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MOLECULAR AND MORPHOLOGICAL REASSESSMENT OF RELATIONSHIPS WITHIN THE *VITTADINIA* GROUP OF ASTEREAE (ASTERACEAE)[1]

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Morphological and ITS (internal transcribed spacer) sequence data for 40 species of the Austral-Pacific genera *Camptacra, Kippistia, Minuria, Peripleura, Tetramolopium,* and *Vittadinia* as well as one semiherbaceous species of *Olearia* were subjected to cladistic analysis, separately and together. *Minuria, Peripleura,* and *Tetramolopium* are paraphyletic as currently defined. *Tetramolopium vagans* from Australia appears to represent an undescribed genus. Both *Kippistia suadefolia* and *Peripleura diffusa* show close affinity to *Minuria* species, and *Minuria macrorhiza* appears to contain two distinct but closely related species. *Vittadinia* and the remaining species of *Tetramolopium* and *Peripleura* form a strong affinity group. The distribution of indels and the combined analysis each provide evidence that the Hawaiian and Cook Island species of *Tetramolopium* are descended from New Guinea species. The combined analysis also suggests that the Cook Island species *T. mitiaroense* is sister to the Hawaiian clade. *Olearia arguta* groups strongly with *Camptacra* and shows no close affinity with either of the arborescent species of *Olearia* used to root these analyses. Marked homoplasy among morphological characters indicates why generic delimitation in the group has been problematic.

Key words: Asteraceae; *Camptacra*; ITS sequence; *Minuria*; *Olearia*; *Peripleura*; phylogeny; *Tetramolopium*; *Vittadinia*.

Vittadinia Rich. and *Tetramolopium* Cass. (Asteraceae; Astereae) are two genera long recognized as being closely related, having been variously merged and segregated by different authors. The two genera encompass diverse morphologies as well as broad and unusual distributional patterns. *Vittadinia* includes 29 species of annual and perennial herbs (Burbidge, 1982), which are mostly Australian, with one species each in New Zealand and New Caledonia. *Tetramolopium* comprises 38 perennial, woody species, of which all but one are insular. The genus has a remarkable distribution extending from the Hawaiian Islands (11 species) and Cook Islands (1 species) to New Guinea (25 species) and Australia (1 species) (Pedley, 1993; Lowrey, 1995).

Richard (1832) first described *Vittadinia* to include one species, *V. australis*, from New Zealand. Nees von Esenbeck (1832) described *Tetramolopium* including one species, *T. tenerrimum*, from the Hawaiian Islands. De Candolle (1836) described several new species of *Vittadinia* and transferred two species from *Brachycome* Cass. to *Vittadinia.* Additionally, he described the monotypic genus *Eurybiopsis* to include *E. macrorhiza* from Australia.

Generic delimitation within this group has been problematic historically. The convoluted history of the two genera since De Candolle (1836) is summarized in Table 1. *Vittadinia* alone has undergone six generic recircumscriptions, and relationships with a number of other Australian genera have been stated or implied (Zhang and Bremer, 1993; Nesom, 1994b; Table 1). Zhang and Bremer (1993) stated, ''There is a need for a more complete analysis of the Astereae with new hypotheses of generic interrelationships among groups from different continents.'' Following this, Nesom (1994b) presented his new hypotheses of generic relationships in the tribe within a new subtribal classification. His *Vittadinia* group (Table 1) differs from previous classifications by the segregation of species previously included in *Vittadinia* to form *Peripleura* (Nesom, 1994a) and by the addition of two very small genera of annuals (*Iotasperma* [two spp.] and *Dichromochlamys* [monotypic]).

In addition to the nomenclatural complexity in the *Vittadinia-Tetramolopium* group, the Cook Islands species of *Tetramolopium* presents an intriguing phylogenetic and biogeographic problem involving the dispersal pathway that led to its disjunct distribution in the South Pacific. The species is morphologically and ecologically similar to species in one of the Hawaiian sections (Sect. *Tetramolopium*) (Lowrey, 1995), suggesting that the species was derived from a recent secondary dispersal event from the Hawaiian Archipelago. However, evidence from nuclear restriction fragment length polymorphism (RFLP) marker analyses indicates that the Cook Island species is a distinct entity forming a sister group to the Hawaiian species suggesting a possible dispersal event from a New Guinea ancestor (Okada, Whitkus, and Lowrey, 1997). Further data are needed to clarify the conflict between the morphology and ecology on the one hand and the molecular data on the other.

Chan et al. (1999) recognized the need to evaluate phylogenetic relationships in this group using molecular sequence

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TABLE 1. Nomenclatural history of the *Vittadinia* group of Astereae. Columns include the genera recognized by each author including the region of occurrence (in boldface). The region is provided for the first citation of the genus and if changed by a revised taxonomic concept of a subsequent author.

Gray (1861)	Bentham (1867)	Hillebrand (1888)	Mattfeld (1929)	Burbidge (1982)	Lander (1987)	Nesom (1994b)
Vittadinia (+ *Tetramolopium* + *Eurybiopsis*) **Australia, New-Zealand and Hawaii**	*Vittadinia*	*Vittadinia* **Australia and New Zealand**	*Vittadinia*	*Vittadinia* **Australia, New Zealand and New Caledonia**	*Minuria* (+ Eurybiopsis + 1 species of Olearia) **Australia**	*Peripleura* (split from Vittadinia) **Australia**
	Tetramolopium **Hawaii**	*Tetramolopium* **Hawaii**	*Tetramolopium* **New Guinea, and Hawaii**	*Eurybiopsis* **Australia** *Camptacra* sp. nov. **Australia** *Tetramolopium*	*Kippistia* (split from Minuria) **Australia**	*Vittadinia, Iotasperma* **Australia** *Camptacra, Dichromochlamys* **Australia** *Tetramolopium*

data in their preliminary investigation of the relationship of the Hawaiian and Cook Island species of *Tetramolopium.* They used the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and included several species of the Australian *Vittadinia* group as outgroup taxa. In agreement with Baldwin et al. (1995), ITS data were shown to be useful for examining phylogenetic relationships among congeneric species and closely related genera. The purpose of this study is to reassess phylogenetic relationships within the Pacific taxa of *Tetramolopium* and among the *Vittadinia-Tetramolopium* group of genera using ITS sequences and morphological data. We feel it is important to include morphological data to assess the phylogenetic usefulness of such data in light of its various historical interpretations. These varying interpretations have produced the complex taxonomic history of the group. Additionally, we desired to: (1) evaluate the hypotheses of Okada, Whitkus, and Lowrey (1997) about the origin of the Cook Island species of *Tetramolopium,* (2) test the monophyly of the genera within the group, (3) use the molecular estimate of phylogeny to evaluate the morphological characters that have been cited as indicators of affinity, and (4) assess phylogenetic relationships among the genera.

MATERIALS AND METHODS

Plant material was obtained from natural populations or from seeds collected from natural populations and grown in the Biology Department greenhouse at the University of New Mexico. Vouchers for original collections and for glasshouse-grown plants are deposited in either the University of New Mexico (UNM) Herbarium or John T. Waterhouse Herbarium (UNSW; Table 2).

Morphological characters were scored on herbarium specimens (1–5 per taxon depending on availability of stages on specimens) and also checked against published literature. Floral characters were scored under a dissecting microscope on capitula softened by soaking in hot water with a few drops of detergent. Data from quantitative characters were plotted on histograms, and states were determined from the apparent discontinuities. The data for *Minuria* sp. nov. were taken from the single known collection. Since no corollas remained on specimens of some taxa, some floral characters are missing.

Total DNA was extracted from fresh leaves, leaves dried in silica gel crystals, or leaves taken from existing herbarium specimens using either the CTAB method (Doyle and Doyle, 1990) or the DNeasy Plant Minikit (QIAGEN, Clifton Hill, Victoria, Australia). The Hawaiian taxa of *Tetramolopium* were sequenced manually; all other taxa were sequenced using an automated sequencer. For manual sequencing, both double-stranded and single-stranded DNA were amplified. Double-stranded DNA was amplified with Taq DNA polymerase from Boehringer Mannheim (Roche, Indianapolis, Indiana) following manufacturer's recommendations. Following an initial melt at 95°C for 2 min, cycling began with 1 min at 95°C, 1 min at 48°C, 45 s at 72°C. The extension time was increased by 4 s for each of 30 subsequent reaction cycles. This was followed by a final extension of 72°C for 7 min. A fragment of 650 base pairs was then isolated from a low-melting agarose gel. Single-stranded DNA was amplified using asymmetric polymerase chain reaction (PCR) and a 1/10 or 1/100 dilution of the double-stranded product. Polymerase chain reaction conditions were identical to those used for double-stranded amplification except that asymmetric primer concentrations were used (500 nmol/L and 20 nmol/L), and the annealing temperature was 54°C. The single-stranded PCR product was purified by differential filtration in a Millipore Ultrafree-MC tube (Millipore UFC3 THK 00–30 000 nmol; Millipore, Bedford, Massachusetts, USA).

Manual sequencing was performed using the SequiTherm cycle sequencing kit (Epicentre Technologies, Madison, Wisconsin, USA) following the procedure for 32P-labeled primers but substituting primers labeled using biotinylated phosphoramidite (Clontech Laboratories Biotin-ON, Palo Alto, California, USA). Cycle sequencing parameters were 95°C for 5 min, 35 cycles at 95°C for 30 s, 54°C for 30 s, and 70°C for 1 min. Upon completion, 3 μL of stop dye solution was added. The sequencing reactions were separated using a 5% Long Ranger (FMC Bioproducts, Rockland, Maine, USA) and visualized using the procedure specified in the Sequenase Images non-isotopic DNA sequence detection kit (US Biochemical, Cleveland, Ohio, USA) with modifications to minimize background. These included a longer blocking step (1 h), decreasing the streptavidin-alkaline phosphatase conjugate (SAAP) concentration to ~1/2850 the volume of the blocking buffer, and performing a 20–30 s distilled water wash after each 1× post-SAAP/SDS wash. Optimal results were obtained by exposing the membrane to Kodak BioMax MR film for 2 h 15 min.

For automated sequencing, double stranded templates were amplified using the universal primers developed from fungal rDNA (White et al., 1990). Samples for automated sequencing were amplified using PCR Supermix (GibcoBRL, Rockville, Maryland, USA) or AmpliTaq Gold (Perkin Elmer, Foster City, California, USA), following manufacturers' recommendations. Polymerase chain reaction conditions included an initial melt at 95°C for 2 min (PCR Supermix) or 10 min (AmpliTaq Gold), 35 cycles of 1 min at 95°C, 1 min at 48°C, and 1 min at 72°C, followed by a final 5-min extension at 72°C. Polymerase chain reaction products were cleaned using QIAquick PCR clean-up kits (Qiagen, Chatsworth, California, USA). Sequence reactions were performed using the Thermosequenase dye-terminator pre-mix kit (Amersham, Cleveland, Ohio, USA) following manufacturer recommendations, ex-

TABLE 2. Voucher details for sequence data included in the analyses. Location of specimens is indicated by Index Herbariorum abbreviation.

Taxon	Voucher no. and location	GenBank no.	Collecting locality
Olearia argophylla (Labill.) Benth.	20059, UNSW	GBAN-326793	New South Wales
O. pannosa Hook	24061, UNSW	GBAN-326801	S. Australia
O. arguta (R. Br.)	Cumming 13918, BRI	GBAN-326806	Queensland
Brachycome ciliaris	Lowrey 1712, UNM	GBAN-326808, 326824	W. Australia
Camptacra gracilis (Benth.) Lander	Lowrey 1758, UNSW	GBAN-326827	Queensland
C. barbata Burbidge	Lowrey 1751, UNSW	GBAN-326828	Queensland
Kippistia suadefolia F. Muell.	Pickard 3657, NSW	GBAN-326830	New South Wales
Minuria cunninghamii (DC.) Benth.	Lowrey 1735, UNSW	GBAN-326831, 326834	S. Australia
M. integerrima (DC.) Benth.	Lowrey 1754, UNSW	GBAN-326836	Queensland
M. macrorhiza (DC.) Lander	Kenneally 7866, PERTH	GBAN-326840	W. Australia
	Adam 23307, UNSW	GBAN-326853	Northern Territory
M. sp. nov.	Lowrey 1713, UNSW	GBAN-326855	W. Australia
Peripleura bicolor (Burbidge) Nesom	Lowrey 1765, UNSW	GBAN-326856	Queensland
P. diffusa (Burbidge) Nesom	Lowrey 1760, NSW	GBAN-326858	Queensland
P. hispidula (F. Muell. ex A. Gray) Nesom	Lowrey 1757, NSW	GBAN-326859, 326860	New South Wales
P. obovata (Burbidge) Nesom	Albrecht 5503, DNA	GBAN-326862	Northern Territory
P. sericea (Burbidge) Nesom	Lowrey 1761, UNSW	GBAN-326863	Queensland
Tetramolopium alinae (Muell.) Mattf.	Lowrey 1536, UNM	GBAN-326866	New Guinea
T. arenarium (A. Gray) Hillebr.	Lowrey 1639, UNM	GBAN-326867	Hawaii
T. consanguineum (A. Gray) Hillebr.	Lowrey 1615, UNM	GBAN-326868	Hawaii
T. filiforme Sherff	Lowrey 1642, UNM	GBAN-326869	Hawaii
T. humile	Lowrey 1616, UNM	GBAN-326870	Hawaii
T. lepidotum (Less.) Sherff	Lowrey 1643, UNM	GBAN-326871	Hawaii
T. macrum (Muell.) Mattf.	Lowrey 1537, UNM	GBAN-326873	New Guinea
T. ''mitiaroense''	Lowrey 1525, UNM	GBAN-326875	Cook Islands
T. pumilum Mattf.	Lowrey 1546, UNM	GBAN-326877	New Guinea
T. remyi (A. Gray) Hillebr.	Lowrey 449, 450, UC	GBAN-326878	Hawaii
T. rockii Sherff	Lowrey 1629, UNM	GBAN-326880	Hawaii
T. sylvae Lowrey	Lowrey 1634, UNM	GBAN-326881	Hawaii
T. vagans Pedley	Lowrey 1755, UNSW	GBAN-326883	Queensland
Vittadinia autralasica (Turcz.) Burbidge	Lowrey 1741, UNSW	GBAN-326885	S. Australia
V. australis	Glenny 7332, UNM	GBAN-326886	New Zealand
V. blackii Burbidge	Lowrey 1749, UNSW	GBAN-326887	S. Australia
V. cervicularis Burbidge	23118d, UNSW	GBAN-326888	New South Wales
V. condyloides Burbidge	23103, UNSW	GBAN-326889	New South Wales
V. cuneata DC.	Lowrey 1753, UNSW	GBAN-326890, 326892	Queensland
V. dissecta (Benth.) Burbidge	Lowrey 1739, UNSW	GBAN-326894	S. Australia
V. eremaea Burbidge	Lowrey 1719, UNSW	GBAN-326895	W. Australia
V. gracilis (Hook. F.) Burbidge	Lowrey 1737, UNSW	GBAN-326896, 326897	S. Australia
V. humerata Burbidge	Lowrey 1711, UNSW	GBAN-326898	W. Australia
V. megacephala (F. Muell. ex Benth.) Black	Lowrey 1746, UNSW	GBAN-326899	S. Australia
V. pterochaeta (F. Muell. ex Benth.) Black	Lowrey 1752, UNSW	GBAN-326900	Queensland
V. pustulata Burbidge	Lowrey 1750, UNSW	GBAN-326901	Queensland
V. sulcata Burbidge	Lowrey 1727, UNSW	GBAN-326902	W. Australia

cept that final reaction volumes were adjusted to 5 μL. Sequence reactions were analyzed using the ABI Model 377 DNA sequencer (Applied Biosystems, Foster City, California, USA) as described in Nelson et al. (1997).

Sequences were assembled and checked using ABI Prism software (Factura and Autoassembler) (Applied Biosystems). The sequences were aligned by determining the boundaries separating the rRNA genes from the ITS1 and ITS2 regions by comparison with the sequences of *Daucus carota* and *Vicia faba* (Yokota et al., 1989), where the limits of mature 18S, 5.8S, and 26S rRNAs have been defined by S1 nuclease mapping. Internal transcribed spacer sequences were either manually aligned, using as a guide the above boundaries, and stored in DAPSA (DNA and protein sequence alignment program; E. Harley, University of Cape Town, Cape Town, South Africa) or were aligned using ClustalW (Thompson, Higgins, and Gibson, 1994) and optimized by eye. Segments affected by insertion/deletion events (indels) were treated as missing data in the analyses, and potentially informative indels were scored as additional characters (present/absent) that were added to the sequence database. All sequences are lodged in GenBank (Table 2).

Heuristic searches were conducted in Phylogenetic Analysis Using Parsimony* (PAUP*) version 4.0b2a (Swofford, 1999) for each data set individually and combined using tree bisection reconnection (TBR) branch-swapping and the MULPARS (save all most parsimonious trees) option. Random taxon addition (100 replicates) was employed to search for multiple islands of trees in all analyses of the molecular and combined data; random taxon addition (20 replicates) was used on the morphological data. Branch lengths for trees were calculated using the accelerated transformation optimization (ACCTRAN) option in PAUP. Relative support for the clades identified by parsimony analysis of the molecular data was estimated by the jackknife option in PAUP using 30% character deletion, fast stepwise addition, and 100 000 replicates, and also by decay analyses (Donoghue and Sanderson, 1992) using PAUP and AutoDecay version 4.0.1 (Eriksson, 1998) with a simple heuristic search on each constraint tree. Output trees were imported into MacClade to explore evolution of nonmolecular characters and to construct constraint trees in order to test alternative hypotheses against the data. Analyses were then performed in PAUP using 100 random addition replicates and the option ''topological constraint enforced.'' The significance of differences between the strict consensus trees obtained in the constraint analysis and that of the most parsimonious trees was determined using the ''compare two trees'' option in PAUP. All analyses were polarized by the outgroup method, using two species of arborescent *Olearia.* A more inclusive analysis of Astereae, polarized on sequences from Calenduleae and Anthemideae, revealed these taxa to belong to an early diverging lineage within the tribe (Noyes and Reiseberg, 1999; E. Cross and C. J. Quinn, unpublished data).

TABLE 3. Location of potentially informative indels within the aligned database of ITS1, 5.8S, and ITS2 totaling 646 positions. The distributions of these are mapped on Fig. 1.

Indel no.	Positions affected
1	15–16
2	50–51
3	58
4	83
5	122
6	127
7	168–169
8	176
9	209
10	605
11	594–595
12	613

Combinability of the morphological and molecular data sets was assessed using the permutation tail probability (PTP) test (Faith and Cranston, 1991) to test for the presence or absence of phylogenetic signal and the partition homogeneity test (the incongruence length difference test of Farris et al., 1994) to test for incongruence between the data sets. These two tests were applied using PAUP* version 4.0b2a. The partition homogeneity test was implemented with invariant characters excluded (Cunningham, 1997) using TBR branch-swapping with 1000 replicates. The maximum of trees held in memory (MAXTREES) option was set to 25 to allow the test to proceed to completion. Analyses performed using an unrestricted MAXTREES setting exceeded the computer memory after only 3 repetitions.

Maximum likelihood (ML) analysis was performed using the stochastic search algorithm of Salter and Pearl (2001) under the molecular clock assumption and the F84 model of substitution, estimating the transition/transversion parameter simultaneously with the tree (ti/tv = 1.47).

RESULTS

Molecular analysis—Sequences were obtained for 50 accessions representing 40 ingroup species (2 *Camptacra,* 1 *Kippistia,* 5 *Minuria,* 5 *Peripleura,* 13 *Tetramolopium,* and 14 *Vittadinia*). A semiherbaceous species of *Olearia* was added because it was considered aberrant, with possible relationships to the *Vittadinia* group (N. S. Lander, personal communication). A preliminary analysis of ITS sequence data for a broad representation of Australasian Astereae (E. Cross and C. J. Quinn, unpublished data) indicated that none of the other genera tested had a close affinity with the members of the above ingroup. The analysis was rooted on two arborescent species of *Olearia,* and one species of *Brachycome* was added as a more closely related outgroup. The latter genus has been associated with *Vittadinia* previously (De Candolle, 1836). Sequences included the complete ITS region except for the Western Australian accession of *Minuria macrorhiza,* where the first 30 bases of ITS-1 were missing, and *Brachycome, Minuria cunninghamii, Vittadinia cuneata,* and *V. gracilis,* for which the 5.8S region was excluded. The aligned data matrix comprised 44 sequences and 634 characters: a single sequence for each species, except *Minuria macrorhiza,* where there was a distinct divergence between those obtained from the Western Australian and Northern Territory accessions. Alignment required 20 indels, 4 affecting two positions and the rest only one. Twelve of these were potentially parsimony informative (Table 3); when these were scored, the matrix comprised 646 characters, 132 of which (20.5%) were parsimony informative, and 109 (16.9%) within the ingroup.

The uncorrected pairwise distance between sequences for the ingroup taxa varies between 13.2% for *Minuria cunninghamii–M. integerrima* and zero for *Tetramolopium rockii–T. sylvae.* The distance between the sequences obtained from the two accessions of *Minuria macrorhiza* is 4%.

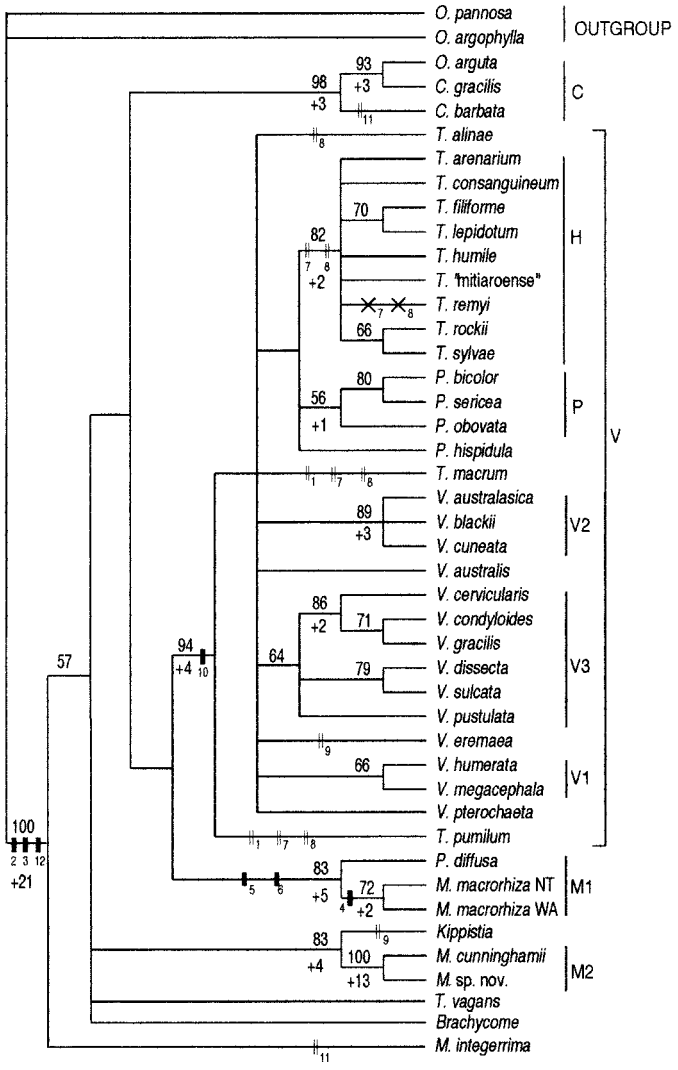

Fig. 1. Strict consensus of a single island of 2520 trees of 490 steps found from a heuristic search of the molecular data base; consistency index (CI) excluding uninformative characters = 0.53; retention index (RI) = 0.68; rescaled consistency index (RC) = 0.44. Jackknife values >50% and decay values >1 are shown on branches. Informative indels numbered as in Table 2 are mapped on the tree; broad bar indicates unique synapomorphy; parallel bars indicate parallelism; cross indicates reversal. Figure abbreviations: C., *Camptacra*; M., *Minuria*; P., *Peripleura*; T., *Tetramolopium*; V., *Vittadinia.*

The heuristic search yielded a single island of 2520 trees of 490 steps having a rescaled consistency (RC) index of 0.44. The strict consensus is shown in Fig. 1 and a phylogram of one of the 2520 most parsimonious trees is shown in Fig. 2. There is no support for the monophyly of any ingroup genus. The two species of *Camptacra* form a very robust clade with the Queensland accession of *Olearia arguta* (98% jackknife support, +3 decay), but *Camptacra gracilis* is much closer to the latter than it is to *C. barbata* (93%, +3). The Hawaiian and Cook Island species of *Tetramolopium* form a well-supported clade (H in Fig. 1; 82%, +2). Four of the five species of the Australian genus *Peripleura* are associated with this

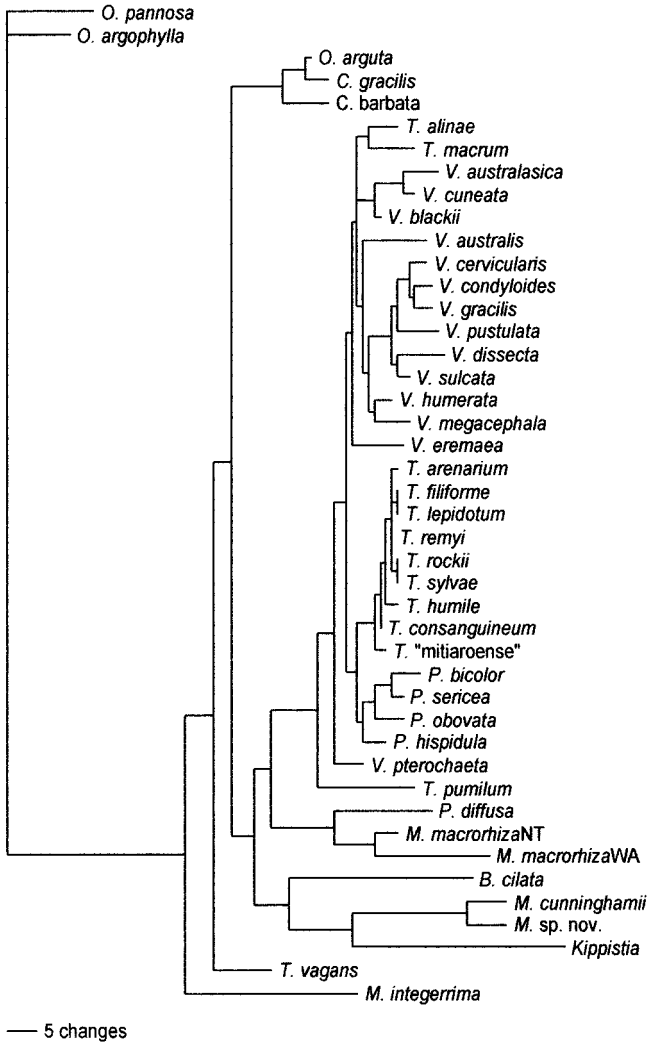

Fig. 2. Phylogram of one of 2520 equally parsimonious trees of 490 steps found from the heuristic search of the molecular data.

clade, although there is low support for this link; *P. bicolor* groups with *P. sericea* and *P. obovata* (clade P; 56%, +1) within a polytomy with *P. hispidula* and clade H. *Peripleura diffusa,* however, is placed at a distance from this grade in M1, being the well-supported sister of one of the clades of *Minuria* species (83%, +5). The two accessions of *M. macrorhiza* are sister taxa within this clade, although support for their association is only weak (72%, +2). *Minuria cunninghamii* and *M.* sp. nov. are strongly nested within (100%, +13) clade M2 along with *Kippistia* as their well-supported (83%, +4) sister group. *Minuria integerrima* is the first lineage to diverge. Three clades of *Vittadinia* species were retrieved (V1, V2, and V3), but other species of this genus are ungrouped within the main polytomy. All species of *Peripleura, Tetramolopium,* and *Vittadinia,* with the exception of *Peripleura diffusa* and *Tetramolopium vagans,* constitute a well-supported major clade (94%, +4; clade V in Fig. 1). The phylogram (Fig. 2) shows that branch lengths are generally very short in the V clade indicative of few changes among the sequences of the taxa in this group.

The distribution of scored indels is mapped on Fig. 1. Six of the 12 have distributions consistent with a single origin.

The best tree produced in the maximum likelihood analysis (log likelihood −3566.32) contained all the labeled clades in Fig. 1. It differed only in some of the unsupported topology, particularly in the placement of *Brachycome* as the first diverging lineage and *Tetramolopium vagans* in a sister relationship with the C clade. Within the H clade, *T.* ''mitiaroense'' and *T. humile* were sequentially the first two taxa to diverge. Again, *T. rockii* grouped with *T. sylvae* and *T. filiforme* grouped with *T. lepidotum.* An examination of the nine next best ML trees showed they also only differed from Fig. 1 in aspects of the unsupported topology.

Morphological analysis—A database of 39 characters was assembled (Appendix). A systematic examination of the receptacle under a scanning electron microscope revealed hitherto unrecognized variation in the surface morphology that permitted the recognition of four states within the study group. After dispersal of the cypselas, the receptacle may appear smooth under the dissecting microscope except for the scar where the floret pedicel was attached (Fig. 3C). In other species the receptacle bears a pattern of single ridges that separate adjoining floret insertions (Fig. 3D). In some cases these ridges extend on the outer side of the insertion to form a pointed scale of variable size (Fig. 3A, B). In *Brachycome ciliaris* each floret is surrounded by the expanded base of a scale, so that there is a double ridge between adjacent insertions. These states are scored under character 36.

A heuristic search with random taxon addition with 20 repetitions obtained two islands of 130 equally parsimonious trees of 252 steps (CI excluding uninformative characters = 0.31; retention index (RI) = 0.64; RC = 0.20). The strict consensus of these is shown in Fig. 4. There is improved resolution in the *Vittadinia* clade (V4), but apart from the distinction between the outgroups and ingroup, virtually all of the topology decayed at +1 step. None of the relationships in the *Vittadinia* clade match those in Fig. 1. Of the clades identified in Fig. 1, only M2 (*Minuria cunninghamii* + *M.* sp. nov.) is retrieved; both representatives of *Minuria macrorhiza* are also grouped as in Fig. 1. *Vittadinia* is the only genus to appear monophyletic. Hawaiian representatives of *Tetramolopium* (except *T. humile*) are grouped (H′) separate from the Cook Island species; New Guinea and Australian species of the genus are placed at a distance from this clade. *Peripleura diffusa* is separated from the other species of the genus that constitute clade P′. The two representatives of *Minuria macrorhiza* are grouped as in Fig. 1.

Combined analysis—The PTP test indicated that each data set had significant phylogenetic structure ($P = 0.001$ for both data sets). The partition homogeneity test showed that the null hypothesis that the two data partitions were homogenous (not incongruent) was rejected ($P = 0.001$). This result indicates statistically significant incongruence between the data sets. Despite this, we have included a combined analysis. A comparison of Figs. 1 and 4 indicates incongruence in the placements of a few taxa, such as *Peripleura diffusa* and *Olearia arguta,* but not wholesale conflict in all clades. In fact when the two taxa were excluded, the combined analysis may reveal some congruence between the data sets in the form of increased support for some parts of the topology or improved resolution of the relationships of some taxa.

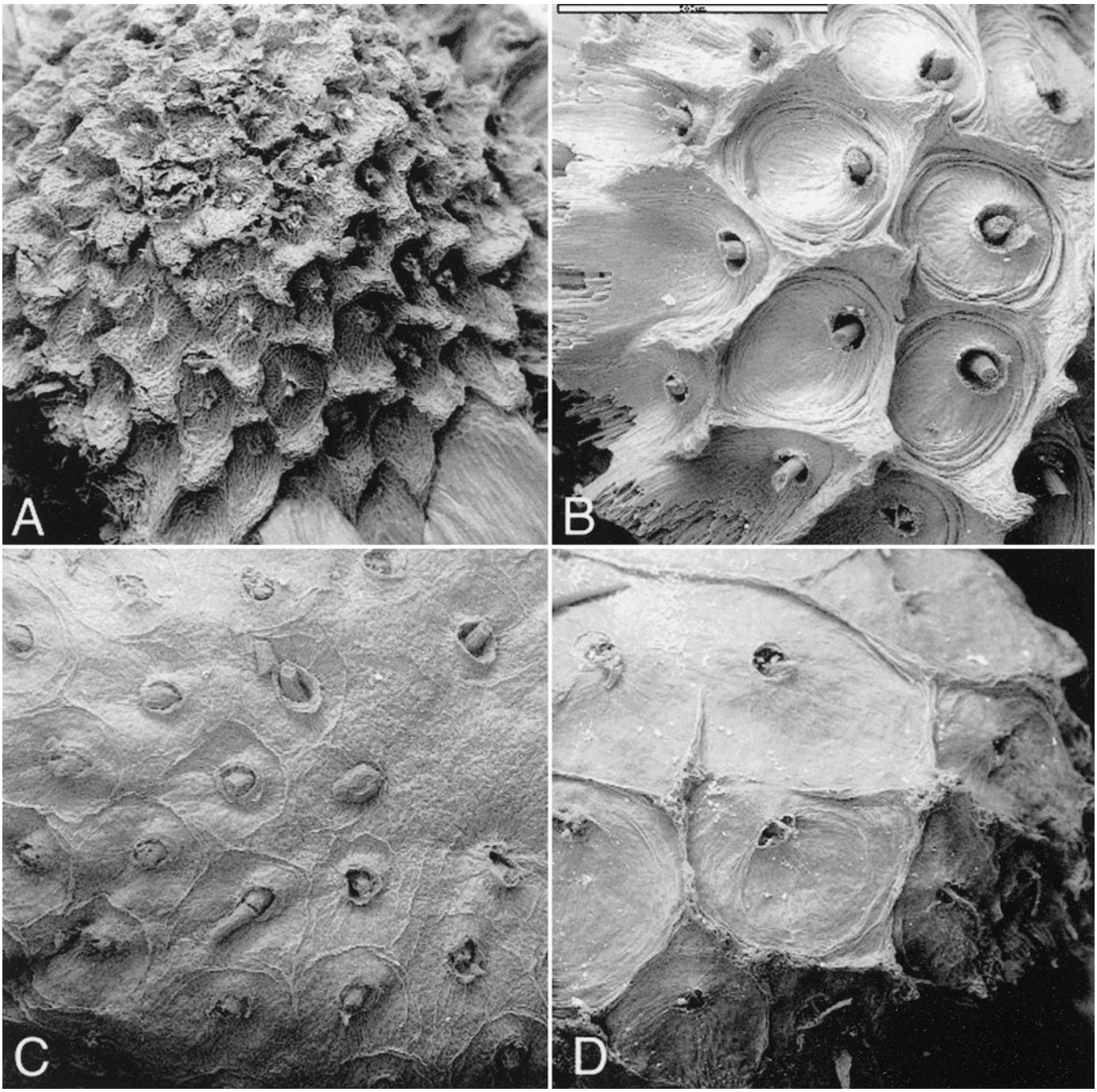

Fig. 3. Scanning electron micrographs of surfaces of receptacles after dispersal of cypselas. Scale bar = 500 μm for all figures. (A) *Kippistia suaedifolia,* showing floret insertions surrounded by bases of reduced receptacular scales; (B) *Vittadinia eremaea*, showing floret insertions outlined by a reticulate pattern bearing small pointed scale tips; (C) *Tetramolopium mitiaroense*, in which the reticulate pattern has been reduced so that the receptacle appears totally smooth under the dissecting microscope; (D) *Peripleura bicolor, in* which the reticulate pattern is visible under the dissecting microscope.

We believe that the more data exploration the better, so we have included both separate and combined analyses in order to get the most information possible to elucidate phylogenetic relationships while keeping in mind the caveat of the partition homogeneity test results.

Analysis of the combined morphological and molecular database gave 32 trees of 756 steps (CI = 0.49; RC = 0.31) in a single island. The strict consensus of these trees is shown in Fig. 5. The topology shows increased resolution of relationships when compared to the tree derived from molecular data only. All the labeled clades in Fig. 1 are present except for V3, which no longer contains *Vittadinia pustulata*, and P, which now also includes *Peripleura hispidula*.

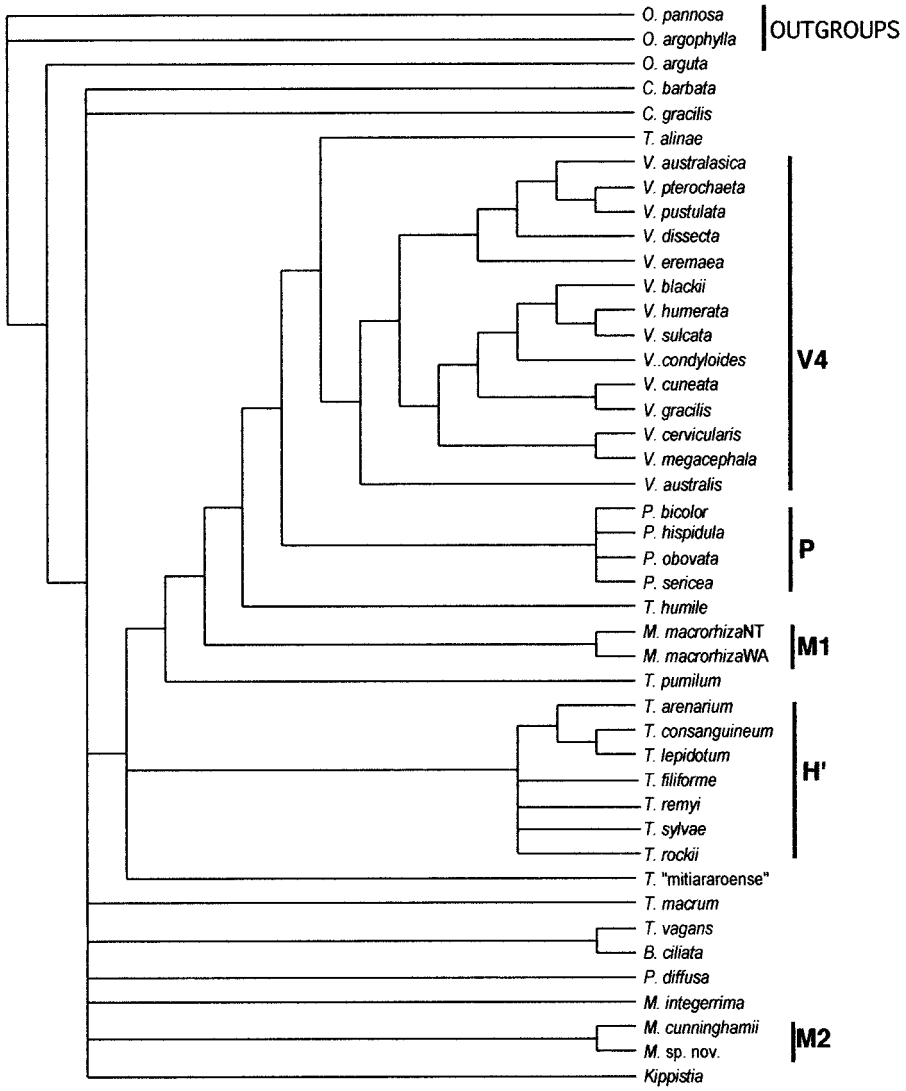

Fig. 4. Strict consensus of 130 equally parsimonious trees of 252 steps found in a heuristic search of the morphological database using 20 replicates; CI = 0.31 excluding uninformative characters; RI = O.64; RC = 0.20.

DISCUSSION

Molecular analysis—The goal of the molecular and morphological analyses is to make progress towards the ultimate delimitation of monophyletic genera in the *Vittadinia* group.

The distributions of the indels are quite informative of relationships within the group. Indels 2, 3, and 12 confirm the distinction between the ingroup and the root on arborescent *Olearia,* 5 and 10 support the M1 and V clades, respectively, and indel 4 supports the sister relationship between the two accessions of *Minuria macrorhiza.*

Tetramolopium vagans is placed at a distance from all other members of the genus in Fig. 1. It shows no close affinity with any included taxon. An undescribed taxon with putative affinity with this species is recorded farther north in Queensland. It seems likely that a new genus may be required for these taxa, but further investigation of their relationships is needed before any final assessment is made. In particular, their relationships to other poorly known members of the tribe in New Guinea need to be considered.

Although all other species of *Tetramolopium* were not grouped in the analysis, when they were constrained in the one clade, tree length was increased by only a single step. Hence the molecular evidence against monophyly of this group is very weak. The inclusion of *T. vagans* within the constrained clade, however, gave a tree length 14 steps longer than the most parsimonious solution ($P < 0.03$ on the compare-two-trees test). This indicates that the molecular data provide very strong evidence against the inclusion of this species within the genus. Again, while the three New Guinea species of *Tetramolopium* were not grouped, and there was no support in the jackknife analysis for any association between them, only a single step was needed to make all the remaining species of the genus monophyletic ($P > 0.9$). Indels 7 and 8 characterize the Hawaiian clade of *Tetramolopium* and also occur in the New Guinea species: indel 8 is present in all three, whereas 7 occurs in only *T. macrum* and *T. pumilum.* These two species also share indel 1. This is direct evidence in favor of the monophyly of the non-Australian members of this genus, despite their not being grouped in the analysis. It further

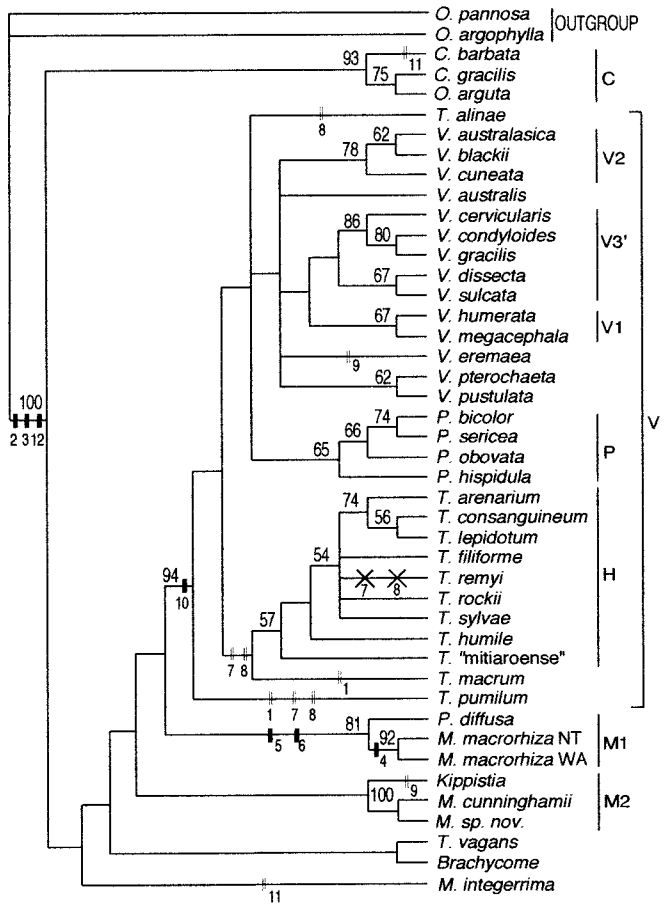

Fig. 5. Strict consensus of 32 trees of 756 steps in one island found from a heuristic search of the combined molecular and nonmolecular data; CI = 0.49; RI = 0.63 excluding uninformative characters; RC = 0.31. Jackknife values >50% and decay values >1 are shown on branches. Informative indels numbered as in Table 2 are mapped on the tree as in Fig. 1.

suggests that *T. alinae* was the first lineage to diverge after indel 8 had arisen but before indel 7, and that the other two New Guinea species share a unique common ancestor, as indicated by indel 1. This hypothesis conflicts with the topology obtained in the analysis, but all the conflicting aspects decay at +1 step. Further data are needed to robustly resolve these relationships.

Although four of the five species of *Peripleura* are grouped with the Hawaiian clade and are strongly supported members of the V clade (94%, +4), *P. diffusa* is the well-supported sister (83%, +5) to the *Minuria macrorhiza* complex (clade M1). This last association is also supported by the distribution of indels 5 and 6 (Fig. 1). Constraint analyses revealed that an extra 19 steps are required to render *Peripleura* monophyletic ($P < 0.03$). Hence the molecular data provide strong evidence against the monophyly of this genus also. The remaining species of *Peripleura* constitute a paraphyletic assemblage, but constraint analyses revealed that only one extra step is required to render the group monophyletic ($P > 0.9$). It is therefore clear from the molecular data that *Peripleura diffusa* has no close affinity with other members of that genus.

Vittadinia s.s. (sensu stricto) is rendered paraphyletic in the molecular analysis: there are several supported groups of species labeled V1, V2, and V3 in Fig. 1, and the relationships of *V. australis*, which is the type species, *V. eremea*, and *V. pterochaeta* are unresolved. When the genus was constrained to a single clade, tree length was increased by four steps. However, the compare-two-trees test showed no significant difference from Fig. 1 ($P > 0.7$).

Species of *Minuria* constitute three separate lineages: the two accessions of *M. macrorhiza* are strongly associated with *P. diffusa* in clade M1, which is the unsupported sister group of the V clade; *M. cunninghamii* is grouped with *M.* sp. nov. in a strongly supported clade (100%, +13), and these are in turn grouped with *Kippistia*; and *M. integerrima* is placed as the first diverging lineage among ingroup taxa. The analysis provides no evidence of any close relationship between these three groups, although all the branches separating them receive little or no support. When all species assigned to the genus were constrained into one clade, tree length increased by 17 steps. The inclusion of *Peripleura diffusa* in the constrained group reduced tree length by four steps; inclusion of *Kippistia* in the group decreased tree length by seven steps (to +10); the inclusion of both these taxa reduced it a further five steps (to +5). Comparisons of the strict consensus trees from these searches exceeded available memory before completion, but it seems highly likely that an extra ten or more steps represents a significant difference from the most parsimonious topology. We conclude that the molecular evidence does not support the current concept of *Minuria* and that there is strong support for the association of *Peripleura diffusa* with the *Minuria macrorhiza* clade and of *Kippistia* with the M2 clade.

Minuria also shows much larger sequence divergences than any other genus in this group, having a maximum pairwise divergence of 13.2% for *M. cunninghamii* to *M. integerrima* (Fig. 2). Neither *Kippistia* nor *Peripleura diffusa* are more divergent from any species of *Minuria.* Divergence within the *M. macrorhiza* complex is 4% and within M2 is 3%. These values can be contrasted with the respective maximum divergences observed in *Peripleura* (excluding *P. diffusa*), *Tetramolopium* (excluding *T. vagans*), and *Vittadinia* of 3.6, 5.4, and 6.1% (Fig. 2). *Minuria* therefore contains taxa that are more divergent in their ITS sequences than any of the other genera. Both *Kippistia* and *Peripleura diffusa* fit well within the group from both a cladistic and sequence divergence point of view. However, further data are needed to establish the monophyly of such an expanded concept.

Constraint analysis of the relationship between *M. integerrima* and the M1 clade revealed that only one extra step is required to render this group monophyletic ($P > 0.9$). Further sampling of taxa, including those outside the ingroup in this study, are needed to see whether this is a fair reflection of the distinction between the two lineages.

Indels 9 and 11 appear to have arisen twice, given the robustness of clades C, V, and M2. The remaining ten indels appear to be reflecting aspects of the phylogeny, although in four cases this is obscured by the lack of resolution and weakness in the topology.

The divergence between the sequences obtained from the two accessions of *Minuria macrorhiza* is of the same order as between the Northern Territory accession and *Peripleura diffusa* (4.4%) and between *Minuria cunninghamii* and *M.* sp. nov. (3%); it also compares favorably with the pairwise divergences observed within *Vittadinia* (1.6–6.1%). The molecular data therefore provide strong support for the morphological distinctions observed between these two populations and for the recognition of a new species.

Analysis of morphological characters—There are several morphological characters that unite the different groups delineated in the molecular analysis. All taxa of *Vittadinia* s.s. possess an apical position of the embryo in the mature cypsela and do not fill the cypsela completely. The Hawaiian *Tetramolopium* clade H and the *Camptacra* clade C are characterized by having a smooth receptacle surface. In addition, clade C is united by the absence of biseriate two-celled hairs with bifurcate tips on the cypsela.

However, when the morphological states are mapped on the strict consensus as well as several representative most parsimonious trees for the combined analysis there remains a high level of homoplasy in most characters. It is instructive to examine some of these, especially characters that have been used in distinguishing taxa. Female sterile disc florets (character 11), a character which has been widely used to distinguish genera in the family (Bremer, 1994), appears to have been gained and lost more than once. Monoecy has arisen in *Tetramolopium* as well as *Minuria,* and there has been a subsequent loss within the Hawaiian clade of the former. Constraint analyses revealed that only two extra steps ($P = 0.92$) are required to group *Tetramolopium humile* with the other species of the Hawaiian group with female fertile disc florets, so that only a single loss is required within this clade. The evidence against monophyly of the ingroup taxa possessing female sterile disc florets, with or without a subsequent reversal, is very strong ($+22$ and $+30$ steps, $P = 0.02$ and 0.01, respectively). Hence the combined data provide strong support for more than one character state change in each direction. Recent genetic analysis of Hawaiian *Tetramolopium* indicates that female disc floret fertility/sterility is largely controlled by just two loci with modifiers (Whitkus, Doan, and Lowrey, 2000). This accords well with the apparent lability of sex expression in the evolution of the group. If the relatively simple genetic control of female fertility/sterility in *Tetramolopium* were found to be a general pattern in the Asteraceae, systematists would do well to be very cautious in using variation in monoclinous sex expression as a taxonomic character for generic delimitation, particularly in the Astereae (Nesom, 2000).

Similar tests of the following characters revealed significant support ($P < 0.01$) for homoplasy in each case: gain/loss of woodiness or a woody rootstock (character 1); ratio of ray to disc florets (9); length of ligule on ray florets (10); corolla form in disc florets (24); filament insertion (25); compression of the cypsela (28); marginal ribs on the cypsela (30); presence and number of facial ribs on the cypsela (31); and loss of scales and/or pattern on the receptacle (36). Characters 1, 24, 28, 30, 31, and 36 have been used rather extensively for generic delimitation as well as for higher ranks in the Asteraceae (Zhang and Bremer, 1993; Bremer, 1994; Nesom, 1994b). We believe that studies similar to those of Whitkus, Doan, and Lowrey (2000) and Bachmann (1991) are needed to elucidate the genetic control of morphological characters in the Asteraceae and provide insight into their lability and potential usefulness as taxonomic and phylogenetic markers.

The morphological data show a very high degree of homoplasy. The CI value of 0.31 in our study is among the lowest (indicative of a high level of homoplasy) of the values reported in recent analyses of homoplasy in published phylogenetic studies (Sanderson and Donaghue, 1996; Givnish and Sytsma, 1997). In addition, the present study has fewer taxa than other studies that have reported high levels of homoplasy correlated with relatively large numbers of taxa being considered (Givnish and Sytsma, 1997). The high level of morphological homoplasy in the *Vittadinia* group explains the poor resolution obtained in the morphological analysis and the lack of support for some of the generic concepts within this assemblage. The probable, main sources of homoplasy are convergence/parallelism and hybridization. Homoplasy in gain/loss of woodiness and length of ray ligules could result from selection for particular states in similar arid environments. There has been no documented case of natural hybridization in this group. However, artificial interspecific hybridization is easily accomplished in Hawaiian *Tetramolopium* and *Vittadinia* (Lowrey, 1986; T. K. Lowrey, C. J. Quinn, and J. Avritt, unpublished data) and artificial intergeneric hybrids of the following crosses *Tetramolopium* × *Vittadinia*, *Vittadinia* × *Camptacra*, and *Tetramolopium* × *Camptacra* have recently been obtained (T. K. Lowrey, C. J. Quinn, and J. Avritt, unpublished data). Therefore it is possible that hybridization has played a role in the evolution of the group. Furthermore past intergeneric hybridization events could explain, in part, the considerable degree of homoplasy in the morphological data. We believe that if it is possible to have reticulate evolution via intergeneric hybridization in this group of genera in the Astereae it may well be an important process elsewhere in the tribe and the family. Future systematic studies of generic groups in the Asteraceae should consider the possibility of reticulate evolution above the species level when high levels of homoplasy are encountered.

Origin of the Cook Island Tetramolopium species—Intensive biosystematic (Lowrey, 1986) and molecular studies (Lowrey and Crawford, 1985; Okada, Whitkus, and Lowrey, 1997) provide strong support for the monophyletic origin of and genetic cohesion among the Hawaiian taxa. The New Guinean species are montane shrublets that occur in submesic or mesic tropic-alpine habitats. The Cook Island species is a dwarf shrub occurring in uplifted, fossilized coral outcrops in coastal scrub vegetation on Mitiaro only. The Hawaiian species exhibit the greatest morphological and ecological diversity, constituting a classic example of adaptive radiation by insular plants (Funk and Wagner, 1995; Lowrey, 1995; Okada, Whitkus, and Lowrey, 1997). They vary from prostrate, rosette perennials to tall, upright shrubs and occur in a diversity of submesic to xeric habitats from sea level to 3000 m on the five main islands (Lowrey, 1995).

Based on morphological similarity and geologic evidence it has been assumed that the Hawaiian taxa developed from a single colonization event from a New Guinean progenitor. Smith (1977) suggested that *Tetramolopium* evolved in the New Guinean highlands as a neo-endemic in the early Pleistocene and was subsequently dispersed to the Hawaiian Islands. The discovery of a new species of *Tetramolopium* in the Cook Islands (Lowrey, 1995) has complicated the previously simple picture of dispersal from New Guinea to the Hawaiian Islands. The Cook Islands taxon is morphologically and ecologically similar to species in one of the Hawaiian sections (section *Tetramolopium*) of *Tetramolopium* (Lowrey, 1995), suggesting that the taxon was derived from a recent secondary dispersal event. However, evidence from nuclear RFLP marker analyses indicates that the Cook Island taxon is a distinct entity forming a sister group to the Hawaiian species (Okada, Whitkus, and Lowrey, 1997). In fact, nearly all analyses of the RFLP data indicate that sect. *Tetramolopium* is paraphyletic with the inclusion of the Cook Island species. Furthermore,

the RFLP trees consistently fail to recognize a close affinity to sect. *Tetramolopium* despite the strong morphological and ecological similarities. This result does not support the hypothesis that the taxon was derived from a relatively recent secondary dispersal event from a Hawaiian founder. However, the RFLP data set used by Okada, Whitkus, and Lowrey (1997) did not use any outgroup taxa nor did it include any New Guinean species of *Tetramolopium.*

Okada, Whitkus, and Lowrey (1997) offered three equally parsimonious hypotheses to account for the two dispersal events necessary to explain the bihemispheric distribution on the Hawaiian and Cook Islands. These hypotheses are: (1) the Cook Islands species is the result of a secondary long-distance founding event from Hawaiian *Tetramolopium,* (2) the original dispersal event was to the Cook Islands with the Hawaiian group resulting from a secondary dispersal event, and (3) the Cook Island species and the Hawaiian group represent independent dispersal events. Okada, Whitkus, and Lowrey (1997) argued that the biogeographic, morphological, and reproductive biology in totality provide support for hypothesis 1 despite the conflicting RFLP data.

Our ITS data alone do not resolve the relationships of *T.* ''mitiaroense'' to the Hawaiian species of *Tetramolopium,* but the combined analysis does place them in a sister relationship. This favors the second hypothesis advanced by Okada, Whitkus, and Lowrey (1997) and is congruent with their RFLP data. However, there is no character support for this pattern of relationship. Clearly further data are required to provide a definitive answer to this question. Nonetheless, both the indel data and the combined analysis support the hypothesis of Smith (1977) that the Pacific Island species of *Tetramolopium* are descended from a New Guinea ancestor.

General conclusions—*Vittadinia* is the only genus to be resolved in both the morphological and combined analysis. None of the analyses provide support for the monophyly of *Camptacra, Minuria, Olearia, Peripleura,* or *Tetramolopium.* The considerable amount of homoplasy in the morphological data set may reflect the influence of past intergeneric hybridization events. *Tetramolopium vagans* is placed at a distance from all other species of that genus. In the molecular and combined analyses *Minuria* is resolved into three separate clades. *Olearia arguta* is strongly grouped with *Camptacra, Kippistia* is grouped with the M1 clade of *Minuria,* and *Peripleura diffusa* is strongly grouped with the *Minuria macrorhiza* complex. Both *Peripleura diffusa* and *Kippistia* appear to fit within an expanded definition of *Minuria.* Indel data suggest this broader concept may be monophyletic, but an analysis with broader taxon sampling is required to demonstrate this unequivocally. In both the molecular and combined analyses, there is strong support for a close association between all taxa belonging to the V clade, i.e., *Vittadinia,* all *Peripleura* except *P. diffusa,* and all *Tetramolopium* except *T. vagans.* The combined analysis places *T.* ''mitiaroense'' as sister to the Hawaiian species of *Tetramolopium.* Although this is congruent with the RFLP data of Okada, Whitkus, and Lowrey (1997), there is not strong character support for this pattern of relationship. Clearly, further data are required to provide a definitive answer to this question. Both the indel data and the combined analysis support the hypothesis of Smith (1977) that the Pacific Island species of *Tetramolopium* are descended from a New Guinea ancestor. *Olearia arguta* is closely related to *Camptacra* and shows no affinity with the arborescent species of *Olearia* included in this analysis. This taxon, which has been collected from both eastern and western parts of northern Australia and reputedly is a complex including several species (N. Lander, personal communication), requires further investigation before any firm conclusions can be drawn.

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APPENDIX. Definition of morphological characters and states.

1. Habit: 0, perennial, shrub or with at least a woody rootstock with herbaceous stems resprouting each year; 1, annual herb.
2. Leaf indumentum: 0, leaves glabrous; 1, leaves with hairs.
3. Glandular hairs on the leaves: 0, absent; 1, present.
4. Arrangement of capitula: 0, capitula borne singly on peduncles; 1, capitula several, grouped on a common peduncle.
5. Mean length of peduncles: 0, <30 mm; 1, >30 mm.
6. Involucral indumentum: 0, bracts glabrous; 1, bracts hairy.
7. Glandular hairs on involucre: 0, absent; 1, present.
8. Maximum length of the ray pappus: 0, <4 mm; 1, >4 mm.
9. Ratio of number of ray/disc florets: 0, <1; 1, $1.1 < x < 3$; 2, >4.
10. Mean length of ligule on ray florets: 0, <6 mm; 1, >6 mm.
11. Female sterile disc florets: 0, absent, disc and ray florets setting seed; 1, present, ray florets only setting seed.
12. Biseriate glandular hairs on the ray corolla: 0, absent; 1, present.
13. Septate hairs on ray corolla: 0, absent; 1, present.
14. Biseriate glandular hairs on disc corolla: 0, absent; 1, present.
15. Septate nonglandular hairs on the disc corolla: 0, absent; 1, present.
16. Color of pappus: 0, white; 1, off-white to brown.
17. Maximum length of ray pappus: 0, <4 mm; 1, >4 mm.
18. Maximum length of disc pappus: 0, <4 mm; 1, 4–6 mm; 2, >6 mm.
19. Dimorphic pappus: 0, absent; 1, present.
20. Number of ray pappus series: 0, 1–2; 1, >2.
21. Mean number of pappus bristles on disc florets: 0, <10; 1, 10–50; 2, 50–90; 3, >90.
22. Glandular hairs on the cypsela: 0, absent; 1, present.
23. Biseriate two-celled hairs with bifurcate tips on the cypsela: 0, absent; 1, present.
24. Shape of the disc floret tube: 0, tubular, diameter not increasing toward top; 1, funnel-shaped, with diameter increasing gradually toward top.
25. Filament attachment in tube: 0, at or below the middle; 1, above the middle.
26. Shape of the anther appendage (not including actual apex): 0, oblong, with more or less parallel sides; 1, triangular, width tapering from a broad base; 2, lanceolate, expanding and then contracting in width.
27. Shape of tip of anther appendage: 0, rounded; 1, acute; 2, acuminate. This character is scored inapplicable [—] in taxa where the appendage is minute.
28. Shape of mature cypsela in median transverse section: 0, not compressed, though sometimes variously angled; 1, weakly or irregularly compressed; 2, markedly compressed.
29. Mean length of the mature cypselae from ray florets: 0, <2.5 mm; 1, 2.5–4.5 mm; 2, >4.5 mm.
30. Marginal ribs on the mature cypsela: 0, absent; 1, present but obscure; 2, present and conspicuous.
31. Number of facial ribs on ray cypselae: 0, absent; 1, one or two only present; 2, 3–7.
32. Shape of mature cypsela in LS: 0, lanceolate; 1, oblanceolate to cuneate; 2, oblong or terete; 3, obcuneate.
33. Bundle sheath extension in the leaf transverse section (Lowrey, 1986): 0, absent; 1, present.
34. Storage parenchyma in the center of the leaf between the mesophyll layers (Lowrey, 1986): 0, absent; 1, present as a single row; 2, present as two or more rows.
35. Shape of the surface of the receptacle: 0, flat or slightly convex; 1, markedly conical.
36. Ornamentation of the receptacle surface: 0, smooth as seen under dissecting microscope; 1, scales with prominent pointed tips borne on single raised ridge separating insertion scars of adjacent; 2, pattern of single raised ridges without tips; 3, pattern of expanded scale bases forming a double ridge between floret insertions.
37. Anther bases with distinct tails: 0, absent; 1, present.
38. Relative length of outermost involucral bracts: 0, not distinctly shorter; 1, distinctly shorter.
39. Position of embryo in mature cypsela: 0, filling the cypsela; 1, apical, not filling cypsela (Burbidge, 1982).