American Journal of Botany 90(3): 496–507. 2003.

PHYLOGENETIC RELATIONSHIPS AMONG SPECIES OF *HYPOCHAERIS* (ASTERACEAE, CICHORIEAE) BASED ON ITS, PLASTID *TRNL* INTRON, *TRNL-F* SPACER, AND *MATK* SEQUENCES[1]

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Nuclear internal transcribed spacer (ITS) regions and chloroplast *trnL* intron and *trnL*/*trnF* spacer and *matK* sequences were used from 86 accessions to assess relationships among 31 European and South American species of *Hypochaeris* plus 18 representatives of related genera of tribe Cichorieae. The ITS tree shows high resolution compared to that of the maternally inherited *trnL* intron, *trnL*/*F* spacer, and *matK* sequences. The ITS and the combined tree reveal clades that agree well with sections of the genus established previously on morphological and cytological grounds, except for *H. robertia*, which groups with *Leontodon helveticus* and *L. autumnalis*. Monophyly of species of *Hypochaeris* from South America is strongly supported by both ITS and the joint matrix of ITS, *trnL*, and *matK* data. European species lie basal to South American taxa, which suggests that species in South America evolved from a single introduction from European progenitors and not from *H. robertia* as suggested previously. Low levels of sequence divergence among South American taxa suggest a pattern of rapid speciation, in contrast to much greater divergence among European representatives. Different species of *Leontodon* form two different clades that are also supported by chromosome numbers and morphology. Both nuclear and chloroplast markers suggest that *Helminthotheca*, *Leontodon*, and *Picris* are closely related to each other as well as to *Hypochaeris*.

Key words: Asteraceae; biogeography; Cichorieae; Hypochaeridinae; *Hypochaeris*; *Leontodon*; phylogenetics.

Hypochaeris L. is a genus of about 60 species (Bremer, 1994) in Asteraceae tribe Cichorieae, with 10 species in Western Eurasia and northern Africa, three species in Eastern Asia, and more than 50 species in South America. Based on morphological and cytological features, the genus previously has been divided taxonomically into five sections (Hoffmann, 1890): *Achyrophorus*, *Hypochaeris* (= *Euhypochaeris*), *Metabasis*, *Robertia*, and *Seriola*. All sections of the genus are found in the Mediterranean region, whereas all of the species in South America have been placed together in sect. *Achyrophorus* (along with several European and all East Asian taxa).

Aside from interest in *Hypochaeris* for developing a more predictive infrageneric classification, the genus is also of significance for understanding evolutionary patterns and processes in the flora of South America. More cytological diversity ($n = 3, 4, 5, 6$) is contained within just the 10 species of this genus in Europe than is found in the more than 50 species in South America, the latter with nearly all the same chromosome number ($n = 4$, except for a few known cases of tetraploidy; Weiss et al., in press) and all with similar asymmetrical and bimodal karyotypes (Stebbins, 1971; Barghi et al., 1989). South American species of *Hypochaeris* are found from sea level to over 3500 m in elevation and in many different habitats. They also demonstrate morphological adaptations to these habitats that involve leaves, roots, and flowering heads. The available data, therefore, suggest an hypothesis that the genus has speciated rapidly, and perhaps recently, in South America into many different environments. To help guide additional evolutionary studies within and among these South American species, therefore, it is essential to learn not only if the group is monophyletic (i.e., originating only once from progenitors in Europe) but also to determine phylogenetically closely related species groups.

Hypochaeris also reveals an interesting biogeographic pattern. Few genera of Asteraceae are known with a broad intercontinental disjunct distribution between Europe and South America (excluding well-known weeds in *Lactuca*, *Matricaria*, *Sonchus*, *Taraxacum*, etc.); hence, interpreting the origin and distributional history of *Hypochaeris* is of significance. The existing hypothesis is that species of the genus in South America have been derived from Mediterranean taxa by long-distance dispersal (Stebbins, 1971; Ruas et al., 1995; Cerbah et al., 1998). Broader sampling and more detailed character comparisons, however, are needed to test this hypothesis.

Critical to resolving issues of biogeography and evolution are detailed phylogenetic analyses using reliable and informative markers. Internal transcribed spacers (ITS) of nuclear ribosomal DNA (rDNA) have proven useful for elucidating phylogenetic relationships among congeneric species and closely related genera in Asteraceae (Baldwin, 1992; Baldwin et al., 1995; Kim et al., 1996) and for suggesting biogeographic affinities (Noyes and Rieseberg, 1999). Usefulness of ITS for phylogeny of *Hypochaeris* among European taxa has already been demonstrated by Cerbah et al. (1998), although

[1] Manuscript received 23 May 2002; revision accepted 26 September 2002.

The authors thank: John Freudenstein (OS) and Brent Mishler (UC) for permission to remove leaf fragments for DNA analysis from selected herbarium sheets; J. Greimler for help with identification of samples of *Leontodon*; M. Weigend for collections from Peru; F. and L. Ehrendorfer for samples from southern Chile; W. Gutermann for permission to extract DNA from specimens in his personal herbarium; M. Chase for selected DNA samples from Kew; FWF (Fonds zur Förderung der Wissenschaftlichen Forschung) grant number P13055-BIO to Tod F. Stuessy for financial support.

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they employed only a limited sampling (four species) of South American taxa.

Plastid noncoding regions are also suitable for phylogenetic investigations. They tend to evolve more rapidly than do coding sequences, by accumulation of insertions/deletions at a rate at least equal to that for nucleotide substitutions (Palmer et al., 1988; Clegg and Zurawski, 1992; Clegg et al., 1994; Kelchner, 2000). The plastid DNA sequences, *trnL* intron, and *trnL*/*trnF* intergenic spacer, have been used for phylogenetic analysis in Asteraceae at the tribal level (Bayer and Starr, 1998; Bayer et al., 2000) and at generic and specific levels in Palmae (Baker et al., 2000). The *matK* gene has become another sequence candidate for phylogenetic analysis. Recent studies have also shown the utility of this gene for resolving lower level relationships (Liang and Hilu, 1996; Xiang et al., 1998; Aoki and Ito, 2000; Soltis et al., 2001).

In the present phylogenetic investigations of *Hypochaeris* we have used both nuclear and plastid sequences individually and in combination to (1) test the present sectional classification by correlation of new molecular data with previously used morphological and cytological information; (2) test monophyly of the South American species; (3) infer geographic origin of the entire genus; and (4) examine relationships among *Hypochaeris* and selected generic relatives of subtribe Hypochaeridinae.

MATERIALS AND METHODS

Collections sampled—We used 86 accessions for the phylogenetic analyses, including 31 species of *Hypochaeris* representing all taxonomic sections and 18 outgroup taxa (http://ajbsupp.botany.org/v90/). Many samples were collected in Chile in 1998–2000, and others were obtained from previously collected herbarium material (with permissions of curators, see acknowledgements). Previously published DNA sequences by Cerbah et al. (1998) were also used. Field-collected material was dried and stored in silica gel prior to DNA extraction.

DNA extraction and amplification—Total DNA was extracted from material stored in silica gel as well as from herbarium specimens following the 2× cetyltrimethyl-ammonium bromide (CTAB) procedure of Doyle and Doyle (1987). The amplification of ITS (Taberlet et al., 1991; Baldwin et al., 1995) and the *trnL* intron and the *trnL*/*F* intergenic spacer and *matK* was done using universal primers. The highest yields of polymerase chain reaction (PCR) products were achieved using the following conditions. The 100-μL PCR reaction contained 72.5 μL of sterile water, 10 μL of 10% (m/v) *Taq* polymerase reaction buffer, 2 mmol/L (4 μL of 50 mmol/L stock) magnesium chloride, 0.2 mmol/L (2 μL of 10 mmol/L stock) of each dNTP (total 8 μL), 0.25 μmol/L (2 μL of 50 μmol/L stock) of each primer (total 4 μL of forward and reverse), 2.5 units of *Taq* DNA polymerase and 2–8 ng (1 μL of 2–8 ng/μL) of template total DNA. Reaction mixtures were sealed with one or two drops of mineral oil to prevent evaporation during thermal cycling. Amplified fragments were checked with 1% agarose gel and the amplified double-stranded DNA fragments were purified using Qiaquick (Qiagen, Valencia, California, USA) gel purification kit. The purified fragments were directly sequenced on an ABI 377 automated sequencer (Perkin Elmer Applied Biosystems, Vienna, Austria) using dye terminator chemistry following manufacturer's protocols. Two cycle sequence reactions were performed for each template using each of the two primers for PCR amplification. The programs Sequence Navigator and AutoAssembler (Perkin Elmer Applied Biosystems) were used to edit and assemble the complementary sequences.

Sequence alignment and phylogenetic analyses—Alignments were obtained using the program Clustal V (Higgins et al., 1992) and adjusted visually. Phylogenetic analysis was done using PAUP beta test version 4.0b8 (Swofford, 1998) for all three data sets: (1) ITS (624 bp excluding the 5.8S); (2) *trnL* (UAA) intron and intergenic spacer between *trnL* (UAA) 3′exon and *trnF* (GAA) (891 base pairs [bp]); and (3) *matK* partial sequence of 853 bp for all the species investigated except *Rhagadiolus edulis* where we had only 524 bp for our analysis. The lengths of the spacers include gaps for obtaining unambiguous alignments. An heuristic search was conducted with 1000 random sequence additions and tree bisection-reconnection (TBR) branch swapping, permitting 10 trees to be held at each step. Successive approximation weighting (SW; Farris, 1969) was performed only for the *trnL* matrix according to the rescaled consistency index using the maximum value (best fit) criterion and base weight of 1.0. Each round of reweighting was followed by 10 replicates of heuristic search with random sequence addition and TBR swapping. After each round, all trees found were used as starting trees in another search to be sure that all shortest trees had been collected. This process was repeated until the same tree length was obtained twice in succession. Confidence limits for trees were assessed by performing 1000 replicates of bootstrapping (Felsenstein, 1985) using equal weigting, TBR swapping, MULTREES on, and holding only 10 trees per replicate.

RESULTS

Results from analyses of (1) nuclear ITS, (2) chloroplast coding *matK*, and (3) noncoding *trnL*/*trnF* sequences give different insights on relationships among species of *Hypochaeris* and near relatives. Analysis of ITS resulted in phylogenies with higher retention index (RI) and clades with good bootstrap support. The plastid noncoding *trnL* intron and *trnL*/*F* spacer and coding *matK*, however, showed poor resolution. Combined analyses of data from all three genes (ITS, *trnL* intron and *trnL*/*F* spacer, *matK*) were congruent with the ITS phylogenetic trees.

ITS—Results were obtained from 78 accessions, including 15 outgroups (Fig. 1). Only spacers ITS1 and ITS2 were included in the analyses; no evidence of multiple rDNA repeat types was observed. The length of ITS1 ranged from 246 to 256 bp and ITS2 about 215 bp, with a highly variable region of 31–55 bp. A total of 624 characters was included in the analysis, of which 299 were parsimony informative. The heuristic search generated 800 equally parsimonious trees with 1028 steps (CI [consistency index] = 0.55; RI = 0.79), one of which is given in Fig. 1. A separate analysis was done removing different accessions for some taxa, leaving only 33 ingroup and 12 outgroup samples. Heuristic search generated 2987 most parsimonious trees with 911 steps (CI = 0.60; RI = 0.68). The specific relationships in this tree did not differ from that with all accessions and are therefore not shown.

The ITS tree (Fig. 1) shows monophyly of the South American taxa with a bootstrap value of 100%. Within the South American clade the Argentinean taxa, i.e., *H. chillensis*, *H. megapotamica*, and *H. microcephala*, appear basal. *Hypochaeris pampasica*, however, the fourth Argentinean species analyzed, falls outside this group. None of the clades among species of South America is well supported. In contrast, the European taxa, i.e., *H. illyrica*, *H. maculata*, and *H. uniflora*, form a very well-substantiated clade (100%); *H. grandiflora* from Russia and China lies basal to this group. The European taxa of sects. *Achyrophorus* and *Metabasis* form a well-supported clade (90%) and are sister to the South American group. The last clade within the genus containing sects. *Seriola* and *Hypochaeris* consists of four additional European species (*H. achyrophorus*, *H. glabra*, *H. laevigata*, and *H. radicata*) and is supported by 95% bootstrap; the two species of sect. *Hypochaeris*, *H. radicata* and *H. glabra*, are well supported (100%), as are *H. achyrophorus* and *H. laevigata* of sect. *Ser-*

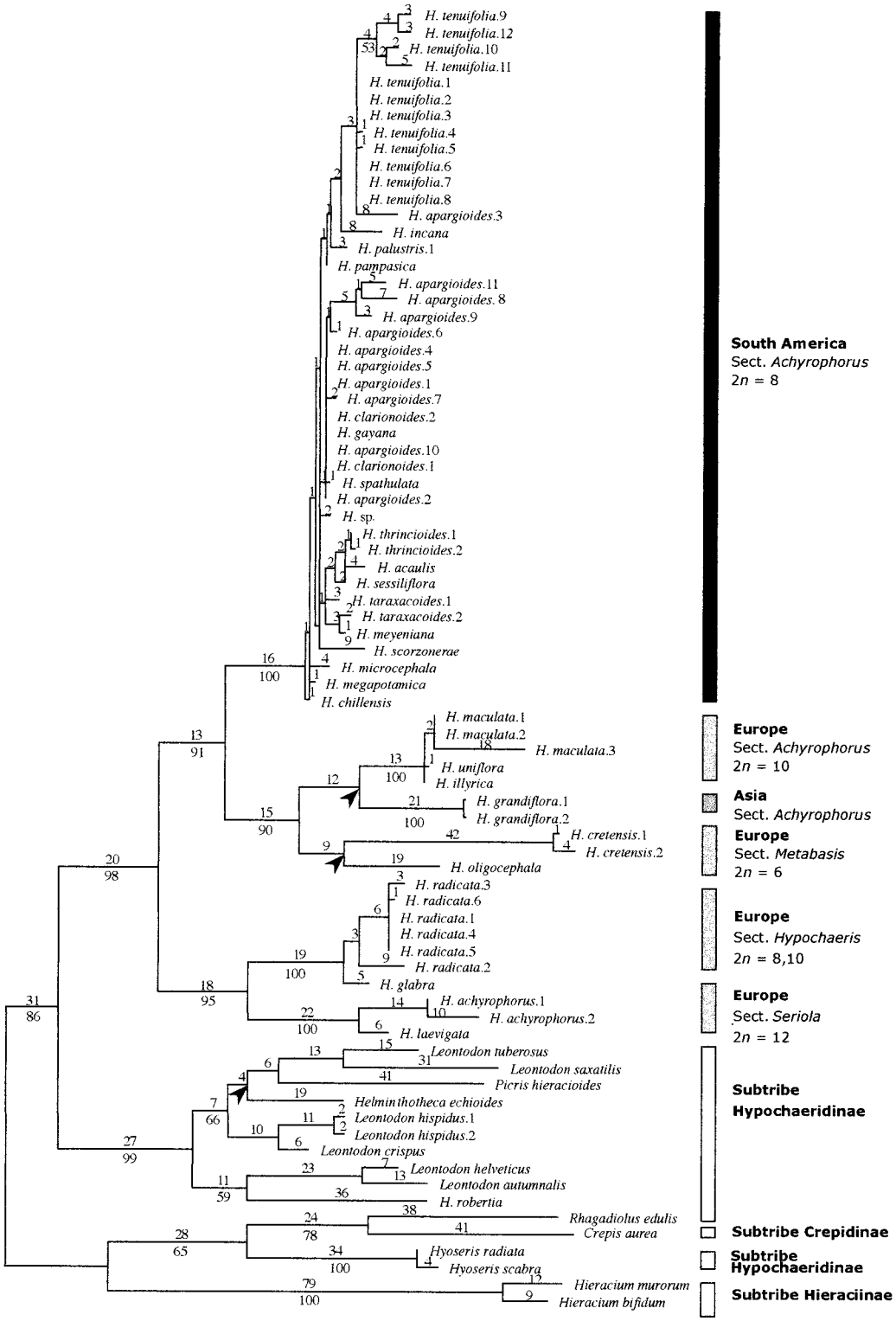

Fig. 1. Phylogram representing one of 800 equally parsimonious trees of *Hypochaeris* and relatives (including different accessions for some taxa) derived from internal transcribed spacer (ITS) nrDNA data. Values above each branch are Fitch lengths (ACCTRAN optimization); those below are bootstrap support (>50%). Arrowheads mark groups not present in strict consensus. None of the clades in the South American species have more than 50% bootstrap value.

iola (100%). The data show that *Hypochaeris* is monophyletic, except for *H. robertia*. *Leontodon* is well supported (99%) but paraphyletic, with a suggestion of two internal units, only weakly supported (66 and 59%). *Leontodon helveticus* and *L. autumnalis* fall into one group (together with *H. robertia*), and *L. hispidus*, *L. crispus*, and *L. tuberosus* combine into another clade, together with species of *Picris* and *Helminthotheca*.

trnL—Analyses included 32 accessions of *Hypochaeris* and 12 outgroups (Fig. 2). Length variation of the *trnL* sequences ranged from 796 to 853 bp. The *trnL*(UAA) intron is about 438 bp including the *trnL*(UAA) exon; the spacer region *trnL*(UAA)-*trnF*(GAA) varies from 358 to 415 bp in length. Gaps ranging from 2 to 40 bp were included for proper sequence alignment, yielding a total length of 891 bp. Of the total characters used for the analysis, 672 characters are constant; 87 variable characters (9.7%) are parsimony informative. Heuristic search generated 8590 most parsimonious trees with 318 steps (CI = 0.77; RI = 0.70). Phylogenetically informative insertions and deletions were observed in the aligned *trnL*/*F* spacer (Table 1).

The *trnL* intron and spacer sequences, in general, do not resolve well the South American and European taxa of *Hypochaeris* (Fig. 2). European taxa such as *H. cretensis*, *H. illyrica*, *H. maculata*, and *H. uniflora* together form a weakly supported clade (54%) within the unresolved major *Hypochaeris* clade. *Hypochaeris radicata*, *H. glabra*, and *H. achyrophorus* also form a fairly well-supported clade (79%). Species of *Leontodon* are held together weakly (<50%) except for the divergent *L. autumnalis*. A 5-bp insertion (GTTCT, positions 635–639) was observed in *H. radicata*, *H. achyrophorus*, *H. robertia*, and all the outgroups included in this study (Table 1). Similarly, a 7-bp insertion (TACAAAA, positions 670–677) is observed in all the South American and European taxa of *Hypochaeris* except for *H. radicata*, *H. glabra*, *H. achyrophorus*, *H. robertia*, and the outgroups (Table 1).

matK—Thirty-one accessions comprising four outgroup taxa and 27 *Hypochaeris* species were used for analysis (Fig. 3). Only part of the *matK* gene was sequenced totaling 853 bp. Of the total characters used, 41 are parsimony informative (7.8%). Heuristic search generated 8860 equally parsimonious trees with 162 steps (CI = 0.58, RI = 0.77; Fig. 3).

Due to problems in PCR amplification and sequencing, the outgroup here is limited to *Leontodon* and *Picris*. The *matK* sequences resolve better than the *trnL* intron and the spacer *trnL-F*. The European taxa, i.e., *H. maculata*, *H. oligocephala*, and *H. cretensis*, form a weakly supported group (56%) basal to the South American clade which is also supported by a weak bootstrap value (65%). Also within the South American clade, *H. hookeri*, *H. scorzonerae*, and *H. barbata* are held together by a bootstrap value of 72%. The other European taxa, *H. radicata*, *H. glabra*, and *H. achyrophorus*, also form a moderately well-supported clade (84%). *Leontodon* and *Picris* form a clade with a bootstrap value of 70%.

ITS + trnL + matK—Joint matrices of sequences from these three genes in taxa for which all data are available yield 2267 characters, of which 339 (15%) are parsimony informative. Fitch parsimony analysis of the combined ITS, *trnL* (intron), *trnL*/*trnF*, and *matK* sequences produced six equally parsimonious trees of 1325 steps (CI = 0.62; RI = 0.56), one of which is shown in Fig. 4.

Monophyly of the South American taxa is shown clearly with maximum bootstrap value (100%). *Hypochaeris oligocephala* and *H. cretensis* form a good clade (100%), with *H. maculata* basal to both (plus Asian *H. grandiflora*). The European taxa appear basal to the South American taxa. *Hypochaeris radicata*, *H. glabra*, and *H. achyrophorus* form a reasonably well-supported clade (72%). *Hypochaeris robertia* joins weakly (<50% bootstrap) with species of *Leontodon*.

DISCUSSION

Infrageneric taxonomy of Hypochaeris—Because no comprehensive recent monograph of *Hypochaeris* exists, some comments on the taxonomic history and sectional structure of the genus are believed helpful. Although a Linnaean genus, *Hypochaeris* was early treated in a very narrow sense to include only the well-known European species, now worldwide weeds, *H. radicata* and *H. glabra*. These were retained in their own genus, *Hypochaeris*, by Cassini (1827) in his classification of Cichorieae, but he placed four additional genera (*Robertia* Cass., *Piptopogon* Cass., *Seriola* L., and *Porcellites* Cass.) into Hypochéridées in his fourth ''Section Scorsonérées.'' This generic grouping provided the basis for subsequent sectional classifications within *Hypochaeris* in a broader sense. Characters distinguishing these taxa were number of rows of pappus bristles, beaking of achenes, and shapes of phyllaries.

De Candolle (1838) built upon the generic distinctions recognized by Cassini (1827), but he took a slightly broader view of *Hypochaeris*, including the former's *Porcellites* as a section (along with sections *Arachnites* and *Euhypochaeris*). Candolle also continued to recognize the related genera *Seriola* and *Robertia*, plus *Achyrophorus* Scop., and his newly described *Metabasis* DC. and *Phalacroderis* DC.

The only monographer of *Hypochaeris*, Schultz-Bipontinus, addressed the genus in two major studies. In the first (1845), he recognized *Hypochaeris* in a narrow sense, treating *Achyrophorus* as a larger genus with subgenera *Geropogonoides*, *Achyrophorus*, *Oreophila*, and *Robertia*. He also included as closely related genera *Piptopogon*, *Seriola*, and *Fabera* Sch.-Bip. Later (1859), Schultz-Bipontinus presented an enlarged concept of *Achyrophorus*, particularly influenced by the new diversity coming from South America by active collectors such as Gardner in Brazil, Weddell in Peru and Bolivia, and Gay in Chile, to include all these previously recognized genera.

Bentham (1873) resuscitated *Hypochaeris* from *Achyrophorus*, based on priority as a Linnaean genus, and included all previously recognized genera as sections: *Achyrophorus*, *Oreophila*, *Amblachaenium* (containing *A. grandiflora* from East Asia), *Arachnites*, *Euhypochoeris*, *Porcellites*, *Serioloides*, *Seriola*, *Metabasis*, and *Robertia*. Hoffmann (1890) followed this basic pattern, but he made amalgamations that resulted in fewer recognized sections (well summarized by de Dalla Torre and Harms [1907] with sectional synonymy): *Achyrophorus* (including European, East Asian, and South American representatives), *Euhypochoeris* (= *Hypochaeris*), *Metabasis*, *Seriola*, and *Robertia*. This is the last comprehensive treatment of the genus.

Since Hoffmann (1890), a number of additional regional taxonomic studies on *Hypochaeris* have been completed. Cabrera initiated several studies on the genus (e.g., 1963, 1976)

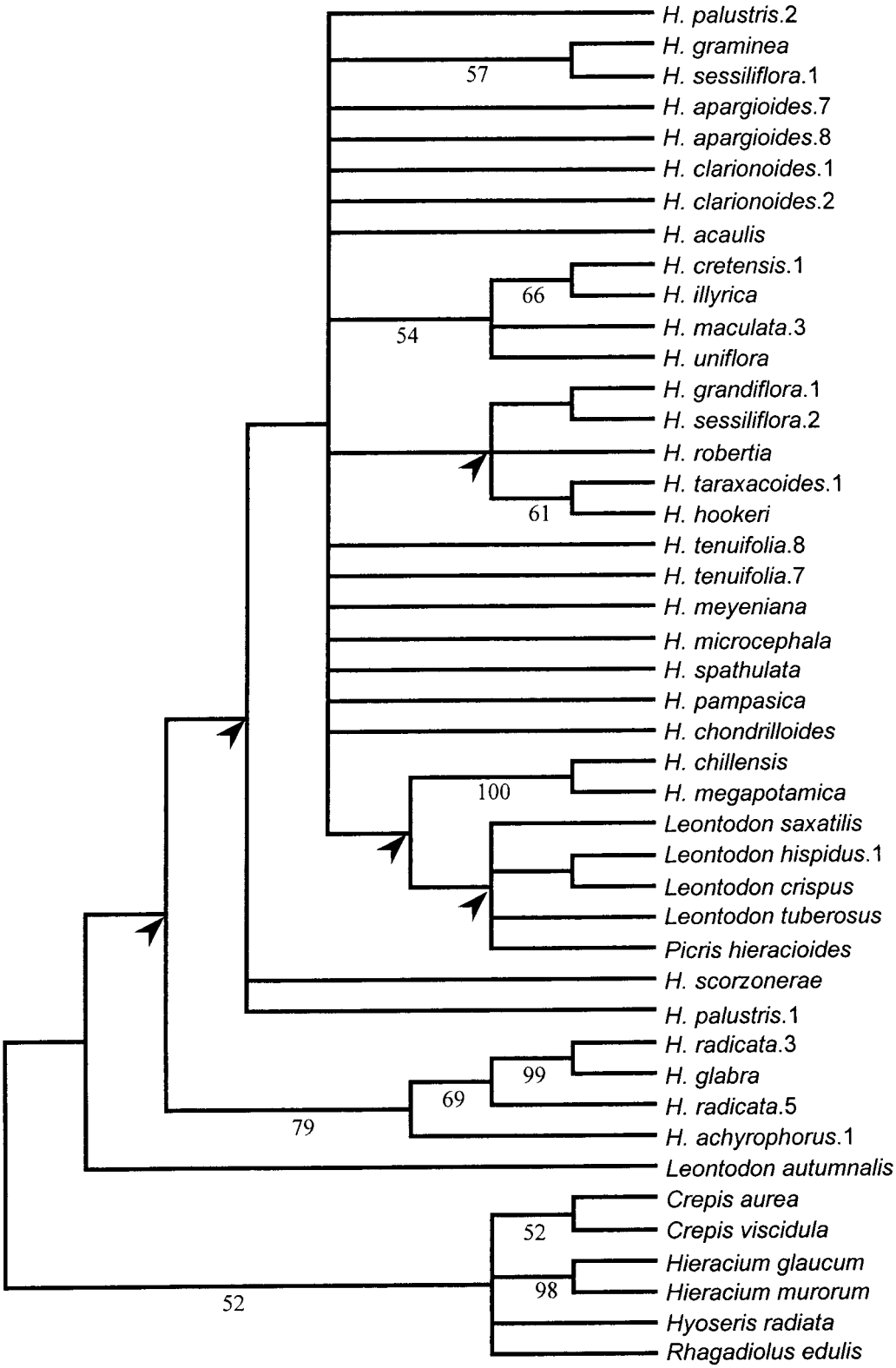

Fig. 2. Bootstrap consensus tree of *Hypochaeris* and relatives from analysis of *trnL* intron and *trnL*/*trnF* spacer data after successive weighting. Bootstrap percentages >50% listed below branches. Arrowheads mark groups not present in all shortest Fitch trees.

TABLE 1. Informative insertions and deletions in the *trnL/F* spacer region of *Hypochaeris* and related taxa.

Taxon	620 | 634 | 648 | 662 | 676 | 690 | 704 |
H. acaulis	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAATGATACATGTACAAAATGATACATGTACAAATGAAC
H. apargioides	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA-----CATGTACAAAATGATACATGTACAAATGAAC
H. clarionoides	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA-----CATGTACAAAATGATACATGTACAAATGAAC
H. chondrilloides	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAATGATACATGTACAAAATCATACATGTACAAATGAAC
H. cretensis	TGAGCGGAAATGCT-----CTTATCACATATGATATATA-GA------TACAAAA-------TGTACAAAATGATACATGTACAAATGAAC
H. graminea	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAATGATACATGTACAAAATGATACATGTACAAATGAAC
H. hookeri	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA-----CATGTACAAA-TGAA-CATGTACAAATGAAC
H. meyeniana	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA-----CATGTACAAAATGATACATGTACAAATGAAC
H. microcephala	TGGAGCGGAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAATGATACATGTACAAAATGATACATGTACAAATGAAC
H. palustris	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAATGATACATGTACAAAATGATACATGTACAAATGAAC
H. pampasica	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA----ACATGTACAAAATGATACATGTACAAATGAAC
H. scorzonerae	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAATGATACATGTACAAAATGATACATGTACAAATGAAC
H. sessiliflora	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA--ATACATGTACAAAATGATACATGTACAAATGAAC
H. spathulata	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA-----CATGTACAAAATGATACATGTACAAATGAAC
H. taraxacoides	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA-----CATGTACAAAATGATACATGTACAAATGAAC
H. tenuifolia	TGAGCGGAAATGCT-----CTTATCACATGTGATATATATGATACATGTACAAAA-----CATGTACAAAATGATACATGTACAAATGAAC
H. grandiflora	TGAGCGGAAATGCT-----CTTATCACATGTGATATATA-GATACATGTACAAAA----------TGATACATGTACAAATGAAC
H. illyrica	TGAGCGGAAATGCT-----CTTATCACATGTGATATA--TGATACATGTACAAAA----------TGATACATGTACAAATGAAC
H. maculata	TGAGCGGAAATGCT-----CTTATCACATGTGATATATAT---ACATGTACAAAA----------TGATACATGTAAAAATGAAC
H. uniflora	TGAGCGGAAATGCT-----CTTATCACATGTGATATATAT---ACATGTACAAAA----------AAAATGATACATGTAAAAATGAAC
H. achyrophorus	TGAGCGGAAATGTTGTTCTCTTATCACATGTAATATATATGATACATG----------TACAAATGAAC
H. glabra	TGAGCGGAAATGCTGTTCTCTTATCACATGTAATATATAT----------ATGTACAAATGAAC
H. radicata	TGAGCGGAAATGCTGTTCTCTTATCACATGTAATATATAT-----ATG----------TACAAATGAAC
H. robertia	TGAGCGGGAATGCTGTTCTCTTATCACATGTGATATATATGATATATG----------TACAAATGAAC
Crepis aurea	TGAGCGGAAATGCTGTTCTCTTATCACATGTGTAATATATATATATAT----------TACAAATGAAC
Hieracium glaucum	TGAGCGGAAATGCTATTTCCGTATCACATGTGATATATATGATACATG----------TACAAATGAAC
Hieracium murorum	TGAGCGGAAATGCTATTCTCGTATCACATGTGATATATATGATACATG----------TACAAATGAAC
Hyoseris radiata	TGAGCGGAAATGCTGTTCTCTTATCACATGTGATATATATGATACATG----------TACAAATGAAC
Leontodon autumnalis	TGAGCGGAAATGCTGTTCTCTTATCACATGTGATATAT--GATACATG----------TACAAATGAAC
Leontodon crispus	TGAGCGGAAATGCTGTTCTCTTATCACATGTGATATATATGATACATG----------TACAAATGAAC
Leontodon hispidus	TGAGCGGAAATGCTGTTCTCTTATCACATGTGATATATATGATACATG----------TCCCAATGAAC
Leontodon saxatilis	TGAGCGGAAATGCTGTTCTCTTATCACATGTGATATATATGATACATG----------TACAAATGAAC
Leontodon tuberosus	TGAGCGGAAATGCTGTTCTCTTATCACATGTGATATATATGATACATG----------TACAAATGAAC
Picris hieracioides	TGAGCGGAAATGCTTTTCTCTTATCACATGT-ATATATATGATACATGTACA----------ACATGTCAAC
Rhagadiolus edulis	TCAGCGGAAATGCTGTTCTCTTATCACATGTGATATATAT----------TACAAATGAAC

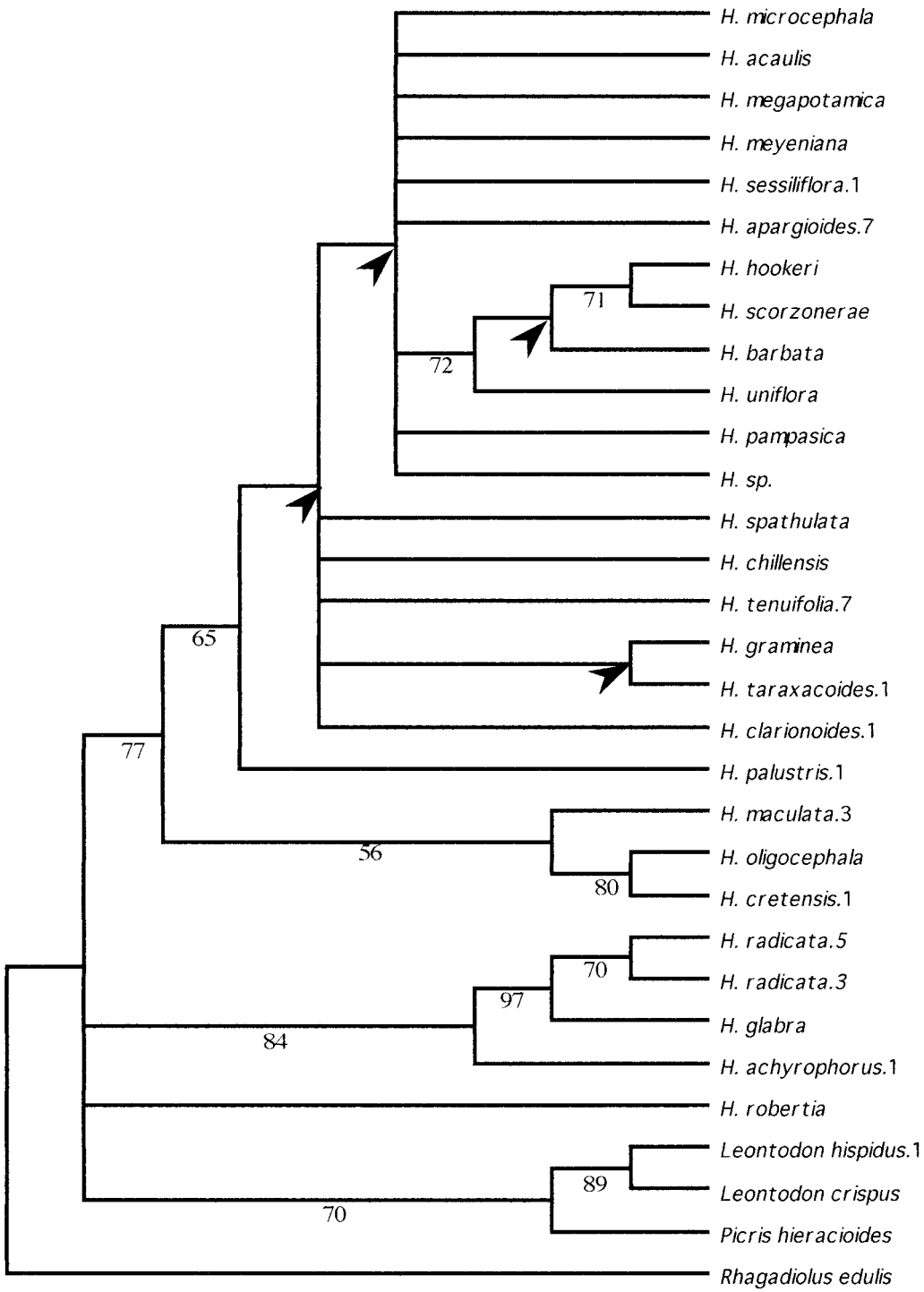

Fig. 3. Strict consensus tree of *Hypochaeris* and outgroups obtained from *matK* sequences. Bootstrap percentages >50% are listed below branches. Arrowheads mark groups not present in all shortest trees.

with the apparent intent of providing a new monograph. He also treated the genus in several detailed floristic treatments of different regions of Argentina (Cabrera, 1971, 1974, 1978). The focus of these studies, however, was on species of southern South America in sect. *Achyrophorus*. Even more recently, Bortiri (1997, 1999) has produced a synopsis of the Argentinean species of the genus. De Fillipps (1976) treated *Hypochaeris* in the context of *Flora Europaea*.

Recent studies by Cerbah et al. (1998), using ITS data for all 10 European species and four South American representatives (GenBank accessions for ITS1 and ITS2 Z-93816–Z93847), have already provided a first test for generic and sectional distinctions involving *Hypochaeris*. Their results with parsimony and neighbor-joining algorithms revealed strong conformity with sectional limits and with 100% bootstrap support values in strict consensus for sects. *Seriola*, *Hy-*

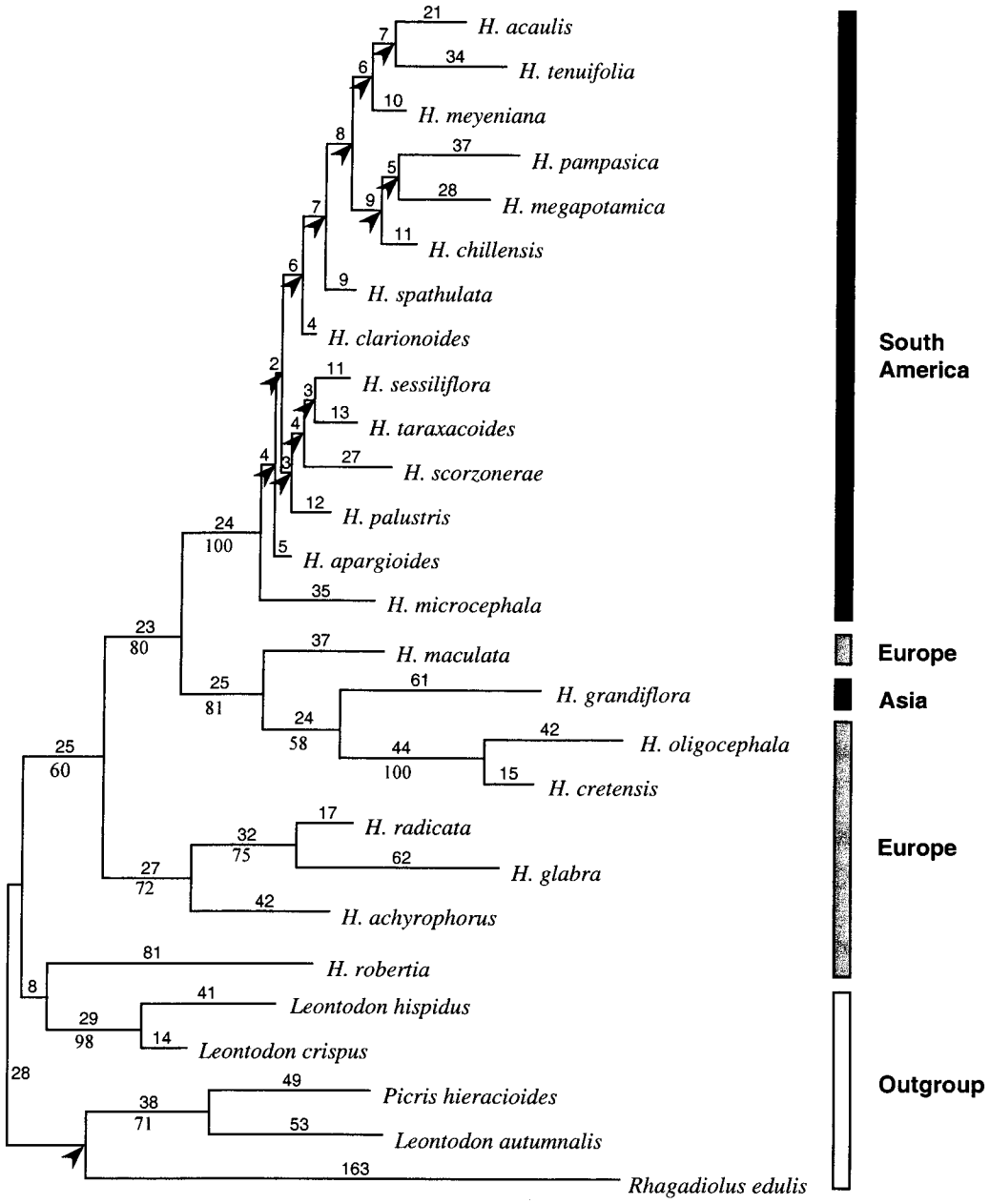

Fig. 4. Phylogram representing one of six equally parsimonious trees of *Hypochaeris* and relatives from combined ITS/*trnL*/*matK* data. Values above each branch are Fitch lengths (ACCTRAN optimization), those below branches are bootstrap percentages (>50%). Arrowheads mark groups not present in all the shortest trees.

pochaeris (= their *Euhypochaeris*), European species of *Achyrophorus*, and South American species of *Achyrophorus* (in a separate clade). Section *Metabasis* was more weakly supported (75%), and sect. *Robertia* was basal to the entire genus.

Our extended analysis with ITS nuclear and chloroplast *trnL* and *matK* genes, including 18 species from South America, one from East Asia, plus European taxa, also agree well with the previous sectional classification of *Hypochaeris* (Figs. 1, 4). Some sections continue to receive strong bootstrap support (100%; Fig. 1): European and South American taxa of sect. *Achyrophorus*, sect. *Hypochaeris*, and sect. *Seriola*. Section *Metabasis* is only weakly supported in the ITS tree (Fig. 1), but it is well supported (100%) in the combined matrix tree analysis (Fig. 4). *Hypochaeris robertia*, the sole representative of sect. *Robertia*, groups outside of *Hypochaeris* within *Leontodon*. Although the tie to *L. autumnalis* and *L. helveticus* is weakly supported (59%; Fig. 1), the total clade including other species of *Leontodon*, *Picris*, and *Helminthotheca* is strongly supported (99%). There seems little doubt, therefore, that *H. robertia* should be excluded from *Hypochaeris*, and a likely position is within *Leontodon*. Because the latter is a large genus of about 50 species distributed throughout Europe, North Africa, and southwestern Asia (especially concentrated in the Mediterranean region; Bremer, 1994), much more sampling

must be accomplished before the more precise relationships of *H. robertia* can be determined (R. Samuel et al., unpublished manuscript).

Overall relationships within and among sections of *Hypochaeris* revealed in cladistic analysis of molecular data merit comment. Focusing first on the more detailed results from ITS (Fig. 1) plus the combined analysis (Fig. 4), the European species of sect. *Achyrophorus* and species from sect. *Metabasis* (*H. cretensis* and *H. oligocephala*) are sister to the South American taxa, having good support (90%). Sections *Seriola* (*H. achyrophorus* and *H. laevigata*) and *Hypochaeris* (*H. radicata* and *H. glabra*) also form sister clades (95% in ITS; Fig. 1). Second, results from the chloroplast marker, *trnL* (Fig. 2), provide no insight on intersectional relationships and all support levels are low. With *matK* (Fig. 3), however, there are useful indications. *Hypochaeris radicata*, *H. glabra* and *H. achyrophorus*, representing sects. *Hypochaeris* and *Seriola*, respectively, tie together at the 84% support level. The European species of sects. *Achyrophorus* and *Metabasis* are basal to the South American taxa with a weak bootstrap (56%) value. Third, *Hypochaeris laevigata* is very close to *H. achyrophorus* in ITS data (Fig. 1). This again is well-supported karyologically, both having $2n = 12$ and a single locus of 5S rDNA at similar positions on chromosome pair 6 (Cerbah et al., 1998). *Hypochaeris glabra* ($2n = 10$) and *H. radicata* ($2n = 8$) form a clade with high bootstrap (100%) support. They are known to have similar karyotypes, and they can easily hybridize in nature (meiotic study of these two species and their hybrid show complete homology of the NOR-bearing chromosomes; Parker, 1975). Chromosomes of *H. uniflora* are similar in morphology to those of its two relatives *H. maculata* and *H. illyrica* (Cerbah et al., 1998), and this relationship is well supported in our ITS and *trnL* trees (Figs. 1, 2). These three species share a common mountain habitat and sequence similarity (pairwise distance in the ITS matrix between *H. illyrica* and *H. uniflora* is 0.002, and that with *H. maculata* is 0.004).

Affinities of two problematical species to sections within *Hypochaeris* are also clarified through molecular analyses. *Hypochaeris oligocephala*, endemic to the Canary Islands, was first described as *Heywoodiella oligocephala* by Sventenius and Bramwell (1971), but later transferred to *Hypochaeris* by Lack (1978). Cerbah et al. (1998), based on their ITS data, revealed this species to have affinities with species of *Hypochaeris* sect. *Metabasis*. Our analyses confirm this placement (Fig. 1). The previously neglected Asian species *H. grandiflora* forms a weakly supported clade with the European species of section *Achyrophorus*. Similarity in chromosome base number ($x = 5$) and karyotype (Stebbins, 1971) would not conflict with this assessment.

Monophyly and evolution of South American species—An important objective of this study has been to determine whether the species of *Hypochaeris* that inhabit the New World (South America) have a single evolutionary origin, i.e., that they are monophyletic. Only with this understanding can detailed phylogenetic, speciational, and biogeographic investigations be conducted within taxa of the continent. Previous morphological studies of the entire genus have concluded that all taxa from South America belong to section *Achyrophorus*, based on having a single series of pappus bristles (Bentham, 1873); these also tie, however, to species of the same section in Europe (*H. illyrica*, *H. maculata*, *H. uniflora*) and Asia (*H. grandiflora*). Preliminary ITS molecular sequence analyses of the genus (Cerbah et al., 1998) suggested that the species from South America do form a monophyletic unit with high support (100% bootstrap), but the sampling included only four species (from approximately 50).

Our results from combined analysis of 14 species of *Hypochaeris* in South America using nuclear and chloroplast genes strongly suggest that they are monophyletic (100% bootstrap support; Fig. 4). Although not all taxa have been sampled from the continent, the species analyzed from Chile, Argentina, Ecuador, and Peru do encompass much morphological, geographic, and ecological variation, including species inhabiting coastal rocky outcrops (*H. spathulata*) to those in páramos over 3000 m (*H. sessiliflora*).

Another argument in favor of monophyly for South American *Hypochaeris* is that the species form a closely knit clade with very little internal sequence divergence (e.g., Fig. 1). Pairwise distances between species are very low (0.007–0.044). The pattern is suggestive of a rapidly speciating group, diverging into new habitats throughout the continent as dispersal and vicariance offered new opportunities for colonization, establishment, and morphological modification. Because of this presumptive rapid, and perhaps recent, adaptive radiation, conspicuous morphological divergence has not been matched by molecular divergence, and it is difficult to see predictive groups of species within the data. Although hints of clades appear in all cladograms, different genes give different results, and all are weakly supported. Sequence variation from these three genes, therefore, is not sensitive enough to resolve phylogenetic relationships among the South American species of *Hypochaeris*. More sensitive markers such as amplified fragment length polymorphisms (AFLP) seem to be promising (Stuessy et al., in press).

Monophyly of South American *Hypochaeris* is also supported by karyological data. Previous karyotypic studies by Cerbah et al. (1998) have shown that considerable cytological divergence exists among taxa of the genus in Europe. Different base numbers occur among the different sections, and much karyotypic variation prevails. Among the four species from South America analyzed previously, all are $n = 4$, and all have a similar asymmetrical karyotype. Recently, we have extended this survey to seven additional species from Chile (Weiss et al., in press, and unpublished data), and the basic pattern holds the same. Some more minor cytological differences prevail, however, that hold potential for suggesting evolutionary groups within South American taxa. The point is that cytological data of consistent base number ($x = 4$; most species, in fact, are $n = 4$, with only a few known tetraploids with $n = 8$; Weiss et al., in press) and reasonably uniform karyotype correlate with lack of sequence divergence in supporting monophyly of the South American species.

Specific relationships among accessions of the South American complex are difficult to assess with certainity, because the degree of differentiation is low. One partcular observation is noteworthy, however. Field sampling of *H. apargioides* on Volcán Llaima in southern Chile (*Stuessy et al. 15602*) was from a population that also contained individuals of *H. tenuifolia*. Based on morphology of several plants seen in the field, they were tentatively suggested to be hybrids (T. F. Stuessy, personal observation). One of these (*H. apargioides*.3; *15603A*) sampled in the present investigation does align near *H. tenuifolia* as an outlier to the rest of *H. apargioides* (Fig. 1), suggesting that it may, indeed, be of hybrid origin. More

sensitive markers are obviously needed to confirm this hypothesis.

Biogeography—Because the distribution of *Hypochaeris* is disjunct between Eurasia and South America, it is useful to consider implications of molecular sequence data for interpreting this biogeographic pattern. Molecular data have the potential of helping reveal taxon phylogenies that can be translated into area cladograms for analysis of relationships among areas (e.g., Humphries and Parenti, 1986). Further, hypothesized rates of sequence divergence may be used to estimate times of divergence of clades (Crawford et al., 1992; Hillis et al., 1996; Nei and Mayamota, 2000). The interesting questions to be addressed in *Hypochaeris*, therefore, concern (1) the origin of the major disjunction in the genus, and (2) estimating the time of such separation. Answering this latter question will also help give a time frame for the rapid adaptive radiation of the group within South America.

Several previous hypotheses have been offered to explain the disjunct distributional pattern within *Hypochaeris*. Stebbins (1971) offered the most complete explanation. He pointed out that two alternatives exist: (1) a South American origin for the genus, supported by the large number of species, the extensive adaptive radiation, and containing what he believed was the primitive section (*Achyrophorus*) of the genus; and (2) a Mediterranean (European) origin, supported by the greater diversity of reproductive features, plus location also of the related genera *Leontodon*, *Picris*, etc. He continued by pointing out that the chromosome number diversity was also higher in the European taxa ($x = 3, 4, 5, 6$) in contrast to uniformity ($x = 4$) in South America. To him the most telling point, however, is the symmetrical karyotype among diverse European taxa (even with different base numbers) and the asymmetrical and bimodal karyotypes among South American species, which strongly suggest a European origin for the genus. To explain the appearance in South America, however, Stebbins (1971, p. 110) hypothesized that *Hypochaeris* first became established in North America by taxa adapted to pioneer habitats and that these migrated southward, perhaps ''in association with larger ungulate mammals, such as camels and deer, during the Pliocene period.'' Change in climate during the Pleistocene may have caused the extinction of the genus from North America, resulting in the pattern of distribution seen today.

The karyological and initial ITS studies of Cerbah and coworkers (1995a, b, 1998) support the hypothesis of a European origin for *Hypochaeris* followed by strong development of a secondary center for the genus in South America. Their detailed analyses, involving not only gross karyological features, but also 18S and 5S rDNA localizations plus CMA3 bands, reveal conspicuous differences among sections of the genus in Europe. The four species of South American taxa analyzed (*H. chillensis*, *H. megapotamica*, *H. microcephala*, and *H. pampasica*), however, are nearly uniform in these features. Chromosomal rearrangements involving translocation of rDNA genes from satellite regions to intercalary sites with secondary constriction of chromosome pair 3 (probably bearing the major locus 18S rDNA) support this, as does the same paracentric position of 5S rDNA signals found in *H. maculata* and in the South American species (Cerbah et al., 1998).

More recent karyological and the present DNA studies confirm the hypothesis that *Hypochaeris* originated in Europe with a secondary spectacular development in South America. Seven additional species of the genus from South America (H. Weiss et al., unpublished data) have been examined for karyological features, including DNA localizations, and the asymmetrical bimodal karyotype prevails. Confirmations that *Leontodon* and *Picris* on molecular grounds are, indeed, close relatives of *Hypochaeris* and from the same general Eurasian region, continue to provide support for this viewpoint. In fact, the data strongly suggest that the South American species have derived from ancestors from sect. *Metabasis* or sect. *Achyrophorus*. Section *Achyrophorus* has been considered previously to be basal within the genus based on analysis of pollen grains (El-Ghazaly, 1980). In the combined tree (Fig. 4), *H. maculata*, a European member of the same section as those taxa of South America, figures closer than other taxa from Europe and Asia. This species is broadly distributed in Europe and the Mediterranean region (De Fillips, 1976), and it would be a good candidate for dispersal and colonization into new habitats. How the genus actually came to South America, however, is still unknown. Of the two likely hypotheses, (1) long-distance dispersal directly to South America or (2) early colonization of North America followed by migration southward and extinction of North American populations, the former is simpler and hence more attractive. That species of Asteraceae are capable of dispersing long distances and successfully colonizing new habitats is amply evidenced by the numerous members of the family in isolated oceanic islands (e.g., in Hawaii, Juan Fernandez, St. Helena, Galapagos; Carlquist, 1981). A particularly good example has recently been documented in *Microseris*, a genus centered in western North America, but also speciating into Australia and New Zealand (Vijverberg et al., 1999).

In ITS analysis (Fig. 1) the Argentinean species, *Hypochaeris chillensis*, *H. megapotamica*, and *H. microcephala*, appear basal to the other species from South America. Although the sample of South American species is still too small for conclusive statements regarding origin of this wide diversity, it is tempting that the basalmost clades in the continent come from the eastern coast taxa (i.e., in Argentina) rather than from the western side of the Andes. This would be consistent with the hypothesis of long-distance dispersal from Europe, resulting in first colonization on the eastern side of the continent (now Argentina). If this were the case, however, one might expect sequence distances between them to be greater than those seen among species now distributed on the western side of the Andes (e.g., in Chile), but the sequence differences are lower (0.007–0.01) among the Argentinean taxa in comparison with those in Chile. Further, with combined data (Fig. 4), only *H. microcephala* is basal to the rest of the South American species, with *H. chillensis*, *H. megapotamica*, and *H. pampasica* being derived.

Also of interest is the time of disjunction of *Hypochaeris* between Europe and South America, or in other words, the age of the South American complex. Dating the origin of groups, at least approximately, may be done by counting accumulation of nucleotide substitutions (Wolfe et al., 1989; Martin et al., 1993). Caution must be exercised (Crawford et al., 1992), however, because the accumulation of mutations varies from gene to gene and from lineage to lineage, especially in response to variation in generation time and also due to metabolic rates. The ITS sequences appear to have evolved more slowly in some ancient woody groups than in herbaceous, primarily annual, groups of comparatively recent origin. For example in Cucurbitaceae the substitution rate is approx-

imately twice (3.62×10^{-9} substitutions per site per year; Jobst et al., 1998) that of Winteraceae (Suh et al., 1993). The average ITS divergence of 34.65 between Heliantheae and Eupatorieae (also Asteraceae), an estimated 14.8 million years (myr) divergence between the two tribes, implies a much faster mutation rate of 2.34% per myr (range 2.27–2.53%; Schmidt and Schilling, 2000). The mutation rates estimated for the Eupatorieae ITS region (1–3% per myr) are relatively high for this gene region in comparison to other plant groups as Cucurbitaceae (Jobst et al., 1998).

Despite difficulties, it is worthwhile attempting an estimate of the age of the South American species of *Hypochaeris*. The fact that only 18 of 50 or more species have been investigated provides an additional handicap because more sampled taxa may increase the degree of divergence seen. Nonetheless, we do know these taxa are herbaceous and reflect a pattern of rapid radiation in comparison to European congeners. It seems reasonable, therefore, to use the rate of sequence divergence of 2.34% per myr from studies on Eupatorieae (Schmidt and Schilling, 2000), because this is also a recently evolved group within the family (Bremer, 1994). Calculations yield an approximation of 6.8 myr (16 mutations in the major clade out of 292 for the entire genus; Fig. 1) for the origin of the South American species of *Hypochaeris*.

Relationships among Hypochaeris and other genera of Cichorieae—Inclusion of selected genera of Cichorieae as outgroups in the phylogenetic analysis of *Hypochaeris* and the resolution of relationships among these taxa give an opportunity to assess results for generic and subtribal insights. Although our sample is small, only 14 species in 7 genera within a large tribe of 98 genera and more than 1550 species (in eight subtribes; Bremer, 1994), some interesting relationships can be seen from ITS analysis (Fig. 1).

First, it is clear that *Hypochaeris robertia* appears better placed within *Leontodon* than within *Hypochaeris*. In both maternally inherited chloroplast markers *matK* and *trnL*, *H. robertia* groups with *Hypochaeris* (Figs. 2, 3), not precluding ancestral hybridization events. In nuclear rDNA (Fig. 1), however, it is closer to *Leontodon*, falling within the clade with *L. helveticus* and *L. autumnalis*, although bootstrap support is weak (59%). Upon examination of herbarium material of *H. robertia*, it is quite similar to some taxa of *Leontodon*, especially *L. saxatilis*. The data disagree with the hypothesis of Barghi et al. (1989) that *H. robertia* is a link between European and South American taxa. The *trnL* sequence matrix (Table 2) shows that *H. robertia* has an insertion of 5 bp (GTTCT; positions 635–639) that is found in sect. *Hypochaeris* (*H. radicata*, *H. glabra*), sect. *Seriola* (e.g., *H. achyrophorus*), and in the outgroups *Leontodon*, *Picris*, and *Helminthotheca*.

Second, the five different species (six accessions) of *Leontodon* analyzed as outgroup taxa separate into two different clades, with *L. helveticus* and *L. autumnalis* in one clade and *L. hispidus*, *L. crispus*, and *L. tuberosus* in the other (also with *Picris hieracioides* and *Helminthotheca echioides*; Fig. 1). Comparing these results with the most recent sectional classification of the genus by Finch and Sell (1976) shows they correlate exactly with sect. *Scorzoneroides* (Moench.) Dumort [= subg. *Oporinia* (Don) Clapham; Widder, 1975] and sect. *Leontodon*, respectively. These two groups also differ in base chromosome numbers, $x = 6, 12$ in the former and $x = 7$ in the latter (Finch and Sell, 1976), as well as nodding flowering heads and presence of 2- or 7-fid branched hairs in the former vs. erect heads and unbranched hairs in the latter (W. Guttermann, University of Vienna, Austria, personal communication). More sampling is needed in this genus of c. 50 species (Bremer, 1994). Widder (1975) also provided a more detailed classification of the genus into five sections within his two subgenera, which will permit direct testing of these hypotheses with broader sampling.

Third, the two most recent analyses of relationships among genera of Cichorieae, that based on morphology by Bremer (1994) and that from cpDNA restriction site data by Whitton et al. (1995), provide the opportunity to examine ITS relationships among *Crepis*, *Helminthotheca*, *Hieracium*, *Hyoseris*, *Hypochaeris*, *Leontodon*, *Picris*, and *Rhagadiolus*. Bremer grouped six of these genera into Hypochaeridinae Less., with *Hieracium* in subtribe Hieraciinae and *Crepis* in Crepidinae. The results suggest a strong relationship between *Leontodon*, *Picris*, and *Helminthotheca* (99% bootstrap support; Fig. 1), in contrast to the other genera in a weakly supported clade, all with long autapomorphic branches. These latter relationships are much less reliable, perhaps suggesting only that *Crepis* and *Rhagadiolus* may be more closely related to each other than to the other genera (also mentioned recently by Gemeinholzer and Bachmann [2002]).

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