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Phylogenetic analysis of ITS sequences suggests a Pliocene origin for the bipolar distribution of *Scleranthus* (Caryophyllaceae)

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Abstract. *Scleranthus* is a genus of about 12 species of herbaceous plants or subshrubs native to Eurasia and Australasia. Here *Scleranthus* is shown to consist of European and Australasian clades, which diverged within the last 10 million years. Biogeographic implications of this dating and alternative hypotheses explaining the disjunct north–south distribution of the genus, are discussed. The trans-Tasman distributions of *S. biflorus* and *S. brockiei* are of recent origin and therefore consistent with long-distance dispersal rather than vicariance explanations. Morphological and ITS sequence data sets are significantly incongruent and trees derived from them differ over relationships among Australasian species. Hybridisation and introgression or lineage sorting are invoked to explain this discordance. Within the family Caryophyllaceae, *Scleranthus* ITS2 sequences have greater similarity to sequences from representatives of the subfamilies Alsinoideae and Caryophylloideae than to sequences from representatives of the subfamily Paronychioideae.

Introduction

The genus *Scleranthus* (Caryophyllaceae) is comprised of 11 named species (Sell 1964; West and Garnock-Jones 1986) of semi-herbaceous annuals or perennials and dwarf woody perennial shrubs. *Scleranthus* plants bear small, mostly inconspicuous flowers without petals. The sepals are green, sometimes with white margins. Perigyny is well-developed in all species and the sepals and floral cup are persistent, forming part of the dispersal unit along with the indehiscent single-seeded fruit.

The genus has been divided into sect. *Scleranthus* and sect. *Mniarum* (Pax and Hoffmann 1934; West and Garnock-Jones 1986). Flowers of sect. *Scleranthus* plants are comparatively large, multi-staminate and borne in clusters, whereas flowers of sect. *Mniarum* plants are smaller, usually single-stamened and borne either singly or in pairs on peduncles that elongate at fruiting. Ants have been implicated in pollination of *S. perennis* (Svensson 1985), which is a comparatively large-flowered species of *Scleranthus* with high pollen production. Ants or other pollinators may also be important in the breeding systems of other multi-staminate *Scleranthus* species. Species with reduced stamen number and flower size, especially members of sect. *Mniarum*, are likely to be highly selfing.

Sect. *Scleranthus* contains three species endemic to Europe, North Africa and western Asia (*S. perennis*, *S. uncinatus* and *S. annuus*: Sell 1964) and three species endemic to Australia (*S. diander*, *S. pungens* and *S. minusculus*: West and Garnock-Jones 1986). Eurasian native *S. annuus* is naturalised in many parts of the world including New Zealand (Garnock-Jones 1981). Sect. *Mniarum* is restricted to Australasia (including New Guinea), with *S. biflorus* and *S. brockiei* occurring in both New Zealand and Australia, *S. singuliflorus* found in Australia and New Guinea, *S. fasciculatus* native to Australia (and naturalised in New Zealand: Garnock-Jones 1981; Webb *et al.* 1988) and *S. uniflorus* endemic to New Zealand. An unnamed endemic Australian species similar in morphology to *S. fasciculatus* (called *S.* '*slender*' in West and Garnock-Jones 1986) is referred to here as *S.* aff. *fasciculatus*.

Recent consideration of morphological character distribution among *Scleranthus* species suggested that sect. *Mniarum* is monophyletic with a number of synapomorphies, but suggested that sect. *Scleranthus* is probably paraphyletic or even polyphyletic as currently circumscribed to include both European and Australasian species (West and Garnock Jones 1986). Pax and Hoffmann

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Fig. 1. World native distribution of *Scleranthus* (redrawn from West and Garnock-Jones 1986).

(1934) had previously included all of the Australasian species of *Scleranthus* in sect. *Mniarum*.

Chloroplast *ndh*F sequence analysis places *Scleranthus* in a clade comprising subfamilies Alsinoideae and Caryophylloideae (Smissen 1999). While *Scleranthus* has sometimes been placed in subfamily Paronychioideae (=family Illecebraceae: e.g. Hutchinson 1974), most authors include the genus in the subfamily Alsinoideae, although it should be noted that this subfamily is possibly paraphyletic with respect to Caryophylloideae (Bittrich 1994). Bittrich (1994) includes *Pentastemonodiscus monochlamydeus* along with *Scleranthus* in tribe Sclerantheae of the subfamily Alsinoideae.

West and Garnock-Jones (1986) formulated four possible historical hypotheses for the Eurasian/Mediterranean–Australasian disjunction (Fig. 1) in *Scleranthus*.

(i) *Scleranthus* is monophyletic and had widespread progenitors throughout Europe–Asia–Australia, which have subsequently disappeared from most of Asia, resulting in the present-day disjunct distribution.

(ii) *Scleranthus* originated from progenitors in Europe and later spread through or across Asia into Australasia.

(iii) *Scleranthus* originated in Australasia and then spread through or across Asia into Europe.

(iv) The Northern and Southern Hemisphere species of *Scleranthus* had different progenitors whose descendants evolved along parallel or convergent lines.

Hypotheses 1, 2 and 3 imply monophyly of *Scleranthus* and require the disappearance of progenitors from Asia or extreme long-distance dispersal, while Hypothesis 4 implies polyphyly of the genus *Scleranthus*. Of critical importance in assessing Hypotheses 1, 2 and 3 is determining whether the Australian species of sect. *Scleranthus* are more closely related to the European species, or to the species of sect. *Mniarum* (*sensu* West and Garnock-Jones 1986) in which case sect. *Scleranthus* as currently circumscribed would be paraphyletic. The possibility of multiple progenitors of the genus *Scleranthus* (Hypothesis 4) can be assessed by investigating the relationships between the genus *Scleranthus* and possible sister taxa within the Caryophyllaceae. The research project reported here aimed to test these four hypotheses and investigate the wider relationships of the genus by answering the following questions:

(i) is the genus *Scleranthus* monophyletic;

(ii) are sections *Mniarum* and *Scleranthus*, as defined in West and Garnock-Jones (1986), monophyletic; and

(iii) what are the closest relatives of *Scleranthus*?

Because evaluation of alternative phylogenetic hypotheses in the group is hampered by the small number of macromorphological characters available as a result of the highly reduced habit and flowers of the plants, we sought molecular characters to provide additional evidence. The internal transcribed spacer regions (ITS1 and ITS2) of the 18S–26S nuclear ribosomal DNA (nrDNA) repeat were sequenced from a number of *Scleranthus* species and potential sister groups. Sequences from this region of DNA have previously been used successfully in phylogenetic studies in a number of angiosperm families, including the Caryophyllaceae (Baldwin *et al.* 1995). The nrDNA repeat unit is present in many thousand copies in the nuclear genomes of plants (Hamby and Zimmer 1992), thereby facilitating its amplification by polymerase chain reaction (PCR) from preserved or ancient material. The DNA-sequence uniformity of the many thousands of copies is maintained by a concerted evolutionary process in which unequal crossing over and gene conversion have been implicated (Baldwin *et al.* 1995). In at least some cases, concerted evolution is incomplete and multiple nrDNA sequence types co-exist in the genome (Zhang and Sang 1999)

Nuclear ITS sequences can also be used at higher taxonomic levels, although alignment becomes difficult due to the accumulation of insertion/deletion events (indels) in which different short strings of nucleotides are lost or gained in a lineage (Baldwin *et al.* 1995). However, phylogenetic information can still be obtained from such highly divergent sequences because conserved sequence regions exist within ITS2 and are shared across taxonomic boundaries (Hershkovitz and Zimmer 1996). Relationships among genera of Caryophyllaceae are poorly understood (Bittrich 1994), with subfamilial placement of several genera disputable. We have compared ITS2 sequences from a wide sample of Caryophyllaceae in order to assess the phylogenetic relationships of *Scleranthus* and assess possible sister-group relationships. Sequences from several other problematic genera (*Spergularia*, *Drymaria*, *Pycnophyllum* and a part of ITS2 from *Pentastemonodiscus*) were also included.

We have also surveyed morphological characters in *Scleranthus*, with the aim of addressing questions about their evolution. Cladistic analysis of these characters is reported elsewhere (Smissen 1999; Smissen and Garnock-Jones 2002) and they are now analysed in conjunction with ITS characters here.

Materials and methods

Species from which ITS sequences were gathered for this study are shown in Table 1. Total cellular DNA was extracted from up to 200 µg fresh or 20 µg dried plant tissue by the CTAB method (Doyle and Doyle 1987) and purified by phenol–chloroform extraction according to Sambrook *et al.* (1989).

Table 1. Samples from which sequence was gathered in this study

Species	Origin	Voucher
Scleranthus annuus L.	Sweden	WELTU 19658
Scleranthus perennis L.	Sweden	WELTU 19659
Scleranthus biflorus J.R.Forst. et G.Forst (NZ)	New Zealand	WELTU 19660
Scleranthus biflorus (AUST)	Australia	AML 2033, CANB
Scleranthus brockiei P.A.Will. (NZ)	New Zealand	WELTU 19661
Scleranthus brockiei	Australia	AML 1974, CANB
Scleranthus uniflorus P.A.Will.	New Zealand	WELTU 19662
Scleranthus fasciculatus R.Br.	Australia	AML 2032, CANB
S. aff. *fasciculatus*	Australia	WELTU 19663
Scleranthus singuliflorus F.Muell. (PNG)	Papua New Guinea	CHR 341457
Scleranthus singuliflorus (AUST)	Australia	CHR 344858
Scleranthus diander R.Br.	Australia	CHR 344856
Scleranthus pungens R.Br.	Australia	CHR 302074
Scleranthus minusculus F.Muell.	Australia	West 5009, CANB
Arenaria benthamii Fenzl. ex Torr. et Gray	Texas	Clement 0021, TEX
Habrosia spinuliflora (Ser.) Fenzl.	Kurdistan	Bornmuller 11506, B
Pycnophyllum bryoides Rohrb.	Chile	Werdmann 1026, B
Pentastemonodiscus monochlamydeus Rech.f.	Afghanistan	Rechinger 117834, B
Cerastium glomeratum Thuill.	New Zealand	WELTU 19664
Sagina procumbens L.	New Zealand	WELTU 19665
Drymaria laxiflora Benth.	Texas	Clement 0223, TEX
Polycarpon tetraphyllum L.	New Zealand	WELTU 19666
Colobanthus brevisepalus Kirk	New Zealand	WELTU 19547
Spergularia marina Griseb.	Texas	Clement 0202, TEX

Table 2. Sequences of primers used in this study

Primer	Sequence (5′ to 3′)
ITS2[A]	gCTgCgTTCTTCATgCATgC
ITS3[A]	gCATCgATgAAgAACgCAgC
ITS4[A]	TCCTCCgCTTATTgATATgC
ITS5[A]	ggAAgTAAAAgTCgTAACAAgg
ITS28cc[B]	CgCCgTTACTAggggAATCCTTgTAAg

[A]Primers based on White *et al.* (1990). [B]Primer from S. Wagstaff (pers. comm.).

Part of the nrDNA repeat unit containing ITS1, ITS2 and the intervening 5.8 s RNA gene was amplified by PCR, using the primers detailed in Table 2 and Fig. 2. For DNA extracted from fresh material the primers ITS4 or ITS28cc and ITS5 were used. The combination of ITS4 and ITS5 produced amplified DNA products that gave unreadable sequence for some specimens but the use of ITS28cc and ITS5 rectified this problem in all cases. Degraded DNA from herbarium specimens was amplified by using primers ITS3 and ITS28cc or ITS2 and ITS5. Single-stranded DNA for dideoxy sequencing was obtained by one of the following two methods:

(i) Asymmetric PCR reactions were carried out directly on genomic DNA samples, with pairs of primers (each supporting amplification from different directions as in Fig. 2) in 1 : 10 molar ratio. PCR reactions contained 1 unit of *Taq*-DNA polymerase (Pharmacia Biotech), 2.5 μL 10 × reaction buffer (500 mM KCl, 15 mM $MgCl_2$, 100 μM Tris–HCl pH 9.0), 10 nM each primer, 42.5 μM $MgCl_2$, 25 ng BSA and H_2O to make up a total volume of 25 μL. Cycling conditions were 30 s at 97°C for denaturation, 45 s at 48°C primer annealing, 45 s increasing by 4 s each subsequent cycle at 72°C for extension, for 30–40 cycles. The mixed double- and single-stranded PCR products were run on 1% agarose gels using TBE buffer (Sambrook *et al.* 1989) to check size, quality and quantity of amplification products.

(ii) Double-stranded PCR reactions were conducted with one 5′ biotinylated primer and a non-biotinylated primer (Hultman *et al.* 1989). Cycling conditions for PCR were as detailed above and 30 cycles were carried out. Double-stranded PCR products were analysed on 1% agarose gels as above. Single-stranded DNA was then recovered by the use of Dynal m-280 Streptavidin Dynabeads, according to the manufacturer's instructions.

Sequencing of amplified single-stranded DNA was conducted using ^{35}S-labelled dATP in conjunction with Sequenase Version 2.0 DNA sequencing kit (P/N 70770, US Biochemicals, Cleveland, Ohio) according to the manufacturer's instructions. Reaction products were separated by electrophoresis on denaturing polyacrylamide gels (Sambrook *et al.* 1989). Following this, gels were fixed and dried then exposed to Kodak Biomax film for up to 3 days. Auto-radiograms were developed in Kodak Xray Developer Number 2.

Sequences were read with a sonic digitiser and checked manually by using the DNAStar Lasergene computer package (DNAStar Inc.). Alignment of sequences was accomplished manually in the case of *Scleranthus*. The ITS2 sequences for Caryophyllaceae species were compared by using the guide tree function of Clustal W 1.7 (Thompson *et al.* 1994). This program constructs a dendrogram (neighbour joining or UPGMA) based on a distance matrix calculated from pairwise sequence comparisons, thus circumventing the need to construct a single alignment for relatively divergent sequences containing indels. Pairs of sequences are assessed for phenetic similarity only. Thus, similarities so revealed are not assessed for homology and are not necessarily synapomorphic. However, where reliable alignment of sequences is not possible the guide-tree provides a method for assessing and communicating sequence similarity (Hershkovitz and Zimmer 1996). The Clustal W parameters for the guide tree shown in Fig. 3 were gap opening penalty 15.00 and gap extension penalty 6.66.

Phylogenetic trees were generated from *Scleranthus* along with outgroup ITS sequences (ITS1 + ITS2) alone and from ITS sequences combined with morphological characters by using PAUP* 4.0b2 (Swofford 1999). Alignment of *Scleranthus* sequences with the outgroup *Colobanthus brevisepalus* and *Stellaria media* (obtained from GenBank) sequences required the introduction of several gaps and in places equally costly alternative alignments were possible. Some sections of ITS 1 were not alignable across all ingroup and outgroup taxa. Where this was the case, part of one or other or both of the outgroup sequences were omitted. All sites at which *Scleranthus* sequences varied were aligned with at least one of the outgroup sequences, except for one site in which *S. minusculus* differed from the remaining *Scleranthus* sequences. Morphological characters are from Smissen and Garnock-Jones (2002). Most-parsimonious trees were found through branch and bound searches with equal character weighting. Bootstrap values were determined from 1000 replicate heuristic searches. Neighbour-joining trees were constructed from *Scleranthus* and outgroup ITS data by a variety of distance correction methods (uncorrected distances, JC69, or Kimura two-parameter). Character evolution was traced by using the MacClade (Maddison and Maddison 1992) software. Uncorrected and JC69 corrected distances used to estimate divergence times for taxa were generated from a Clustal W alignment of ITS2 sequences of *Scleranthus*, *Drymaria* and *Polycarpon* (parameters as above) by using PAUP* 4.0b2 and excluding sites with gaps or missing data in pairwise calculations. The JC-69 model (Jukes and Cantor 1969) was used because it makes few explicit assumptions about the pattern of nucleotide substitution.

Results

High-quality sequences were readily obtained from most samples by using both DNA strands. Ambiguous sites were rare in most sequences and when encountered were coded as unknown. However, only a small amount of readable sequence could be generated from the herbarium material of *Pentastemonodiscus monochlamydeus*. This is a contiguous ITS2 region of 82 nucleotides long, which includes eight

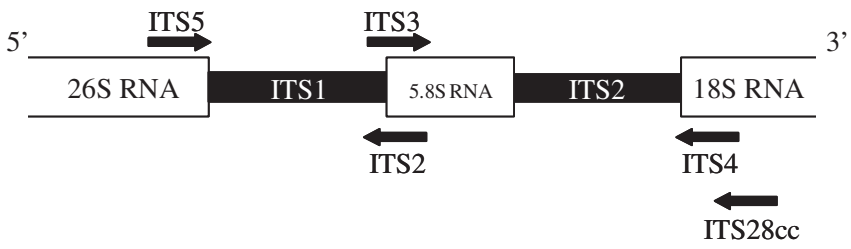

Fig. 2. Position of primers used for PCR and sequencing.

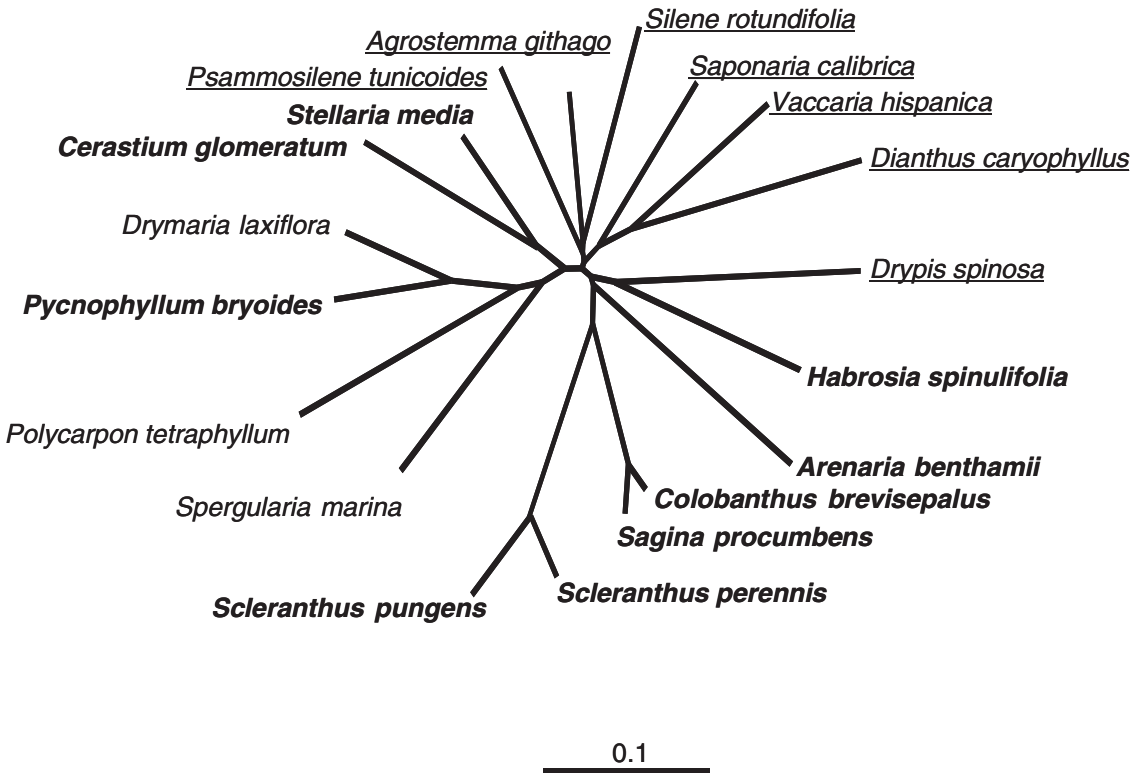

Fig. 3. Clustal W guide tree for Caryophyllaceae ITS2 sequences. Scale is proportion of nucleotides different in pairwise alignment. Alsinoideae are shown in bold, Caryophylloideae are underlined and remaining taxa are Paronychiodeae (following Bittrich 1994).

ambiguous sites and was aligned with other Caryophyllaceae sequences with up to 75% nucleotide similarity. The presence of multiple sequence signals prevented reading any more of the ITS sequence from auto-radiograms and insufficient sample of *P. monochlamydeus* was available to attempt further sequencing experiments.

Complete, or near complete, ITS2 sequences were obtained for representative species of seven genera of Alsinoideae and three genera of Paronychioideae and these were analysed along with published sequence data from other Caryophyllaceae as shown in Table 3.

Evidence relating to intergeneric relationships within Caryophyllaceae from ITS sequences

It was not possible to confidently align sequences for ITS2 across most generic boundaries, even with the aid of Clustal W. Variation in length of ITS2 is the result of indels. Other types of structural rearrangement within the nrDNA repeat and the high proportion of substituted nucleotide sites in variable regions, have also contributed to difficulty experienced in aligning highly divergent sequences.

The dendrogram produced by the guide tree function of Clustal W, with the alignment parameters described in the methods section, is shown in Fig. 3. Varying either the values of alignment parameters, or the taxa included in the analysis, was found to affect the tree produced, especially the ordering of the short internal branches. However, the process does provide an objective method for comparing and communicating sequence similarities.

Table 3. Sequences included in guide tree

Taxon	GenBank accession no.
Arenaria benthamii[A]	AY286524
Cerastium glomeratum[A]	AY286525
Colobanthus brevisepalus[A]	AY286531
Habrosia spinulifolia[A]	AY286523
Pycnophyllum bryoides[A]	AY286527
Sagina procumbens[A]	AY286526
Scleranthus pungens[A]	AY286537
Scleranthus perennis[A]	AY286532
Drymaria laxiflora[A]	AY286528
Polycarpon tetraphyllum[A]	AY286530
Spergularia marina[A]	AY286529
Stellaria media[B]	X86899
Drypis spinosa	X86900
Agrostemma githago[B]	X86895
Dianthus seguieri	U30973
Psammosilene tunicoides[B]	X86897
Silene rotundifolia[B]	X86887
Saponaria calibrica[B]	X86898
Vaccaria hispanica[B]	X86896

[A]Sequences generated in this study. [B]Sequences from Oxelman and Liden (1995).

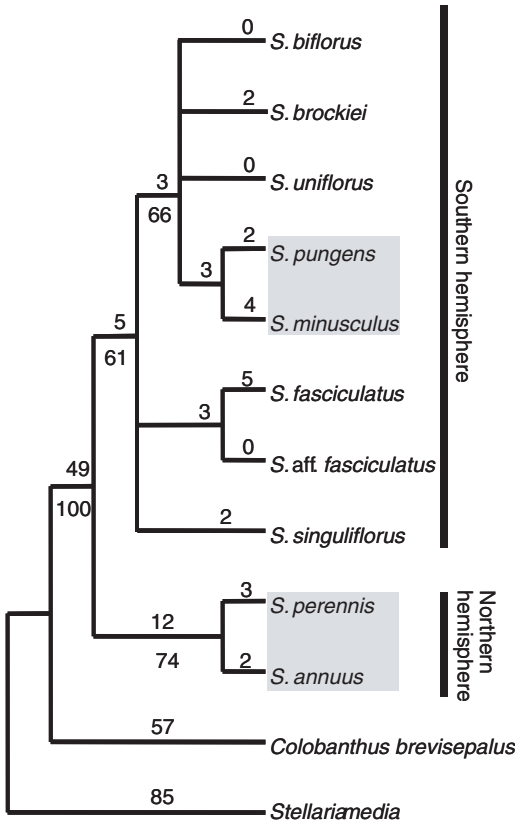

Fig. 4. One of nine shortest parsimony trees for *Scleranthus* and outgroup ITS sequences. Numbers above branches are inferred number of character-state changes, numbers below branches are bootstrap values. Internal branches of length zero have been collapsed; all remaining clades are also present in the consensus of all nine shortest trees. Northern and Southern Hemisphere clades are indicated by vertical lines and sect. *Scleranthus* (*sensu* West and Garnock-Jones 1986) by shading.

The star-like pattern of Fig. 3 shows that little hierarchy exists in ITS2 sequence variation among genera belonging to the subfamilies Alsinoideae and Caryophylloideae. Most groupings became unstable if alignment parameters were altered. However, several close similarities are evident in the dendrogram and these were resistant to changes in alignment parameters. The two *Scleranthus* sequences included (one European, one Australian) are very similar to each other, but dissimilar from all the other sequences. The *Colobanthus brevisepalus* and *Sagina procumbens* sequences are also very similar to each other. The ITS2 sequence of *Pycnophyllum bryoides* has higher sequence identity in pairwise alignments with Polycarpeae sequences, especially *Drymaria laxiflora* (with which it groups in Fig. 3), than with Alsinoideae sequences. The *Spergularia marina* sequence has similarities to sequences of both the Alsinoideae and Caryophylloideae on one hand and with the sequences of the Polycarpeae and *Pycnophyllum* on the other.

Analysis of Scleranthus *ITS sequences*

Complete ITS1 and ITS2 sequences were obtained for 10 of the 11 named *Scleranthus* species recognised by West and Garnock-Jones (1986), an unnamed Australian *Scleranthus* species referred to as *S.* aff. *fasciculatus* and the outgroup *Colobanthus brevisepalus*. This latter species belongs to the group that had ITS2 sequences most similar to *Scleranthus* sequences in the guide tree analysis above. The ITS1 and ITS2 sequences gathered from *Scleranthus* species were readily aligned requiring no gaps.

Sequence from *S. diander* had five sites at which double signal was present indicating that at least two different DNA products had been amplified from the sample. All of these sites correspond with sites where nucleotides vary between *S. minusculus* and *S. biflorus/uniflorus*. At three of these five sites where double signal occurred, the signal was notably stronger for one base than for the other. Cross-contamination is an unlikely explanation for the double signal because no samples of *S. minusculus* were in our possession at the time the *S. diander* sequence was gathered. The *S. diander* sequence has been excluded from the tree-building analyses outlined below.

Scleranthus singuliflorus samples from Papua New Guinea and from Australia differed by one (uninformative) substitution, as did *S. biflorus* and *S. brockiei* specimens from New Zealand and Australia. In each case, only one sequence was included in the final analysis.

A branch-and-bound search revealed nine shortest trees. One of these, with zero-length internal branches collapsed, is shown in Fig. 4. Note the large number of substitutions inferred along the branch separating the outgroup *Colobanthus* and *Stellaria* sequences from those of the *Scleranthus* samples. All shortest ITS trees nest the Australian species that have been previously included in sect. *Scleranthus* within the species of sect. *Mniarum*. Neighbour-joining trees for the same data using different distance corrections were consistent with the shortest parsimony tree.

Combined analysis of ITS and morphological characters

The morphological data set of Smissen and Garnock-Jones (2002) was reanalysed with *S. diander* excluded. Four shortest parsimony trees were revealed by a branch-and-bound search, one of which is shown in Fig. 5. The consensus of these shortest trees is entirely congruent with the consensus of shortest morphology trees reported in Smissen and Garnock-Jones (2002).

The degree of congruence between morphological and ITS character sets was assessed by a partition homogeneity test as implemented in PAUP* 4.0b2. The two data sets (partitions) were found to be significantly ($P = 0.0095$ from 10000 bootstrap replicates) incongruent. If ITS1 and ITS2 are treated as separate partitions, then ITS1 is not

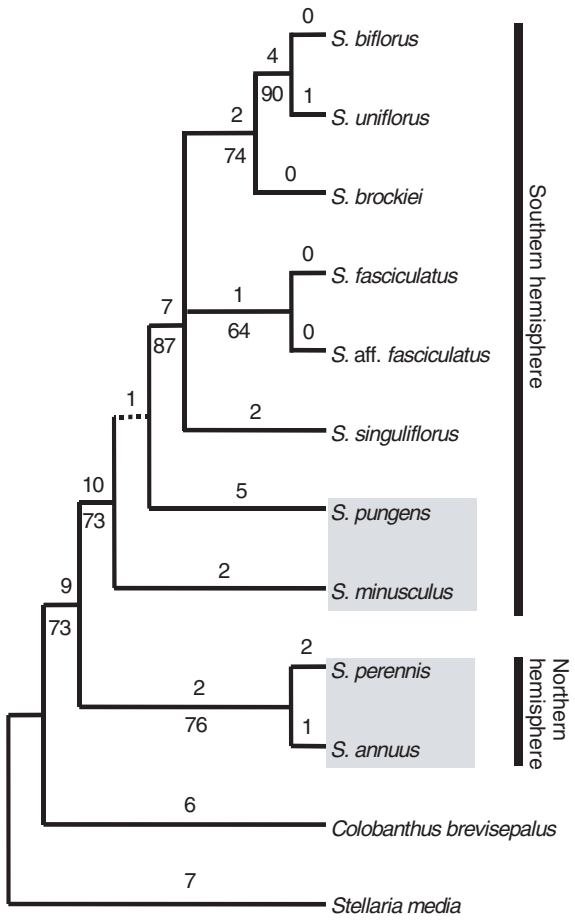

Fig. 5. One of two shortest trees for *Scleranthus* morphology data with *S. diander* excluded. Numbers above branches are inferred number of character-state changes, numbers below branches are bootstrap values. Branches collapsing in the consensus of shortest trees are shown as dotted lines. Northern and Southern Hemisphere clades are indicated by vertical lines and sect. *Scleranthus* (*sensu* West and Garnock-Jones 1986) is indicated by shading.

significantly (P = 0.2000 when compared with ITS2, P = 0.2300 when compared with the morphological data) incongruent with either of the other data sets. In contrast, the ITS2 and morphological data are significantly (P = 0.0100) incongruent. This apparent discrepancy arises because ITS1 does not vary sufficiently among Australian species of *Scleranthus* to provide a statistically significant phylogenetic signal when treated alone. Consequently, the two ITS regions are considered together in all further analyses.

The incongruence between the morphological and molecular data sets may arise because they do not share a common evolutionary history or because one or both are positively misleading. As shown by Bull *et al.* (1993), combining data from incongruent partitions can reduce the probability of returning the correct tree. However, under certain conditions combined analysis can perform better than separate analysis, even when data partitions reflect different histories, particularly when data sets are small and incongruence is localised to part of the phylogeny (Wiens 1998). In this case, neither data set alone fully resolves relationships within the Australasian species of *Scleranthus*, essentially because there are too few characters. Also the data sets (ITS1 + ITS2 and morphology) are both small and include little homoplasy, so their relative support for different clades in the combined tree can readily be assessed through character mapping. Further, incongruence in the partitions reflects their support of alternative positions for the Australian species sometimes included in sect. *Scleranthus* (as sister group to sect. *Mniarum* or nested within sect. *Mniarum*). If these species are excluded, the morphological and molecular data are congruent (partition homogeneity test score of P = 1.0000 from 10000 replicates with *S. pungens* and *S. minusculus* excluded), indicating that the data sets are congruent at least over the basal dichotomy in the genus. In fact, incongruence can be attributed to three transition substitutions in ITS2, which support a split among Southern Hemisphere taxa not suggested by any morphological characters.

Combined morphological and ITS sequence data for *Scleranthus* were analysed by a branch-and-bound search with equal character weighting. The single shortest tree found (shown in Fig. 6) is similar to the four shortest trees found for the morphological data alone. It differs in that *S. singuliflorus* is sister to *S. fasciculatus* and *S.* aff. *fasciculatus*, rather than sister to a clade comprised of all the other single-stamened species, or as part of an unresolved polytomy. Like the morphology and ITS trees, the combined tree divides *Scleranthus* into Northern and Southern Hemisphere clades. Not surprisingly, the combined data provide greater confidence in these clades than either data set alone, with 100% bootstrap support for the Northern Hemisphere clade and 98% for the Southern Hemisphere clade. More surprisingly, bootstrap support for the monophyly of the single-stamened species group (sect. *Mniarum*, *sensu* West and Garnock-Jones 1986) is also significantly better in the combined data set (96%) than in the morphology data set (87%). In the ITS trees, an incongruent clade of *S. pungens*, *S. diander*, *S. biflorus*, *S. brockiei* and *S. uniflorus* appears in all the shortest trees, with 66% bootstrap support. This increased confidence can be attributed to the additional support for the two basal clades in *Scleranthus* obtained by the combined data set. A bootstrap analysis of the morphological character set where monophyly of the Northern and Southern Hemisphere groups was constrained reported 100% bootstrap support for a clade including all the single-stamened species.

A consensus of shortest trees produced from separate analysis of ITS and morphology data sets is shown in Fig. 7. In this conservative summary of evidence, no relationships are resolved within the Southern Hemisphere species of

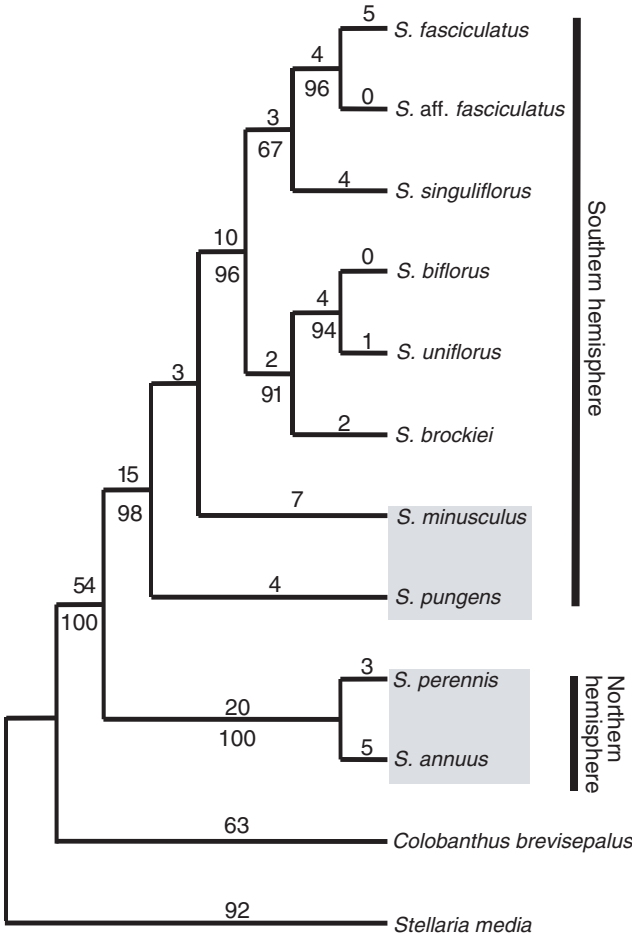

Fig. 6. Shortest parsimony tree for *Scleranthus* combined ITS sequence and morphological characters. Numbers above branches are inferred number of character-state changes, numbers below branches are bootstrap values. Northern and Southern Hemisphere clades are indicated by vertical lines and sect. *Scleranthus* (*sensu* West and Garnock-Jones 1986) by shading.

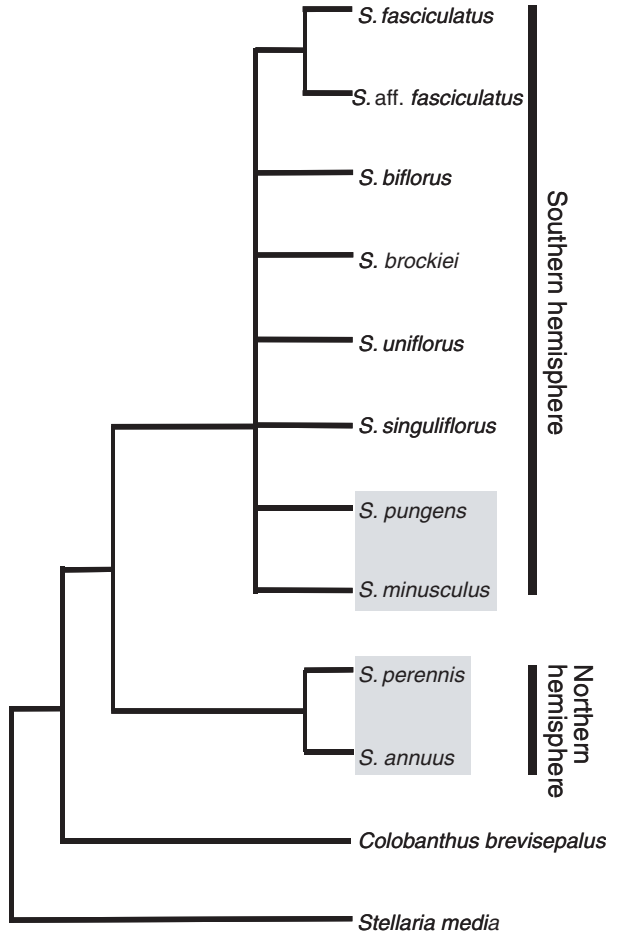

Fig. 7. Strict consensus of trees from separate analysis of ITS and morphology data sets. Northern and Southern Hemisphere clades are indicated by vertical lines and sect. *Scleranthus* (*sensu* West and Garnock-Jones 1986) by shading.

Scleranthus, except for the sister relationship of *S. fasciculatus* and *S.* aff. *fasciculatus*.

Date estimates

Estimates for the divergence date of European and Australasian *Scleranthus* clades and for the earliest divergence among extant Australasian species were made by comparing ITS sequence divergence within *Scleranthus* with the divergences between *Scleranthus* sequences and those of *Polycarpon tetraphyllum* and *Drymaria laxiflora* (Polycarpeae). According to *ndh*F sequence analysis, *Drymaria* and *Polycarpon* are part of a clade that is the sister group to a clade comprised of the genera that make up subfamilies Alsinoideae and Caryophylloideae, including *Scleranthus* (Smissen *et al.* 2002). Although a number of lineages belonging to subfamilies Alsinoideae and Caryophylloideae are closer to *Scleranthus* than *Drymaria* or *Polycarpon*, they are not useful for dating purposes because no independent information is available as to when these lineages may have diverged from the lineage leading to *Scleranthus*. Figure 8 shows the phylogenetic relationships used to estimate dates for divergence events between *Scleranthus* lineages.

In the shortest (i.e. maximum parsimony) tree for *Scleranthus* ITS data (Fig. 4), the earliest dichotomy within the Australasian group is between the *S. fasciculatus*–*S.* aff. *fasciculatus* clade and the remaining Australasian species. In Fig. 8, distances between ITS2 sequences of plants belonging to these Australasian clades are defined as d_1, the distances between sequences of European and Australasian species are d_2 and the distances between sequences of *Scleranthus* species and the two Polycarpeae species are d_3. Because of the stochastic nature of molecular evolution and possibly because of rate variation between lineages, observed nucleotide substitution rates for ITS2 differ between different pairs of species across each node. For example, d_1 calculated as the distance between

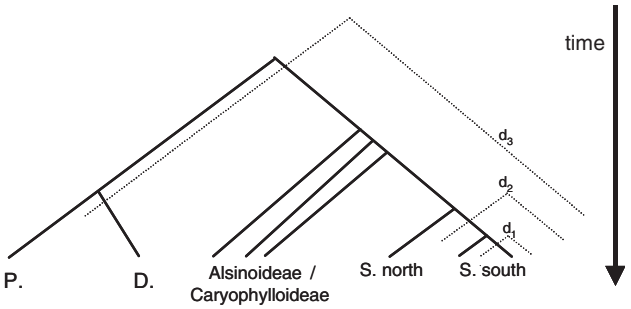

Fig. 8. Relationship of taxa used to estimate times since divergence of *Scleranthus* lineages. P. = *Polycarpon*, D. = *Drymaria*, S. north. = Northern Hemisphere *Scleranthus* species, S. south = Southern Hemisphere *Scleranthus* species (eight sequences included). Distances used in calculations of divergence event date estimates, as discussed in text, are shown as d_1, d_2 and d_3.

S. singuliflorus (PNG) and *S. fasciculatus* is different from d_1 calculated as the distance between *S.* aff. *fasciculatus* and *S. biflorus*, despite of these lineages diverging from each other at the same node in the ITS tree. For this reason, a range of values has been given here for each set of comparisons (Table 4). Averaging these values is inappropriate because some terminal taxa will have shared histories for some of the time since their divergence from the node in question. Consequently, different calculations of d_1, d_2 and d_3 cannot provide independent estimates of the rate of nucleotide substitution or the time elapsed since a divergence event. As a result, mean values of d_1, d_2, or d_3 would differ widely depending on which terminal taxa were included in calculations.

It is known that ITS2 is subject to considerable among-sites rate variation (Hershkovitz and Zimmer 1996). Distance estimates of highly divergent ITS2 sequences, calculated as number of sites varying (n) divided by the total number of sites in the alignment (n_t), may be underestimates because of the presence of several short regions of relatively conserved sequence. The number of sites free to vary in a sequence (n_v) can be estimated by simply excluding all sites invariant over all taxa in the alignment. Distances can then be calculated as the number of sites varying, divided by the number of sites free to vary (n/n_v). However, Jukes–Cantor corrected distances cannot be calculated from uncorrected distances greater than 0.75. For this reason PAUP* 4.0b2 returned 'undefined' distance estimates for two of the pairwise comparisons between Polycarpeae and *Scleranthus* species. The range of distance estimates of d_3 shown for 'variable sites only' in Table 4 includes only those for which PAUP calculated a result.

The rate at which substitutions accumulate in a DNA molecule may be estimated as half of the distance between sequences from two lineages divided by the time elapsed since the lineages diverged (where this time is known and where rates of substitution are assumed to be the same in each lineage). Fossil pollen attributable to the Caryophyllaceae appears in the middle Oligocene (Muller 1981). Pollen described as similar to modern Alsinoideae–Caryophylloideae and pollen similar to modern Paronychioideae are also found in deposits of similar age (Bittrich 1994). This palaeontological evidence provides the basis for assuming that Paronychioid and Alsinoid–Caryophylloid lineages diverged about 35 million years ago, just prior to the appearance of these pollen types in the fossil record. If this is taken as the true date for divergence of Alsinoideae and Paronychioideae, then nucleotide substitution rates derived from corrected pairwise distances between *Scleranthus* and the Polycarpeae species vary from 5.4 to 5.6×10^{-9} substitutions per site per year for all sites (n/n_t) and from 27 to 52×10^{-9} substitutions per site per year for variable sites only (n/n_v: 19–22 $\times 10^{-9}$ for uncorrected distances).

If substitutions have accumulated at a more or less constant rate along all Caryophyllaceae lineages, then the length of time elapsed since any two lineages within *Scleranthus* diverged can be estimated by multiplying this substitution rate by half the distance between species representing each lineage. Table 4 summarises the range of estimated dates derived from the substitution rates above, with all possible pairs of species separated by the divergence events considered (with the exclusions mentioned above).

Discussion

Relationships among subfamilies of Caryophyllaceae

ITS sequences could not be unambiguously aligned along their entire length across most genera of Caryophyllaceae

Table 4. Evolutionary distance and divergence date estimates from ITS sequences for taxa as shown in Fig. 8
Values in parentheses for 'variable sites only' are uncorrected distances

	All sites in alignment (n/n_t)		Variable sites only (n/n_v)	
	Distance estimate	Date estimate (million years ago)	Distance estimate	Date estimate (million years ago)
d_1[A]	0.028–0.052	2.1–4.9	0.065–0.125 (0.031–0.115)	0.6–2.3 (1.4–5.9)
d_2[B]	0.052–0.082	4.0–7.7	0.124–0.205 (0.104–0.178)	1.2–3.8 (4.5–9.1)
d_3[C]	0.375–0.451	35[D]	1.890–3.64 (0.688–0.788)	35[D]

[A]Divergence of European and Australasian *Scleranthus* lineages. [B]First divergence within extant Australasian *Scleranthus* species. [C]Divergence of Polycarpeae and Caryophylloideae–Alsinoideae. [D]Assumption based on fossil record.

because of the accumulation of indels and the high divergence of variable regions. However, similarity information can be recovered through pairwise comparisons. This allows sequences to be grouped according to possession of shared strings of nucleotides. Within the Caryophyllaceae, this approach has allowed us to identify closely similar sequences and to attribute taxa of uncertain position to major groupings previously identified. Thus, Fig. 3 should not be interpreted as a phylogenetic tree, but as a network of sequence similarities, from which relationships can be reasonably inferred for taxa whose sequences are closely similar. The ITS2 sequences of Alsinoideae (excluding *Pycnophyllum*) and Caryophylloideae species are more similar to each other than to *Polycarpon* or *Drymaria* of the Paronychioideae. Within the Alsinoideae–Caryophylloideae group, little hierarchical structure in ITS2 similarity is evident. This may be an artifact in part due to 'saturation' of variable nucleotide sites, but may also reflect a rapid radiation of these lineages from a common ancestor some time in the relatively distant past. This latter suggestion is in keeping with the results of an analysis of Caryophyllaceae *ndh*F sequence variation (Smissen 1999; Smissen *et al.* 2002). The sequences obtained from Caryophyllaceae for this gene are much more conserved than their ITS2 sequences, but are nevertheless unable to resolve relationships fully between most genera of Caryophylloideae and Alsinoideae.

The short length of ITS2 sequence that was obtained for *Pentastemonodiscus* was not enough to place this genus firmly in any subfamily. We suggest that *Pentastemonodiscus* is probably not closely related to *Scleranthus* as its ITS2 sequence was not found to be recognisably more similar to *Scleranthus* ITS2 sequence than to those of other Caryophyllaceae by inspection or pairwise comparison. However, the sequence is broadly similar to those of the other Caryophyllaceae sequences, suggesting it does properly belong to the family. Plants of *Pentastemonodiscus* and *Scleranthus* do not share any notable morphological apomorphies and their suggested grouping in the tribe *Scleranthea*e (Bittrich 1994) appears to be based on their sharing a reduced vegetative habit and small, apetelous, uniovulate flowers with two-parted gynoecia. Unlike *Scleranthus*, *Pentastemonodiscus* is described as having anatropous ovules and is not perigynous. Further, *Pentastemonodiscus* has connate styles, which amongst Alsinoideae are otherwise found only in *Pycnophyllum* (Bittrich 1994) whose placement in this subfamily is also called into question by its ITS2 sequence (Fig. 3).

Relationships of Scleranthus *species*

ITS sequences are consistent with the monophyly of *Scleranthus*. The ITS2 sequences of *Scleranthus* show much greater similarity to each other than to sequences from species belonging to other genera in the guide tree network (Fig. 3). There is a very large number of character-state changes inferred along the branch, separating the outgroup species from the *Scleranthus* species in the parsimony analysis of ITS sequences (Fig. 4). This close relationship of all the *Scleranthus* species is further supported by the fact that no gaps or other rearrangements were required to align their sequences. Aspects of floral anatomy, especially gynoecial morphology and ontogeny, provide additional support for the monophyly of *Scleranthus* (Smissen 1999; Smissen and Garnock-Jones 2002).

Parsimony analysis of ITS and morphological characters divides Scleranthus *into European and Australasian clades*

Within *Scleranthus*, both ITS sequence and morphology data sets suggest that there is a basal dichotomy between European and Australasian clades (Figs 4 and 6). Sect. *Scleranthus* (*sensu* West and Garnock-Jones 1986) is shown by these results to be at least paraphyletic (as in the morphology-only and combined analyses) or polyphyletic (ITS alone). The Australasian members of sect. *Scleranthus* should therefore be included in sect. *Mniarum* (Table 5). Morphological support for the grouping of *Scleranthus* into European and Australasian clades is based on characters of floral anatomy, pollen and inflorescence.

Data-combining and incongruence

The combined morphology and ITS analysis conducted in this study must be interpreted with caution, as the two data partitions were significantly incongruent. The consensus of ITS and morphology trees (Fig. 7) provides a conservative summary of agreement between the two data sets. Although we are conscious of the strong arguments against combining incongruent data, as Messenger and McGuire (1998) we feel that full exploration of the data is facilitated by a combined analysis, even in the presence of partition incongruence. Further, we note that the otherwise compelling argument of Bull *et al.* (1993) against combining incongruent data sets begins with the remark:

'*For any modest set of well-known taxa, the systematist may have access to nucleic acid sequence from many different genes plus a variety of morphological, biochemical and physiological characters*.'

The present study is of a poorly known group where only a limited set of morphological characters and a single set of

Table 5. Revised classification of *Scleranthus*

Scleranthus L.
- subgenus *Scleranthus*
  - *S. annuus*; *S. perennis*; *S. uncinatus*
- subgenus *Mniarum* (J.R.Forst. et G.Forst.) Pax
  - *S.* aff. *fasciculatus*; *S. biflorus*; *S. brockiei*; *S. diander*; *S. fasciculatus*;
  - *S. minusculus*; *S. pungens*; *S. singuliflorus*; *S. uniflorus*

nucleotide sequence characters are available. The choice here is not which data to combine in order to give the best chance of obtaining a totally correct species tree, but rather how best to combine two partially resolved and partly complementary phylogenetic hypotheses. The data partitions (ITS and morphological data sets) are only incongruent in supporting alternative relationships among Australian species (a matter only summarily examined in previous publications: West and Garnock-Jones 1986). They are congruent with regard to the most significant questions posed at the outset of the study (i.e. regarding monophyly or otherwise of Australasian species and possible non-monophyly of sect. *Scleranthus*) and confidence in the basal clades is greatly increased when data are analysed together. Justification for the approach adopted here of separate analysis followed by combined analysis is provided by Wiens (1998) with the proviso that contested parts of the combined tree be viewed as questionable. In presenting a combined analysis, we do not suggest that it is inherently more likely to have produced a more correct tree than either separate analysis. Therefore, we view the discordance between ITS- and morphology-based trees as competing hypotheses, which require further testing with independent data.

However, we note that the inclusion of ITS characters has increased bootstrap support at all well-supported branches when the combined tree is compared with the consensus of shortest morphology trees. The main effect of combining data has been to greatly increase confidence in the basal clades of the tree, which in turn has led to greater confidence in more terminal clades outweighing the contradictory signal introduced with the ITS data. Bootstrap support for alternative clades in the ITS analysis is low (i.e. <70%).

The large increase in support for monophyly of the Southern Hemisphere species of *Scleranthus* in the combined data analysis over either separate analysis appears to be due to more than a simple addition of signal from the two data partitions. Suboptimal trees generated from the morphology data set show a tendency to group the large-flowered Australasian species with the European species of *Scleranthus*. As longer and longer trees are examined, all the single-stamened species (i.e. sect. *Mniarum*, *sensu* West and Garnock-Jones 1986) begin to group with *Colobanthus brevisepalus*, in some trees. However, in suboptimal trees generated from the ITS data, single-stamened *S. fasciculatus* and *S.* aff. *fasciculatus* (and in slightly longer trees *S. singuliflorus*) show a tendency to group with the European species of *Scleranthus*, while the remaining Southern Hemisphere species (both large- and small-flowered) continue to appear as a clade. When the two data sets are combined, they act synergistically to provide strong support for a clade comprised of all the Southern Hemisphere species.

Relationships among Australasian species of Scleranthus

In any case, several hypotheses of relationship within the Southern Hemisphere species of *Scleranthus* are supported by, or at least consistent with, both data sets. The sister group relationship of *S. fasciculatus* and *S.* aff. *fasciculatus* appears in the consensus of trees produced from separate analysis of each data set. This reflects the very similar morphologies of these species and their two nucleotide substitution synapomorphies. The three *Scleranthus* species with compact cushion habit (*S. brockiei*, *S. biflorus* and *S. uniflorus*) appear as a monophyletic group in the shortest trees derived from the morphology and the combined data sets. However, their relationships are unresolved in the ITS trees and therefore also unresolved in the consensus of trees from each separate analysis. The ITS sequences of these cushion species are very similar and their lack of ITS synapomorphies is not evidence against their monophyly. In this case, the taxonomic congruence approach appears unnecessarily conservative (at least if strict consensus is used), as it obscures relationships that are well-supported by one data set and consistent with the other. Within this group, *S. biflorus* and *S. uniflorus* share several morphological synapomorphies, implying the derivation of *S. uniflorus* from the more widespread *S. biflorus* (West and Garnock-Jones 1986; Smissen 1999; Smissen and Garnock-Jones 2002).

In contrast to morphological characters, the ITS data suggest that large-flowered *S. pungens* and *S. minusculus* are nested among the species with single-stamened flowers. Paraphyly of the single-stamened species (sect. *Mniarum*, *sensu* West and Garnock-Jones) is unlikely in the light of the considerable morphological similarity among these species that share a number of derived characters of flowers and fruiting structures not shared by *S. pungens* and *S. minusculus*. Paraphyly of sect. *Mniarum* with respect to *S. minusculus* and *S. pungens*, if accepted, would imply either the convergent evolution of several single-stamened lineages or, more parsimoniously, a reversion to larger infloresences, larger flower size and increased stamen number in the *S. minusculus–S. pungens* clade. Such a series of reversals is difficult to exclude because many of the characters involved (stamen number, calyx size, sepal margins and inflorescence flower number) are probably functionally related to pollination and are unlikely to be independent. However, not all the characters linking the single-stamened species have obvious functional connection. For example, the rough texture of cells at the top of ovaries in most species of this group and the elongation of the peduncles in the fruit are unlikely to be developmentally or selectively linked and probably constitute independently evolving characters. No synapomorphies were found linking *S. pungens* or *S. minusculus* with any subset of the single-stamened species in a morphological study (Smissen

1999; Smissen and Garnock-Jones 2002). Likewise, convergence of the single-stamened species as a result of adaptation to autogamy would explain some, but not all, of the morphological characters supporting their monophyly.

Hybridisation and introgression

The *S. diander* ITS sequence obtained in this study included five sites (four in ITS2 and one in ITS1) where double-banding indicated the presence of multiple sequence types. The pattern can be interpreted as an additive combination between those sequences found in *S. minusculus* and *S. biflorus/uniflorus*, perhaps suggesting a hybrid origin for *S. diander* with members of these species or their progenitors as parents. West and Garnock-Jones (1986) described *S. diander* as providing '*morphological... and ecological links*' between Australasian species they included in sect. *Scleranthus* and sect. *Mniarum*. Such links might be explained by hybridisation. Hybridisation can also explain the observed discordance between morphological and ITS-based phylogenies. In addition to the evidence discussed above relating to a possible hybrid origin of *S. diander*, hybrids between other species occur naturally in Europe (Sell 1964) and perhaps New Zealand (Williamson 1956). Plant species of recent hybrid origin are known to display additive patterns of parental ITS characters (Rieseberg 1991). Over time, processes of concerted evolution can result in homogenisation of ITS sequence types in stabilised hybrids (Wolfe and Elisens 1994). The variation in signal strength of the additive pattern of ITS sequence in *S. diander* observed in this study suggests that this process has already begun acting on independent nucleotide sites. The final outcome would most likely be a single sequence displaying a non-additive combination of parental characters. This same process could account for the distribution of ITS characters in Australasian *Scleranthus*, particularly the three nucleotide apomorphies shared between the *S. minusculus–S. pungens* clade and the *S. biflorus/brockiei/uniflorus* group. Specifically the sequences observed in *S. brockiei*, *S. biflorus* and *S. uniflorus* could have arisen through homogenisation of sequence types similar to those observed in *S. gracilis* and *S. minusculus* or *S. pungens*. This could have occurred either in an ancestral population containing polymorphic ITS sequence types, or after divergence of lineages. If so, then a chloroplast DNA phylogeny should resemble more closely the morphology-derived trees, especially as the single-stamened species are much more likely to have provided the female parent than the male parent of any hybrids (see Smissen 1999 and Smissen and Garnock-Jones 2002, for a discussion of pollen ovule ratios). If ITS nucleotide sequence data reflect the true pattern of relationships among *Scleranthus* species and reticulate evolution or lineage sorting have not been major factors, then a chloroplast DNA nucleotide sequence phylogeny should resemble the ITS phylogeny. Full elucidation of the evolutionary history of the Australasian species of *Scleranthus* will likely require population-level sampling of highly variable nuclear and chloroplast molecular markers.

Lineage sorting

An alternative explanation of the ITS nucleotide sequence tree topology, that also rejects paraphyly of the group of single-stamened species, is that different members of a suite of ancestral ITS polymorphisms have become variously fixed in different Australasian *Scleranthus* lineages, with the result that the gene tree and species tree are discordant (lineage sorting). Because the ITS regions for relatively few individuals of each species (in most cases only one) have been sequenced, the level of variation within each species remains unknown. However, the ITS sequences determined for two *S. biflorus* specimens, one from New Zealand the other from Australia, differed by only one substitution. Likewise, *S. brockiei* specimens from Australia and New Zealand differed by only one substitution.

Chromosome counts

Chromosome counts for the Australian endemic species remain an important gap in knowledge of the genus *Scleranthus*. European species include diploid and tetraploid examples on a base of $x = 22$ (Sell 1964), while the three New Zealand species of sect. *Mniarum* and Australian *S. fasciculatus* all have $2n = 48$ (Beuzenberg and Hair 1983). If all the Australasian species share $2n = 48$, then the additional counts would be of limited phylogenetic value (especially as these cannot be polarised unambiguously by available outgroups, Smissen and Garnock-Jones 2002). Other numbers, if present, could provide evidence of relationships among the Australasian *Scleranthus* species. It would certainly be worth examining *S. diander* in this regard, in order to test the possibility that it is an allopolyploid.

Divergence times

We have estimated the time of the divergence of European and Australasian *Scleranthus* clades and of the radiation of Australasian *Scleranthus* species on the basis of ITS nucleotide substitution rates. The use of ITS2 nucleotide substitution rates calculated from other angiosperm groups was rejected because evolutionary rates vary by an order of magnitude or more among groups of plants with differing biology (Suh *et al.* 1993). The estimates given here are entirely reliant on assumptions about the timing of the divergence of the lineage ancestral to *Drymaria* and *Polycarpon* from the lineage ancestral to subfamilies Alsinoideae and Caryophylloideae and must, therefore, be regarded as provisional. The figure of 35 million years ago for this event is subject to considerable error, including ambiguity of the relationships of fossil taxa to extant lineages. However, the fossil record of the Caryophyllaceae simply does not allow more reliable dating.

Further, the alignment of ITS sequences that was used to estimate distances is arbitrary in places. Thus, some nucleotide sites compared in distance calculation may not have been genuinely homologous. Substitution-rate variation between lineages can also introduce inaccuracy to estimates of divergence times, as a strict molecular clock is not evident for the Caryophyllaceae ITS2 data. For this reason, ranges of values based on the minimum and maximum observed distances for different pairs of species separated at a node have been given as a precautionary measure.

Simple calculation of genetic distances as the number of sites varying between two sequences divided by the total number of sites is unrealistic because the substitution rate is known to vary markedly among different nucleotide positions in ITS2. Therefore, distances based on all ITS2 sites rather than just variable sites alone will certainly underestimate the true distances between the Polycarpeae species and *Scleranthus*, causing our estimates of the times since divergence events within *Scleranthus* to be too great. For this reason, distances calculated from variable sites only probably give more realistic estimates of the time elapsed since divergence events. However, an additional problem arises because correction of distances for multiple substitutions (by JC69 or similar method) may be unreliable when uncorrected distances are large (Kumar *et al.* 1993). This is evident in part from the greater range of date estimates derived from corrected distances versus uncorrected distances, when invariant sites are excluded. However, in the absence of more realistic models of ITS sequence evolution, more-accurate distance corrections are not possible. In any case, the date estimates presented here do allow sufficient confidence to exclude hypotheses invoking relatively ancient events for the explanation of *Scleranthus* distribution.

Biogeographical history

The extant distribution of the genus must be explained as either an extreme case of long-distance dispersal, or through extinction of progenitor species from Asia. Extinction of *Scleranthus* from Asia seems unlikely because habitats exist throughout this region that are similar to those inhabited by the genus today and it is likely that this has been the case for millions of years. Clearly, *Scleranthus* is capable of long-distance dispersal despite lacking any obvious adaptations to facilitate it. Two well-distinguished species, *S. biflorus* and *S. brockiei*, occur in both Australia and New Zealand, indicating that *Scleranthus* propagules have crossed the Tasman Sea at least twice. Vicariance explanations for the shared species would be inconsistent with the lack of ITS divergence between samples of *S. biflorus* specimens from Australia and New Zealand (these lands having been separated by around 2000 km for 60 million years: Pole 1994). Sequences gathered for all three species native to New Zealand differed by no more than five sites for ITS1 and ITS2 combined. However, it is much harder to imagine direct dispersal occurring between Europe and Australasia. Further, even if such cross-global dispersal should be possible, it remains hard to explain the absence of *Scleranthus* from so much of the rest of the world.

Scleranthus is not unique in having a bipolar distribution with disjunt Australasian and European ranges. The predominantly Northern Hemisphere genus *Gypsophila* (Caryophyllaceae) has a single Australian species, *G. australis*, that is also introduced to New Zealand (Webb *et al.* 1988). Also, *Ceratocephala* (Ranunculaceae) has two species native to Eurasia and a single species endemic in New Zealand (Garnock-Jones 1984, as *Ceratocephalus*). Like that of *Scleranthus*, these distributions may be the result of relatively recent long-distance dispersal events.

The species *S. uniflorus* and *S. biflorus* share several morphological apomorphies (nectary shape, gynoecium without overgrowth of styles by ovary cells and four- rather than five-parted calyx). The more restricted *S. uniflorus* is endemic to New Zealand, whereas *S. biflorus* is found in New Zealand and Australia. It is parsimonious to conclude that *S. uniflorus* has evolved from *S. biflorus* ancestors since the dispersal of the latter to New Zealand from Australia. This leaves *S. biflorus* as a paraphyletic species. Several justifications can be advanced for recognising paraphyletic groups at species level within a cladistic framework (Donoghue and Cantino 1988). Perhaps the most powerful of these is when the organisms concerned form an interbreeding population. Although gene flow through continual seed dispersal of *S. biflorus* from Australia to New Zealand cannot be excluded, there is no current evidence to support it is happening either. However, *S. biflorus* is morphologically indistinguishable in Australia and New Zealand and probably constitutes a biological species (*sensu* Mayr 1963) in that the plants are potentially interbreeding. We will, therefore, not attempt to recognise strictly monophyletic species groups in place of a wider definition of *S. biflorus*, that includes allopatric populations. ITS and morphology provide no evidence that refutes a hypothesis of conspecifity of Australian and New Zealand populations of *S. biflorus*.

West and Garnock-Jones (1986) suggested a Miocene radiation of Australasian sect. *Scleranthus* and a Pleistocene origin of sect. *Mniarum*. While a Quaternary origin of sect. *Mniarum* is consistent with the ITS data, a significantly older radiation of sect. *Scleranthus* in Australasia is not. The small divergence among ITS sequences for Australasian *Scleranthus* species in general indicates that all the extant species probably had a common ancestor more recently than the Miocene, which ended about 5 million years ago.

In summary, the divergence of extant European and Australasian clades within the genus probably occurred no more than 10 million years ago and may have been much more recent. In the time between the divergence of the progenitors of *Scleranthus* from other Alsinoideae and the

divergence of the extant European and Australasian clades, *Scleranthus* may either have had a much greater range than at present, or may have been restricted to part of its present range, or may even have occurred only in an area where it is no longer found. This period in *Scleranthus* history is not reflected in the phylogeny of the extant species because they are apparently the result of more recent radiations in Europe and Australasia. Fossil evidence is unlikely to be forthcoming because the output of pollen from *Scleranthus* is low. However, even if *Scleranthus* had contributed significantly to the pollen record, then its similar appearance to pollen of many other species of Caryophyllaceae would preclude its recognition. The extant Australasian species of *Scleranthus* probably all evolved in response to climate change in the Quaternary and may not have a long history in the area, as was suggested by West and Garnock-Jones (1986). Hybridisation and introgression seem to have played a role in the distribution of morphological and molecular characters among extant Australasian species and have been invoked here to explain the discordance of their phylogenies.

Classification

Our results suggest that two monophyletic groups within *Scleranthus* can be recognised with confidence, a European-centred group and an Australasian group. These groups correspond with sect. *Euscleranthus* and sect. *Mniarum* of Pax and Hoffman (1934) and the earlier subgenera of the same name recognised by Pax (1889). Here we follow Pax (1889) in recognising two subgenera in *Scleranthus*, as summarised in Table 5.

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