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Phylogeny and Generic Interrelationships of the Stylidiaceae (Asterales), with a Possible Extreme Case of Floral Paedomorphosis

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ABSTRACT. Cladistic analyses of Stylidiaceae (Asterales), using Donatiaceae as outgroup and with both morphological and molecular characters, produced two equally parsimonious cladograms. The analyses used morphological characters for 26 species and molecular characters from the chloroplast DNA genes *rbcl* and *ndhF* for 12 species. The cladograms indicate that *Levenhookia* and *Stylidium* are sister groups and that *Oreostylidium* is nested within *Stylidium*. The latter result is remarkable because *Stylidium* has several significant flower specialisations that *Oreostylidium* lacks and because *Oreostylidium* is endemic to New Zealand, where *Stylidium* is missing. The simple flowers of *Oreostylidium* may have evolved by reduction and paedomorphosis of the zygomorphic and sensitive flowers of a *Stylidium*-like ancestor, a change caused by adaptation to a new environment lacking a suitable pollinator. In connection with a switch to unspecialised pollinators or self-fertilisation, the flowers of *Oreostylidium* apparently became fertile at a morphologically immature or reduced stage.

The trigger plant family Stylidiaceae is well-known for its peculiar floral column (Fig. 1a-c), which in the main genus *Stylidium* is triggered in a bent-back position, suddenly springing forward releasing a cloud of pollen on visiting pollinators. The Stylidiaceae are primarily Australian, except for a few species in New Zealand, tropical Asia, and South America (Good 1925). The family includes five genera and ca. 150 species (Cronquist 1981). The largest genus by far is *Stylidium*. The four remaining genera *Forstera*, *Phyllachne*, *Levenhookia*, and *Oreostylidium* have eight or fewer species each. *Oreostylidium* is monotypic. The closest relative is *Donatia*, a genus of one South American and one New Zealand-Tasmanian species usually classified in a separate family, Donatiaceae (Gustafsson and Bremer 1995). *Donatia* and *Phyllachne* are small, woody, cushion-plants and *Levenhookia* consists exclusively of ephemeral annuals. In *Stylidium* there is a wide range of habit; the majority are perennial herbs of which a few are geophytes with subterranean bulbs, and a small group consists of ephemeral annuals. There are several diagnostic family-features of which the floral column consisting of the fused stamens and style is the most prominent. *Stylidium* and *Levenhookia* have a zygomorphic flower (Fig. 1a-c) with one minute petal called a labellum. In *Stylidium* the column is irritable, while *Levenhookia* has an irritable labellum. The remaining three genera do not possess the sensitive pollination mechanism and the flowers are actinomorphic.

The present family classification was provided by Mildbraed (1908). Mildbraed distinguished 16

sections of *Stylidium* assigned to six subgenera. Most subgenera are circumscribed according to the shape of the calyx or the capsule and the existence and appearance of an ovary septum.

The disjunct distributions along with floral specialisations provide many interesting implications at the generic level. The purpose of this study is to determine the generic status and interrelationships within the Stylidiaceae by means of cladistic analyses and to explore the evolution of the flower. We do not attempt to solve relationships at the species level.

MATERIALS AND METHODS

The cladistic analyses are based both on morphological characters and on sequence data derived from the chloroplast DNA genes *rbcl* and *ndhF*. The morphological characters have to a large extent been extracted from literature, particularly from Mildbraed (1908) and Erickson (1958), but almost all characters have been checked in studies of herbarium material and specimens preserved in 70% ethanol. Twenty-four species of Stylidiaceae and two outgroup species of Donatiaceae are included in the morphological part; of these, twelve have been sequenced.

Outgroup Choice. Among taxonomists the opinions have been divided about the position of the Stylidiaceae. The family has been placed in such widely different positions as near Saxifragaceae (Thorne 1976), in the Cornales (R. Dahlgren 1980, 1983) and in the Ericales (G. Dahlgren 1989; Lambers 1992). More common, though, has been to fol-

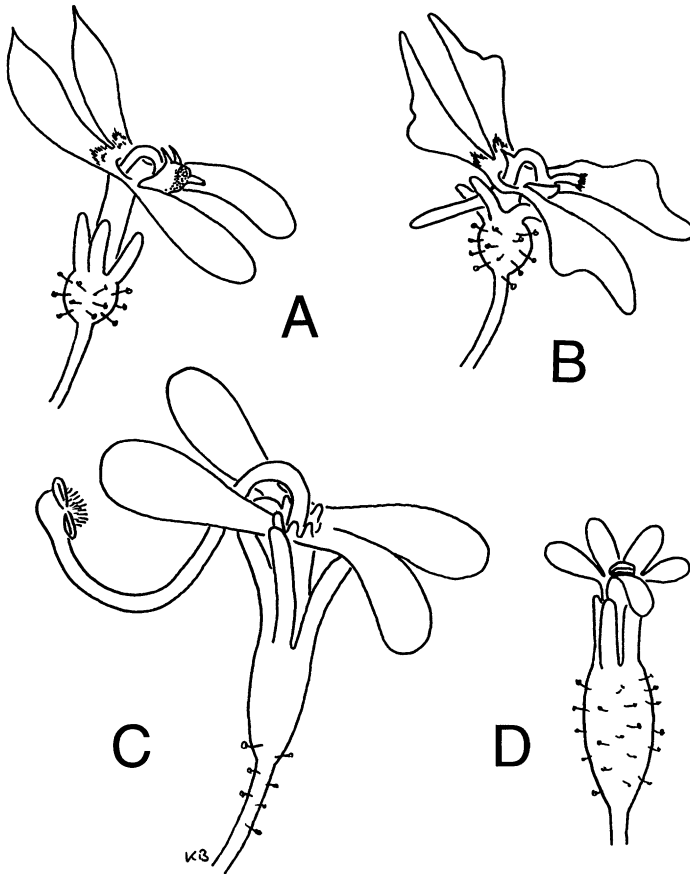


FIG. 1. Flowers of Stylidiaceae, ca. 4x. A: *Levenhookia pauciflora*, B: *Stylidium calcaratum*, C: *Stylidium brunonianum*, and D: *Oreostylidium subulatum*. The presence of a spur is autapomorphic for *S. calcaratum* (B) and not included among the characters in Table 2. Redrawn after Erickson (1958; A-C) and Berggren (1878; D).

low de Candolle (1839) and place Stylidiaceae within or close to Asterales or Campanulales. Recently, some high-level taxonomic studies have treated the problem (Cosner et al. 1994; Gustafsson and Bremer 1995; Gustafsson et al. 1996). All found Stylidiaceae to belong within Asterales s.l.

The monotypic family Donatiaceae has sometimes been considered close to Stylidiaceae (Cronquist 1981; Takhtajan 1987). Gustafsson and Bremer (1995) pointed out several characters supporting this relationship: reduced number of stamens, extrorse anther dehiscence opening by longitudinal slits, and extrastaminal nectaries. Additional similarities are found in ovule development and configuration of megagametophyte as well as in endosperm haustoria (Philipson and Philipson 1973). Because of the shared characters and the ecological, habitual, and geographic similarities, *Donatia* has sometimes been included within Stylidi-

aceae (Von Mueller 1873; Mildbraed 1908; R. Dahlgren 1983). Mildbraed (1908) divided the family into Stylidioideae and Donatioideae, the latter including *Donatia* alone. Nevertheless, *Donatia* is distinct from Stylidiaceae in having free stamens and petals, paracytic stomata, and smooth, tricolporate pollen (Erdtman 1952; Lammers 1992). There are also differences in a number of anatomical characters, such as vessel and endodermis type, sclerenchyma, and the general vascular plan (Rapsion 1953). Some authors have distinguished *Donatia* as sufficiently distinct to merit recognition as its own family (Chandler 1911; Skottsberg 1915). Hutchinson (1969, 1973) even had the two families widely separated in his system.

In a broad *rbcL* analysis of Campanulales by Cosner et al. (1994), Stylidiaceae turned out close to Campanulaceae. A new *rbcL* study of Asterales including Campanulales was performed by Gus-

Table 1. Voucher or reference and EMBL association numbers for all species of Stylidiaceae sequenced in the study.

Sequenced species	Voucher or reference	Herb.	EMBL-number	
			<i>rbcl</i>	<i>ndhF</i>
<i>Donatia fascicularis</i>	Swenson and Martinsson 301	UPS	X87385	AJ225074
<i>Donatia novae-zelandiae</i>	NG Walsh 3492	MEL	AJ225058	AJ225072
<i>Forstera bellidifolia</i>	NG Walsh 3491	MEL	AJ225056	AJ225082
<i>Forstera bidwillii</i>	EK Cameron 7263	AK, UPS	AJ225055	AJ225083
<i>Levenhookia leptantha</i>	Bremer and Gustafsson 38	UPS	AJ225051	AJ225081
<i>Levenhookia pauciflora</i>	Bremer and Gustafsson 122	UPS	AJ225050	AJ225080
<i>Oreostylidium subulatum</i>	EK Cameron 7273	AK, UPS	AJ225057	
<i>Oreostylidium subulatum</i>	Destroyed in customs			AJ225073
<i>Phyllachne uliginosa</i>	Swenson and Martinsson 305	UPS	X87393	AJ225075
<i>Stylidium bulbiferum</i>	Bremer and Gustafsson 44	UPS	AJ225054	AJ225077
<i>Stylidium calcaratum</i>	Bremer and Gustafsson 157	UPS	AJ225053	AJ225079
<i>Stylidium emarginatum</i>	Bremer and Gustafsson 14	UPS	AJ225052	AJ225078
<i>Stylidium graminifolium</i>	Cosner, M. E. et al., 1994.	OS	L18790	
<i>Stylidium graminifolium</i>	Mjd 368	UPS		AJ225076

tafsson et al. (1996). In some of the most parsimonious reconstructions, *Donatia* was the sister group of Stylidiaceae but in the consensus tree the clade collapsed. However, after adding the sequences from the present study to the matrix of Gustafsson et al. (1996), the position of *Donatia* as sister group of Stylidiaceae is corroborated. Considering both the morphological study by Gustafsson and Bremer (1995) and the latest molecular results, *Donatia* is the closest outgroup choice for Stylidiaceae.

Sampling. The morphological matrix consists of 26 taxa, two of which are outgroups. The species are chosen to reflect as much variation as possible, regarding characters and the geographic distribution. In *Stylidium* we have sampled 16 species with representatives from all the subgenera. Of these, 12 have also been used in the *rbcl* and *ndhF* analyses. Some interesting species are unfortunately very rare, not recently collected and not available for sequencing even on herbarium material. Nevertheless, we maintain that our sampling is adequate for the scope of this paper. The *rbcl* sequences of *Donatia fascicularis*, *Phyllachne uliginosa*, and *Stylidium graminifolium* were accessioned through European Molecular Biology Laboratory database (EMBL). The remaining species have been sequenced for this study. EMBL accession numbers and vouchers are in Table 1. For the molecular study, species were chosen with the goal to represent as far as possible variation within the whole family. *Levenhookia pauciflora* was selected because the morphology indicates a position between *Levenhookia* and *Stylidium* (Erickson 1958). Recently collected material was preferred when it was available.

Morphological Data. We used herbarium material from K, S, and UPS; a list of the specimens examined is provided in Table 2. Some specimens

were collected for this study by K. Bremer and M. H. G. Gustafsson in September 1993 in the southwestern part of Australia, and by U. Swenson and K. Martinsson in Tierra del Fuego, Chile, in December 1993. During these field trips, inflorescences were preserved in ethanol.

To be able to examine plant parts under light microscope, the material was placed in water with Aerosol added. The material was heated for softening and mounted on slides in Hoyer's solution (Anderson 1954). Before mounting, thick leaves were bleached in 5% sodium hydrate for 2–7 days and subsequently in 50% chloralhydrate for 1 day.

A total of 48 morphological characters is used, about half of which were floral characters. Of the morphological informative character states, only 3% are coded as either unknown or unapplicable. Two autapomorphies (characters 13 and 39) and two ingroup synapomorphies (characters 14 and 20) were included in the matrix. The autapomorphies were, however, excluded from all the analyses. The character states with comments are listed in Table 3. The data matrix with the characters scored against taxa is presented in Table 4. In most cases we also studied herbarium material to confirm character states recorded in literature. From such studies we also obtained some additional characters.

Seven of 16 multistate characters (Table 4) were treated as ordered, instead of unordered. The idea of Hennig's Auxiliary Principle (Hennig 1966) for homology is in our opinion also applicable to ordered characters because ordering of character states is a hypothesis of homology. The principle is to assume a character evolution in the absence of contrary evidence, that is to make a character ordered whenever possible. The assumption is tested, a posteriori, against the obtained cladogram.

Table 2. Specimens studied of Stylidiaceae.

- Donatia* J.R.Forst. & G.Forst.
D. fascicularis J.R.Forst. & G.Forst.: Dusén 603 (UPS); Dusén 1896–04 (UPS); Dusén 1897–04 (UPS); Skottsberg 201 (UPS); Smith 95 (K); Swenson and Martinsson 301 (UPS); Wirgarhi 533 (UPS).
D. novae-zelandiae: Van Balgooy 4408 (K); Berggren 1874–02 (UPS); Melville 5919 (K).
Forstera L.f.
F. bellidifolia Hook.f.: Comber 2239 (K); Curtis 1970–01–11 (K).
F. bidwillii Hook.f.: Haines 34 (K); Herb. Hookerianum 4139 (K).
Levenhookia R.Br.
L. leptantha Benth.: Bremer and Gustafsson 38 (UPS); Bremer and Gustafsson 75 (UPS); Morrison 1898–11–09 (S).
L. pauciflora Benth.: Bremer and Gustafsson 110 (UPS); Bremer and Gustafsson 122 (UPS).
L. stipitata (Benth.) F.Muell.: Herb. Morrison, Kelmscott 1898–11–09 (S); Pritzel 1900–11 (S); Strid 21518 (S); Wall 260 (S).
Oreostylidium Berggr.
O. subulatum (Hook.f.) Berggr.: Berggren 1874–06 (UPS); Berggren 1875–01 (S); Travers 1861, Herb. Hookerianum (K).
Phyllachne J.R.Forst. & G.Forst.
P. clavigera (Hook.f.) F.Muell.: Berggren 1874 (UPS); Given 9162 (K); Philipson 10165 (K); Vallin 1924–03–24 (UPS).
P. uliginosa J.R.Forst. & G.Forst.: Dusén 694 (UPS); Godley 619 (K); Godley 1031 (K); Moore 2727 (K, UPS); Skottsberg 1908–05–25 (UPS); Swenson and Martinsson 305 (UPS).
Stylidium Sw.
S. alsinoides R.Br.: Blake 21836 (K); Clarkson 2138 (K); Clarkson 2444 (K); Helms 1896 (K); Loher 3724 (K); Loher 5188 (K); Pullen 6631 (K).
S. brunonianum Benth.: Bremer and Gustafsson 49 (UPS); Nordenstam and Anderberg 42 (S); Strid 21110 (S).
S. bulbiferum Benth.: Bremer and Gustafsson 44 (UPS); Bremer and Gustafsson 65 (UPS); Bremer and Gustafsson 108 (UPS); Nordenstam and Anderberg 713 (S); Strid 21509 (S).
S. calcaratum R.Br.: Bremer and Gustafsson 45 (UPS); Bremer and Gustafsson 58 (UPS); Bremer and Gustafsson 157 (UPS).
S. elongatum Benth.: Bremer and Gustafsson 26 (UPS).
S. emarginatum Sond.: Bremer and Gustafsson 14 (UPS).
S. fasciculatum R.Br.: Nyman 1839 (S); Strid 21384 (S); Strid 21769 (S).
S. graminifolium Sw.: Boorman 1904–11–19 (UPS); Caley 1804 (UPS); Caley 1805 (UPS); Ladiges PYL1403 (UPS); Muir 270 (UPS); Muir 1071 (UPS); Muir 1806 (UPS); O. D. E 2431 (UPS); Skewes 52–01–19 (UPS); Swartz s.n. (UPS).
S. junceum R.Br.: Herb. Morrison 1900–10–03 (S).
S. kunthii Wall.: Beuskom, Geesing, Phengkhlay and Wongwan 4498 (K); Clarke 26567 (K); Gamble 7406 (K); Jackson, herb. Lamb (UPS); Nordenstam and Anderberg 656 (S); Nehu 5456 (K); Wall s.n. (S, UPS).
S. laricifolium Rich.: Caley s.n. (UPS); Willis and Wakefiel 51–12–30 (UPS).
S. petiolare Sond.: Bremer and Gustafsson 56 (UPS); Bremer and Gustafsson 64 (UPS); Pritzel 1901–10 (S).
S. preissii (Sond.) F.Muell.: Carlquist 4013 (K); Strid 21194 (S).
S. repens R.Br.: Morrison 1899–05–04 (S); Reehinger 60127 (S).
S. scandens R.Br.: Bremer and Gustafsson 169 (UPS); Reehinger 59912 (S).
S. schoenoides DC.: Nordenstam and Anderberg 10 (S).

Molecular Data. Total DNA was extracted in accordance with the methods by Saghai-Marouf et al. (1984) and Doyle and Doyle (1987). The DNA's were purified with Qiaquick PCR kit (Qiagen) according to the instructions from the manufacturer. Most PCR reactions were carried out using earlier described primers (*rbcl*—Olmstead et al. 1992 or G. Zurawski, DNAX Research Institute; *ndhF*—Olmstead and Sweere 1994 or Oxelman et al. unpubl. data) or for a few *rbcl* sequences a new primer corresponding to a region ca. 800 nucleotides downstream of the coding region in tobacco (BB47 5'GAGTTTTCTTGCCCCCTATTGC) and for a few of the *ndhF* amplifications two new primers (2188r 5'GACCAACACCATTCGTAATTC-CAT and 2230r 5'CTACTGATTTGATACCCTCTC-CTA) were used at the 3' end of the gene. For *rbcl*, the procedure was to first amplify double-stranded DNA and then single-stranded DNA (Kaltenboeck et al. 1992). Efforts to amplify the *rbcl* gene of *Sty-*

lidium petiolare failed for all attempted primer combinations. The amplified single-stranded DNA was purified with the Qiaquick PCR kit. The *rbcl* sequencing was done manually (Sanger et al. 1977) while *ndhF* was obtained with automated sequencing. Double-stranded DNA was cycle-sequenced using FS and GeneAmp PCR Systems 9600 kits (Perkin-Elmer) and analysed on an ABI 377 automated sequencer (Applied Biosystems). All obtained sequences were aligned manually to the reading frames of the corresponding proteins of *rbcl* and *ndhF* in *Nicotiana tabacum* L. (Shinozaki et al. 1986; GenBank accession number Z00044).

The *rbcl* gene was sequenced for nine species within the Stylidiaceae and *ndhF* for 12 species. The sequences have been submitted to the EMBL database (Table 1). Some of the taxa were not completely sequenced for the *rbcl* gene. *Forstera bellidifolia*, *Phyllachne uliginosa*, *Donatia novae-zelandiae*, and *Stylidium bulbiferum* have a large number of

Table 3. The 48 morphological characters and the character states used in the morphological and the combined analyses of the Stylidiaceae.

Vegetative structures

1. Plants perennial (0); plants annual (1). *Stylidium* is variable in this respect, consisting of both ephemeral annuals, perennial herbs, or, more rarely, shrublets. The other genera are perennials except for *Levenhookia*, which only consists of annuals.
2. Subterranean bulbs absent (0); subterranean bulbs present (1). A small number of the perennial species in *Stylidium* have subterranean bulbs instead of rhizomes. These species are habitually very similar to the ephemeral annual species but they have entirely different life histories.
3. Plants pulvinate (0); plants not pulvinate (1). *Donatia* and *Phyllachne* both have a very dense cushion-like (pulvinate) habit that could be an example of parallel adaption; both genera occur in the same cold environment. In the absence of any a priori evidence supporting such an interpretation, we regard the pulvinate condition as a potential homology.
4. Stems not stoloniferous (0); stems partly stoloniferous (1). Some *Stylidium* species have stolons ending in new leaf rosettes, the whole plant assuming a mat-like habit. In *Oreostylidium* the stolons are not as obvious but still recognizable.
5. Thickened nodes absent (0); thickened stem nodes present (1). Thickened nodes occur in some species of *Stylidium* and they are usually distinct and obvious.
6. Aerial roots present (0); aerial roots absent (1). In Stylidiaceae and Donatiaceae only the ephemeral species of *Stylidium* and *Levenhookia* lack adventitious aerial roots. In the cushion plants aerial roots grow downward from young stems through older parts of the cushion. In other perennial species the aerial roots are usually easily seen but we have not been able to confirm their presence or absence in *S. laricifolium*, which is coded as unknown for this character.

Leaves

7. Leaves densely set throughout the stem (0); leaves sparsely set (1); leaves basally rosetted (2); leaves in whorls (3); leaves in an apical whorl, often with alternate leaves beneath (4). The leaf arrangement is here described in terms of densely or sparsely set, alternate leaves, leaf rosettes, and leaf whorls. The tightly adpressed leaf arrangement in *Stylidium preissii* might be an autapomorphy, different from the other species coded for leaves densely set throughout the stem.
8. Leaves <2 cm long (0); leaves 2–5 cm long (1); leaves 5–40 cm long (2). This character is treated as ordered. *Oreostylidium* is variable in leaf length, with <2 cm up to 5 cm long leaves. All other species are unequivocally coded with one of the three states.
9. Leaves ovate to lanceolate to linear (0); leaves obovate to spatulate (1). The leaf shape is rather variable and difficult to code into distinct character states and we distinguish only two states.
10. Leaf point absent (0); leaf point present (1). Sometimes the leaf point is almost mucronate, for example in *Stylidium repens*.
11. Marginal leaf-membrane absent (0); marginal leaf-membrane present (1). In *Stylidium* a distinct marginal leaf-membrane occurs in some species; it varies from narrow to broad as in *S. preissii*.
12. Cells of leaf epidermis short (0); cells of leaf epidermis elongated (1). Mildbraed (1908) reported extensively on cell shape variation in the leaf epidermis. The short cells can be either six-to eight-cornered or rectangular, sometimes with wavy cell walls but we have not been able to code this variation into discrete character states.
13. Scale leaves absent (0); scale leaves present (1). Of the studied species only *Stylidium schoenoides* has scale leaves sheathing the petioles of the long and green leaves, but the character is taken into account here because it has been used for circumscribing species-groups (Erickson 1958).
14. Stomata paracytic (0); stomata anomocytic (1). Donatiaceae have two subsidiary cells parallel with the the pore, paracytic stomata, while Stylidiaceae have no subsidiary cells, anomocytic stomata (Rapson 1953).

Inflorescence

15. Flowers few or solitary (0); flowers in cymose corymbs or umbels (1); flowers in helicoid cymes (2); flowers in elongated thyrses (3); flowers in racemes, corymbs or spikes (4). Carolin (1967) recognized a series of different inflorescence types and their supposed interrelationships. We find his scheme difficult to apply. The inflorescence variation cannot be neglected, however, and we have recognized five different states, easily applied to the species included in this study.
16. Peduncle absent (0); peduncle present (1). An elongated leaf-less part of the stem, the peduncle, between the foliage and the inflorescence is characteristic of *Forstera* and some species of *Stylidium*.

Flowers

17. Pedicels absent or shorter than flowers (0); pedicels longer than flowers (1). Distinct flower stalks or pedicels occur in some species of *Levenhookia* and *Stylidium*.
18. Calyx actinomorphic (0); calyx zygomorphic (1). The *Stylidium* species with a zygomorphic calyx have the 5 calyx lobes in a 2+3 arrangement as if composed of a lower 2-lobed and an upper 3-lobed lip. The lobes need not be united, however (next character). Berggren (1878) stated that *Oreostylidium* has a slightly zygomorphic calyx but we have coded it as actinomorphic because we found it almost regular.
19. Calyx lobes free (0); calyx lobes 2 united (1); calyx lobes 2+3 united (2). Some *Stylidium* species with a zygomorphic calyx (former character) have partly united calyx lobes. Either the two lower lobes only are united (state 1), or the two lower as well as the three upper lobes resulting in a truly 2-lipped calyx (state 2). This character is treated as ordered.

Table 3. Continued.

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20. Corolla choripetalous (0); corolla sympetalous (1). Fig. 1.
21. Petals in actinomorphic arrangement (0); petals pseudoactinomorphic (1); petals paired vertically (2); petals paired laterally (3). The corolla is actinomorphic in *Phyllachne*, *Oreostylidium*, and one species of *Forstera*. In *Levenhookia* and *Stylidium* one petal is modified to a small labellum. The other four petals are equal in size and regularly arranged in most species of *Levenhookia* and because of the erect position of the labellum, the corolla looks regular and tetramerous; we code it as pseudoactinomorphic. In *Stylidium* (Fig. 1b-c) and *Levenhookia pauciflora* (Fig. 1a) the four main petals are normally paired giving an irregular appearance to the flower. The pairing may be either vertical or lateral. In the former case the two petals adjacent to the labellum form one pair (Fig. 1a-c), the other two petals another pair. In lateral pairing the four main petals form two pairs at each side of the labellum.
22. Corolla lobes variable 5–9 (0); corolla lobes regularly 5 (1). *Levenhookia* and *Stylidium* consistently have five corolla lobes or petals, in the other genera the number is variable.
23. Corolla orientation with labellum down (0); corolla orientation with labellum up (1). This character is not applicable in the species with actinomorphic corolla. In *Levenhookia* and *Stylidium calcaratum* the labellum is oriented downwards (Fig. 1a-b), in the other *Stylidium* species it is oriented upwards (Fig. 1c). Some species of *Stylidium* have to be coded as unknown for this character, because complicated inflorescences make corolla orientation difficult to see.
24. Corolla tube shorter than lobes (0); corolla tube longer than lobes (1). This character is not applicable in *Donatia* because the corolla of *Donatia* is choripetalous (character 20). A long corolla tube is shown in Fig. 1a.
25. Labellum/fifth petal direction spreading (0); labellum direction reflexed (1); labellum direction erect (2). In the taxa with actinomorphic flowers, the fifth petal corresponds to the labellum in the zygomorphic genera and its direction is spreading as for the other petals. In *Stylidium* and *Levenhookia pauciflora* the labellum is reflexed and positioned beneath the petals (Fig. 1a-c). In most species of *Levenhookia* the labellum is instead erect, positioned alongside the straight column.
26. Labellum/fifth petal flat (0); labellum sac-like (1). The labellum is flat or somewhat convex in most species of *Stylidium*; it is apically sac-like or hooded in *Levenhookia* and *Stylidium calcaratum* (Fig. 1a-b).
27. Labellum/fifth petal stalk absent (0); labellum stalk present (1). *Levenhookia* is characterized by a long labellum stalk.
28. Labellum/fifth petal texture thin (0); labellum texture thick (1). Most of the *Stylidium* species have a thick labellum structure not present in the other taxa.
29. Labellum/fifth petal insensitive (0); labellum sensitive (1). In *Levenhookia* the labellum is sensitive, suddenly springing back from the insensitive column. Because the column is enclosed by the apically sac-like or hooded labellum, the movement nevertheless releases a burst of pollen from the column on to visiting pollinators.
30. Labellum/fifth petal without basal appendages (0); labellum with basal appendages (1). In *Stylidium* there are sometimes appendages at the base of the labellum, usually large and obvious. *Stylidium brunonianum* and *S. petiolare* are variable in this respect. The interpretation of the labellum in *F. bellidifolia* is uncertain; the labellum basally has narrow lobes, but they do not resemble those of the *Stylidium* species and are therefore regarded as an autapomorphy not included in the data.
31. Petal night movement present (0); petal night movement absent (1). Erickson (1958) made many observations on night movements of the petals and she regarded this as an important similarity between *Stylidium calcaratum* and *Levenhookia*. From the other genera we have no information.
32. Petals single-veined (0); petals many-veined (1). This character has proved to be useful in the family classification of *Asterales* (Gustafsson 1995). The character is constant within the genera. *Donatia*, *Phyllachne*, and *Levenhookia* all have single-veined petals, the other genera have many-veined petals.
33. Paracorolla absent (0); paracorolla of scales (1); paracorolla of knobs (2); paracorolla of hairs (3); paracorolla of humps (4); paracorolla of sheaths (5). The paracorolla is the throat-appendages appearing in species of *Levenhookia* and *Stylidium* (Fig. 1c). In *Stylidium* the paracorolla is most often formed by 2–8 small teeth or membranous scales, more rarely of knob-, hair-, or “hump”-like, presumably homologous, structures. The sheath around the basal part of the column in *Levenhookia* is here regarded as an elongated paracorolla. Erickson (1958) observed that the paracorolla is often variable, but among the species studied here we find variation only in *Stylidium calcaratum*, which sometimes lacks a paracorolla.
34. Glands absent (0); glands with unicellular heads (1); glands with multicellular heads (2). Glandular hairs may occur in virtually all organs, but when present they are usually found on floral parts. The heads of the glands in *Levenhookia* and most ephemeral *Stylidium* species are unicellular; the other species have multicellular heads. The glands can be long or short and either wide- or slender-headed, but we find this variation hard to code into discrete character states.
35. Epigynous nectaries absent (0); epigynous nectaries short (1); epigynous nectaries long (2). Two short or long epigynous nectaries are found in some species, best developed in *Phyllachne* and some species of *Forstera*. This character is treated as ordered.
- Column**
36. Column absent (0); column insensitive (1); column sensitive (2). The flowers of Stylidiaceae are characterised by having the stamens and style fused to a single column. In *Stylidium* the column is sensitive and bent back in a triggered position, suddenly springing forward releasing a burst of pollen upon visiting pollinators. The sensitivity is located to the lowest of two bends in the column. Also *Levenhookia* has a slightly sensitive column, at least in *L. pauciflora* (Erickson 1958). This character is treated as ordered.
37. Column absent or present but not associated with labellum (0); column with head inside labellum (1); column with stalk attached to labellum (2). In *Stylidium* the column rests against the labellum in the triggered position, the upper portion of the column being bent below the petals (Fig. 1c). In *Levenhookia* and *Stylidium calcaratum* the column head is positioned inside the labellum (Fig. 1a-b). This character is treated as ordered.

Table 3. Continued.

38. Column hairs absent (0); column hairs present (1). Claviform hairs are found beneath the thecae on the column in some species of *Stylidium*.

Androecium

39. Stamens 3 (0); stamens 2 (1). In Stylidiaceae there are 2 stamens adnate to the style at the apex of the column. In Donatiaceae the stamens are 2 or 3 and free. The small number of stamens and styles could be a step towards their fusion into a column (Rapson 1953).
40. Thecae distinct (0); thecae fused (1). The thecae in *Forstera* and *Phyllachne* are fused or disporangiate, opening horizontally, and this must be regarded as a strong synapomorphy. *Stylidium*, *Oreostylidium*, and *Levenhookia* have distinct and tetrasporangiate thecae.

Gynoecium

41. Stigma head-shaped (0); stigma two-lobed (1); stigma brush-shaped (2); stigma cushion-shaped (3); stigma finger-shaped (4); stigma tongue-shaped (5). The stigma develops after dehiscence and withering of the anthers. Usually it is cushion-shaped (Fig. 1c) or head-shaped but it can also assume other, more elongated, shapes. In *Levenhookia* the stigma consists of two hairy projections. A brush-shaped stigma is shown in Fig. 1b.
42. Ovary cone-shaped (0); ovary obovoid to oblong (1); ovary almost globose (2); ovary long and slender (3). The ovary (or fruit) was used by Mildbraed (1908) as an important source of diagnostic characteristics for circumscribing subgenera of *Stylidium*. Ovary shape was one of his characters although it is variable even within some species.
43. Ovary cross-section rounded (0); ovary cross-section flattened (1). A flattened ovary usually has a very narrow septum separating the two locules and in cross-section it often assumes the shape of the figure 8 (Mildbraed 1908).
44. Ovary 3-locular (0); ovary 2-locular (1); ovary basally 2-locular (2); ovary 1-locular (3). There is a gradual reduction of the septum so that the ovary may be 2-locular (3-locular only in one *Donatia* species), only basally 2-locular, or 1-locular. The septum may be long, incomplete, or very short making the ovary bilocular, basally bilocular, or bilocular only at the base. The septum variation is found in *Stylidium* and *Oreostylidium* whereas the other genera of the Stylidiaceae are unilocular. This character is treated as ordered.
45. Placenta short (0); placenta elongated (1). In *Stylidium* the placenta is of variable length; the other genera have short placentas.
46. Fruit indehiscent (0); fruit a bilobed capsule (1); fruit a deeply bilobed capsule (2). The fruit is indehiscent in *Donatia*, *Oreostylidium*, and *Phyllachne*. The remaining genera have a variously deeply bilobed capsule with the valves coherent at base (Mildbraed 1908). This character is treated as ordered.

Pollen and cytology

47. Pollen spines equal (0); pollen spines dimorphic (1). Different studies of the pollen show that the pollen-grains are very variable (Bronckers and Stainier 1972; Erickson 1958; Willis and Ash 1990). Two or three forms of pollen may be found even within species of *Stylidium*; for example, the colpi can vary from 3 to 8 (Cronquist 1981). The character is coded as suggested by Bronckers and Stainier (1972). We lack pollen information for some species, coded with question marks.
48. Chromosome number: $n=8$ (0); $n=9$ (1); $n=11$ (2); $n=13$ (3); $n=14$ (4); $n=15$ (5); $n=16$ (6); $n=30$ (7). In chromosome numbers *Stylidium* constitutes a complex example of variation with $n = 5-16, 26, 28,$ and 30 (not all of these numbers are represented in our sample of species). The haploid numbers are taken from the literature (Coates 1982; James 1979; Hair and Beuzenberg 1959).

missing nucleotides (253, 224, 112, and 83 respectively). The sequence at the end of the gene is missing for the first two species, while the other two lack sequences of nucleotides in the middle of the gene. For the other sequenced species there are no or only few (<10) missing entries.

Separate and Combined Data. The molecular and morphological data sets for Stylidiaceae were analysed both separately and combined. Discussions of separate versus combined analyses are found in various papers, e.g. de Queiroz et al. (1995) and B. Bremer (1996). There are obviously clear advantages to have a large number of characters when phylogenetic groups are to be determined. In practice this usually leads to different data sets. The comparison of different resulting cladograms may be problematic if the cladograms

are incongruent with each other, and the decision of how to weigh one data set against the other may be difficult as well. We will give the combined cladogram most significance because the combined data summarize all the available information in a parsimonious way. To clarify the differences between the morphological and molecular results, the separate cladograms are also shown and discussed.

The combined analysis was performed with two different sizes of the data matrices, one including only the 12 sequenced species and one including all 26 examined species. The small matrix was complete for both morphological and molecular characters while the large matrix was incomplete, with question-marks in all the molecular positions for species not sequenced. No contradictory relationships were obtained when the small and large com-

TABLE 4. The coding of the morphological characters listed in Table 3. Polymorphic or variable taxa are coded with letters: A=0/1, B=1/2, C=1/3, D=2/3, E=5/7. Unknown and variable states are coded as ? and -, respectively. Ordered characters are marked with arrows.

Ordered characters	Character numbers											
	↓			↓			↓ ↓ ↓			↓ ↓		
	1	11111	11112	22222	22223	33333	33334	44444	444			
Included taxa	12345	67890	12345	67890	12345	67890	12345	67890	12345	678		
<i>Donatia fascicularis</i>	00000	00000	00000	00000	00--0	00000	?0000	00000	00000	00000	0??	
<i>Donatia novae-zelandiae</i>	00000	00000	00000	00000	01--0	00000	?0020	00010	00010	00010	0??	
<i>Forstera bellidifolia</i>	00100	00010	00010	10001	00--0	00000	?1000	10011	11030	1??	1??	
<i>Forstera bidwillii</i>	00100	00010	00010	10001	00--0	00000	?1002	10011	11030	10?	10?	
<i>Levenhookia leptantha</i>	10100	11010	00011	00001	11012	11010	00510	11010	12030	10?	10?	
<i>Levenhookia pauciflora</i>	10100	11010	00010	01001	21011	11010	00110	21010	12030	10?	10?	
<i>Levenhookia stipitata</i>	10100	11010	00011	01001	11002	11010	00510	11010	12030	11?	11?	
<i>Oreostylidium subulatum</i>	00110	02A01	01010	00001	01--0	00000	?1021	10010	32020	005	005	
<i>Phyllachne clavigera</i>	00000	00000	00010	00001	00--0	00000	?0001	10011	11030	01?	01?	
<i>Phyllachne uliginosa</i>	00000	00001	00010	00001	00--0	00000	?0001	10011	11030	01?	01?	
<i>Stylidium alsinoides</i>	10100	11010	00012	00111	31?11	00100	11010	22010	33011	20?	20?	
<i>Stylidium brunonianum</i>	00101	03101	01013	11001	21101	0010A	11220	22010	3B020	101	101	
<i>Stylidium bulbiferum</i>	00111	04001	11010	10101	31?01	00101	11020	22110	3C111	104	104	
<i>Stylidium calcaratum</i>	10100	12010	00011	01001	21001	10000	01A10	21010	22030	10D	10D	
<i>Stylidium elongatum</i>	00100	02200	00014	10001	31101	00101	11320	22110	11111	103	103	
<i>Stylidium emarginatum</i>	01100	12010	00010	01001	21101	00100	11120	22010	31121	1?4	1?4	
<i>Stylidium fasciculatum</i>	00101	04100	00014	10001	31101	00101	11020	22110	33111	1?5	1?5	
<i>Stylidium graminifolium</i>	00100	02201	01013	10121	31101	01101	11121	22110	31020	115	115	
<i>Stylidium junceum</i>	00101	02101	11013	11101	31101	00100	11120	22010	41030	106	106	
<i>Stylidium kunthii</i>	10100	12010	00012	00111	31?11	00100	11010	22110	33011	20?	20?	
<i>Stylidium laricifolium</i>	00100	?0101	00014	11001	31111	00101	11020	22110	31020	1??	1??	
<i>Stylidium petiolare</i>	01100	12010	00011	01001	21101	0010A	11110	22010	31121	104	104	
<i>Stylidium preissii</i>	00100	00000	11010	00111	31?01	00000	11020	22110	32031	104	104	
<i>Stylidium repens</i>	00111	04001	11010	01121	31?01	00100	11120	22010	31021	10E	10E	
<i>Stylidium scandens</i>	00101	03101	01013	11001	31101	00101	11120	22110	4B030	100	100	
<i>Stylidium schoenoides</i>	00101	02201	01110	11001	21101	00101	11420	22110	51020	1?0	1?0	

bined matrices were analysed and therefore the larger matrix with its larger sample of species is the one used henceforth. Wiens and Reeder (1995) recommended inclusion of incomplete taxa, even when the accuracy of the resulting trees slightly decreases, because of the gain of a phylogenetic hypothesis for all the taxa.

Character Weighting. All characters are treated as equally reliable indicators of phylogeny in the analyses, and hence weighted equally. This assumption may not be correct, but in the absence of a priori information about the relative importance of characters, they are best treated as equally important.

Cladistic Analyses. The computer program PAUP (Phylogenetic Analysis Using Parsimony) version 3.1.1 (Swofford 1993) was used for the analyses. All analyses excluded uninformative characters. Unknown and inapplicable character states (coded as ? and - in the data matrix in Table 4) were treated equally. Molecular cladograms were generated using the exact branch-and-bound algorithm in PAUP, guaranteed to find all the most par-

simonious cladograms. For the morphological and combined matrices heuristic search options were employed. The searches were performed using 1,000 randomized stepwise addition sequences and TBR (tree bisection-reconnection) was chosen as branch swapping routine. In order to choose among equally parsimonious cladograms (Carpenter 1988), the morphological and the combined matrices were also analysed using Farris's (1969) successive approximations approach to character weighting. When successive weighting has been applied we have pointed out the clades that are collapsed in the consensus tree from the unweighted analyses to avoid an impression of more resolution than given by the original data. Tree lengths, consistency indices (CI; Kluge and Farris 1969) and retention indices (RI; Farris 1989) were calculated automatically by PAUP.

To estimate the stability of particular nodes, Bremer support (K. Bremer 1988, 1994) and bootstrap frequencies (Felsenstein 1985) were used. Bremer support is sometimes also called either decay index or branch support. Both Bremer support and boot-

strap values were calculated in PAUP, the former with the aid of the program AutoDecay 2.9 HyperCard stack (Eriksson 1996). The bootstrap analyses were performed with 10,000 replicates and a randomised stepwise addition without branch swapping. The total support index (TI; K. Bremer 1994) was determined by summarising all Bremer support values in a particular cladogram and then dividing the sum by the obtained tree length. The indices (CI, RI, and TI) are different ways of comparing tree fitness (K. Bremer 1994). The MacClade program 3.03 (Maddison and Maddison 1992) was used to trace character state evolution.

The congruence between the two data sets was estimated both by using the data program Arn by Farris et al. (1994), and by means of the tree lengths (Mickevich and Farris 1981; Endress et al. 1996). Arn makes a statistical test of the congruence by generating new combined matrices randomly, by moving the existing characters between the data sets. The new matrices are of the same size as the original combined matrix. The searches were performed 1,000 times. Mickevich and Farris's (1981) way of measuring the congruity is an easy, but statistically less sophisticated way. For each data set the tree length of the most parsimonious tree is needed as well as the minimum possible length specified by the data set; these numbers are given by PAUP. The first number minus the second gives the homoplasy (H), i.e. the number of extra steps on the tree. The incongruity (I), caused by having the morphological and the molecular data sets combined instead of separated is calculated as:

$$I = \frac{H_{\text{comb}} - (H_{\text{morph}} + H_{\text{mol}})}{H_{\text{comb}}}$$

RESULTS

The morphological analysis generated ten equally parsimonious cladograms. The different morphological results are due to various arrangements of the basal taxa *Phyllachne*, *Forstera*, and *Oreostylidium* and the different positions of the *Stylidium preissi* to *S. bulbiferum* clade. When successive weighting was applied, two of the original cladograms remained (Fig. 2). The molecular data set resulted in one most parsimonious cladogram (Fig. 3). Two most parsimonious cladograms were obtained when the data sets were combined. After successive weighting just one of these cladograms (Fig. 4) was left. The combined cladogram with all morphological characters mapped is given in Fig. 5.

When successive weighting is applied the characters get different weights according to their fit on the cladograms. The morphological characters which contribute most to the result are various corolla and labellum characters.

The number of cladograms found in each analysis together with number of steps as well as values for consistency index (CI), retention index (RI), and the total support index (TI) from the three cladistic analyses are listed in Table 5. Also, the number of phylogenetically informative characters is shown in that table. Two of the seven ordered characters (19 and 44) did not turn out in an ordered way, indicating another evolutionary history than a priori assumed. When the analyses were run with characters 19 and 44 unordered the topology did not change, even though the indices increased slightly.

The Bremer support values and the bootstrap frequencies are not always correlated. For example, in the combined cladogram (Fig. 4) two clades have a high Bremer support of 4 while the bootstrap frequencies are below 50%. The reasons for such discrepancies are unknown (cf. K. Bremer 1994).

The data program Arn did not find any better combined matrices than the one used and thus the morphological and the molecular data sets have a significant congruence of 100%, compared to Arn's null hypothesis. The number of steps required in the combined analysis is 15 (ca. 5% incongruity) more compared to having the morphological and the molecular analyses separated. When the combined analysis instead is compared to having all three (morphology, *rbcL*, and *ndhF*) data sets separated, then 16 (ca. 5% incongruity) more steps are required. The discrepancy is mainly due to differences in the positions of *Oreostylidium subulatum* and the *Levenhookia* clade.

DISCUSSION

As previously discussed, the family Styliaceae is clearly monophyletic with a strong synapomorphy in the fusion of the two stamens and the style. The generic classification, however, is more uncertain and Styliaceae have quite a high level of homoplasy at generic level. The molecular data presented here provide new, interesting insights into the generic interrelationships of Styliaceae. The molecular cladogram contradicts the morphological cladogram regarding the position of the *Levenhookia* clade as well as the position of *Oreostylidium*. In the combined analysis, the molecular result is retained in both these aspects.

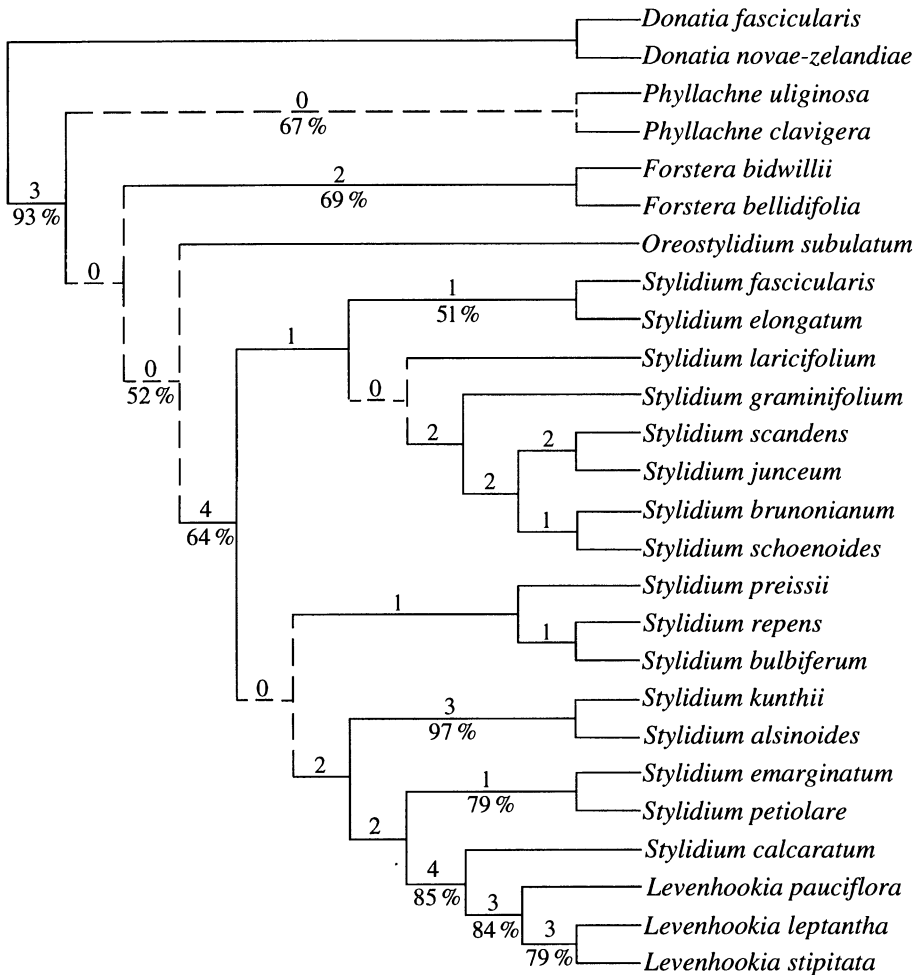


FIG. 2. One of ten most parsimonious trees resulting from the morphological analysis of Styliaceae. This tree is also one of two trees obtained using the successive approximations approach to character weighting. Bremer support values are indicated above branches; bootstrap frequencies are below branches. Bootstrap values below 50% are not indicated. The strict consensus tree is obtained if the branches with Bremer support of zero (branches of dashed lines) are collapsed.

Forstera and Phyllachne. *Forstera* and *Phyllachne* have always been regarded as the most plesiomorphic genera of the family, an interpretation supported also in the present study. The apically fused thecae forming a curved anther with a single cell (character 40) constitute a strong synapomorphy connecting *Forstera* and *Phyllachne* (Fig. 5). Another possible synapomorphy is the commonly occurring epigynous nectaries (character 35), which however are lacking in *F. bellidifolia*. A character shared with *Levenhookia* is the two-lobed stigma (character 41). Both Bremer support and bootstrap values are high for the *Forstera* and *Phyllachne* clade supporting the close relationship of these two gen-

era. The combined analysis even shows *Forstera* to be paraphyletic, with *Phyllachne* nested inside it.

Many early writers have suggested that these genera should be united but Bentham and Hooker (1873–1876) kept them separate. Von Mueller (1878) considered that the generic separation between *Forstera* and *Phyllachne* mainly or entirely rests on habitual differences, *Phyllachne* having a cushion-like habit that *Forstera* lacks (character 3). However, there are other distinguishing characters such as the shape of the leaves (character 9) and the long peduncle in *Forstera* (character 16), which is absent in *Phyllachne*. These characters provide a convenient means of distinguishing the two genera.

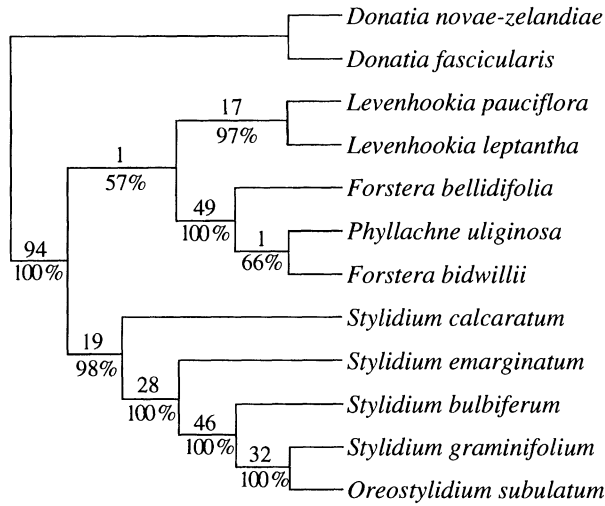


FIG. 3. Single most parsimonious tree from the molecular analyses of *rbcL* and *ndhF* in Stylidiaceae. Bremer support values are indicated above all branches, bootstrap frequencies are below the branches.

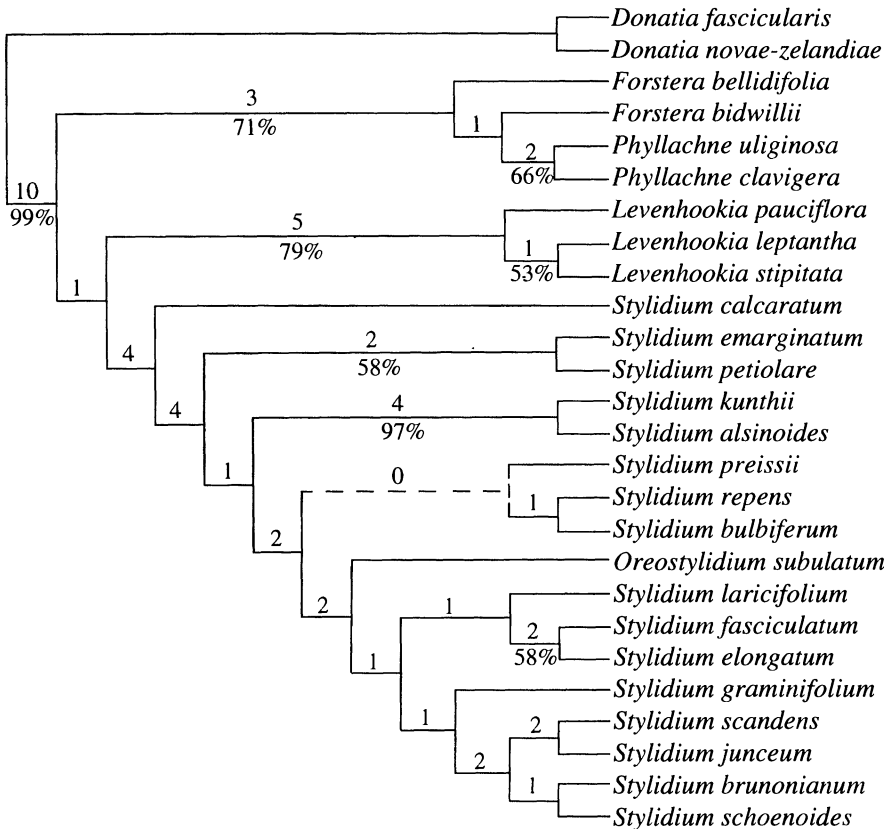


FIG. 4. The combined morphological and molecular data of Stylidiaceae gave two trees of equal length, one of these was also obtained when successive weighting was applied and this tree is shown here. Bremer support values are indicated above branches, bootstrap frequencies are below branches. Bootstrap values below 50% are not indicated. The strict consensus tree is obtained if the branches with Bremer support of zero (branches of dashed lines) are collapsed.

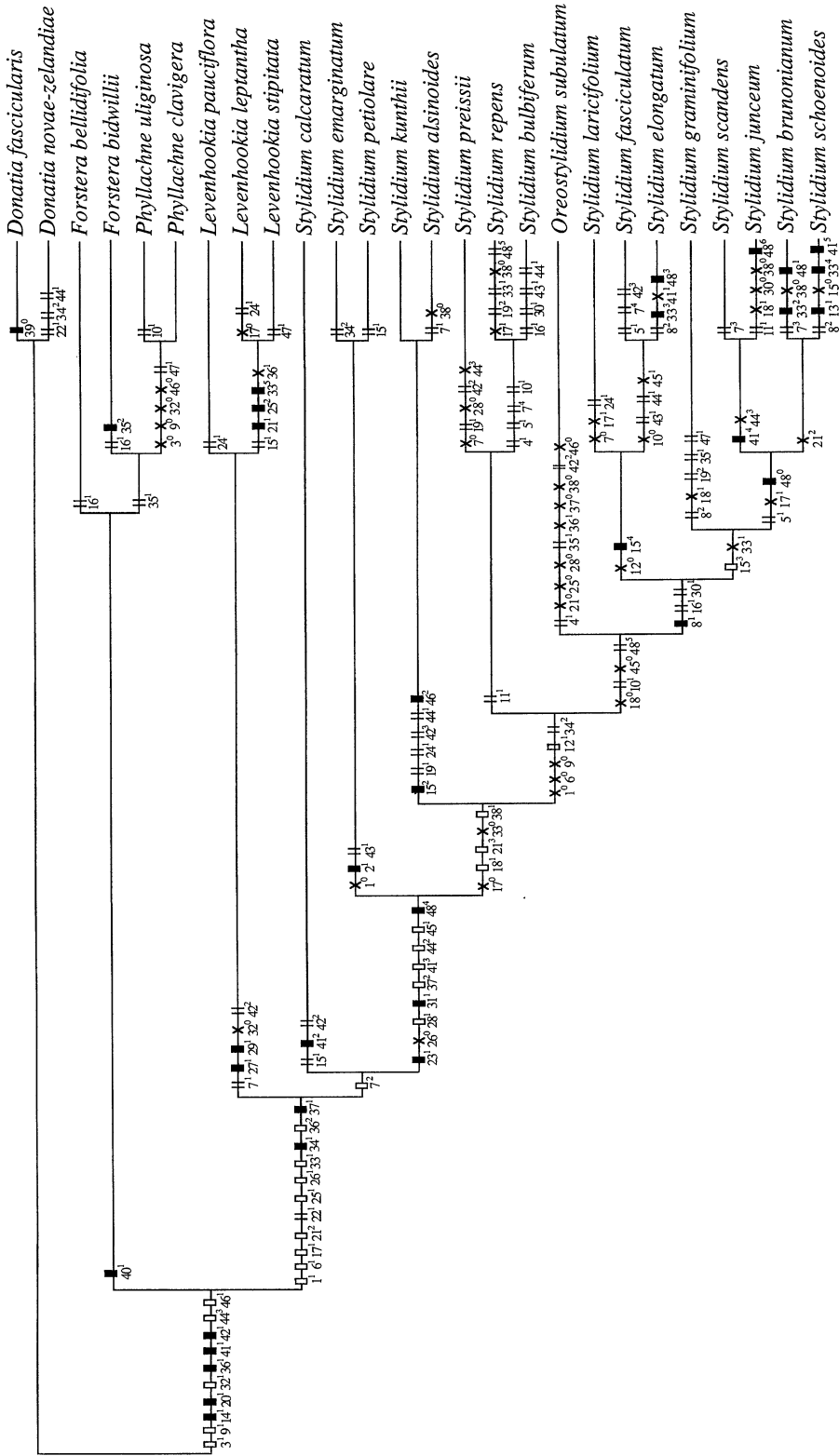


FIG. 5. The combined tree from Fig. 3 with all morphological character changes mapped, the numbers referring to Table 3. Filled rectangles represent apomorphies without reversals and open rectangles represent apomorphies that reverse, sometimes repeatedly. Parallel bars represent parallelisms and crosses represent reversals.

Table 5. Results from the morphological, molecular, and combined cladistic analyses and the number of informative characters used in the analyses. MPT = most parsimonious trees, CI = consistency index (all autapomorphies are excluded), RI = retention index, and TI = total support index.

Analysis	Inf. char.	MPT	Steps	CI	RI	TI
Morphological	46	10	167	0.52	0.73	0.23
Molecular (<i>rbcl+ndhF</i>)	431	1	762	0.73	0.77	0.38
Combined	477	2	937	0.68	0.75	0.06

Given the commonly held principle that taxa should be monophyletic, the status of *Forstera* needs to be reconsidered based on the results from the combined analysis (Figs. 4, 5). *Forstera* and *Phyllachne* could be merged but it seems premature to unite them based only on the very weak support for a sister group relationship between one of the two *Forstera* species (*F. biduillii*) and *Phyllachne* (Fig. 4; Bremer support 1 and bootstrap frequency <50%). Further studies are needed to corroborate the possible paraphyly of *Forstera* and a reduction of *Phyllachne* to synonymy under *Forstera*.

Some of the characters in *Phyllachne* are optimized as reversals in Fig. 5, i.e. the cushion-like habit (character 3), the shape of the leaves (character 9), single-veined petals (character 32), and an indehiscent fruit (character 46). Equally parsimoniously, they may be interpreted as parallelisms to conditions occurring in *Donatia*, representing a case of convergent evolution.

Levenhookia and Stylidium. In the morphological cladogram (Fig. 2) *Levenhookia* is nested within *Stylidium* with a close affinity to *Stylidium calcaratum*. The morphological similarities between *Levenhookia* (particularly *Levenhookia pauciflora*) and *Stylidium calcaratum* are striking, especially in the corolla orientation with the labellum oriented downwards (character 23), in the appearance of the sac-like labellum (character 26), and in the way the column head is positioned inside the sac-like labellum when posed (character 37). Other similarities between *Levenhookia* and *Stylidium calcaratum* are the inflorescence (character 15), the petal night movement (character 31), and the globose shape of the ovary (character 42). Furthermore, the column of *Levenhookia* is slightly sensitive even if the labellum is the most responsive part (Erickson 1958). Hence, one might suspect that *Levenhookia* is a specialized derivative of *Stylidium* and nested inside that genus with *S. calcaratum* as its sister group, as indicated by the morphological cladogram. However, in the molecular cladogram *Levenhookia* is placed at a more basal position in the phylogeny as sister group of *Stylidium*, a solution that also remains in the combined cladogram. Because the

combined cladogram supports both *Stylidium* (including *Oreostylidium*) and *Levenhookia* as monophyletic, no reclassification of these genera seems justified (except for the inclusion of *Oreostylidium* in *Stylidium* as discussed below).

The combined cladogram suggests that the common ancestor of *Levenhookia* and *Stylidium* had zygomorphic flowers, with vertically paired petals (character 21), with a reflexed and sac-like labellum oriented downwards (characters 25, 26 and 23), and with a sensitive column positioned inside the sac-like labellum (characters 36 and 37). Flowers with a sac-like labellum oriented downwards and enclosing the column are retained as symplesiomorphies in *Levenhookia* and *S. calcaratum*, whereas other species of *Stylidium* have a flat labellum oriented upwards, not enclosing the column (characters 26, 23 and 37). Flowers with vertically paired petals, a reflexed labellum, and a sensitive column are retained as symplesiomorphies in *Stylidium* and *L. pauciflora*, whereas other species of *Levenhookia* have pseudoactinomorphic flowers, an erect labellum, and an insensitive column (characters 21, 25 and 36). The retention of all these symplesiomorphies in *S. calcaratum* and *L. pauciflora* make the two species deceptively similar, but they belong in two different monophyletic groups, as judged from the molecular and combined cladograms.

The current delimitation of *Stylidium* into subgenera (Mildbraed 1908) is not in accordance with the results from any of the presented cladograms. Mildbraed's subgeneric classification is mainly based on the appearance of the capsule, but when all aspects of the plants are considered this delimitation does not seem to be justified. It is necessary to do some rearrangement within *Stylidium* in the future but this is beyond the scope of the present work.

Oreostylidium. *Oreostylidium* is found close to the base in the morphological cladogram but nested within *Stylidium* in both the molecular and the combined cladograms. The bootstrap values in the combined analysis are low, but in the molecular analysis the result is unequivocal and strongly supported. As far as the flowers are concerned this is an unexpected result. The flowers are actinomorphic and

lack sensitivity and they resemble the flowers of the two plesiomorphic genera *Forstera* and *Phyllachne*. Furthermore, *Oreostylidium* is endemic to New Zealand where *Stylidium* is absent. *Stylidium* has diversified within Australia, in particular in the southwestern part. To eliminate all suspicions of laboratory mistakes, the DNA-extraction and the sequencing procedure were performed independently three times, twice using the same voucher material and once using new fresh material. All three times, the sequences were identical. The molecular data indicate that *Oreostylidium* should not be considered as a separate genus and that the floral construction is an example of reduction and extreme paedomorphosis. This is demonstrated by the many reversals on the branch leading to *Oreostylidium* in Fig. 5, i.e. actinomorphic flowers (characters 21, 25, 28) with an insensitive and glabrous column not attached to the labellum (characters 36, 37, 38) and the indehiscent fruit (character 46).

We interpret these reversals as a case of floral paedomorphosis, which may have been caused by adaptation to an environment (New Zealand) where, for example, an earlier used pollinator was lacking. Erickson (1958) observed that most *Stylidium* species attract only one specific insect. The variation concerning the column and the nectar-tube within *Stylidium* has also been interpreted as a specialization towards a more efficient intraspecific exchange of pollen (Armbruster et al. 1994). If the zygomorphic and column-sensitive ancestor of *Oreostylidium* dispersed to a location where there was no pollinator available, this might be the explanation of the simplification. In connection with a switch to unspecialized pollinators or self-fertilization the flowers then became fertile at a morphologically immature stage because a complicated flower was no longer necessary. The simplification may have occurred in such a short time span, that strong differentiations in the gene sequences have not yet occurred. The postulated simplification of *Oreostylidium* makes good sense in the New Zealand context. The New Zealand flora is characterised by a considerable proportion of insignificant white flowers that lack complicated adaptations such as strong zygomorphy (Lloyd 1985; Webb and Kelly 1993). *Oreostylidium* parallels other groups that elsewhere are zygomorphic and/or colorful. Representatives of the families Orchidaceae, Scrophulariaceae, and Violaceae are more close to actinomorphic and the usually conspicuously colored genera *Gentiana* L. and *Myosotis* L. are white-flowered in New Zealand (Lloyd 1985; Webb and Kelly

1993). Generally, the explanation is considered to be connected to the paucity of specialized pollinators (Lloyd 1985) and the long isolation of New Zealand (Webb and Kelly 1993). Thus, the flowers have to rely on unspecialized insects that visit a variety of different flowers. Some of the main pollinators of *Stylidium* are nectar-seeking solitary bees (Armbruster et al. 1994) but in New Zealand there are only a few species of bees, all with a short tongue and unrestricted in their search for nectar (Godley 1979). The question whether the white-colored flowers and their simple features are developed locally or as the result of dispersal selection is discussed by Godley (1979) and Lloyd (1985). As was stated above the most plausible scenario for *Oreostylidium* is that the simplified flowers have evolved within New Zealand. In some genera white flowers are associated with autogamy (Garnock-Jones 1976). However, there is no information from reproductive biology of *Oreostylidium* itself.

The idea of *Oreostylidium* belonging within *Stylidium* is not completely new. In fact the species was originally described as *Stylidium subulatum* Hook. by Hooker (1864). Hooker based his classification on the fruit morphology and the habitual similarities but he did not have any complete flowers available. Small formations (hard epigynic glands) at the top of the fruit resemble those of several *Stylidium* species, especially from the subg. *Tolypangium* Endl. (Hooker 1864; Berggren 1878). Berggren (1878) also found the calyx to be slightly zygomorphic (in this study coded as actinomorphic) with the lobes united as in some *Stylidium* species and the column slightly curved like a reduced form of the column in *Stylidium*. On the corolla tube, beneath the petal corresponding to the labellum, Berggren (1878) saw a small enlargement. The enlargement corresponds to the place where the column in *Stylidium* is located during the bud-stage. It is desirable to make further morphological investigations of *Oreostylidium* to study Berggren's observed similarities between *Stylidium* and *Oreostylidium*, to see if the hypothesis of extreme paedomorphosis resulting in a very reduced flower in *Oreostylidium* can be confirmed. Von Mueller (1878) changed Hooker's classification and transferred *Oreostylidium* to *Phyllachne* based on the appearance of the flowers, while Berggren (1878) considered *Oreostylidium* to have a position together with *Levenhookia* between *Stylidium* and the actinomorphic genera *Phyllachne* and *Forstera*. He (Berggren 1878) established the monotypic genus *Oreostylidium* for the species and this classification has been the most accepted one. Our data show that

Oreostylidium is firmly nested inside *Stylidium*. Hence, *Oreostylidium* should be reduced to synonymy under *Stylidium* and the species should be known under its original name *Stylidium subulatum*.

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