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AFLP Phylogeny of South American Species of *Hypochaeris* (Asteraceae, Lactuceae)

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ABSTRACT. *Hypochaeris* is thought to have arrived in South America by dispersal over the Atlantic Ocean from NW Africa during the Pliocene or Pleistocene. We used amplified fragment length polymorphism (AFLP) to unravel specific limits and relationships in the South American group of *Hypochaeris* (c. 45 species). The Moroccan endemic *Hypochaeris angustifolia*, which is sister to the entire South American group, was used as outgroup. Our AFLP analysis comprises 415 individuals from 32 South American species and is based on six primer combinations with 670 fragments scored. It provides important information for the delimitation of species and detection of closely related species pairs or groups. Most species are monophyletic and supported with > 90% bootstrap proportion. Hybridization is suggested between *Hypochaeris chillensis* and *H. microcephala* in Brazil. The internal nodes (or ''backbone'') of the tree are not highly supported, but six major phylogenetic groups (also showing similarity in distribution and growth form) center around *Hypochaeris apargioides*, *H. chondrilloides*, *H. microcephala*, *H. pampasica*, *H. sessiliflora*, and *H. tenuifolia*. These results suggest that rapid migration into different geographical regions played an important role in the initial diversification of *Hypochaeris* in South America.

KEYWORDS: amplified fragment length polymorphism, Compositae, *Hypochaeris*, South America.

The herbaceous genus *Hypochaeris* L. has a disjunct distribution, with more than 15 species in the Mediterranean region, the Canary Islands, Europe, and Asia, and c. 45 species in South America. It originated in the Old World and most probably arrived in South America after long-distance dispersal from NW Africa (Tremetsberger et al. 2005). After arrival, *Hypochaeris* has colonized most parts of South America except the tropical rainforest and the extreme arid areas of the coastal deserts, growing in the Subantarctic domain (southern tip of the continent), the Andean-Patagonian domain, the domain of the Chaco, and the Amazonian domain (Cabrera and Willink 1980), from sea level to Andean habitats of more than 5,000 m (Urtubey et al. in press). It reaches Colombia and Venezuela in the N and has diversified morphologically and ecologically (e.g., Bortiri 1999). Most species have upright or ascending stems, but acaulescent rosulate species with a short or lacking peduncle of the head also exist. The corollas are usually yellow, though white or pink in some species. Most species are diploids ($2n = 8$), but some species have both diploid and tetraploid individuals, sometimes occurring in mixed populations (Weiss et al. 2003 and unpubl.). Low DNA sequence divergence among species of this monophyletic South American group of *Hypochaeris* suggests that it is very young (Samuel et al. 2003), trans-Atlantic dispersal having taken place ~3.5 or fewer million years ago (i.e., during the Pliocene or Pleistocene; Tremetsberger et al. 2005).

Radiations such as that seen in *Hypochaeris* in South America represent an important evolutionary phenomenon. Examples of adaptive radiation in plants most commonly involve groups on islands or in island-like scenarios, such as *Dendroseris* on the Juan Fernández Islands in the S Pacific ocean (Sang et al. 1994), the woody *Sonchus* alliance in Macaronesia (Kim et al. 1999), and the Hawaiian silversword alliance (Baldwin 1997; Baldwin and Sanderson 1998). On continents, fewer examples are known, one of them being the *Espeletia* complex (Asteraceae) in the Andean desert páramo (Monasterio 1986; Rauscher 2002). Our focus has been on the recently evolved and species-rich group of *Hypochaeris* in South America. Intraspecific phylogeographies have been published for the Argentinian and Chilean species *H. acaulis* (J. Rémy) Britton (Tremetsberger et al. 2003a), *H. palustris* (Phil.) De Wild. (Muellner et al. 2005), and *H. tenuifolia* (Hook. et Arn.) Griseb.

(Tremetsberger et al. 2003b), which shed light on the biogeographical effect and stimulus for speciation and intraspecific differentiation of the Pleistocene glaciations in the S Andes.

Detailed knowledge of specific limits and relationships in *Hypochaeris* of South America is needed to address questions regarding mechanisms of speciation. Previous taxonomic treatments have been delimited by regional or national frontiers (e.g., Bortiri 1999 and Cabrera 1937, 1941, 1963, 1976 for Argentinian species; Azevêdo-Gonçalves 2004 for Brazilian species). Highly resolving molecular markers may provide sufficient information to unravel relationships among closely related species. However, DNA sequences widely used for analysis of closely related plant species, such as ITS and *matK* have only provided limited information for revealing specific relationships among *Hypochaeris* in South America (Samuel et al. 2003). The highly resolving DNA fingerprinting technique, amplified fragment length polymorphism (AFLP; Vos et al. 1995), has proven useful for resolving interspecific relationships in diverse plant groups (e.g., Abdalla et al. 2001; Beardsley et al. 2003; Després et al. 2003; Hodkinson et al. 2000; Kardolus et al. 1998; Koopman et al. 2001; Pelser et al. 2003; Pfosser et al. 2002; Spooner et al. 2005) as well as in a rapidly evolving clade of cichlid fishes of Lake Malawi (Albertson et al. 1999). Despite concern that AFLP fragments of the same length seen in two species might not be homologous, several authors suggest that the AFLP method can be used for phylogenetic reconstructions, especially for species groups that have diverged recently or radiated within a short period of time (e.g., Bensch and Åkesson 2005; Bussell et al. 2005; Koopman 2005). AFLPs have already been successfully applied to a reduced sample of *Hypochaeris* in South America (14 species; Stuessy et al. 2004) and to Old World species of *Hypochaeris* sect. *Hypochaeris* (Tremetsberger et al. 2004). The results of both studies suggested that AFLPs are appropriate markers at the interspecific level and Stuessy et al. (2004) recommended continued sampling throughout the South American continent.

We therefore analyzed AFLPs of 415 individual plants in populations of 32 species of South American species of *Hypochaeris*. We have attempted to provide a basic framework of relationships among these species, which would allow further detailed investigations on processes of adaptation and evolution in this group. Thus, the aims of the present study among the South American species of *Hypochaeris* were to (1) test specific limits based previously on morphology, and (2) determine phylogenetic relationships among these species.

Materials and Methods

Plant Material. A total of 32 South American species has been analyzed with AFLP (Appendix 1) with a minimum of three and a maximum of 31 individuals per species (in the polymorphic *Hypochaeris sessiliflora* Kunth). We analysed individuals from throughout the distributional area of many species. As an outgroup we used the Moroccan *Hypochaeris angustifolia* (Litard. and Maire) Maire, which has been shown to be sister to the entire South American group (Tremetsberger et al. 2005). Leaves were placed in silica-gel in the field and brought to the laboratory for DNA extraction.

AFLP. Total DNA was extracted from dry leaf material according to a modified CTAB-protocol and quality-checked on 1% TAE-agarose gels as in Tremetsberger et al. (2003b). Analysis of amplified fragment length polymorphism (Vos et al. 1995) was carried out according to the PE Applied Biosystems (1996) protocol (see also Tremetsberger et al. 2004). To obtain higher specificity of amplified fragments, we conducted two preselective amplifications. The *Eco*RI-primer had one additional base in both cases (*Eco*RI+A). The *Mse*I-primer had one [*Mse*I+C; used in the next step, the selective amplification, with primers with three selective bases (*Mse*I+CNN)] or two additional bases [*Mse*I+CT; used in the selective amplification with primers with four selective bases (*Mse*I+CTNN)]. The six selective primer combinations used are *Mse*I+CAG/*Eco*RI+ACT (FAM), *Mse*I+CAG/*Eco*RI+AGC (NED), *Mse*I+CTC/*Eco*RI+ACG (HEX), *Mse*I+CTGA/*Eco*RI+ACT (FAM), *Mse*I+CTCG/*Eco*RI+ATC (NED), and *Mse*I+CTTC/*Eco*RI+ACG (HEX). The fluorescence-labelled selective amplification products were run on a 5% denaturing polyacrylamide gel on an automated sequencer (ABI 377, Perkin Elmer).

Genetic Data Analysis. The internal size standard (GeneScan 500 ROX; Applied Biosystems) was adjusted for each sample with GeneScan ver. 3.1.2 (Applied Biosystems, 1989–2000). Due to the large number of individuals analysed (415), presence or absence of fragments from 50 to 500 bp in each individual was scored with Genographer ver. 1.1.0 (Montana State University, 1998) in two steps. A first analysis included a few individuals from all putative taxonomic units. This analysis also included *Hypochaeris angustifolia* as an outgroup and was used to determine major phylogenetic groups among the South American species. The second series of analyses was carried out for each major group determined in the first step separately and included all individuals from the respective phylogenetic group. The presence/absence matrices from each step were exported for further analyses and deposited in TreeBASE (study accession number S1288).

Koopman (2005) demonstrated the presence of phylogenetic signal in AFLP data despite several drawbacks such as possible non-independence of fragments, problems of homology assignment of fragments, asymmetry in the probability of losing and gaining fragments, and problems in distinguishing heterozygote from homozygote bands. Although AFLP phenograms based on the neighbour-joining clustering algorithm do not necessarily represent phylogenetic relationships, the phenograms can be interpreted in context of phylogenetic relationships among the taxa (e.g., Futuyma, 1998, p. 94). This interpretation rests on the assumption that similarities among taxa due to shared derived character states outnumber similarities due to shared ancestral or homoplasious character states (Futuyma, 1998). Sequencing of AFLP fragments and determination of their genomic location yielded very different levels of non-homology (e.g., Rouppe van der Voort et al. 1997; Santos and Simon 2002; Mechanda et al. 2004). However, Adams and Rieseberg (1998) demonstrated for RAPD bands in *Brassica* and *Helianthus* that non-homology introduces random errors in the data set, which merely reduce the absolute similarities, but not the relative similarities nor the relationships among the taxa. This finding should also apply to other arbitrarily amplified DNA markers such as AFLP (Bussell et al. 2005).

O'Hanlon and Peakall (2000) experimentally demonstrated that the amount of homoplasy of AFLP fragments is less among congeners of *Cynara* and *Onopordum* than at higher taxonomic level in the Carduinae thistles. In general, it is intuitively obvious that the amount of homoplasy will be less the more closely related the genotypes are. In other words, higher confidence in inferred phylogenetic relationships is to be expected for the terminal, rather than for the basal, nodes of the tree. For instance, in Lactuceae,

strong support was obtained for the terminal branches in an AFLP study of 20 species of *Lactuca* s.l. and related genera, but the basal nodes were resolved rather poorly (Koopman et al. 2001; see Bussell et al. 2005 for a review). In a literature survey, in which Koopman (2005) tested the congruence of AFLP and ITS tree topologies, the author found that AFLP markers are likely to be phylogenetically informative at a level of ITS sequence divergence of up to 30 bp in plants. Internal transcribed spacer (ITS1 and ITS2) sequence divergence among 13 South American species of *Hypochaeris* analysed in Tremetsberger et al. (2005) varies from a minimum of 0 bp (among *H. apargioides*, *H. clarionoides*, *H. gayana*, and *H. thrincioides*) to a maximum of 15 bp (between *H. acaulis* and *H. microcephala*). Minimum and maximum ITS sequence divergence between *H. angustifolia* and any South American species is 33 and 37 bp, respectively, counting each indel as one difference, irrespective of its length. Thus, AFLP should be an adequate tool for detecting phylogenetic relationships among South American *Hypochaeris*.

We therefore used the neighbour-joining method with Nei-Li distances implemented in PAUP* ver. 4.0b10 (Swofford 2003) for the construction of trees from the AFLP matrices. The robustness of specific nodes was assessed using the bootstrap method (Felsenstein 1985) using 10,000 replicates also with PAUP* ver. 4.0b10 (Swofford 2003). The phenograms show overall genetic relationships among all individuals analysed; only in Figs. 1 and 3, which were rather crowded, clusters with all individuals belonging to the same species or population were condensed using TreeExplorer ver 2.12 (Tamura 1997–1999). In addition, we also carried out a Principal Coordinates Analysis with R package ver. 4.0 (Casgrain and Legendre 2000; not shown; the first four Eigenvectors contain 11.7%, 9.0%, 7.1%, and 5.3% of variance, respectively), but it did not provide any additional information that was not contained in the neighbour-joining trees.

Results

In the first analysis (including all putative South American taxa of *Hypochaeris* plus the outgroup *H. angustifolia*), six primer combinations generated 670 unambiguously scorable fragments between 50 and 500 bp with 665 fragments (99.3%) being polymorphic [620 out of 630 fragments (98.4%) polymorphic when *H. angustifolia* is excluded]. AFLP analysis of individuals from populations throughout the distributional ranges of South American species allowed determination of mono- or paraphyly (sensu Hennig 1966) of these species. Twenty-four out of the 32 studied species are monophyletic and receive more than 90% bootstrap support (BS; Table 1).

Phylogenetic Relationships. Twenty-six species of *Hypochaeris* in South America can be grouped into six clusters (Figs. 1–6; Table 2). In addition to their genetic similarity, the species in these clusters are also united by other shared characters (e.g., their geographical distributions and/or growth forms; see below). The Apargioides group and Pampasica group cluster together (28% BS), and this latter cluster together with the associated *H. petiolaris* (Hook. et Arn.) Griseb. and *H. variegata* (Lam.) Baker clusters with the Microcephala group (41% BS), a relationship that is also supported by DNA sequence analysis (ITS and ETS; Tremetsberger et al. unpubl.). Two species, *Hypochaeris lutea* (Vell.) Britton and *H. scorzonerae* (DC.) F. Muell., branch off from deep nodes of the tree and are not associated with any of the groups (Fig. 1). *Hypochaeris caespitosa* Cabrera is weakly associated with the Tenuifolia group (22% BS) and *H. elata* (Wedd.) Griseb. is weakly associated with the Sessiliflora group (38% BS; see below).

Discussion

Delimitation of Species. Our AFLP results corroborate a recent morphological analysis of Brazilian species of *Hypochaeris* (Azevêdo-Gonçalves 2004), which led to taxonomic rearrangements at the specific level (Azevêdo-Gonçalves and Matzenbacher 2005). These include the recognition of *Hypochaeris rosengurttii* Cabrera var. *rosengurttii* as a synonym of *H. lutea* (Vell.) Britton, *Hypochaeris pinnatifida* (Speg.) Azevêdo-Gonçalves & Matzenbacher as a distinct species rather than as a variety of *H. rosengurttii* (Cabrera, 1941), and *Hypochaeris albiflora* (Kuntze) Azevêdo-Gonçalves & Matzenbacher also as a distinct species rather than as a variety of *H. microcephala* (Sch. Bip.) Cabrera (Cabrera 1937). The AFLP phenograms clearly show that *Hypochaeris lutea* is not closely related to *H. pinnatifida* (Fig. 1) and *H. albiflora* not close to *H. microcephala* (Figs. 1, 4).

Eight out of the 32 studied species are not monophyletic. *Hypochaeris palustris* is one of them. It is represented in two separate groups and paraphyletic (monophyletic only if *H. acaulis* was included; Figs. 1, 2). Andean populations of *H. palustris* are genetically differentiated from those of the Coastal Cordillera in Chile (forming two monophyletic groups with 100% BS each). The observation of a large genetic differentiation between populations of *H. palustris* from the Andes and the Coastal Cordillera, which are separated by the Longitudinal Valley, was already made by Muellner et al. (2005) in an infraspecific phylogeographical study that aimed to elucidate potential Pleistocene refugia and recolonization routes in the S Andes. The authors concluded that the Coastal Cordillera served as a refugium for *H. palustris* during the last glacial maximum, but that these populations did not contribute to the recolonization of the central Andean ranges after their deglaciation. Rather, the central ranges of the S Andes were recolonized from several nearby refugia. Our results show that Andean populations of *H. palustris* are sister to *H. acaulis* (82% BS; Fig. 2) and thus the likely extant progenitors of the latter species. On morphological grounds, there is no reason to taxonomically separate the two populational systems of *H. palustris*. This species can be easily recognized by thick adventitious roots among other features, but *H. acaulis* is very different from *H. palustris*, in growth form (acaulescent vs. erect), head (hemispheric vs. cylindric-campanulate), involucral bracts (ovate vs. linear-lanceolate), and other features.

In the Andes, *H. acaulis* grows largely sympatrically with *H. palustris*, but *H. palustris* is more widespread.

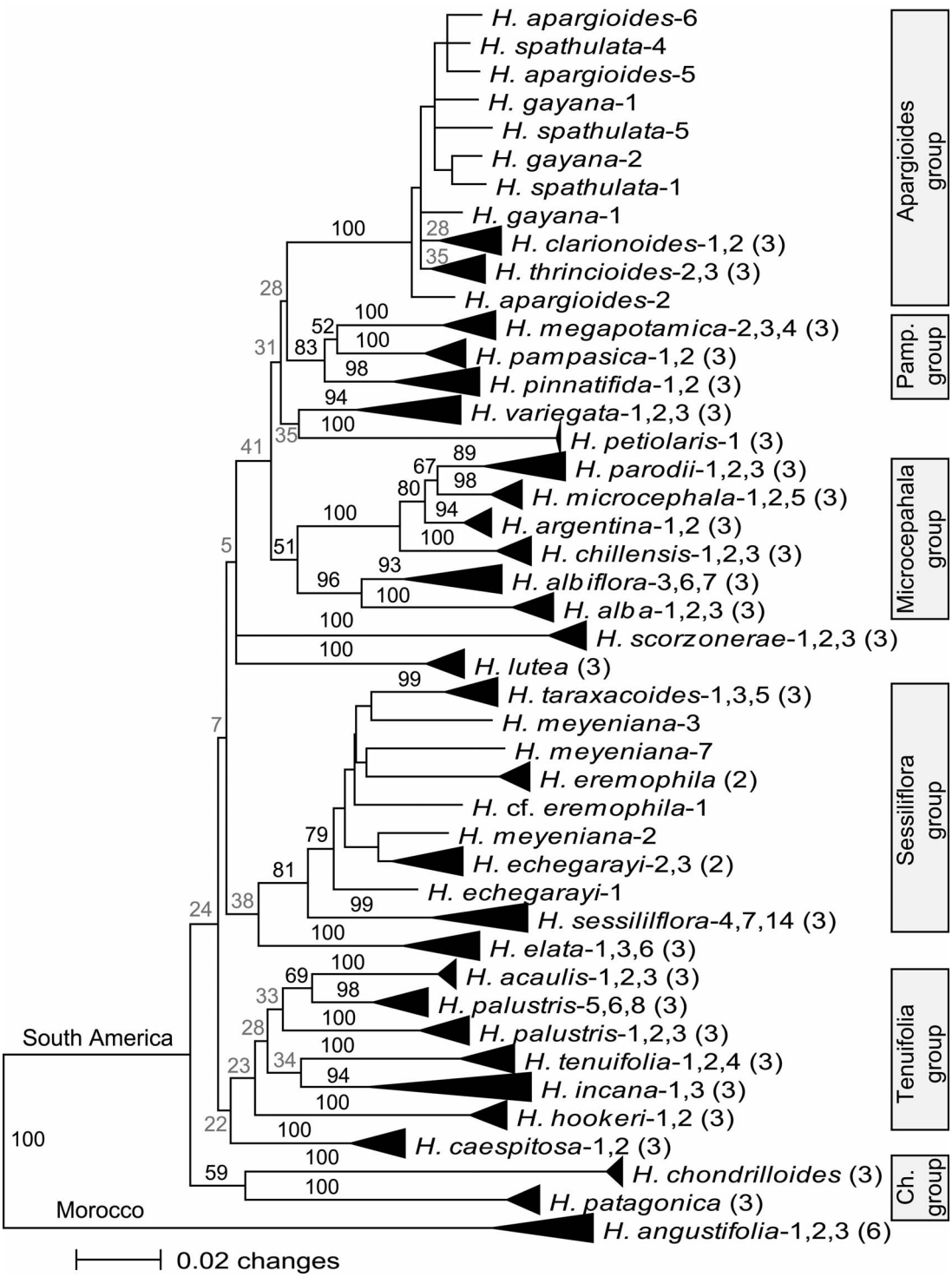

FIG. 1. 50% majority rule consensus tree including groups compatible with it of 10,000 bootstrap replicates of South American species of *Hypochaeris* (each represented by three individuals) and *H. angustifolia* as outgroup based on neighbour-joining analysis of 670 AFLP fragments. Numbers on branches are bootstrap proportions (below 50% in grey). Numbers after names of species give populations (see Appendix 1) from which the sampled individuals (number in parentheses) in a condensed cluster have come. Scale bar indicates 2% character difference (c. 13 fragments). Ch. group—Chondrilloides group; Pamp. group—Pampasica group.

TABLE 1. South American species of *Hypochaeris* that have been resolved as monophyletic entities by AFLP analysis (Figs. 1–6) and their bootstrap support (BS).

Species	BS (%)
H. acaulis (J. Rémy) Britton	100
H. alba Cabrera	100
H. albiflora (Kuntze) Azevêdo-Gonçalves & Matzenbacher	86–93
H. argentina Cabrera	84–94
H. caespitosa Cabrera	100
H. chillensis (Kunth) Hieron.	92–100
H. chondrilloides (A. Gray) Cabrera	100
H. clarionoides (J. Rémy) Reiche	28–91
H. elata (Wedd.) Griseb.	100
H. hookeri Phil.	100
H. incana (Hook. et Arn.) Macloskie	93–94
H. lutea (Vell.) Britton	100
H. megapotamica Cabrera	100
H. microcephala (Sch. Bip.) Cabrera	98
H. pampasica Cabrera	100
H. parodii Cabrera	89–99
H. patagonica Cabrera	100
H. petiolaris (Hook. et Arn.) Griseb.	100
H. pinnatifida (Speg.) Azevêdo–Gonçalves & Matzenbacher	98–99
H. scorzonerae (DC.) F. Muell.	100
H. sessiliflora Kunth	99–100
H. taraxacoides (Walp.) Benth. & Hook. f.	98–99
H. tenuifolia (Hook. et Arn.) Griseb.	99–100
H. variegata (Lam.) Baker	94–99

The latter grows from sea-level (in the subantarctic southernmost part of the continent, where it reaches the southern tip of the continent) to ~2100 m. Northwards, it reaches ~35°S. It grows in humid places, such as river banks, moist alpine meadows, and *Araucaria* and *Nothofagus* forests. *Hypochaeris acaulis* grows in the Andes from ~1400 to ~3000 m and from ~34° to ~39°S latitude in similar habitats as *H. palustris* (bogs, moist alpine meadows, river banks and *Araucaria* forests; Urtubey unpublished). Tremetsberger et al. (2003a) studied the infraspecific genetic variation in *H. acaulis*. The authors report low within-populational variation consistent with the facultatively autogamous breeding system and high among-populational differentiation and suggest that restricted gene flow among isolated populations might result from the species' limited dispersal capability. Since the present localities of *H. acaulis* are all situated north of the continuous ice-shield that covered the Andes from 39° southwards during the last glacial maximum, it might have outlived local Pleistocene glaciations in situ without having expanded its range after deglaciation. Without hybridizing, *H. palustris* and *H. acaulis* may grow together in the same locality in the overlapping parts of their distributional ranges (i.e., from ~35° to ~39°S). Sympatric populations have been found in Chile, region VII, Laguna del Maule in a moist alpine meadow at 2390 m (*H. acaulis TS & CB 15571* and *H. palustris TS & CB 15572*), in region VIII, Termas de Chillán in a moist alpine meadow at 2020 m (*H. acaulis TS, CB &*

TABLE 2. Major phylogenetic groups among the South American species of *Hypochaeris* resolved by AFLP analysis along with their bootstrap support (BS).

Groups	BS (%)
Apargioides group: *H. apargioides*, *H. clarionoides*, *H. gayana*, *H. spathulata*, *H. thrincioides*	100
Chondrilloides group: *H. chondrilloides*, *H. patagonica*	59
Microcephala group: *H. alba*, *H. albiflora*, *H. argentina*, *H. chillensis*, *H. microcephala*, *H. parodii*	51
Pampasica group: *H. megapotamica*, *H. pampasica*, *H. pinnatifida*	83
Sessiliflora group: *H. echegarayi*, *H. eremophila*, *H. meyeniana*, *H. sessiliflora*, *H. taraxacoides*	81
Tenuifolia group: *H. acaulis*, *H. hookeri*, *H. incana*, *H. palustris*, *H. tenuifolia*	23

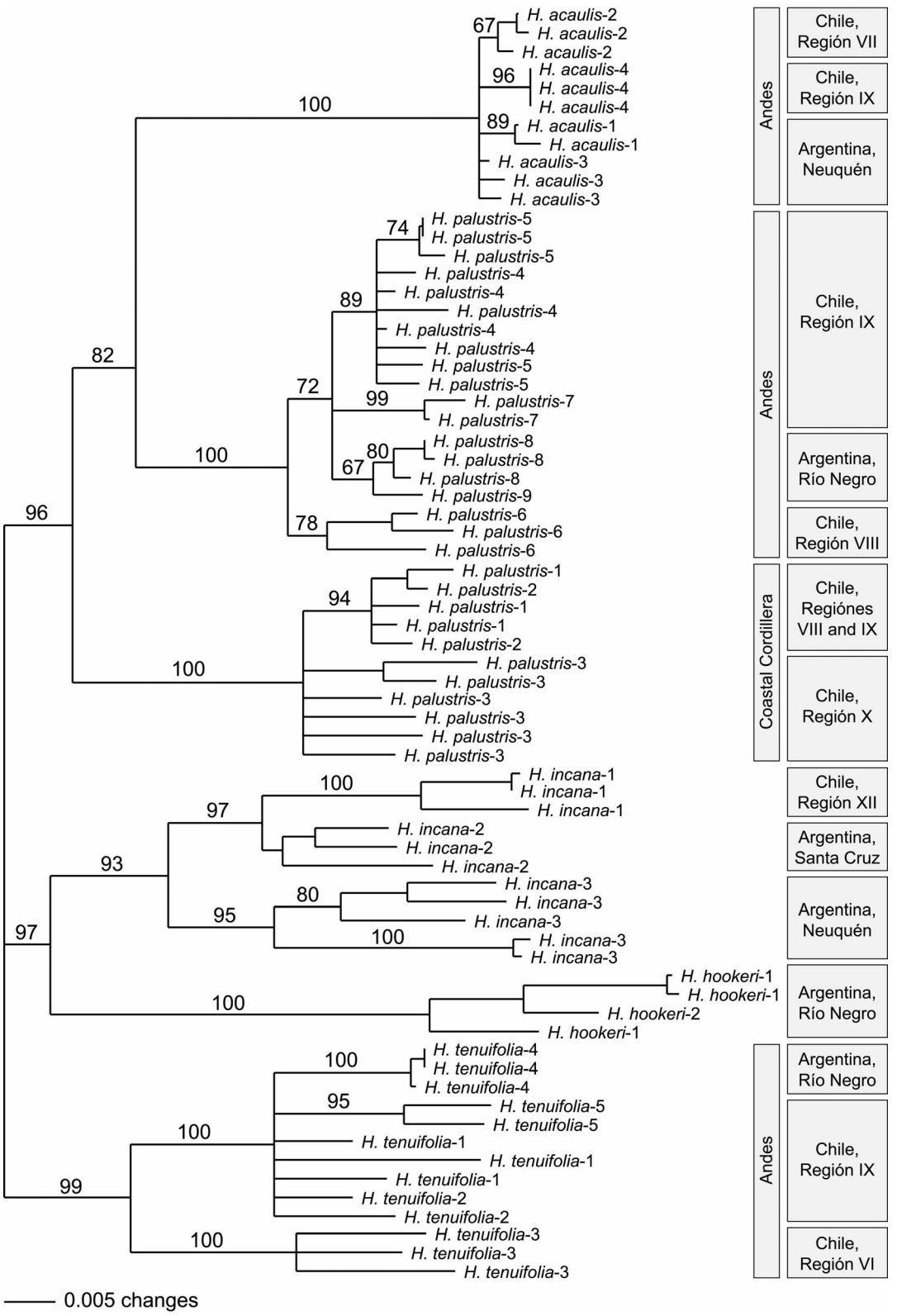

FIG. 2. 50% majority rule consensus tree of 10,000 bootstrap replicates of individuals belonging to the Tenuifolia group based on neighbour-joining analysis of 435 AFLP fragments. Numbers are bootstrap proportions. Scale bar indicates 0.5% character difference (c. 2 fragments).

GK 15565 and *H. palustris TS, CB & GK 15566*), and in region IX, Paso Pino Hachado in wet alpine vegetation along a creek at 1790 m (*H. acaulis TS & CB 15587* and *H. palustris TS & CB 15588*; all vouchers in CONC and WU).

Rieseberg and Brouillet (1994) argue that many species should be paraphyletic, because founder effect, area effect, and most models of sympatric speciation generate a monophyletic derivative species and a paraphyletic or at least metaphyletic (i.e., unresolved) progenitor. With low levels of gene flow and geographic differentiation, paraphyly or metaphyly of the progenitor species will remain over long periods of time (Rieseberg and Brouillet 1994). Our results suggest that *H. acaulis* may have evolved from *H. palustris* by one of these modes of speciation. The acaulescent growth form of *H. acaulis* can be seen as an adaptation to higher elevation.

Three closely related species, *Hypochaeris echegarayi* Hieron., *H. eremophila* Cabrera, and *H. meyeniana* (Walp.) Griseb., are also not monophyletic (Fig. 3). Rather, individuals of the three species are intermingled in the tree, in particular the individuals of *H. echegarayi* and *H. meyeniana* from La Paz department (Bolivia). Close relationship of the three species is also suggested by a morphological cladistic analysis, in which *H. echegarayi*, *H. eremophila*, and *H. meyeniana* (together with *H. eriolaena* (Sch.-Bip.) Reiche, which was not analysed with AFLP) form an unresolved group (Urtubey et al. unpubl.).

Likewise, four closely related species, *H. apargioides* Hook. & Arn., *H. gayana* (DC.) Cabrera, *H. spathulata* (J. Rémy) Reiche, and *H. thrincioides* (J. Rémy) Reiche, form an interesting, not monophyletic complex with their individuals being partly intermingled. *Hypochaeris gayana*, which grows in the Coastal Cordillera in Chile, forms a distinct cluster, but with no bootstrap support (2%; Fig. 6). *Hypochaeris spathulata*, found on coastal rocks along the Pacific coast, also forms a distinct cluster with no bootstrap support (1%), but this cluster also includes an individual of *H. thrincioides* (population 1), which is growing sympatrically with *H. spathulata* (population 4) in Quidico (region IX, Chile). Hybridization between *H. spathulata* and *H. thrincioides* possibly exists there (individuals of collection *KT & RH 55*). *Hypochaeris apargioides* and *H. thrincioides* are paraphyletic. *Hypochaeris apargioides* forms two main clusters, which are separated geographically [individuals of one cluster (populations 2 and 3) grow in the Longitudinal Valley at low elevation (< 150 m), whereas individuals of the other cluster (populations 1, 5, and 6) grow in the Andes at higher elevation (> 950 m)]. From a morphological standpoint *H. spathulata* is clearly a distinct entity, but some blurring and clinal variation does occur between *H. apargioides* and *H. gayana* as well as between *H. apargioides* and *H. thrincioides*. Morphological characters need to be re-evaluated across the ranges of the latter three species in order to detect possible conspecificity among them. As the populations of this complex might represent a stage in the actively ongoing process of speciation (e.g., Wu 2001), they are an interesting case for the study of speciation.

Groupings. AFLP proved useful for the delimitation of six groups among *Hypochaeris* in South America. The basal phylogenetic relationships (or the ''backbone'') of the tree are not reliably resolved due to the shortness of the branches connecting the basal nodes, i.e., the basal nodes receive only low bootstrap support (mostly below 50%; Fig. 1). A too high proportion of non-homologous fragments could possibly obscure phylogenetic signal at this level of divergence. Alternatively, the initial branching events in South America could have occurred in a very short period of time, as it would be expected with a rapid diversification. Addition of more AFLP primer combinations and/or analysis of other molecular markers (e.g., sequencing of highly variable introns of single- or low-copy nuclear genes) might help to provide additional insight on the basal relationships among the major phylogenetic groups.

Groups among 14 South American species of *Hypochaeris* obtained by Stuessy et al. (2004) using AFLP with three selective primer combinations and neighbour-joining analysis largely correlate with groups obtained in this study. In the Stuessy et al. (2004) study, the sister species *H. acaulis* and *H. palustris* form a group together with *H. incana* (Hook. et Arn.) Macloskie and *H. tenuifolia*, which corresponds to the Tenuifolia group of this study, but in the former study *H. scorzonerae* is also included. *Hypochaeris chillensis* (Kunth) Hieron., *H. megapotamica* Cabrera, *H. microcephala*, and *H. pampasica* Cabrera form a group in the Stuessy et al. (2004) study, species represented in the Microcephala group and Pampasica group of the present study. The Apargioides group was resolved in both studies.

Chondrilloides Group (Fig. 1). Both species of this group, which branches off at the basal node of South American *Hypochaeris*, inhabit the southern part of the continent. *Hypochaeris chondrilloides* (A. Gray) Cabrera grows in humid salty marshes in provinces Jujuy to Santa Cruz (Argentina) and in regions II and III (Chile). *Hypochaeris patagonica* Cabrera grows in open places of the Subantarctic forest, in provinces Chubut to Tierra del Fuego (Argentina).

Tenuifolia Group (Fig. 2). The five species included [four erect and one acaulescent (*H. acaulis*)] share the same general distributional area, mainly along the S Andean chain (central-W to SW Argentina and central to S Chile; from the southern tip of the continent to approximately 34°S, the northern distributional limit of *H. tenuifolia*). This provides an additional reason for

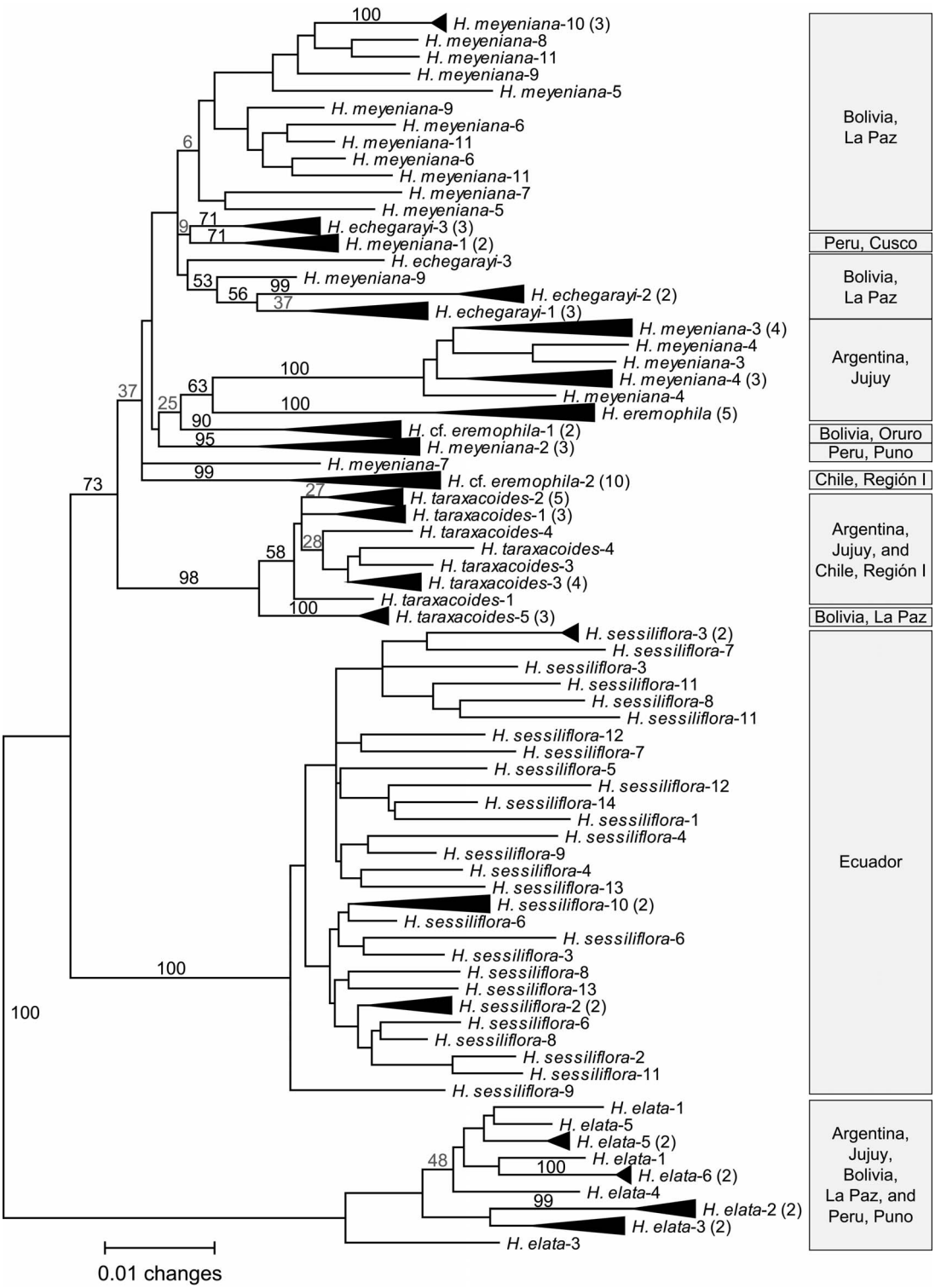

FIG. 3. 50% majority rule consensus tree including groups compatible with it of 10,000 bootstrap replicates of individuals belonging to the Sessiliflora group plus *H. elata* based on neighbour-joining analysis of 408 AFLP fragments. Numbers are bootstrap proportions (below 50% in grey). Numbers in parentheses refer to number of individuals in a condensed cluster. Scale bar indicates 1% character difference (c. 4 fragments).

grouping them despite their very low support (23% BS). *Hypochaeris caespitosa*, which is sister to the Tenuifolia group (22% BS), grows in a different area, the Sierra de Córdoba and Sierra de San Luis (center of Argentina), and was therefore not included in the group. *Hypochaeris palustris* also inhabits the Falkland Islands (Islas Malvinas) in the S Atlantic ocean (Cabrera 1963). These islands are situated on the South American continental shelf, which is presently submerged between the islands and the continent. The species of the Tenuifolia group grow in mountain habitats (Andes to Patagonian Argentina and the Coastal Cordillera in Chile) and down to sea level in the S Subantarctic zone (Cabrera and Willink 1980). Phylogenetic relationships among *H. hookeri* Phil., *H. incana*, and *H. tenuifolia* are unresolved; Fig. 1 shows *H. incana* and *H. tenuifolia* as sister species (34% BS), whereas Fig. 2 shows *H. hookeri* and *H. incana* as sister species (97% BS).

In a comparative approach, infraspecific phylogeographical studies have been carried out for three species with partly overlapping distributions in the S Andes (*H. acaulis*, Tremetsberger et al. 2003a; *H. palustris*, Muellner et al. 2005; *H. tenuifolia*, Tremetsberger et al. 2003b) in order to elucidate the effect of Pleistocene separation of populations in refugia for speciation and intraspecific differentiation. The studies suggest that Andean populations of the three species north of ~38–39°S may have survived the last glacial maximum in refugia isolated by glacial tongues. These populations are more strongly genetically differentiated from each other than populations in the formerly completely glaciated part of the Andes south of 39°S. Recolonization of this area by *H. palustris* and *H. tenuifolia* probably occurred rapidly from close refugia as indicated by large genetic coherence of populations in this region [see also Fig. 2; the northern population 6 of *H. palustris* (36°80′S, near Termas de Chillán in Chile) is differentiated from the central populations 4, 5, 7, 8, and 9 (all S of 38°S, in region IX, Chile, and Río Negro province, Argentina, which are more similar genetically; and the northern population 3 of *H. tenuifolia* (34°90′S, Termas del Flaco in Chile) is differentiated from the southern populations 1, 2, 4, and 5 (all S of 38°S, in region IX, Chile, and Río Negro province, Argentina), which are also more similar genetically]. Muellner et al. (2005) also show evidence for a refugium of *H. palustris* in the Chilean Coastal Cordillera (see above) and raise the possibility of an additional southern or southeastern refugium, since the eastern and very southernmost parts of southern Patagonia were partly ice-free during the last glacial maximum.

Sessiliflora Group (Fig. 3). The taxa included grow in the high altitudinal vegetation (puna, páramo, and up to the nival zone) of the central and N Andes, from San Juan province (Argentina) and region II (Chile) to Venezuela (Sierra Nevada de Santa Marta and Cordillera de Mérida), and have an acaulescent growth form (peduncle of the single head short or lacking). *Hypochaeris elata*, which is sister to the Sessiliflora group, shares the same distributional area, but has an erect growth form. DNA sequences (ITS and ETS; Tremetsberger et al. unpubl.) also show it to be closely related to *H. sessiliflora*. This latter species branches off at the basal node of the acaulescent species. The sister group of *H. sessiliflora* contains *H. echegarayi*, *H. eremophila*, *H. meyeniana*, and *H. taraxacoides* (Walp.) Benth. & Hook. f. Within this group, only *H. taraxacoides* is monophyletic. Two species, *H. echegarayi* and *H. taraxacoides*, have white corollas. Like in *H. acaulis*, the acaulescent growth form can be seen as an adaptation to high altitudes.

There is no geographical structure among individuals of *H. sessiliflora* in Ecuador. This species is morphologically highly variable (e.g., flowers yellow, white or pink; involucral bracts varying in shape, color and vestiture), but this variation is not reflected in genetic similarities. However, we must keep in mind that our collections of *H. sessiliflora* only cover the central part of the distributional area of *H. sessiliflora* in Ecuador (the total area reaches from Peru to Venezuela). Two possible explanations might account for the great morphological variability paired with genetic uniformity: (1) the region could be a secondary contact zone between more differentiated taxa in the north (Colombia, Venezuela) and south (Peru); (2) the populations are presently in the process of adaptation and differentiation. A more complete sampling of populations throughout the distributional area of *H. sessiliflora* is needed for further evaluation of the two hypotheses.

Hypochaeris taraxacoides shows infraspecific differentiation between (1) populations from Jujuy province (Argentina) and region I (Chile) and (2) the population from La Paz department (Bolivia). Some populations or regional groups of *H. echegarayi*, *H. eremophila*, and *H. meyeniana* are differentiated, such as *H. eremophila* from Jujuy province (Argentina), *H.* cf. *eremophila* from Oruro department (Bolivia), *H.* cf. *eremophila* from region I (Chile), *H. meyeniana* from Jujuy province (Argentina), and *H. meyeniana* from Puno province (Peru). Individuals of other populations are intermingled, namely those of *H. echegarayi* and *H. meyeniana* from La Paz department (Bolivia). Morphologically, these taxa are not usually difficult to distinguish, suggesting that the occasional morphological and AFLP intergradation may signal interspecific hybridization.

Microcephala Group (Fig. 4). This group mainly contains species from the hills and plains of the eastern part of the continent, from Buenos Aires province (Argentina) to Río de Janeiro state (Brazil), and Paraguay (Urtubey and Stuessy 2003; Urtubey et al. in press), viz., *H. alba* Cabrera, *H. albiflora, H. chillensis*

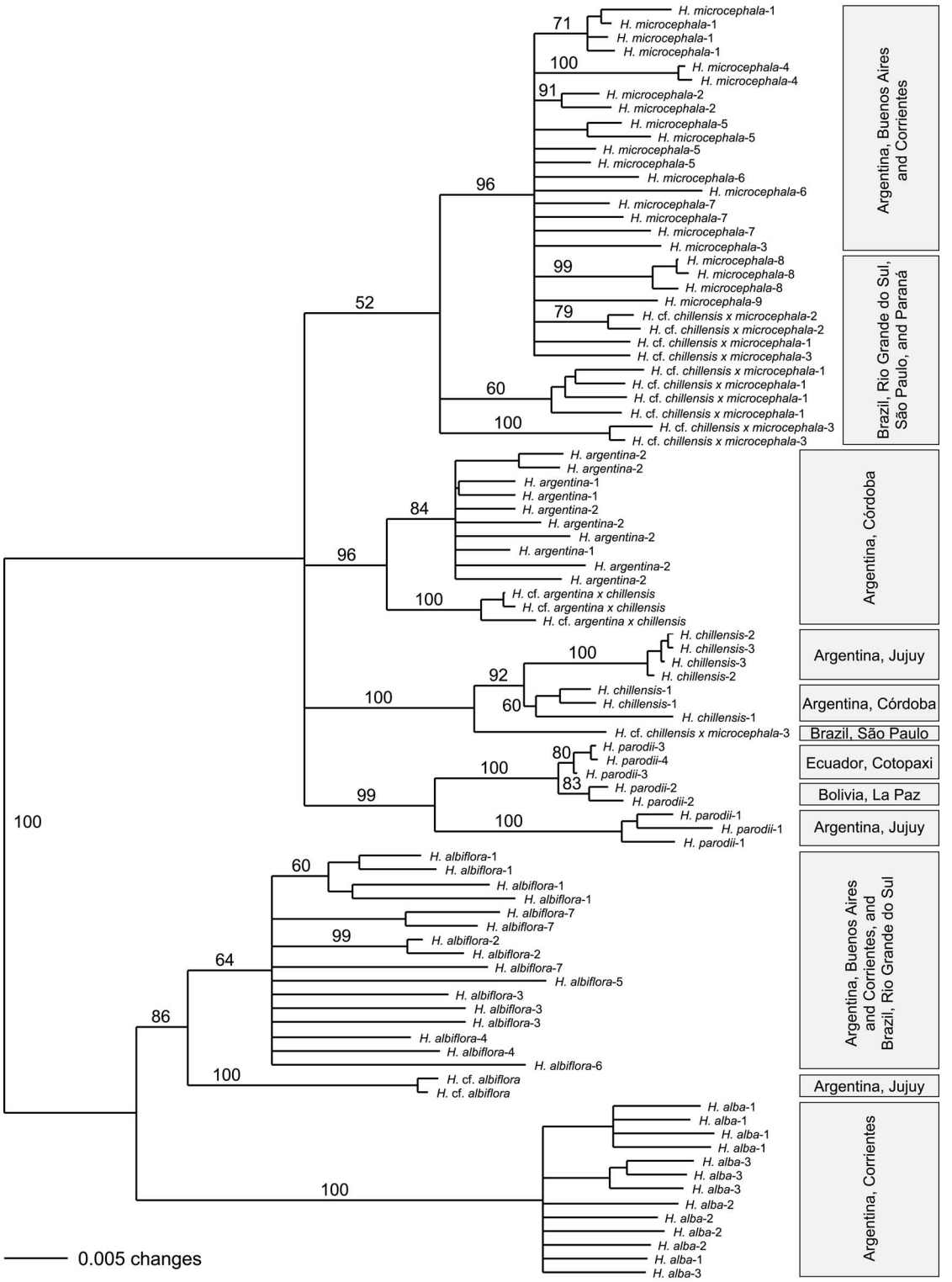

FIG. 4. 50% majority rule consensus tree of 10,000 bootstrap replicates of individuals belonging to the Microcephala group based on neighbour-joining analysis of 391 AFLP fragments. Numbers are bootstrap proportions. Scale bar indicates 0.5% character difference (c. 2 fragments).

(see map in Cabrera 1976), and *H. microcephala*. Three of these extend westward to the Sierras de Córdoba (*H. chillensis*) and the Andes (*H. albiflora, H. chillensis*, and *H. microcephala*). *Hypochaeris chillensis* has the broadest distribution (as far as Colombia in the northern Andes; Cabrera 1976). *Hypochaeris argentina* Cabrera grows from Jujuy province to Sierras de Córdoba (Córdoba province) in Argentina. *Hypochaeris parodii* Cabrera grows in high-altitudinal habitats in the central Andes [Catamarca to Jujuy provinces (Argentina) and La Paz department (Bolivia)]. This is the first report of *H. parodii* from the N Andes (Ecuador).

Hypochaeris alba and *H. albiflora*, both with white corollas, group together and are sister to *H. argentina*, *H. chillensis*, *H. microcephala*, and *H. parodii*, all with yellow or yellow-orange corollas. Phylogenetic relationships among the latter four species are unresolved; Fig. 1 shows *H. chillensis* as sister to *H. argentina*, *H. microcephala*, and *H. parodii*, whereas the four species are unresolved in Fig. 4.

Infraspecific differentiation is apparent in species of the Microcephala group. In *H. albiflora*, the lowland populations from central-eastern Argentina (Buenos Aires and Corrientes provinces) and SE Brazil (Rio Grande do Sul state), are separated from a higher elevational population from NW Argentina (Jujuy province, 1910 m). This latter population (*TS, EU & KT 18059*) was determined by AFLP analysis, since it was past flowering in the field (February). Within Argentinean *H. chillensis*, populations from Jujuy province are separated from populations from Córdoba province. Populations of *H. parodii* from Jujuy province (Argentina) are differentiated from those of Bolivia and Ecuador.

AFLP analysis allows suggestion of previously unsuspected hybrid origins of some populations of the Microcephala group. It shows that the only ''pure'' populations of *H. chillensis* in our sample are from Jujuy (populations 2 and 3; at 1640 m) and Córdoba provinces (population 1; at 915 m) in Argentina. All material sampled from Buenos Aires province (Argentina) and Brazil and previously determined as *H. chillensis* is *H. microcephala* or of presumptive hybrid origin (*H. chillensis* × *microcephala*). Several populations from São Paulo (*ST & PO BRA31, BRA33,* and *BRA34*) and Paraná states (*CR s. n.*) in Brazil might be hybrid populations between *H. chillensis* and *H. microcephala* based on the co-occurrence of fixed diagnostic fragments of the putative parental species in these populations and the calculation of maximum likelihood hybrid indices (Tremetsberger et al. unpubl.). We can observe a geographical and ecological separation between *H. chillensis*, which is growing in the high-altitudinal regions of central and NW South America (Sierra de Córdoba and eastern side of Andes), and *H. microcephala*, which is growing in the lowlands of the eastern part of the continent [plains and hills of Buenos Aires and Corrientes provinces (Argentina) and Brazil] in a humid temperate and subtropical climate. High incidence of hybridization in Brazil has also been reported by Azevêdo-Gonçalves (2004), who describes a hybrid taxon *H.* × *microcephala* (Sch. Bip.) Azevêdo-Gonçalves & Matzenbacher, and co-occurrence of a hybrid (*H. albiflora* × *chillensis*) with its parents has been observed by Wulff (1992) in Buenos Aires, Argentina. The hybrid had lowered pollen viability and reduced seed production, but normal meiosis (Wulff 1992). Hybridization might also be partly responsible for the great variability of characters (e.g., pubescence and leaf shape) in *H. chillensis* recognized by Cabrera (1976), who noted that glabrous or hairy involucral bracts can occur in the same population and even in the same plant. These reports and our AFLP results make it quite clear that hybridization is very common, even though all species involved are at the diploid level (Weiss et al. 2003 and literature cited therein). A denser sampling throughout the complete distributional ranges of the species and re-evaluation of morphological characters is needed to clarify further the specific limits of *H. chillensis* and *H. microcephala* and to assess their geographical ranges and ecological requirements as well as their taxonomy and dynamics of hybridization. Population *EU & KT 152* might be a hybrid population between *H. argentina* and *H. chillensis* based also on co-occurrence of fixed diagnostic fragments of the parental species and calculation of a maximum likelihood hybrid index (Tremetsberger et al. unpubl.).

Pampasica Group (Fig. 5). The species included together with the associated *H. petiolaris* and *H. variegata* grow in the hills and plains of the central-eastern part of the continent, from Buenos Aires province (Argentina) to Santa Catarina state (Brazil; sympatric with species of the Microcephala group). *Hypochaeris megapotamica* and *H. pampasica* (together with *H. grisebachi* Cabrera) also group together in a RAPD study (Ruas et al. 2005; *H. pinnatifida* was not analysed in that investigation). Phylogenetic relationships among *H. megapotamica, H. pampasica*, and *H. pinnatifida* are unresolved; Fig. 1 shows *H. megapotamica* and *H. pampasica* as sister species (52% BS), whereas Fig. 5 shows *H. pampasica* and *H. pinnatifida* as sister species (66% BS). In *H. variegata*, there is a pronounced differentiation between populations from Buenos Aires province (Argentina) and those from Rio Grande do Sul state (Brazil).

Apargioides Group (Fig. 6). The species included grow in coastal to Andean habitats of the SW part of the continent, from regions Metropolitana [*H. clarionoides* (J. Rémy) Reiche] to XI (*H. spathulata*) in Chile and from Mendoza to Río Negro provinces in Argentina (sympatric with species of the Tenuifolia group). AFLP data clearly show *H. clarionoides* to be a distinct entity

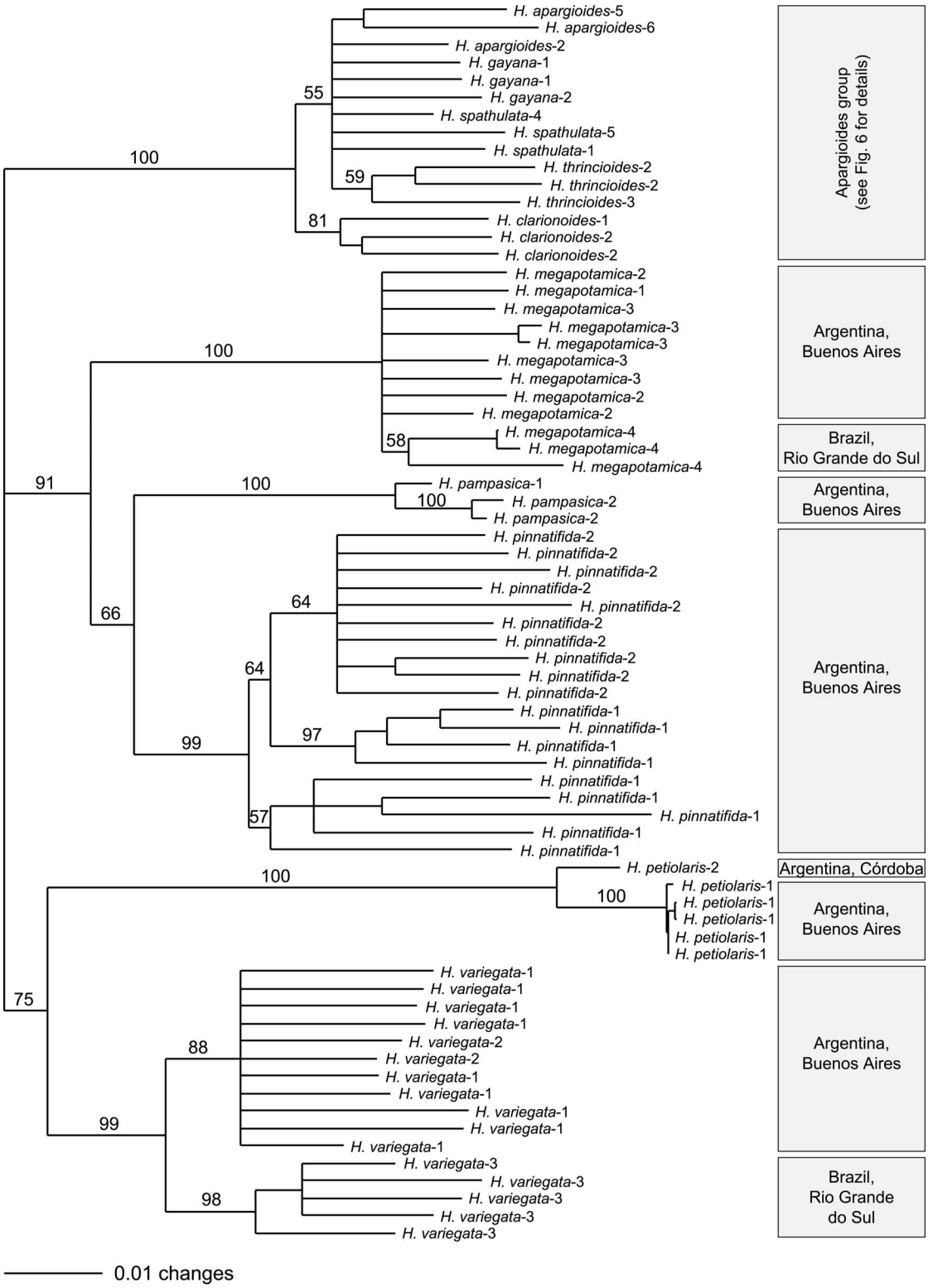

FIG. 5. 50% majority rule consensus tree of 10,000 bootstrap replicates of individuals belonging to the Pampasica group plus *H. petiolaris*, *H. variegata*, and the Apargioides group (species of the latter group are represented by three individuals each; see Fig. 6 for details) based on neighbour-joining analysis of 606 AFLP fragments. Numbers are bootstrap proportions. Scale bar indicates 1% character difference (c. 6 fragments).

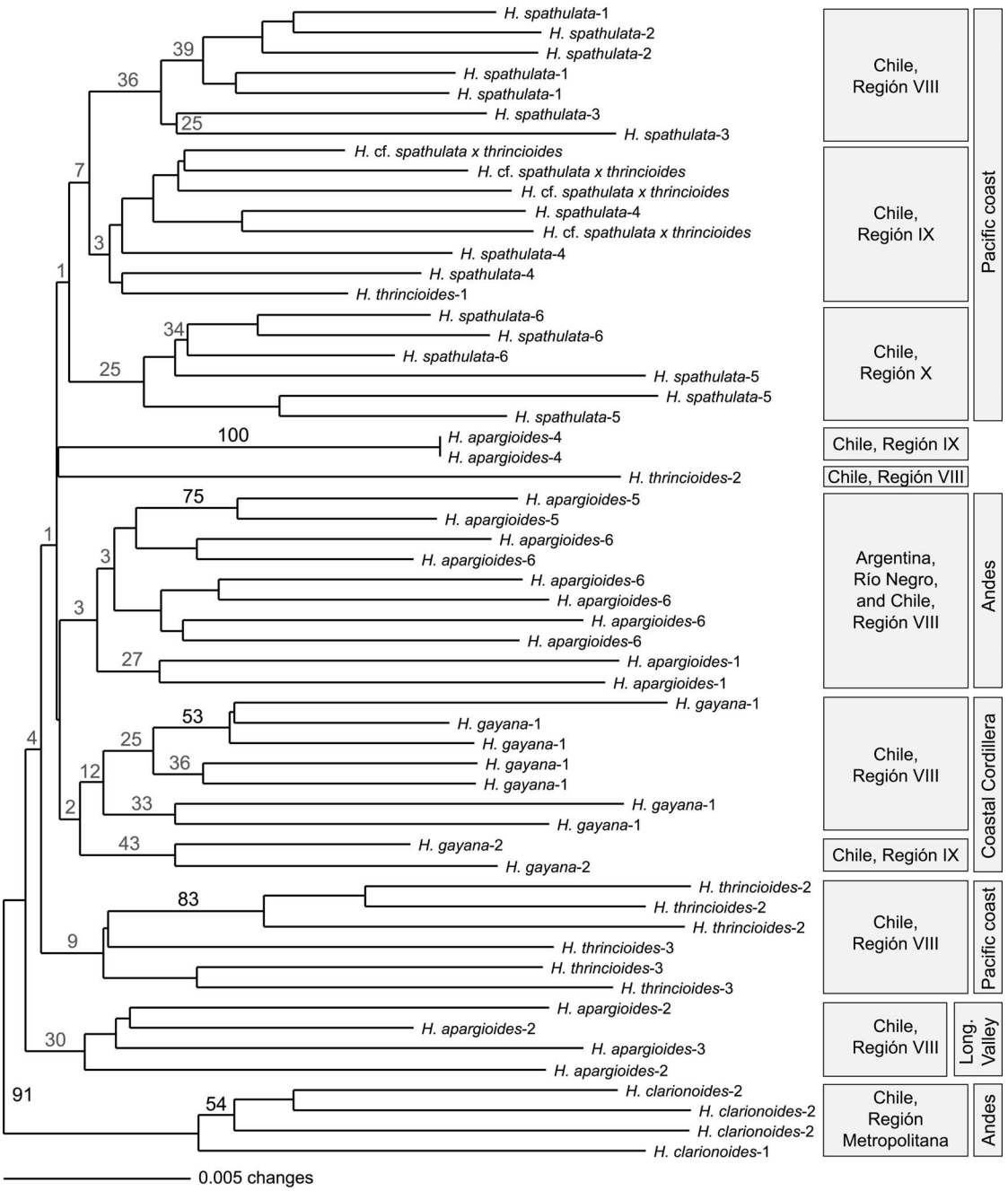

FIG. 6. 50% majority rule consensus tree including groups compatible with it of 10,000 bootstrap replicates of individuals of the Apargioides group based on analysis of 412 AFLP fragments. Numbers are bootstrap proportions (below 50% in grey). Scale bar indicates 0.5% character difference (c. 2 fragments). Long. Valley—Longitudinal Valley.

and member of the Apargioides group, despite its recent combination as a variety of *H. tenuifolia* (Bortiri 1997). *Hypochaeris clarionoides*, which has the northernmost distribution within the group, is sister to the other four species with more southern, partly overlapping distributions (see above).

Synthesis. Our results suggest that after its initial arrival in South America ~3.5 or fewer million years ago (Tremetsberger et al. 2005), the spread of *Hypochaeris* over the continent occurred rapidly and that geographic isolation was important for initial diversification. This is shown by very short branches con-

necting the basal nodes of the tree among South American species and distinct distributional areas occupied by species of the major phylogenetic lineages that are distributed across the entire range occupied today (e.g., the southern Andes by the Tenuifolia group; the northern Andes by the Sessiliflora group; the Mediterranean Pacific coast in Chile, regions III to VI, by the ungrouped species *H. scorzonerae*; the eastern part of the continent from NE Argentina to SE Brazil by the Pampasica group). Adaptation to different habitats and changes in the reproductive biology (e.g., switch to selfing, change of pollinator possibly associated with change of flower color) might have played important roles in subsequent speciation within lineages. Polyploidy occurs only rarely and, if so, mixed with diploidy within species (Weiss-Schneeweiss et al. unpubl.), and, therefore, does not seem relevant for speciation. Neither are there major chromosomal rearrangements, which could have driven reproductive isolation (Weiss-Schneeweiss et al. 2003). Pleistocene climate changes might also have been an important factor for geographical isolation and thus promoting speciation and intraspecific differentiation, as it has been demonstrated in species of the Tenuifolia group.

This study provides a basic framework of cohesive genetic entities and taxonomic relationships. Morphological analysis of the group is in progress (Urtubey et al. unpubl.) and will enable a taxonomic revision together with results of the present study, karyotypic data (Weiss et al. 2003; Weiss-Schneeweiss et al. 2003, and unpubl.), genome size data (König et al. unpubl.), and information about reproductive biology (Ortiz et al. unpubl.).

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APPENDIX 1. Plant material of *Hypochaeris* analysed for AFLP listed alphabetically with name, locality, collector(s) and number, and location of voucher specimens indicated by acronyms of Index Herbariorum. The numbers in parentheses refer to the numbers of individuals analysed in each population. Abbreviations: CB—C. Baeza; FE—F. Essl; SG—S. Gómez; RH—R. Hössinger; AJ—A. Jiménez; GK—G. Kottirsch; PL—P. López; AM—A. Marticorena; NM—N. Matzenbacher; RM—R. Meneses; MO—M. Á. Ortiz; PO—P. Ortiz; MP—M. J. Parra; PP—P. Peñailillo; LR—L. Reyes; CR—C. Ruas; PS—P. Schönswetter; PSi—P. Simón; MS—M. Stau-

dinger; TS—T. Stuessy; ST—S. Talavera; KT—K. Tremetsberger; AT—A. Tribsch; EU—E. Urtubey; HV—H. Valdebenito.

Ingroup. ***H. acaulis*-1** (J. Rémy) Britton, Argentina, Neuquén, Volcán Copahue, *KT, AJ & SG 1038,* WU (2). ***H. acaulis*-2,** Chile, Región VII, Laguna Maule, *TS & CB 15571,* CONC, WU (3). ***H. acaulis*-3,** Argentina, Neuquén, Paso Pino Hachado, *TS & CB 15593,* CONC, WU (3). ***H. acaulis*-4,** Chile, Región IX, below Volcán Tolhuaca, *TS & CB 15817,* CONC, WU (3). ***H. alba*-1** Cabrera, Argentina, Corrientes, National Park Mburucuyá, *EU & KT 156,* LP, WU (5). ***H. alba*-2,** Argentina, Corrientes, S of Bella Vista, *EU & KT 157,* LP, WU (4). ***H. alba*-3,** Argentina, Corrientes, Bella Vista, Parque Cruz de los Milagros, *EU & KT 159,* LP, WU (4). ***H. albiflora*-1** (Kuntze) Azevêdo-Gonçalves & Matzenbacher, Brazil, Rio Grande do Sul, Guaíba, *NM s. n., ICN 59803* (4). ***H. albiflora*-2,** Argentina, Buenos Aires, La Plata, Jardín Botánico C. Spegazzini, *EU 110,* LP (2). ***H. albiflora*-3,** Argentina, Buenos Aires, Diagonal 74 from Punta Lara to La Plata, *EU & KT 116,* LP, WU (3). ***H. albiflora*-4,** Argentina, Buenos Aires, Sarmiento, km 139 of road 8, *EU & KT 135,* LP, WU (2). ***H. albiflora*-5,** Argentina, Corrientes, Bella Vista, Calle Sarmiento and Buenos Aires, *EU & KT 158,* LP, WU (1). ***H. albiflora*-6,** Argentina, Buenos Aires, La Plata, Museo de Historia Natural, *KT 1010,* WU (1). ***H. albiflora*-7,** Argentina, Buenos Aires, Ensenada, Parque Siderar near Río de La Plata, *TS, EU & KT 18015,* LP, WU (3). ***H.* cf. *albiflora*,** Argentina, Jujuy, 1.3 km S of Arroyo Horquetas down from Laguna Yala, *TS, EU & KT 18059,* LP, WU (2). ***H. apargioides*-1** Hook. & Arn., Chile, Región VIII, between Volcán Lonquimay and Casa Lolco, *KT & RH 89,* WU (2). ***H. apargioides*-2,** Chile, Región VIII, Yumbel, *KT & CB 1013,* WU (3). ***H. apargioides*-3,** Chile, Región VIII, between Cabrero and Cerro Negro, *KT & CB 1015,* WU (1). ***H. apargioides*-4,** Chile, Región IX, Río Blanco, *TS & CB 15809,* CONC, WU (2). ***H. apargioides*-5,** Chile, Región IX, Volcán Villarrica, *TS & CB 15612,* CONC, WU (2). ***H. apargioides*-6,** Argentina, Río Negro, Cerro Buitrero, *TS, EU & KT 18021, 18023,* LP, WU (6). ***H. argentina*-1** Cabrera, Argentina, Córdoba, Sierra de Córdoba: Champaquí, *EU & KT 142, 143,* LP, WU (3). ***H. argentina*-2,** Argentina, Córdoba, Sierra de Córdoba, Cerro Los Gigantes, *EU & KT 144, 146,* LP, WU (7). ***H.* cf. *argentina* × *chillensis*,** Argentina, Córdoba, Sierra de Córdoba, Cerro Los Gigantes, *EU & KT 152,* LP (3). ***H. caespitosa*-1** Cabrera, Argentina, Córdoba, Sierra de Córdoba, Champaquí, *EU & KT 138,* LP, WU (1). ***H. caespitosa*-2,** Argentina, Córdoba, Sierra de Córdoba, Cerro Los Gigantes, *EU & KT 145, 148,* LP, WU (2). ***H. chillensis*-1** (Kunth) Hieron., Argentina, Córdoba, road from Yacanto de Calamuchita to Champaquí, *EU & KT 137,* LP, WU (3). ***H. chillensis*-2,** Argentina, Jujuy, c. 7 km N of Yala on route to León, *TS, EU & KT 18060,* LP, WU (2). ***H. chillensis*-3,** Argentina, Jujuy, c. 1 km S of León, puente León, *TS, EU & KT 18061,* LP, WU (2). ***H.* cf. *chillensis* × *microcephala*-1,** Brazil, Paraná, Curitiba, *CR s. n.,* WU (5). ***H.* cf. *chillensis* × *microcephala*-2,** Brazil, São Paulo, Guarulhos airport, *ST & PO BRA31,* HUFU, SEV (2). ***H.* cf. *chillensis* × *microcephala*-3,** Brazil, São Paulo, Parque do Estado, *ST & PO BRA33, BRA34,* HUFU, SEV (4). ***H. chondrilloides*** (A. Gray) Cabrera, Argentina, Jujuy, Iturbe, *TS, EU & KT 18072,* LP, WU (3). ***H. clarionoides*-1** (J. Rémy) Reiche, Chile, Región Metropolitana, Santuario de la Naturaleza Yerba Loca, *T. F, P, D. & M. Stuessy 15532,* WU (1). ***H. clarionoides*-2,** Chile, Región Metropolitana, El Colorado, *T. F. & M. Stuessy 15636,* WU (3). ***H. echegarayi*-1** Hieron., Bolivia, Depto. La Paz, Laguna Cañuma, *TS, KT & RH 18516,* LPB, WU (3). ***H. echegarayi*-2,** Bolivia, Depto. La Paz, up from village Cañuma, *TS, KT & RH 18517,* LPB, WU (2). ***H. echegarayi*-3,** Bolivia, Depto. La Paz, 3–5.5 km E of Kaluyo (Valle de Achachicala), *TS, KT & RH 18527, 18529,* LPB, WU (4). ***H. elata*-1** (Wedd.) Griseb., Peru, Prov. Puno, Puno, Azojini, *KT & RH 1099,* WU (2). ***H. elata*-2,** Argentina, Prov. Jujuy, 2 km NE of route 9 on gravel road to Iruya, *TS, EU & KT 18070,* LP, WU (2). ***H. elata*-3,** Argentina, Prov. Jujuy, 22.6 km W of Humahuaca on road 14 toward El Aguilar between Coraya and Casa Grande, *TS, EU & KT 18080,* LP, WU (3). ***H. elata*-4,** Bolivia, La Paz, Cota-Cota, Universidad Mayor de San Andrés, Botanical Garden, *TS, KT & RH 18500,* LPB, WU (1). ***H. elata*-5,** Bolivia, Depto. La Paz, c. 3 km on dirt road toward Peñas from main motorway La Paz-Huarina, *TS, KT & RH 18506,* LPB, WU (3). ***H. elata*-6,** Bolivia, Depto. La Paz, 15 km W of La Huarina on road to Achacachi, *TS, KT & RH 18511,* LPB, WU (2). ***H. eremophila*** Cabrera, Argentina, Prov. Jujuy, 33 km W of Purmamarca on gravel road near Saladillo, *TS, EU & KT 18064,* LP, WU (5). ***H.* cf. *eremophila*-1,** Bolivia, Depto. Oruro, Sajama, *RM 602–6,* LPB (2). ***H.* cf. *eremophila*-2,** Chile, Región I, along road 2 between Socoroma and Putre, *TS & KT 18094, 18097,* WU (10). ***H. gayana*-1** (DC.) Cabrera, Chile, Región VIII, Cordillera de Nahuelbuta, *KT & MP 40, 41, 42, 50,* WU (7). ***H. gayana*-2,** Chile, Región IX, Villa Araucaria, *KT & RH 56,* WU (2). ***H. hookeri*-1** Phil., Argentina, Prov. Río Negro, c. 6 km S of Bariloche airport, *TS, EU & KT 18019,* LP, WU (3). ***H. hookeri*-2,** Argentina, Prov. Río Negro, Estancia Rayhuao c. 29 km S of Pilcaniyeu, *TS, EU & KT 18044,* LP, WU (1). ***H. incana*-1** (Hook. et Arn.) Macloskie, Chile, Región XII, Punta Arenas: in front of airport, *PS, MS & AT 5640,* WU (3). ***H. incana*-2,** Argentina, Prov. Santa Cruz, Parque Nacional Los Glaciares, *PS, MS & AT 5641,* WU (3). ***H. incana*-3,** Argentina, Prov. Río Negro, Cerro Buitrero, *TS, EU & KT 18022, 18024,* LP, WU (5). ***H. lutea*** (Vell.) Britton, Brazil, Rio Grande do Sul, Guaíba, *NM s. n., ICN 63890,* (3). ***H. megapotamica*-1** Cabrera, Argentina, Prov. Buenos Aires, Sarmiento, *EU & KT 134,* LP, WU (1). ***H. megapotamica*-2,** Argentina, Prov. Buenos Aires, La Plata, *TS & EU s. n. (27.10.2001), KT 1008,* LP, WU (3). ***H. megapotamica*-3,** Argentina, Prov. Buenos Aires, Sierra de Tandil, *KT & PSi 1000, 1004,* WU (5). ***H. megapotamica*-4,** Brazil, Rio Grande do Sul, Guaíba, *NM s. n., ICN 59004* (3). ***H. meyeniana*-1** (Walp.) Griseb., Peru, Prov. Cusco, Inca ruins Puca Pucara, *KT & RH 1095,* WU (2). ***H. meyeniana*-2,** Peru, Prov. Puno, Puno, Azojini, *KT & RH 1100,* WU (3). ***H. meyeniana*-3,** Argentina, Prov. Jujuy, 20.1 km W of Purmamarca on gravel road into the mountains, *TS, EU & KT 18062,* LP, WU (5). ***H. meyeniana*-4,** Argentina, Prov. Jujuy, 21 km N of Humahuaca on route 9, *TS, EU & KT 18068,* LP, WU (5). ***H. meyeniana*-5,** Bolivia, Depto. La Paz, 10 km NE of La Paz, below dam Incachaca, *TS, KT & RH 18502,* LPB, WU (2). ***H. meyeniana*-6,** Bolivia, Depto. La Paz, c. 3 km on dirt road toward Peñas from main motorway La Paz-Huarina, *TS, KT & RH 18507,* LPB, WU (2). ***H. meyeniana*-7,** Bolivia, Depto. La Paz, 15 km W of La Huarina on road to Achacachi, *TS, KT & RH 18510,* LPB, WU (2). ***H. meyeniana*-8,** Bolivia, Depto. La Paz, 25.6 km N of Escoma on road to Ulla-Ulla, by large Christ statue, *TS, KT & RH 18512,* LPB, WU (1). ***H. meyeniana*-9,** Bolivia, Depto. La Paz, 12.2 km N of Huilacala, *TS, KT & RH 18514,* LPB, WU (3). ***H. meyeniana*-10,** Bolivia, Depto. La Paz, up from village Cañuma, *TS, KT & RH 18520,* LPB, WU (3). ***H. meyeniana*-11,** Bolivia, Depto. La Paz, 3 km E of Kaluyo (Valle de Achachicala), *TS, KT & RH 18524,* LPB, WU (3). ***H. microcephala*-1** (Sch. Bip.) Cabrera, Argentina, Buenos Aires, Magdalena, Reserva Parque Costero del Sur, *EU & KT 113, 114,* LP, WU (4). ***H. microcephala*-2,** Argentina, Buenos Aires, Sierra de la Ventana, *EU & KT 118, 130,* LP, WU (2). ***H. microcephala*-3,** Argentina, Buenos Aires, Sarmiento, *EU & KT 136,* LP, WU (1). ***H. microcephala*-4,** Argentina, Corrientes, Bella Vista, Parque Cruz de los Milagros, *EU & KT 160,* LP, WU (2). ***H. microcephala*-5,** Argentina, Buenos Aires, Sierra de Tandil, *KT & PSi 1002, 1005,* WU (4). ***H. microcephala*-6,** Argentina, Buenos Aires, La Plata, Museo de Historia Natural, *TS & EU s. n. (27.10.2001), KT 1009,* LP, WU (2). ***H. microcephala*-7,** Argentina, Buenos Aires, La Plata: Barrio Aeropuerto c. 5 km SE of city, *TS, EU & KT 18007,* LP, WU (3). ***H. microcephala*-8,** Brazil, Rio Grande do Sul, Guaíba, *NM s. n., ICN 28260* (3). ***H. microcephala*-9,** Brazil, São Paulo, Guarulhos airport, *ST & PO BRA32,* HUFU, SEV (1). ***H. palustris*-1** (Phil.) De Wild., Chile, Región VIII, Cordillera de Nahuelbuta: Laguna Las Totoras, *KT & RH 49,* WU (3). ***H. palustris*-2,** Chile, Región IX, drained remnant of a bog W of Villa Araucaria, *KT & RH 57,* WU (2). ***H. palustris*-3,** Chile, Región X, Cordillera Pelada, *KT & RH 102, 103,* WU (6). ***H. palustris*-4,** Chile, Région IX, Cor-

dillera de los Raices, *KT, AJ & SG 1048,* WU (5). ***H. palustris*-5,** Chile, Région IX, road from Las Mellizas to Cerros de Lanco, *KT, AJ & SG 1060,* WU (5). ***H. palustris*-6,** Chile, Región VIII, Termas de Chillán, Valle de las Nieblas, *TS, CB & GK 15566,* CONC, WU (3). ***H. palustris*-7,** Chile, Región IX, Paso Pino Hachado, *TS & CB 15588,* CONC, WU (2). ***H. palustris*-8,** Argentina, Río Negro, Cerro López, *TS, EU & KT 18030,* LP, WU (3). ***H. palustris*-9,** Argentina, Río Negro, Monte Tronador, *TS, EU & KT 18048,* LP, WU (1). ***H. pampasica*-1** Cabrera, Argentina, Buenos Aires, Magdalena, *EU & KT 112,* LP, WU (1). ***H. pampasica*-2,** Argentina, Buenos Aires, Sierra de la Ventana, *EU & KT 124,* LP, WU (2). ***H. parodii*-1** Cabrera, Argentina, Jujuy, Laguna Yala, c. 2 km down from main Laguna, *TS, EU & KT 18057,* LP, WU (3). ***H. parodii*-2,** Bolivia, La Paz, Cota-Cota, Universidad Mayor de San Andrés, Botanical Garden, *TS, KT & RH 18522,* LPB, WU (2). ***H. parodii*-3,** Ecuador, Prov. Cotopaxi, 5.5 km E of Pujilí, *TS, HV, KT & RH 18551,* QUSF, WU (2). ***H. parodii*-4,** Ecuador, Prov. Cotopaxi, Pujilí, petrol station of E side of town, *TS, HV, KT & RH 18555,* QUSF, WU (1). ***H. patagonica*** Cabrera, Argentina, Santa Cruz, valley of Río Pinturas near Cueva de las Manos, *FE 6202,* WU (3). ***H. petiolaris*-1** (Hook. et Arn.) Griseb., Argentina, Buenos Aires, Sierra de la Ventana, *EU & KT 122,* LP, WU (5). ***H. petiolaris*-2,** Argentina, Córdoba, Sierra de Córdoba, Cerro Los Gigantes, *EU & KT 153,* LP (1). ***H. pinnatifida*-1** (Speg.) Azevêdo-Gonçalves & Matzenbacher, Argentina, Buenos Aires, Sierra de la Ventana, *EU & KT 120, 125,* LP, WU (9). ***H. pinnatifida*-2,** Argentina, Buenos Aires, Sierra de Tandil, *KT & PSi 1003, 1006,* WU (10). ***H. scorzonerae*-1** (DC.) F. Muell., Chile, Región IV, 5 km E of Huentelauquén along path to Mincha, *PL, AM, PP & LR 503,* CONC, WU (1). ***H. scorzonerae*-2,** Chile, Región IV, 3 km N of Puerto Oscuro, *PL, AM, PP & LR 505a,* CONC, WU (1). ***H. scorzonerae*-3,** Chile, Región IV, 6 km N of Puerto Oscuro, *PL, AM, PP & LR 507,* CONC, WU (1). ***H. sessiliflora*-1** Kunth, Ecuador, Prov. Pichincha, c. 5 km E of San Juan on old road from Quito to Santo Domingo de los Colorados, *TS, HV, KT & RH 18533,* QUSF, WU (1). ***H. sessiliflora*-2,** Ecuador, Prov. Pichincha, 9.5 km S of San Juan on road to Volcán Atacazo, near the antennas, *TS, HV, KT & RH 18536,* QUSF, WU (3). ***H. sessiliflora*-3,** Ecuador, Prov. Pichincha, 26 km E of Pifo, Papallacta, La Virgen, *TS, HV, KT & RH 18539,* QUSF, WU (4). ***H. sessiliflora*-4,** Ecuador, Prov. Pichincha, 23 km E of Pifo on road to Papallacta, *TS, HV, KT & RH 18540,* QUSF, WU (2). ***H. sessiliflora*-5,** Ecuador, Prov. Cotopaxi, c. 20 km S of Aloag, then c. 5 km E toward Volcán Cotopaxi, NASA tracking station, *TS, HV, KT & RH 18541,* QUSF, WU (1). ***H. sessiliflora*-6,** Ecuador, Prov. Cotopaxi, along road toward Volcán Cotopaxi, *TS, HV, KT & RH 18543,* QUSF, WU (3). ***H. sessiliflora*-7,** Ecuador, Prov. Cotopaxi, c. 6 km S from Laguna Limpiopongo on dirt road toward Volcán Cotopaxi, *TS, HV, KT & RH 18545,* QUSF, WU (2). ***H. sessiliflora*-8,** Ecuador, Prov. Cotopaxi, 6.9 km SW of Chaupi on dirt road to Illinizas (in Reserva Ecologica), *TS, HV, KT & RH 18547,* QUSF, WU (3). ***H. sessiliflora*-9,** Ecuador, Prov. Cotopaxi, 9.6 km SW of Chaupi on dirt road to Illinizas (in Reserva Ecologica): parking area at the end of the road, *TS, HV, KT & RH 18548,* QUSF, WU (2). ***H. sessiliflora*-10,** Ecuador, Prov. Cotopaxi, 5.5 km E of Pujilí, *TS, HV, KT & RH 18549,* QUSF, WU (2). ***H. sessiliflora*-11,** Ecuador, Prov. Cotopaxi, 15.9 km E of Pujilí, *TS, HV, KT & RH 18552,* QUSF, WU (3). ***H. sessiliflora*-12,** Ecuador, Prov. Cotopaxi, 0.3 km W of Zumbahua, *TS, HV, KT & RH 18554,* QUSF, WU (2). ***H. sessiliflora*-13,** Ecuador, Prov. Imbabura, near crossroads to Laguna San Marcos, *KT & RH 18556,* WU (2). ***H. sessiliflora*-14,** Ecuador, Prov. Imbabura, road passing S of Laguna Cuicocha, *KT & RH 18557,* WU (1). ***H. spathulata*-1** (J. Rémy) Reiche, Chile, Región VIII, Caleta Rumena, *KT & RH 43,* WU (3). ***H. spathulata*-2,** Chile, Región VIII, Caleta Yani, *KT & RH 44,* WU (2). ***H. spathulata*-3,** Chile, Región VIII, Lebu, *KT & RH 45,* WU (2). ***H. spathulata*-4,** Chile, Región IX, Quidico, *KT & RH 52,* WU (3). ***H. spathulata*-5,** Chile, Región X, Hueicolla, *KT & RH 104,* WU (3). ***H. spathulata*-6,** Chile, Región X, Puente Palo Muerto between Chaihuín and Corral, *KT & RH 105,* WU (3). ***H.* cf. *spathulata* × *thrincioides*,** Chile, Región IX, Quidico, *KT & RH 55,* WU (4). ***H. taraxacoides*-1** (Walp.) Benth. & Hook. f., Argentina, Jujuy, 0.8 km NW of Chaupi Rodeo on dirt road to Iruya, *TS, EU & KT 18074,* LP, WU (4). ***H. taraxacoides*-2,** Argentina, Jujuy, 31.4 km W of Humahuaca on road 14 toward El Aguilar between Coraya and Casa Grande, *TS, EU & KT 18089,* LP, WU (5). ***H. taraxacoides*-3,** Chile, Región I, c. 15 km E of Putre on road to Chucuyo, *TS & KT 18095,* WU (5). ***H. taraxacoides*-4,** Chile, Región I, c. 18 km E of Putre on road to Chucuyo, *TS & KT 18096,* WU (2). ***H. taraxacoides*-5,** Bolivia, Depto. La Paz, 3 km E of Kaluyo (Valle de Achachicala), *TS, KT & RH 18526,* LPB, WU (3). ***H. tenuifolia*-1** (Hook. et Arn.) Griseb., Chile, Región IX, Volcán Lonquimay, *TS & CB 15577(1),* CONC, WU (3). ***H. tenuifolia*-2,** Chile, Región IX, Volcán Lanín, *TS & CB 15617,* CONC, WU (2). ***H. tenuifolia*-3,** Chile, Región VI, Termas del Flaco, *TS & CB 15700,* CONC, WU (3). ***H. tenuifolia*-4,** Argentina, Río Negro, Villa Cerro Catedral: saddle between Co. Princesa and Co. Tronador, *TS, EU & KT 18025,* LP, WU (3). ***H. tenuifolia*-5,** Chile, Región IX, Volcán Lonquimay, *TS, CB & KT 18099,* WU (2). ***H. thrincioides*-1** (J. Rémy) Reiche, Chile, Región VIII, Quidico, *KT & RH 54,* WU (1). ***H. thrincioides*-2,** Chile, Región VIII, Concepción, *TS 15453, TS, CB & GK 15635,* WU (4). ***H. thrincioides*-3,** Chile, Región VIII, Talcahuano, *TS & KT 18092,* WU (3). ***H. variegata*-1** (Lam.) Baker, Argentina, Buenos Aires, Sierra de la Ventana, *EU & KT 117, 126, 131, 133,* LP, WU (9). ***H. variegata*-2,** Argentina, Buenos Aires, Sierra de Tandil, *KT & PSi 1001,* WU (2). ***H. variegata*-3,** Brazil, Rio Grande do Sul, Guaíba, *NM s. n., ICN 59001* (5).

Outgroup. ***H. angustifolia*-1** (Litard. & Maire) Maire, Morocco, Moyen Atlas, Meknès, *ST, TS et al. 270/03M, 282/03M,* SEV, WU (2). ***H. angustifolia*-2,** Morocco, Moyen Atlas, Meknès, *ST et al. 676/03M, 693/03M, 703/03M,* SEV (3). ***H. angustifolia*-3,** Morocco, Taza: Jbel Tazzeka between Bab-Azhar and Bab-Bou-Idir, *ST et al. 633/03M,* SEV (1).