Chapter 4 Phylogenetic Relationships of *Carpha* and its Relatives (Schoeneae, Cyperaceae) as Assessed by Cladistic Analyses of Morphology

4.1 Introduction

The genus *Carpha* belongs to the tribe Schoeneae of the family Cyperaceae (Clarke 1908; Kükenthal 1940b, 1944; Goetghebeur 1986, 1998; Bruhl 1995). Schoeneae has been defined differently by different authors; the definition used in this study is after Bruhl (1995; see Chapter 1 for reasons). Several morphological characters are common to members of the Schoeneae including a restricted number of bisexual flowers per spikelet, often provided with a well-developed perianth, and sympodial spikelet structure (see Chapter 2). The genera of the tribe Schoeneae were previously included in the tribe Rhynchosporeae of Bentham (1883), Koyama (1961), Schultze-Motel (1964) and Hooper (1973) (see Chapter 1).

Within the tribe Schoeneae, the relationships of *Carpha* are not yet well understood. Clarke considered *Carpha* to be closely related to *Schoenus* (Clarke 1902) and *Ecklonea* (*Trianoptiles*) (Clarke 1897–1898). Kükenthal (1939c) included *Trianoptiles* as a subgenus in *Carpha* and thought *Carpha* was closely related to *Costularia* and *Schoenus*. Kükenthal (1939d, 1940a; also see Wilson 1981) also considered that some features of *Carpha* were similar to those of *Ptilothrix* (*Ptilanthelium* auct.), *Gymnoschoenus* and *Mesomelaena*. The species of the monotypic genus *Ptilothrix* was initially included in *Carpha* (Brown 1810; Kunth 1837; Steudel 1855; Boeckeler 1874; Clarke 1908, 1909; Pfeiffer 1931) and subsequently segregated from *Carpha* (Kükenthal 1939d; Wilson 1994b). The results of recent phylogenetic studies of Cyperaceae based on morphology do not agree on the relationships of *Carpha* (Goetghebeur 1986; Bruhl 1995). Goetghebeur's (1986) results indicated that *Carpha*, *Costularia, Oreobolus* and *Trianoptiles* formed a monophyletic clade, in which *Trianoptiles* was sister to *Carpha*, while Bruhl's (1995) analyses revealed *Oreobolus*, *Schoenoides*, *Ptilothrix, Trianoptiles* and *Carpha* to be a robust group and *Trianoptiles* was most likely the sister to *Ptilothrix*.

Since *Carpha* was first described by Brown (1810), over 40 names have been applied to species in the genus (see Chapter 1). The definition of *Carpha* was modified as species were moved in and out the genus. Generic definitions and species changes related to *Carpha* have been reviewed in detail in Chapter 1. Until now, the limits of *Carpha* have been unclear and definition of the genus controversial (see Chapter 1 for details), with two main definitions of *Carpha* based on flower number per spikelet and whether the hypogynous bristles are plumose or not (see Chapter 1). However, no test of the monophyly of *Carpha* for either definition has been carried out until this present study.

In addition to problems in generic circumscription, the relationships among the species of *Carpha* have not been clear. There has been no species level cladistic analysis of this genus. Reid and Arnold (1984) once used morphological observation to infer the relationships among five species (*C. filifolia*, *C. bracteosa, C. capitellata*, *C. schlechteri* and *C. glomerata*).

Hence, it is necessary to make a complete and systematic study of *Carpha* and relatives, to test generic limits and to estimate the phylogeny in and around *Carpha*.

Morphology has traditionally been the most important source of information in plant taxonomy. Most taxonomic groups recognized today are defined mainly on morphological characters (e.g. Stuessy 1990; Sennblad et al. 1998; Stevens 2000). Although many of these groups are challenged by the recent addition of molecular data together with a phylogenetic concept, morphology still plays a most important role in systematic studies (e.g. Stuessy 1990; Stevens 2000).

The aims of this study are to address the following three questions using the cladistic analyses of morphology data.

- Is *Carpha* monophyletic, and if so, what characters support it?
- What are the relationships of *Carpha* and its relatives?
- What are the relationships among the different species within *Carpha*?

4.2 Materials and Methods

4.2.1 Species Sampled

The sample included all species that were described in *Carpha* at various time (for details see section 1.6) with an exception of *Carpha schweinfurthiana* Boeck. *Carpha schweinfurthiana* Boeck. has very different features to other species in *Carpha* (Nelmes 1953), and is now placed in *Coleochloa* which is very distantly related to *Carpha* (Muasya et al. 1998). The sample also included some species from all genera that were considered to be close relatives of *Carpha* (also see section 1.5) and from other genera of the tribe Schoeneae, as well as from more remotely related genera outside Schoeneae. The latter were included as outgroups.

Ingroups: Within Schoeneae, 16 species of *Carpha* recognized by phenetic analyses (Chapter 3) and 29 species of relatives of *Carpha* were chosen as ingroups for cladistic analysis. A total of 262 herbarium specimens of 45 species sampled for ingroups are listed in Appendix 1. Two specimens of *Tetraria capillaris* (Appendix 1) showed great differences in their morphology. For example, one had flowers with a perianth and the other had flowers without a perianth. Hence they were treated separately in the cladistic analyses. A specimen of *Costularia pilisepala* (*L. J. Brass 8802*) from New Guinea showed some differences from the other two specimens (*W. L. Chew 4966*, *M. S. Clemens 51062*) from Borneo such as in having curling leaves and a dense inflorescence. This specimen (*L. J. Brass 8802*) was also treated separately in the cladistic analyses and labelled as *Costularia pilisepala*2.

Outgroups: Two species (six specimens) of *Rhynchospora* in the tribe Rhynchosporeae, which is considered to be closely related to Schoeneae, and two species (five specimens) of *Scleria* in the tribe Sclerieae, which is supposed to be more distantly related (Bentham 1883; Koyama 1961; Schultze-Motel 1964; Hooper 1973; Goetghebeur 1998; also see Fig. 1.1), were chosen as outgroups (Appendix 1).

4.2.2 Characters

Ninety-four characters (37 quantitative and 57 qualitative) were included in this cladistic analysis. The characters and character states are given in Table 4.1. The annotated

characters are listed in DELTA format (Dallwitz et al. 1999) in Appendix 2. The characters represent aspects of growth habit, and vegetative and reproductive morphology. Measurements of the characters are the same as those described in Chapter 3 (Section 3.2.3). The value of each of the quantitative characters for each species was the mean value of sampled specimens. For qualitative characters, states recorded for each species were used.

4.2.3 Character Coding and Weighting

The 37 quantitative characters (i.e. characters 3, 5, 6, 17 18 22–24 27 29–31 34 38 40 42–47 53 55 59 63 64 71 74–77 80–82 91 92 94) were coded using the gap weighting method (Thiele 1993) because this coding method uses range-standardization and retains information on both the rank order of states and the sizes of the gaps between states (Thiele 1993; Kitching et al. 1998). Among currently available methods for coding quantitative data, gap weighting is among the best at retaining phylogenetic information as argued by Lee et al. (2001).

To maximize the utilization of the raw data, a suitable state number of each quantitative character for gap weighting should be a maximum that must be within the maximum number of states allowed by the computer program (Kitching et al. 1998). In this study, the state number was determined to be 24 for the following reasons. The first is that it is under the limit of 26 and 32 imposed by MacClade (Maddison and Maddison 1992) and by PAUP* (Swofford 2000) respectively. The second is that after gap weighting, qualitative characters must be weighted to maintain parity with quantitative characters, i.e. the qualitative character should be weighted to have the same maximum state value (i.e. 24) as quantitative characters, and the number ensures an integer weight for each qualitative character for convenience in weighting. This number is 24 because it is the least common multiple of 2, 3, 4 and 5 states of the qualitative characters.

The binary-state qualitative characters (0, 1) were weighted by 24, three-state characters (0, 1, 2) by 12, four-state characters (0, 1, 2, 3) by 8 and five-state characters (0, 1, 2, 3, 4) by 6. The full dataset for cladistic analysis is presented in Appendix 4.

Table 4.1. Morphological characters and coded character states used in the cladistic analyses. Ordered characters are indicated. See Appendix 2 for character explanation and units.

1. Rhizome: absent (0), present (1).
2. Lifeform: annual (0), perennial (1).
3. Plant height from ground level to top of plant, including inflorescence (ordered).
4. Culm shape in cross-section: triangular (0), narrow-elliptical or fusiform (1), obtusangular-circular (2).
5. Fertile node number (see Reid and Arnold 1984) (ordered).
6. Sterile node number (cauline leaves; see Reid and Arnold 1984) (ordered).
7. Leaf sheath colour: brownish includes yellow-green to brown (0), reddish includes red to dark red (cf. *Schoenus andinus, S. rhynchosporoides, S. antarcticus*) (1).
8. Ligule: absent (0), present (1).
9. Ligule whether ciliate: glabrous (0), ciliate (1).
10. Contraligule: absent (0), present (cf. *Scleria levis*) (1).
11. Pseudopetiole: absent (0), present (1).
12. Leaf blade whether curling (see Curtis 1984): curled for at least one third of its length (0), only leaf tips curled (1), not curled (2).
13. Leaf blade whether spirally twisted: not spirally twisted (0), spirally twisted (e.g. the leaves of *Cyathochaeta diandra, C. avenacea* and some of *C. clandestina*) (1).
14. Leaf whether rigid: not rigid (0), rigid (1).
15. Leaf blades with a median stomate-less longitudinal band adaxially between two faint or obvious veins (Wilson 1993): absent (0), present (1).
16. Leaf blade shape in cross-section at mid-third: V-shaped (0), subcircular–circular (1), thinly crescentiform or flat (includes shallowly corrugate) (2), thickly crescentiform (includes sub-triangular, thickly V-shaped and subhemispherical) (3).
17. Mature longest leaf blade length (ordered).
18. Mature widest leaf blade maximum width (ordered).
19. Involucral bract sheath colour: brownish includes yellow-green to brown (0), reddish includes red to dark red (cf. *Schoenus andinus, S. rhynchosporoides, S. antarcticus*) (1).
20. Involucral bract type: ovate (i.e. bract-like) (0), linear-lanceolate (i.e. leaf-like) (1).
21. Ovate involucral bracts type: ovate without long apices (0), ovate with long leaf-like apices (1).
22. Proximal involucral bract length including sheath (ordered).
23. Proximal involucral bract blade maximum width (ordered).
24. Inflorescence length (ordered).
25. Spikelets, whether all enclosed by involucral bracts: not all enclosed by involucral bracts (0), all enclosed by involucral bracts (1).
26. Spikelets, whether densely clustered: densely clustered (0), loosely clustered (1).
27. Head(s) number per inflorescence (see Clarke 1897–1898) (ordered).
28. Shape of the head(s) formed by spikelets: ovoid (0), oblong-ellipsoid (1), globose (2), obovoid or obconical or fan-shaped (3).
29. Spikelet pedicel length (spikelet pedicel is enclosed by primary involucral bract sheaths) (ordered).
30. Spikelet secondary pedicel length (spikelet pedicel is not enclosed by primary involucral bract sheaths) (ordered).

Table 4.1. (Continued)

31. Spikelet number per inflorescence (ordered).
32. Basal spikelets: absent (0), present (cf. the species of *Trianoptiles*) (1).
33. Male only spikelets: absent (0), present (1).
34. Female-fertile spikelet length (excluding pedicel) (ordered).
35. Glume colour: brownish includes yellow green to brown (0), reddish includes red to dark red (cf. *Schoenus andinus, S. rhynchosporoides*) (1).
36. Glume arrangement: spiralled (0), distichous (1).
37. Lower glumes relative length to upper glumes within a spikelet: shorter than upper glumes (0), longer than upper glumes (1).
38. Glume number per spikelet (ordered).
39. Glumes whether persistent: all glumes persistent (0), lower sterile glumes persistent (1), all glumes deciduous (2).
40. Proximal sterile glumes number (ordered).
41. Uppermost glume: fertile (0), sterile (1).
42. Proximal fertile glume length (including any awn) (ordered).
43. Proximal fertile glume maximum width (ordered).
44. Second fertile glume length (including any awn) (ordered).
45. Second fertile glume maximum width (ordered).
46. Third fertile glume length (including any awn) (ordered).
47. Third fertile glume maximum width (ordered).
48. 'Rachilla', whether elongated above fertile flower: not elongated above fertile flower (0), elongated above fertile flower (1).
49. 'Rachilla', whether adnate to fertile glume base: not adnate to fertile glume base (0), adnate to fertile glume base (1).
50. Bisexual flowers: absent (0), present (1).
51. Female only flowers: absent (0), present (1).
52. Male only flowers: absent (0), present (1).
53. Flower number per female-fertile spikelet (including all kind of flowers: bisexual, male and female flower in spikelet) (ordered).
54. Perianth: absent (0), present (1).
55. Perianth member number (ordered).
56. Perianth whorls: one whorl (0), two whorls (1).
57. Perianth members whether inner whorl and outer whorl more or less equal in length: inner whorl more or less equal in length to outer whorl (0), inner whorl much longer than outer whorl (1).
58. Perianth members whether more or less equal in length within a whorl: obviously unequal in length within a whorl (0), more or less equal in length within a whorl (1).
59. Maximum perianth length (ordered).
60. Perianth member type: bristles (0), scales (1).
61. Perianth members, whether glabrous: glabrous (0), not glabrous (scabrous or with some hairs) (1).
62. Perianth bristles whether plumose: plumose (0), scabrous (1).

Table 4.1. (Continued)

63. Plumose perianth trichomes maximum length (ordered).
64. Plumose perianth scabrous zone maximum length (ordered).
65. Perianth members whether trifid: not divided (0), trifid (1).
66. Perianth members whether twisted at maturity: not twisted (0), twisted (1).
67. Perianth scales whether with a dense tuft of hairs on the adaxial surface: without a dense tuft of hairs on the adaxial surface (0), with a dense tuft of hairs on adaxial surface (cf. *Trianoptiles stipitata*) (1).
68. Perianth members whether base fused into a band: base not fused into a band (0), base fused into a band (cf. *Cyathocoma hexandra*) (1).
69. Perianth member, whether forming a disc at base of fruit: absent (0), present (cf. *Scleria*, there is a disc at base of fruit, usually falling with the mature nut) (1).
70. Perianth: deciduous from spikelet (0), persistent on spikelet (1).
71. Stamen number per flower (ordered).
72. Stamen filaments whether persistent around fruit: deciduous separately from fruit (0), persistent around fruit (1).
73. Anthers colour: anthers green-yellow (0), anthers red-brown (1).
74. Anther length excluding apical appendage (ordered).
75. Anther apical appendage length (ordered).
76. Anther apical appendage maximum width (ordered).
77. Stigma number (ordered).
78. Style base: not enlarged (0), enlarged (1).
79. Style base: deciduous (0), persistent (1).
80. Persistent style base length (ordered).
81. Persistent style base maximum width (ordered).
82. Fruit number per spikelet (ordered).
83. Fruit shape in the broadest lateral view: elliptic (0), obovate (1), ovate (2), subcircular-circular (3), lanceolate to narrow-oblong (4).
84. Fruit shape in cross-section: trigonous (1), subcircular to circular (2), biconvex (3), crescentiform (cf. species of *Cyathochaeta*) (4).
85. Fruit colour at maturity: white (1), red (2), brown (pale brown to dark brown) (3).
86. Fruit whether with tapered apex: without tapered apex (0), with tapered apex (cf. *Oreobolus oxycarpus*) (1).
87. Fruit whether with loose outermost layer (see Wilson 1993): without loose outermost layer (0), with loose outermost layer (cf. *Gymnoschoenus sphaerocephalus*) (1).
88. Fruit surface whether reticulate: not reticulate (0), reticulate (1).
89. Fruit surface whether rugose (see Bruhl 1995): not rugose (0), rugose (1).
90. Fruit surface whether punctulate: not punctulate (0), punctulate (1).
91. Fruit length (excluding stalk and persistent style base) (ordered).
92. Fruit maximum diameter (ordered).
93. Gynophore: absent (0), present (cf. *Mesomelaena graciliceps*) (1).
94. Fruit stalk length (ordered).

4.2.4 Cladistic Analyses

The data set including all ingroups and outgroups were analysed phylogenetically using PAUP* 4.0b8 (Swofford 2000) for Windows. The quantitative characters and qualitative characters were treated as ordered and unordered respectively in the analysis. The characters were polarized by the outgroup method. Heuristic searches were conducted using parsimony with TBR branch swapping. Random-taxon addition (1000 replicates) was employed to search for multiple islands of trees. Branch length for trees was calculated using the accelerated transformation optimization (ACCTRAN; the default). Bootstrapping analysis (Felsenstein 1985) using random addition 2000 replicates of fast bootstrap was performed in PAUP* to determine relative support for various clades found in the parsimony analysis. Jackknife analysis (Farris et al. 1996) using random addition 2000 replicates of fast-heuristic search with 37% of characters deleted per run was also performed in PAUP*. In this study, the support levels are defined as no support <50%, weak support 51% to 74%, moderate support 75 to 84%, and strong support 85 to 100% (e.g. Muasya et al. 1998; Oxelman et al. 1999) to assist the interpretation of the results and discussion.

After analysis, the position of the outgroup taxon *Rhynchospora* violated the initial assumption of ingroup monophyly (see Fig. 4.1). To investigate whether this result is affected by character polarity, i.e. outgroup taxa, an analysis excluding two species of *Scleria* and using only two species of *Rhynchospora* as outgroups was conducted using the same character coding and weighting and cladistic analysis methods described above.

4.3 Results

Maximum parsimony analysis of the full data set including all ingroups and outgroups resulted in one most parsimonious tree (Fig. 4.1), 6821 steps long, with a retention index of 0.6036 and consistency indices of 0.3272 including, and 0.3127 excluding, autapomorphies. To easily describe results, the major clades are labelled as A to H in Fig. 4.1.

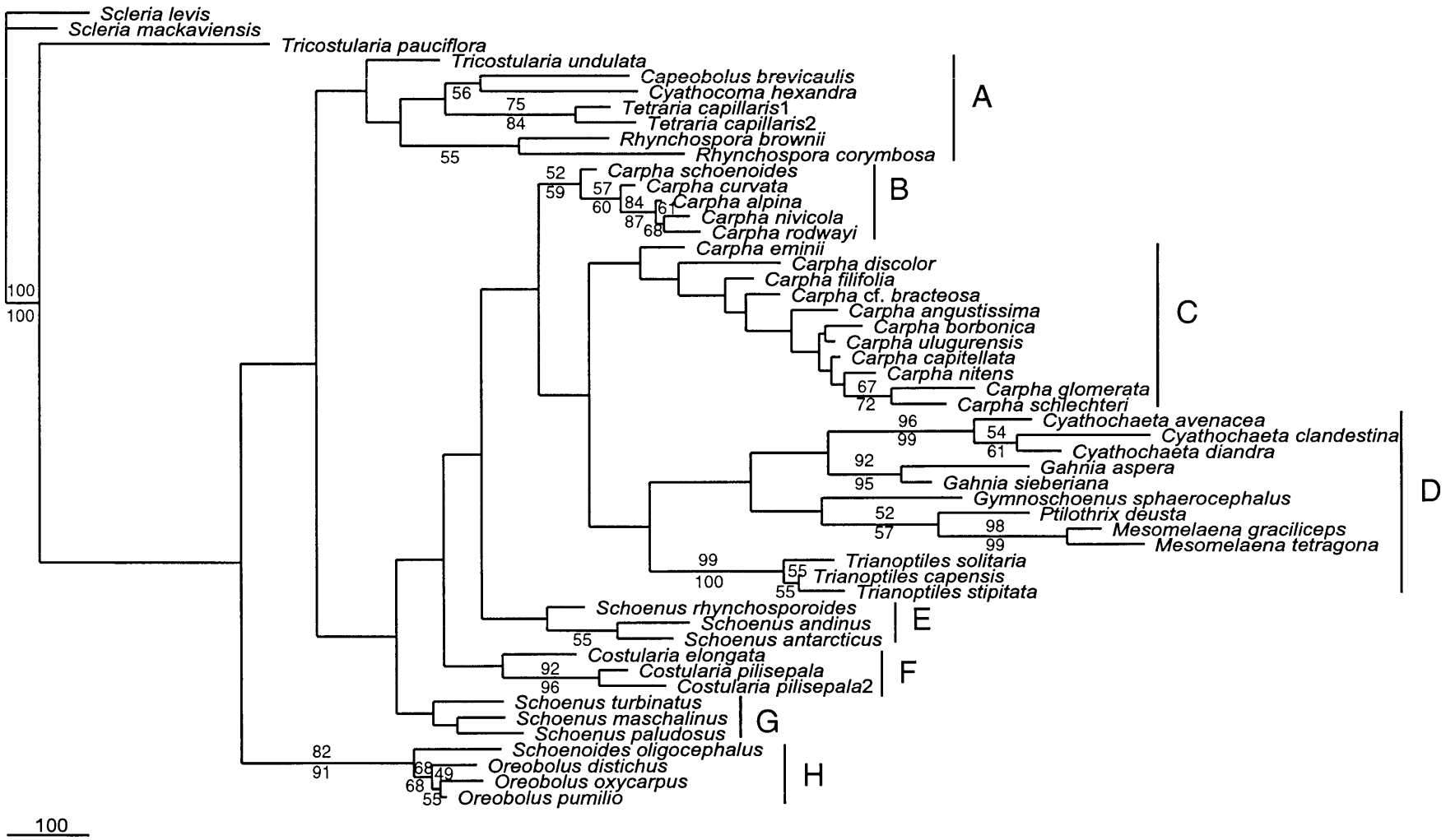

Fig. 4.1. The most parsimonious tree (length = 6821 steps, CI = 0.3127, RI = 0.6036) from the cladistic analysis included all ingroups (within Schoeneae) and outgroups (two species of *Scleria* and *Rhynchospora* respectively). Bootstrap values (> 50%), for 2000 replicate analyses, are presented above the branches. Jackknife values (> 50%), for 2000 replicate analyses, are presented below the branches. The major clades are labelled as A–H (for details see text).

The position of the outgroup taxon *Rhynchospora* violated the initial assumption of ingroup monophyly; it nested in Schoeneae. The clade formed by *Rhynchospora* and all the sampled species of Schoeneae has 100% bootstrap and jackknife support (Fig. 4.1).

The cladistic analysis of the data set that excluded two species of *Scleria* and used only two species of *Rhynchospora* as outgroups also resulted in one most parsimonious tree (Fig. 4.2), 6319 steps long, with a retention index of 0.6058 and consistency indices of 0.3298 including, and 0.3142 excluding, autapomorphies. This tree presented the same topology as in Fig. 4.1, but rooted with different taxa. The bootstrap and jackknife analyses also give essentially the same results as in Fig 4.1. Thus, all subsequent descriptions and discussions on bootstrap and jackknife values are based on the results presented in Fig. 4.1.

The bootstrap and jackknife analyses indicated that, while some clades were strongly supported, others were relatively to very weakly supported by the data (Figs 4.1, 4.2). All traditional genera sampled formed monophyletic clades except for *Carpha*, *Schoenus* and *Tricostularia*. Most of these clades, such as *Tetraria*, *Cyathochaeta*, *Gahnia*, *Mesomelaena* and *Trianoptiles*, had over 70% bootstrap and jackknife support. Only the *Costularia* clade was without support (Figs 4.1, 4.2).

Within clade A in Fig. 4.1, *Capeobolus* and *Cyathocoma* came together in a group (56% jackknife support), sister to which was *Tetraria* and then *Rhynchospora* (Figs 4.1, 4.2) but without support. The two specimens of *Tetraria capillaris* came together with 75% bootstrap and 84% jackknife support. Clade A in Fig 4.2 consists of *Capeobolus Cyathocoma* and *Tetraria* where *Rhynchospora* was used as an outgroup.

Carpha was separated into two clades, B and C (Figs 4.1, 4.2). Clade B consists of *C. alpina, C. curvata, C. nivicola, C. rodwayi* and *C. schoenoides* with 52% bootstrap and 59% jackknife support. Within this clade, the phylogenetic relationships among the five species were fully resolved with 57% or more bootstrap and jackknife support for each relationship (Figs 4.1, 4.2). *Carpha schoenoides* was sister to the rest of the species in this clade. *Carpha curvata* was closer to the other three species than was *C. schoenoides. Carpha alpina* was sister to the clade formed by *C. nivicola* and *C. rodwayi.*

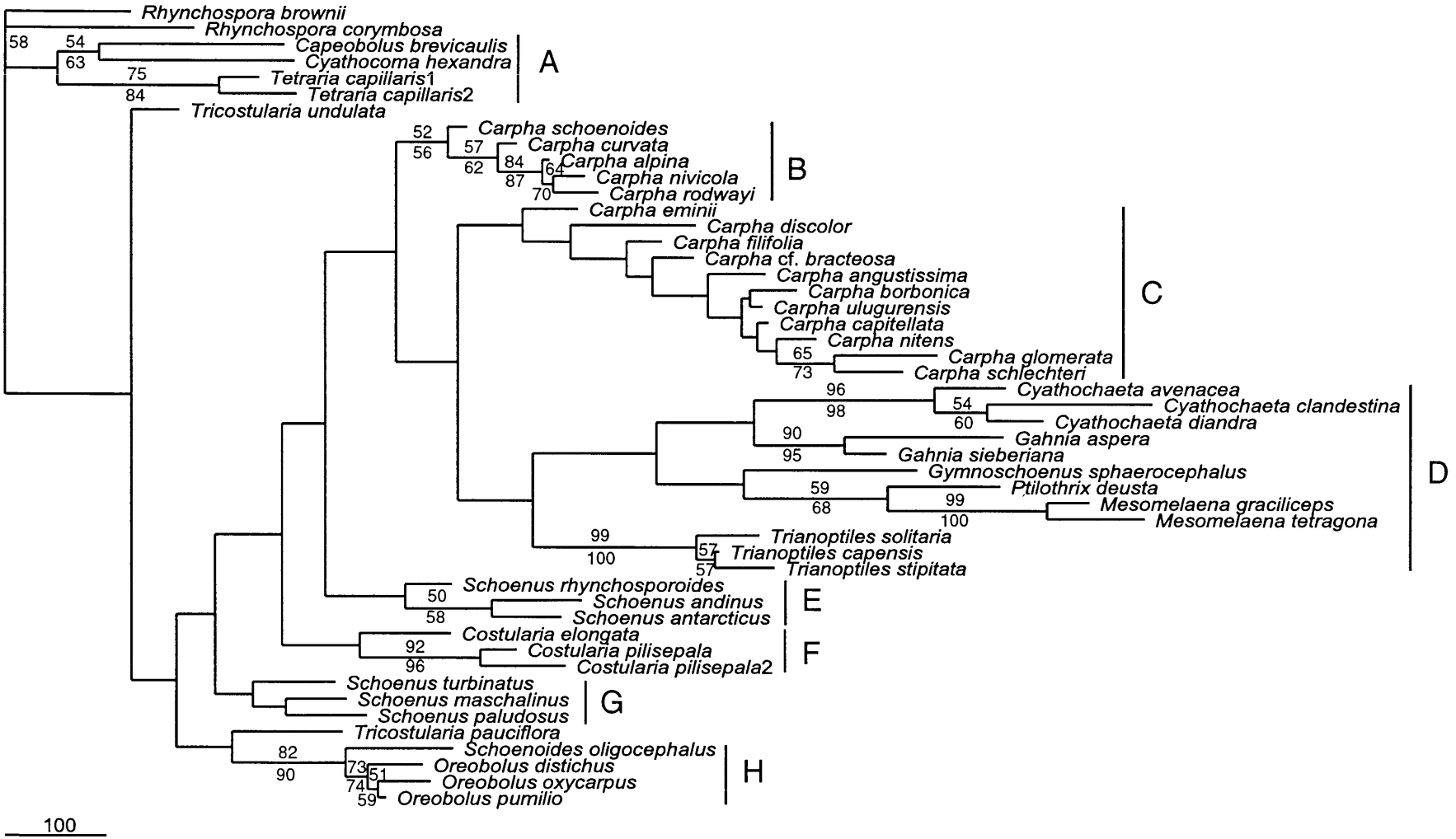

Fig. 4.2. The most parsimonious tree (length = 6319 steps, CI = 0.3142, RI = 0.6058) from the cladistic analysis included all ingroups (within Schoeneae) and outgroups (two species of *Rhynchospora*). Bootstrap values (> 50%), for 2000 replicate analyses, are presented above the branches. Jackknife values (> 50%), for 2000 replicate analyses, are presented below or on the right of the branches. The major clades (except A) are labelled the same as in Fig. 4.1 (for details see text).

Clade C was formed by all the other species of *Carpha*. The phylogenetic relationships among these species within clade C were no support except the relationship of *C. glomerata* and *C. schlechteri*, which were sister species with 67% bootstrap and 72% jackknife support. *Carpha capitellata* and *C. nitens* with *C. glomerata* and *C. schlechteri* formed a clade, and this was sister to the clade formed by *C. borbonica* and *C. ulugurensis*, and then, *C. angustissima*, *C.* cf. *bracteosa*, *C. filifolia* and *C. discolor* joined successively. *Carpha eminii* was basal to all other species within this clade.

According to the topologies (Figs 4.1, 4.2), clades D, E and F were closer to *Carpha* (i.e. clade B and C) than other clades but without support.

Within clade D (Figs 4.1, 4.2), *Ptilothrix* and *Mesomelaena* were sisters (52% bootstrap and 57% jackknife support), sister to which were *Gymnoschoenus* (no support) and then the clade formed by *Cyathochaeta* and *Gahnia* (also no support). *Trianoptiles* was sister to the clade formed by *Cyathochaeta, Gahnia, Gymnoschoenus, Mesomelaena* and *Ptilothrix*, but without support.

Species of *Schoenus* formed two clades E and G (Figs 4.1, 4.2) indicating that *Schoenus* was polyphyletic.

Species of *Costularia* formed clade F, but without support. Within this clade, *C. pilisepala*2 and *C. pilisepala* formed a well-supported (92% bootstrap and 96% jackknife support) clade.

Clade H was a well-defined monophyletic group formed by all the sampled species of *Schoenoides* and *Oreobolus* (82% bootstrap and 91% jackknife support), and within this clade *Schoenoides* was sister to *Oreobolus*.

The analyses (Figs 4.1, 4.2) also indicated that *Tricostularia* was polyphyletic, with its two representatives in different clades.

Performance of qualitative characters: character state changes for 57 qualitative characters were plotted on the most parsimonious tree of the full data set analysis (Fig. 4.3) to show character support for each clade.

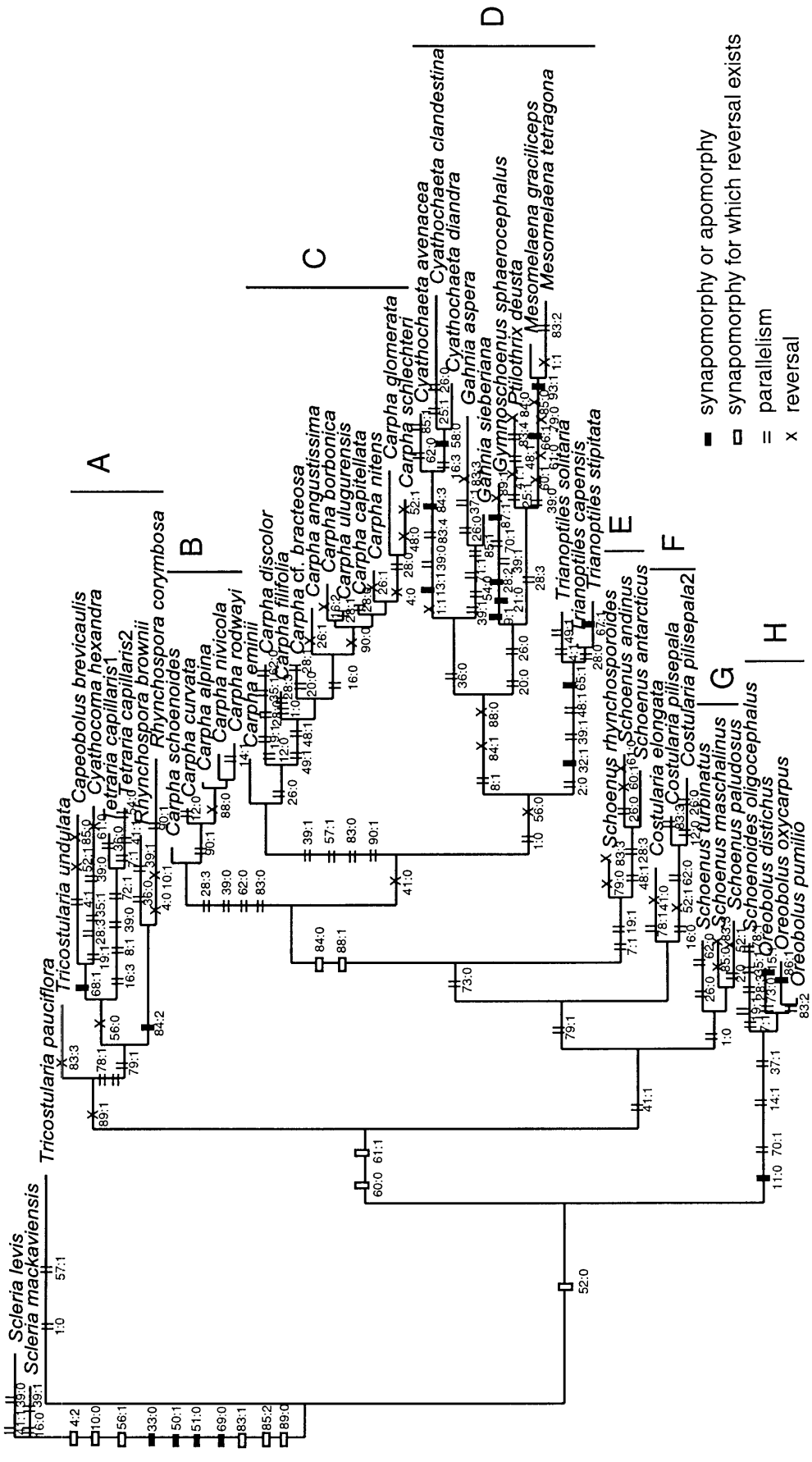

Fig. 4.3. The same most parsimonious tree from the cladistic analysis as in Fig. 4.1. The position of all character state changes for the 57 qualitative characters are indicated. Numbers are character:character state (Table 4.1).

4.4 Discussion

The morphological data show a high degree of homoplasy as indicated by CI values of 0.3127 (51 taxa) and 0.3142 (49 taxa) in the two analyses respectively of this study, which are among the lowest (indicative of a high level of homoplasy) of the values reported in previous studies with a comparable number of taxa (Givnish and Sytsma 1997; Lowrey et al. 2001). The high level of morphological homoplasy of Schoeneae in this study is consistent with a previous morphological analysis on Cyperaceae with a comparable number of taxa (Muasya et al. 2000). Muasya et al. (2000) reported a CI of 0.28 (64 taxa) in their analyses on Cyperaceae. The high level of morphological homoplasy of Schoeneae may explain the lack of strong support for some of the clades within this assemblage.

4.4.1 Tribal Limits

Goetghebeur (1986) and Bruhl (1995) divided the tribe Rhynchosporeae of Bentham (1883), Koyama (1961), Schultze-Motel (1964) and Hooper (1973) into three tribes: Arthrostylideae, Rhynchosporeae and Schoeneae (also see Fig. 1.1 in Chapter 1). Their Arthrostylideae is a small tribe of three monotypic genera (*Arthrostylis*, *Trachystylis* and *Trichoschoenus*) and one with three species (*Actinoschoenus*). Their Rhynchosporeae is composed of *Micropapyrus*, *Pleurostachys*, *Rhynchospora* and *Syntrinema*. The remaining genera of the tribe Rhynchosporeae of Bentham (1883), Koyama (1961), Schultze-Motel (1964) and Hooper (1973) are in Schoeneae (see Chapter 1 for these genera of Schoeneae). The cladistic analysis of Muasya et al. (1998) recovered the Rhynchosporeae of Goetghebeur (1986) and Bruhl (1995) as a monophyletic group using a sample of only *Rhynchospora* and *Pleurostachys*, and Schoeneae as a paraphyletic group because *Cladium* was sister to most genera in Cyperaceae. In their analysis, *Capeobolus*, *Cyathocoma* and *Tetraria* were not included. Goetghebeur (1998) combined the two tribes Arthrostylideae and Rhynchosporeae of Goetghebeur (1986) and Bruhl (1995) in Schoeneae.

The analysis of the full data set including all ingroups and outgroups has *Rhynchospora* nested in Schoeneae (Fig. 4.1). The clade formed by *Rhynchospora* and all sampled species of Schoeneae in Fig 4.1 is monophyletic with 100% bootstrap and jackknife support. There are four synapomorphies (character states 33:0, 50:1, 51:0 and 69:0; Table 4.1; Fig. 4.3) to

support this monophyletic clade. The cladistic analysis of the data set that excluded two species of *Scleria* and used only two species of *Rhynchospora* as an outgroup (Fig. 4.2) provided the same topology (i.e, the same relationship between species of *Rhynchospora* and species of Schoeneae) as in Fig. 4.1, indicating that the relationship between *Rhynchospora* and Schoeneae is not affected by outgroup taxa. Therefore, this study indicates that Schoeneae is not monophyletic if *Rhynchospora* is separated from this tribe; it provides support for Goetghebeur's (1998) inclusion of the Rhynchosporeae of Goetghebeur (1986) and Bruhl (1995) in Schoeneae. However, the current study does not focus on tribal limits, so the genera sampled are not enough to determine the tribal limits of Schoeneae. A further study on the Schoeneae of Goetghebeur (1998) is needed to determine the tribal limits.

4.4.2 Generic Limits and Relationships

4.4.2a Generic Limits

The present analyses (Figs 4.1, 4.2) indicate that, except for *Carpha*, *Schoenus* and *Tricostularia*, all currently recognized genera sampled form monophyletic clades. Most of these clades have strong bootstrap and jackknife support.

Carpha is paraphyletic and forms two clades B and C (Figs 4.1, 4.2) in the present analyses, supporting the separation of *Carpha* into two genera, i.e. *Carpha sensu stricto* (Hooker 1860, 1867; Bentham 1878, 1883; Wilson 1986, 1993, 1994a, 1994b) and *Asterochaete* (Nees 1834; Kunth 1837; Steudel 1855; Levyns 1950). Clade B includes the five species of *Carpha sensu stricto* (Hooker 1860, 1867; Bentham 1878, 1883; Wilson 1986, 1993, 1994a, 1994b). This clade is supported by 52% bootstrap and 59% jackknife support (Figs 4.1, 4.2), and character states 28:3, 39:0, 62:0 and 83:0 (Table 4.1; Fig. 4.3). Although these character states are parallelisms, they are shared by all members of this clade. Clade C is consistent with the definition of *Asterochaete* (Nees 1834; Kunth 1837; Steudel 1855; Levyns 1950) and includes the remaining species of *Carpha sensu lato*, but without support. The supported character states for this clade are 39:1, 57:1, 83:1 and 90:1 (Table 4.1; Fig. 4.3). Although these character states are also parallelisms, they are shared by all members of this clade.

Schoenus is a large genus and the character state that has been used to distinguish this genus from other genera is having the internodes above the fertile nodes of the 'rachilla' elongated and prominently zigzag (Clarke 1902; Kern 1974; Wilson 1993; Goetghebeur 1998). However, this character state (48:1; Table 4.1) is homoplasious in the analyses (Fig. 4.3), and is not shared by all species of *Schoenus*. Three species in the present analyses, *Schoenus rhynchosporoides* (also see Fig 2.6 b-c for 'rachilla'), *S. paludosus* and *S. turbinatus*, do not have elongated and prominently zigzag upper internodes of the 'rachilla'. The present study indicates that the genus is polyphyletic (Figs 4.1, 4.2). Thus, to reliably resolve the relationships and determine the limits of *Schoenus*, more thorough sampling and further analyses are required.

Tricostularia is distinguished from other genera by its deciduous scale perianth and lower flower functionally male (Kern 1974; Wilson 1993; Goetghebeur 1998). In this study, the character state of spikelets with a male flower (character state 52:1; Fig. 4.3) appears to be a reversed parallelism and occurs in *Capeobolus brevicaulis, Carpha schlechteri, Costularia pilisepala, Schoenus paludosus and Tricostularia pauciflora*. This character state is not shared by all species in *Tricostularia*. *Tricostularia undulata* does not have male flowers at all. A perianth of scales (character state 60:1; Fig. 4.3) is not a unique character for *Tricostularia* either, and is not shared by all members of the genus; for example, the perianth of *Tricostularia undulata* is composed of bristles rather than scales, although there are conflicting descriptions. Bentham (1878) described the perianth members of *T. undulata* as hypogynous bristles while Kern (1974) called them scales. According to the definitions in this study (see character 60 in appendix 2), the perianth members of *T. undulata* are composed of bristles. Bruhl (1995, p. 210) noted that 'the generic limits of *Tricostularia* warrant further attention'. The present analyses (Figs 4.1, 4.2) indicate that *Tricostularia* is polyphyletic. More sampling and further studies are needed to define its limits and clarify its phylogenetic relationships.

Koyama (1961) sank *Costularia* in *Tetraria*, and this was followed by Gordon-Gray (1995). The present study showed both genera formed well-separated clades (Figs 4.1, 4.2). This result is consistent with previous cladistic analyses (Goetghebeur 1986; Bruhl 1995) and anatomical studies (Metcalfe 1971), and indicates that both genera should be maintained.

The South African endemic species *Capeobolus brevicaulis* was previously treated as *Costularia brevicaulis* (Clarke 1897–1898; Kükenthal 1939b; Browning and Gordon-Gray 1996a) or *Tetraria brevicaulis* (Clarke 1894; Levyns 1947, 1950). It was amalgamated with *Costularia* in Seberg's (1986, 1988b) cladistic analyses, but it failed to pair with *Tetraria* or *Costularia* in Bruhl's (1995) analyses. Browning and Gordon-Gray (1999, p. 218) established a new genus, *Capeobolus*, for it because it differs from *Costularia* and *Tetraria* by its 'low growth form, reduced cryptic inflorescence, short non-plumose perianth outgrowths and shape and positioning of the embryo within the fruit'. The present study indicates this species is sister to *Cyathocoma* and does not group with *Tetraria* or *Costularia* directly. This result is consistent with Bruhl's (1995) analyses, i.e. it indicates support for the separation of *Capeobolus brevicaulis* from *Costularia* and *Tetraria*.

Schoenoides oligocephalus was first described by Curtis (1984) as *Oreobolus oligocephalus*. It resembles *O. pumilio* in habit. Later, Seberg (1986) separated it from *Oreobolus* and set up a monotypic genus *Schoenoides* for it because it differs from *Oreobolus* in that it has almost capitate inflorescences and usually has two flowers per spikelet, rarely three or one, rather than a single flower per spikelet. Recently Goetghebeur (1998) merged *Schoenoides* back into *Oreobolus* again. The results in this study are consistent with both hypotheses. *Schoenoides* forms the sister clade to *Oreobolus*; this supports Seberg's (1986) results. *Schoenoides* and *Oreobolus* together form a single well-supported clade (82% bootstrap and 91% jackknife support) that supports the merging of *Schoenoides* into *Oreobolus*.

Although two specimens of *Tetraria capillaris* grouped together as expected with 75% bootstrap and 84% jackknife support, the evident differences in their morphology suggest a further study, especially phenetic analysis with more samples, to determine species limits. Similarly, *Costularia pilisepala* and *C. pilisepala*2 formed a well-supported (92% bootstrap and 96% jackknife support) clade as expected, but heterogeneities in their morphology might suggest a further study using phenetic analysis with more samples to determine species limits.

4.4.2b Generic Relationships

Within clade A, *Capeobolus* and *Cyathocoma* formed a clade with 56% jackknife support and with their synapomorphy 68:1 (Fig. 3; Table 4.1), sister to which is *Tetraria* (Figs 4.1, 4.2). All three species have flowers with perianth members in one whorl (or without perianth) (character state 56:0; Fig. 4.3; Table 4.1). *Tetraria* being closer to *Cyathocoma* is consistent with the results of Goetghebeur's (1986) analysis.

Within clade D (Figs 4.1, 4.2), *Cyathochaeta* is sister to *Gahnia*, as was indicated by Bruhl's (1995) analyses, supported here by character state 36:0 (Fig. 4.3; Table 4.1). *Gymnoschoenus, Ptilothrix* and *Mesomelaena* formed a clade supported by character states 20:0 and 26:0 (Fig. 4.3; Table 4.1), and this is consistent with Goetghebeur's (1986) analysis. The close relationship of these three genera was previously recognized by Bentham (1878), who included the two monotypic genera *Gymnoschoenus* and *Ptilothrix* in *Mesomelaena*, and Kükenthal (1939d, 1940a). Two clades formed by *Cyathochaeta* + *Gahnia,* and *Gymnoschoenus* + *Mesomelaena* + *Ptilothrix* are sister in the present analyses. This agrees with Goetghebeur's (1986) analysis with the exception of *Gahnia*, which failed to form a clade with the other four genera in his analysis.

Clade D forms a clade with *Carpha* (clades B and C) supported by character states 84:0, 88:1 (synapomorphy for which reversals exist; Fig 4.3; Table 4.1). Although without bootstrap and jackknife support, the topologies indicate that the genera *Trianoptiles*, *Gymnoschoenus, Mesomelaena, Ptilothrix, Cyathochaeta* and *Gahnia* within Clade D are close to *Carpha.* That *Trianoptiles* has a close relationship with *Carpha* agrees with the analyses of Goetghebeur (1986) and Bruhl (1995). *Trianoptiles* was once treated as a subgenus of *Carpha* by Kükenthal (1939c). The close relationship of *Gymnoschoenus* and *Mesomelaena* with *Carpha* was supported by previous observations of Kükenthal (1940a). The close relationship of *Ptilothrix* (*Ptilanthelium* auct.) with *Carpha* was previously recognized by Kükenthal (1939d) and Bruhl (1995). Many systematists (Brown 1810; Kunth 1837; Steudel 1855; Boeckeler 1874; Clarke 1908, 1909; Pfeiffer 1931) included the single species of *Ptilothrix* in *Carpha.*

One of the clades (E) formed by some species of *Schoenus* is close to *Carpha* although without bootstrap and jackknife support, while the other clade (G) formed by the remaining

species of *Schoenus* is more distant from *Carpha* (Figs 4.1, 4.2). Clarke (1902) and Kükenthal (1939c) previously noted that *Carpha* had a close relationship with *Schoenus*. Clarke (1902, p. 483) wrote that 'This genus differs from *Schoenus* only by the lowest nut-bearing glume having the next glume close over it, not separated by an elongate curved joint of the rachilla as is the case in *Schoenus*'. In fact, the three species in clade E were once included in *Carpha* (Philippi 1857–58, 1873; Clarke 1901; Pfeiffer 1927; also see Chapter 1). This study indicates that some species (clade E) in the polyphyletic genus *Schoenus* have a closer relationship to *Carpha*.

Costularia (clade F) is a close relative of *Carpha* (Figs 4.1, 4.2) and this agrees with the result of Goetghebeur (1986). Kükenthal (1939c, p.101) recognized this and wrote 'Die Blütenverhältnisse zeigen annähernd dasselbe Bild wie bei *Costularia*. ...Wie *Costularia* hat *Carpha* fast durchweg gestauchte, gerade und schmale Scheinachsen mit dicht übereinanderstehanden Gipfelblüten.' (The floral features show nearly the same picture as in *Costularia*. ...Like *Costularia*, *Carpha* has nearly always congested, straight and narrow pseudo-axes with closely superposed terminal flowers.)

In contrast with the analyses of Goetghebeur (1986) and Bruhl (1995), the topologies indicate that *Oreobolus* and *Schoenoides* are more distant from *Carpha* than are *Costularia, Cyathochaeta, Gahnia, Gymnoschoenus, Mesomelaena, Ptilothrix, Schoenus* and *Trianoptiles* (Figs 4.1, 4.2). This is not surprising because these two genera have very different features from *Carpha*, especially in the morphology of their flowers.

Although the topologies present many aspects of relationships of *Carpha* and its relatives to be in agreement with previous studies, the weak support or lack of support on some clades requires other sources of data, such as molecular data (DNA sequences) and embryo morphology, to re-evaluate and test these relationships.

4.4.3 Relationships within *Carpha*

Species of *Carpha* formed two paraphyletic clades, B and C (Figs 4.1, 4.2). Within clade B, the phylogenetic relationships of five species *C. alpina, C. curvata, C. nivicola, C. rodwayi* and *C. schoenoides* were fully resolved and with more than 50% support (Figs 4.1, 4.2).

Within clade C, although the phylogenetic relationships among these species were fully resolved, there is no support within this clade except for the relationship of *C. glomerata* and *C. schlechteri*, which are sister species with 67% bootstrap and 72% jackknife support. This shows that the morphological data presented here do not provide strong evidence to support recognition of these relationships. Thus, other sources of data, such as molecular data (DNA sequences) or ontogenetic development data, are obviously needed to re-evaluate and test the relationships of these species.

4.5 Conclusions

This study represents the first detailed cladistic analysis of *Carpha* and its relatives. The results allow several important conclusions.

1. The morphological data showed a high degree of homoplasy, which may explain the lack of strong support for some of the clades within this assemblage.
2. The present analyses show *Rhynchospora* nested in Schoeneae (Fig. 4.1). *Rhynchospora* and all sampled species of Schoeneae formed a monophyletic group with 100% bootstrap and jackknife support. This indicates that Schoeneae is not monophyletic if *Rhynchospora* is separated from it. However, the current study does not focus on tribal limits, so the genera sampled are not enough to determine the tribal limits of Schoeneae. A further study on the Schoeneae of Goetghebeur (1998) is needed to determine the tribal limits.
3. *Carpha* is paraphyletic and formed two clades B and C (Figs 4.1, 4.2) in the present analyses. Clade B is consistent with the definition of *Carpha sensu stricto* (Hooker 1860, 1867; Bentham 1878, 1883; Wilson 1986, 1993, 1994a, 1994b). Clade C is consistent with the definition of *Asterochaete* (Nees 1834; Kunth 1837; Steudel 1855;

Levyns 1950). Thus, the present analyses supports dividing *Carpha* into two genera, i.e. *Carpha sensu stricto* and *Asterochaete*.

4. The present analyses (Figs 4.1, 4.2) indicate that *Schoenus* and *Tricostularia* are polyphyletic. More samples and further studies are needed because sample size in this study is too small to define their limits and clarify their phylogenetic relationships.

5. This study supports separation of *Capeobolus brevicaulis* from *Costularia* or *Tetraria*.

6. This study is consistent with either separation of *Schoenoides* from *Oreobolus* or combination of these two genera (Figs 4.1, 4.2).

7. The study also indicates relationships between *Carpha* and its relatives, but most of these relationships have no support, although many aspects of these relationships are in agreement with previous studies. Lack of support for these clades highlights the need for other sources of data, such as molecular data (DNA sequences) and embryo morphology, to re-evaluate and test these relationships.

8. The phylogenetic relationships of the five species *C. alpina, C. curvata, C. nivicola, C. rodwayi* and *C. schoenoides* were fully resolved (Figs 4.1, 4.2).

9. Although the phylogenetic relationships within clade C (all other species of *Carpha*) were fully resolved, lack of support within this clade except for the relationship of *C. glomerata* and *C. schlechteri*, indicates that other sources of data, such as molecular (DNA sequences) and ontogenetic development data, are needed to re-evaluate and test the relationships of these species.

Chapter 5 Phylogenetic Relationships of *Carpha* and its Relatives (Schoeneae, Cyperaceae) Inferred from Chloroplast *trn*L Intron and *trn*L-*trn*F Intergenic Spacer Sequences

5.1 Introduction

Molecular data are helpful in reconstructing phylogenetic relationships. Chloroplast DNA has proven to be well suited for evolutionary and phylogenetic studies, and has been a focus of research in plant molecular evolution and systematics (Clegg and Zurawski 1992; Clegg et al. 1994; Olmstead and Palmer 1994; Sang et al. 1997; Palmer et al. 1998; Kelchner 2000; Brouat et al. 2001). Among the different approaches using chloroplast DNA in plant molecular systematics, DNA sequencing has become one of the most used for inferring phylogenies (Clegg and Zurawski 1992; Clegg et al. 1994; Olmstead and Palmer 1994; Hillis et al. 1996; Bayer and Starr 1998; Brouat et al. 2001). The most common chloroplast gene used to provide sequence data for cladistic analyses in plants is the large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbc*L) (Clegg et al. 1994; Olmstead and Palmer 1994; Steele and Vilgalys 1994). Molecular data from the *rbc*L gene have been used successfully to resolve phylogenetic relationships at family or higher taxonomic levels (e.g. Zurawski et al. 1984; Chase et al. 1993). In combination with other sequences, *rbc*L has been useful at lower taxonomic levels (Muasya et al. 2001; Muasya et al. 2002). However, when used alone *rbc*L appears to be less suitable at lower taxonomic levels than more rapidly evolving genes, introns and spacers such as the non-coding region of the chloroplast DNA *trn*L intron and *trn*L-*trn*F intergenic spacer (Taberlet et al. 1991; Gielly and Taberlet 1994, 1996).

The *trn*L intron and *trn*L-*trn*F intergenic spacer exhibit a higher level of sequence variation among closely related species than the coding region, and vary in length and substitution rates (Taberlet et al. 1991; Clegg et al. 1994; Kelchner and Clark 1997; McDade and Moody 1999). Therefore, they are more useful at lower taxonomic levels (Gielly and Taberlet 1994). The *trn*L intron and *trn*L-*trn*F intergenic spacer have three advantages over the other commonly used gene regions: (1) they are easy to amplify across a wide taxonomic range because the 'universal' primers designed by Taberlet et al. (1991) are available; (2) the primers used to amplify the region can also be used to sequence it

entirely; and (3) the numerous large indels provide additional phylogenetic information (Bayer and Starr 1998). Moreover, the *trn*L intron and *trn*L-*trn*F intergenic spacer have demonstrated they are highly phylogenetically informative at generic, species and infraspecific levels (Gielly and Taberlet 1994, 1996; Bayer and Starr 1998; McDade and Moody 1999; Lledo et al. 2000; Brouat et al. 2001). Within Cyperaceae, the *trn*L intron and *trn*L-*trn*F intergenic spacer are also informative and have been used effectively in addressing systematic questions in the tribe Cariceae (Yen and Olmstead 2000a, 2000b; Roalson et al. 2001). Therefore, the *trn*L intron and *trn*L-*trn*F intergenic spacer were chosen as the molecular data source in this study.

Given strictly limited time and funds available for this study, the aims are to use the *trn*L intron and *trn*L-*trn*F intergenic spacer to:

- estimate the phylogeny of *Carpha* and its relatives;
- define generic limits of *Carpha*; and
- estimate the phylogeny of species within *Carpha.*

5.2 Materials and Methods

5.2.1 Plant Samples

Sampling was designed to include all the species of *Carpha* for which material could be obtained and species of the genera considered by various authors to be related to *Carpha.* The 35 specimens of 25 species sampled are listed in Table 5.1. These included 17 specimens of eight species of *Carpha*, 11 specimens of 10 species of genera thought to be close to *Carpha—Costularia, Oreobolus, Ptilothrix, Schoenoides, Schoenus, Trianoptiles* (Clarke 1897–1898, 1902; Kükenthal 1939c, 1939d; Goetghebeur 1986; Bruhl 1995)—and five species from genera more distant from *Carpha* within the tribe Schoeneae (Goetghebeur 1986; Bruhl 1995). Two species of *Rhynchospora* in tribe Rhynchosporeae (Bruhl 1995), which is considered to be closely related to Schoeneae (Bentham 1883; Koyama 1961; Schultze-Motel 1964; Hooper 1973; Goetghebeur 1998; also see Fig. 1.1), were included as outgroups.

Table 5.1. Taxa included in molecular cladistic analyses of *Carpha* and its relatives. The herbarium abbreviation and sheet number, location, first collector, collection number, and date of collection are given.

Taxon	Herbarium abbreviation & number	Location	Collector & No.	Collection date
Carpha alpina	NE 71804	Mt Field National Park, Tasmania, Australia	J. J. Bruhl 1878B	15 Feb. 2000
Carpha alpina	NE 71813	Mt Field National Park, Tasmania, Australia	J. J. Bruhl 1880B	15 Feb. 2000
Carpha alpina	NE 72986	West of Snowy River, 1km from Snowy River bridge, Koscuiszko National Park, NSW, Australia	X. Zhang 13	24 Jan. 2000
Carpha capitellata	NE 76468	Roadside seepage to farm dam, 200m on Langkloof road off route 323 between Riversdale and Ladismith, Cape Province, S. Africa	J. J. Bruhl 1718	6 Dec. 1996
Carpha capitellata var. *bracteosa*	NE 80080	Roadside, route 323 between Riversdale and Ladismith, Cape Province, S. Africa	J. J. Bruhl 1725	6 Dec. 1996
Carpha curvata	NE 71843	Mt Field National Park, Tasmania, Australia	J. J. Bruhl 1894	16 Feb. 2000
Carpha curvata	NE 71847	Mt Field National Park, Tasmania, Australia	J. J. Bruhl 1896C	16 Feb. 2000
Carpha filifolia	NE 76454.	Giant's Castle, Drakensberg, Kwa-Zulu Natal, S. Africa	J. J. Bruhl 1700	24 Nov. 1996
Carpha glomerata	NE 76453	Vlei to N side of Robinson's Fall Ck., at top of Falls, E. Cape, S. Africa	J. J. Bruhl 1706	1 Dec. 1996
Carpha glomerata	NE 76469	Along stream at beginning of Bristalkloof trail off route 323 between Riversdale and Ladismith, Cape Province, S. Africa	J. J. Bruhl 1719	6 Dec. 1996
Carpha glomerata	NE 76464	Along R102, N of Witelsbos, E. Cape, S. Africa	J. J. Bruhl 1711	4 Dec. 1996
Carpha glomerata	NE 76460	Along R102, S of Witelsbos, E. Cape, S. Africa	J. J. Bruhl 1712	4 Dec. 1996
*Carpha nitens**	K *s.n.*	Brulé-Sentier de la Roche Ecrite, Réunion	M. J. E. Coode 4186	25 Nov. 1973
Carpha nivicola	NE 70646	75m W of bridge near Snowy River, Kosciuszko National Park, NSW, Australia	J. J. Bruhl 1868A	23 Dec. 1999
Carpha nivicola	NE 72984	W of Snowy River, 1km NW of Snow River bridge, Kosciuszko National Park, NSW, Australia	X. Zhang 11	24 Jan. 2000
Carpha rodwayi	NE 71816	Mt Field National Park, Tasmania, Australia	J. J. Bruhl 1881B	15 Feb. 2000
Carpha rodwayi	NE 71834	Mt Field National Park, Tasmania, Australia	J. J. Bruhl 1890A	16 Feb. 2000

*DNA of *Carpha nitens* was provided by Royal Botanic Gardens Kew.

Table 5.1. (Continued)

Taxon	Herbarium abbreviation & number	Location	Collector & No.	Collection date
Costularia arundinacea	NSW 479976	Rivière Bleu Provincial Park, New Caledonia	K. L. Wilson 9935	12 June 2001
Costularia nervosa	NSW 479994	Col de Plum, New Caledonia	K. L. Wilson 9939	12 June 2001
Costularia pubescens	NSW 479997	Col de Plum, New Caledonia	K. L. Wilson 9940	12 June 2001
Cyathochaeta diandra	NE 72997	South Coast, NSW, Australia	X. Zhang 24	26 Jan. 2000
*Gahnia clarkei***	NSW *s.n.*	Cult. Royal Botanic Gardens Sydney; wild souce: Cowan Ck., 1.8 km E. of Berowra Railway Stn., NSW, Australia	A. Rodd 1621	6 Mar. 1971
Gahnia sieberiana	NSW 472135	c. 100 m along Darkes Forest road from Wollongong-Sydney road, NSW, Australia	K. L. Wilson 9913	21 May 2001
Gymnoschoenus sphaerocephalus	NE 72981	New England National Park, Northern Tablelands, NSW, Australia	X. Zhang 8	13 Dec. 1999
Oreobolus distichus	NE 72990	Kosciuszko National Park, NSW, Australia	X. Zhang 17	24 Jan. 2000
Oreobolus pumilio	NE 72985	Kosciuszko National Park, NSW, Australia	X. Zhang 12	24 Jan. 2000
Ptilothrix deusta	NE 70548	Single National Park, Northern Tablelands, NSW, Australia	X. Zhang 1	12 Nov. 1999
Schoenoides oligocephalus	NE 71832	Mt Field National Park, Tasmania, Australia	J. J. Bruhl 1889A	16 Feb. 2000
Schoenus paludosus	NSW 441060	c. 0.5 km S of Darkes Forest road on 10A Management Trail, Dharawall State Recreation Area, NSW, Australia	K. L. Wilson 9858	12 Feb. 2000
Schoenus turbinatus	NSW *s.n.*	3–4 km from Great Western Highway, Mount Victoria, NSW	L. McLaughlin 35	19 Feb. 2001
Trianoptiles solitaria	NE 80084	Rondebosch, Cape Province, S. Africa	J. J. Bruhl 1756	21 Dec. 1996
Trianoptiles solitaria	NE72056	Municipal Park alongside Winfield Road, North Balwyn, Gippsland Plain, Victoria, Australia	J. R. Hosking 1765	3 Oct. 1999
Tricostularia pauciflora	NSW 472132	Junction of Mt Keira and Picton/Wilton roads, NSW, Australia	K. L. Wilson 9910	21 May 2001
Outgroups				
Rhynchospora brownii	NSW 472131	Menangle Park area, just N of junction of Fitzpatrick Street and Cummins Road, NSW, Australia	K. L. Wilson 9909	21 May 2001
Rhynchospora corymbosa	NE *s.n.*	1.9 km N of Rossville near small creek, Qld, Australia	K. L. Clarke 75	23 June 1998

**Sequence of the *trn*L intron and *trn*L-*trn*F intergenic spacer region of *Gahnia clarkei* was provided by Dr A. Marchant.

Four samples of *Carpha glomerata*, three samples of *Carpha alpina*, and two each of *C. capitellata, C. curvata, C. nivicola, C. rodwayi* and *Trianoptiles solitaria* from different localities were used to examine the length polymorphism of simple sequence repeats found in the *trn*L intron and *trn*L-*trn*F intergenic spacer. Variation between specimens in the *trn*L intron and *trn*L-*trn*F intergenic spacer did not affect species positions in the most parsimonious and maximum likelihood trees because the trees did not change when alternative samples of these species were used in analyses. Even when all samples were used, species position in the most parsimonious and maximum likelihood trees still did not change (Appendices 6 and 7). Therefore, of these species with more than one sample, only the samples *J. J. Bruhl 1719* (*Carpha glomerata*), *J. J. Bruhl 1880B* (*Carpha alpina*), *J. J. Bruhl 1718* (*C. capitellata*), *J. J. Bruhl 1894* (*C. curvata*), *J. J. Bruhl 1868A* (*C. nivicola*), *J. J. Bruhl 1890A* (*C. rodwayi*) and *J. R. Hosking 1765* (*Trianoptiles solitaria*) were used in cladistic analyses.

5.2.2 Plant Material, DNA Isolation, PCR Amplification, Sequencing and Sequence Alignment

5.2.2a Plant Material

Healthy leaves and/or culms for DNA isolation were obtained, either:

1) fresh,
2) after being dried with silica gel (Chase and Hills 1991), or
3) preserved in CTAB solution (Rogstad 1992) modified by Thomson (2002).

5.2.2b DNA Isolation

DNA was isolated from 0.65–1.0 g fresh material or material preserved in CTAB solution or from 0.1–0.18 g silica gel material according to the following procedure.

- The material was ground to a fine powder in a mortar with about half a teaspoon of sand, and liquid nitrogen was added when the fresh material or material preserved in CTAB solution was ground.
- The powder was transferred to a 50 ml centrifuge tube containing 40 ml ice-cold rinse buffer (50 mM Tris, 100 mM EDTA, 100 mM NaCl, 1% PVP, pH 7.5), then mixed and stored in refrigerator at 4°C for at least 30 minutes.

- The mixture was centrifuged in a cooled centrifuge (Sigma 4K-10 centrifuge, rotor #11140) (5°C, 10 mins at 5000 rpm = 4400×g), and the supernatant was discarded.
- 10 ml preheated (65°C) digestion buffer (50 mM Tris, 100 mM NaCl, 100 mM EDTA, 0.5% SDS, pH 7.5) was added to the sediment, and mixed, and then incubated at 65°C for 30 mins, with gentle shaking at 5 min intervals.
- 3 ml of 3 M potassium acetate was added.
- The mixture was then stored in ice/water for half an hour.
- The mixture was centrifuged at 5°C at 5000 rpm for 15 mins.
- The supernatant was transferred to a new 50 ml tube, to which twice the supernatant volume of cold 95% ethanol (-20°C) was added. Usually an obvious nucleic acid precipitate should appear, but for my material it did not, so the sample was placed in a freezer (-20°C) overnight, then centrifuged at room temperature at 5000 rpm for 30 mins.
- The supernatant was discarded.
- The pellet was washed with 70% ethanol: added to 10 ml of 70% ethanol, and left for 10 mins, after which it was centrifuged at room temperature for 7 mins at 5000 rpm.
- The supernatant was discarded, and the pellet was dried by putting the tube upside down on a paper hand towel until all visible traces of ethanol had gone. The DNA was dissolved in 500 µl of Tris-EDTA buffer (TE: 10 mM Tris, 1 mM EDTA, pH 7.6).

5.2.2c DNA Purification

The "Dicalite" method of Gilmore et al. (1993) modified by A. Marchant (unpublished) was used for DNA purification:

- 1500 µl of binding buffer (50 mM Tris, 6 mM $NaClO_4$, 1 mM EDTA, pH 7.5, warmed to room temperature before use) was added to DNA solution, mixed and allowed to stand for 20 mins.
- 300 µl of diatomite (Diatomaceous earth from Sigma #D-3877, suspended in water, according to the protocol of Gilmore et al. 1993) was added, and the contents was mixed for 20–30 mins by regular gentle inversion to allow the DNA to bind to the diatomite.

- The mixture was centrifuged at room temperature at 3000 rpm (= 1600 ×g) for 5 mins, the supernatant was discarded, and the tube was put upside down on a paper hand towel.
- 1.5 ml of wash buffer 1 (3 vols binding, 1 vol water, warmed to room temperature before use) was added and shaken. The mixture was centrifuged at room temperature at 3000 rpm for 5 mins. The supernatant was discarded, and the tube was put upside down on a paper hand towel.
- 1.5 ml of wash buffer 2 (1 vol 40 mM Tris, 4 mM EDTA, 0.8 M NaCl, 1 vol 95% ethanol, warmed to room temperature before use) was added and shaken. The mixture was centrifuged at room temperature at 3000 rpm for 5 mins. The supernatant was discarded, and the tube was put upside down on a paper hand towel. This step was repeated.
- 300 µl TE buffer (10 mM Tris, PH 8.0, 1 mM EDTA) was added and shaken, then the mixture was incubated, with regular inversion, at 40–50°C for 20–30 mins.
- The mixture was centrifuged at 3000 rpm for 5 mins, and the supernatant was collected as much as possible into a 2 ml microfuge tube.
- 200 µl TE buffer was added to the pellet, shaken, then centrifuged at 3000 rpm for 5 mins. All of the supernatant was collected into the same 2 ml microfuge tube.
- 10 µl of the sample (i.e. the supernatant) were run on the gel at this stage and the sample should be stored in a freezer (at -20°C).

5.2.2d PCR Amplification

Amplifications of the *trn*L intron and *trn*L-*trn*F intergenic spacer were accomplished via the polymerase chain reaction (PCR). The primers used for amplification are listed in Table 5.2. For the PCR amplification, each reaction mixture (25 µl) was composed of 2.5 µl of 10x Taq buffer (Promega #M1910), 1.5 µl of 25 mM $MgCl_2$, 2 µl of 4dNTPs 2.5 mM each, 5 µl of each of two primers (2 µM), 0.1 µl of Taq polymerase (Promega #1661) and 1 µl of genomic DNA template (10–100 ng).

Double-stranded DNA amplifications were performed in a HYBAID Omm-E Thermal Sequencer. The conditions for PCR were: 5 mins at 94°C for initial denaturation, followed by 30 cycles of 30 sec at 94°C for denaturation, 30 sec at 60°C for annealing, 60 sec at 72°C for primer extension and then held at 24°C (Kidd and Ruano 1995).

Table 5.2. List of primers for amplification and sequencing of the *trn*L intron and *trn*L-*trn*F intergenic spacer.

Primer	5' to 3' primer sequence	Designer
PCR primers		
B49317	CGAAATCGGTAGACGCTACG	Taberlet et al. 1991
A50272	ATTTGAACTGGTGACACGAG	Taberlet et al. 1991
A49855	GGGGATAGAGGGACTTGAAC	Taberlet et al. 1991
CalTabF	GTCCTCTGCTCTACCAACTG	Andrew Perkins (NSW; unpublished)
Sequencing Primers		
A50272	ATTTGAACTGGTGACACGAG	Taberlet et al. 1991
A49855	GGGGATAGAGGGACTTGAAC	Taberlet et al. 1991
B49317	CGAAATCGGTAGACGCTACG	Taberlet et al. 1991
B49873	GGTTCAAGTCCCTCTATCCC	Taberlet et al. 1991
AdTabA2#2	ATTGACATGTAGAATGGGACTC	Briggs et al. 2000
AdTabA3	TTCCGTTGAGTCTCTGCACCTATC	Briggs et al. 2000
AdTabB2	AGAGTCCCATTCTACATGTC	Briggs et al. 2000
CalTabF	GTCCTCTGCTCTACCAACTG	Andrew Perkins (NSW; unpublished)

Positive and negative controls were included in each set of amplifications. PCR products were analysed by 1% agarose gel electrophoresis, and purified by using a Concert™ Rapid PCR Purification System (Gibco BRL Products, Life Technologies) to remove excess primer and unincorporated nucleotides.

5.2.2e Sequencing

All sequencing reactions were performed by 'SUPAMAC' (Sydney University and Prince Alfred [Hospital] Molecular Analysis Centre) using the ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kits (ABI Biosystems). Both strands were sequenced and primers are listed in Table 5.2. Sequencing reactions were Cycle Sequencing on the GeneAmp 9700 with conditions of 25 cycles of 10 sec at 96°C, 5 sec at 50°C and 4 mins at 60°C, according to the manufacturer's specifications. Following this step, excess dye terminators were removed by a Spin-Column Purification using Multiscreen 96-Well Filter Plates (Millipore, P/N MADYEKIT1). Then, sequencing reaction products were electrophoresed on a ABI PRISM 3700 DNA Analyzer (ABI Biosystems). Data were collected using ABI PRISM DNA Sequencing Analysis software 3.6.1.

5.2.2f Sequence Alignment

DNA sequences for each taxon were edited using the computer package 'Sequencher' 3.1.1. (Genes Codes Corporation 1995). The *trn*L intron and *trn*L-*trn*F intergenic spacer ranged in size from 712 bp (*Carpha nitens*) to 1122 bp (*Oreobolus pumilio* and *Rhynchospora brownii*). Sequence alignment was done using ClustalX (Thompson et al. 1997) for the Macintosh computer with 'Slow-Accurate' option, gap creation penalty of 15 and gap extension penalty of 1, and subsequently refined by eye according to the criteria listed below.

- 'Indels [insertion/deletion events] were placed so as to keep the number of substitutions within an aligned region to a minimum' (Wikström et al. 1999, p. 227). In cases where a gap was the result of an insertion or a deletion of a repeat unit (suggesting alternative positioning of the gap), the indel was placed to minimize nucleotide mismatches (Golenberg et al. 1993; Kelchner and Clark 1997; Wikström et al. 1999).
- 'Overlapping gaps were treated as multiple-event length mutations and positioned to minimize the number of required mutational events for creation of the indel' (Kelchner and Clark 1997, p.388).

The aligned sequences of all samples listed in Table 5.1 are presented in Appendix 5. Postulated insertions/deletions ranged from 1 to 238 bp in length. The aligned sequences, with length variations that introduced gaps, had a length of 1502 bp.

5.2.3 Cladistic Analyses

Parsimony methods have been shown to produce inconsistent estimates of the phylogeny under some situations when dealing with molecular sequence data, and other methods, such as likelihood and distance methods, also have their Achilles' heels (Hillis and Huelsenbeck 1995; Nei et al. 1995; Swofford et al. 1996; Sanderson and Kim 2000; de Queiroz and Poe 2001). Because different methods have strengths in different areas and are sensitive to different biases in the data sets, analysing data with a variety of methods, such as maximum parsimony, minimum evolution and maximum likelihood, is desirable. The variety of methods can cover most potential pitfalls and detect potentially weakly

supported lineages (Baum et al. 1994; Lewis 2000). In this study, a variety of model-based methods, in addition to maximum parsimony, was employed to search for phylogenetic relationships and the results were evaluated using the likelihood ratio test (Yang et al. 1994; Sokal and Rohlf 1995; Swofford et al. 1996; Huelsenbeck and Crandall 1997; Sullivan et al. 1999; Goldman et al. 2000; Goldman and Whelan 2000), the Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999; Goldman et al. 2000) and the Kishino-Hasegawa test (implemented in PAUP*). All analyses were performed using the computer software PAUP* 4.0b8 (Swofford 2000) for Windows. The data sets were polarised by the outgroup method, using two species of *Rhynchospora*. Gaps were treated as missing data in the analyses.

5.2.3a Maximum Parsimony

The maximum parsimony analyses were conducted using a heuristic search with TBR branch swapping, collapse of zero-length branches and accelerated transformation (ACCTRAN); characters were equally weighted; character states were specified as unordered. Random taxon addition (100 replicates) was employed to search for multiple islands of trees. In previous studies 10–1000 replicates of random addition were used (e.g. Sennblad et al. 1998; Bell and Patterson 2000; Eldenäs and Linder 2000; Lewis 2000; O'Brien et al. 2000; Swofford 2000; Lowrey et al. 2001). For this study, the same results were generated for 100 and 1000 replicates, so 100 replicates were chosen for this study. The strict consensus tree was generated based on the equally most parsimonious trees produced by heuristic search. Bootstrapping analysis (Felsenstein 1985) using random addition 2000 replicates of fast bootstrap was performed to determine relative support for various clades found in the parsimony analysis. Jackknife analysis (Farris et al. 1996) using random addition 2000 replicates of fast-heuristic search with 37% of characters deleted per run was also performed.

5.2.3b Minimum Evolution

The mimimum evolution criterion was used in conjunction with LogDet distances (Lockhart et al. 1994; implemented in PAUP*). The analyses used a heuristic search with TBR branch swapping and repeated 100 times with the random addition; characters were equally weighted, character states were specified as unordered. The reason for choosing the

LogDet model among distance models is that this model together with parsimony and likelihood analyses can cover most potential pitfalls that would be likely to be encountered (Lewis 2000).

5.2.3c Maximum Likelihood

To assess which model best fits the data, maximum likelihood analyses were performed under two nucleotide substitution models of Hasegawa-Kishino-Yano (HKY85; Hasegawa et al. 1985) and general time-reversible (GTR; Yang 1994a) with the following rate heterogeneity:

(1) equal heterogeneity rates (HKY85 and GTR);

(2) rates at all sites assumed to follow a gamma distribution (HKY85 + Γ, GTR + Γ; Yang 1994b); and

(3) a mixture of invariable sites plus gamma-distributed rates (HKY85 + P_{inv} + Γ, GTR + P_{inv} + Γ; Gu et al. 1995).

All searches were heuristic, with TBR branch swapping and the random addition (100 replicate) option. Parameters needed for each model were estimated on one of the most parsimonious trees, then estimated parameters were used to search each model tree. Once the search was finished, the parameters needed for each model were estimated again on the tree resolved from each model respectively, and the search was rerun using the new, better estimates. After searching, the parameters were estimated again to see if they changed. If so, the process was repeated until all parameters did not change, i.e. the best tree was reached.

After searching, likelihood scores were calculated for the topologies under each model by using re-estimated parameters. Then likelihood scores of the best topologies under the given model were used to conduct likelihood ratio tests (Yang et al. 1994; Sokal and Rohlf 1995; Swofford et al. 1996; Huelsenbeck and Crandall 1997; Sullivan et al. 1999; Goldman et al. 2000; Goldman and Whelan 2000) to judge which model fitted the data better. The results of the tests are shown in Table 5.3. It was immediately evident that the GTR + Γ and GTR + P_{inv} + Γ models fitted the data significantly better than any of the other models considered. Both models produced a single best topology and these two topologies were completely identical. The topology under the GTR + P_{inv} + Γ model had a

better likelihood score than that under the GTR + Γ model. Therefore, the GTR + P_{inv} + Γ model was chosen in this study.

Table 5.3. The likelihood scores of the best tree under given models and likelihood ratio test.

Model	ln Likelihood	Free Parameters	df	G	p
HKY85*	-6765.248	4	6	281.620	0.000
GTR*	-6754.861	8	2	260.846	0.000
HKY85 + Γ	-6633.226	5	5	17.576	0.006
GTR + Γ	-6626.036	9	1	3.196	0.072
HKY85 + P_{inv} + Γ	-6631.670	6	4	14.464	0.004
GTR + P_{inv} + Γ	-6624.438	10			

*The χ^2 distribution was used for these two models instead of $\overline{\chi}^2$ distribution (Sullivan et al. 1999; Goldman and Whelan 2000), because HKY85 and GTR models had much smaller likelihood scores which showed that they were not better models to fit the data and χ^2 distribution was easy to calculate.

5.2.3d Maximum Likelihood and Parsimony Tests

Both maximum likelihood and parsimony tests were then used to evaluate competing phylogenetic hypotheses obtained from the maximum parsimony, minimum evolution and maximum likelihood searches. Shimodaira-Hasegawa tests (maximum likelihood tests) (Shimodaira and Hasegawa 1999; Goldman et al. 2000) were performed (on a variety of tree topologies) to see whether one tree was supported significantly more by the data than other trees calculated by estimating the rate matrix, proportion of invariable sites and shape parameter α of gamma distribution simultaneously with four rate categories and empirically observed base frequencies. Kishino-Hasegawa tests were used to complete the parsimony test.

5.3 Results

5.3.1 Maximum Parsimony Analysis

Maximum parsimony analysis revealed two equally most parsimonious trees of 948 steps, a consistency index (CI) of 0.7690 (0.6656 excluding uninformative characters) and retention index (RI) of 0.7882. The difference between the two most parsimonious trees was that *Costularia nervosa* and *Oreobolus pumilio* exchanged positions. The strict consensus tree is shown in Fig. 5.1 with bootstrap values (> 50%) above each branch and jackknife values (> 50%) below each branch. Using two species of *Rhynchospora* as

outgroups to root the phylogeny, a well-supported clade (100% bootstrap and jackknife support) formed by all species sampled in Schoeneae was revealed. *Gymnoschoenus* appeared to be sister to the clade formed by all other species sampled in Schoeneae but without support. Four monophyletic, well-supported clades, which are labelled A, B, C and D in Fig 5.1, were revealed. Clade A was formed by species of *Carpha* and *Trianoptiles solitaria* with 100% bootstrap and jackknife support. Clade B was composed of one species of *Costularia* (*C. nervosa*), and all sampled species of *Oreobolus* and *Schoenoides* with 100% bootstrap and jackknife support. Clade C contained the other two species of *Costularia*, one species each of *Schoenus* and *Tricostularia* with 99% bootstrap and 100% jackknife support. Clade D was formed by the species of *Cyathochaeta*, *Gahnia* and *Ptilothrix* with 94% bootstrap and 97% jackknife support.

Group D appeared to be the sister to the clade of B and C plus *Schoenus paludosus*, but without support, then the clade of B and C plus *Schoenus paludosus* and D formed the sister group to group A (without support).

Within Clade A, all species of *Carpha* sampled formed a monophyletic group, sister to *Trianoptiles solitaria*, with 99% bootstrap and jackknife support. Two clades were resolved within *Carpha*. One included all species sampled in *Carpha sensu stricto* (*C. alpina*, *C. curvata*, *C. nivicola*, *C. rodwayi*), while the other clade was composed of species previously placed in *Asterochaete* (*C. capitellata*, *C. filifolia*, *C. glomerata* and *C. nitens*). The relationships of the species within both clades were resolved although with weak or no support: the clade of *Carpha rodwayi* and *C. nivicola* was sister to the clade of *C. alpina* and *C. curvata*; *C. rodwayi* and *C. nivicola*, and *C. alpina* and *C. curvata*, were sister species respectively; the clade of *C. filifolia* and *C. glomerata* was sister to the clade of *C. nitens* and *C. capitellata*; *C. filifolia* and *C. glomerata*, and *C. nitens* and *C. capitellata*, were sister species respectively.

The two species of *Schoenus* were not recovered as a monophyletic group. Nor were the species of *Costularia*. *Costularia nervosa* with sampled species of *Oreobolus* and *Schoenoides* formed a well-supported group (100% bootstrap and jackknife support), rather than with the other two species of *Costularia*. *Oreobolus distichus* was sister to *Schoenoides oligocephalus*, i.e. *Schoenoides* is nested within *Oreobolus*.

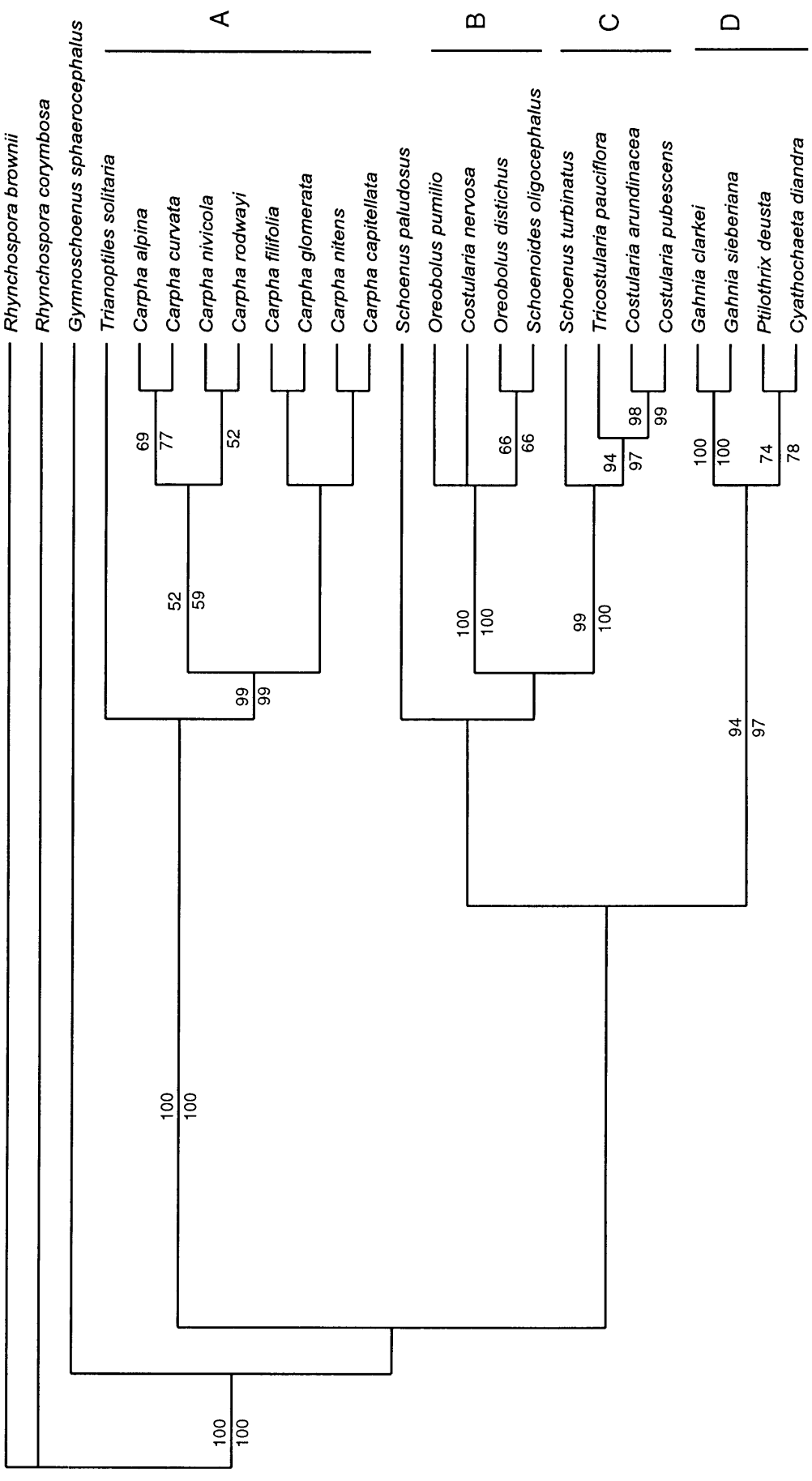

Fig. 5.1. Strict consensus of two equally most parsimonious trees with bootstrap values (> 50%) above each branch and jackknife values (> 50%) below each branch. The well-supported clades are labelled as A–D (for details see text).

Within group D, the clade of *Ptilothrix* and *Cyathochaeta* was sister to *Gahnia*.

5.3.2 Distance Analysis

A distance analysis using the minimum evolution optimality criterion, LogDet model resulted in one tree topology and with a minimum evolution score of 0.91791. This tree is not shown, because this tree was rejected in both maximum likelihood (Shimodaira-Hasegawa test) and parsimony (Kishino-Hasegawa test) tests (see 5.3.4 below).

5.3.3 Maximum Likelihood Analysis

The single best tree resulted from the maximum likelihood analysis under GTR + P_{inv} + Γ model (Fig. 5.2) was consistent with the strict consensus parsimony tree (Fig. 5.1) in many respects. It revealed the same four well-supported (long branch) clades A–D (and B as the sister clade to C) as in the strict consensus parsimony tree, grouped all the sampled species of Schoeneae in one well-supported (long branch) clade, indicated *Gymnoschoenus* to be sister to the clade formed by all other sampled species of Schoeneae, and indicated the polyphyletic status of *Schoenus*. One difference between these two trees (Figs 5.1, 5.2) was the relationships of the four clades of A, B + C, and D. The maximum likelihood tree placed clade B + C as sister to A, then the clade formed by A and B + C was sister to D, whereas in the strict consensus tree the clade formed by B + C + *Schoenus paludosus* was the sister to D but without bootstrap and jackknife support, then the clade formed by the group B + C + *Schoenus paludosus* and D was sister to group A, also without support. Another difference was that the maximum likelihood tree placed *C. nitens* and *C. capitellata* in an unresolved trichotomy with the clade formed by *C. filifolia* and *C. glomerata*, while *C. nitens* and *C. capitellata* were sister species in a clade in the parsimonious tree, and sister to the clade formed by *C. filifolia* and *C. glomerata*.

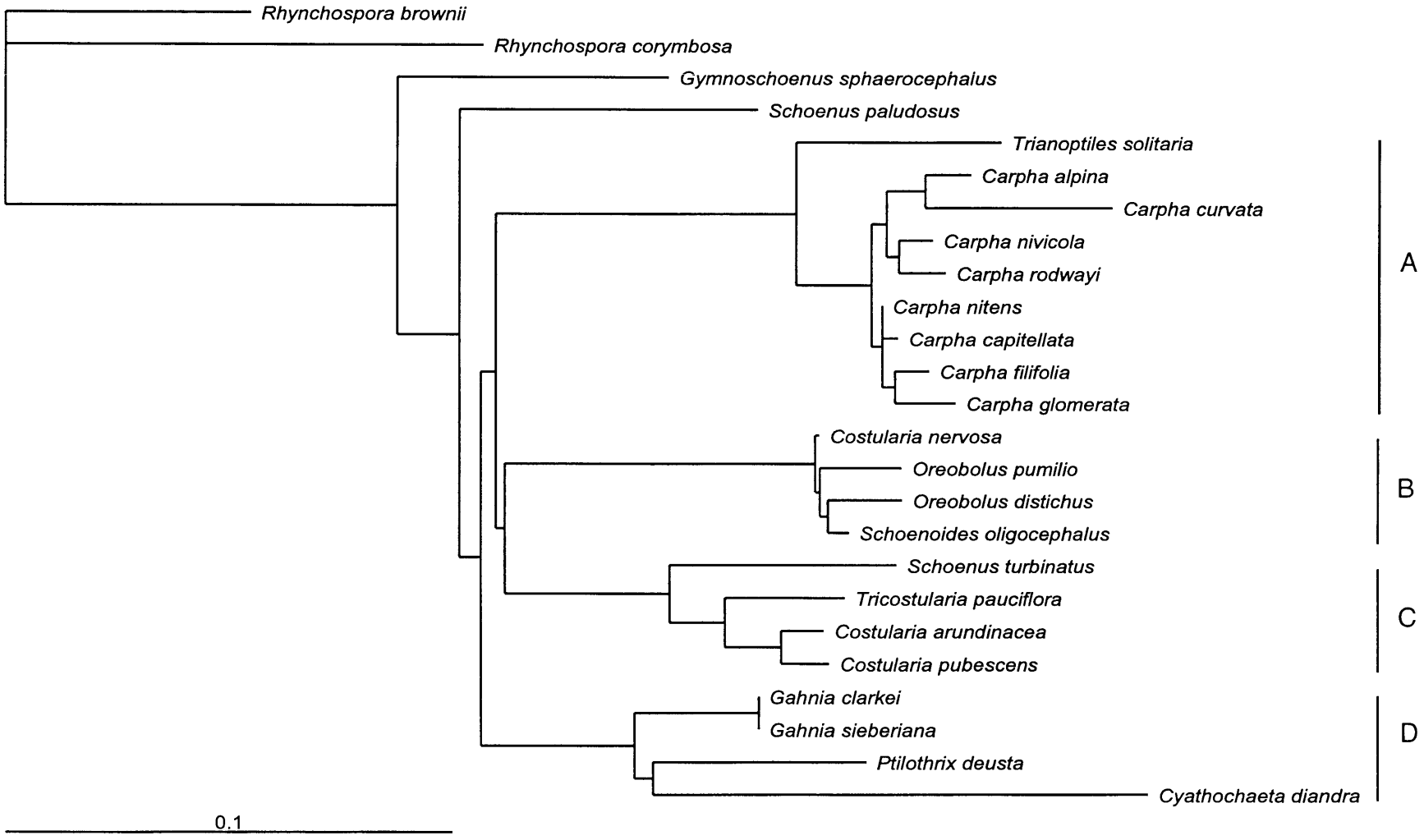

Fig. 5.2. Maximum likelihood reconstruction under GTR + P_{inv} + Γ model. See Materials and Methods for the selection and description of the model. The clades A–D are labelled the same as in Fig. 5.1.

5.3.4 Testing Taxonomic Hypotheses

The results of the maximum likelihood evaluation of tree topologies from the maximum parsimony, minimum evolution and maximum likelihood searches are shown in Table 5.4. A total of five tree topologies (two trees produced by maximum parsimony searches plus one strict consensus tree, one tree produced by minimum evolution and one tree produced by maximum likelihood under GTR + P_{inv} + Γ model) were included in Shimodaira-Hasegawa tests. Only the topology produced by the minimum evolution search was rejected. The two most parsimonious trees, the strict consensus tree and the maximum likelihood tree (under GTR + P_{inv} + Γ model) were not significantly different.

Table 5.4. Maximum likelihood evaluation of tree topologies from the maximum parsimony, minimum evolution and maximum likelihood searches using Shimodaira-Hasegawa tests.

Tree	-ln likelihood	δ	p
Maximum parsimony tree 1	6626.187	1.749	0.721
Maximum parsimony tree 2	6627.718	3.279	0.546
Strict consensus	6627.718	3.280	0.547
Minimum evolution	6649.827	25.389	0.039
GTR + P_{inv} + Γ model	6624.438	(best)	

Parsimony evaluation using Kishino-Hasegawa tests for the same five tree topologies is given in Table 5.5. Only the topology produced by the minimum evolution search was rejected. The two most parsimonious trees, the strict consensus tree and the maximum likelihood tree (under GTR + P_{inv} + Γ model) were not significantly different.

Table 5.5. Parsimony evaluation of tree topologies from the maximum parsimony, minimum evolution and maximum likelihood searches using Kishino-Hasegawa tests

Tree	Length	Length difference	t	p
Maximum parsimony tree 1	948	(best)		
Maximum parsimony tree 2	948	0	0.000	1.000
Strict consensus	949	1	1.000	0.318
Minimum evolution	959	11	2.297	0.022
GTR + P_{inv} + Γ model	950	2	0.447	0.655

5.4 Discussion

This study revealed a monophyletic group formed by all the species sampled in Schoeneae (Figs 5.1, 5.2), a finding consistent with other recent cladistic analyses of Cyperaceae, based on morphology (Goetghebeur 1986; Bruhl 1995; Simpson 1995) and on DNA sequence data (Muasya et al. 1998). However, the current study does not focus on tribal limits, so the genera sampled are not enough to determine the tribal limits of Schoeneae.

Species now included in the genus *Trianoptiles* were once included in *Carpha* and subsequently segregated into their own genus on morphological grounds. They have three hairy scales, each with three bristles at the apex, and female 1-flowered spikelets at the base of the plant, whereas the perianth of *Carpha* consists of six simple bristles, and female 1-flowered spikelets at the base of the plant are absent from *Carpha* (Levyns 1943). The genus *Ptilothrix* was also once included in *Carpha* and subsequently segregated from *Carpha* because it differs in various features including: (1) having a membranous leaf ligule; (2) having a perianth of three bristles; (3) the elongated style-base of *Ptilothrix* is thickened, unlike that of *Carpha*, which is rigid but remains relatively slender; and (4) the inflorescence of *Ptilothrix* consists of numerous spikelets clustered within two very large involucral bracts, with the bases of the bracts very broad and about as long as the spikelets (Wilson 1994b). Besides these two genera, *Costularia* and *Schoenus* were thought to be close to *Carpha*. Clarke (1902, p. 483) indicated that *Carpha* 'differs from *Schoenus* only by the lowest nut-bearing glume having the next glume close over it, not separated by an elongate curved joint of rachilla as is the case in *Schoenus*'. Kükenthal (1939c) observed that the floral features of *Carpha* showed nearly the same picture as in *Costularia*. Recent phylogenetic studies by Goetghebeur (1986) indicated that *Carpha*, *Costularia, Oreobolus* and *Trianoptiles* formed a monophyletic clade, in which *Trianoptiles* was closer to *Carpha* than to the other two genera, while Bruhl (1995) found *Oreobolus*, *Schoenoides*, *Ptilothrix, Trianoptiles* and *Carpha* to be a robust group, and *Trianoptiles* was much closer to *Ptilothrix* than to others. In this study, the results of both maximum parsimony and likelihood analyses of the *trn*L intron and *trn*L-*trn*F intergenic spacer sequences (Figs 5.1, 5.2) indicated that *Trianoptiles* is sister to *Carpha*, in agreement with Goetghebeur's findings. The maximum likelihood tree and the strict consensus parsimony tree show differences as to the next closest relatives of *Carpha*. *Costularia, Oreobolus*, *Schoenoides*, *Schoenus* and *Tricostularia* are closer to *Carpha* than *Cyathochaeta*, *Gahnia* and *Ptilothrix*

according to the maximum likelihood tree (Fig. 5.2), while the strict consensus parsimony tree shows that all these eight genera form the sister clade to the *Carpha* and *Trianoptiles* clade although without support (Fig. 5.1).

Ptilothrix, in this study, was grouped with *Cyathochaeta* and *Gahnia* (Figs 5.1, 5.2) rather than with *Carpha*. The association between *Ptilothrix* and *Cyathochaeta* agrees with Goetghebeur's (1986) conclusion, and the association between *Cyathochaeta* and *Gahnia* agrees with Bruhl (1995). Goetghebeur's (1986) association of *Gymnoschoenus* with *Ptilothrix* was not supported by the present study, nor by Wilson's (1981) embryological data. *Gymnoschoenus* appears to be distant from all species of Schoeneae sampled in this study (Figs 5.1, 5.2), which may correlate with its special morphological characters, for example, globose inflorescence, leaf sheaths long-ciliate on upper margins, nut with the loose, thin and easily removed outermost layer, and other features.

That the two species of *Schoenus* are biphyletic (Figs 5.1, 5.2) is not unexpected, because *Schoenus paludosus* differs from other species of *Schoenus* in having a lower male flower and upper bisexual flower at each spikelet, and non-zigzag 'rachilla' (as opposed to the usual states for the genus of bisexual flowers and upper internodes of the 'rachilla' elongated and prominently zig-zag) (Wilson 1993). Wilson (1993, p. 304) considered that *Schoenus paludosus* might be 'perhaps better placed in another genus such as *Tricostularia*'. This study shows that *S. paludosus* is isolated from other sampled taxa, especially in the maximum likelihood tree. The correct placement of this species requires additional sampling across the Schoeneae to assess its relationships.

Schoenus turbinatus is grouped with *Tricostularia* and some species of *Costularia* in this study. It is consistent with *Schoenus* being close to *Tricostularia* (Goetghebeur 1986) and *Schoenus* being somewhat similar to *Costularia* (Kükenthal 1939c). However, a greatly expanded sample of *Schoenus* is needed to reliably estimate the limits and relationships of this large and morphologically diverse genus.

This study revealed the non-monophyletic status of *Costularia* because one species (*C. nervosa*) formed a well-supported clade (100% bootstrap and jackknife support) with *Oreobolus* and *Schoenoides*, while the other two species formed a separate well-supported clade (99% bootstrap and100% jackknife support) with *Schoenus* and *Tricostularia* (Figs

5.1, 5.2). The polyphyly of *Costularia* is consistent with Seberg's (1986, 1988a, 1988b) studies. Therefore, the morphological variation of *Costularia* needs to be reassessed and a molecular phylogenetic study is needed to assess its generic limits.

Schoenoides oligocephalus was originally described by Curtis (1984) under *Oreobolus*, and was subsequently segregated as a monotypic genus by Seberg (1986). In Seberg's phylogeny, *Schoenoides* formed a monophyletic clade with *Oreobolus* but was sister to *Oreobolus* using *Costularia* as an outgroup (Seberg 1986). Later, Bruhl (1995, p. 212) found that most autapomorphies for *Schoenoides* observed by Seberg were in fact more widely distributed in *Oreobolus* and wrote 'the justification for maintaining the monotypic genus *Schoenoides* appears weak'. Recently, Goetghebeur (1998) merged *Schoenoides* back into *Oreobolus* in his taxonomic treatment. His view is supported by this study; *Schoenoides oligocephalus* nested within *Oreobolus* in both maximum likelihood and parsimony analyses (Figs 5.1, 5.2) providing evidence for restoring *Schoenoides* to its original place within *Oreobolus.*

Maximum parsimony and likelihood analyses reveal the same groups A, B, C, and D, and B as the sister clade to C. But the phylogenetic relationships of clades A, B plus C, and D differ (Figs 5.1, 5.2). There are no bootstrap and jackknife values on the strict consensus tree for grouping them, and branches grouping them on the maximum likelihood tree are short. Therefore, the data do not provide direct means for the interpretation of relationships among these groups.

Within *Carpha*, the definition and limits of the genus are controversial (see Chapter 1 for details). Using the narrow definition of *Carpha* (Hooker 1860, 1867; Bentham 1878, 1883; Wilson 1986, 1993, 1994a, 1994b), the genus *Asterochaete* whose species occur in South Africa, Réunion and Madagascar is separated from *Carpha.* However, according to the alternative broad definition of *Carpha* (Boeckeler 1874; Clarke 1894, 1902, 1904, 1908; Chermezon 1922; Pfeiffer 1931; Chermezon 1935; Kükenthal 1939c, 1939d; Haines and Lye 1983; Bruhl et al. 1992; Bruhl 1995; Goetghebeur 1998), *Asterochaete* is included in *Carpha.* In this study both the strict consensus parsimony and the maximum likelihood trees show that *Carpha* is divided into two clades (Figs 5.1, 5.2): one is a clade of *C. alpina*, *C. curvata*, *C. nivicola* and *C. rodwayi* which do not occur in South Africa, Réunion and Madagascar and belong to *Carpha sensu stricto,* the other clade consists of

C. capitellata, *C. filifolia*, *C. glomerata* and *C. nitens*, which are the species of *Asterochaete*. However, (1) there is weak or no support in the strict consensus parsimony tree for these two clades and branch lengths in the maximum likelihood tree are not long; (2) there is strong bootstrap and jackknife support for the clade formed by these two clades. Intriguingly the molecular data presented here mimic the ambiguous treatment of *Carpha* and *Asterochaete*.

This study revealed the relationships of some species in *Carpha*. The relationships among the species *C. alpina*, *C. curvata*, *C. nivicola* and *C. rodwayi* are identical in both maximum parsimony and likelihood analyses (Figs 5.1, 5.2). *Carpha alpina* occurs in Australia (NSW, Vic. and Tas.), New Zealand and New Guinea, while the other three species only occur in Australia. *Carpha nivicola* occurs on subalpine areas in NSW (Mt Kosciuszko) and Victoria, and its morphology is similar to *C. alpina* but it is bigger than *C. alpina*; *C. rodwayi* only occurs in Tasmania and resembles small forms of *C. alpina* but with rigid leaves; *C. curvata* only occurs in Tasmania and resembles *C. alpina* but with curled leaves. This study indicated that *C. alpina* is closer to *C. curvata*, and *C. nivicola* is closer to *C. rodwayi*.

Reid and Arnold (1984) used morphological observations to infer that *C. filifolia* was much closer to *C. capitellata* than to *C. glomerata*. The results from this study (Figs 5.1, 5.2) indicated that *C. filifolia* is closer to *C. glomerata* than to *C. capitellata*.

The strict consensus parsimony tree placed *C. nitens* as the sister of *C. capitellata* (Fig. 5.1). In the maximum likelihood tree, they were placed in an unresolved trichotomy with the clade formed by *C. filifolia* and *C. glomerata* (Fig. 5.2). Maximum parsimony and likelihood analyses provide different placement of these two species, and there is no support on the strict consensus parsimony tree, and branches grouping them on the maximum likelihood tree are short. So, the statements of relationships must await additional data and analysis. Here, as in other studies (e.g. Murphy et al. 2000), *trn*L intron and *trn*L-*trn*F intergenic spacer data fail to resolve relationships of some species groups.

This study is the first cladistic analysis of *Carpha* and its relatives using molecular data. It identified several well-supported lineages at the generic level within the tribe Schoeneae (Clades A, B, C and D; Figs 5.1, 5.2), revealed *Trianoptiles* as sister genus to *Carpha*,

indicated the non-monophyletic status of *Costularia* and *Schoenus*, and found that *Ptilothrix* is sister to *Cyathochaeta* (then both to *Gahnia*) rather than to *Carpha*, that *Gymnoschoenus* is distant from *Carpha* and its close relatives, and that *Schoenoides* should be sunk in *Oreobolus*. The study also revealed some relationships within *Carpha*, such as (1) the relationships of *C. alpina* to *C. curvata*, and *C. nivicola* to *C. rodwayi* and (2) the close relationship of *C. filifolia* and *C. glomerata*. The study also interpreted some phylogenetic patterns in and around *Carpha*, and an assessment of previous hypotheses is offered in light of the present study. Parsimony and maximum likelihood analyses also reveal differences in the branching order for some taxa or taxa groups. The weak internal support and short branches within these taxa or groups indicate a need for more data. Both sampling of a wider range of taxa and studies of other regions of genomes are needed.