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PHYLOGENETICS OF THE SUBTRIBE PLEUROTHALLIDINAE (EPIDENDREAE: ORCHIDACEAE) BASED ON COMBINED EVIDENCE FROM DNA SEQUENCES

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Subtribe Pleurothallidinae (Epidendreae: Orchidaceae) comprises an estimated 4000 Neotropical species in about 30 genera (Luer 1986), accounting for 15-20% of the species in the entire family. The vast majority are dipteran-, deceit-pollinated epiphytes with sympodial growth, unifoliate non-pseudobulbous stems or "ramicauls," conduplicate leaves, velamentous roots, and an articulation between the pedicel and ovary. Genera have been circumscribed on the basis of number of pollinia– eight, six, four or two – although there can be either eight or six in *Brachionidium* Lindl. (Luer 1986) and two or four (one large pair and one small pair) in *Myoxanthus* Poepp. & Endl. and *Lepanthes* Sw. (Stenzel 2000).

To evaluate the monophyly of subtribe Pleurothallidinae (Epidendreae: Orchidaceae) and the component genera and to reveal evolutionary relationships and trends, we sequenced the nuclear ribosomal DNA internal transcribed spacers (ITS1 and ITS2) and 5.8S gene for 185 taxa (Pridgeon, Solano & Chase 2001), later increased to 187 taxa (Pridgeon & Chase 2001). *Dilomilis montana, Neocogniauxia hexaptera, Arpophyllum giganteum*, and *Isochilus amparoanus* were used as outgroups. All but seven of the 32 subgenera of the megagenus *Pleurothallis* are represented by one or more taxa; those subgenera not represented are monospecific or comprise only a few species. As a result, the overall morphological diversity was sampled to minimize spurious attractions, a strategy recommended for large study groups in particular (Hillis 1998).

To resolve internal nodes in the ITS topology and offer additional evidence from another genome, we also sequenced the plastid gene *matK* and the *trnL* intron with the *trnL-F* intergenic spacer (hereafter simply *trnL-F*) for a representative subset of the taxa in the ITS study (Pridgeon, Solano & Chase 2001, Pridgeon & Chase 2001). Sequences of *rbcL* (Chase *et al.* 1994, Kores *et al.* 1997, Cameron *et al.* 1999, van den Berg 2000), *matK* (Ryan *et al.* 2000, van den Berg 2000, Kores *et al.* 2001; Whitten, Williams & Chase 2000), and *trnL-F* (van den Berg 2000, Kores *et al*. 2001, Whitten, Williams & Chase 2000) have been useful in evaluating higher-level relationships in Orchidaceae by virtue of the relatively conservative evolution of the plastid genome.

Finally, we combined the plastid data with the corresponding ITS sequences for a separate analysis of 58 representative taxa to assess congruence among the separate and combined data sets (Pridgeon, Solano & Chase 2001, Pridgeon & Chase 2001). In this way we were able to compare topologies of DNA regions with different functional constraints (e.g., coding vs. noncoding, nuclear vs. plastid, concerted evolution in ribosomal ITS sequences) before combining them to limit spurious results in the separate analyses (Johnson & Soltis 1998, Soltis, Soltis and Chase 1999, Wiens 1998).

There is strong support in most analyses (Pridgeon, Solano & Chase 2001, Pridgeon & Chase 2001) for the monophyly of Pleurothallidinae and, as in other analyses (van den Berg *et al.* 2000), for inclusion of *Dilomilis* and *Neocognauxia* in Pleurothallidinae. Taking into account the highly supported molecular evidence from multiple DNA regions, the shared number of pollinia in some taxa (eight) and leaf anatomy, the ancestral reed-stem condition in other clades of Epidendroideae (van den Berg 2000), and evolutionary remnants thereof in present-day Pleurothallidinae, we proposed (Pridgeon & Chase, 2001) that Pleurothallidinae be expanded to include *Dilomilis*, *Neocogniauxia*, and presumably the monospecific *Tomzanonia*, thereby forming a more natural unit. Furthermore, recognition of a new subtribe comprising only three genera that are collectively sister to Pleurothallidinae would result in unnecessary taxonomic inflation.

Although most genera in the several clades identified in the analyses are monophyletic, all data sets are highly congruent in revealing the polyphyly of *Pleurothallis* and its constituent subgenera as presently understood, falling into seven clades with generally strong bootstrap support (Pridgeon, Solano & Chase, 2001, Pridgeon & Chase, 2001). The high degree of homoplasy in anatomical/morphological characters, especially floral characters, limits their usefulness in phylogenetic reconstruction of the subtribe.

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