

# MOLECULAR SYSTEMATICS OF THE TRANS-PACIFIC ALPINE GENUS *OREOMYRRHIS* (APIACEAE): PHYLOGENETIC AFFINITIES AND BIOGEOGRAPHIC IMPLICATIONS<sup>1</sup>

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The alpine ecosystem is the only terrestrial biogeographic unit that is distributed globally. Studying phylogenetics of the plant species in this widespread ecosystem can provide insights into the historical biogeographic processes that have shaped the global biodiversity. The trans-Pacific disjunct alpine genus *Oreomyrrhis* (Apiaceae) was investigated using nrDNA ITS sequences to test the taxonomic and biogeographic hypotheses. Phylogenetic analyses using maximum parsimony, maximum likelihood, and Bayesian inference revealed that species of *Oreomyrrhis* form a weakly supported monophyletic clade that is nested within *Chaerophyllum* sect. *Chaerophyllum* (subtribe Scandicinae, tribe Scandiceae). The optimal solutions of dispersal-vicariance analysis indicate that the ancestor of *Chaerophyllum* sect. *Chaerophyllum* (including *Oreomyrrhis*) was distributed in Eurasia and subsequently dispersed to North America and southern Pacific Rim. Based on dating using ITS sequence variation, these dispersal events were most likely recent, probably during late Tertiary to Quaternary. The structure of the ITS haplotype network suggests that a rapid range expansion via long-distance dispersal had been crucial in generating the trans-Pacific disjunction of *Oreomyrrhis*. Furthermore, evolution toward smaller mericarp size and a transition from outcrossing to selfing during *Oreomyrrhis*'s evolution might have increased the chances for long-distance dispersal, facilitating its range expansion and occupation on alpine environments.

**Key words:** alpine plants; Apiaceae; *Chaerophyllum*; internal transcribed spacer (ITS); long-distance dispersal; *Oreomyrrhis*; Pleistocene glaciations; South Pacific disjunction.

The alpine ecosystem is the only terrestrial biogeographic unit that is distributed globally (Körner, 2003). Studying phy-

logenetics of the plant species in this widespread ecosystem presents a unique opportunity to understand the historical biogeographic processes that have shaped global biodiversity. Recent studies in Europe suggest that dispersal dynamics, Pleistocene glaciations, and landscape heterogeneity have been the most influential factors shaping the geographic distribution and genetic variation in alpine plants (Tribusch and Stuessy, 2003). However, few studies have attempted to understand how these historical, geological, and biological factors have affected the alpine biodiversity outside of Europe, on a global scale. The trans-Pacific disjunct genus, *Oreomyrrhis*, found in isolated alpine life zones across different southern latitudes around the southern Pacific Basin, presents an ideal system to test these biogeographic hypotheses.

*Oreomyrrhis* Endl. (Apiaceae subfamily Apioideae), as circumscribed in the seminal work of Mathias and Constance (1955) and with novelties described subsequently by Mathias and Constance (1977) and Chen and Wang (2001), consists of ca. 25 species distributed in alpine and subalpine areas and sub-Antarctic islands around the southern Pacific Rim (Mexico, Central American highlands, northern South American Andes, Tierra del Fuego, Falkland Islands, New Zealand, SE Australia, Tasmania, New Guinea, Borneo, and Taiwan; Fig. 1). It is one of the few genera in Apioideae with a mainly Southern Hemisphere distribution (Mathias, 1965). Known as an “exemplary demonstration of the sub-Antarctic-South Pacific pattern of distribution” (van Steenis, 1963), its unique geographic pattern has intrigued generations of phytogeographers (Hayata, 1911; Merrill, 1918; Mathias and Constance,

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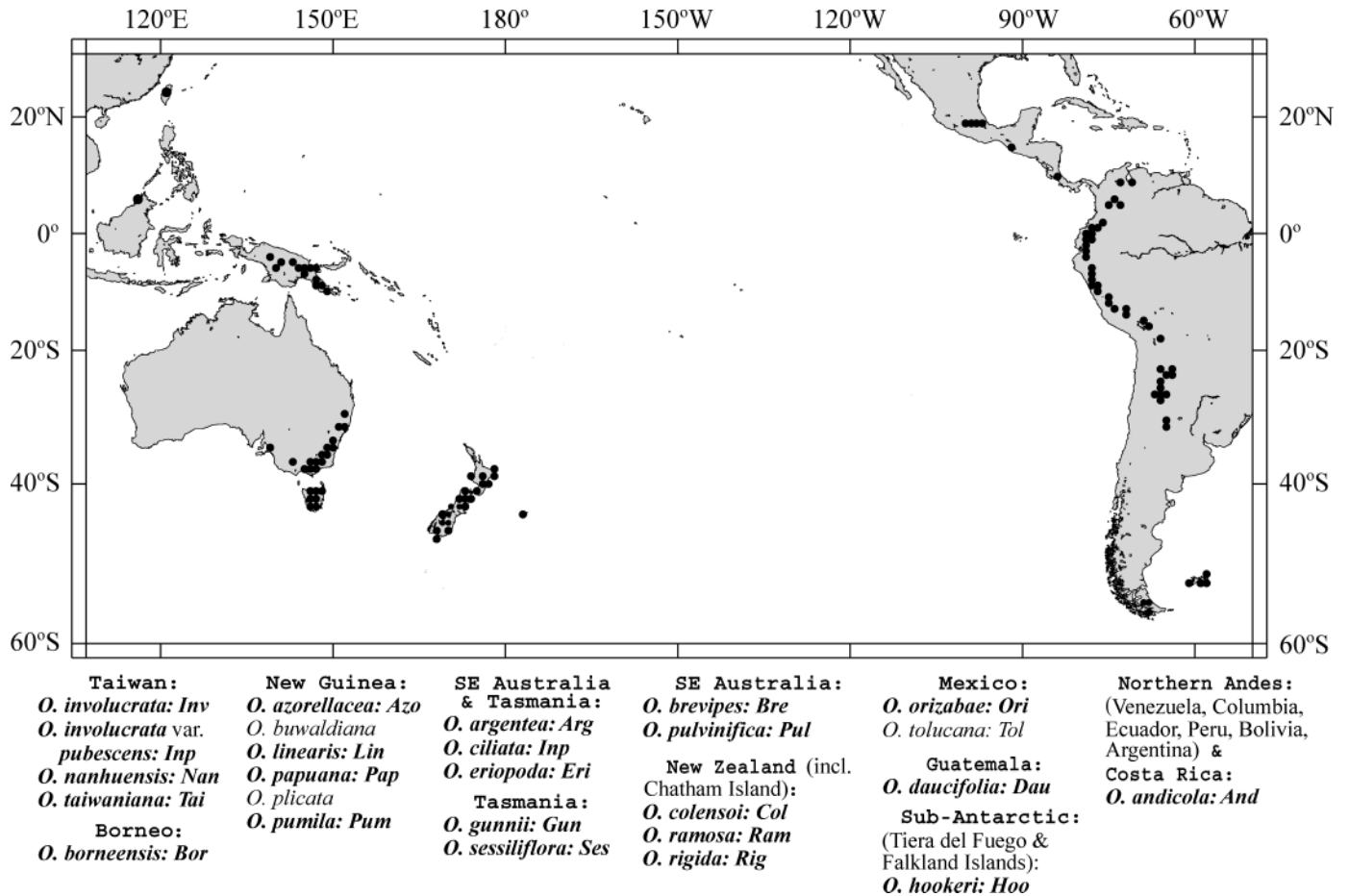


Fig. 1. Distribution of *Oreomyrrhis* Endl. (Apiaceae). Species that were sampled for ITS sequences in the current study are in bold-italic face. Specific geographic distributions and abbreviations for each taxon are below the map. Localities were mapped based on a database compiled from collections at A, B, CANB, F, G, GH, HO, K, L, LA, LAE, NSW, NY, P, UC, US, W, and WELT (herbarium acronyms follow Holmgren et al., 1990).

1955; van Steenis, 1962, 1963, 1964; Raven, 1973; Melville, 1981).

Biogeographic explanations for the trans-Pacific disjunction of *Oreomyrrhis* range from recent late Tertiary to Quaternary long-distance dispersal events (Dawson, 1971; Thorne, 1972; Raven, 1973) to ancient vicariance such as Tertiary land-bridges (van Steenis, 1962, 1964) and plate tectonic movements dating to the Jurassic (Melville, 1981). These competing hypotheses predict different evolutionary relationships among *Oreomyrrhis* species that are readily testable with phylogenetic data. Elucidating the evolutionary history of *Oreomyrrhis* will also contribute to our understanding of broad patterns of Pacific and global alpine biogeography.

The taxonomic history of *Oreomyrrhis* began with the description of *Myrrhis andicola* by Kunth in 1821, based on a Humboldt and Bonpland collection from Ecuador (Humboldt et al., 1821). Later that year, a new genus/species *Caldasia chaerophylloides* Lag. was described from Peru (La Gasca, 1821). De Candolle (1829, 1830) accepted this new genus and transferred *M. andicola* to *Caldasia*. However, because *Caldasia* Lag. was a later homonym, Endlicher (1839) proposed to replace it with the new generic name *Oreomyrrhis*, which has been accepted since that time (Mathias and Constance, 1955).

Morphologically, *Oreomyrrhis* is characterized by an inflo-

rescence consisting of a simple umbel borne terminally on peduncles that arise from the bases of sheathing and often rosetted leaves (Mathias and Constance, 1955). Such a simple umbel is extremely rare in Apioideae, appearing consistently only in *Oreomyrrhis*, *Lilaeopsis* Greene, and *Neogoezia* Hemsl. (Constance, 1987). The first tribal classification that included *Oreomyrrhis* placed the genus (as *Caldasia*) in tribe Scandiceae Spreng. (de Candolle, 1830), which was challenged by Hooker (1856) but followed by Bentham (1867) and Koso-Poljansky (1916). Emphasizing the presence or absence of vittae (secretory canals) and secondary ridges on the mericarps, Bentham (1866) suggested that, among the genera of Scandiceae, *Oreomyrrhis* is most similar to *Chaerophyllum* L. of the subtribe Scandicinae Tausch.

In one of the most comprehensive and influential taxonomic works on Apiaceae, Drude (1898), whose taxonomy emphasized microscopic structure of the mericarps, removed *Oreomyrrhis* from Scandiceae on the basis of mericarp shape and the absence of crystal druses around the carpophore (Mathias and Constance, 1955). Drude placed *Oreomyrrhis* in tribe Smyrnieae Spreng., that included other simple or irregularly compound-umbelled genera, such as *Neogoezia*, *Apiastrum* Nutt. ex Torr. et A. Gray, *Erigenia* Nutt., and *Orogenia* S. Watson (Mathias and Constance, 1955). Drude's treatment has been followed by many subsequent authors (e.g., Shan and

Sheh, 1979) and was adopted with minor changes in the most recent synopsis of Apiaceae (Pimenov and Leonov, 1993). However, the phylogenetic position of *Oreomyrrhis* was left unanswered by Mathias and Constance (1955, p. 349) as they concluded "... We are nevertheless inclined to regard *Oreomyrrhis* as having a vague relationship to *Chaerophyllum* and probably no close affinity with other genera bearing simple umbels. Although *Oreomyrrhis* is clearly referable to the subfamily Apioideae, we have been unable to recognize any close relative among the living Umbelliferae of either the northern or southern hemisphere."

All 13 species of *Oreomyrrhis* investigated to date have a base chromosome number of  $n = 6$  (Mathias and Constance, 1955; Moore, 1971; Chen and Wang, 2001; Pimenov et al., 2003). This number is rare in Apioideae, representing only 4% of the 1461 species with published chromosome counts in the subfamily (Moore, 1971; Pimenov et al., 2003). The rarity of its base number has stimulated much speculation regarding *Oreomyrrhis*'s phylogenetic position (Moore, 1971; Raven, 1975; Pimenov et al., 2003). Recently, serological (Shneyer et al., 1992) and molecular phylogenetic studies (Downie and Katz-Downie, 1996; Downie et al., 2001) have shown that Drude's tribe Smyrnieae is a heterogeneous and polyphyletic assemblage. Unfortunately, *Oreomyrrhis* has not been included in any modern systematic study and its phylogenetic affinity remains puzzling.

In addition to its dubious tribal position, the infrageneric taxonomy of *Oreomyrrhis* has been equally contentious (Mathias and Constance, 1955; Stevens, 1990). The majority of these controversies have centered on the delimitation of *O. andicola* (Kunth) Endl. ex Hook. f. (Mathias and Constance, 1955). First described from the Ecuadorian Andes (as *Myrrhis andicola*), subsequent collections extending from the northern Andes north to the Mexican highlands indicate that *O. andicola* is an extremely polymorphic complex. These populations display continuous morphological variation in habit, size, leaf shape, color, and pubescence that nearly spans the range of morphological diversity of the entire genus (Mathias and Constance, 1955; Melville, 1981). While there have been attempts to divide the Andean *Oreomyrrhis* into multiple species (e.g., Urban, 1880–1882; Johnston, 1938), some authors have extended the species boundary of *O. andicola* beyond South America to include populations in New Zealand (Kirk, 1899), Australia (Bentham, 1866), and New Guinea (Buwalda, 1951).

By restricting the name *Oreomyrrhis andicola* to encompass only the Andean populations and by accepting most regional endemics, Mathias and Constance (1955) recognized 23 species of *Oreomyrrhis* (Fig. 1). Similar species recognition criteria were later adopted in Mathias and Constance (1977) and Chen and Wang (2001) to describe new species from New Guinea and Taiwan, respectively. However, the species delimitation by Mathias and Constance (1955) was later criticized by Kern (1958) as being too narrow. Kern (1958) surmised that *Oreomyrrhis* comprises only a few species, with *O. andicola* being a species with a circum-Pacific distribution from Mexico to Taiwan. Despite Kern's criticism, Mathias and Constance's delimitations have been widely adopted in most local floras (e.g., Allan, 1961; van Royen, 1983; Walsh and Entwistle, 1999). Based on the work of Mathias and Constance (1955), Melville (1981) reconstructed the infrageneric relationships of *Oreomyrrhis* and, based on his own hypothesis of *Oreomyrrhis*'s phylogeny, proposed a vicariance scenario that is linked with ancient geological events. Melville's phyloge-

netic hypothesis was later adopted by Crisci et al. (1991) for a historical biogeographic analysis of southern South America.

In recent years, DNA sequence data have proven to be a promising and powerful tool for elucidating relationships within Apiaceae that have been difficult to resolve with morphological data alone (Downie et al., 2001). In particular, the large quantity of data in GenBank from the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region for numerous taxa of Apiaceae are a valuable asset for testing taxonomic hypotheses surrounding *Oreomyrrhis*. Moreover, ITS sequence data are useful for reconstructing infrageneric relationships (e.g., Downie et al., 2004; Neves and Watson, 2004) and for inferring the biogeographic histories of Apiaceae genera (e.g., Wen et al., 2002).

In an ongoing project of global alpine biogeography, *Oreomyrrhis* was investigated using a combination of phylogenetic, phylogeographic, and population genetic approaches. In this study, ITS sequence data were analyzed by various phylogenetic methods to address the following questions: (1) Is *Oreomyrrhis* monophyletic? (2) What are its closest relatives? (3) Does vicariance or long-distance dispersal better explain the present distribution of *Oreomyrrhis*?

## MATERIALS AND METHODS

**Sampling strategy for *Oreomyrrhis***—Species of *Oreomyrrhis* were sampled to optimize coverage of the geographic distribution of the genus (Fig. 1) and intraspecific morphological variation. Leaves were collected and preserved on silica gel by the first author during fieldwork in Ecuador (*O. andicola*), Taiwan (*O. involucrata* var. *pubescens*, *O. nanhuensis*, and *O. taiwaniana*), SE Australia (*O. brevipes*, *O. ciliata*, *O. eriopoda*, and *O. pulvinifolia*), Tasmania (*O. argentea*, *O. ciliata*, *O. eriopoda*, and *O. sessiliflora*), New Zealand (*O. colensoi*, *O. ramosa*, and *O. rigida*), and Guatemala (*O. daucifolia*) and through the courtesy of local researchers in Taiwan (*O. involucrata* and *O. taiwaniana*), Tierra del Fuego (*O. hookeri*), the Falkland Islands (*O. hookeri*), and Mexico (*O. orizabae*). DNA was also extracted from herbarium specimens for some taxa (*O. azorellacea*, *O. borneensis*, *O. gunnii*, *O. involucrata*, *O. linearis*, *O. papuana*, *O. pumila*, and *O. sessiliflora*), including two of the most unique New Guinea species, *O. azorellacea* (moss-like cushion plant) and *O. linearis* (characterized by grass-like linear leaves). The species not sampled in this study include the rare New Guinean species *O. buwaldiana* and *O. plicata*, that are known only by type collections, and the Mexican high alpine species *O. toluicana* for which no ITS sequence was amplified after several trials using herbarium material.

To evaluate the monophyly of species with broad distributions and substantial morphological variation, multiple individuals (number in parentheses) from different localities were sampled for *O. andicola* (5), *O. argentea* (2), *O. ciliata* (5), *O. colensoi* (4), *O. eriopoda* (6), *O. hookeri* (2), *O. involucrata* (4), *O. linearis* (2), *O. ramosa* (4), *O. rigida* (4), *O. sessiliflora* (2), and *O. taiwaniana* (4). *Oreomyrrhis involucrata* var. *pubescens*, a taxon not recognized by Mathias and Constance (1955) and Chen and Wang (2001) but readily recognizable in the field (K. Chung, personal observation), was also surveyed. One individual from Awahokomo karstland, Otago, New Zealand, previously identified as *O. rigida* (Molloy et al., 1999), but likely a new species (P. Heenan, Landcare Research, personal communication), was also investigated. In total, 57 accessions of *Oreomyrrhis*, representing 22 of the 25 species recognized and/or later described by Mathias and Constance (1955, 1977) and Chen and Wang (2001), were included. The taxa sampled, taxon authorities, voucher information, and Genbank accession numbers are listed in the Appendix. Throughout this study, Mathias and Constance's (1955) species circumscription was closely followed for plant identification, with further verification based on examination of type specimens and/or specimens annotated by Mathias and Constance (1955).

**DNA extraction, PCR amplification, and sequencing**—Total genomic DNA was isolated from silica-dried leaf material using the CTAB procedure

of Doyle and Doyle (1987). For herbarium specimens, DNA was isolated using a VIOGENE Plant Genomic DNA extraction miniprep system (Viogen U.S.A., Sunnyvale, California) following the manufacturer's protocol. The entire ITS region (ITS-1, 5.8S, and ITS-2) was amplified using primers ITS1 and ITS4 described in White et al. (1990). For older herbarium material, the internal primers ITS2 and ITS3 (Downie and Katz-Downie, 1996) or primers ITS-C and ITS-D (Blattner, 1999), in combination with the external primers ITS1 and ITS4, were used to amplify the entire ITS region. All amplifications were performed in a 50- $\mu$ L reaction. Polymerase chain reaction (PCR) conditions were set for an initial 4 min denaturation at 94°C, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min and a final incubation at 30°C for 10 min. PCR products (templates) were visualized via agarose gel electrophoresis and were further purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, California). Sequence reactions were carried out in both directions for each purified PCR template using the ABI PRISM dGTP BigDye Terminator Ready Reaction kit (Applied Biosystems, Foster City, California, USA) following the manufacturer's protocol. Excessive BigDye in the sequence reactions was removed using CENTRI-SEP columns (Princeton Separations, Adelphia, New Jersey, USA). Sequence reaction products were visualized using either an Applied Biosystem 373 automated DNA sequencer or a BaseStation DNA Fragment analyzer (MJ Research, South San Francisco, California, USA). Contigs were assembled using SEQMAN (Swindell and Plasterer, 1997). All newly acquired sequences have been archived in Genbank (Appendix).

**Molecular cloning**—To examine the extent of sequence homogenization among reiterated ITS copies, molecular cloning was conducted on 10 accessions of nine *Oreomyrrhis* species (*O. andicola* [Chung 1493], *O. daucifolia* [Chung 1650], *O. eriopoda* [Chung 1554 and Chung 1612], *O. hookeri* [D. Broughton, no voucher], *O. involucreta* [Juan 53], *O. nanhuensis* [Chung 1510], *O. orizabae* [Avenidaño R. 5349], *O. ramosa* [Chung 1630], and *O. rigida* [Chung 1618]). The purified ITS templates were ligated to the pGEM-T Easy vector (Promega, Madison, Wisconsin, USA) following the manufacturer's protocol and subsequently transformed into competent cells. After an overnight culture at 37°C on the LB ampicillin/IPTG/X-gal selective plate, colonies carrying the ITS insert were identified by color (white) and further verified by PCR using the T7 and SP6 promoter primer pairs (Promega, Madison, Wisconsin, USA). For each accession, up to 20 white colonies were checked for inserts. The amplified PCR products were purified and subsequently sequenced using the ITS1 and ITS4 primers as described in the previous section.

**Tests of sequence evolution**—The rate constancy of the ITS sequence evolution of *Oreomyrrhis* and its closest relatives *Chaerophyllum procumbens* and *C. tainturieri* (see Figs. 3 and 4) were inferred from relative rate tests (Wu and Li, 1985) based on Kimura two-parameter distance (K2P; Kimura, 1980), implemented by the program K2WuLi (Jermini, 1996). *Chaerophyllum temulum* was used as a reference taxon. Given a constant ITS evolution rate, approximate times of diversification with 95% confidence intervals between *Oreomyrrhis* and its outgroup and among *Oreomyrrhis* species were estimated as  $\pm 2$  SE of the K2P distances, calibrated with an ITS sequence divergence rate of 0.79% per million years (Sang et al., 1994; Kropf et al., 2002). This more conservative ITS divergence rate was chosen because we were mainly interested in testing competing biogeographic scenarios related to recent (late Tertiary-Quaternary) vs. old (Jurassic or early Tertiary) diversification of *Oreomyrrhis*, as predicted by long-distance dispersal (e.g., Dawson, 1971; Raven, 1973) vs. vicariance hypotheses (Melville, 1981; van Steenis, 1964), respectively. Pairwise K2P distances and their SEs were calculated using MEGA2 (Kumar et al., 2001).

**Phylogenetic analyses**—Three data matrices were compiled and analyzed to test taxonomic hypotheses and to elucidate phylogenetic relationships within *Oreomyrrhis* and between *Oreomyrrhis* and allied Apiaceae.

**Analysis 1**—To elucidate *Oreomyrrhis*'s phylogenetic position within Apiodeae, the data matrix of Downie and Katz-Downie (1996) was adopted with

some modifications. This matrix was composed of ITS-1 and ITS-2 sequences of representatives of tribe Smyrnieae, subtribes Scandicinae, Daucinae, and Torilidinae of tribe Scandiceae, and several major clades of Apiodeae identified by recent molecular systematic studies (Downie et al., 2001). *Pleurospermum foetens* Franch. (tribe Symrnieae, sensu Drude [1898]) was chosen as outgroup (cf. Downie et al., 2001). ITS sequences of eight *Oreomyrrhis* species from different geographic regions [*O. orizabae* (Mexico), *O. andicola* (South American Andes), *O. hookeri* (Tierra del Fuego), *O. colensoi* (New Zealand), *O. eriopoda* (Australia), *O. papuana* (New Guinea), *O. borneensis* (Borneo), and *O. taiwaniana* (Taiwan)] were added to the matrix. *Chaerophyllum temulum* L., the type species of the largest genus of Scandiceae (Spalik and Downie, 2001), was added to test Bentham's (1866) proposition that *Oreomyrrhis* is most similar to *Chaerophyllum*. Other additions included selected taxa of Drude's (1898) tribe Smyrnieae that possess irregularly compound umbels (*Eriogenia*, *Neogoezia*, and *Orogenia*), and *Lilaeopsis*, another predominantly Southern Hemispheric distributed genus also possessing a simple umbel (Constance, 1987; Petersen et al., 2001). Sequences were aligned using DAMBE (Xia and Xie, 2001) and were manually adjusted in the alignment editor Se-Al (Rambaut, 1996). Only those positions that were aligned unambiguously were used in the phylogenetic analyses; the aligned data matrix is available in TreeBASE (study accession number S1350, matrix accession number M2384).

Phylogenetic analyses were conducted using maximum parsimony (MP) algorithm implemented in PAUP\* version 4.0b10 (Swofford, 2003). All characters were treated as unordered, and all character transformations were weighted equally. A heuristic search with 1000 random addition replicates was performed with the following options in effect: MULTTREES, TBR branch swapping, and gaps treated as missing data. Clade support was assessed by bootstrap (Felsenstein, 1985) and decay (Bremer, 1988) analyses. One hundred bootstrap replicates were analyzed by PAUP\* using the heuristic search option with 10 random sequence additions. Decay indices were calculated using the program TreeRot.v2 (Sorenson, 1999). To evaluate the phylogenetic content of the data, the  $g_1$  statistic of tree-length distribution skewness was calculated from 10000 random MP trees generated by PAUP\*, and was subsequently compared to the critical values provided in Hillis and Huelsenbeck (1992).

**Analysis 2**—The results of analysis 1 unambiguously placed *Oreomyrrhis* in subtribe Scandicinae of tribe Scandiceae, with *Chaerophyllum* being its closest relative (Fig. 2). To further elucidate their relationships within Scandicinae, ITS sequences of 57 *Oreomyrrhis* accessions (22 species) were combined with all available ITS sequences (ITS-1 and ITS-2) of the genus *Chaerophyllum* (22 species) and representatives of other major clades in the subtribe (*Anthriscus*, *Kozlovia*, *Myrrhis*, *Osmorhiza*, *Scandix*, *Sphallerocarpus*; see Spalik and Downie, 2001). *Athamanta cretensis* and *Conopodium ramosum* were selected as outgroups based on recent phylogenetic analysis (Downie et al., 2000). Voucher information of sampled taxa is shown in the Appendix.

Sequences were aligned as in analysis 1. To facilitate analysis, identical sequences were only included once in the data set. The aligned data matrix is available in TreeBASE (study accession number S1350, matrix accession number M2385). To evaluate the phylogenetic utility of alignment gaps (indels), two separate matrices were compiled for MP analysis. In the first matrix (gap-excluding matrix), gaps were treated as missing data. In the second matrix (gap-including matrix), parsimony-informative gaps were coded as binary characters and were added at the end of the matrix using the "simple indel coding" method of Simmons and Ochoterena (2000). Both matrices were analyzed using the same criteria described in analysis 1. Bootstrap support values, decay indices, and the  $g_1$  statistic for the gap-excluding matrix were calculated based on methods in analysis 1.

The gap-excluding data matrix was then analyzed using maximum likelihood (ML). Modeltest 3.5 (Posada and Crandall, 1998) was used to identify the appropriate evolutionary model of nucleotide substitution. Based on the model selected by Modeltest 3.5, 10 heuristic searches were performed in PAUP\* using random addition sequence and TBR branch swapping. To assess clade support, a bootstrap analysis of 1000 replicates was conducted using

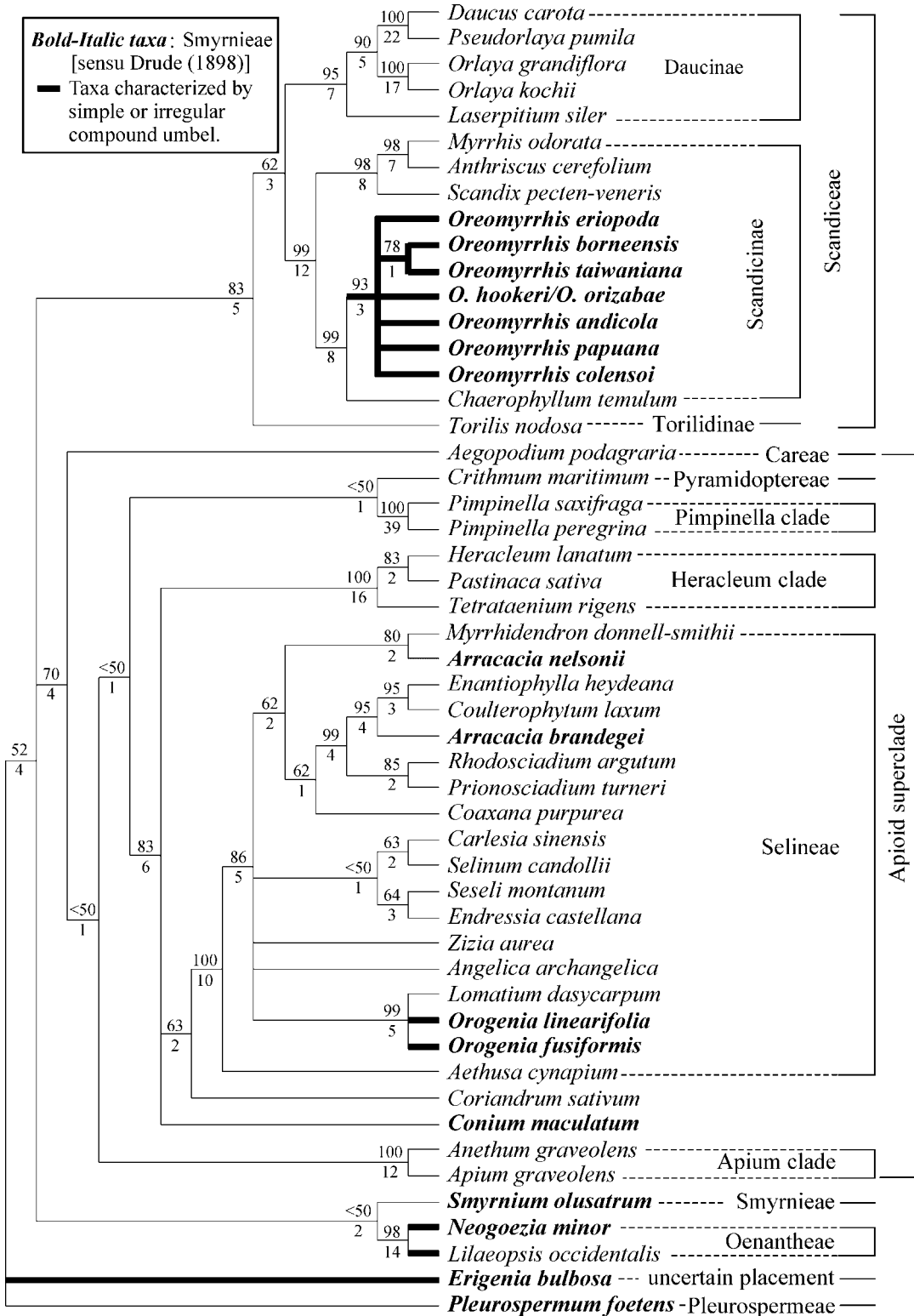


Fig. 2. Strict consensus of 144 minimal length 1218-step trees (corrected CI = 0.44, RI = 0.72) with bootstrap values (above clades) and decay indices (below clades). Members of tribe Smyrnieae (sensu Drude [1898]) are in bold-italic face. Clades indicated by thick lines denote taxa that possess simple or irregular compound umbels. Names for tribes and clades are based on Downie et al. (2001) and Spalik et al. (2004).

the neighbor-joining method with ML distance (e.g., Downie et al., 2004; Sun et al., 2004).

A Bayesian phylogenetic analysis was also performed using the program MrBayes 3 (Ronquist and Huelsenbeck, 2003) based on the same nucleotide substitution model used in ML analysis. In MrBayes, parameters were set as follows: random starting tree, 2,000,000 generations runs with sampling occurring every 100 generations, and a 4000-tree “burn-in” period. PAUP\* was used to generate a 50% majority consensus tree of the output tree file from MrBayes.

**Analysis 3**—Results of analysis 2 revealed that *Oreomyrrhis* accessions formed a poorly resolved polytomous clade probably resulting from low sequence divergences. Such levels of sequence variation suggest ongoing speciation processes in *Oreomyrrhis*. To better elucidate relationships among *Oreomyrrhis* accessions, a haplotype network was constructed. Theoretically, haplotype networks can straddle population–species interfaces where speciation occurs, providing a powerful tool to investigate evolutionary processes (Templeton et al., 2000; Schaal and Leverich, 2001). We compiled an ITS sequence (ITS-1, 5.8S, and ITS-2) matrix of 57 *Oreomyrrhis* accessions. Based on the results of analysis 2 (see Figs. 3 and 4), *Chaerophyllum procumbens* and *C. tainturieri* were selected as outgroups. The haplotype network was calculated using statistical parsimony (Templeton et al., 1992) in the program TCS (Clement et al., 2000), with alignment gaps treated as a fifth state.

**Biogeographic analysis**—A dispersal–vicariance analysis (DIVA; Ronquist, 1997) was performed to infer biogeographic history of *Oreomyrrhis* and subtribe Scandicinae. Given a phylogeny, DIVA reconstructs distribution of ancestral areas that minimize dispersal and extinction events under a parsimony criterion (Ronquist, 1997), presenting an ideal tool to identify sources and directions of dispersal for *Oreomyrrhis* and its close relatives. For implementing DIVA, a fully resolved phylogeny is demanded. Because parts of the Scandicinae phylogeny are unresolved, clades composed of taxa distributed in the same general unit area were compressed to generate a simplified bifurcating phylogeny that is required by DIVA. Information of taxon distribution of subtribe Scandicinae was gathered from Spalik and Downie (2001; Fig. 3). *Athamanta* and *Conopodium* were chosen as outgroups (Spalik et al., 2001a). Since we were mainly interested in identifying the ancestral source of dispersal for *Oreomyrrhis*, not the exact locality of initial diversification, the unresolved clade of *Oreomyrrhis* (Fig. 4A) was also compressed to a single terminal and designated with a Pacific distribution (D). Three additional general unit areas were defined to cover the distributional range of Scandicinae: Eurasia (including North Africa; A), North America (B), and South America (C).

## RESULTS

**Characteristics and evolution of *Oreomyrrhis* ITS sequences**—From 57 accessions of 22 *Oreomyrrhis* species, 26 distinct ITS sequences (haplotypes) were obtained. The alignment of the polymorphic sites, haplotype designations, and their frequencies are shown in Table 1. The length of the entire ITS region for the 26 *Oreomyrrhis* haplotypes ranged from 602 to 607 bp (ITS-1: 217–218 bp; 5.8S: 163–164 bp; ITS-2: 221–226 bp). Alignment of the 26 haplotypes resulted in a matrix of 610 positions (ITS-1: 219 bp; 5.8S: 164 bp; ITS-2: 227 bp), including two 1-bp gaps in ITS-1, a single 1-bp gap in 5.8S, and three 1-bp gaps and a 4-bp gap in ITS-2.

In addition to the ITS sequence types known from direct sequencing, molecular cloning for all 10 accessions of *Oreomyrrhis* revealed intra-individual ITS polymorphisms. Within each accession, those additional ITS clones differ from direct-sequenced ITS (dsITS) by one to four substitutions. Gene tree reconstructions (Bailey et al., 2003) were conducted to evaluate if intra-individual paralogous ITS sequences are present

and shared with other species and thereby would mislead phylogenetic inferences. These ITS clones were added to the matrices used in analyses 1, 2, and 3, respectively. Phylogenetic as well as haplotype network reconstructions resulted in congruent tree topologies as the ITS clones were excluded (data not shown). In particular, ITS clones within each accession coalesced to the dsITS, suggesting that paralogous ITS copies within each accession originated after speciation, a condition of sequence homology known as “shallow paralogy” (Bailey et al., 2003). Because shallow paralogy does not interfere with the phylogeny reconstruction (Hershkovitz et al., 1999; Bailey et al., 2003), only dsITS were used in all subsequent phylogenetic analyses.

Within the 378 relative rate tests among *C. procumbens*, *C. tainturieri*, and the 26 *Oreomyrrhis* ITS haplotypes, there were only two tests where rate constancy of ITS sequence evolution was rejected significantly ( $P < 0.05$ ;  $z$  score  $> |1.96|$ ). This figure is much lower than the expected number by chance alone (one of every 20 comparisons will be rejected significantly at the 5% level by chance; Sytsma and Schaal, 1985; Abbott and Comes, 2004). Given the apparent rate constancy of ITS evolution, times of speciation and diversification were approximated based on pairwise sequence divergences, calibrated with an ITS sequence divergence rate of 0.79% per million years. The average pairwise sequence divergence between *C. temulum* and *Oreomyrrhis* and its closest relatives (*C. procumbens* and *C. tainturieri*) was  $6.45 \pm 1.18\%$ , corresponding to a separation time of 8.16 (95% confidence intervals = 5.18–11.15) million years ago (mya). Between *Oreomyrrhis* and its closest relatives (*C. procumbens* and *C. tainturieri*), the average pairwise sequence divergence is  $1.76 \pm 0.49\%$ , indicating that *Oreomyrrhis* diverged from its most recent common ancestor at 2.23 (95% confidence intervals = 0.99–3.47) mya. Within *Oreomyrrhis*, the average pairwise sequence divergence is  $0.80 \pm 0.18\%$ , corresponding to a diversification time of 1.01 (95% confidence intervals = 0.56–1.47) mya.

**Analysis 1**—Alignment of the ITS-1 and ITS-2 sequences of the seven *Oreomyrrhis* ITS sequences (*O. hookeri* and *O. orizabae* are identical) of eight *Oreomyrrhis* species and 44 representatives of Apioideae resulted in a data matrix of 475 positions. Due to ambiguous alignment, 42 characters (70–74, 102–104, 249–258, 268–285, 388–389, and 409–412) were excluded. The final 433-bp matrix contained 264 parsimony-informative characters. Parsimony analysis resulted in 144 equally most parsimonious trees (MPTs) of 1218 steps. The uncorrected consistency index (CI) was 0.48 and the corrected CI was 0.44, the latter of which was higher than the expected CI (0.31) for 52 taxa (Sanderson and Donoghue, 1989). The retention index (RI) was 0.72. The strict consensus tree of the 144 MPTs with bootstrap support values (BV) and decay indices (DI) for each clade is presented in Fig. 2. The  $g_1$  statistic for 10,000 random trees was  $-0.39$ , which is significantly more skewed than random data ( $g_1 = -0.09$  for 250 variable position and 25 or more taxa;  $P < 0.01$ ; Hillis and Huelsenbeck, 1992).

Compared to the results of Downie and Katz-Downie (1996), whose sampling strategy was basically adopted here, relationships among taxa that were both sampled by previous and current studies are highly concordant (Fig. 2). One of the major differences was the placement of *Smyrniolum olusatrum*, which was placed sister to tribe Scandiceae with strong support

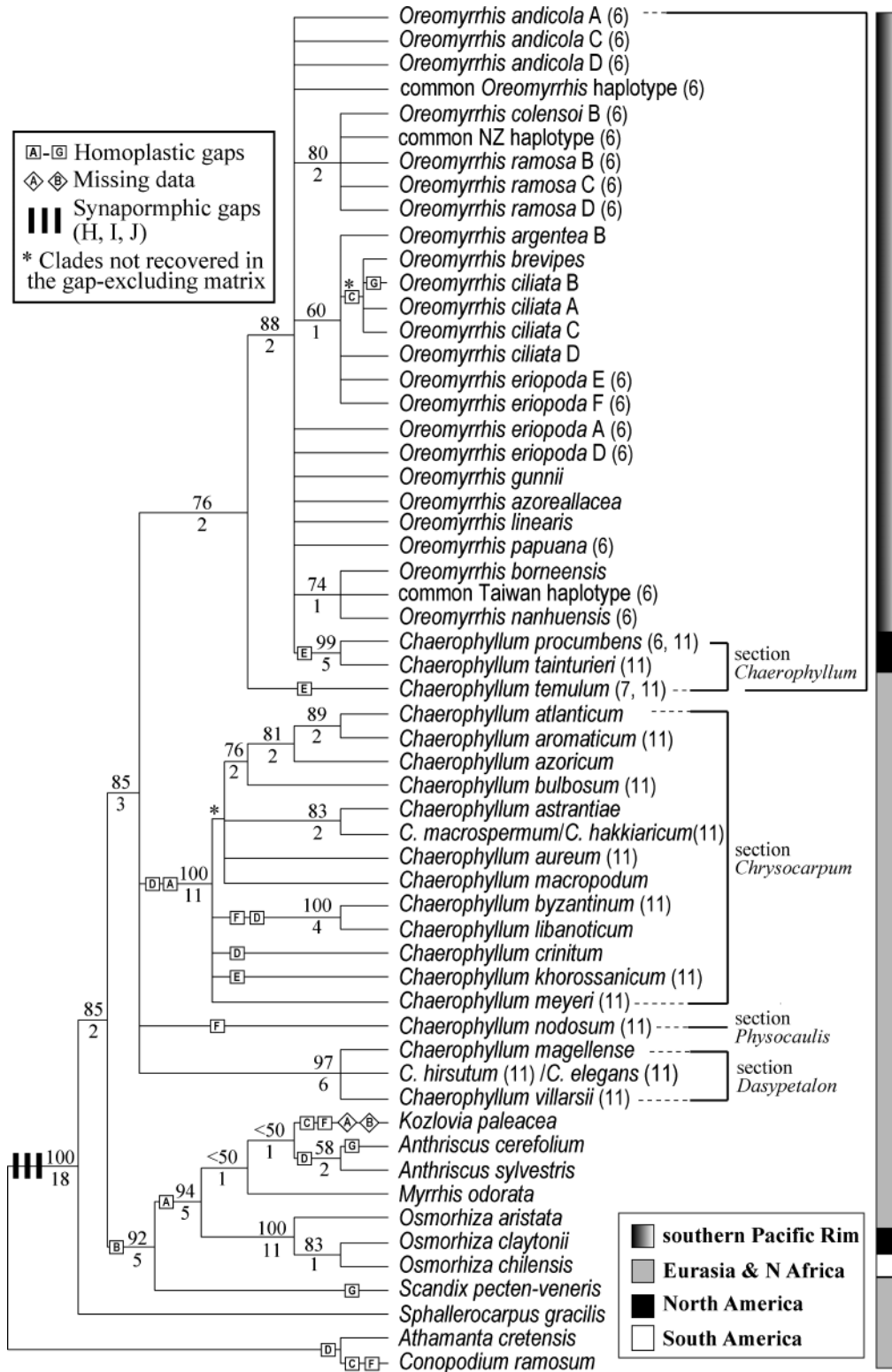


Fig. 3. Strict consensus of 1580 minimal length 490-step trees (corrected CI = 0.60, RI = 0.83) derived from the gap-including matrix of analysis 2. Bootstrap values (above clades) and decay indices (below clades) were calculated based on gap-excluding matrix. Asterisks denote the two clades that were not recovered in the gap-excluding matrix. Parsimony-informative gaps (see Materials and Methods, Analysis 1) are optimized onto the clades. Letters after *Oreomyrrhis* species denote haplotype designation (cf. Table 1 and Appendix). Known chromosome counts of *Oreomyrrhis* and *Chaerophyllum* are shown in parentheses after taxon names. Sectional treatment of *Chaerophyllum* follows Spalik and Downie (2001). Different gray-scaled bars on the far right indicate geographic distribution of the taxa (cf. Spalik and Downie, 2001).

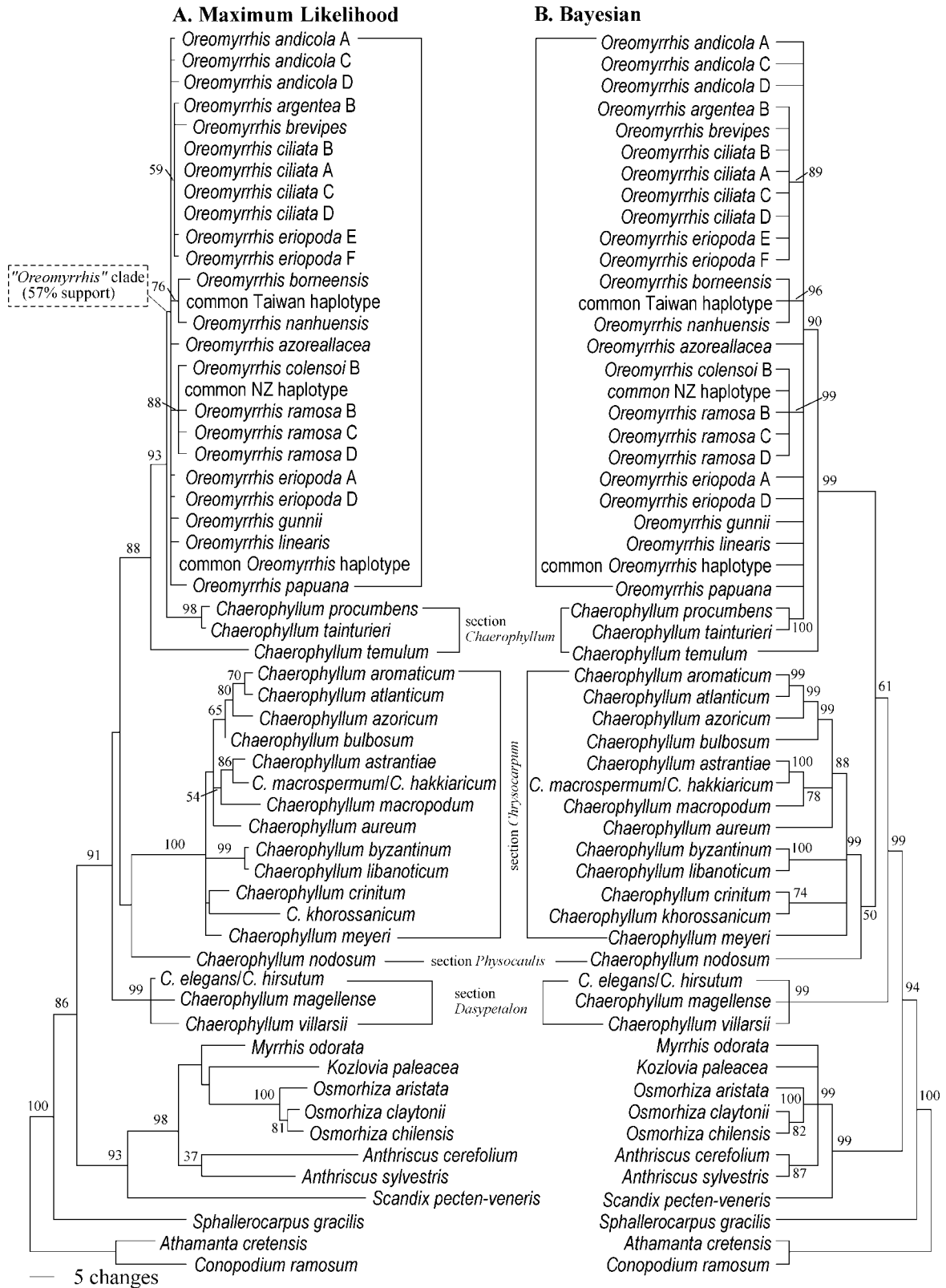


Fig. 4. Maximum likelihood (ML) and Bayesian phylogenies. (A) Single ML tree derived under the GTR + G model of nucleotide substitution ( $-\ln L = 3153.10211$ ). Numbers at nodes indicate bootstrap support values calculated from 1000 replicate neighbor-joining analyses using ML distances. (B) Bayesian inference tree. Bayesian posterior probabilities that are greater or equal to 50% are shown around nodes. Sectional treatment of *Chaerophyllum* follows Spalik and Downie (2001).





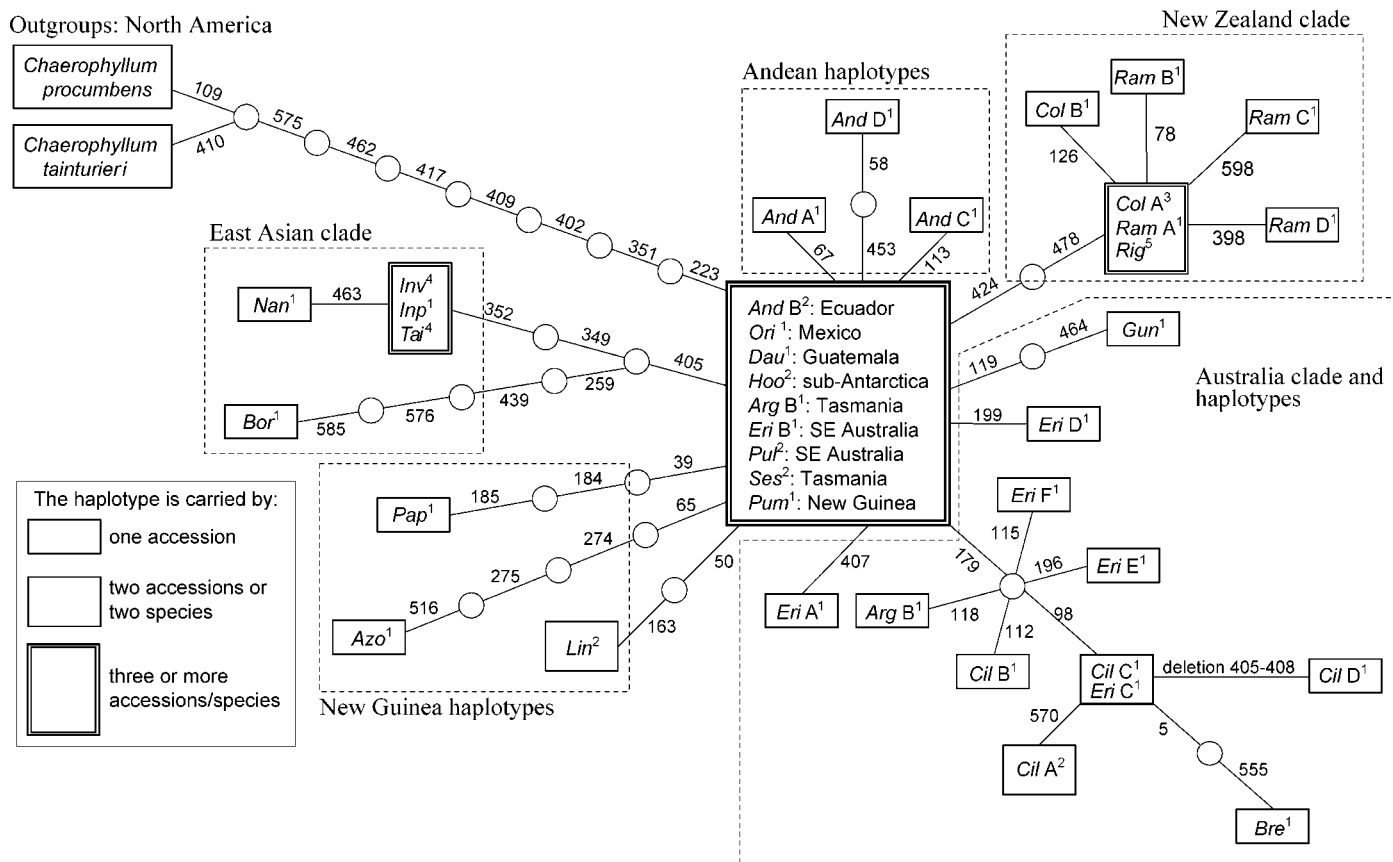


Fig. 5. ITS haplotype network. Each haplotype is represented by a rectangle. Double-lined boxes indicate that an identical haplotype is found in three or more accessions per species. Taxon abbreviations and haplotype identities correspond to those in Fig. 1 and Table 1, respectively. Superscripts following the haplotype name indicate frequency of the given haplotype. The relative frequency is also suggested by the size of the rectangle. Open circles are intermediate haplotypes not observed in the present study. Lines connecting haplotypes represent single point mutations or indel events, with numbers on lines indicating aligned positions where mutations occurred (cf. Table 1). The relative placement of the mutations along the branches possessing more than one mutational step is arbitrary.

consensus tree with Bayesian posterior probabilities (PP) is shown in Fig. 4B.

Topologies uncovered from the three phylogenetic inference methods were very similar, with only minor differences in resolution between some terminal taxa (Figs. 3 and 4). In all analyses, monophyly of the *Chaerophyllum-Oreomyrrhis* clade was well to strongly supported (BV = 85% in MP and 91% in ML; DI = 3; PP = 99%). Within this clade, four strongly supported clades were uncovered. Three of these corresponded perfectly to *Chaerophyllum* sect. *Chrysocarpum* Spalik & S. R. Downie, sect. *Dasypetalon* Neilr., and sect. *Physocaulis* DC. [a monotypic section composed of only *C. nodosum* (L.) Crantz], respectively, that were identified by recent molecular systematic studies (Spalik and Downie, 2001; Spalik et al., 2001a). Section *Chaerophyllum*, however, was rendered paraphyletic by *Oreomyrrhis*.

In all analyses, *Oreomyrrhis* was placed within *Chaerophyllum* sect. *Chaerophyllum* with moderate to strong support (BV = 76% in MP and 88% in ML; DI = 2; PP = 99%). Within this clade, all 26 *Oreomyrrhis* accessions and the two North American *Chaerophyllum* species (*C. procumbens* and *C. tainturieri*) formed a strongly supported subclade (BV = 88% in MP and 93% in ML; DI = 2; PP = 90%) that was sister to *C. temulum*. Relationships within the subclade, however, were polytomous, with four moderate to strongly sup-

ported clades nested within it. These four clades are the North American *Chaerophyllum* clade (BV = 99% in MP and 98% in ML; DI = 5; PP = 100%), the New Zealand *Oreomyrrhis* clade (BV = 80% in MP and 88% in ML; DI = 2; PP = 99%), the East Asian *Oreomyrrhis* clade (BV = 74% in MP and 76% in ML; DI = 1; PP = 96%), and the Australian endemic clade consisting of several Australian *Oreomyrrhis* taxa (BV = 60% in MP and 59% in ML; DI = 1; PP = 89%).

In the ML analysis (Fig. 4A), all *Oreomyrrhis* accessions were grouped together in a weakly supported monophyletic group (BV = 57%) that was sister to the North American *Chaerophyllum* clade. However, supports for the monophyletic *Oreomyrrhis* were weak in both MP and Bayesian analysis (BV = 36% in MP; PP = 38%). Whether or not the *Oreomyrrhis* species that were represented by multiple accessions were monophyletic was inconclusive.

**Analysis 3**—Alignment of the 57 *Oreomyrrhis* accessions and the two North American *Chaerophyllum* species resulted in a matrix of 611 positions (Table 1). Alignment position 568 was excluded due to its potential homoplasy (Table 1; see Discussion). With alignment gaps treated as a fifth state, a single, fully resolved network was reconstructed (Fig. 5). The ITS network has a starlike topology, within which the most frequent haplotype (common *Oreomyrrhis* haplotype) is linked

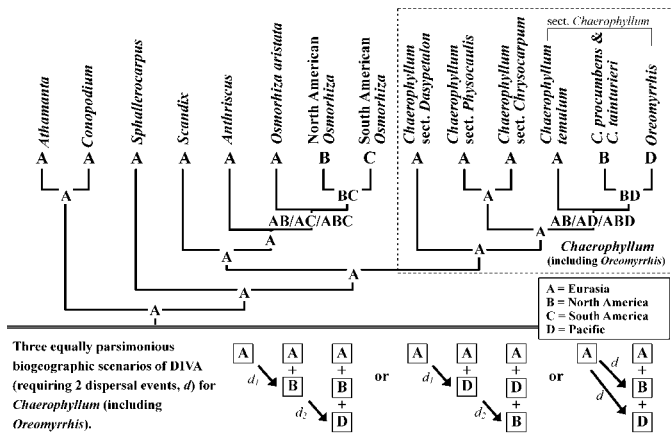


Fig. 6. Dispersal vicariance analysis (DIVA) optimization of the ancestral distributions of subtribe Scandicinae and inferred biogeographic scenarios for *Chaerophyllum* (including *Oreomyrrhis*). The Scandicinae phylogeny is simplified based on results of Spalik and Downie (2001), Wen et al. (2002) and the current study (Fig. 4A). The three equally parsimonious biogeographic scenarios of *Chaerophyllum* (including *Oreomyrrhis*) postulated by DIVA are shown schematically below the double line. Each scenario depicts distributional changes through time (from left to right) via dispersal ( $d$  and arrows).

to the outgroup taxa (*Chaerophyllum* clade) and connected to 12 relatively low frequency haplotypes and clades, including the New Zealand clade, the East Asian clade, and the Australian clade that were also recovered in analysis 2 (Figs. 3 and 4). For species represented by multiple haplotypes (*O. argentea*, *O. ciliata*, *O. colensoi*, *O. eriopoda*, and *O. ramosa*), these constituent haplotypes were not grouped into separate haplotype lineages (Fig. 5).

**Dispersal-vicariance analysis (DIVA)**—By consolidating the published molecular phylogenies and distributional data of subtribe Scandicinae (Spalik and Downie, 2001; Spalik et al., 2001a; Wen et al., 2002) and results of the current study, a simplified phylogeny composed of 14 terminals (Fig. 6) was generated. The optimal solution of DIVA indicates that the ancestor of Scandicinae was distributed in Eurasia and four dispersal events were required to explain the current biogeographic pattern of this subtribe (Fig. 6). For our focal taxa (*Chaerophyllum*-*Oreomyrrhis*), three equally parsimonious biogeographic scenarios were obtained (Fig. 6). In all three scenarios, Eurasia was shown to be the ancestral area, from which two dispersal events to North America and Pacific were required (Fig. 6). Two scenarios suggest that the dispersal events were sequential, with either North America or the Pacific region each being equally probable of being reached first. Alternatively, the third scenario suggests two independent dispersal events to North America and Pacific, with their order unknown.

## DISCUSSION

**Utilization of nrDNA ITS region for phylogenetic inference**—The properties of ITS loci that have attracted and facilitated their application in plant phylogenetic studies (Baldwin et al., 1995) were recently reexamined by Álvarez and Wendel (2003). Although ITS loci are generally assumed to be homogenized by concerted evolution, Álvarez and Wendel (2003) cautioned that, under circumstances such as hybridization, polyploidization, or pseudogene formation, separate ITS

sequences might be present and persistent in a genome. This could interfere with sequencing, distort assessment of sequence homology, and subsequently mislead phylogenetic inference.

The ITS region was chosen primarily because it is the only molecular marker for which a comprehensive sampling of Apiaceae (Downie et al., 2000, 2001) is available for testing taxonomic hypotheses regarding the tribal placement of *Oreomyrrhis*. The ITS region is also the fastest evolving sequence known in Apiaceae (Downie et al., 2001) and therefore is more likely to resolve infrageneric relationships. In *Oreomyrrhis*, a low base chromosome number precludes its origin through polyploidization. The autogamous nature (discussed later) and currently isolated distribution of most *Oreomyrrhis* species assure that interspecific hybridization and introgression are probably rare. Plant species characterized by these biological attributes are frequently assumed to have had their ITS paralogues homogenized by concerted evolution (Denduangboripant and Cronk, 2000).

However, molecular cloning of all 10 selected *Oreomyrrhis* accessions revealed intra-individual ITS polymorphisms that were imperceptible from the electropherogram of direct sequencing. The persistence of intra-individual ITS polymorphism in *Oreomyrrhis* suggests that concerted evolution might not have proceeded fast enough to homogenize different ITS sequence types (Denduangboripant and Cronk, 2000; Hughes et al., 2002). One possible scenario for the failure of concerted evolution in this non-hybrid diploid genus is the existence of multiple nrDNA arrays in the genome (O'Kane et al., 1996), which can be detected by fluorescent in situ hybridization (FISH) and genomic in situ hybridization (GISH) (Álvarez and Wendel, 2003). Alternatively, the existence of different ITS paralogues within each accession of *Oreomyrrhis* may suggest that intra-individual ITS polymorphism might be more widespread than previously assumed (Hughes et al., 2002). Nevertheless, since the revealed intra-individual ITS polymorphisms present shallow paralogy that does not impede phylogenetic inference, ITS sequences remain a credible molecular marker for phylogenetic reconstruction in *Oreomyrrhis*.

**Phylogenetic affinities of *Oreomyrrhis***—The ITS data presented here unambiguously placed *Oreomyrrhis* in the subtribe Scandicinae of tribe Scandiceae (Fig. 2), supporting the taxonomic hypotheses of de Candolle (1830), Bentham (1867), and Koso-Poljansky (1916), and rejecting Drude's (1898) placement of *Oreomyrrhis* in tribe Smyrnieae. Drude's tribe Smyrnieae (encompassing *Arracacia*, *Conium*, *Erigenia*, *Neogoezia*, *Orogenia*, *Oreomyrrhis*, *Pleurospermum*, and *Smyrnum*) is polyphyletic in the present analysis (Fig. 2), confirming relationships suggested by previous studies using serological (Shneyer et al., 1992) and molecular data (Downie and Katz-Downie, 1996; Katz-Downie et al., 1999). Our ITS phylogenies also indicate that taxa characterized by simple or irregular compound umbels (e.g., *Oreomyrrhis*, *Orogenia*, *Neogoezia*, *Lilaeopsis*, and *Erigenia*) do not form a monophyletic group (Fig. 2), indicating that this trait has had multiple independent origins in Apioideae and that its phylogenetic utility has been overemphasized.

The close relationship between *Oreomyrrhis* and *Chaerophyllum* proposed by Bentham (1866) was verified by our ITS data (Figs. 2–4). *Chaerophyllum* is the largest (ca. 30 species) and most diverse genus in subtribe Scandicinae. It is distributed mainly in Eurasia (including North Africa) and is most

diversified in the Mediterranean and the Caucasus regions, with two species native to North America (Spalik and Downie, 2001). Recent molecular studies support the monophyly of *Chaerophyllum* and reveal four well-supported clades within it (Spalik and Downie, 2001). Lacking morphological synapomorphies, these four clades were each treated as section by Spalik and Downie (2001).

Based on the ITS data, *Oreomyrrhis* is nested within the sect. *Chaerophyllum* (Figs. 3 and 4) that comprises one species native to Europe (*C. temulum*) and two to North America (*C. procumbens* and *C. tainturieri*). Within this section, *Oreomyrrhis* is most closely related to the two North American species. The phylogenetic relationships revealed by the current data indicate the paraphyletic nature of *Chaerophyllum* if *Oreomyrrhis* is treated as a separate genus. Because *C. temulum* is the type species of the genus, preservation of the generic status of *Oreomyrrhis* would simultaneously mandate the erection of four small genera (the North American *Chaerophyllum* clade, sect. *Chrysocarpum*, sect. *Dasyptalon*, and the monotypic sect. *Physocaulis*), leaving *Chaerophyllum* a monotypic genus composed of only *C. temulum*. Before proposing formal nomenclatural changes, further study by employing additional molecular markers of chloroplast genome will be conducted to test the result reached by ITS data.

**Monophyly of *Oreomyrrhis***—The monophyly of *Oreomyrrhis* was only weakly supported in the ML analysis (BV = 57%; Fig. 4A). The bootstrap support in MP analysis and Bayesian posterior probability for this clade are both less than 50%. Examination of the aligned ITS matrix revealed a likely homoplasious character at position 409 (position 568 in Table 1 and analysis 3). To evaluate how this character had affected phylogeny reconstruction, we excluded this character and performed a MP analysis. Using the same parameters for the gap-excluding matrix in analysis 2, a heuristic search resulted in 536 equal length MPTs of 458 steps, two steps shorter than the original trees (460 steps). We used the “fast stepwise addition” option of PAUP\* to perform a bootstrap analysis with 100 000 replicates (e.g., Sun et al., 2004). The strict consensus of the 536 MPTs was almost identical to the ML tree (Fig. 4A), and the support for the monophyly of *Oreomyrrhis* accessions increased to 66%, compared to 36% when character 568 was included.

Although the ITS data provide only weak supports, the monophyly of *Oreomyrrhis* is unambiguously defined by its morphological synapomorphy (simple umbel) and apparent cytological and ecological distinctiveness. The monophyly indicates a single origin of *Oreomyrrhis*, suggesting that the trans-Pacific disjunction of the genus reflects a genuine biogeographic process, not a taxonomic artifact.

**Biogeographic implications of the ITS phylogenies**—Because a great proportion of the distribution range of *Oreomyrrhis* parallels the Gondwanan disjunction in the South Pacific (e.g., Sanmartín and Ronquist, 2004), this genus had often been assumed to have a Southern Hemispheric and/or ancient origin (e.g., van Steenis, 1963; Dawson, 1963; Raven, 1973; Melville, 1981; Smith, 1986; Wu, 1998). However, our ITS data strongly support that *Oreomyrrhis* is closely related to *Chaerophyllum* of subtribe Scandicinae, that is mainly distributed in the Northern Hemisphere. Within *Chaerophyllum*, *Oreomyrrhis* is nested within sect. *Chaerophyllum* and, together with the two North American species of the section (*C. pro-*

*cumbens* and *C. tainturieri*), forms a clade that is sister to the European species *C. temulum* (Figs. 3 and 4). Spalik and Downie (2001) hypothesized that *Chaerophyllum* arrived in North America by the incidental dispersion of seeds from Europe, probably by vagrant birds. Based on the optimization of DIVA, the ancestral area for sect. *Chaerophyllum* (including *Oreomyrrhis*) was suggested to be Eurasia, and the disjunction in North American and Pacific involved two dispersal events (Fig. 6). Because the distribution range of *Oreomyrrhis* and *Chaerophyllum* do not overlap, DIVA optimization indicates that the current distribution could have resulted from dispersal from Europe to North America followed by a second dispersal to Pacific, to Pacific first and second dispersal to North America, or two independent dispersals to Europe and Pacific (Fig. 6).

Based on our time estimates, these dispersal events probably took place around late Miocene to early Pliocene ([5.18–] 8.16 [–11.15] mya), when dispersals from Europe to North America through island hopping via the North Atlantic Land Bridge (Tiffney and Manchester, 2001) were still possible. However, since the basal lineage of *Oreomyrrhis* cannot be identified confidently by our data, the initial dispersal event that gave rise to subsequent *Oreomyrrhis* diversification remains unclear.

The estimated times for the separation of the North American *Chaerophyllum* and *Oreomyrrhis* (2.23 mya [95% confidence intervals = 0.99–3.47 mya]) and diversification within *Oreomyrrhis* (1.06 mya [95% confidence intervals = 0.56–1.47 mya]) are relatively recent, corresponding to a late Tertiary to Quaternary evolution of the group. Note that the 0.79% sequence divergence rate is at the lower end of the range reported for flowering plants (Zhang et al., 2001), indicating that the dates inferred here might have been overestimated and the actual times for *Oreomyrrhis* diversification might be more recent. Even under this more conservative estimate, our results strongly support a late Tertiary to Quaternary origin and diversification of *Oreomyrrhis*, and most likely long-distance dispersal as the major biogeographic mechanism underlying the trans-Pacific disjunction of *Oreomyrrhis* (Dawson, 1971; Thorne, 1972; Raven, 1973). The vicariant scenarios that invoke early Tertiary land-bridge (van Steenis, 1962, 1964) or plate tectonic movements dating back to the Jurassic (Melville, 1981) can be rejected confidently because their predicted temporal contexts significantly predate the origin of *Oreomyrrhis* inferred from the ITS data.

Recent phylogenetic studies suggest that long-distance dispersal underlies the distributions of many trans-Pacific disjunct plant groups (Winkworth et al., 2002; Sanmartín and Ronquist, 2004). Comparisons of these Pacific taxa have revealed concordant phylogenetic relationships, suggesting that recent long-distance dispersal events might not occur randomly but have followed certain dispersal routes, probably associated with ancient vegetation types that may have served as migratory corridors (Winkworth et al., 2002; Sanmartín and Ronquist, 2004). Unfortunately, the ITS data contain only limited information regarding the pattern of dispersal that have shaped the trans-Pacific disjunction of *Oreomyrrhis*.

**Biogeographic implications of the ITS haplotype network**—To better understand genealogical processes that underlying the Pacific disjunction, an ITS haplotype network was constructed (Fig. 5). The predominant feature of the network is a single widespread and high frequency haplotype (common *Oreomyrrhis* haplotype) linked to numerous low-frequency

haplotypes by a few mutational steps, a pattern that is commonly seen in temperate taxa in Europe and North America that have experienced drastic distributional changes during the Pleistocene (Hewitt, 1996; Schaal et al., 1998; Milá et al., 2000). This type of network structure could have resulted from (1) a postglacial range expansion from refugium populations that had experienced genetic bottleneck, (2) gene flow, or (3) a selective sweep (Milá et al., 2000). In *Oreomyrrhis*, trans-Pacific gene flow seems an unlikely explanation for the wide distribution of the common *Oreomyrrhis* haplotype (Abbott and Comes, 2004). Because the ITS region is known to subject little functional constraint (Baldwin et al., 1995), unless this region is closely linked to a gene favored by natural selection, a selective sweep is a less likely explanation than range expansion (Milá et al., 2000).

However, unlike low-elevation temperate taxa whose distribution ranges expanded after the retreat of the ice sheet (Hewitt, 1996), alpine plants such as *Oreomyrrhis* would have actually experienced postglacial range contraction, as rising global temperatures forced alpine plants to migrate up toward mountain tops (Raven, 1973; Larena et al., 2002). During the glacial maxima, alpine plant populations would have actually expanded as the alpine zone moved downward. This reverse scenario of glacial population expansion and interglacial population contraction is known as the "displacement refugia model" (Kropf et al., 2003). Under this model, the common *Oreomyrrhis* haplotype likely achieved its broad distribution by "glacial" range expansion as more suitable alpine habitats became available during glacial maxima. Since the common *Oreomyrrhis* haplotype is the only haplotype distributed in more than one geographic region, its glacial range expansion probably had occurred before other haplotypes arose.

**Species circumscription and speciation in *Oreomyrrhis***—Although many of the species circumscribed by Mathias and Constance (1955) and Chen and Wang (2001) are characterized by distinct ITS haplotypes (e.g., *O. azorellacea*, *O. borneensis*, *O. brevipes*, *O. gunnii*, *O. linearis*, *O. nanhuensis*, and *O. papuana*), suggesting that they have evolved as separate evolutionary lineages (Templeton et al., 2000), such genetic distinctness is absent in the majority of the species investigated (Fig. 5). For instance, the common *Oreomyrrhis* haplotype is carried by nine species that are distributed throughout most of the range of *Oreomyrrhis* (Fig. 5). The sharing of haplotypes by recently differentiated species could result from interspecific hybridization or incomplete lineage sorting of ancestral haplotypes that had been present prior to the divergence of the species (Olsen and Schaal, 1999). In the current study, the ancestral position of the common *Oreomyrrhis* haplotype suggested by its high mutational connectedness and high frequency (Castelloe and Templeton, 1994) strongly favors lineage sorting, for interspecific hybridizations between disjunct taxa across Pacific seem unlikely (Abbott and Comes, 2004). On the other hand, gene flows among species in the same geographic area (e.g., *O. argentea* A, *O. eriopoda* B, and *O. pulvinifera* in SE Australia; *O. involucrata* and *O. taiwaniana* in Taiwan; *O. colensoi* A, *O. ramosa* A, and *O. rigida* in New Zealand) are possible for haplotype sharing, especially during glacial maxima when alpine plant populations underwent range expansion into lower elevations (Larena et al., 2002). Furthermore, the recency of species divergences within *Oreomyrrhis* suggests that there probably has not been enough time for achieving reciprocal monophyly of the newly evolved

lineages (Avice, 2000). The preliminary results from ITS data provide an exciting first step toward an in-depth understanding of the speciation in *Oreomyrrhis*. In the ongoing phylogeographic and population genetic studies, we are employing chloroplast sequence data and AFLP markers to explore the intriguing evolutionary history and biogeography of *Oreomyrrhis*.

**Cytological and morphological considerations**—The base chromosome number for the genus *Chaerophyllum* is  $n = 11$ , with deviant and intraspecific variable counts only being reported in sect. *Chaerophyllum* (*C. temulum* [ $2n = 14$  and  $22$ ] and *C. procumbens* [ $2n = 12$  and  $22$ ]) (Pimenov et al., 2003). Mapping base chromosome numbers onto the ITS phylogeny (Fig. 3) indicates that the numbers  $n = 6$  and  $n = 7$  occur only in the sect. *Chaerophyllum* clade (including *Oreomyrrhis*).

Although mericarps of most species of *Oreomyrrhis* are not equipped with structures such as hooks or nutrient tissues (Fig. 7) that could facilitate dispersal (Mathias and Constance, 1955; van Steenis, 1963), Raven (1973) suggested that, during glacial maxima when high latitude alpine plants were forced to migrate to lower elevations closer to sea levels, chances for seed dispersals by transoceanic wanderers (e.g., albatross) would greatly increase. Compared to other species in *Chaerophyllum*, members of sect. *Chaerophyllum* (including *Oreomyrrhis*) possess relatively smaller mericarps (Spalik et al., 2001b), with a further size reduction in *Oreomyrrhis* (Fig. 7). The evolution toward smaller mericarps in *Oreomyrrhis* might have been advantageous in enhancing their dispersibility (Howe and Smallwood, 1982), especially when carried in mud adhering to the legs of wanderers (Salisbury, 1970; Raven, 1973).

*Chaerophyllum procumbens* and *C. tainturieri* are common eastern North American winter annuals in forest and ruderal communities (Baskin and Baskin, 1990; Baskin et al., 2004). They differ from their Eurasian congeners by the absence of male flowers and by the possession of reduced corollas, traits also characterized by *Oreomyrrhis* and typically associated with self-pollination (Spalik and Downie, 2001). Selfing was reported in the sub-Antarctic species *O. hookeri* (Moore, 1968). Ongoing greenhouse bagging experiments indicate that the Australian species *O. eriopoda* is autogamous (K. Chung, unpublished data). In *Oreomyrrhis*, self-pollination is also indicated by the very inconspicuous basal umbels of *O. andicola*, *O. taiwaniana*, and *O. eriopoda*, which are nearly sessile during early anthesis (K. Chung, personal observations). The same trait has also been noted in specimen labels of *O. linearis* (M. Coode & P. Stevens NGF 46182) and *O. papuana* (J. F. Veldkamp 6579).

Despite inbreeding depression, the genetic benefits (e.g., reproductive assurance and purge of genetic load) associated with the shift from outcrossing to selfing promote successful long-distance colonization in plants, an observation known as Baker's Rule (Baker, 1955). Recent studies suggest that a rapid transition from outcrossing to self-pollination in the early evolution of *Arabidopsis thaliana* could have contributed to its rapid postglacial range expansion around 17 000 years ago (Shimizu et al., 2004). In conjunction with these lines of evidence, our results suggest that the evolution in chromosome number and transition in reproductive syndrome, along with reduction in mericarp size, probably played pivotal roles during the early evolution of sect. *Chaerophyllum*, followed by

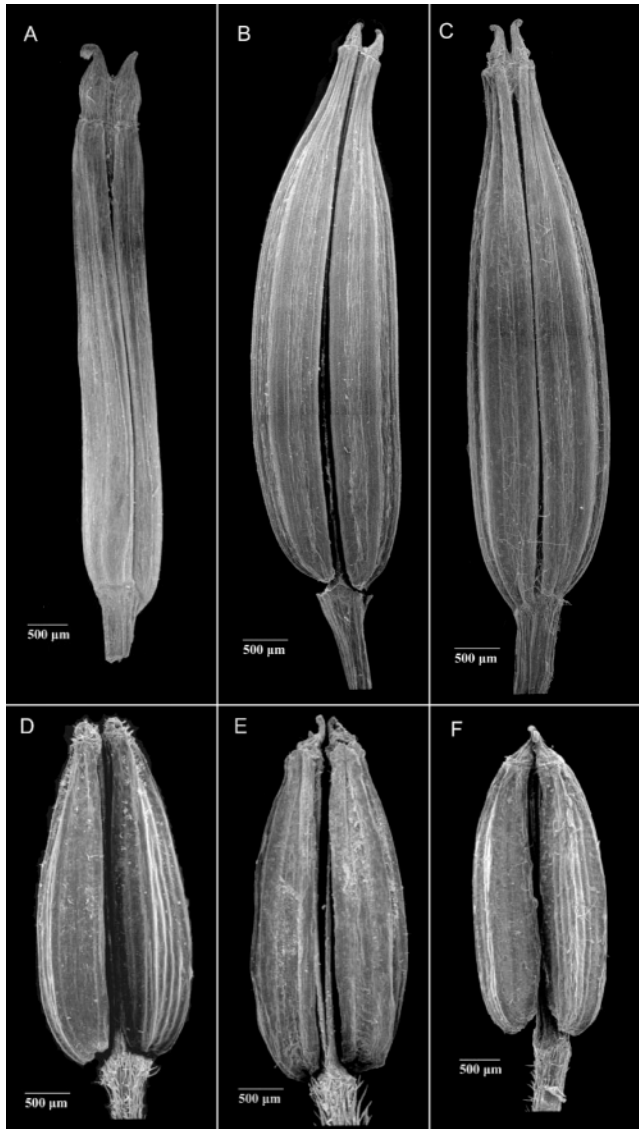


Fig. 7. Scanning electron micrographs (SEMs) of mericarps in *Chaerophyllum* sect. *Chaerophyllum* (sensu Spalik and Downie, 2001) and *Oreomyrrhis* species. A, B, and C are composed of two SEMs (upper and lower part of the mericarps). All voucher specimens are deposited at the Missouri Botanical Garden. (A) *Chaerophyllum temulum* (B. Wurzell 263); (B) *Chaerophyllum tainturieri* (J.R. Laatsch s.n.); (C) *Chaerophyllum procumbens* (B.F. Bush 14859); (D) *Oreomyrrhis andicola* (K. Chung 1490); (E) *Oreomyrrhis taiwaniana* (K. Chung 1507); (F) *Oreomyrrhis involucrata* (K. Chung 1530).

further diversification and occupation of cold and alpine environments by *Oreomyrrhis*.

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APPENDIX. Taxa, sources of sequences, geographic and voucher information, and GenBank accession numbers for *Oreomyrrhis* and species of Apiaceae subfamily Apioideae examined for nuclear rDNA ITS sequence variation. Two GenBank accession numbers are given when ITS-1 and ITS-2 sequences were registered separately (no intervening 5.8S sequence); a single GenBank accession number indicates a contiguous ITS-1–5.8S–ITS-2 sequence is registered. For haplotypes carried by multiple individuals within a species, the GenBank accession number corresponds to the first individual listed in the appendix. Herbarium acronyms follow Holmgren et al. (1990).

**Taxon;** Reference or Source/voucher; GenBank accession numbers.

*Aegopodium podagraria* L.; Downie and Katz-Downie (1996); U30536/U30537. *Aethusa cynapium* L.; Downie & Katz-Downie (1996); U30582/U30583. *Anethum graveolens* L.; Downie & Katz-Downie (1996); U30550/U30551. *Angelica archangelica* L.; Downie & Katz-Downie (1996); U30576/U30577. *Anthriscus cerefolium* (L.) Hoffm.; Downie & Katz-Downie (1996); U30532/U30533. *Anthriscus sylvestris* (L.) Hoffm. subsp. *sylvestris*; Downie et al. (1998); U79603/U79604. *Apium graveolens* L.; Downie & Katz-Downie (1996); U30576/U30577. *Arracacia braddegei* J. M. Coult. & Rose; Downie & Katz-Downie (1996); U30570/U30571. *Arracacia nelsonii* J. M. Coult. & Rose; Downie & Katz-Downie

(1996); U30556/U30557. *Athamanta cretensis* L.; Downie et al. (2000); AF073685/AF073686. *Carlesia sinensis* Dunn.; Downie & Katz-Downie (1996); U30562/U30563. *Chaerophyllum aromaticum* L.; Downie et al. (2000); AF073631/AF073632. *Chaerophyllum astrantiae* Boiss. & Balansa; Downie et al. (2000); AF073653/AF073654. *Chaerophyllum atlanticum* Coss.; Downie et al. (2000); AF073633/AF073634. *Chaerophyllum aureum* L.; Downie et al. (2000); AF073655/AF073656. *Chaerophyllum azoricum* Trel.; Downie et al. (2000); AF073657/AF073658. *Chaerophyllum bulbosum* L.; Downie et al. (2000); AF073659/AF073660. *Chaerophyllum byzantinum*



- Boiss.; Downie et al. (2000); AF073635/AF073636. *Chaerophyllum crinitum* Boiss.; Downie et al. (2000); AF073661/AF073662. *Chaerophyllum elegans* Gaudin; Downie et al. (2000); AF073663/AF073664. *Chaerophyllum hakkiaricum* Hedge & Lamond; Downie et al. (2000); AF073649/AF073650. *Chaerophyllum hirsutum* L.; Downie et al. (2000); AF073665/AF073666. *Chaerophyllum khorassanicum* Czerniak. ex Schischk.; Downie et al. (1998); U78366/U78426. *Chaerophyllum libanoticum* Boiss. & Kotschy; Downie et al. (2000); AF073637/AF073638. *Chaerophyllum macropodium* Boiss.; Downie et al. (2000); AF073671/AF073672. *Chaerophyllum macrospermum* (Willd. ex Spreng.) Fisch. & C.A. Mey.; Downie et al. (2000); AF073651/AF073652. *Chaerophyllum magellense* Ten.; Downie et al. (2000); AF073669/AF073670. *Chaerophyllum meyeri* Boiss. & Buhse; Downie et al. (2000); AF073639/AF073640. *Chaerophyllum nodosum* (L.) Crantz; Downie et al. (2000); AF073675/AF073676. *Chaerophyllum procumbens* (L.) Crantz; USA, Missouri, St. Louis, 8 Apr 2003, *Chung 1551* (MO); AJ854344. *Chaerophyllum tainturieri* Hook. & Arn.; USA, Missouri, St. Louis, 21 May 2001, *Chung 1495* (MO); AJ854345. *Chaerophyllum temulum* L.; Downie et al. (2000); AF073641/AF073642. *Chaerophyllum villarsii* W.D.J. Koch; Downie et al. (2000); AF073667/AF073668. *Coaxana purpurea* J.M. Coult. & Rose; Downie & Katz-Downie (1996); U30572/U30573. *Conium maculatum* L.; Downie & Katz-Downie (1996); U30556/U30557. *Conopodium ramosum* Costa; Downie et al. (2000); AF073693/AF073694. *Coriandrum sativum* L.; Downie & Katz-Downie (1996); U30586/U30587. *Coulterophytum laxum* Robins.; Downie & Katz-Downie (1996); U30560/U30561. *Crithmun maritimum* L.; Downie & Katz-Downie (1996); U30540/U30541.
- Daucus carota* L.; Downie & Katz-Downie (1996); U27589/U30315.
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Kinabalu, 12000 ft, 21 Jun 1932, *Clemens 29809* (A); AJ854312. *Oreomyrrhis brevipes* Mathias & Constance; AU, NSW, Kosciuszko NP, 2057 m, 13 Dec 2003, *Chung 1571* (MO); AJ854313. *Oreomyrrhis ciliata* Hook. f.; AU, NSW, Kosciuszko NP, 1890 m, 9 Jan 1997, *Plunkett 1554* (VCU); AJ854314 (haplotype A); AU, Victoria, Alpine NP, 1570 m, 24 Dec 2003, *Chung 1588* (MO); AJ854314 (haplotype A); AU, NSW, Barrington Tops NP, 1440 m, 4 Dec 2003, *Chung 1555* (MO); AJ854315 (haplotype B); AU, TS, Middlesex Plain, 805 m, 8 Jan 2004, *Chung 1602* (MO); AJ854316 (haplotype C); AU, TS, Mt. Barrow, 1300 m, 15 Jan 2004, *Chung 1614* (MO); AJ854317 (haplotype D). *Oreomyrrhis colensoi* Hook. f.; New Zealand (NZ), North Island, Taupo, *Chung 1641* (MO); AJ854318 (haplotype A); NZ, South Island (SI), Westland NP, 1200 m, 24 Feb 2004, *Chung 1642* (MO); AJ854318 (haplotype A); NZ, SI, Old Man Range, 1360 m, 4 Feb 2004, *Chung 1631* (MO); AJ854318 (haplotype A); NZ, SI, Arthur's Pass NP, 1730 m, 25 Feb 2004, *Chung 1643* (MO); AJ854319 (haplotype B). *Oreomyrrhis daucifolia* I.M. Johnst.; Guatemala, Huehuetenango, 3230 m, 31 Aug 2004, *Chung 1650* (MO); AJ854320. *Oreomyrrhis eriopoda* (DC.) 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Broughton; no voucher); AJ854328. *Oreomyrrhis involucreta* Hayata; Taiwan (TW), Chuntashan, 3000 m, 26 Jun 1996, *Liu 949* (HAST); AJ854329; TW, Tienchi, 2800 m, 21 May 1993, *Liao 1361* (MO); AJ854329; TW, Hokuashan, 3100 m, 16 Jun 1999, *Peng 17371* (HAST); AJ854329; TW, Hsiangyangshan, 3350 m, 1 Jul 2001, *Juan 53* (HAST); AJ854329. *Oreomyrrhis involucreta* var. *pubescens* Masam.; TW, Yushan National Park, 3700 m, 24 Jul 2002, *Chung 1523* (MO); AJ854330. *Oreomyrrhis linearis* Hemsl.; PNG, Mt. Giluwe, 12300 ft, 14 Aug 1961, *Schodde 1825* (CANB); AJ854331; PNG, Mt. Wilhelm, Lake Piunde, 23 Apr 1965, *Eichler 18210* (CANB); AJ854331. *Oreomyrrhis nanhuensis* C.H. Chen & J.C. Wang; TW, Nantutashan, Taroko NP, 3470 m, 13 Jul 2001, *Chung 1511* (MO); AJ854332. *Oreomyrrhis orizabae* I.M. Johnst.; Mexico, Cofre de Perote, 3800 m, 28 Jul 2001, *Avenidaño R. 5349* (MO); AJ854333. *Oreomyrrhis papuana* Buwalda ex Steenis; Indonesia, Irian Jaya, Valentijn Mt, 3400 m, 8 Sep 1988, *Mangen 2349* (A); AJ854334. *Oreomyrrhis pulviniflora* F. Muell.; AU, NSW, Kosciuszko NP, Lake Cootapatamba, 2055 m, 13 Dec 2003, *Chung 1572* (MO); AJ854335; AU, NSW, Charlottes Pass, 1900–2200 m, 17 Jan 1983, *Strid 22112* (MO); AJ854335. *Oreomyrrhis pumila* Ridl.; PNG, Mt. Wilhelm, 14250 ft, May 1965, *Walker ANU 5241* (CANB); AJ854336. *Oreomyrrhis ramosa* Hook. f.; NZ, SI, Porter's Pass, 970 m, 24 Jan 2004, *Chung 1617* (MO); AJ854337 (haplotype A); NZ, SI, Nelson Lakes NP, 950 m, 29 Jan 2004, *Chung 1621* (MO); AJ854338 (haplotype B); NZ, SI, Rahu Saddle, 470 m, 30 Jan 2004, *Chung 1624* (MO); AJ854339 (haplotype C); NZ, SI, Ottago, Dunedin, 700 m, 3 Feb 2004, *Chung 1630* (MO); AJ854340 (haplotype D). *Oreomyrrhis rigida* (Kirk) Allan ex Mathias & Constance; NZ, SI, Lake Hawea, 920 m, 5 Feb 2004, *Chung 1635* (MO); AJ854341; NZ, SI, Nelson Lakes NP, 950 m, 29 Jan 2004, *Chung 1622* (MO); AJ854341; NZ, SI, Ward, 20 m, 26 Jan 26 2004, *Chung 1618* (MO); AJ854341; NZ, SI, MacKenzie Pass, 670 m, 5 Feb 2004, *Chung 1636* (MO); AJ854341. *Oreomyrrhis sessiliflora* Hook. f.; AU, TS, Ben Lomond NP, 1515 m, 30 Dec 2003, *Chung 1592* (MO); AJ854342; AU, TS, Walls of Jerusalem NP, 4400 ft, 28 Jan 1973, *D. A. & A. V. Ratkovsky 70* (MO); AJ854342. *Oreomyrrhis* sp.; NZ, SI, Ottago, Awahokomo, 485 m, 8 Feb 2004, *Chung 1638* (MO); AJ854341. *Oreomyrrhis taiwaniana* Masam.; TW, Nantutashan, 3470 m, 13 Jul 2002, *Chung 1510* (MO); AJ854343; TW, Yushan NP, 3660 m, 25 Jul 2002, *Chung 1525* (MO); AJ854343; TW, Hsiukuluanshan, 3700 m, 22 Apr 2003, *Huang 1326* (HAST); AJ854343 TW, Sheipa NP, 3700 m, 12 Sep 2002, *Huang 1289* (HAST); AJ854343. *Orlaya grandiflora* (L.) Hoffm.; Downie & Katz-Downie (1996); U30524/U30525. *Orlaya kochii* Heywood; Downie & Katz-Downie (1996); U30526/U30527. *Orogenia fusiformis* S. Watson; Sun et al. (2004); AY372879. *Orogenia linearifolia* S. Watson; Downie et al. (2002); AF358531/AF358580. *Osmorhiza aristata* (Thunb.) Rydb.; Downie et al. (2000); AF073609/AF073610. *Osmorhiza chilensis* Hook. & Arn.; Downie et al. (2000); AF073619/AF073620. *Osmorhiza claytonii* (Michx.) C.B. Clarke; Downie et al. (2000); AF073615/AF073616.
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