponthieva* (see Appendix 2).

Ponthieva is customarily distinguished from other genera by the basal adnation of petals and labellum to the column. In addition, the petals are often distinctly broadened above the narrow base forming an obliquely triangular-ovate blade, and the two petals are close to one another forming a pseudolabellum (Dressler, 1993), whereas the inconspicuous true labellum stands in an upright position (Fig. 1E, F). In many instances, the petals adhere to the dorsal sepal at their apices. Neither *Exalaria* nor *Ocampoa* shows these features, which may be an indication of a different pollination mechanism (and likely autopollination in the former). There are other species of *Ponthieva* in which one or more of the above-mentioned 'diagnostic' features may be absent; for instance, in *P. schaffneri* the petals are free from the column, and they are narrowly oblanceolate-spathulate and do not form a pseudolabellum. Mapping of this last character on the Bayesian tree (Fig. 4B) reveals variation even among closely related species. All the above suggests that flower morphology is labile in the whole '*Ponthieva* complex' and emphasizes the need for detailed comparative studies of floral morphology and development in this group, coupled with pollination studies. Dressler (1993) noted that the labellum of *P. racemosa* produces oil instead of nectar and suggested that this species might be pollinated by oil-gathering anthophorid bees, but otherwise there is no published information on pollination of any representative of core Cranichidinae.

No material of *Nothostele* Garay, *Pseudocentrum* Lindl. and *Solenocentrum* Schltr. has been available for molecular study. *Nothostele* includes a single species restricted to the Brazilian Plateau that was originally placed in Spiranthinae by Garay (1982), but the non-resupinate flowers, pointed rostellum and four clavate pollinia with hamular viscidium (Szlachetko and Rutkowski, 2000) support its inclusion in Cranichidinae. *Pseudocentrum* and *Solenocentrum*, on the other hand, are found in southern Central America and the Andes and include about six and two species, respectively. Plants are similar to those of *Baskervilla*, but their flowers differ from the latter in having distinct floral 'spurs', which in *Pseudocentrum* is formed by the partially connate sepals and in *Solenocentrum* by the labellum.

CONCLUSIONS

Although the present analyses included most of the genera currently recognized in Cranichidinae *s.l.*, there are still some important gaps, including the puzzling Brazilian genus *Nothostele*. The inclusion of *Pseudocentrum*, *Solenocentrum* and other representatives of *Baskervilla* and its look-alikes in *Ponthieva* would permit attainment of a clearer picture of generic limits and establish a framework to investigate evolution of floral morphology in the complex by means of detailed comparative (including developmental) studies. This sort of study would also benefit greatly from data on the natural pollination of the taxa to attain a better understanding of the functional role of the floral structures.

Lack of clear patterns of support for the divergence of the four core spiranthid clades in the present parsimony analysis suggests the possibility of a rapid succession of lineage divergences or a slowdown in the rate of nucleotide substitution. Phylogenetic analyses based on morphological characters, both by themselves and in combination with DNA sequence data, might improve resolution and shed light on the kind of structural changes that accompanied, if not promoted, the early divergence of these groups.

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APPENDIX 1

Taxa studied, voucher information and GenBank accessions

		GenBank accession			
Taxon	Voucher	*rbcL*	*trnL-F*	*matK*	ITS
Subtribe Achlydosinae M.A.Clem. & D.L.Jones					
Achlydosa glandulosa (Schltr.) M.A.Clem. & D.L.Jones	New Caledonia, *Clements D-285*, CANB	AJ542401	AJ544506	AJ543950	AJ539525
Subtribe Chloraeinae Rchb.f.					
Chloraea magellanica Hook.f.	Chile, *Ryan 1*, K (spirit)	AJ542403	AJ544504	AJ543948	AJ539523
Gavilea lutea (Pers.) M.N.Correa	Chile, *Ryan 3*, K (spirit)	AJ542402	AJ544505	AJ543949	AJ539524
Subtribe Cranichidinae Lindl.					
Baskervilla colombiana Garay	Colombia, *Niessen 5*, MEXU (spirit)	AM778157	AM412714	AM900826	AM419791
Cranichis apiculata Lindl.	Mexico, *Ruiz 21*, MEXU	AM778148	AM412717	AM900819	AM419784
Cranichis ciliata (Kunth) Kunth	Mexico, *Salazar 7375*, MEXU (spirit)	AM778142	AM412724	AM900811	AM419776
Cranichis ciliilabia C.Schweinf.	Mexico, *Soto 8735*, MEXU (spirit)	AJ542419	AJ544488	AJ543934	AJ539506
Cranichis cochleata Dressler	Mexico, *Salazar et al. 6547*, MEXU	AM778146	AM412719	AM900817	AM419782
Cranichis diphylla Sw.	Venezuela, *Munich Bot. Gard. 92/3063*, M	AM778144	AM412722	AM900813	AM419778
Cranichis engelii Rchb.f.	Ecuador, *Schott s.n.*, K (spirit)	AM778145	AM412721	AM900814	AM419779
Cranichis muscosa Sw.	Costa Rica, *Pupulin 1792*, USJ	AM778143	AM412723	AM900812	AM419777
Cranichis revoluta F.Hamer & Garay	Mexico, *Soto 10097*, AMO	AM778147	AM412718	AM900818	AM419783
Cranichis subumbellata A.Rich. & Galeotti	Mexico, *Suárez 2094*, MEXU (spirit)	AM778149	AM412720	AM900815	AM419780
Cranichis sylvatica A.Rich. & Galeotti	Mexico, *Suárez 2443*, MEXU (photograph)	AM778150	AM412734	AM900816	AM419781
Exalaria parviflora (C.Presl) Garay & G.A.Romero	Ecuador, *Chase O-401*, K	AF074137	AJ409392	AJ310013	AJ000137
Ocampoa mexicana (A.Rich.& Galeotti) Schltr.	Mexico, *López s.n.*, MEXU	AM778156	AM412715	AM900825	AM419790
Ponthieva brittoniae Ames	Mexico, *Álvarez 4142*, MEXU	AM778153	AM412712	AM900822	AM419787
Ponthieva elata Schltr.	Colombia, *Salazar s.n.*, MEXU (photograph)	AM778158	AM412708	AM900827	AM419792
Ponthieva formosa Schltr.	Mexico, *Salazar et al. 6250*, MEXU	AM778159	AM412707	AM900828	AM419793
Ponthieva ephippium Rchb.f.	Mexico, Salazar *et al.* 6440, MEXU	AM778155	AM412709	AM900824	AM419789
Ponthieva guatemalensis Rchb.f.	Central America (cultivated specimen), *Salazar s.n.*, MEXU (spirit)	AM778152	AM412713	AM900821	AM419786
Ponthieva parvula Schltr.	Mexico, *Soto 10021*, AMO	AM778151	AM412710	AM900820	AM419785
Ponthieva racemosa (Walt.) C.Mohr	Mexico, *Salazar 6049*, MEXU	AJ542417	AJ544490	AJ543936	AJ539508
Ponthieva schaffneri (Rchb.f.) E.W.Greenw.	Mexico, *Salazar 6051*, MEXU	AJ542418	AJ544489	AJ543935	AJ539507
Ponthieva triloba Schltr.	Mexico, Soto 10022, AMO	AM778154	AM412711	AM900823	AM419788
Pontieva trilobata (L.O.Williams) L.O.Williams	Mexico*, Nava et al. 1747*, MEXU	AM901012	AM901010	AM901011	AM901013
Ponthieva tuerckheimii Schltr.	Mexico, *Salazar et al. 6512*, MEXU	AM778160	AM412716	AM900829	AM419794
Pterichis galeata Lindl.	Ecuador, *Schott s.n*, K (spirit)	AM778162	AM412732	AM900831	AM419796
Pterichis habenarioides Schltr.	Colombia, *Aldana 12*, COL	AJ542416	AJ544491	AJ543937	AJ539509
Pterichis triloba (Lindl.) Schltr.	Ecuador, *Schott s.n*, K (spirit)	AM778161	AM412733	AM900830	AM419795
Subtribe Galeottiellinae Salazar & M.W.Chase					
Galeottiella sarcoglossa (A.Rich. & Galeotti) Schltr.	Mexico, *Jiménez 2334*, AMO	AJ542407	AJ544500	AJ543945	AJ539518
Subtribe Goodyerinae Klotzsch					
Dossinia marmorata (Lindl.) E.Morr.	Tropical Asia (cultivated specimen),, *Munich Bot. Gard. 94/1190*, M	AJ542405	AJ544502	AJ543947	AJ539521
Goodyera pubescens (Willd.) R.Br.	USA, *Chase 212*, NCU	AF074174	AM419815	AJ543954	AJ539519
Ludisia discolor (Ker-Gawl.) A.Rich.	Tropical Asia (cultivated specimen), *Salazar 6354*, K (spirit)	AJ542395	AJ544466	AJ543911	AJ539483
Pachyplectron arifolium Schltr.	New Caledonia, *Chase 529*, K	AJ542404	AJ544503	AJ310051	AJ539522
Platylepis polyadenia Rchb.f.	Madagascar, *Salazar 6352*, K (spirit)	AJ542406	AJ544501	AJ543946	AJ539520
Subtribe Manniellinae Schltr.					
Manniella cypripedioides Salazar, T.Franke, Zapfack & Benkeen	Cameroon, *Salazar et al. 6323*, YA	AJ542409	AJ544498	AJ543943	AJ539516
Manniella gustavi Rchb.f.	Cameroon, *Etuge 4515R*, YA	AJ542408	AJ544499	AJ543944	AJ539517
Subtribe Prescottiinae Dressler					
Aa colombiana Schltr.	Colombia, *Aldana 2*, ANDES	AM778133	AM412731	AM900802	AM419766
Aa hartwegii Garay	Ecuador, *Schott s.n.*, K (spirit)	AM778134	AM412730	AM900803	AM419767
Aa palacea (Kunth) Rchb.f.	Ecuador, *Chase 535*, K	AJ542410	AJ544497	AJ309989	AJ539515

Continued

Appendix 1 *Continued*

Taxon	Voucher	GenBank accession			
		rbcL	*trnL-F*	*matK*	ITS
Altensteinia fimbriata Kunth	Ecuador, *Salazar 6789*, MEXU (spirit)	AM778132	AM412737	AM900801	AM419765
Gomphichis bogotensis Renz	Colombia, *Bello 86*, ANDES	AJ542412	AJ544495	AJ543941	AJ539513
Gomphichis caucana Schltr.	Colombia, *Díaz 159*, ANDES	AM778136	AM412736	AM900805	AM419770
Gomphichis costaricensis (Schltr.) Ames, F.T.Hubb. & C.Schweinf.	Costa Rica, *Soto s.n.*, AMO	AM778135	AM412729	AM900804	AM419769
Porphyrostachys pilifera Rchb.f.	Peru, *Whalley s.n.*, K (photograph)	AJ542411	AJ544496	AJ543942	AJ539514
Prescottia cordifolia Lindl.	Panama, *Salazar et al. 6225*, PMA	AM778138	AM412727	AM900807	AM419772
Prescottia aff. *oligantha* (Sw.) Lindl.	Brazil, *da Silva 877*, MG	AJ519445	AJ519451	AJ519449	AJ519447
Prescottia petiolaris Lindl.	Peru, *Munich Bot. Gard. 00/2013*, M	AM778137	AM412728	AM900806	AM419771
Prescottia plantaginea Lindl.	Brazil, *Salazar 6350*, K (spirit)	AJ542414	AJ544493	AJ543939	AJ539511
Prescottia stachyodes (Sw.) Lindl.	Mexico, *Salazar 6092*, MEXU	AM778139	AM412735	AM900808	AM419773
Prescottia aff. *stachyodes* (Sw.) Lindl.	Mexico, *Salazar et al. 7312*, MEXU	AM778140	AM412726	AM900809	AM419774
Prescottia tubulosa (Lindl.) L.O.Williams	Mexico, *Salazar 6054*, MEXU	AJ542415	AJ544492	AJ543938	AJ539510
Pseudocranichis thysanochila (B.L.Rob. & Greenm.) Garay	Mexico, *Tenorio 17900*, MEXU	AM778141	AM412725	AM900810	AM419775
Stenoptera ecuadorana Dodson & C.Vargas	Ecuador, *Salazar 6357*, K (spirit)	AJ542413	AJ544494	AJ543940	AJ539512
Subtribe Pterostylidinae Pfitz.					
Pterostylis curta R.Br.	Australia, *Chase 572*, K	AJ542400	AJ544507	AJ543951	AJ539526
Subtribe Spiranthinae Lindl.					
Aulosepalum tenuiflorum (Greenm.) Garay	Mexico, *Salazar 6017*, MEXU	–	–	AJ543919	–
Aulosepalum tenuiflorum (Greenm.) Garay	Mexico, *Salazar et al. 6150*, MEXU	AJ542433	AJ544474	–	AJ539591
Beloglottis costaricensis (Rchb.f.) Schltr.	Mexico, *Soto 8129*, MEXU	AJ542432	AJ544475	AJ543920	AJ539492
Coccineorchis cernua (Lindl.) Garay	Panama, *Salazar et al. 6249*, MEXU (spirit)	AJ542422	AJ544485	AJ543930	AJ539502
Cyclopogon epiphyticus (Dodson) Dodson	Ecuador, *Salazar 6355*, K	AJ542425	AJ544482	AJ543927	AJ539499
Deiregyne diaphana (Lindl.) Garay	Mexico, *Salazar et al. 6172*, MEXU	AJ542440	AJ544467	AJ543912	AJ539484
Dichromanthus aurantiacus (La Llave & Lex.) Salazar & Soto Arenas	Mexico, *Salazar 6351*, K (spirit)	AJ542439	AJ544468	AJ543913	AJ539485
Dichromanthus cinnabarinus (La Llave & Lex.) Garay	Mexico, *Linares 4469*, MEXU	AJ542438	AJ544469	AJ543914	AJ539486
Eltroplectris calcarata (Sw.) Garay & H.R.Sweet	Brazil, *Soares s.n.*, K (photograph)	AJ519446	AJ519452	AJ519450	AJ519448
Eurystyles borealis A.H.Heller	Mexico, *Soto 9149*, AMO	AJ542427	AJ544480	AJ543925	AJ539497
Funkiella hyemalis (A.Rich. & Galeotti) Schltr.	Mexico, *Salazar et al. 6128*, MEXU	AJ542429	AJ544478	AJ543923	AJ539495
Mesadenella petenensis (Standl. & L.O.Williams) Garay	Mexico, *Salazar 6069*, MEXU	AJ542421	AJ544486	AJ543931	AJ539503
Mesadenus lucayanus (Britt.) Schltr.	Mexico, *Salazar 6043*, MEXU	AJ542436	AJ544471	AJ543916	AJ539488
Microthelys minutiflora (A.Rich. & Galeotti) Garay	Mexico, *Salazar et al. 6129*, MEXU	AJ542430	AJ544477	AJ543922	AJ539494
Odontorrhynchus variablis Garay	Chile, *Wallace 130/85*, CANB	AJ542426	AJ544481	AJ543926	AJ539498
Pelexia adnata (Sw.) Poit. ex Spreng.	Mexico, *Salazar 6012*, MEXU	AJ542423	AJ544484	AJ543929	AJ539501
Sacoila lanceolata (Aubl.) Garay	Brazil, *Da Silva 874*, MG	AJ542441	AJ544529	AJ543933	–
Sacoila lanceolata (Aubl.) Garay	Panama, *Förther 2545*, M	–	–	–	AJ539504
Sarcoglottis acaulis (J.E.Sm.) Schltr.	Trinidad, *Salazar 6356*, K (spirit)	AJ542424	AJ544483	AJ543928	AJ539500
Schiedeella faucisanguinea (Dod) Burns-Bal.	Mexico, *Jiménez s.n.*, AMO	AJ542428	AJ544479	AJ543924	AJ539496
Schiedeella llaveana (Lindl.) Schltr.	Mexico, *Salazar 6073*, MEXU	–	AJ544470	–	–
Schiedeella llaveana (Lindl.) Schltr.	Mexico, *Salazar 6105*, MEXU	AJ542437	–	AJ543915	AJ539487
Spiranthes cernua (L.) Rich.	USA, *Nickrent 4188*, MEXU	AJ542435	AJ544472	AJ543916	AJ539489
Spiranthes spiralis (L.) Cheval.	UK, *Bateman s.n.*, K (spirit)	AJ542434	AJ544473	AJ543918	AJ539490
Stenorrhynchos glicensteinii Christenson	Mexico, *Salazar 6090*, MEXU	AJ542420	AJ544487	AJ543532	AJ539505
Svenkoeltzia congestiflora (L.O.Williams) Burns-Bal.	Mexico, *Salazar 6143*, MEXU	AJ542431	AJ544476	AJ543921	AJ539493

APPENDIX 2

Nomenclatural changes

Ponthieva fertilis (F.Lehm. & Kraenzl.) Salazar, **comb. nov.**

Basionym: *Goodyera fertilis* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26: 498. 1899.

Other synonyms: *Cranichis fertilis* (F.Lehm. & Kraenzl.) Schltr., Repert. Sp. Nov. Regni Veg. Beih. 8: 115. 1921; *Ophrys parviflora* Presl, Reliq. Haenk. 2: 92. 1827, non *Ponthieva parviflora* Ames & C.Schweinf., 1936; *Exalaria parviflora* (Presl) Garay & G.A.Romero, Harvard Papers in Botany 4: 480. 1999 (for a complete synonymy of this species refer to Garay and Romero-González, 1999).

Ponthieva mexicana (A.Rich. & Galeotti) Salazar, **comb. nov.**

Basionym: *Ocampoa mexicana* A.Rich. & Galeotti, Ann. Sci. Nat., Bot., ser. 3, 3: 31. 1845.

Synonym: *Cranichis mexicana* (A.Rich. & Galeotti) Schltr., Beih. Bot. Centralbl. 36: 430. 1918.