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PHYLOGENETIC ANALYSIS OF *PINGUICULA* (LENTIBULARIACEAE): CHLOROPLAST DNA SEQUENCES AND MORPHOLOGY SUPPORT SEVERAL GEOGRAPHICALLY DISTINCT RADIATIONS[1]

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The genus *Pinguicula* is one of the three genera of the carnivorous Lentibulariaceae, comprising approximately 80 species. Phylogeny inference using nucleotide sequences of the chloroplast gene *matK* and the *trnK* group II intron, as well as a set of 32 morphological characters revealed five well-supported, major lineages within the genus. These lineages largely reflect radiations in clearly defined geographic regions, whereas most previously recognized sections of the genus are shown to be para- or polyphyletic. A species-rich Mexican-Central American-Caribbean clade has the Eurasian *P. alpina* and an East Asian clade as successive sisters. All three are characterized by a production of flower buds on winter-resting plants, a specific corolla hair structure and a very large corolla lower central lobe. Another diverse clade is composed of species with primarily European distribution including the widespread type species *P. vulgaris*. For this clade, vegetative reproduction during dormancy is synapomorphic. Species native to SE North America and the South American Andes and a group of Mediterranean and NE Atlantic coast species together appear in a fifth well-supported clade, that is characterized by a tropical-type growth habit. It is the only clade that has reached temperate zones of the southern hemisphere.

Key words: Lamiales; Lentibulariaceae; *matK/trnK*; morphology; northern hemisphere biogeography; *Pinguicula*.

The Butterworts (*Pinguicula*) constitute the second most diverse genus of the carnivorous Lentibulariaceae, with 85 currently accepted species (Steiger, 1998; Legendre, 2000). With its extant diversity and distribution throughout Eurasia, North America, Mexico, Central America, the Caribbean, and the South American Andes (Casper, 1966), phylogenetic patterns in *Pinguicula* provide relevant data to understand the historical biogeography of the northern hemisphere where, so far, mostly intercontinental disjuncts (e.g., Guo, 1999; Wen, 1999, 2001; Quian and Ricklefs, 2000) and woody lineages with a wider distribution (e.g., Donoghue et al., 2001; Tiffney and Manchester, 2001) have been studied.

In *Pinguicula*, insects are caught and digested by sessile and short-stalked mucilage-producing glands on the upper side of rosette leaves (''flypaper traps''). This causes the shiny appearance of the leaves that led to the common name ''butterwort.'' The closest relatives of *Pinguicula* are in the suction-trap-bearing genus *Utricularia* (Taylor, 1989) and the eel-trap-bearing genus *Genlisea* (Fromm-Trinta, 1981; Taylor, 1991). While the monophyly of the Lentibulariaceae has been substantiated by molecular data (Müller et al., 2000, 2004; Jobson et al., 2003), the position of the family, and related ones, within the order Lamiales remains obscure despite recent analyses based on molecular data that included noncoding DNA (Olmstead et al., 2001; Bremer et al., 2002; Müller et al., 2004; Rahmanzadeh et al., 2005). Nevertheless, statistical data derived from Kishino-Hasegawa-tests suggested that the ''protocarnivores'' (Byblidaceae, Martyniaceae) evolved independently from Lentibulariaceae (Müller et al., 2004) in agreement with the observation that digestive glands in all three genera of Lentibulariaceae are attached to vessels unlike secretory glands of Byblidaceae and Martyniaceae that rest on at least two epidermal cells (Müller et al., 2004). In an attempt to unravel infrageneric relationships, most lineages of *Utricularia* and *Genlisea* have been sampled and the monophyly of the two genera has been established (Müller et al., 2004; Müller and Borsch, 2005). Reconstruction of morphological character evolution within the Lentibulariaceae (Müller et al., 2004) indicated that the more complex pitcher traps of *Utricularia* and *Genlisea* have evolved via the folding of leaves of a *Pinguicula*-like ancestor. Nevertheless, the inclusion of species from *Pinguicula* in molecular studies was limited. Jobson et al. (2003) sequenced the *rps16* intron and the *trnL* intron and *trnL-F* spacer of 12 species, and Müller et al. (2004) analyzed sequences of *matK* and *trnK* of 26 taxa, covering only few sections, while Zamora et al. (1996) conducted RAPD-based comparisons of four European species. Additionally, the implications of phylogenetic results for character evolution, infrageneric classification, or phytogeography of *Pinguicula* have never been discussed. Therefore, a more complete sampling is needed, to test if *Pinguicula*, as currently circumscribed, is monophyletic or paraphyletic to the *Genlisea-Utricularia* clade and whether there are morphological characters synapomorphic for *Pinguicula*.

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The first description of a member of *Pinguicula* was made more than 400 years ago by Gesner (1555) who introduced the genus name later studied by Ray (1660) and Linné (1737), and formalized by Linné (1753). De Candolle (1844) introduced the first infrageneric classification, which had three sections and increased the number of species to 32. De Candolle emphasized morphological characters such as spur size, floral symmetry, corolla tube size, and corolla lobe color. These sections were *Orcheosanthus* (all Central American species), *Brandonia* (only the yellow-flowered North American *P. lutea*), and *Pionophyllum* (the remaining species). Due to varying importance assigned to different morphological characters states, the subdivision of the genus has been the subject of controversy. Barnhart (1916) raised section *Pionophyllum* to subgeneric level and removed all species from subg. *Pionophyllum* that have corolla lobes of equal size to group them with *P. lutea* in a novel subgenus *Isoloba*. Similarly, *P. crenatiloba* was removed from section *Pionophyllum* to become the sole member of a further new subgenus called *Temnoceras* (Barnhart, 1916). None of the early classification systems included the nominate entity, subgen. *Pinguicula*, what was formally corrected by Casper (1966). A careful examination of flower morphology throughout *Pinguicula* showed the presence of a palatum in *P. lutea* and allies that further supported the afore-mentioned circumscription of subg. *Isoloba* (Wood and Godfrey, 1957; Godfrey and Stripling, 1961). In contrast, Ernst (1961) used flower throat hair structures for his classification and merged subg. *Isoloba* with subg. *Pionophyllum*. The use of flower throat hair morphology as taxonomic character was further modified by Casper (1966) and complemented by life forms and cytological data to generate the most thorough revision of *Pinguicula* to date. It recognizes 48 species in three subgenera (*Isoloba*, *Temnoceras*, *Pinguicula*) and 12 sections that, in most cases, contain a mix of species occurring on different continents. This system was based on the relatively small number of seven morphological characters, some of which had never been observed on living specimens under comparable growing conditions. The number of described species has also doubled since the publication of this work with new taxa originating from many parts of the world including Mexico (e.g., Zamudio and Rzedowski, 1986; Zamudio, 1999, 2005), Cuba (Casper, 2003, 2004) and Europe (Blanca et al., 1999; Zamora et al., 1996; Casper and Steiger, 2001).

Sequences of the chloroplast gene *matK* and adjacent parts of the *trnK* intron were very informative in asserting phylogenetic relationships among the different species of the genera *Utricularia* and *Genlisea* (Müller et al., 2004; Müller and Borsch, 2005). It, therefore, seemed reasonable to extend further the *matK/trnK* datasets for *Pinguicula*. The mutational dynamics of *matK* differ from other protein coding genes by exhibiting high rates of nonsynonymous substitutions and a high frequency of indels (Johnson and Soltis, 1995; Hilu et al., 2003; Müller and Borsch, 2005). The *trnK* intron, in which *matK* is located, is a group II intron that provides good amounts of potentially phylogeny informative characters, including many indels that appear even less homoplastic than substitutions (Müller and Borsch, 2005).

Goals of this study are to (1) test the monophyly of the genus *Pinguicula* based on an extended sampling, (2) infer relationships within *Pinguicula* using molecular data, also as a basis for a revised classification and an understanding of extant phytogeographic patterns and (3) reevaluate and extend the number of morphological characters potentially providing synapomorphies for clades found with molecular data.

MATERIALS AND METHODS

Plant material and sampling—Appendix lists all taxa included in this study with voucher information, GenBank accession numbers, and geographic origin. Many plants were collected in the wild and then cultivated in a glasshouse and in vitro to allow comparative observation and scoring of characters relating to growth types, reproduction mode (both spontaneous and after cutting leaves), prey capture, and hybridization partners. Growing conditions were empirically optimized (results will be published elsewhere). A description and list of morphological characters, character states and character scores are respectively given in appendices 2, 3 and 4. For phylogenetic analysis, two different matrices were compiled: one that consisted of *Pinguicula* only plus outgroup and a second that included *Utricularia* and *Genlisea* (extended data set). Thus, the influence of highly accelerated mutation rates in the *Utricularia–Genlisea* lineage on the internal topology of the slower evolving *Pinguicula* clade (Jobson and Albert, 2002; Müller et al., 2004) could be tested. Considering existing trees of Lamiales (Olmstead et al., 2001; Müller et al., 2004; Rahmanzadeh et al., 2005), *Kigelia africana* (Bignoniaceae) and *Antirrhinum majus* (Plantaginaceae) were used as outgroup. Sequences of *Genlisea*, *Utricularia* and outgroup taxa were already available (Appendix).

DNA extraction, PCR amplification, and sequencing—Fresh leaves were washed under a stream of water to remove dead insects and plant debris. Then, a cotton swab was rolled on the surface of the leaves to activate mechanically the secretion of hydrolytic enzymes (includes DNase activity: Heslop-Harrison and Knox, 1971; Heslop-Harrison, 1975). Leaves were subsequently rinsed again under a stream of water until all mucilage was washed off before being gently blotted on paper. About 1 g of leaf was then ground in liquid nitrogen and incubated at 65°C for 15 min in 5 mL extraction buffer (2% hexadecyltrimethyl ammonium bromide, 100 mM Trisma-HCl, 20 mM ethylene diaminetetracetic acid, 1.4 M NaCl, 1% polyvinylpyrrolidone, 60 μL β-mercaptoethanol, pH 8.0) followed by 30 min and 2 h incubation steps at 37°C with RNase A (0.4 mg) and proteinase K (0.4 mg), respectively. After sequential extractions with phenol : chlorophorm : isoamylic acid (25 : 24 : 1) and chlorophorm : isoamylic acid (24 : 1), DNA was precipitated with isopropanol. The pellet was washed with 70% ethanol, dried, dissolved in 50 μL of water, cleaned with a QIAquick PCR purification kit (QIAGEN, Clifton Hill, Victoria, Australia,) according to the manufacturer's instructions and stored at −20°C.

Amplification of *trnK* including *matK* was made by PCR using primers 3914F and 2R (Johnson and Soltis, 1995) with DyNAzyme EXT (Finnzymes, Thebarton, South Australia, Australia) under the following conditions: initial melting (3 min, 95°C) followed by 25 cycles of melting (1 min, 95°C), annealing (1 min, 52°C) and elongation (3 min, 72°C) and a termination step of 10 min at 72°C. The PCR reaction mixtures were cleaned using a QIAquick PCR purification kit (QIAGEN) according to the manufacturer's instructions and sequenced. Sequencing was conducted at the Strasbourg (France) or Millenium Institute of Westmead (NSW, Australia) DNA sequencing centers using the primers listed in Table 1.

Alignment, indel coding, and phylogenetic analyses—All *trnK/matK* sequences were manually aligned using QuickAlign (Müller and Müller, 2003) following the principles detailed in Borsch et al. (2003) and Löhne and Borsch (2005). Six highly variable sequence regions (hotspots: H1 = alignment positions 242–253, H2 = 396–402, H3 = 512–528, H4 = 743–765, H5 = 1436–1448, H6 = 2461–2486) were excluded from analyses of the extended data set. A single hotspot (H5) is within the *matK* CDS. Because initial separate calculations using noncoding intron and coding *matK* sequences, respectively, yielded congruent but incompletely resolved topologies, both partitions were combined in all subsequent analyses.

Indels were coded according to the ''simple indel coding'' method (Simmons and Ochoterena, 2000). This was achieved automatically with the IndelCoder function of the program SeqState (Müller, 2005). The matrix is

TABLE 1. Primers used in this study.

Primer name	5′ to 3′ Primer sequence	5′ Primer position[a]	Primer source
3914F	GGG GTT GCT AAC TCA ACG G	Start	Johnston and Soltis, 1995
2R	AAC TAG TCG GAT GGA GTA G	Finish	Johnston and Soltis, 1995
TrnK 1R	CGG CTT ACT AAT GGG ATG CC	1951	This study
TrnK 2F	CGG GCT GAT TTA GCA GAT TC	1955	This study
TrnK 3R	GCA AAG AAG AAG GGT CTT TTA CC	1268	This study
TrnK 4R	CGG ATC CTC ATT CCA TGA TA	474	This study
TrnK 5F	TAT CAT GGA ATG AGG ATC CG	455	This study
TrnK 8F	TTG CTC ATG ATG GTG GTT TC	840	This study
TrnK 9R	GAA ACC ACG ATC ATG AGC AA	859	This study
TrnK 10F	TGG TCA AGG AAC CTT GCA TAC	1581	This study
TrnK 11R	GTA TGC AAG GTT CCT TGA CCA	1601	This study
TrnK 12F	CTT ACC CGT TGA GGG CAG TA	2214	This study
TrnK 13R	TAC TGC CCT CAA CGG GTA AG	2233	This study
TrnK 19R	CAT TGC ACA CGG CTT TAC CTA	2494	This study

[a] Position of the head of the primer on the *P. grandiflora* f. *pallida trnK* sequence determined in this study.

provided in Appendix S4 (see Supplemental Data with online version of article). Maximum parsimony (MP) analyses were carried out using PAUP* (Swofford, 2000). Heuristic analyses were conducted using 1000 random addition replicates and tree-bisection-reconnection (TBR) branch swapping, saving multiple trees. With 10 random addition cycles each, 500 bootstrap (Felsenstein, 1985) replications were performed to evaluate internal branch support.

Bayesian inference (BI) of phylogeny was performed with MrBayes3 (Ronquist and Huelsenbeck, 2003). The model of best fit was (GTR + Γ + I) as determined with Modeltest (Posada and Crandall, 1998). This model was specified for the analysis, and posterior probabilities were estimated by sampling trees from the posterior probability distribution using the Metropolis-coupled Markov chain-Monte Carlo approach (MCMCMC) implemented in MrBayes3 (Ronquist and Huelsenbeck, 2003), using default priors. The temperature of the heated chain was set to 0.2. Three times, four chains were run for 1 million generations. As verified using the plot command in MrBayes3, the likelihood scores of the sampled trees reached stationarity approximately after generation 20 000 in all three runs. Consensus trees, clade posterior probabilities, and mean branch lengths were based upon the trees sampled (every 10 generations) after the burn-in and graphed with TreeGraph (Müller and Müller, 2004).

Relative rate tests—Major clades within *Pinguicula* determined via phylogenetic analysis were subjected to relative rate tests using maximum likelihood estimates of substitutions per site between taxon groups according to the model of best fit (GTR + Γ + I) determined with Modeltest (Posada and Crandall, 1998). For the tests, standard errors were estimated using the bootstrap option in the program G-Rate (Müller, 2002; see also Müller et al., 2004). Relative rates were intended to provide evidence of whether certain longer branches within *Pinguicula* were the result of rate accelerations of individual species. Furthermore, rates should at least give some hints on whether shallow clades may be explained by rapid diversification rather than putative rate decrease. In the absence of Lentibulariaceae fossil records, knowledge of Lentibulariaceae relatives, and in the light of the extreme rate heterogeneity within this plant family, molecular dating was not attempted here.

Optimization of indel and morphological characters—Optimization of indel and morphological characters was performed with WinClada (Nixon, 1996) using the fast optimization scheme, assuming accelerated transitions. The tree used to plot character state transformations was the Bayesian majority rule consensus tree resulting from the analysis of the extended matrix.

RESULTS

Variability of trnK/matK DNA sequences—Among the 43 species of *Pinguicula* of this study (contains sequences already included in Müller et al., 2004), the length of the *trnK* intron including the *matK* gene ranged from 2514 to 2560 bp (Table 2). *MatK* stop codons were positionally homologous for all *Pinguicula* species except for *P. sharpii* (10 bp downstream), unlike the extreme divergence found in *Genlisea* and *Utricularia* (Müller et al., 2004). A perfect reading frame was found in all *matK* sequences of *Pinguicula*. Table 2 summarizes sequence characteristics of the two *trnK* intron parts and *matK*. A total of 95 indel characters were coded (60 potentially parsimony-informative; Appendix S4, see Supplemental Data with online version of article). Within *Pinguicula* only 27 were variable (9 potentially parsimony-informative), and the majority was due to the *Utricularia–Genlisea* lineage. The 5′ noncoding intron part comprised 51 indels, the *matK*-coding region 22, and the 3′ noncoding intron part 22. Some of the indels in *Pinguicula* were extremely large for what is known in *matK* (a 36-bp deletion and a 54-bp insertion in all *Pinguicula* sequences, Appendix S4). An optimization of the indel characters on the Bayesian majority rule tree shown as a phylogram (Fig. 3) revealed that eight indels are synapomorphic for *Pinguicula*, thereby providing additional support for the monophyly of the genus. An additional eight indels were found to be synapomorphic for clades within *Pinguicula*. The illustration also indicates that substitution-based branch length and indel frequencies largely correlate.

Parsimony analyses—MP analysis of the extended data set yielded nine shortest trees of 1716 steps (CI = 0.769; RI = 0.807; RC = 0.621). The analysis that excluded *Genlisea* and *Utricularia* resulted in three trees of 907 steps (CI = 0.830; RI = 0.770; RC = 0.728). Maximum support was found for a lineage comprising all species of *Pinguicula* (Fig. 1). The strict consensus obtained when including *Genlisea* and *Utricularia* did not resolve the node sister to clade V (marked ''n.p.'' in Fig. 1), but was otherwise of identical topology. Both analyses supported a grouping of *Pinguicula* species into five distinct clades (I–V). Bootstrap support decreased for several nodes when *Genlisea* and *Utricularia* were added (Fig. 1). However, there are also a few nodes where it increased such as for the sister-group relationship of *P. antarctica* to the southeastern North American species.

Clade I comprises all Central American and Caribbean species and is sister to clade II, that only contains the Eurasian *P. alpina*. Clade III is made up of East Asian species. Within

TABLE 2. Sequence statistics for the coding and noncoding parts of the *trnK* intron. % divergence = overall pairwise sequence dissimilarity (uncorrected *p* distance × 100), standard error in parentheses (calculated using 500 bootstrap replicates).

Region	Sequence length[a]		No. of characters		% Divergence[b] (SE)		Variable[b] (%)		% Parsimony informative[b] (%)		Nucleotides[a] (%)			
	Ping.	All	Incl. hotspots	Without hotspots	*Ping.*	All	*Ping.*	All	*Ping.*	All	T	C	A	G
trnK/matK	2514–2560	2447–2560	2762	2589	2.333 (0.168)	5.165 (0.195)	16	39	7	21	34.0	17.0	31.6	17.4
matK-Coding region	1542–1554	1497–1554	1600	1587	2.174 (0.200)	5.114 (0.228)	17	42	7	23	35.5	17.9	29.8	16.8
Noncoding regions	972–1012	936–1012	1162	1002	2.588 (0.351)	5.214 (0.340)	16	36	7	18	31.6	15.5	34.5	18.4
5′ noncoding	693–729	669–729	796	736	2.476 (0.338)	5.075 (0.368)	16	39	7	20	31.7	16.4	33.6	18.3
3′ noncoding	268–293	255–307	366	266	2.908 (0.756)	5.935 (0.838)	14%	26%	7%	14%	31.3	13.5	36.6	18.6

[a] Calculated with hotspot regions included (see Materials and Methods; Alignment, indel coding and phylogenetic analyses). Note that for *P. ehlersiae*, *P. ionantha*, *P. medusina*, *P. primuliflora* and *P. variegata* only partial sequences of 1148 bp, 2089 bp, 1951 bp, 2408 bp and 2537 bp, respectively, could be obtained.

[b] Calculated based on the alignment with hotspot regions excluded.

this clade, *P. villosa* has a near-circumboreal distribution (absent from Western Norway to Eastern Canada; Torbjorn, 2000) and can only be found at lower latitudes on the Siberian East coast down to the Korean peninsula (Lee, 1993). All mountain-inhabiting European species appear in clade IV. This clade includes the widespread *P. vulgaris*, that is found throughout Europe and the northern part of North America, and two closely related species, *P. macroceras* (range around the northern pacific rim) and *P. fontiqueriana* (small range in Northern Morocco near Gibraltar). The New World species originating from coastal regions of the southeastern USA and South America are grouped together in a lineage (clade V) that is characterized by a tropical growth type (character nos. 3 and 4) and also contains coastal European (Atlantic and north Mediterranean) species as a subclade. MP depicts this clade V as sister to all remaining species albeit with low support.

Relationships among the Mexican/Central American/Caribbean species are well resolved with *trnK/matK* (Fig. 1). The Cuban *P. filifolia* is found sister to the northern Mexican *P. gracilis* and *P. rotundifolia* (BP 92/78) in a lineage sister to the remainder of clade I (BP 100/100). Under the current sampling sections *Crassifolia* (*P. ehlersiae* and *P. esseriana*) appears monophyletic, whereas other sections are poly- or paraphyletic (Fig. 1). Clade III shows *P. ramosa* and *P. villosa* (BP = 94), plus *P. variegata* as successive sisters. Clade IV comprises two well-supported subclades. One of these comprises species with a distribution restricted to Italy (BP = 96/95; *P. leptoceras*, *P. longifolia* subsp. *reichenbachiana*, and *P. poldinii*). The other subclade (BS 97/98) contains a group of European species, some of which extending into Asia, North Africa, and North America, and which are not well resolved. Within clade V, *P. antarctica* is found sister (BP = 100) to the species from the southeastern USA.

Bayesian analyses—The Bayesian tree (Fig. 2) is identical to the MP topology (Fig. 1). In clade IV two more nodes were resolved, revealing *P. corsica* and *P. vallisneriifolia* as sisters, and, together with *P. longifolia* subsp. *longifolia*, as sister to the remaining non-Italian taxa. Posterior probabilities (Fig. 2) were generally much higher than bootstrap values (Fig. 1) and were less affected by the addition of *Genlisea* + *Utricularia*. Bayesian analyses only weekly (38/40 PP) support the placement of clade V as sister to the remainder of the genus. Figure 3 shows branch lengths of the Bayesian majority rule tree of the extended sampling of this study.

Relative rate tests—Members of clade IV (the European clade, sect. *Pinguicula* according to Casper, 1966, Fig. 1) exhibit significantly lower rates than all other clades (Table 3). Differences were particularly striking between the tropical-growth-type clade (V) and the European clade (IV). The Mexican/Central American/Caribbean clade also displays clearly higher substitution rates than the European clade.

Optimization of morphological characters—The optimization reveals that 13 of 32 morphological characters are synapomorphies and that one is autapomorphic (Fig. 4). The presence of digestive glands (no. 1-1) is synapomorphic for Lentibulariaceae and of mucilage glands (no. 2-1) for *Pinguicula*. Moreover, *Pinguicula* differs from *Utricularia* and *Genlisea* by possessing glands only on the upper side of the leaves (no. 14-0; except for *P. longifolia* subsp. *longifolia* and *P. gigantea*). The ability to multiply vegetatively in winter (no. 6-2)

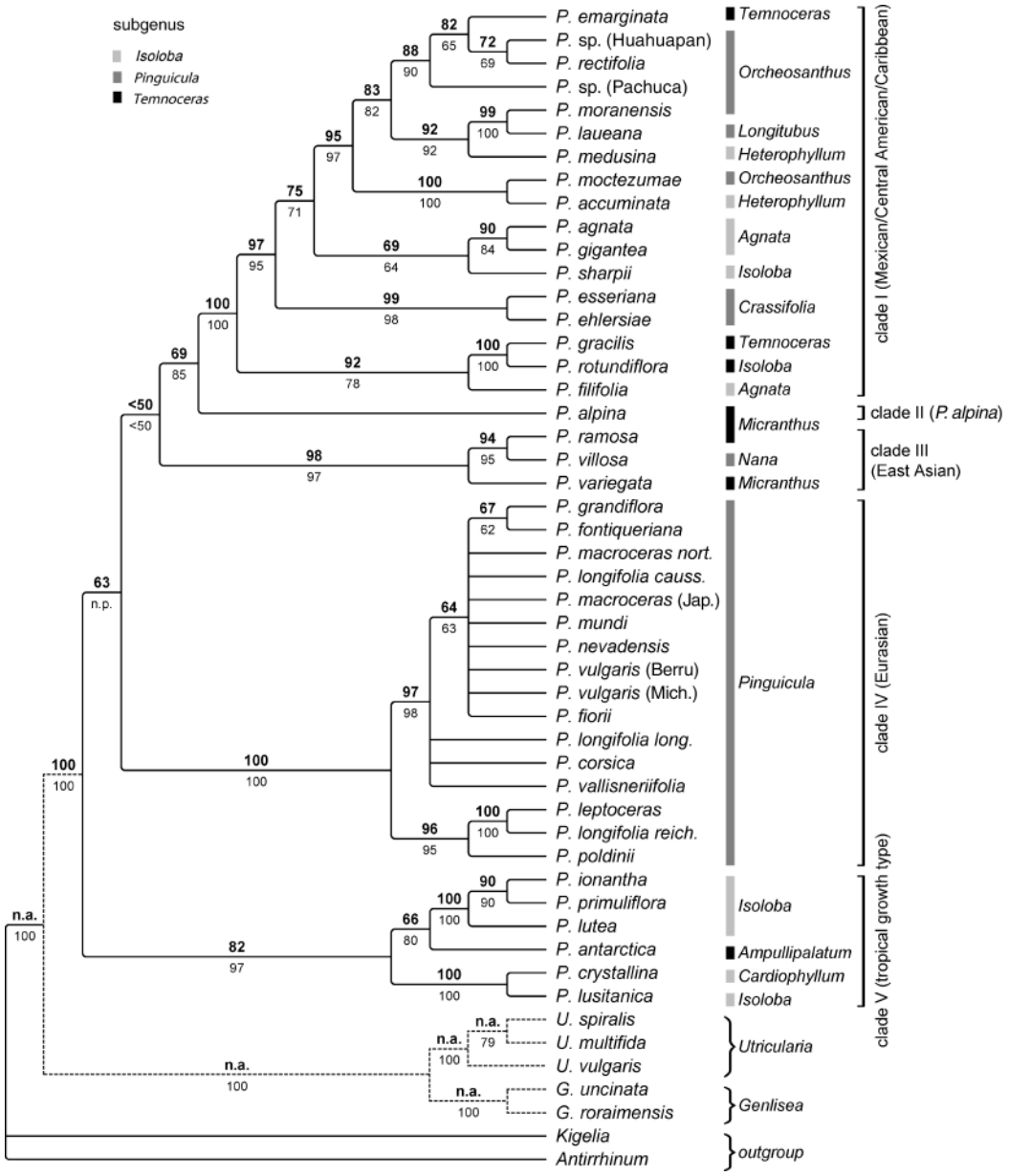


Fig. 1. Strict consensus tree of the most parsimonious trees of the *trnK* intron analysis. The classification of Casper (1966) is shown to the right. The three subgenera are indicated by lines with different grey shades, accompanied by the names of sections. To the far right clade, names are annotated that are used throughout the text. Bold numbers above branches show bootstrap support when *Utricularia*/*Genlisea* are excluded; plain face letters below branches indicate support when the full matrix is analyzed. Branches not present in the first analysis are dashed. n.p. = not present: this node collapses in the strict consensus tree. n.a. = not applicable: respective nodes not present because terminal taxa not included in analysis.

and for detached leaves to generate new plants (no. 7-1) are synapomorphic to members of clades IV and I, respectively. Members of clade I are also characterized by corolla hairs that are made of strings of single cells (no. 19-0). Clades II and III are supported by the presence of a white corolla tube (no. 21-0) and a high density of mucilage glands on the flower stems (no. 16-1), respectively. The production of bifid flower stems (no. 17-1) on the one hand and of a purple speck at the base of leaves (no. 13-1), a spur that accounts for two thirds of the size of the length of the corolla (no. 31-2) and twisted lateral corolla lobes (no. 27-0) on the other appear to be recently acquired characters that are synapomorphic within *Pinguicula* to subclades in clades III and I, respectively.

A few taxa dispersed throughout the tree are responsible for the fact that so many characters are homoplasic (Fig. 4). These include most species of clade I, *P. villosa* in clade III and *P. crystallina* in clade VI. They differ from other species of the same subclade by at least four, and by up to 12 (*P. medusina*), character states that are otherwise only expressed by all the species of one of the other five major clades.

DISCUSSION

Evolution of the Pinguicula clade—Members of the genus *Pinguicula* are traditionally distinguished from all other Lentibulariaceae by their ability to produce mucilage glands on

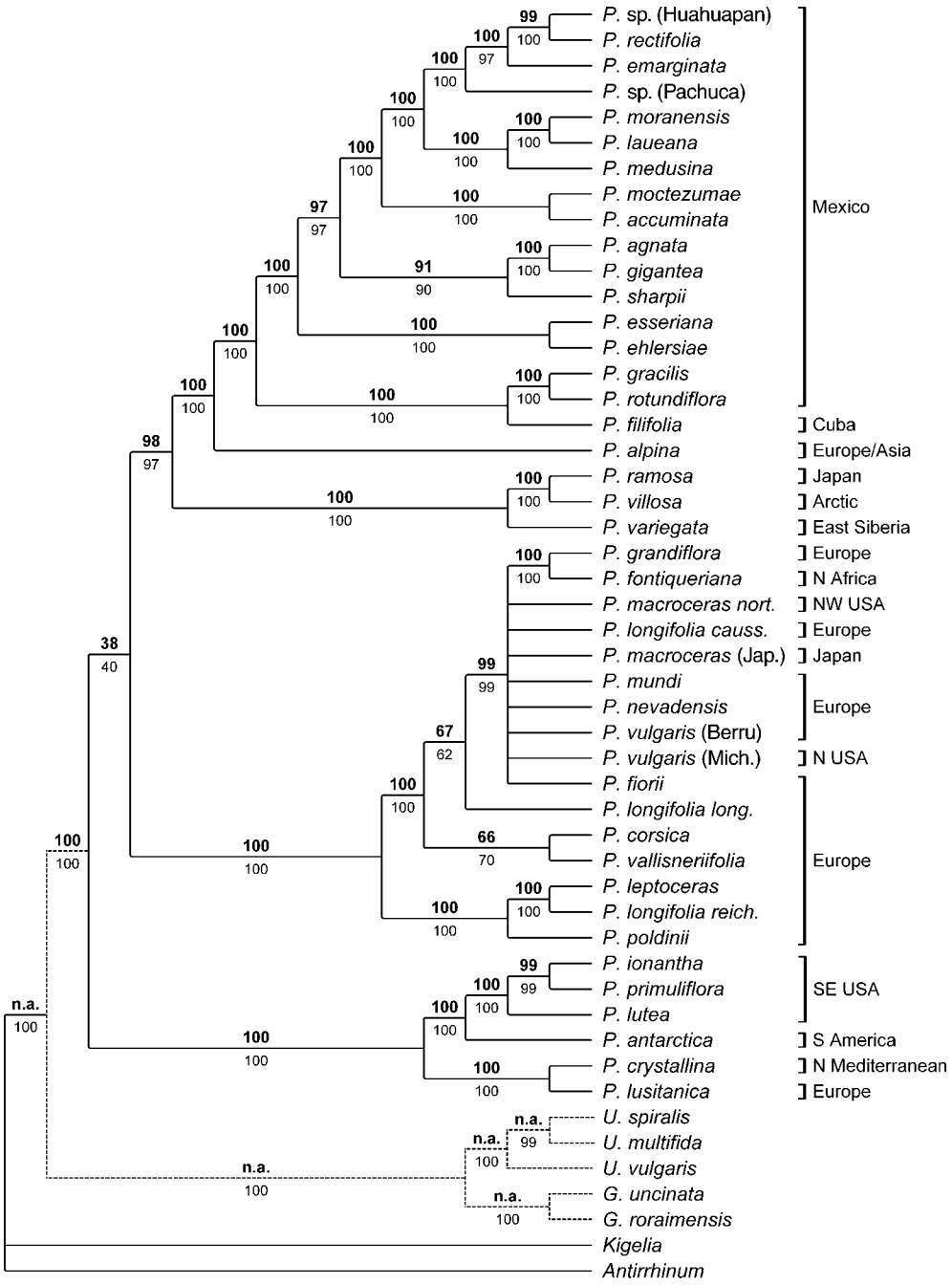


Fig. 2. Consensus tree of the Bayesian analysis of the *trnK* intron. The geographical distribution of species is shown to the right. Bold numbers above branches show posterior probabilities when *Utricularia*/*Genlisea* are excluded; plain numbers below branches indicate posterior probabilities for the extended matrix. Branches not present in the first analysis are dashed. n.a. = not applicable: respective nodes not present because terminal taxa not included in analysis.

their leaves (Casper, 1966; Taylor, 1989, 1991; Legendre, 2000). Because the bladder and eel traps in *Utricularia* and *Genlisea* have a more complex anatomy than flypaper traps, it has been hypothesized that they evolved via the folding of a *Pinguicula*-looking (already carnivorous) ancestor (Juniper et al., 1989; Taylor, 1989). Our molecular analysis is consistent with earlier hypotheses of *Pinguicula* monophyly that were based on a much narrower taxon sampling (Jobson et al., 2003; Müller et al., 2004). This implies that mucilage glands (no. 2-1) are a synapomorphy to *Pinguicula*. These glands have the unique characteristic within the plant kingdom of secreting large quantities of water and losing turgor upon mechanical stimulation by the prey to assist in prey digestion. Nevertheless, they secrete small quantities of digestive enzymes and are not involved in the absorption of digestive fluid from the prey. The absorption is achieved via an additional type of gland, a non-mucilaginous sessile gland synapomorphic to all Lentibulariaceae (Lloyd, 1942; Legendre, 2000; Appendix S1, character 2). *Pinguicula* also differs from *Utricularia*, *Genlisea*, and other Lamiales in the absence of trichomes on abaxial leaf

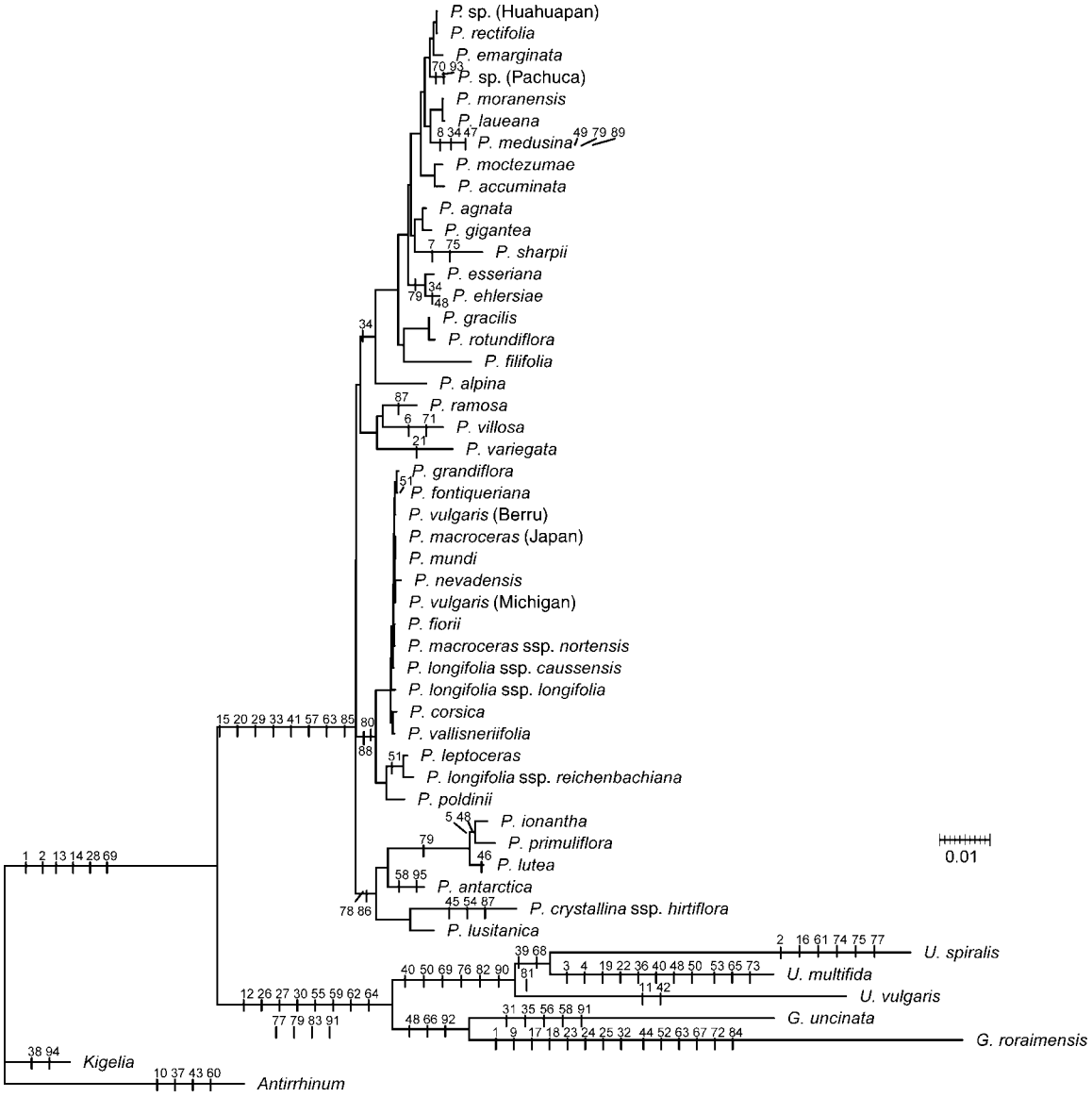


Fig. 3. Phylogram of the Bayesian analysis of the *trnK* intron, showing mean branch lengths of all trees samples after the burn-in. The scale bar represents 0.01 substitution per site. Indel character numbers are plotted on the branches when a state transformation occurs in this character (online Appendix S4).

surfaces. Therefore, it seems more likely that *Pinguicula* traps have specialized independently from a common ancestor of Lentibulariaceae that was equipped with digestive glands and where the current mucilage glands were absent. More detailed analyses on gland anatomy and function are required to better understand character state changes.

Major clade diversification within Pinguicula—Parsimony and Bayesian analyses group all Mexican and Caribbean species in a clade sister to the Eurasian *P. alpina* (clades I and II). This relationship is also supported by an 8-bp deletion (indel character 34). Nevertheless, no morphological synapomorphy is currently known for these two clades. Inference of clade III as successive sister is congruently made with MP and BI although only the later yields support (98/97 PP). Several morphological synapomorphies such as character states 9-0 (blooming initiation during winter dormancy), 19-0 (corolla hairs made of strings of single cells) and 26-1 (very large central lower lobe) provide additional evidence for close relationships between clades I, II, and III. The sole species of clade II, *P. alpina*, has several autapomorphies such as a pointed/tubular winter resting bud and the lack of pigments in the corolla tube (no. 21-0) It differs from clades I and II by a lack of spontaneous vegetative multiplication during vegetative growth or resting (no. 6-0) and the absence of spade-shaped leaves (no. 12-0). Additionally, *P. alpina* exhibits fleshy roots that stay alive in the winter (no. 5-2), a character state independently present in members of clade V.

The respective positions of the Eurasian (clade IV) and tropical growth type (clade V) lineages are less clear. The node placing clade V as sister to all remaining species generally receives weak support (Figs. 1, 2) and was not resolved when *Genlisea* and *Utricularia* were included in MP reconstruction. The analysis of Lentibulariaceae by Müller et al. (2004) did not include representatives of clade V. Nevertheless, with the exception of single isolated taxa, character states 3-0 (non-

TABLE 3. Relative rate tests among the five *Pinguicula* clades (cf. Fig. 1), calculated using maximum likelihood estimates of sequence distances (GTR + G + I model of DNA substitution) and assessing standard errors via 100 bootstrap replicates (see Materials and Methods; Relative rate tests for details). Above diagonal: *d*, the difference of the mean group distance (row minus column), significance level in brackets (insign. = insignificant, >0.05). Below diagonal: standard error and number of individual sequence comparisons invoked to estimate the overall group rate divergence.

	Clade I	Clade II	Clade III	Clade IV	Clade V
clade I	—	0.009 (<0.05)	−0.002 (insign.)	0.009 (<0.05)	−0.009 (insign.)
clade II	0.004 (17)	—	−0.011 (<0.05)	−0.001 (insign.)	−0.018 (<0.0001)
clade III	0.004 (51)	0.004 (3)	—	0.010 (<0.05)	−0.007 (insign.)
clade IV	0.004 (272)	0.004 (16)	0.004 (48)	—	−0.018 (<0.0001)
clade V	0.005 (102)	0.005 (6)	0.005 (18)	0.004 (96)	—

carnivorous winter leaves), 4-2 (resistance to near-freezing temperatures), 30-1 (cylindrical spur) and 32-0 (spur forming no angle with the tube of the corolla) were found to be synapomorphic to clades I–IV (see Appendix S3, see Supplemental Data with online version of article, and Fig. 4), while character states 5-1 (roots alive in the winter), 20-0 (no color spot on corolla), 21-1 (yellow-brown corolla tube), 23-2 (crenated lobes), 29-1 (long corolla tube), and 31-0 (short spur) were synapomorphic to clade V.

An understanding of the early diversification of *Pinguicula* would greatly beneficiate from information on whether the tropical growth pattern in clade V represents a plesiomorphic condition (common with *Utricularia* and *Genlisea*) or was adopted independently after early diversification of the genus. However, additional markers that could support deep nodes in the *Pinguicula* or define the sister-group of Lentibulariaceae are lacking. Interestingly, the otherwise well-supported clade V (Figs. 1, 2) shows a deep split into a New World lineage and a European-Mediterranean lineage that is also evident from the rather long branches separating the respective terminals of each lineage (Fig. 3). This suggests that *Pinguicula* may have diversified from a paratropical laurophyllous vegetation that was present in the Tertiary under warmer and more humid climatic conditions (Mai, 1995; Collinson and Hooker, 2003) followed by early Laurasian migrations, similar to what was recently inferred for a number of extant lineages with both a western and eastern Gondwanan distribution (Davis et al., 2002). Axelrod (1975) estimated this vegetation to have occurred approximately 65–54 mya, whereas the separation of Western Gondwana had began much earlier (~105 mya; McLoughlin, 2001). Further research will be needed to infer the age of clade V.

Relationships among Mexican/Central American/Caribbean species (clade I)—Recognizing a clade that comprises all Central American, Mexican and Caribbean species disagrees with infrageneric classifications by Barnhart (1916) and Casper (1966) but supports De Candolle's (1844) section *Orcheosanthus*. Nevertheless, character state 7-1 (detached leaves generating plantlets) was reconstructed as a synapomorphy (Fig. 4), and the potential for successful hybridization (unpublished data) further substantiates close relationships of the species composing this clade. At the species level, several morphological character states are in line with relationships hypothesized with *matK*/*trnK* despite deviating sectional classification. For example, *P. laueana* and *P. moranensis* share a long spur (no. 31-2) while *P. emarginata*, *P. rectifolia* and *P. species* (from Huahuapan) have flowers with lateral corolla lobes possessing a twist (no. 27-0) and leaves with a purple speck at their base (no. 13-1). In the contrary, resistance to drought in the winter (no. 4-1) and the ability of leaf cuttings to strike (no. 7-1) appear plesiomorphic. A unique blooming behavior with flowers arising twice during the year, once from the resting rosette (no. 9-0) and once from the summer rosette (no. 9-1), occurs in *P. moctezumae*, *P. moranensis*, *P. emarginata*, *P. species* 'Huahuapan' and *P. rectifolia*. Although this feature is confined to a terminal group among the Mexican species, it is homoplastic within this group. Flowers in all other species either arise from the resting or from the summer rosette.

The lineage resolved as sister to all other species of clade I includes *P. filifolia* from Cuba along with two species from Mexico (*P. gracilis* and *P. rotundifolia*). These occur in the northern part of the Sierra del Orientale in the states of Tamaulipas and Nuevo Leon. The inclusion of a Cuban species in this subclade agrees with the known affinities of the floras from these two regions (Briggs, 1987). It is probable that an ancestor of *P. filifolia* was transferred from northern Mexico to Cuba via ocean drafting as hypothesized for a majority of Cuban plant species (Briggs, 1987). The placement of species within clade I follows a geographical north to south gradient in Mexico on the Sierra del Orientate and the Sierra del Sur, starting with a subclade composed of *P. ehlersiae* and *P. esseriana*, which has members located just south from the earlier subclade, on the same mountain formation, in the states of San Louis Potosi and Tamaulipas. This is followed by (1) a subclade formed by *P. agnata*, *P. gigantea*, and *P. sharpii* occurring on the southern tip of the Sierra del Orientale in the state of Hidalgo and northern Sierra del Sur in southern Mexico; (2) a clade of *P. accuminata* and *P. moctezumae* from the states of Hidalgo and Queretaro; (3) a clade consisting of *P. laueana* and *P. medusina*, both in the state of Oaxaca on the northern fringe of Sierra del Sur, as well as the very widespread *P. moranensis*; (4), a clade consisting of *P. spec* 'Pachuca' from the state of Hidalgo and, (5) a clade consisting of exclusively Southern Mexican members (*P. rectifolia*, *P. emarginata*, and *P. species* 'Huahuapan') from the states of Oaxaca, Veracruz, and Puebla (Casper, 1966).

Results of this study do not support the inclusion of *P. moranensis* in section *Orcheosanthus* (Casper, 1966) and suggest that this species should be included in section *Longitubus* together with *P. laueana*, with which it shares a long spur as synapomorphy (no. 31-2). Our results also suggest that the taxonomy of the *P. moranensis* complex needs to be revisited. Several *Pinguicula* populations that are widely distributed for horticulture are marketed under the name "*P. moranensis*," for example *P. species* 'Pachuca' and *P. species* 'Huahuapan' (Meyers-Rice and Schlauer, 2000). According to our sequence data, they do not belong to *P. moranensis*, and consequently their naming is incorrect. Phylogenetic data suggest that the

P. moranensis and *P. rectifolia* complexes can be differentiated on the base of spur size (>50% longer than the rest of the corolla in *P. moranensis*, no. 31), shape of the lateral corolla lobe (exhibiting a twist in *P. rectifolia*, no. 27), and corolla color (*P. moranensis* and *P. laueana* have pink to red lobes with no blue tinge while *P. rectifolia*, *P. species* 'Huahuapan' and *P. species* 'Pachuca' have pure blue or purple/blue lobes, no. 22). *P. rectifolia* also shares with *P. emarginata* and *P. species* 'Huahuapan' the presence of a purple speck at the base of its leaves. A complete revision of section *Orcheosanthus* and allies to which these species belong is currently underway (Zamudio, 1999; S. Zamudio, Universidad Nacional Autonoma de Mexico, personal communication) and is, therefore, essential.

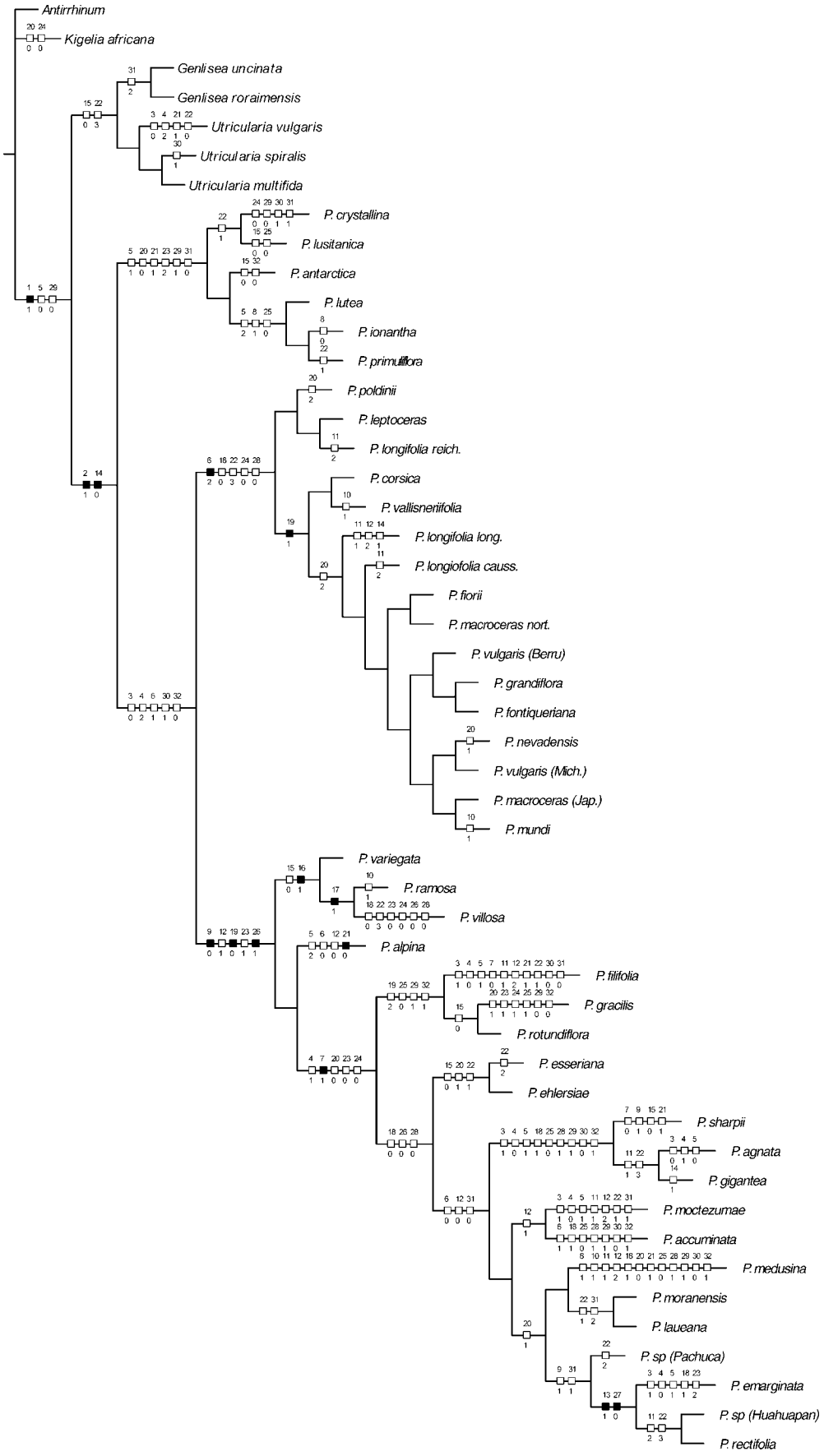
Relationships among northeastern Asian species (clade III)—All three northeastern Asian species of *Pinguicula* (*P. ramosa*, *P. villosa*, and *P. variegata*) belong to a single clade (III), for which peduncles densely covered by mucilage glands (no.16-1) is inferred as synapomorphy (Fig. 4). This feature was already used in the first treatment of the genus (Sprengel, 1825) to differentiate *P. villosa* from western European species (clade III and IV) even though disregarded by later authors (De Candolle, 1844; Barnhart, 1916; Ernst, 1961; Casper, 1966). At least in cultivation, these three species catch more than 90% of their prey with their peduncles (unpublished data). The sister group relationship of *P. villosa* and *P. ramosa* is supported by the ability to generate forked flower stems (no. 17-1). From the other two species, *P. villosa* differs in having a nearly circumboreal distribution (only absent from the western part of Norway to eastern Canada; Torbjorn, 2000). Outside the boreal regions, it has only been reported in Korea (Lee, 1993). Interestingly, *P. villosa* shares six floral morphology character states (nos. 18-0, 22-3, 23-0, 24-0, 26-0, 28-0) with members of clade IV but not with members of its own clade (Appendix S3, see Supplemental Data with online version of article). This explains why previous taxonomic treatments (De Candolle, 1844; Casper, 1966) grouped *P. villosa* with members of clade IV. Further molecular and cytological analyses are needed to clarify if events such as ancient hybridization have played a role in such striking patterns of homoplasy.

Relationships among European Pinguicula species (clade IV)—Section *Pinguicula* (Casper, 1966) comprises all Eurasian mountain species except *P. alpina*. It is well supported by *matK/trnK* sequence data and morphology and is the only species-rich section found to be monophyletic. A synapomorphy for this clade (IV) is the ability of its species to reproduce vegetatively during winter dormancy (no. 6-2). Unlike other *Pinguicula* species, members of clade IV frequently undergo vegetative reproduction in their native habitats. This is a mode for rapid range extension (this clade indeed comprises the most widespread species) that, at least partly, explains the short branch lengths within the Eurasian clade. Morphological differences within clade IV are mainly found in leaf characters. *Pinguicula nevadensis*, for example, has prostrate rosettes of ovate leaves that are 4–6 cm in diameter, while *P. vallisneriifolia*, which occurs a few hundred kilometers more south in Spain, has 20–25 cm long, linear, and erect leaves.

Both MP and BI suggest that the Eurasian species belong to two lineages differing by the shape of hairs in their flower throats (no. 19) and their geographic distribution patterns. The subclade of *P. leptoceras*, *P. longifolia* subsp. *reichenbachiana* and *P. poldinii* is centred to northern Italy, whereas the other members of clade IV have a distribution that is either restricted to Spain, or is extremely wide in the northern hemisphere sometimes only extending into the Iberian peninsula. In addition, there are some narrow endemics, such as *P. fiorii*, *P. longifolia* subsp. *caussensis*, and *P. Corsica*, which are found in Italy, southern France, and Corsica, respectively. *Pinguicula fontiqueriana* which grows in northern Africa, South Gibraltar (Romo et al., 1996) has close affinities to *P. grandiflora* (Figs. 1, 2). A similar split between lineages into one with an eastern (eastern Alps, Italy) and another with a western (western Alps and Iberian Peninsula) range of distribution has been observed in several other herbaceous genera of the high altitude European alpine regions, such as *Anthyllis*, *Soldanella*, and *Primula* (Comes and Kadereit, 2003). These patterns have been hypothesized to result from diversification during the Pleistocene period (Comes and Kadereit, 2003; Kropf et al., 2003) following a range restriction into refugia in Italy and Spain during the glaciations of the Quaternary (Taberlet et al., 1998; Hewitt, 2000). This partly accounts for the known differences between the floras present on each side of the ''Alpes Maritimes'' state on the western end of the French Alps (Kropf et al., 2002). The current wide distribution of *P. vulgaris* (Appendix) suggests that a rapid colonization of the North Atlantic region took place (Gugerli and Holderegger, 2001; Brochmann et al., 2003). The present range of the closely allied *P. macroceras* in north Eastern Asia (Japan) is also most likely to be explained by ''short-distance-dispersed'' circumpolar spreading (Bronken et al., 2001). The occurrence of *P. vulgaris* and *P. macroceras* in North America similarly appears to result from recent spreading via a Beringian connection (Miocene to Oligocene; Tiffney and Manchester, 2001), contrary to a supposedly much older New and Old-World split within clade V. A better understanding of migration patterns, however, has to await a more detailed analysis with high resolving molecular markers.

Furthermore, *matK/trnK* data support a reclassification of the three subspecies of *P. longifolia* into distinct species (Figs. 1, 2). This is also based on earlier finding that *P. leptoceras* and *P. longifolia* subsp. *reichenbachiana* on the one hand (Casper, 1966) and *P. longifolia* subsp. *longifolia* and *P. vallisneriifolia* on the other (Blanca et al., 1999) have closer affinities than the three subspecies of *P. longifolia* to each other.

Relationships among species with a tropical growth type (clade V)—The relationship between species from southeastern North America and the South American Andes with the Mediterranean *P. crystallina* and the northeastern Atlantic coast dweller *P. lusitanica* (tropical growth type clade; Figs. 1, 2) is well supported by molecular and also substantiated by morphological data. Our results confirm the addition of *P. crystallina* (sect. *Cardiophyllum*) to subgenus *Isoloba* by Casper (1966) despite the non-isolobic corollas of its flowers. *Pinguicula antarctica* is resolved within a clade consisting of members of subg. *Isoloba* (Fig. 1) and not subg. *Temnoceras* as classified by Casper (1966). *Pinguicula crystallina* differs from other members of clade V by four floral attributes (nos. 24-0, 29-0, 30-1, 31-1), but these appear homoplastic with respect to members of clade IV (Fig. 4). This species also shows a long branch in the phylogram and three autapomorphic indels (Fig. 3). The range of *P. crystallina* covers the North Mediterranean coast from southeastern France to Lebanon. Its


Antirrhinum
Kigelia africana
Genlisea uncinata
Genlisea roraimensis
Utricularia vulgaris
Utricularia spiralis
Utricularia multifida
P. crystallina
P. lusitanica
P. antarctica
P. lutea
P. ionantha
P. primuliflora
P. poldinii
P. leptoceras
P. longifolia reich.
P. corsica
P. vallisneriifolia
P. longifolia long.
P. longiofolia causs.
P. fiorii
P. macroceras nort.
P. vulgaris (Berru)
P. grandiflora
P. fontiqueriana
P. nevadensis
P. vulgaris (Mich.)
P. macroceras (Jap.)
P. mundi
P. variegata
P. ramosa
P. villosa
P. alpina
P. filifolia
P. gracilis
P. rotundiflora
P. esseriana
P. ehlersiae
P. sharpii
P. agnata
P. gigantea
P. moctezumae
P. accuminata
P. medusina
P. moranensis
P. laueana
P. sp (Pachuca)
P. emarginata
P. sp (Huahuapan)
P. rectifolia

sister taxon *P. lusitanica* grows on the European Atlantic coast from Ireland to Portugal and is only found inland in southeastern France near its border with Italy where it overlaps with *P. crystallina* north of the city of Marseilles (Casper, 1966). The current gap in distributions between species with SE USA ranges and the southern Andean *P. antarctica* is, therefore, to be explained by a lack of sampling of neotropical species.

Utility of matK/trnK sequence data for resolving Pinguicula relationships—In *Pinguicula*, *matK/trnK* data allow a robust identification of major clades. Contrary to *Utricularia–Genlisea*, where relationships among species were largely resolved even using intron or coding region sequences alone, combined *matK/trnK* sequences do not provide sufficient information within some clades (e.g., clade IV). At the species level, this is mainly explained by much higher substitutional rates in *Utricularia–Genlisea* (Müller and Borsch, 2005).

Within *Pinguicula*, several species exhibit conspicuously longer branch lengths than others (Fig. 3). These include *P. cystallina* subsp. *hirtiflora* (clade VI) and *P. villosa* (clade III). Moreover, branch lengths among most members of clade I (most obvious for *P. filifolia*, *P. sharpii*, and *P. medusina*) are higher than among the Eurasian taxa (clade IV). Most likely the lower genetic distances in the Eurasian clade (sect. *Pinguicula* according to Casper, 1966, Fig. 1) can in part be explained by a young radiation and rather rapid recent range extensions. However, relative rate tests that account for differences in divergence times, indicate that clade IV also exhibits significantly lower substitution rates compared to all other clades (Table 3). Differences are most significant when compared to the tropical-growth-type clade or the Mexican/Caribbean clade. Species with high substitution rates also account for most of the species-specific indels (Fig. 3) and, interestingly, are responsible for most of the homoplasy among morphological characters. A detailed discussion of rates and potential causes underlying the rate discrepancies is beyond the scope of this paper and will be presented elsewhere.

Evolution of morphological characters in Pinguicula—Diversity in *Pinguicula* includes growth patterns. An early diversification event has placed in sister clades species with a continuous growth type (termed tropical in this study, no. 3-0) under constant, and nonfreezing, temperatures all year (clade V and some members of clade I plus *Utricularia* and *Genlisea*) and species with noncarnivorous winter leaves that are reduced in size (termed temperate in this study, no. 3-1, clades I–IV). The latter can be seen as an adaptation to resist frost. Further differentiation of the winter buds in clade I has led them to resist drought (no. 4-1) in response to their Central American/Mexican environment. Temperate species have also further differentiated into those that multiply vegetatively during the winter and bloom from their summer rosette (clade IV) and those that do the reverse (clades II–III and many members of clade I). Unlike most other families of the Lamiales, species belonging to the Lentibulariaceae do not generate any central root system and give rise to plants that grow at one end and die on the other (no. 5). Within Lentibulariaceae, only *Pinguicula* species generate adventitious roots. Ancestral *Pinguicula* species most likely had nonfleshy roots, which evolved as more fleshy at least twice (clades II and V). These disappear in the winter in parallel with the expression of a temperate growth type (Appendix S3, see Supplemental Data with online version of article).

Pinguicula species exhibit a variety of vegetative reproduction modes that include the production of plantlets on the central veins near the tip of leaves or roots (no. 8-1, homoplastic between two members of clade V and one of clade I) or near the base of the plant (no. 6-1/2, synapomorphic for clades I, III, IV). Some members of clade I that were not accessible for this study (*P. stolonifera* for example) and *P. vallisneriifolia* (clade IV) bear their daughter plants on long stems (stolons). Only a few members of clades V and clade II do not reproduce vegetatively in the wild or after artificial induction. The number of plantlets produced by the rosettes of members of clade I wanes off in more terminal lineages to be replaced by a novel trait, the formation of plantlets at the base of detached leaves (no. 7-1).

A spade-shaped leaf type with round tips is most likely a more recent attribute to the genus *Pinguicula* that evolved once (present in clades I and III) unlike the ability to generate different types of leaves through the vegetative period (no. 10), which appeared in two species independently. However, the origin of characters such as a vertical leaf orientation (no. 11, with the accompanying loss of leaf edge curling) and the linearity of the leaf blade (no. 12) is hard to assess at this stage since they were found to be homoplastic based on the *trnK* trees (present in two *Pinguicula* clades. The production of digestive glands on the underside of the leaves (no. 14) in two species with erect leaves (*P. longifolia* subsp. *longifolia* and *P. gigantea*) appears to result from two separate evolutionary events according to our phylogeny. It is most likely an adaptation to increase trapping efficiency when leaves are held upright vertically.

The flowers of the outgroup species used in this study (and of other Lamiales families potentially related to Lentibulariaceae) differ from the flowers in Lentibulariaceae by having non-isolobic corollas with long cylindrical tubes, non-crenated lobes, and by lacking a spur. Members of the *Pinguicula* clades V and some species of clade I were the only species of our sampling to have isolobic corollas (no. 25-0) a trait that, therefore, appears as being derived several times in parallel. However, several other attributes of the corolla of members of clades V are plesiomorphic within *Pinguicula* such as the presence of an angle between the corolla tube and the spur (no. 32-1 shared with *Utricularia* and *Genlisea*), spurs with a bag shape (no. 30-0, shared with *Utricularia* and *Genlisea*), a long (no. 29-1, shared with outgroup species), and cylindrical (no. 28-1, only lost in clade IV and some members of clade I), tube and a palatum (no. 24-1, only lost in clade IV and some members of clade I). The evolution and origin of lobe crenation, a character that is only found in few *Pinguicula* species within the Lentibulariaceae, is more difficult to assess even though

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Fig. 4. State transformations of selected characters on the *Pinguicula* phylogeny, based on an optimization of morphological characters on the Bayesian tree topology assuming accelerated transitions. Numbers indicate the characters (according to Appendices S1 and S2, see Supplemental Data with online version of article; first number) and state (according to Appendix S3; second number after the dash). Black boxes represent non-homoplastic characters, open ones represent characters changing their state more than once.

used previously to support the creation of subgenus *Isoloba* and the grouping of southeast USA species (Wood and Godfrey, 1957; Godfrey and Stripling, 1961). Members of clades V harbour five crenated lobes except *P. crystallina*, which only has three (lower lip ones). The other *Pinguicula* species that have crenated lobes are *P. alpina* (clade II) and *P. gracilis* (clade I) and all members of clade III except *P. villosa*. All of these species have a single crenated lobe, the lower central one, which is at the same time significantly larger than the other lobes (no. 26-1).

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APPENDIX. Information on the species used in this study.

Species; Abbreviations used in Appendix S2 and S3 (in Supplemental Data with online version of this article); *Subgenus/Section* (Casper, 1966; Legendre, 2000) or family for outgroup; Origin (field/garden); *Voucher*; GenBank accession no.; Authors; Geographic distribution.

P. accuminata Benth.; Paccu; *Isoloba*/*heterophyllum*; Mexico; *L. Legendre 1081 (NSW);* DQ010652; this study; Mexico. ***P. agnata*** Casper; Pagna; *Isoloba*/*agnata*; Mexico; *L. Legendre 1021 (NSW)*; AF531782; Müller et al. (2004); Mexico. ***P. alpina*** L.; Palpi; *Temnoceras*/*micranthus*; Switzerland; *J. Steiger 17 (JE)*; AF531783; Müller et al. (2004); Europe/Asia.[1] ***P. antarctica*** Vahl; Panta; *Temnoceras*/*ampullipalatum*; Argentina; *L. Legendre 1076 (NSW)*; DQ010653; this study; South America. ***P. corsica*** Bern. & Gren. ex Gren. & Godr.; Pcors; *Pinguicula*/*pinguicula*; France; *J. Steiger 18 (JE)*; AF531784; Müller et al. (2004); Europe (Corsica). ***P. crystallina*** Sibth. ex Sibth. & Smith subsp *hirtiflora* (Ten.) Strid; Pcrys; *Isoloba*/*cardiophyllum*; Italy; *L. Legendre 1079 (NSW)*; DQ010654; this study; North Mediterranean.[2] ***P. ehlersiae*** Speta & Fuchs; Pehle; *Pinguicula*/*crassifolia*; Mexico; *L. Legendre 1082 (NSW)*; DQ010655; this study; Mexico. ***P. emarginata*** Zamudio Ruiz & Rzedowski; Pemar; *Temnoceras*/*temnoceras*; Mexico; *L. Legendre 1026 (NSW)*; AF531785; Müller et al. (2004); Mexico. ***P. esseriana*** Kirchner; Pesse; *Pinguicula*/*crassifolia*; Mexico; *L. Legendre 1083 (NSW)*; DQ010656; this study; Mexico. ***P. filifolia*** Wright ex Griseb.; Pfili; *Isoloba*/*agnata*; Cuba; *L. Legendre 1022 (NSW)*; AF531786; Müller et al. (2004); Cuba. ***P. fiorii*** Tammaro & Pace; Pfior; *Pinguicula*/*pinguicula*; Italy; *J. Steiger 45 (JE)*; AF531787; Müller et al. (2004); Europe (Italy). ***P. fontiqueriana*** Romo, Peris & Stuebing; Pfont; *Pinguicula*/*pinguicula*; Morocco; *J. Steiger 72 (NSW)*; AF531788; Müller et al. (2004); North Africa.[3] ***P. gigantea*** Luhrs; Pgiga; *Isoloba*/*agnata*; Mexico; *L. Legendre 1023 (NSW)*; AF531789; Müller et al. (2004); Mexico. ***P. gracilis*** Zamudio Ruiz; Pgrac; *Temnoceras*/*temnoceras*; Mexico; *L. Legendre 1027 (NSW)*; AF531790; Müller et al. (2004); Mexico. ***P. grandiflora*** Lam. f. pallida (Gaud.) Casper; Pgran; *Pinguicula*/*pinguicula*; France; *J. Steiger 7b (JE)*; AF531791; Müller et al. (2004); Europe (France–Switzerland). ***P. ionantha*** Godfr.; Piona; *Isoloba*/*isoloba*; USA; *L. Legendre 1077 (NSW)*; DQ010658; this study; SE USA. ***P. laueana*** Speta & Fuchs; Plaue; *Pinguicula*/*longitubus*; Mexico; *L. Legendre 1095 (NSW)*; DQ010659; this study; Mexico. ***P. leptoceras*** Rchb.; Plept; *Pinguicula*/*pinguicula*; Italy; *J. Steiger 30a (JE)*; AF531792; Müller et al. (2004); Europe (Alps in Italy-France-Switzerland-Austria). ***P. longifolia*** Ram. ex DC. subsp *caussensis* Casper; Plcau; *Pinguicula*/*pinguicula*; France; *J. Steiger 20 (JE)*; AF531793; Müller et al. (2004); Europe (France). ***P. longifolia*** Ram. ex DC. subsp longifolia; Pllon; *Pinguicula*/*pinguicula*; France; *L. Legendre 1032 (NSW)*; AF531794; Müller et al. (2004); Europe (France-Spain). ***P. longifolia*** Ram. ex DC. subsp *reichenbachiana* (Schindler) Casper; Plrei; *Pinguicula*/*pinguicula*; Italy; *L. Legendre 1078 (NSW)*; DQ010660; this study; Europe (Italy–France). ***P. lusitanica*** L.; Plusi; *Isoloba*/*isoloba*; France; *L. Legendre 1094 (NSW)*; DQ010661; this study; Europe (Atlantic in Ireland, UK, France, Spain, Portugal). ***P. lutea*** Walt.; Plute; *Isoloba*/*isoloba*; USA; *L. Legendre 1084 (NSW);* DQ010662; this study; SE USA. ***P. macroceras*** Link 'Japan'; Pmjap; *Pinguicula*/*pinguicula*; Japan; *J. Steiger 29b (JE)*; AF531796; Müller et al. (2004); North pacific (Japan-Russia-Canada-USA). ***P. macroceras*** Link subsp nortensis Steiger ex Steiger & Rondeau; Pmno; *Pinguicula*/*pinguicula*; NW USA; *L. Legendre 1033 (NSW)*; AF531795; Müller et al. (2004); North Pacific (Japan-Russia-Canada-USA). ***P. medusina*** Zamudio Ruiz & Studnicka; Pmed; *Isoloba*/*heterophyllum*; Mexico; *L. Legendre 1080 (NSW)*; DQ010663; this study; Mexico. ***P. moctezumae*** Zamudio Ruiz & R. Z.Ortega; Pmoc; *Pinguicula*/*orcheosanthus*; Mexico; *L. Legendre 1025 (NSW)*; AF531797; Müller et al. (2004); Mexico. ***P. moranensis*** H. B. K.; Pmor; *Pinguicula*/*orcheosanthus*; Mexico; *L. Legendre 1029 (NSW)*; AF531798; Müller et al. (2004); *Mexico*. ***P. mundi*** Blanca, Jamilena, Ruiz-Rejon & Zamora; Pmun; *Pin-*

guicula/*pinguicul*; Spain; *J. Steiger 22 (JE)*; AF531800; Müller et al. (2004); Europe (Spain). ***P. nevadensis*** (Lindbg.) Casper; Pneva; *Pinguicula*/*pinguicula*; Spain; *L. Legendre 1086 (NSW)*; DQ010664; this study; Europe (Spain). ***P. poldinii*** Steiger & Casper; Ppold; *Pinguicula*/*pinguicula*; Italy; *J. Steiger 49 (JE)*; AF531804; Müller et al. (2004); Europe (Italy). ***P. primuliflora*** Wood & Godfr.; Pprim; *Isoloba*/*isoloba*; USA; *L. Legendre 1090 (NSW)*; DQ010666; this study; SE USA. ***P. ramosa*** Miyoshi ex Yatabe; Pram; *Temnoceras*/*micranthus*; Japan; *L. Legendre 1085 (NSW)*; DQ010667; this study; Japan. ***P. rectifolia*** Speta & Fuchs; Prect; *Pinguicula*/*orcheosanthus*; Mexico; *L. Legendre 1031 (NSW)*; AF531801; Müller et al. (2004); Mexico. ***P. rotundiflora*** Studnicka; Protu; *Isoloba*/*heterophyllum*; Mexico; *L. Legendre 1028 (NSW)*; AF531802; Müller et al. (2004); Mexico. ***P. sharpii*** Casper & Kondo; Pshar; *Isoloba*/*isoloba*; Mexico; *L. Legendre 1024 (NSW)*; AF531803; Müller et al. (2004); Mexico. ***P. vallisneriifolia*** Webb; Pvall; *Pinguicula*/*pinguicula*; Spain; *J. Steiger 23 (JE)*; AF531805; Müller et al. (2004); Europe (Spain). ***P. variegata*** Turcz.; Pvari; *Temnoceras*/*micranthus*; Russia; *L. Legendre 1087 (NSW)*; DQ010668; this study; E Siberia. ***P. villosa*** L.; Pvill; *Pinguicula*/*nana*; Norway; *L. Legendre 1088 (NSW)*; DQ010669; this study; Arctic.[4] ***P. vulgaris*** L. 'Berru'; Pvber; *Pinguicula*/*pinguicula*; France; *L. Legendre 1034 (NSW)*; AF531806; Müller et al. (2004); All Europe-USA-Canada. ***P. vulgaris*** L. 'Michigan'; Pvmic; *Pinguicula*/*pinguicula*; USA; *L. Legendre 1035 (NSW)*; AF531807; Müller et al. (2004); All Europe-USA-Canada. ***P.* sp 'huahuapan'**[5]; Phuah; *Pinguicula*/?; Mexico; *L. Legendre 1089 (NSW)*; DQ010657; Müller et al. (2004); Mexico. ***P.* sp 'pachuca'**[6]; Ppach; *Pinguicula*/?; Mexico; *L. Legendre 1091 (NSW)*; DQ010665; this study; Mexico. ***G. uncinata*** Taylor and Fromm-Trinta; Gunci; *Lentibulariaceae*; *K. Müller 730*; AF531819; Müller et al. (2004); Africa. ***G. roraimensis*** Brown; Grora; *Lentibulariaceae*; *K. Müller 729*; AF531817; Müller et al. (2004); South America. ***U. multifida*** Br.; Umult; *Lentibulariaceae*; *K. Müller 719*; AF531848; Müller and Borsch (subm.); Australia. ***U. spiralis*** Sm.; Uspir; *Lentibulariaceae*; *S. Porembski 3853 (ROST)*; AF531851; Müller and Borsch (subm.); Africa. ***U. vulgaris*** L.; Uvulg; *Lentibulariaceae*; *K. Müller 743*; AF531831; Müller and Borsch (subm.); Europe. ***Antirrhinum majus* L.**; Antir; *Plantaginaceae*; —; —; AF051978; Young et al. (1999); Europe. ***Kigelia Africana*** Benth.; Kigel; *Bignoniaceae*; —; —; AF051988; Young et al. (1999); Africa.

[1] *P. alpina* distribution: Pyrenees, Alps, Scotland, Norway, Sweden, Finland, Ural, Lapland, Siberia, Caucasus, Himalaya
[2] *P. crystallina* subsp. *hirtiflora* distribution: Eastern France, Italy, Balkans, Greece, Turkey, Lebanon
[3] *P. fontiqueriana* distribution: northern tip of Morocco
[4] *P. villosa* distribution: Norway to eastern Siberia, Alaska, Canada (except Quebec)
[5] Described under the name *P. moranensis* by A. Slack (Meyers-Rice and Schlauer, 2000)
[6] No formal description. Horticultural trade name. Marketed under the name *P. moranensis*