

## Molecular Phylogenetics of Lentibulariaceae Inferred from Plastid *rps16* Intron and *trnL-F* DNA Sequences: Implications for Character Evolution and Biogeography

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**ABSTRACT.** Phylogenetic relationships among 75 species of Lentibulariaceae, representing the three recognized genera, were assessed by cladistic analysis of DNA sequences from the plastid *rps16* intron and the *trnL-F* region. Sequence data from the two loci were analyzed both separately and in combination. Consensus trees from all analyses are congruent, and parsimony jackknife results demonstrate strong support for relationships both between and within each of the three demonstrably monophyletic genera. The genus *Pinguicula* is sister to a *Gentlisea-Utricularia* clade, the phylogenetic structure within this clade closely follows Taylor's recent sectional delimitations based on morphology. Three principal clades are shown within *Utricularia*, with the basal sections *Polypompholyx* and *Pleiochasia* together forming the sister lineage of the remaining *Utricularia* species. Of the fundamental morphological specializations, the stoloniferous growth form apparently arose independently within *Gentlisea* and *Utricularia* three times, and within *Utricularia* itself, perhaps more than once. The epiphytic habit has evolved independently at least three times, in *Pinguicula*, in *Utricularia* section *Phyllaria*, and within the two sections *Orchidioides* and *Iperua* (in the latter as bromeliad tank-epiphytes). The suspended aquatic habit may have evolved independently within sections *Utricularia* and *Vesiculina*. Biogeographic optimization on the phylogeny demonstrates patterns commonly associated with the boreotropics hypothesis and limits the spatial origin of Lentibulariaceae to temperate Eurasia or tropical America.

The Lentibulariaceae are carnivorous herbs currently placed within the order Lamiales s. l., in the subclass Asteridae (Albert et al. 1992; Chase et al. 1993; Olmstead et al. 1993; Cosner et al. 1994; Williams et al. 1994; Hedrén et al. 1995), that until recently comprised five genera with around 200 recognized species (Cronquist 1981; Taylor 1989). Currently, there are ~284 taxa among the three genera, *Pinguicula* L., *Gentlisea* St. Hil. and *Utricularia* L. (Casper 1966; Fromm-Trinta 1981, 1989; Speta and Fuchs 1982; Studnička 1985; Zamudio 1988; Taylor 1989; Fischer et al. 2000).

Casper's (1966) monograph included 46 species of *Pinguicula* distributed among three subgenera: *Isoloba* with five sections, seven subsections and 20 species, *Tenmoceras* with three sections, two subsections and nine species, and *Pinguicula* with six sections, five subsections and 17 species. The geographic distribution of *Pinguicula* stretches from Europe through Northern Asia, North America and tropical America, with disjunctive occurrences also in tropical Asia and the southern Andes (Casper 1966; Legendre 2000).

*Gentlisea* is the smallest genus with 21 species placed in two subgenera: *Tayloria*, with three tropical American species, and *Gentlisea*, with 18 species, 10 of which are found in Africa and Madagascar, and eight in tropical America (Fromm-Trinta 1979, 1981, 1989; Taylor 1991; Fischer et al. 2000).

Two frequently recognized genera, *Biovularia* and *Polypompholyx*, are currently synonymized under the largest genus *Utricularia*, which has 214 species divided into the two subgenera *Polypompholyx* and *Utricularia*, the former containing two sections with three species, and the latter containing 33 sections with 211 species (Taylor 1989). *Utricularia* have a cosmopolitan distribution, although most species are found in tropical to sub-tropical regions with a general absence on oceanic islands and arid regions (Taylor 1989; Dörrestock et al. 1996).

To date, there have been no morphological or molecular phylogenetic studies demonstrating evolutionary relationships within and between all three genera of Lentibulariaceae. In the current study, we generated a phylogenetic hypothesis for Lentibulariaceae using DNA sequence data from the predominantly non-coding chloroplast region *trnL-F* and the *rps16* intron. These two chloroplast regions have been used together in the past to determine phylogenetic relationships among other Lamiales s.l., for example Oleaceae (Waldlander and Albert 2000).

Our study aimed to achieve the following: (i) to demonstrate the lamialean affinities of Lentibulariaceae, (ii) to determine internal phylogenetic relationships among the three principal genera of Lentibulariaceae.

TABLE 1. Accessions sampled for molecular phylogenetic analyses of Lentibulariaceae. Holdings in specific herbaria are so indicated, with abbreviations following Holmgren et al. (1990). (N.S. = not sequenced). Specimen information for all other taxa presented in the current study are listed in Jobson and Albert (2002) (GenBank no. AF482531–AF482681).

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<i>Genlisea hispidula</i> Stapf (R. W. Jobson and D. W. Darnowski 285 (NY); <i>rps16</i> AF488523, <i>trnL-F</i> AF488528)
<i>Genlisea lobata</i> Fromm-Trinta (R. W. Jobson and S. Ippenberger 295 (NY); <i>rps16</i> AF488524, <i>trnL-F</i> AF488529)
<i>Utricularia delicatula</i> Cheeseman (R. W. Jobson and B. Salmon 41 (BRI); <i>rps16</i> N.S., <i>trnL-F</i> AF488530)
<i>Utricularia inflata</i> Walter (R. R. Haynes et al. 10211 (UNA); <i>rps16</i> AF488525, <i>trnL-F</i> AF488531)
<i>Utricularia leptoplectra</i> F. Muell. (R. W. Jobson and R. Gilliland 174 (BRI); <i>rps16</i> AF488526, <i>trnL-F</i> AF488532)
<i>Utricularia resupinata</i> Greene ex Bigelow (B. R. Keener 1948 (UNA); <i>rps16</i> AF488527, <i>trnL-F</i> AF488533)

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iaceae, (iii) to examine how many times the fundamental specializations, such as carnivorous strategies and vegetative forms, habits, capsule dehiscence types, seed shape characters, inflorescence features, pollen aperture numbers, cytological counts and generation time, arose independently within the family, especially among species of the highly variable genus *Utricularia* and, (iv) to elucidate the biogeographic implications of the phylogenetic hypotheses.

#### MATERIALS AND METHODS

**Taxon Sampling and DNA Extraction.** All three recognized genera of the Lentibulariaceae were sampled. Most *rps16* (AF482531–AF482603) and *trnL-F* (AF482604–AF482681) sequences used in the current study have been published in the work of Jobson and Albert (2002). The current study includes the six previously unpublished sequences for taxa *Genlisea lobata* (subgenus *Tayloria*), *G. hispidula* (subgenus *Genlisea*), *Utricularia delicatula*, *U. inflata*, *U. leptoplectra* and *U. resupinata* (Table 1). GenBank accession numbers and voucher information are listed in Table 1. Taxonomy of Lentibulariaceae species follows Casper (1966), Fromm-Trinta (1979, 1981), Speta and Fuchs (1982), Studnička (1985), Zamudio (1988), Taylor (1989, 1991) and Fischer et al. (2000).

DNA extractions were performed using the micro-prep protocol described in Struwe et al. (1989) or maxi-prep method of Doyle and Doyle (1987). There are no morphological or molecular systematic studies of the Lentibulariaceae and therefore selection of ingroup taxa was based on recent sectional delimitations (Casper 1966; Fromm-Trinta 1979, 1981; Taylor 1989, 1991; Fischer et al. 2000). Outgroup taxa were selected to root the tree based on molecular investigations of angiosperms, using the plastid gene *rbcL* (Albert et al. 1992; Chase et al. 1993; Olmstead et al. 1993).

**Amplification, Sequencing, and Sequence Alignment.** Methods used to amplify and sequence the intron of *rps16* (Oxelman et al. 1997), and the *trnL-F* region (Taberlet et al. 1991), were performed according to standard protocols described in Struwe et al. (1998).

Separation of the fragments was done using a 4.5% ABI<sup>®</sup> (29:1 acrylamide) gel on an ABI Prism<sup>®</sup> 377XL DNA Sequencer (Perkin Elmer Applied Biosystems), or using the ABI Prism<sup>®</sup> 3100 Genetic Analyzer (Applied Biosystems, Hitachi). The forward and reverse sequences were edited using the Sequencher<sup>®</sup> software version 3.1.1 (Genes Codes Corporation). Alignment for both data sets was done manually using criteria described in Andersson and Rova (1999). The taxon *Pinguicula rotundiflora* was represented by *rps16* data only. *Melampyrum lineare*, *Utricularia chrysantha*, *U. delicatula*, *U. endresii*, *U. geminiloba*, *U. simplex* and *U. vulgaris* were represented by *trnL-F* data only due to difficulty amplifying the *rps16* intron.

The *trnL-F* alignment is available from GenBank as a Popset, and the *rps16* alignment is available upon request from R.W.J. or V.A.A. For alignment and phylogenetic analysis purposes, two AT-rich regions (with highly variable base composition) were excluded from the *rps16* sequence matrix: (1) nucleotide positions 247–262, and (2) nucleotide positions 643–679 (both with reference to *Pinguicula agnata* GBAF482543).

**Cladistic Analysis.** The combined matrix of 75 ingroup and 10 outgroup taxa was assessed by cladistic analysis. All characters were analyzed using equal weights (=1) and gaps were treated as missing data. The data were subjected to parsimony analysis both separately and combined. Phylogeny reconstruction was performed using the NONA (Goloboff 1998) and PAUP\* 4.0b2a (Swoford 1998) applications. Trees resulting from approximate searches (100 random replicates with TBR branch swapping) were further enumerated using the Parsimony Ratchet method (Nixon 1999) as implemented in NONA. Parsimony jackknife support for internal branches (Farris et al. 1996) was estimated using SPR branch swapping on each of 5 random entry orders for 1000 jackknife replicates using Xac (J. S. Farris, Swedish Museum of Natural History, Stockholm, Sweden). Jackknife values at ca. 63% (given statistical error around the number of replicates performed) or greater indicate support by the equivalent of one uncontradicted synapomorphy or more, whereas the range between 50% and ca. 63 percent indicates some robustness to extra steps. Branches with values below 50%, the point of equivocation, are not supported and so are not shown. The *trnL-F* and *rps16* sequence data were analyzed as a combined matrix (2096 sites) since separate phylogenetic analyses of the regions produced extremely compatible topologies. A strict consensus tree was calculated from all most-parsimonious trees (Kluge and Farris 1969; Farris 1989) for which all branches with potential zero-length were collapsed. The parsimony jackknife tree and strict consensus tree were not totally congruent.

**Character-State Reconstruction.** Patterns of phenotypic evolution and biogeographic diversification were assessed by optimization reconstructions overlaying ecological, morphological, and biogeographic area data onto the terminal taxa of the molecular phylogeny using MacClade version 3.07 (Maddison and Maddison 1997). The character-state tracing used all unambiguous changes along each branch (Figs. 3A–D). Character data for each taxon is shown in the Appendix.

#### RESULTS

The *trnL-F* data set contained 1122 characters of which 543 were informative, while the *rps16* contained 974 characters of which 508 were informative. The combined matrix contained 2096 characters of which 1051 were informative.

Consensus trees of most parsimonious trees from each analysis were largely congruent, and therefore analysis was performed on a combined matrix, which resulted in 24 most parsimonious trees of length 5336, consistency index 0.493 and retention index 0.656. The consensus of the combined data is shown in Figure 1 with parsimony jackknife values greater than 50% indicated above branches. Jackknife analysis gave excellent support to all major clades of the parsimony analysis (Fig. 1).

Long branch lengths for the *Utricularia-Genlisea* clade, relative to significantly shorter branch lengths

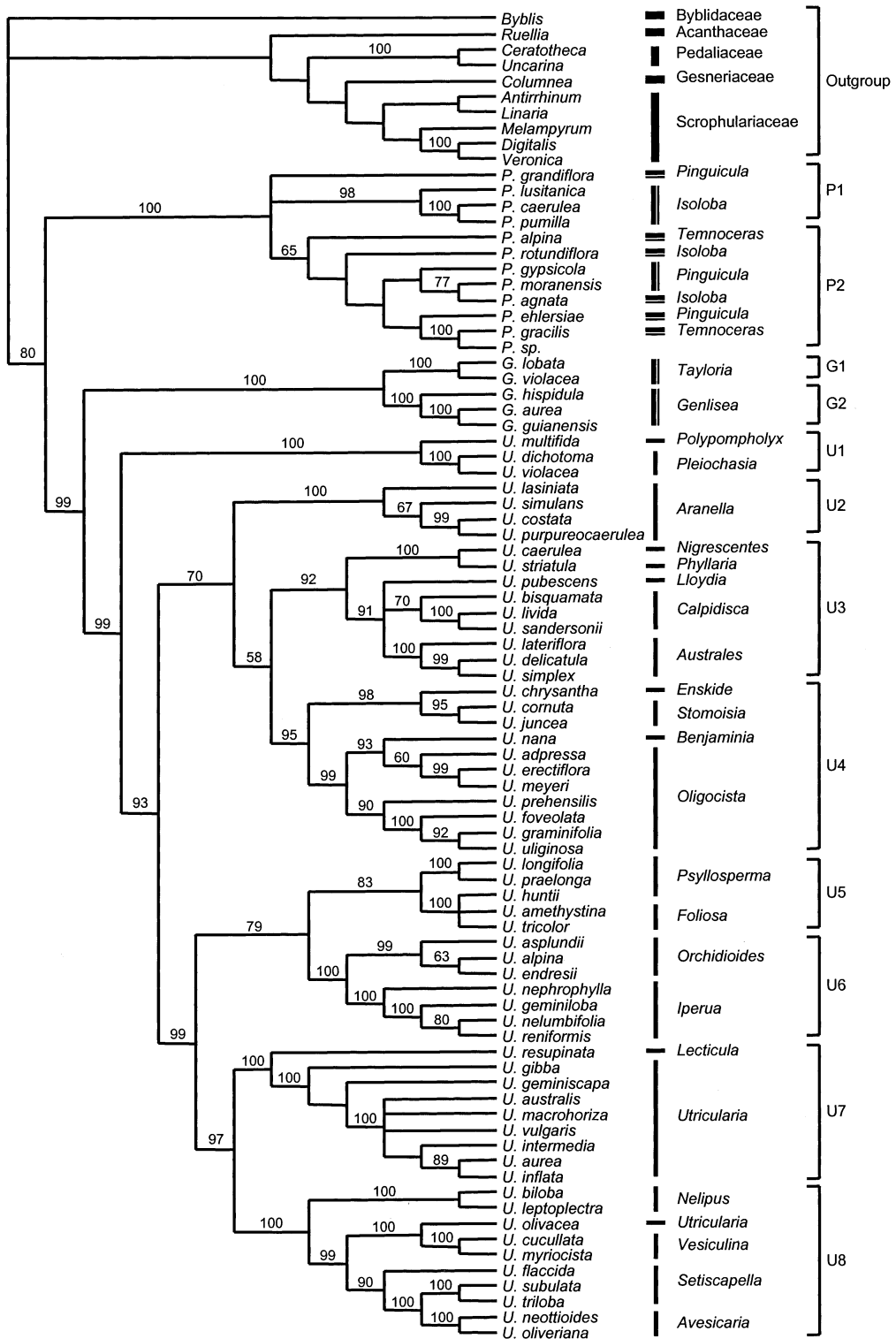


FIG. 1. The strict consensus of 24 most-parsimonious trees from the analysis of the combined data set. Jackknife support values over 50% are shown above branches. Thick solid bars indicate outgroup families; split bars denote delimitation of genera *Pinguicula* and *Genlisea* at subgeneric level; and thin solid bars denote delimitation of *Utricularia* at the sectional level. Descriptive clades are shown behind square brackets; P1-2 = *Pinguicula*, G1-2 = *Genlisea*, and U1-8 = *Utricularia*. (RI = 0.656; CI = 0.493; 1051 sites were parsimony informative).

for the *Pinguicula* clade where also observed (Fig. 2). Following parsimony jackknife analysis of the combined data matrix, polytomies are evident. For the strict consensus tree (Fig. 1), polytomies were found for the *Pinguicula* clade, as well as between the *Utricularia* sections *Lloydia*, *Australes* and *Calpidisca* (U3 clade), the two species of section *Foliosa* and one species of section *Psyllosperma* (U5 clade), and within species of the U7 clade (Fig. 1).

The subgenus *Utricularia* was found to be paraphyletic due to the inclusion of subgenus *Polypompholyx* as sister to section *Pleiochasia* (Fig. 1). Four *Utricularia* sections (sensu Taylor 1989) were resolved as paraphyletic: section *Oligocista* containing section *Benjaminia*, section *Setiscapella* containing section *Avesicaria*, and *U. huntii* of section *Psyllosperma* including section *Foliosa* (Fig. 1). One section was resolved as polyphyletic: section *Utricularia*, with *U. olivacea* resolved as sister to section *Vesiculina* (Fig. 1).

#### DISCUSSION

**Phylogenetic Relationships Among Major Clades of Lentibulariaceae.** The data supports the hypothesis for the placement of the family within the order Lamiales s.l., and is consistent with molecular studies using single species of *Pinguicula* and *Utricularia* (Albert et al. 1992; Chase et al. 1993; Olmstead et al. 1993; Cosner et al. 1994; Williams et al. 1994 and Hedrén et al. 1995) (Fig. 1).

Our strongly supported molecular phylogeny within the monophyletic Lentibulariaceae demonstrates full support for the three genera *Utricularia*, *Genlisea* and *Pinguicula*. Substantial support also occurs for recent sectional delimitations of *Utricularia* and *Genlisea* based on morphology (Fromm-Trinta 1979, 1981, 1989; Taylor 1989, 1991; Fischer et al. 2000), but less so for *Pinguicula* (Casper 1966; Speta and Fuchs 1982; Studnička 1985; Zamudio 1988). Sufficient levels of variation for resolution at infra-generic levels in the family Lentibulariaceae were especially evident in the *Genlisea-Utricularia* clade (Figs. 1, 2).

A monophyletic *Pinguicula* clade is resolved as sister to a *Genlisea/Utricularia* clade (Fig. 1). Internally, the *Pinguicula* clade is relatively unresolved, with more data being required and possibly the use of less conserved molecular markers to evaluate the sectional and subsectional delimitations of Casper (1966). The 12 *Pinguicula* taxa selected for this study (one of the *Pinguicula* taxa is unidentified) are circumscribed by the three subgenera, *Isoloba* (*P. lusitanica*, *P. caerulea*, *P. pumila*, *P. rotundiflora*, *P. agnata*), *Temnoceras* (*P. alpina*, *P. gracilis*) and *Pinguicula* (*P. grandiflora*, *P. gypsicola*, *P. ehlersiae*, *P. moranensis*) (Fig. 1). The results show that three taxa from subgenus *Isoloba* and one from *Pinguicula* (P1) are sister to a clade of mixed taxa from all three subgen-

era, with the temperate Eurasian *P. alpina* from subgenus *Temnoceras* at the base (P2) (Fig. 1).

The genus *Genlisea* contains 21 species of which we represent five (Fig. 1). Our results demonstrate that *Genlisea* is monophyletic and that the two circumscribed subgenera *Genlisea* (G1) and *Tayloria* (G2) form two independent clades (Fischer et al. 2000).

The hierarchic structure of the *Utricularia* clade closely follows the recent sectional delimitations of Taylor (1989), based on morphology. Within *Utricularia*, subgenus *Polypompholyx* and section *Pleiochasia* together form a lineage (U1) sister to two other *Utricularia* groups (subgenus *Utricularia*) consisting of the clades (U2–4) and (U5–8) respectively (Fig. 1). The position of section *Pleiochasia* within subgenus *Polypompholyx* makes Taylor's (1989) delimitation of subgenus *Utricularia* paraphyletic (Fig. 1). This had been previously suggested by Lobreau-Callen et al. (1999) based on studies of pollen morphology, and we therefore recommend the revision of subgenus *Polypompholyx* to include section *Pleiochasia* (Taylor 1989).

These molecular results for the Lentibulariaceae demonstrate excellent support for Taylor's (1989) sectional delimitation of *Utricularia* based on morphology (Fig. 1), especially given that no explicit effort was made to identify monophyletic groups. Apomorphic derivatives yielding largely paraphyletic sections could have been equally likely outcomes. Our molecular results generally support optimized reconstructions of non-molecular characters such as: carnivorous strategy (no figure presented), vegetative growth form (no figure presented; Appendix), habit (Fig. 3A; Appendix), capsule dehiscence (Fig. 3B; Appendix), seed shape (no figure presented; Appendix), floral form (raceme appendages) (no figure presented; Appendix), sepal number (no figure presented; Appendix), pollen features (no figure presented; Appendix), generation time habit or plant duration (annual versus perennial) (Fig. 3C; Appendix); cytological counts ( $n = 1$ ) (no figure presented; Appendix) and, biogeographic area (Fig. 3D; Appendix).

#### CHARACTER EVOLUTION AND BIOGEOGRAPHY

**Trap Morphology.** Each of the three currently recognized genera has its own unique prey trapping strategy (Givnish 1989; Juniper et al. 1989; Albert et al. 1992). The flypaper traps of *Pinguicula* form a trapping surface mostly consisting of mucilage (stalked) and enzyme producing (sessile) glands that are usually situated on the upper surface of the leaf mainly for the capture of arthropods (Heslop-Harrison and Heslop-Harrison 1981; Juniper et al. 1989). The carnivorous glands of *Pinguicula* are homologous across the genus (Juniper et al. 1989) and therefore diverge from the basal node of the Lentibulariaceae into a homologous P1–2 clade (Fig. 1).

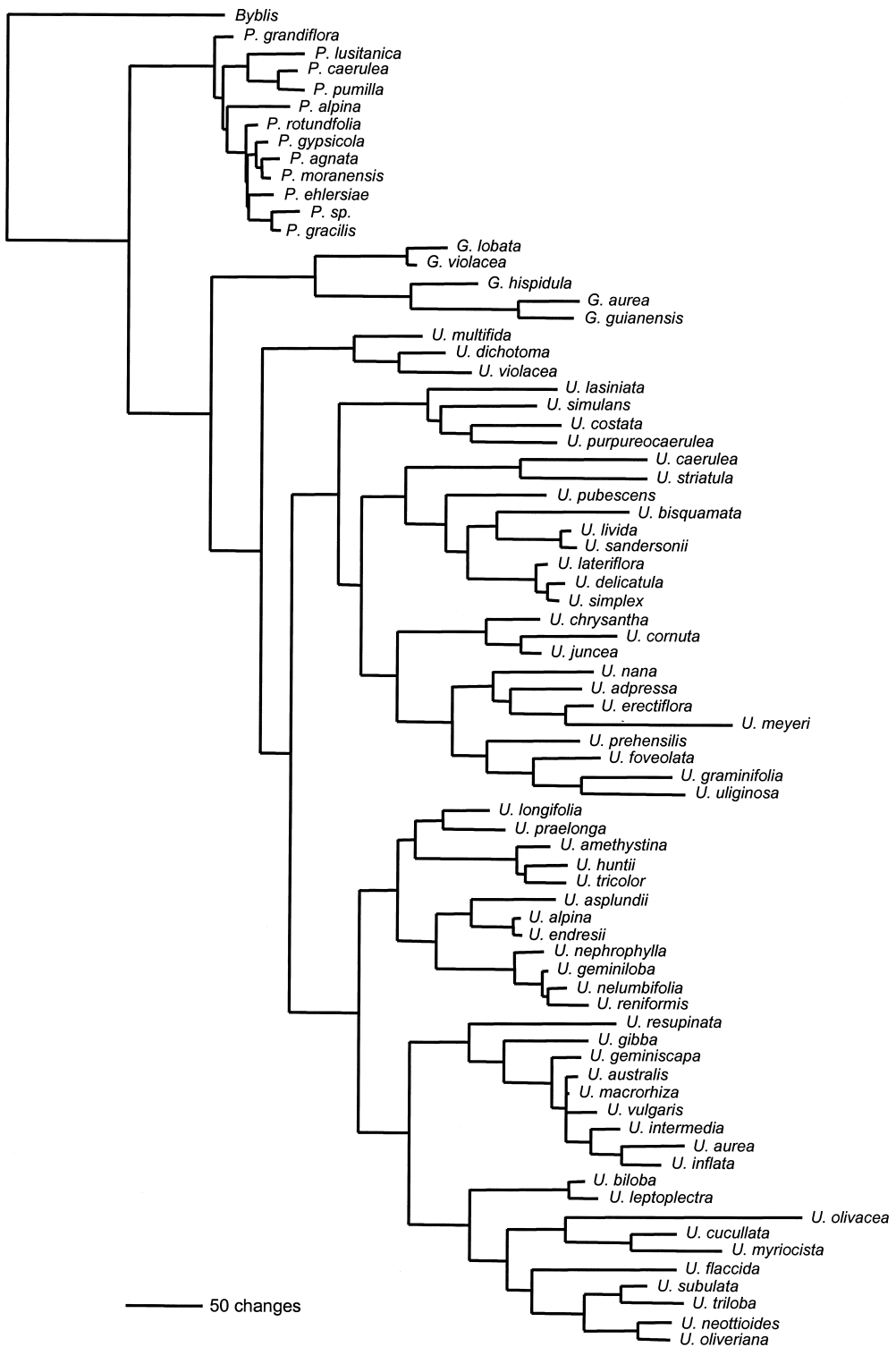
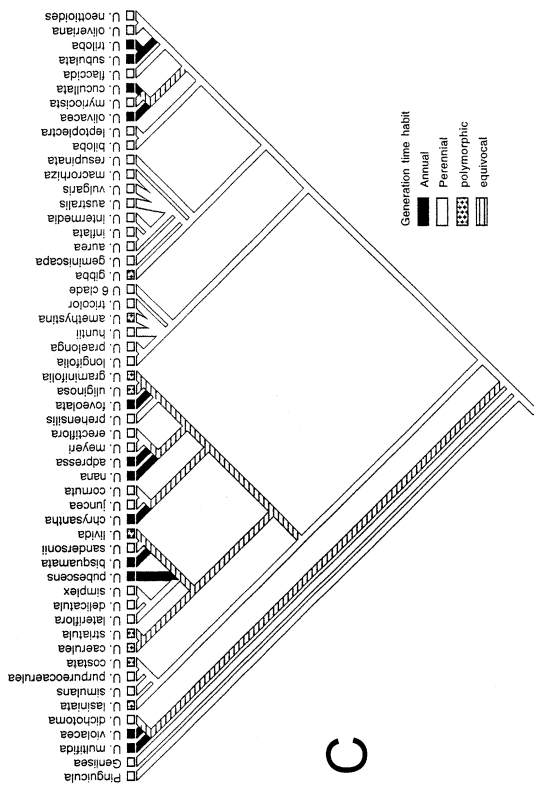
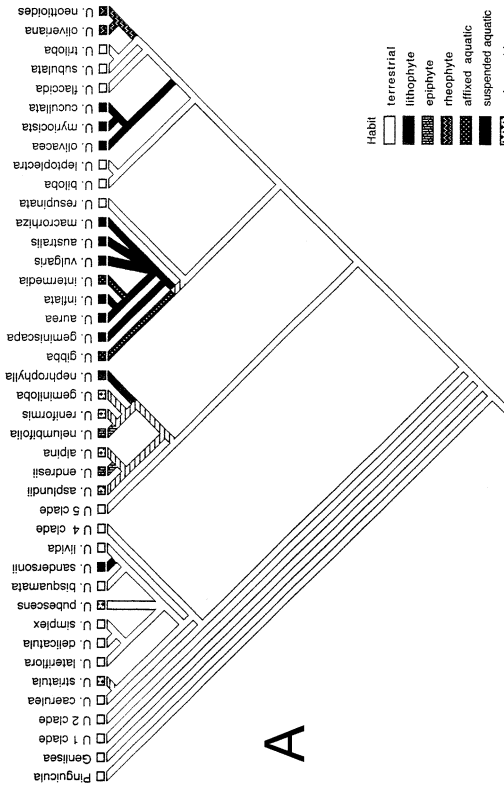
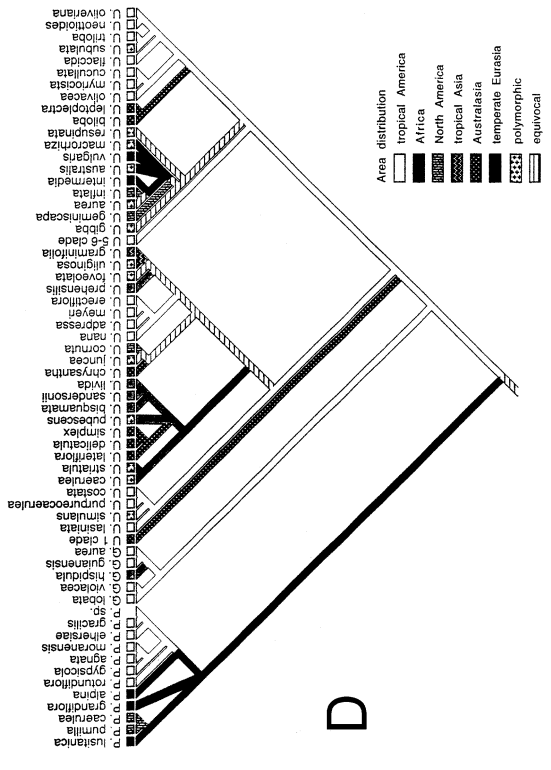
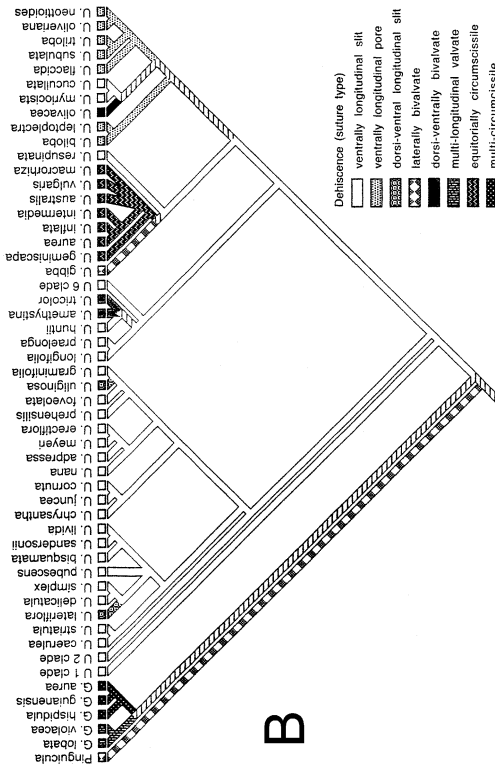


FIG. 2. A randomly selected phylogram from the analyses of the combined data set. The outgroup is represented by the outgroup taxon *Byblis*.



At the Lentibulariaceae basal node, the primordial *Utricularia*/*Genlisea* trapping system diverged and then split into the *Utricularia* suction bladders and *Genlisea* lobster-pot traps. The traps of both *Utricularia* and *Genlisea* are apparently episcidiate leaves (inward-rolling of the adaxial leaf surface with subsequent marginal fusion), which in *Genlisea* form cylindrical primordia with an invaginated tip, whereas in *Utricularia* a spherical invagination forms the primordial trap (Lloyd 1932, 1942; Juniper et al. 1989; Albert et al. 1992; Reut 1993). Several later stages of *Genlisea* trap development are also homologous with that of *Utricularia*, and the trapping structures across *Genlisea* are homologous (Reut 1993; Juniper et al. 1989).

The large variation found for the gross external morphology of the *Utricularia* trap, which can be of one or more of several appendage types (Lloyd 1932, 1942; Taylor 1989), has been correlated with a version of the phylogeny presented in the work of Jobson and Albert (2002). Suggestions of adaptive advantage gained by different trap appendage forms range from (i) exclusion of soil particles from around the trap mouth by overhanging wings (mainly in terrestrial species) (Lloyd 1942; Taylor 1989), (ii) support for functionally vital surface films of water at the trap mouth by thickly set dorsal wings in some epiphytic species (e.g., the U6 clade) (Taylor 1989) and, (iii) formation of funnels which may guide potential prey organisms to the trap door (e.g., suspended aquatic taxa of the U7 clade) (Darwin 1875; Lloyd 1942; Meyers and Strickler 1979; Juniper et al. 1989; Taylor 1989). Appendage variation across the genus may have permitted *Utricularia* to become specialized carnivores, leading to novel niche occupation (Jobson and Albert 2002), with utilization of particular meiofaunal prey in both aquatic and terrestrial species observed (Harms 1999; Jobson and Morris 2001). Similarly, the lobster-pot traps of *Genlisea* may be specialized for microfaunal prey (Barthlott et al. 1998).

**Vegetative Growth Form.** The rosulate growth form is found in all species of *Pinguicula* and *Genlisea*, and in some species of the U1 clade. This growth form also coincides with stolonifery in *Utricularia simulans* of the U2 clade, *U. striatula* of the U3 clade, and *U. amethystina* and *U. tricolor* of the U5 clade (Fig. 1; Appendix). Stolonifery occurs in all species of *Utricularia* except the rosulate subgenus *Polypompholyx* and *U. vio-*

*lacea* of section *Pleiochasia* (Fig. 1) (Taylor 1989). Stolonifery was optimized as having arisen independently within the U1 clade (e.g., *U. dichotoma*), and the rest of *Utricularia* (no figure presented; Appendix). The alternative, though less parsimonious hypothesis, is that a lack of stolons in species of the U1 clade is a reversal to a more primitive state (Fig. 1; Appendix).

**Habit and Seed Dispersal.** Morphological characters within Lentibulariaceae often seem to be linked to the particular habit of each species (Robins and Subramanyam 1980; Taylor 1989; Givnish 1989; Lobreau-Callen et al. 1999). The terrestrial habit is the most common for all three genera, and we have characterized this mode as being either rooted, in the case of *Pinguicula*, or having stolons and rhizoids for *Genlisea* and *Utricularia*, growing into a substrate (Casper 1966; Fromm-Trinta 1979; Taylor 1991) (Fig. 3A; Appendix). About 60% of species of *Utricularia* have the terrestrial habit, with most species also having short root-like extensions, termed rhizoids, produced from the stolons or peduncle base, apparently for anchoring the plant (Taylor 1989).

At the basal Lentibulariaceae node, the terrestrial habit was optimized as the state from which all the other habit types evolved (Fig. 3A; Appendix). The terrestrial habit in *Pinguicula* involves the standard leaf-shoot-root morphology typical of angiosperms (Casper 1966; Brugger and Rutishauser 1989; Rutishauser 1999; Jobson and Albert 2002). The rooted plants usually occupy boggy habitats, but several species are epiphytic (Casper 1966, 1987). Across the family fruiting capsules are globose or ovoid with free basal placentation (Casper 1966; Fromm-Trinta 1979, 1981; Cronquist 1981; Taylor 1989). All terrestrial species of *Pinguicula* have laterally bivalvate dehiscence and ellipsoid, cylindrical to fusiform seeds with rugose coats (Casper 1966) (Fig. 3B; Appendix).

*Genlisea* is also terrestrial, though sometimes sub-aquatic (e.g., *G. aurea*), and concerning dispersal modes, the equivocal ancestral node splits into two unique dehiscence types that correlate with phylogeny (Figs. 1, 3B; Appendix). Across the genus, seeds of the tropical American species are prismatic/conical whereas the African species are globose/ovoid (Fromm-Trinta 1979; Taylor 1991; Fischer et al. 2000). Our present sampling of one African and four tropical American species of *Genlisea* shows the ancestral node

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FIGS. 3. A–D. Cladograms of the strict consensus tree showing parsimoniously optimized analyses of (A) habit (c. f., character key); (B) capsule dehiscence (suture type); (C) generation time habit (annual versus perennial); and, (D) biogeographic area (c. f., area distribution key). All trees describe representatively patterned branches of characters shown in Appendix. Dashed equivocal branches indicate parsimoniously optimized plesiomorphic characters/area. Regional area and morphological character data was adopted from Casper (1966), Steenis (1978), Robins and Subramanyam (1980), Fromm-Trinta (1979, 1981), Speta and Fuchs (1982), Studnička (1985), Zamudio (1988), Taylor (1989, 1991), Dörrstock et al. (1996), Lobreau-Callen et al. (1999) and Fischer et al. (2000).

for seed shape optimized as equivocal (no figure presented; Appendix).

Within *Utricularia* a vast range of capsule dehiscence types are present, and these seem to correlate strongly with both habit (Taylor 1989) and phylogeny (Figs. 1, 3A, B; Appendix). The basal node of the *Utricularia* clade was optimized as dehiscent from a single ventral longitudinal suture that varies in length depending upon the species (Taylor 1989) (Fig. 3B; Appendix). Most species with this dehiscence type have the similarly primitive terrestrial habit, and therefore it may be that all other *Utricularia* dehiscence types are derived from it (Taylor 1989) (Figs. 3A, B; Appendix). Considering that both *Pinguicula* and *Genlisea* have the seemingly more complex laterally bivalvate and multi-suture dehiscence, respectively, it seems that initially, dehiscence in *Utricularia* may have become simplified (Fig. 3B; Appendix). The basal node of the *Utricularia* clade is also optimized as having seeds that are globose/ovoid in shape, mostly with raised anticlinal walls (Taylor 1989) (not shown; Appendix). This surface, which may permit seeds to remain afloat for many weeks, is similar to that found in seeds of *Genlisea* (Robins and Subramanyam 1980; Taylor 1989), and this flotation may aid dispersal via water birds (Arber 1920; Beal and Quay 1968; Kondo et al. 1978; Taylor 1989).

An extreme habit within the family is epiphytism in which the entire life cycle is specialized for growth on a host tree (Benzing 1989). This habit has arisen in *Pinguicula* at least once in several species (not included in this study), which have specialized adventitious rootlets that adhere to host surfaces (Casper 1966, 1987; Benzing 1989; Zamudio and Rzedowski 1991).

In our optimized experiment the epiphytic habit in *Utricularia* evolved from the terrestrial habit at least twice: in the African, tropical Asian and Australasia section *Phyllaria*, within the tropical American sections *Iperua* and *Orchidioides* (Fig. 3A, D; Appendix), and probably also in the mono-specific section *Chelidion* (though this species was not included in this study) (Taylor 1989).

Lithophytism is a similar habit type found mainly in sections *Iperua* and *Phyllaria* (Figs. 1, 3A; Appendix). Many lithophytic species can also occupy terrestrial and epiphytic habits, and specializations for the habit (e.g., seed form and production of tubers) can sometimes be similar (Robins and Subramanyam 1980; Taylor 1989).

The basal node for the *Iperua/Orchidioides* clade was optimized as equivocal for terrestrial, lithophytic and epiphytic habits, due to *Utricularia nephrophylla*, *U. geminiloba* and *U. reniformis* being able to occupy more than one habit type (Fig. 3A; Appendix). Epiphytism within section *Iperua* is a specialization for growth in the tanks of epiphytic bromeliads (*U. nelumbifolia*, *U. humboldtii*)

and is termed 'aquatic epiphytism' (Taylor 1989). There is also one instance of typical epiphytism in the 'sometimes terrestrial' *U. reniformis* (Benzing 1989; Taylor 1989) (Fig. 3A; Appendix). The sections *Phyllaria*, *Iperua* and *Orchidioides* are specialized for the epiphytic habit by having water-storage tubers (Taylor 1989). Members of sections *Phyllaria* and *Iperua* also have seed coats with multicellular outgrowths (glochidia-like in the former section), although they are less pronounced in the aquatic-epiphytic and viviparous *Iperua* taxon *U. nelumbifolia* (Taylor 1989). These appendages may be a specialization for attaching to a host (Robins and Subramanyam 1980; Taylor 1989). Seeds of the epiphytic/terrestrial sister section *Orchidioides* do not possess the appendages of section *Phyllaria* and *Iperua*, but rather all species have seeds that are cylindrical or fusiform in shape and mainly with smooth coats (Taylor 1989) (Appendix). These seeds are very numerous and the shape may be a specialization for wind dispersal (Robins and Subramanyam 1980; Taylor 1989).

In accordance with our optimization analysis, the invasion of terrestrial *Utricularia* into aquatic habitats has occurred with the rheophytic habit as well as with suspension in the water column (Taylor 1989) (Fig. 3A; Appendix). Rheophytic *Utricularia* are specialized for growth in fast-flowing waters (Steenis 1978; Taylor 1989), with ventrally papillate, claw-like rhizoids for adherence to rock surfaces and reduced or pinnate leaves (Taylor 1989). The rheophytic section *Avesicaria* (*U. neottioides*, *U. oliveriana*) is derived within the mainly terrestrial section *Setiscapella* (Figs. 1, 3A), both sections having similar dehiscence types and seed shapes and surfaces (Taylor 1989) (Fig. 3B; Appendix). In section *Avesicaria* seeds are covered in mucilage, which presumably aids dispersal in fast flowing streams (Robins and Subramanyam 1980; Taylor 1989). Due to difficulty obtaining material for the African and other tropical American rheophytic sections, their affinity within section *Avesicaria/Setiscapella* and whether or not the habit has evolved more than once remains uncertain.

In the mostly suspended aquatic U7 clade (Fig. 1), equatorially circumscissile dehiscence predominates (Figs. 3A, B; Appendix). In this dehiscence type, the equatorial plane of the capsule is separated, presumably giving greater space for dispersal of the large and usually winged seeds (Taylor 1989). However, the basal subaquatic/terrestrial species *U. resupinata* dehisces via a longitudinal slit similar to that of the U8 clade (Figs. 1, 3A, B; Appendix). The seeds of the U7 clade have a diverse array of shapes ranging from globose/ovoid, prismatic/conical, to discoid/lenticular, with many species having multicellular outgrowths such as wings (e.g., *U. gibba*) (Robins and Subramanyam 1980; Taylor 1989).

The terrestrial sections *Nelipus* and *Setiscapella* oc-



copy the U8 clade along with the suspended aquatic section *Vesiculina* (Figs. 1, 3A). Dehiscence within this clade is mostly from a single ventral longitudinal slit, and seeds are generally ovoid/globose in shape with varying forms of testa ornamentation, including multicellular outgrowths (Taylor 1989) (Fig. 3B; Appendix). The one exception within the U8 clade is *Utricularia olivacea* (classified by Taylor (1989) to section *Utricularia*), which is indehiscent and produces a single ovoid and smooth seed, adnate to the capsule wall (Robins and Subramanyam 1980; Taylor 1989) (Figs. 3A, B; Appendix).

In our data, the node between the U8 and U7 clades was optimized as terrestrial (Fig. 3A; Appendix). This is due to the juxtaposition of the subaquatic/terrestrial *U. resupinata* at the base of the U7 clade, and terrestrial section *Nelipus* and other species of the U8 clade separating the suspended aquatic sections *Vesiculina* (including *U. olivacea*) and *Utricularia* (Figs. 1, 3A; Appendix). This separation suggests that the suspended aquatic habit has evolved from terrestrial ancestors independently in the U7 and U8 clades (Figs. 1, 3A; Appendix). However, the basic vegetative ontogeny of the suspended aquatic sections *Utricularia* and *Vesiculina* seems to be largely homologous, although overall morphologies differ (e.g., capsule dehiscence, seed shape) (Rutishauser and Sattler 1989; Taylor 1989) (Fig. 3B; Appendix).

The affixed aquatic habit is found in the section *Utricularia* (e.g., *U. gibba*), and could be an intermediate step between the terrestrial (e.g., *U. resupinata*) and the suspended aquatic habit (Taylor 1989; Rutishauser 1999) (Fig. 3A; Appendix). Dehiscence in the affixed aquatic *U. gibba* is via a laterally bivalvate suture, which is not characteristic of the U7 clade (Taylor 1989) (Figs. 1, 3A, B; Appendix).

**Floral and Pollen Morphology.** Phylogenetic affinity within Lentibulariaceae is strongly characterized by zygomorphic flowers with persistent calyces that form an upper and lower lip, a sympetalous corolla tube, a basally spurred or saccate lower lip, two anthers, four sporangia and one or two confluent thecae, two fused carpels, and a unilocular ovary (Casper 1966; Fromm-Trinta 1979, 1981; Cronquist 1981; Taylor 1989).

For flower number per inflorescence there is a split to a single flowered scape in all species of *Pinguicula*, and to racemes of few to many flowers in the genera *Genlisea* and *Utricularia* (Casper 1966; Fromm-Trinta 1981; Taylor 1989). The basal Lentibulariaceae node is equivocal for either a naked scape or a raceme, with racemes having evolved once at the *Pinguicula/Genlisea* node (no figure presented; Appendix). Along with racemes has come an array of different arrangements of bracts, bracteoles and scales upon the peduncle (Casper 1966; Taylor 1989, 1991; Fischer et al. 2000) (Appendix).

Bracts, bracteoles, and scales are absent on scapes of *Pinguicula* (Casper 1966). In contrast, bracts are present in all species of *Genlisea* and *Utricularia*, while adjacent bracteoles are present in both genera except for species of *Utricularia* from the U7 and U8 clades (Fig. 1; Appendix). Scales are present in all species of *Genlisea* and *Utricularia* except those *Utricularia* circumscribed to the U1 clade, section *Vesiculina* (including *U. olivacea*), and some species of the U7 clade (*U. resupinata*, *U. aurea*, *U. geminiscapa* and *U. inflata*) (Fromm-Trinta 1981; Taylor 1989, 1991; Fischer et al. 2000) (Fig. 1; Appendix).

*Pinguicula* and *Genlisea* have a calyx consisting of five sepals, while there is a divergence at the *Utricularia* basal node with the subgenus *Utricularia* having two sepals and subgenus *Polypompholyx* having four (Taylor 1989) (no figure presented; Appendix). Taylor (1989) suggests that the four-parted calyx of subgenus/section *Polypompholyx* may be an intermediate form between *Genlisea* and subgenus *Utricularia*, although optimization of the U1 basal node as having two sepals suggests a reversal to a *Genlisea/Pinguicula* type (no figure presented; Appendix).

During the initial developmental stages of the floral bud of subgenus *Utricularia*, five sepals are evident; the anterior part has two sepals and the posterior has three, these sets becoming fused and forming two sepals later in development (Lang 1901; Subramanyam and Narayana 1978). Apart from the reduction in sepal number, it is evident that inflorescences in Lentibulariaceae have become more complex within the *Genlisea/Utricularia* clades, which has subsequently lead to an increase in the number of seed bearing capsules (Taylor 1989).

Pollen varies within the family, especially in *Utricularia* (Thanikaimoni 1966; Huynh 1968; Sohma 1975; Taylor 1989; Lobreau-Callen et al. 1999), although in general, the pollen for both *Pinguicula* and *Genlisea* is similar to that of *Utricularia* (Lobreau-Callen et al. 1999). Lobreau-Callen et al. (1999) described nine pollen types for *Utricularia* based mainly on external and internal features, including overall external form and aperture number. Pollen aperture number varies across the family from three to 23 (Taylor 1989; Lobreau-Callen et al. 1999) (Appendix).

Morphological variation of pollen within Lentibulariaceae is complex, although the exines of most species within the family are generally smooth or rugose, with the exception being the rheophytic *Avesicaria* (*U. neotitoides*, *U. oliveriana*), having an exine covered with micro-spines (Casper 1966; Sohma 1975; Fromm-Trinta 1981; Taylor 1989; Lobreau-Callen et al. 1999) (Fig. 3A; Appendix). A general trend for lower aperture numbers of between three and eight is seen in the mostly terrestrial *Pinguicula* (4–7), *Genlisea* (3–4) and also *Utricularia* clades U1–4 (3–8) (Fig. 1; Appendix). Apart from low numbers of three to four apertures in section

*Orchidioides*, clades U5–8 range from eight to 23 apertures (Casper 1966; Fromm-Trinta 1979, 1981; Taylor 1989) (Fig. 1; Appendix).

The overall morphology of pollen in *Pinguicula* and *Genlisea* is similar to that of the *Utricularia* taxa in the U1 clade (Lobreau-Callen et al. 1999). Taylor (1989) suggests that, with a few exceptions, pollen and vegetative morphology are strongly correlated, while Lobreau-Callen et al. (1999) suggest that pollen variation in general is more related to the evolutionary history of each species rather than to habitat specialization.

**Cytology.** There are 20 available cytological counts for *Pinguicula*, with seven counts among the 12 taxa of this study ranging from  $n = 6$  to  $n = 22$  (Casper 1966; Legendre 2000) (Appendix). *Genlisea* is devoid of cytological data, this being an item for future study (Taylor 1991; Fromm-Trinta 1979). For *Utricularia*, however, 28 species have been studied but do not follow a discernable pattern apart from the general trend of low, seemingly aneuploid counts of  $n = 7, 9, 10, 11, 14$  within the non-suspended aquatic clades (U1–4) (with the exception being the terrestrial *U. livida* and *U. caerulea* with  $n = 18$  and 20 respectively), to counts for the U7 suspended aquatics clade, in which the number may be greater (e.g.,  $n = 22$  in the majority of the clade) (Kondo 1966; Taylor 1989; Krahulcová and Jarolímová 1991). An optimization analysis suggests that the basal nodes of each genus, and the family as a whole, are  $n = 14–16$  (no figure presented; Appendix). It seems evident that aneuploidy and polyploidy occurs within *Utricularia* although it is probable that many of the extant species have evolved reproductive barriers and therefore rarely hybridize, many being autogamous (Kondo 1972; Jérémie & Jeune 1985; Taylor 1989). The status is similar within *Genlisea* of which there are a few unsubstantiated accounts of hybridization between African species and possibly a trend toward autogamy (Taylor 1991; Fischer et al. 2000). In contrast, natural hybrids are known within temperate Eurasian and tropical American *Pinguicula* (Casper 1966).

**Generation Time Habit and Substitution Rate Asymmetry Between Major Clades.** Generation time habit or plant duration (i.e. annual versus perennial), varies across the family with *Pinguicula* being exclusively perennial (Casper 1966). For *Utricularia*, ~56% of circumscribed species are perennial, although for many species both conditions are found (Taylor 1989). Annual species occur regularly in most *Utricularia* sections, although no section is exclusively annual (Taylor 1989). However, several sections only contain perennial species (e.g., *Meionula*, *Australes*, *Orchidioides*, *Iperua*, *Stomoisia*, *Avesicaria*, *Nelipus* and several other monotypic sections) (Taylor 1989) (Figs. 1, 3C; Appendix). In *Genlisea*, generation time is uncertain in several species, but there seems to be about 50% frequency of

perennials in the tropical American and African taxa (Fromm-Trinta 1979, 1981; Fischer et al. 2000). The *Genlisea/Utricularia* node was optimized as perennial with a split into an equivocal U1 clade, and apart from the U3–4 clade, all higher nodes are optimized as perennial (Fig. 1, 3C; Appendix). In our phylogenetic data, generation time differences between the *Pinguicula* and *Genlisea/Utricularia* clades have been shown to have no significant correlation with amplified nucleotide substitution rates (Jobson and Albert 2002). Instead, the accelerated nucleotide substitution rate observed for the *Genlisea/Utricularia* clade (Fig. 2), has been correlated with the speciation rate hypothesis that posits a relationship between increased nucleotide substitution and increased cladogenesis (Mayr 1954; Barraclough et al. 1996; Barraclough and Savolainen 2001; Jobson and Albert 2002).

**Biogeographic Implications and Spatial Origin of The Family.** A hypothesis for the center of origin for each of the genera, and the family as a whole, was developed using an area optimization analyses that traces biogeographic distributions of species and clades as unordered character states (Mickey 1981; Bremer 1992; Ronquist 1994). This attempt employed a limited number of total species, with 16% of total *Pinguicula*, 25% of *Genlisea* and 32% of *Utricularia* included. The P1 clade of *Pinguicula* is made up of the subgenera *Isoloba* and *Pinguicula*, including members of the former from North America (*P. caerulea*, *P. pumila*) and temperate Eurasia (*P. lusitanica*), and the latter also from temperate Eurasia (*P. grandiflora*) (Figs. 1, 3D; Appendix). The most basal taxon of the P2 clade consists of the temperate Eurasian *P. alpina*, of subgenus *Tennoceras*, which is sister to a clade of tropical American (Mexican) taxa that are taxonomically derived from all three subgenera (Casper 1966; Speta and Fuchs 1982; Studnička 1985; Zamudio 1988) (Figs. 1, 3D; Appendix). This grouping of tropical American taxa may or may not be an indication that the true delimitation for *Pinguicula* is more strongly related to geography rather than to the morphological features commonly used to place these species into disparate subgeneric groups (Casper 1966; Speta and Fuchs 1982; Studnička 1985; Zamudio 1988). Regardless, area optimization analysis demonstrates a temperate Eurasia or tropical American origin as being equivocal at the Lentibulariaceae nodal root (Fig. 3D; Appendix). This is followed by a split into the *Pinguicula* clade P1–2, which was optimized as originating in temperate Eurasia, whereas the origin of the *Genlisea/Utricularia* clade (G1–U8) was optimized as tropical American (Fig. 3D; Appendix). For *Genlisea*, we include two tropical American taxa for subgenus *Tayloria* (G1), and one African and two tropical American taxa of the subgenus *Genlisea* (G2) (Figs. 1, 3D; Appendix). These taxa are optimized as originating from tropical America

with subsequent dispersal into Africa (Fig. 3D; Appendix). Subgenus *Genlisea* does not include taxa found on both continents (Fromm-Trinta 1979; Taylor 1991; Fischer et al. 2000).

Optimization analysis tentatively demonstrates three dispersion and/or vicariance events for *Utricularia* from tropical America (Fig. 3D; Appendix). A *Polypompholyx*-like ancestor may have dispersed from tropical America to Australasia, suggested by the split at the basal *Utricularia* node that includes the endemic Australasian U1 clade, which consists of the subgenus *Polypompholyx* and section *Pleiochasia* (Taylor 1989) (Figs. 1, 3D; Appendix).

From the optimization, the initial dispersion and/or vicariance event of the U1 clade was followed by a subsequent segregation of a rosulate/stoloniferous (e.g., *U. simulans*) *Aranella*-like ancestor between the American tropics and Africa (diverging into the U3 clade), and likewise a *Nelipus*-like ancestor to/from Australasia (U8 clade) (Figs. 1, 3D; Appendix). These events were followed by subsequent separations such as an *Enskide*-like ancestor (U3) split among either Africa, tropical America or North America versus Australasia, an *Australes*-like ancestor (U3) between Africa versus Australasia (with directionality to Australasia indicated by the optimization), and an *Oligocista*-like ancestor (U4) between Africa and tropical America versus Asia and Australasia (Figs. 1, 3D; Appendix).

The mainly suspended aquatic section *Utricularia* (U7) are optimized as probably dispersing from either Australasia or tropical America to all areas; these species may be pan-tropical (e.g., *U. gibba*), though some are also boreal, being widely distributed throughout temperate Eurasia and North America (Taylor 1989) (Fig. 3D; Appendix). Many of the temperate suspended aquatic species from section *Utricularia* produce turions over colder months; these may aid in long distance dispersal, resisting mild desiccation, and thereby may easily survive being transported by water birds over long distances (Taylor 1989).

Within *Genlisea* and *Utricularia* there tends to be species affinity between tropical America and Africa, and further association for *Utricularia* between Africa and tropical Asia (especially India) and between tropical Asia/Australasia. Biogeographic optimization on our phylogeny limits the spatial origin of Lentibulariaceae to temperate Eurasia and/or tropical America. These biogeographic patterns are commonly associated with the boreotropics hypothesis, which assumes an early Tertiary (Oligocene-Eocene) tropical interchange of flora and fauna between temperate Eurasia and North America (Lavin and Luckow 1993). Distributions in tropical Africa and Australasia, like those in sub-tropical to tropical America, can be considered relictual from subsequent Miocene cooling and drying.

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APPENDIX 1. Character information for all taxa used in the study consisting of: **Growth form** [(1) rosulate, (2) stoloniferous, (3) rosulate with stolons]; **Habit** (Fig. 3A) [(1) terrestrial, (2) lithophyte, (3) epiphyte, (4) rheophyte, (5) affixed aquatic, (6) suspended aquatic]; **Capsule dehiscence type** (Fig. 3B) [(1) ventrally longitudinal slit, (2) ventrally longitudinal pore, (3) dorsi-ventral longitudinal slit, (4) laterally bivalvate, (5) dorsi-ventrally bivalvate, (6) multi-longitudinal valvate, (7) equatorially circumscissile, (8) multi-circumscissile, (9) indehiscent]; **Seed shape** [(1) globose/ovoid, (2) cylindrical/fusiform, (3) conical/prismatic, (4) discoid/lenticular]; **Pollen aperture number** [actual range shown; (? = no count available)]; **Sepal number** [actual number shown]; **Floral form** [(1) scape, (2) raceme with bracts, bracteoles, scales, (3) raceme with bracts, bracteoles, (4) raceme with bracts, scales, (5) raceme with bracts]; **Cytological counts** ( $n = 1$ ) [actual range shown; (? = no count available)]; **Generation time habit** (Fig. 3C) [(1) annual, (2) perennial]; and, **Biogeographic area** (Fig. 3D) [(1) tropical America, (2) Africa, (3) North America, (4) tropical Asia, (5) Australasia, (6) temperate Eurasia]. Authors of area/character data are shown in the text. Characters states are separated by & = AND, / = OR, and - = continuous range.

Taxon	Growth form	Habit	Capsule dehisc.	Seed shape	Pollen aperture	Sepal no.	Flower form	Gen. time	Cytol. $n = 1$	Area distrib
<i>G. aurea</i>	1	1	8	3	3-4	5	2	2	?	1
<i>G. guianensis</i>	1	1	8	3	3-4	5	2	2	?	1
<i>G. hispidula</i>	1	1	8	1	3-4	5	2	2	?	2
<i>G. lobata</i>	1	1	6	3	3	5	2	2	?	1
<i>G. violacea</i>	1	1	6	3	3	5	2	2	?	1
<i>P. agnata</i>	1	1	4	2	4-5	5	1	2	?	1
<i>P. alpina</i>	1	1	4	2	6-7	5	1	2	16	6
<i>P. caerulea</i>	1	1	4	2	4-6	5	1	2	16	3
<i>P. ehlersiae</i>	1	1	4	2	?	5	1	2	?	1
<i>P. gracilis</i>	1	1	4	2	?	5	1	2	?	1
<i>P. grandiflora</i>	1	1	4	2	?	5	1	2	16	6
<i>P. gypsicola</i>	1	1	4	2	6	5	1	2	11	1
<i>P. lusitanica</i>	1	1	4	2	5-6	5	1	2	6	6
<i>P. morenensis</i>	1	1	4	2	5-6	5	1	2	22	1
<i>P. pumila</i>	1	1	4	2	5-6	5	1	2	11	3
<i>P. rotundiflora</i>	1	1	4	2	?	5	1	2	?	1
<i>U. adpressa</i>	2	1	1	1	4	2	2	1	7	1
<i>U. alpina</i>	2	1 & 3	1	2	4-6	2	2	2	9	1
<i>U. amethystina</i>	3	1	5	1	12-14	2	2	1/2	?	1 & 3
<i>U. asplundii</i>	2	1 & 3	1	2	3-4	2	2	2	?	1
<i>U. aurea</i>	2	6	7	4	15-23	2	5	2	21	4 & 5
<i>U. australis</i>	2	6	7	3	11-14	2	4	2	18-22	2 & 4-6
<i>U. biloba</i>	2	1	2	1	9	2	4	2	?	5
<i>U. bisquamata</i>	2	1	1	1	5-6	2	2	1	?	2
<i>U. caerulea</i>	2	1	1	1	3	2	2	1/2	20	2 & 4 & 5
<i>U. chrysantha</i>	2	1	1	1	4-5	2	2	1	?	5
<i>U. cornuta</i>	2	1	1	1	4	2	2	2	9	3
<i>U. costata</i>	2	1	1	1	3	2	2	1	?	1
<i>U. cucullata</i>	2	6	1	1	8-9	2	5	1	?	1
<i>U. delicatula</i>	2	1	1	1	6	2	2	2	?	5
<i>U. dichotoma</i>	2	1	1	1	3-4	2	3	2	14	5
<i>U. endresii</i>	2	3	1	2	4-5	2	2	2	?	1
<i>U. erectiflora</i>	2	1	1	1	5-6	2	2	2	?	1
<i>U. flaccida</i>	2	1	2	1	15-17	2	4	2	?	1
<i>U. foveolata</i>	2	1	1	1	4-5	2	2	1	10	2 & 4 & 5
<i>U. geminiloba</i>	2	1 & 2	1	2	5-6	2	2	2	?	1
<i>U. geminiscapa</i>	2	6	7	1	10-12	2	5	2	?	3
<i>U. gibba</i>	2	5	4	4	13	2	4	1/2	14	1-6
<i>U. graminifolia</i>	2	1	1	1	4-5	2	2	1/2	?	4
<i>U. huntii</i>	2	1	1	1	9-11	2	2	2	?	1
<i>U. inflata</i>	2	6	7	1	13-17	2	5	2	9/18	3
<i>U. intermedia</i>	2	5	7	3	14-19	2	4	2	22	3 & 6
<i>U. juncea</i>	2	1	1	1	3-4	2	2	2	9	1-3
<i>U. laciniata</i>	2	1	1	1	4-5	2	2	1	?	1
<i>U. lateriflora</i>	2	1	3	1	6	2	2	2	14	5
<i>U. leptoplectra</i>	2	1	2	1	10-12	2	4	2	?	5
<i>U. livida</i>	2	1	1	1	6-8	2	2	1/2	18	1 & 2
<i>U. longifolia</i>	2	1 & 2	1	1	8-10	2	2	2	?	1
<i>U. macrorhiza</i>	2	6	7	3	14-16	2	4	2	22	3 & 6
<i>U. meyeri</i>	2	1	1	1	4-5	2	2	2	?	1
<i>U. multifida</i>	1	1	1	1	3	4	3	1	?	5

## APPENDIX 1. Continued.

Taxon	Growth form	Habit	Capsule dehisc.	Seed shape	Pollen aperture	Sepal no.	Flower form	Gen. time	Cytol. $n = 1$	Area distrib
<i>U. myriocista</i>	2	6	1	1	10–11	2	5	2	?	1
<i>U. nana</i>	2	1	1	1	6–7	2	2	1	?	1
<i>U. nelumbifolia</i>	2	3	1	2	8	2	2	2	?	1
<i>U. neottioides</i>	2	4	2	1	9	2	4	2	?	1
<i>U. nephrophylla</i>	2	2	1	1	5–6	2	2	2	?	1
<i>U. olivacea</i>	2	6	9	1	9–10	2	5	1	?	1 & 3
<i>U. oliveriana</i>	2	4	2	1	9–10	2	4	2	?	1
<i>U. praelonga</i>	2	1	1	1	12–13	2	2	2	?	1
<i>U. prehensilis</i>	2	1	1	1	5–7	2	2	2	?	2
<i>U. pubescens</i>	2	1 & 2	1	1	7–8	2	2	1	?	1 & 2 & 4
<i>U. purpureocaerulea</i>	2	1	1	1	4	2	2	2	?	1
<i>U. reniformis</i>	2	1 & 3	1	2	7–8	2	2	2	?	1
<i>U. resupinata</i>	2	1	1	3	10–13	2	5	2	18	1 & 3
<i>U. sandersonii</i>	2	2	1	1	6–7	2	2	2	?	2
<i>U. simplex</i>	2	1	1	1	6	2	2	2	?	5
<i>U. simulans</i>	3	1	1	1	4–5	2	2	2	?	1 & 2 & 3
<i>U. striatula</i>	3	2 & 3	1	1	3	2	2	1/2	14	2 & 4 & 5
<i>U. subulata</i>	2	1	2	1	8–9	2	4	1	15	1–5
<i>U. tricolor</i>	3	1	5	2	12–14	2	2	2	14	1
<i>U. triloba</i>	2	1	2	1	8–10	2	4	1	?	1
<i>U. uliginosa</i>	2	1	3	1	4–5	2	2	1/2	?	4 & 5
<i>U. violacea</i>	1	1	1	1	3	2	3	1	?	5
<i>U. vulgaris</i>	2	6	7	3	17–19	2	4	2	22	6