Molecular Phylogeny and Character Evolution of
Cymbidium (Orchidaceae)

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Abstract   Molecular phylogenetic analyses using two data sets, derived from DNA sequences of *matK*, the maturase-encoding gene located in an intron of the chloroplast gene *trnK*, and ITS (internal transcribed spacer) region of the 18S–26S nuclear ribosomal DNA, were performed to examine relationships among 37 taxa in *Cymbidium*. Although each data set did not provide conclusive evidence in itself, the data sets combining the two regions yielded the following insights: (1) *Cymbidium* subgenus *Cyperorchis* and section *Pachyrhizanthe*, often treated as independent genera, were nested within the remaining members of *Cymbidium*. (2) All the three subgenera of *Cymbidium*, namely, *Cymbidium*, *Cyperorchis*, and *Jensoa*, turned to be para-/polyphyletic. (3) Among sections of *Cymbidium*, *Austrocymbidium*, *Cyperorchis*, *Iridorchis*, and *Maxillarianthe* did not show monophyly. (4) At specific level, our results indicated the polyphyly of *Cymbidium bicolor*, although bootstrap supports for the clades including *C. bicolor* were not robust.
Key words :   *Cymbidium*, ITS, *matK*, molecular, Orchidaceae, phylogeny

Introduction

The genus *Cymbidium* Sw. comprises about 50 species. Geographic distribution extends from the northwestern Himalaya to Japan and south through Indochina and Malesia to northern and eastern Australia. This group exhibits distinctive ecological diversification and occurs as terrestrial and epiphytic life forms. Moreover, one section, *Pachyrhizanthe* Schltr. lacks foliage leaves and has a strongly mycoparasitic existence.

Swartz (1799) established the genus *Cymbidium* with a broad generic delimitation. Circumscription of the genus has been variously defined by later workers: Lindley (1833), Blume (1848, 1849, 1858), Reichenbach (1852, 1864), Hooker (1890), Schlechter (1924), Hunt (1970), Seth & Cribb (1984), and Du Puy & Cribb (1988). At

generic level, there have been inconsistencies on the delimitation. For example, Blume (1848, 1849, 1858), Reichenbach (1852), Hooker (1890), and Schlechter (1924) treated *Cyperorchis* as an independent genus. Another example is *Pachyrhizanthe*. As mentioned above, this group shows very distinctive vegetative morphology and nutritional habit, and Nakai (1931) thus elevated *Pachyrhizanthe* to generic rank.

The first major revision of *Cymbidium* was undertaken by Schlechter (1924) where he proposed sectional limits within the genus. His framework has been modified by Hunt (1970), and Seth & Cribb (1984). So far, the most extensive infrageneric classification of *Cymbidium* is that of Du Puy & Cribb (1988), because their system was based on the most recent information on morphological and anatomical characters.

However, satisfying conclusions have not been affirmed for the infrageneric relationships and circumscriptions of several species in *Cymbidium*.

Recently, DNA sequences of *matK*, the maturase-encoding gene located in an intron of the chloroplast gene *trnK*, and ITS (internal transcribed spacer region) of the 18S–26S nuclear ribosomal DNA have proven to be powerful tools for phylogenetic reconstruction within angiosperm families and genera (e.g., Baldwin, 1992; Johnson and Soltis, 1994; Steele and Vilgalys, 1994). In this study we compared and combined DNA sequences of *matK* and ITS to establish more concrete relationships in *Cymbidium*.

Material and Methods

In this study we selected 37 representative taxa from all the subgenera and sections in *Cymbidium*. Results of *matK* and ITS analyses for tribe Cymbidieae have indicated that the sister group of *Cymbidium* becomes the clade comprising *Acriopsis*, *Grammatophyllum*, and *Thecostele* (Yukawa, unpublished). Consequently, these genera were chosen as outgroup of this study. Materials used in the phylogenetic analyses are shown in Table 1. Voucher specimens are deposited at TNS.

Experimental methods were described in Yukawa *et al.* (1993, 1996). Sequences were determined by amplifying the *matK* (including parts of *trnK* introns) and ITS regions (including 5.8S rDNA and parts of 18S and 26S rDNA) via the polymerase chain reaction (PCR) from a total DNA extract. All of the primers were from Yukawa *et al.* (1999) for *matK* and Douzery *et al.* (1999) for ITS. DNA sequences were aligned manually. Gaps were treated as missing characters. The aligned data file is available from the first author upon request. Parsimony and distance analyses were conducted with PAUP* version 4.0b6 (Swofford, 2001). The huristic option was used to perform Fitch parsimony analyses (Fitch, 1971). Distance trees were obtained using the

neighbor-joining (NJ) method (Saitou & Nei, 1987) with a Kimura two-parameter correction (Kimura, 1980). To assess the relative robustness for branches, the bootstrap method (Felsenstein, 1985) was used with 1000 replicates.

Results

Phylogenetic analyses

Figure 1 shows a strict consensus of 28,800 most parsimonious (MP) trees derived from the *matK* sequences. The tree had a consistency index (CI) of 0.7726 (0.6115 excluding uninformative characters) and a retention index (RI) of 0.8033. The NJ topology with more than 50% bootstrap support was identical (Fig. 1). A strict consensus of 325 MP trees on the basis of ITS data set is shown in Fig. 2. CI was 0.6848 (0.5606 excluding uninformative characters) and RI was 0.7790. In this data set the NJ topology with more than 50% bootstrap support was identical (Fig. 2). On the other hand, analyses of *matK* sequences provided results substantially concordant with those achieved via analyses of ITS sequences except for positions of *Cymbidium erythrostylum* and *Cymbidium sanderae*. In such cases, analyses of combined data sets excluding problematic taxa provide more resolution and internal support for relationships than do the individual data sets (e.g., Olmstead and Sweere, 1995). We thus conducted a combined analysis of the information derived from *matK* and ITS sequences. In this analysis *C. erythrostylum* and *C. sanderae* were excluded. The length of aligned sequence was 2829 base pairs. Figure 3 shows a strict consensus of 168 MP trees from the combined data set. CI was 0.7283 (0.5931 excluding uninformative characters) and RI was 0.7788. In this data set the clades revealed by NJ analyses with more than 50% bootstrap support was also identical (Fig. 3).

In the combined analysis, the following insights were provided (Fig. 3): (1) *Cymbidium* subgenus *Cyperorchis* and section *Pachyrhizanthe*, often treated as independent genera, were nested within the remaining members of *Cymbid-*

Table 1.   Taxa of *Cymbidium* and outgroups used for sequencing.

Species	Voucher
Subgenus *Cymbidium*	
Section *Cymbidium*	
Cymbidium aloifolium (L.) Sw.	TBG118253*
C. bicolor Lindl. subsp. *obtusum* Du Puy & P. J. Cribb	TBG124328
C. bicolor Lindl. subsp. *pubescens* (Lindl.) Du Puy & P. J. Cribb	TBG118509
C. finlaysonianum Lindl.	TBG122826
C. rectum Ridl.	Leiden Bot. Gard. 913298
Section *Hymantophyllum* Schltr.	
C. dayanum Rchb. f.	TBG127899
Section *Borneense* Du Puy & P. J. Cribb	
C. borneense J. J. Wood	Suzuki s.n.
Section *Austrocymbidium* Schltr.	
C. canaliculatum R. Br.	TBG135991
C. chloranthum Lindl.	TBG127438
C. hartinahianum J. B. Comber & Nasution	Cibodas Bot. Gard. s.n.
C. madidum Lindl.	TBG119076
Section *Floribundum* Seth & P. J. Cribb	
C. floribundum Lindl.	TBG127357
C. suavissimum Sander ex C. Curtis	TBG128817
Section *Bigibbarium* Schltr.	
C. devonianum Paxton	TBG119073
Subgenus *Cyperorchis* (Blume) Seth & P. J. Cribb	
Section *Iridorchis* (Blume) P. Hunt	
C. erythraeum Lindl.	TBG135399
C. hookerianum Rchb. f.	TBG133783
C. insigne Rolfe	TBG135526
C. iridioides D. Don	TBG118251
C. lowianum (Rchb. f.) Rchb. f.	TBG54943
C. sanderae (Rolfe) Du Puy & P. J. Cribb	Hiroshima Bot. Gard. 5138
C. tracyanum L. Castle	TBG124406
Section *Eburnea* Seth & P. J. Cribb	
C. eburneum Lindl.	Suzuki s.n.
C. roseum J. J. Sm.	TBG133724
Section *Annamaea* (Schltr.) P. Hunt	
C. erythrostylum Rolfe	TBG126650
Section *Cyperorchis* (Blume) P. Hunt	
C. elegans Lindl.	Karasawa s.n.
C. cochleare Lindl.	Ohba s.n.
Section *Parishiella* (Schltr.) P. Hunt	
C. tigrinum Parish ex Hook.	TBG133781
Subgenus *Jensoa* (Raf.) Seth & P. J. Cribb	
Section *Jensoa* (Raf.) Schltr.	
C. ensifolium (L.) Sw.	TBG118255
C. kanran Makino	TBG133782
C. sinense (Jackson in Andr.) Willd.	Iwagawa s.n.
Section *Maxillarianthe* Schltr.	
C. aliciae Quisumb.	TBG119071
C. faberi Rolfe	TBG133593
C. goeringii (Rchb. f.) Rchb. f.	TBG115919
C. tortisepalum (Fukuyama) Y. S. Wu & S. C. Chen	TBG133595
Section *Geocymbidium* Schltr.	
C. lancifolium Hook.	TBG56111
Section *Pachyrhizanthe* Schltr.	
C. aberrans (Finet) Schltr.	Yagame s.n.
C. macrorhizon Lindl.	Yagame s.n.
Acriopsis liliifolia (Koen.) Ormerod	Kyoto Bot. Gard. s.n.
Grammatophyllum scriptum (L.) Blume	TBG118874
G. speciosum Blume	Shinjukugyoen s.n.
G. stapeliiflorum (Teijsm. & Binn.) J. J. Sm.	TBG124396
Thecostele alata (Roxb.) Par. & Rchb. f.	TBG118540

*TBG series indicate accession numbers in living collection database at Tsukuba Botanical Garden.

Tomohisa Yukawa *et al.*

	Subgenus	Section

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                    ┌─ C.aloifolium              Cymbidium    Cymbidium
                    ├─ C.finlaysonianum          Cymbidium    Cymbidium
              100   ├─ C.bicolor subsp.pubescens Cymbidium    Cymbidium
              97    ├─ C.bicolor subsp.obtusum   Cymbidium    Cymbidium
          95  ┤     └─ C.rectum                  Cymbidium    Cymbidium
          92  │ 98  ┌─ C.aliciae                 Jensoa       Maxillarianthe
     72       │ 92  └─ C.borneense               Cymbidium    Borneense
     51       │  98 ┌─ C.canaliculatum           Cymbidium    Austrocymbidium
              │  92 └─ C.madidum                 Cymbidium    Austrocymbidium
           90 ┌─ C.dayanum                       Cymbidium    Himantophyllum
           84 └─ C.erythrostylum                 Cyperorchis  Annamaea
                    ─ C.cochleare                 Cyperorchis  Cyperorchis
                    ─ C.elegans                   Cyperorchis  Cyperorchis
   82          99   ┌─ C.sanderae                Cyperorchis  Iridorchis
   64    93    98   └─ C.insigne                 Cyperorchis  Iridorchis
         95         ─ C.erythraeum                Cyperorchis  Iridorchis
                 87 ┌─ C.iridioides              Cyperorchis  Iridorchis
         93   86 91 ├─ C.lowianum                Cyperorchis  Iridorchis
         86   85    └─ C.hookerianum             Cyperorchis  Iridorchis
              100   ┌─ C.eburneum                Cyperorchis  Eburnea
              100   └─ C.roseum                  Cyperorchis  Eburnea
                    ─ C.tracyanum                 Cyperorchis  Iridorchis
                    ─ C.tigrinum                  Cyperorchis  Parishiella
                    ─ C.devonianum                Cymbidium    Bigibbarium
           93       ┌─ C.hartinahianum           Cymbidium    Austrocymbidium
           93       ├─ C.chloranthum             Cymbidium    Austrocymbidium
                 99 ├─ C.floribundum             Cymbidium    Floribundum
   59            98 └─ C.suavissimum             Cymbidium    Floribundum
   56         88    ┌─ C.goeringii               Jensoa       Maxillarianthe
         86   87    └─ C.faberi                  Jensoa       Maxillarianthe
         70      99 ┌─ C.tortisepalum            Jensoa       Maxillarianthe
                100 ├─ C.ensifolium              Jensoa       Jensoa
              87    ├─ C.kanran                  Jensoa       Jensoa
              80    └─ C.sinense                 Jensoa       Jensoa
              96    ┌─ C.lancifolium             Jensoa       Geocymbidium
              96 100├─ C.macrorhizon            Jensoa       Pachyrhizanthe
                 100└─ C.aberrans                Jensoa       Pachyrhizanthe
                 100┌─ Grammatophyllum speciosum
           100   100└─ Grammatophyllum scriptum
           100      ┌─ Grammatophyllum stapeliaeflorum
   100           100└─ Thecostele alata
   99               ─ Acriopsis lilifolia
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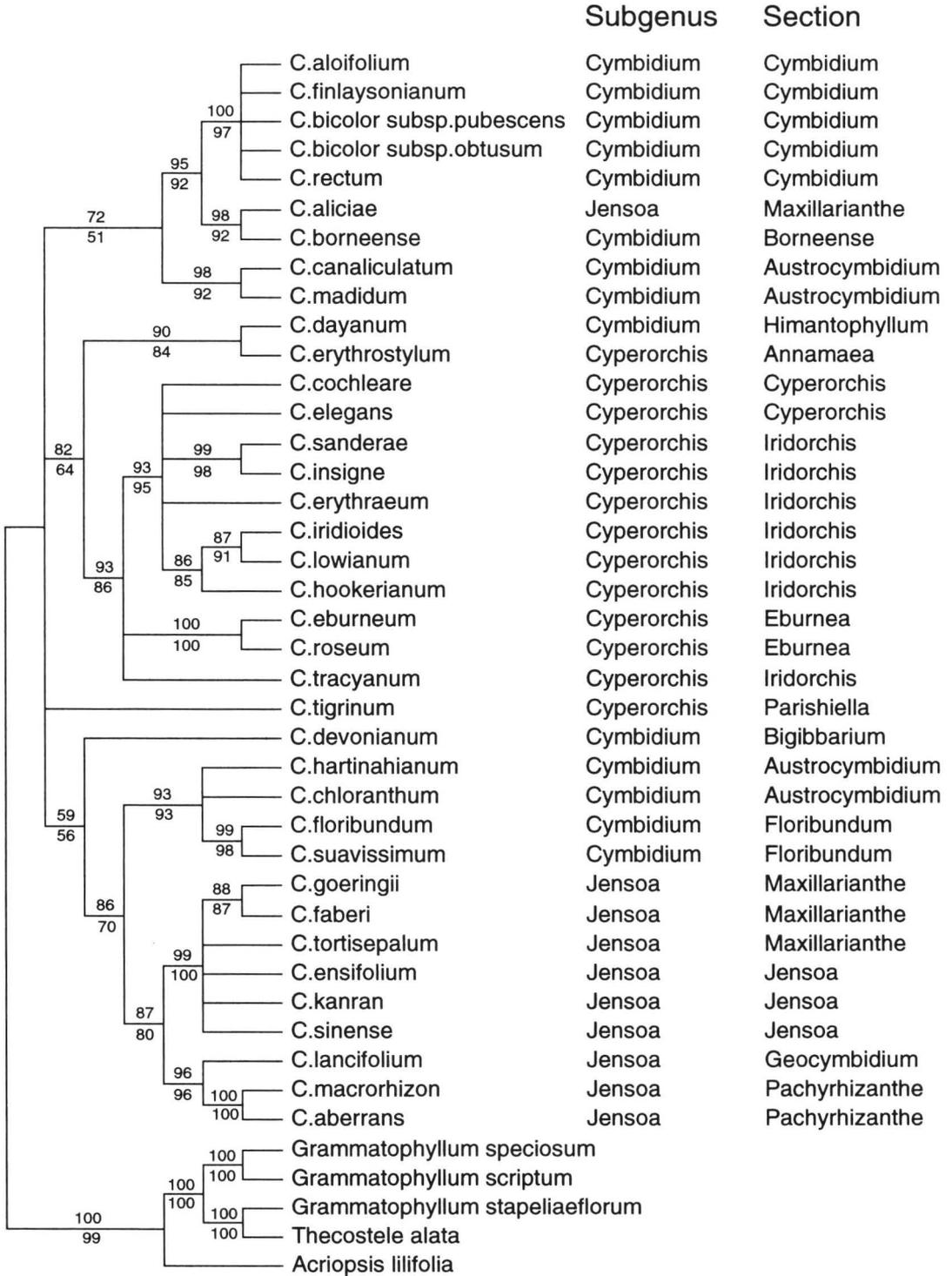
Fig. 1. Strict consensus of 28,800 most-parsimonious Fitch trees based upon *matK* sequences: length=475, consistency index=0.7726 (0.6115 excluding uninformative characters), retention index of 0.8033. Numbers above internodes indicate bootstrap values from 1,000 replicates of Fitch parsimony analysis (maxtree limit of 200 per replicate). Numbers below internodes indicate bootstrap values from 1,000 replicates of neighbor-joining distance analysis.
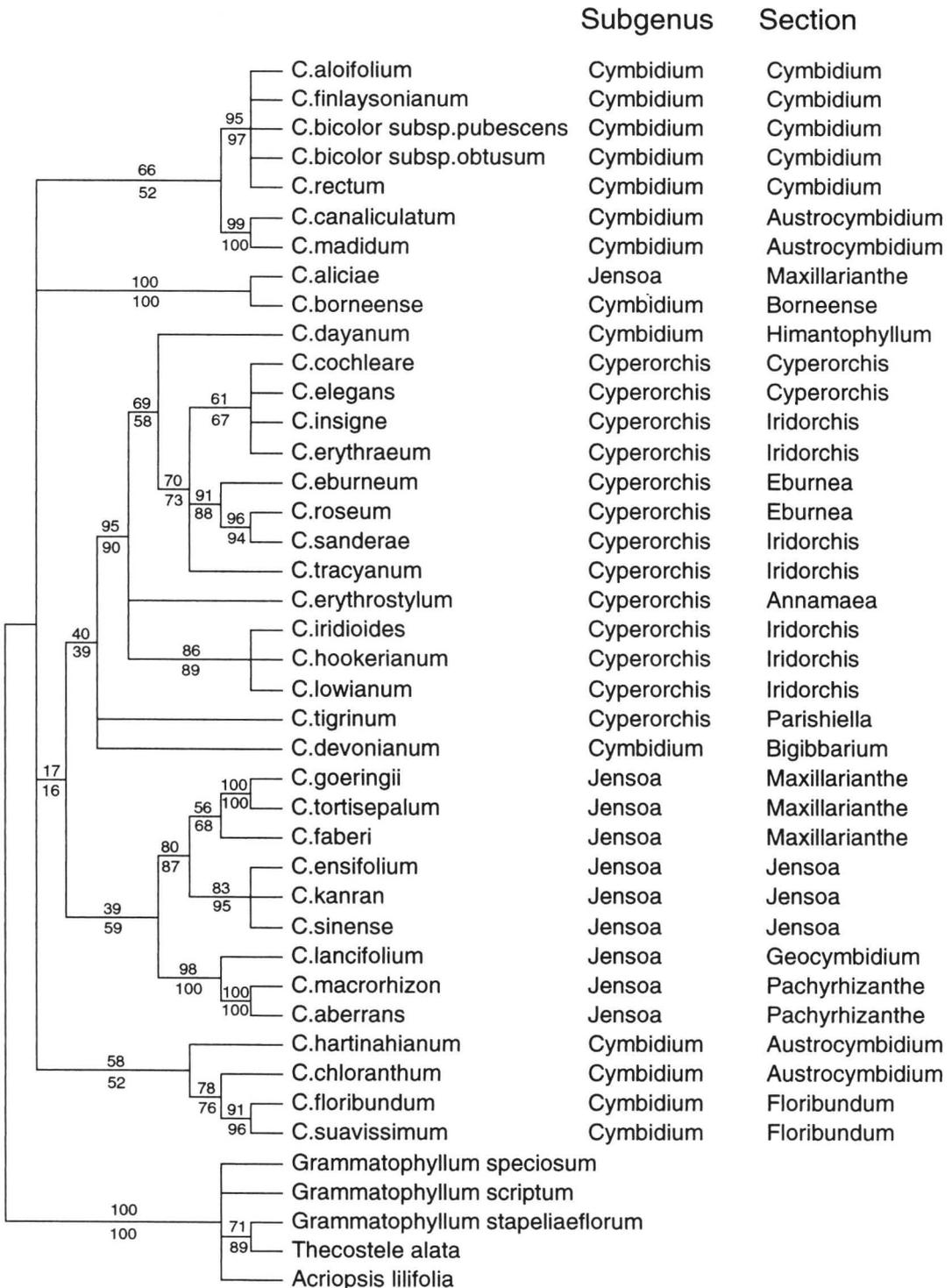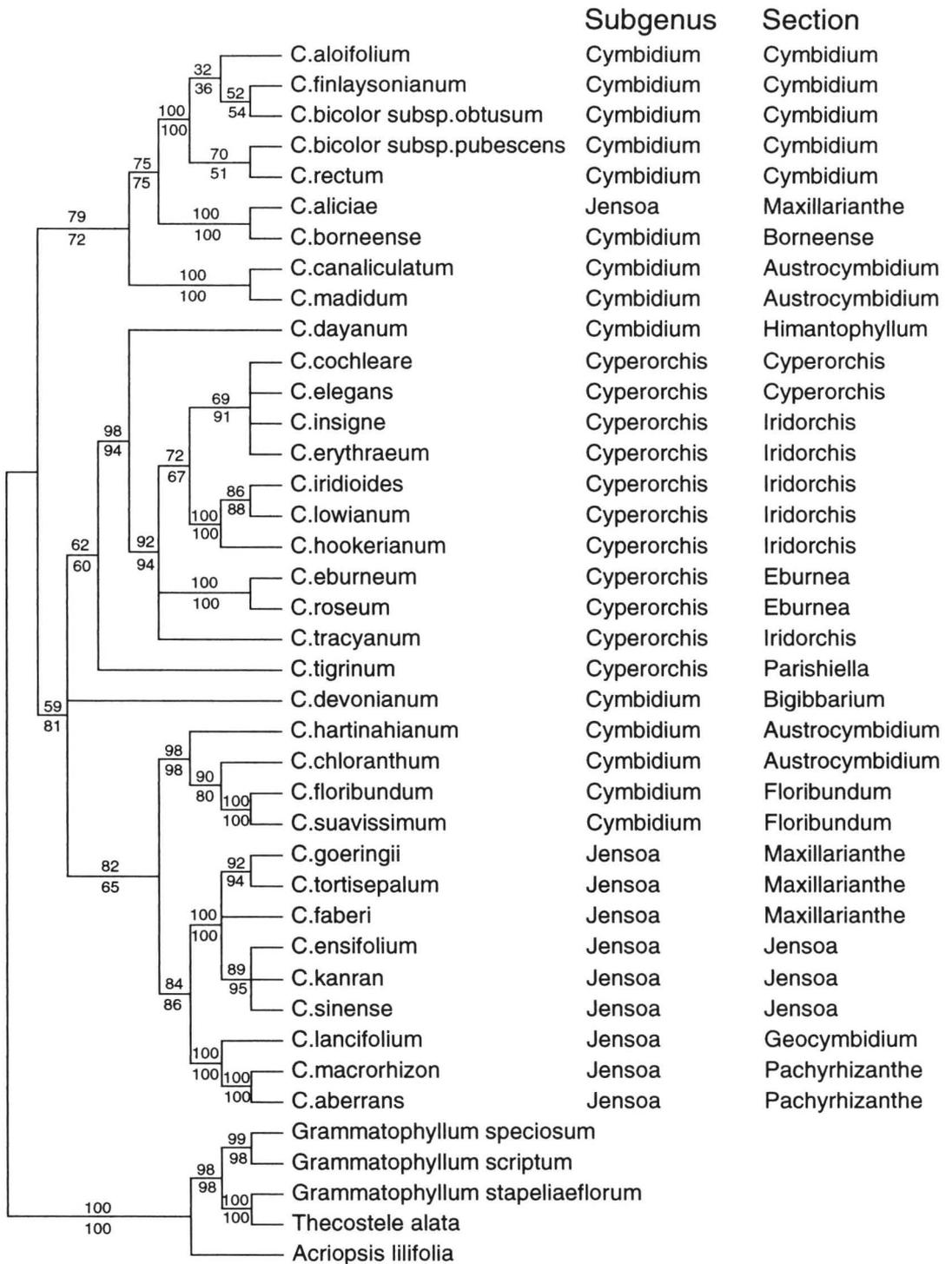
Fig. 2.   Strict consensus of 325 most-parsimonious Fitch trees based upon ITS sequences: length=368, consistency index=0.6848 (0.5606 excluding uninformative characters), retention index of 0.7790. Numbers above internodes indicate bootstrap values from 1,000 replicates of Fitch parsimony analysis (maxtree limit of 200 per replicate). Numbers below internodes indicate bootstrap values from 1,000 replicates of neighbor-joining distance analysis.

	Subgenus	Section
C.aloifolium	Cymbidium	Cymbidium
C.finlaysonianum	Cymbidium	Cymbidium
C.bicolor subsp.obtusum	Cymbidium	Cymbidium
C.bicolor subsp.pubescens	Cymbidium	Cymbidium
C.rectum	Cymbidium	Cymbidium
C.aliciae	Jensoa	Maxillarianthe
C.borneense	Cymbidium	Borneense
C.canaliculatum	Cymbidium	Austrocymbidium
C.madidum	Cymbidium	Austrocymbidium
C.dayanum	Cymbidium	Himantophyllum
C.cochleare	Cyperorchis	Cyperorchis
C.elegans	Cyperorchis	Cyperorchis
C.insigne	Cyperorchis	Iridorchis
C.erythraeum	Cyperorchis	Iridorchis
C.iridioides	Cyperorchis	Iridorchis
C.lowianum	Cyperorchis	Iridorchis
C.hookerianum	Cyperorchis	Iridorchis
C.eburneum	Cyperorchis	Eburnea
C.roseum	Cyperorchis	Eburnea
C.tracyanum	Cyperorchis	Iridorchis
C.tigrinum	Cyperorchis	Parishiella
C.devonianum	Cymbidium	Bigibbarium
C.hartinahianum	Cymbidium	Austrocymbidium
C.chloranthum	Cymbidium	Austrocymbidium
C.floribundum	Cymbidium	Floribundum
C.suavissimum	Cymbidium	Floribundum
C.goeringii	Jensoa	Maxillarianthe
C.tortisepalum	Jensoa	Maxillarianthe
C.faberi	Jensoa	Maxillarianthe
C.ensifolium	Jensoa	Jensoa
C.kanran	Jensoa	Jensoa
C.sinense	Jensoa	Jensoa
C.lancifolium	Jensoa	Geocymbidium
C.macrorhizon	Jensoa	Pachyrhizanthe
C.aberrans	Jensoa	Pachyrhizanthe
Grammatophyllum speciosum		
Grammatophyllum scriptum		
Grammatophyllum stapeliaeflorum		
Thecostele alata		
Acriopsis lilifolia		

Fig. 3. Strict consensus of 168 most-parsimonious Fitch trees based upon *matK* and ITS sequences: length=828, consistency index=0.7283 (0.5731 excluding uninformative characters), retention index of 0.7788. Numbers above internodes indicate bootstrap values from 1,000 replicates of Fitch parsimony analysis (maxtree limit of 1,000 per replicate). Numbers below internodes indicate bootstrap values from 1,000 replicates of neighbor-joining distance analysis.

ium. (2) All the three subgenera of *Cymbidium*, namely, *Cymbidium*, *Cyperorchis*, and *Jensoa*, turned to be para-/polyphyletic. (3) Among sections of *Cymbidium*, *Austrocymbidium*, *Cyperorchis*, *Iridorchis*, and *Maxillarianthe* did not show monophyly. (4) At specific level, our results indicated the polyphyly of *Cymbidium bicolor*, although bootstrap supports for the clades including *C. bicolor* were not robust.

Discussion

Classification

Generic circumscription of *Cymbidium* by modern taxonomists was supported in this study. *Cyperorchis*, sometimes treated as an independent genus (e.g. Blume, 1848, 1849, 1858; Reichenbach, 1852; Hooker, 1890; and Schlechter, 1924) was nested within other clades of *Cymbidium*. *Pachyrhizanthe*, another problematic group with reduced vegetative morphology and strongly mycoparasitic nutritional existence, was elevated to a new genus by Nakai (1931). The results of this study showed that *Pachyrhizanthe* makes a sister group relationship with section *Geocymbidium* and that it is reasonable to treat this group as a section of *Cymbidium*. Moreover, these two sections have the following synapomorphies: (1) underground rhizomes that survive throughout their life history, (2) whitish perianth lobes with purple striations and markings.

We clarified that all the three subgenera of *Cymbidium* proposed by Seth & Cribb (1984) represent para-/polyphyly. Subgenus *Cymbidium* comprises four clades and these clades become polyphyletic assemblage. It is apparent that the subgenus was defined by plesiomorphic characters such as a lip without any fusing structures and two pollinia. The monophyly of subgenus *Cyperorchis* was broken by a nested position of subgenus *Cymbidium* section *Himantophyllum*. Transfer of section *Himantophyllum* to a member of subgenus *Cyperorchis* results in the loss of a prominent synapomorphic character of subgenus *Cyperorchis*, that is, a fused basal part of the lip with the column. Subgenus *Jensoa* will become a

monophyletic group, if *Cymbidium aliciae* is removed. The four pollinia is a principal synapomorphic character of subgenus *Jensoa*, but *C. aliciae* and *Cymbidium borneense*, distantly related to subgenus *Jensoa*, also share this character. Taking the phylogenetic relationships and the morphological characters into consideration, subdivisions of *Cymbidium* at subgeneric level are not useful. In other words, we did not find any stable synapomorphic characters that feature each major clade clarified in this study.

Our results further indicated that sectional delimitation of *Cymbidium* also has several problems. Among members of subgenus *Cymbidium*, section *Austrocymbidium* exhibits paraphyly and comprises the following three clades: (1) *Cymbidium canaliculatum* and *Cymbidium madidum*; (2) *Cymbidium hartinahianum*; (3) *Cymbidium chloranthum*. The first clade includes the type of this section (*C. canaliculatum*) and corresponds to a revised circumscription of section *Austrocymbidium*. Since the remaining two clades further form another major clade together with section *Floribundum* and morphological characters of the two clades are consistent with those of section *Floribundum*, we extend the limits of this section to include members of the two clades, namely, *C. hartinahianum* and *C. chloranthum*.

In subgenus *Cyperorchis*, the monophyly of the clade including sections *Cyperorchis*, *Eburnea*, and *Iridorchis* was supported by high bootstrap values. However, support on several relationships among members of sections *Cyperorchis* and *Iridorchis* was weak due to few synapomorphic nucleotide substitutions for these groups. It is likely that section *Iridorchis* was established on the basis of plesiomorphic characters such as widely-opened perianth lobes and an operculum without an obvious backwards-pointing beak, but explicit relationships on the basis of more molecular signals are needed to rearrange the classification for these taxa.

As mentioned above, *Cymbidium aliciae* broke the monophyly of subgenus *Jensoa*, more specifically section *Maxillarianthe*. Du Puy & Cribb (1988) placed *C. aliciae* into subgenus *Jensoa*

section *Maxillarianthe* as a synonym of *Cymbidium cyperifolium* Wall. ex Lindl. Our results did not support this placement and suggested a sister group relationship of *C. aliciae* to *Cymbidium borneense*, a sole representative of subgenus *Cymbidium* section *Borneense*. Observations of morphological and anatomical characters (Yukawa & Stern, 2002; Yukawa, unpublished) of *C. aliciae* also supported the affinity of these two species and a distant relationship of section *Maxillarianthe* to them. For example, *C. aliciae* and *C. borneense* have hypodermal fiber strands both in abaxial and adaxial surfaces, but members of section *Maxillarianthe* lack this character at least in abaxial surface. Du Puy & Cribb (1988) established section *Borneense*, because *C. borneense* shows mixed diagnostic characters of subgenus *Cymbidium* section *Cymbidium* and subgenus *Jensoa*. The present results corroborated the soundness of section *Borneense* and the two species, *C. borneense* and *C. aliciae*, constitute the section.

Relationships at species level also may include problems. Our results indicated the polyphyly of *Cymbidium bicolor*, although bootstrap supports for the clades including *C. bicolor* were not robust. On the other hand, a species complex including *Cymbidium ensifolium*, *Cymbidium kanran*, and *Cymbidium sinense* has low resolution. Accumulation of more molecular signals is needed to clarify these relationships.

On the other hand, we found discrepant positions for *Cymbidium erythrostylum* and *Cymbidium sanderae* between *matK* and ITS trees. Since maternal inheritance of plastid DNA was confirmed in Orchidaceae (Corriveau & Coleman, 1988; Sears, 1980), inconsistent phylogenetic positions for these two species in *matK* trees can be caused by introgressions. Interestingly, collection records of *C. erythrostylum* and *C. sanderae* are very few, and this fact also may account for their hybrid origin. Moreover, Du Puy and Cribb (1988) also indicated a hybrid nature of *C. sanderae*. Further studies by use of more markers both from nuclear and plastid genomes can confirm this hypothesis.

Pollinia number

The most controversial morphological character of *Cymbidium* is different numbers of pollinia (two or four) in the genus. Most members of *Cymbidium* have two pollinia, but subgenus *Jensoa* and subgenus *Cymbidium* section *Borneense* exhibit four pollinia. On the other hand, the sister group of *Cymbidium* (*Acriopsis*, *Grammatophyllum*, and *Thecostele*) and the sister group to these taxa (*Bromheadia*: Yukawa, unpublished) share two pollinia. Since the pollinia number of Orchidaceae is usually considered to be stable and has been treated as a cardinal character among taxonomists, variations in this genus have provoked much discussion (e.g. Du Puy & Cribb, 1988). The results of this study showed that the four pollinia are an apomorphic character in *Cymbidium* and likely evolved twice on the lineages leading to subgenus *Jensoa* and subgenus *Cymbidium* section *Borneense* (both in ACCTRAN and DELTRAN optimizations). Our results also indicated that the conservative nature of pollinia number is not a rule, but it is a kind of "dogma" in orchid classification. Actually, variations of pollinia numbers are found in other small- to middle-sized monophyletic groups such as *Thunia* (e.g. Seidenfaden, 1986) and *Phalaenopsis* (e.g. Christenson, 2001).

Life form

As mentioned before, *Cymbidium* exhibits a great variation of life forms. In this study we define epiphytes in a broad sense, that is, a group of species inhabiting trees and/or rocks. In *Cymbidium*, many species grow on trees and some of them also have potentials to live on rocks. However, accurate figures on habitats of each species are not known due to the lack of observations in situ. Besides, there are no *Cymbidium* species that grow exclusively on rocks. We thus use a broad concept of epiphyte.

The sister group taxa of *Cymbidium* (*Acriopsis*, *Grammatophyllum*, and *Thecostele*) are epiphytes and the sister group to these taxa (*Bromheadia*: Yukawa, unpublished) comprises both epiphytic and terrestrial species. The most
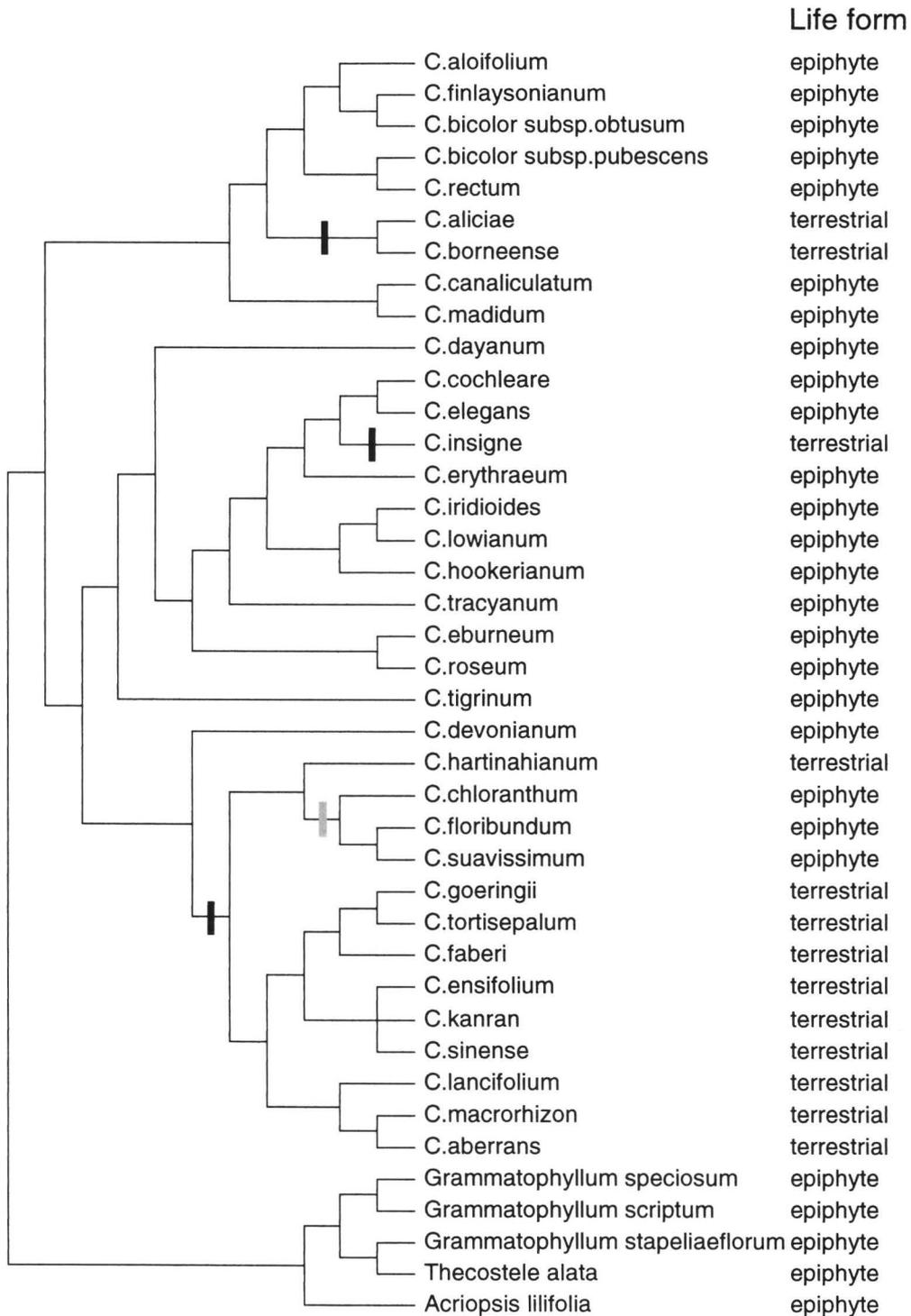
Life form

C.aloifolium	epiphyte
C.finlaysonianum	epiphyte
C.bicolor subsp.obtusum	epiphyte
C.bicolor subsp.pubescens	epiphyte
C.rectum	epiphyte
C.aliciae	terrestrial
C.borneense	terrestrial
C.canaliculatum	epiphyte
C.madidum	epiphyte
C.dayanum	epiphyte
C.cochleare	epiphyte
C.elegans	epiphyte
C.insigne	terrestrial
C.erythraeum	epiphyte
C.iridioides	epiphyte
C.lowianum	epiphyte
C.hookerianum	epiphyte
C.tracyanum	epiphyte
C.eburneum	epiphyte
C.roseum	epiphyte
C.tigrinum	epiphyte
C.devonianum	epiphyte
C.hartinahianum	terrestrial
C.chloranthum	epiphyte
C.floribundum	epiphyte
C.suavissimum	epiphyte
C.goeringii	terrestrial
C.tortisepalum	terrestrial
C.faberi	terrestrial
C.ensifolium	terrestrial
C.kanran	terrestrial
C.sinense	terrestrial
C.lancifolium	terrestrial
C.macrorhizon	terrestrial
C.aberrans	terrestrial
Grammatophyllum speciosum	epiphyte
Grammatophyllum scriptum	epiphyte
Grammatophyllum stapeliaeflorum	epiphyte
Thecostele alata	epiphyte
Acriopsis lilifolia	epiphyte

Fig. 4.   Reconstruction of diversification in life form in *Cymbidium* under ACCTRAN optimization. The tree is one of 168 most-parsimonious Fitch trees based upon *matK* and ITS sequences. Black bars=change of states from epiphytic to terrestrial habitat; grey bars=change of states from terrestrial to epiphytic habitat.

parsimonious reconstruction of character evolution on life forms indicated changes of character states at internodes shown by bars (Fig. 4). The ancestor of *Cymbidium* was likely an epiphyte. Under ACCTRAN optimization, the epiphytic *Cymbidium* evolved terrestrial plants three times; moreover, a single reversal from terrestrial to epiphytic condition likely occurred in *Cymbidium*. Under DELTRAN optimization, the epiphytic ancestor of *Cymbidium* evolved into terrestrial plants four times, that is, (1) in the common ancestor of *Cymbidium* section *Borneense*, (2) in the ancestor of *Cymbidium insigne*, (3) in the ancestor of *Cymbidium hartinahianum*, (4) in the ancestor of *Cymbidium* subgenus *Jensoa* (results not shown in figures).

Recent phylogenetic analyses clarified that Orchidaceae had a terrestrial ancestry (Neyland and Urbatsch, 1995; Cameron *et al.*, 1999). Therefore, evolution from epiphytic to terrestrial habits in *Cymbidium* represents reversal in Orchidaceae as a whole. These results indicated that the changes of habitats appeared repeatedly in the lineage of *Cymbidium* and are not rare evolutionary events in Orchidaceae.

Nutritional habit

Cymbidium section *Pachyrhizanthe* Schltr. keeps strong mycotrophy throughout life history and lacks foliage leaves as well as roots. Albeit Nakai (1931)'s suggestion on its separate taxonomic status from *Cymbidium*, we found that this group belongs to *Cymbidium*. The present results also showed that the state, mycotrophy throughout life history, evolved only once within a clade of terrestrial *Cymbidium* species. All the terrestrial species of *Cymbidium* have obligate mycotrophy in their juvenile stage of life history. During this stage, the rhizome only represents the body and functions as the home of symbiotic fungi. Undoubtedly, the existence of obligate mycotrophy during juvenile stage in terrestrial species of *Cymbidium* acted as preadaptation of complete mycotrophy throughout life history in section *Pachyrhizanthe*. Furthermore, the growth habit of section *Pachyrhizanthe* only composed

of rhizomes and inflorescences represents a case of heterochrony.

Our molecular data further showed low genetic divergence between the mycotrophic clade and its autotrophic sister clade, namely, *Cymbidium lancifolium* (section *Geocymbidium*). The number of base differences and sequence divergences within the ITS region was as follows: *Cymbidium macrorhizon-C. lancifolium*: 9, 0.0118; *Cymbidium aberrans-C. lancifolium*: 9, 0.0106. This fact indicated that these apparently drastic shifts of nutritional habit and morphological characters were established within relatively short period of time.

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