American Journal of Botany 90(1): 123–130. 2003.

PHYLOGENY OF THE SUNDEWS, *DROSERA* (DROSERACEAE), BASED ON CHLOROPLAST *RBCL* AND NUCLEAR 18S RIBOSOMAL DNA SEQUENCES[1]

FERNANDO RIVADAVIA,[2,3] KATSUHIKO KONDO,[4] MASAHIRO KATO,[3] AND MITSUYASU HASEBE[2,5,6]

[2]National Institute for Basic Biology, Okazaki 444-8585, Japan; [3]Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan; [4]Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University, Higashi-Hiroshima 739-8526, Japan; and [5]Department of Molecular Biomechanics, School of Life Science, The Graduate University for Advanced Studies, Okazaki 444-8585, Japan

The sundew genus *Drosera* consists of carnivorous plants with active flypaper traps and includes nearly 150 species distributed mainly in Australia, Africa, and South America, with some Northern Hemisphere species. In addition to confused intrageneric classification of *Drosera*, the intergeneric relationships among the *Drosera* and two other genera in the Droseraceae with snap traps, *Dionaea* and *Aldrovanda*, are problematic. We conducted phylogenetic analyses of DNA sequences of the chloroplast *rbcL* gene for 59 species of *Drosera*, covering all sections except one. These analyses revealed that five of 11 sections, including three monotypic sections, are polyphyletic. Combined *rbcL* and 18S rDNA sequence data were used to infer phylogenetic relationships among *Drosera*, *Dionaea*, and *Aldrovanda*. This analysis revealed that all *Drosera* species form a clade sister to a clade including *Dionaea* and *Aldrovanda*, suggesting that the snap traps of *Aldrovanda* and *Dionaea* are homologous despite their morphological differences. MacClade reconstructions indicated that multiple episodes of aneuploidy occurred in a clade that includes mainly Australian species, while the chromosome numbers in the other clades are not as variable. *Drosera regia*, which is native to South Africa, and most species native to Australia, were clustered basally, suggesting that *Drosera* originated in Africa or Australia. The *rbcL* tree indicates that Australian species expanded their distribution to South America and then to Africa. Expansion of distribution to the Northern Hemisphere from the Southern Hemispere occurred in a few different lineages.

Key words: *Aldrovanda*; biogeography; carnivorous plants; *Dionaea*; *Drosera*; Droseraceae; *rbcL*; 18S rDNA.

Carnivorous plants have long attracted the attention of botanists, because of their highly specialized morphology and curious trapping mechanisms (Juniper, Robins, and Joel, 1989). The carnivorous plant family Droseraceae includes four genera historically: the sundews *Drosera*, *Drosophyllum*, *Aldrovanda*, and the Venus's flytrap *Dionaea*, the last three of which are monotypic (Cronquist, 1981; Takhtajan, 1997). A phylogenetic analysis based on the sequences of two plastid genes, *rbcL* and *matK*, indicated that *Drosophyllum lusitanicum*, a perennial subshrub native to the Iberian coastal fringe and northern Morocco (Juniper, Robins, and Joel, 1989), does not form a clade with other members of the Droseraceae, but is sister to the Dioncophyllaceae-Ancistrocladaceae clade (Fay et al., 1997; Meimberg et al., 2000). The exclusion of *Drosophyllum* is also supported by some morphological characters (Takahashi and Sohma, 1982; Juniper, Robins, and Joel, 1989; Conran, Jaudzems, and Hallam, 1997).

Aldrovanda vesiculosa and *Dionaea muscipula* share a similar trapping mechanism, called a snap trap, exclusive to these two taxa (Juniper, Robins, and Joel, 1989). *Aldrovanda vesiculosa* is a floating aquatic species that is found throughout the Old World and northern and eastern Australia, while *Dionaea muscipula* is a terrestrial plant that is endemic to marshy habitats on the coastal plains of North and South Carolina, USA (Juniper, Robins, and Joel, 1989). The genus *Drosera* includes nearly 150, mostly perennial, species (Juniper, Robins, and Joel, 1989; Lowrie, 1998). Although *Drosera* has a worldwide distribution, the vast majority of species are found in the Southern Hemisphere, especially in southwestern Australia. *Drosera* have active flypaper traps and capture their prey with mobile glandular hairs that are present on the adaxial leaf surface.

The evolution of leaves with trap systems from noncarnivorous ones is mysterious, and there are no widely accepted hypotheses. Active flypaper traps are believed to have evolved from a leaf only with adhesive glands after acquisition of nastic and tropic gland tentacles with touch receptors and mobility (Juniper, Robins, and Joel, 1989). However, the correlations between active flypaper traps and snap traps are ambiguous, because there are no reports of morphologically intermediate leaves. Furthermore, the relationships among the three genera have not been solved with high statistical confidence in either *rbcL* or *matK* trees, although the monophyly of *Drosera*, *Dionaea*, and *Aldrovanda* is widely accepted, based on the morphological and molecular data (Williams, Albert, and Chase, 1994; Fay et al., 1997; Meimberg et al., 2000).

[1] Manuscript received 23 April 2002; revision accepted 19 July 2002.

The authors thank Ryosuke Sano, Yukiko Tanikawa, and Tomoaki Nishiyama for experimental supports and phylogenetic analyses, Yoshikazu Hoshi, Misako Mishima, and two anonymous reviewers for helpful comments on this manuscript, Joe Mullins, Jan Schlauer, and Stephen Williams for discussion on this study, Paul Burkhardt, the Caeppart family, Marcos R. F. Cardoso, Mark Edwards, Dave Evans, Robert Gibson, Eric Green, Sadashi Komiya, Ivo Koudela, Robert and Michelle Kunitz, Robert Kunitz, Jay Lechtman, Laurent Legendre, Allen Lowrie, David Mellard, Peter Northcote, Fabio Pinheiro, Chiaki Shibata, Isao Takai, and Kunihiko Ueda for plant material and DNA. This research was partly supported by grants from the Ministry of Education, Science, Culture, and Sports, Japan (MESCSJ) to M.H.; Japan Society for the Promotion of Science to K.K., M.K., and M.H.; and the Fujiwara Natural History Foundation to M.H. F.R., a MESCSJ scholarship student, was awarded a Sasakawa Scientific Research Grant from the Japan Science Society.

[6] Author for reprint requests (FAX: +81-564-55-7546; e-mail: mhasebe@nibb.ac.jp).

Historically, the characters most used for the taxonomy of *Drosera* have been habit, leaf shape, style number and morphology, and the presence or absence of stipules and specialized organs, such as tubers and gemmae (Candolle, 1824; Planchon, 1848; Diels, 1906, 1936). In recent decades, new information, such as chromosome numbers (e.g., Kondo, 1976), pollen morphology (Takahashi and Sohma, 1982), secondary compounds (Culham and Gornall, 1994), and seed germination types (Conran, Jaudzems, and Hallam, 1997), has been added. Although new systems have been proposed recently (Marchant, Ashton, and George, 1982; Seine and Barthlott, 1994; Schlauer, 1996), the delimitations of the subgenera and sections of *Drosera* are controversial. Williams, Albert, and Chase (1994) inferred the phylogenetic relationship of 12 *Drosera* species covering most sections sensu Seine and Barthlott (1994), but the phylogenetic relationships within *Drosera*, which is morphologically divergent and includes more than 150 species, are still ambiguous, because the few species selected from each section have not been sufficient for an overview of the general phylogenetic relationships of *Drosera*.

The purposes of this study were (1) to investigate the delimitations and phylogenetic relationships of the subgenera and sections in *Drosera*, (2) to infer the origin and dispersal of *Drosera*, (3) to infer the evolution of chromosome number, and (4) to infer the phylogenetic relationships among *Aldrovanda*, *Dionaea*, and *Drosera* using *rbcL* and 18S rDNA sequences.

MATERIALS AND METHODS

Plant materials—Dried leaf materials of taxa were collected preferentially in their natural habitats or otherwise obtained from tissue culture and cultivation, for all subgenera and sections of *Drosera* sensu Seine and Barthlott (1994) except sect. *Meristocaules* (http://ajbsupp.botany.org/v90/). Total DNA extraction and sequencing generally followed Hasebe et al. (1994).

DNA isolation and sequencing—Three overlapping fragments, which cover most of the *rbcL* gene, were amplified by the polymerase chain reaction (PCR). The primers used for the amplification followed Hasebe et al. (1994). The amplified products were electrophoresed on 1% agarose gels and purified with GeneClean III (BIO 101, La Jolla, California, USA). The purified double-stranded DNA was sequenced in both directions using an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, California, USA) with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

Based on the resulting *rbcL* tree, eight species of *Drosera*, *Aldrovanda*, and *Dionaea* were selected (http://ajbsupp.botany.org/v90/), and their 18S rDNA was sequenced to analyze the basal relationships of the Droseraceae. The PCR amplification of partial 18S rDNA using primer N-NS1, N-18G, N-18H, C-18G, C-18H, and C-18L (Bult, Källersjö, and Suh, 1992) was performed. The DNA was purified and sequenced as for the *rbcL* gene.

Phylogenetic analyses—The *rbcL* and 18S rDNA nucleotide sequences were aligned using Clustal W version 1.8 (Thompson, Higgins, and Gibson, 1994) and then revised manually. Phylogenetic analyses were conducted using the parsimony method. To search for the most-parsimonious (MP) tree, PAUP* version 4.0b4a (Swofford, 2000) was used with the heuristic search option, saving all minimal length trees (MULPARS on) with tree bisection-reconstruction (TBR) branch-swapping, and 10 000 replicates of random taxon addition. Characters were equally weighted. Bootstrap (Felsenstein, 1985) and decay (Bremer, 1988; Donoghue et al., 1992) analyses were used to obtain a measure of support for each branch. Ten thousand bootstrap replications were carried out using ''Fast'' stepwise addition. The decay indices for representative branches were calculated with PAUP* in conjunction with the program AutoDecay version 5.0 (provided by T. Eriksson, Stockholm University, Stockholm, Sweden). In the decay analyses, the MP trees were searched for under the reverse-constraint option of PAUP* with 100 replications of random sequence addition using TBR branch swapping. The computations were done on a SUN Enterprise 3000 or an SGI Origin 2000 in the Computer Laboratory of the National Institute for Basic Biology (NIBB).

Species of Ancistrocladaceae, Frankeniaceae, Nepenthaceae, Plumbaginaceae, Polygonaceae, Simmondsiaceae, Tamaricaceae, and *Drosophyllum* were selected as outgroup taxa of the *rbcL* tree based on previously published broadscale analyses (Williams, Albert, and Chase, 1994; Meimberg et al., 2000). Because 18S rDNA sequences are not available in the DNA database for most of these taxa, *Simmondsia chinensis* and *Nepenthes alata* were used as outgroup taxa.

We also conducted maximum likelihood (ML) analyses for both *rbcL* and the combined data sets with PAUP* version 4.0 using a heuristic search, TBR branch-swapping with 1000 random sequence additions, and the HKY85 model (Hasegawa, Kishino, and Yano, 1985) with a transition/transversion ratio of 2.0, empirical base frequencies, and assuming an equal rate of evolution for all sites.

Character evolution—The phylogenetic distribution of chromosome numbers and geographic distribution were investigated. Chromosome numbers were obtained from the references listed in http://ajbsupp.botany.org/v90/, and operational taxonomic units (OTUs) whose chromosome numbers were counted were included in this analysis. Using MacClade 3.05 (Maddison and Maddison, 1992), we traced data onto the strict consensus of the shortest trees obtained in the *rbcL* analysis. Outgroup taxa were excluded, because information on chromosome number for these taxa is scarce. The phylogenetic positions of *Aldrovanda* and *Dionaea* were followed for the tree using the combined data set of *rbcL* and 18S rDNA (Fig. 2). Species with intraspecific polymorphisms of chromosome number were treated as a clade including polytomy OTUs with each chromosome number. *Drosera trinervia* ''A'' and ''B'' formed a sister group (Fig. 1) and merged into a single OTU. To gain insights into the geographic history of *Drosera* from a phylogenetic perspective, information on the geographic distribution of *Drosera* obtained from the Carnivorous Plant Database (http://www2.labs.agilent.com/bot/cp_home) was traced on the strict consensus tree of the shortest *rbcL* trees modified as mentioned above. Species distributed in different geographic areas were treated as a clade, including OTUs with different distributions.

RESULTS

The 1227-base pair (bp) region of the *rbcL* gene between base pairs 64 and 1290, numbered from the initial methionine codon of *Nicotiana tabacum* (Shinozaki et al., 1986), was used for the phylogenetic analyses. The nucleotide sequences were aligned without any insertions or deletions. The data matrix for the 75 taxa including 16 outgroup taxa contained 395 variable sites, of which 262 were phylogenetically informative. Parsimony analysis produced 4608 MP trees of 1087 steps in 12 islands (Maddison, 1991). One of the islands included 4597 MP trees, while every other island contained a single tree. The MP trees had a consistency index of 0.501 (0.421 excluding uninformative sites), a retention index of 0.800, and a rescaled consistency index of 0.400. One of the 4620 MP trees is shown in Fig. 1 with decay indices and bootstrap values. The ML analysis yielded a single ML tree ($-\ln = 8789.030$) in a single island that is concordant with the strict consensus tree of the MP analysis (data not shown).

As the relationships of *Dionaea*, *Aldrovanda*, *Drosera regia*, *D. arcturi*, and other *Drosera* species were not well-resolved, partial 18S rDNAs of the former four species and *D. pygmaea*, *D. glanduligera*, *D. anglica*, *D. montana* var. *tomentosa*, *D. sessilifolia*, and *D. platypoda* were sequenced (http://ajbsupp.botany.org/v90/). The nucleotide sequences of

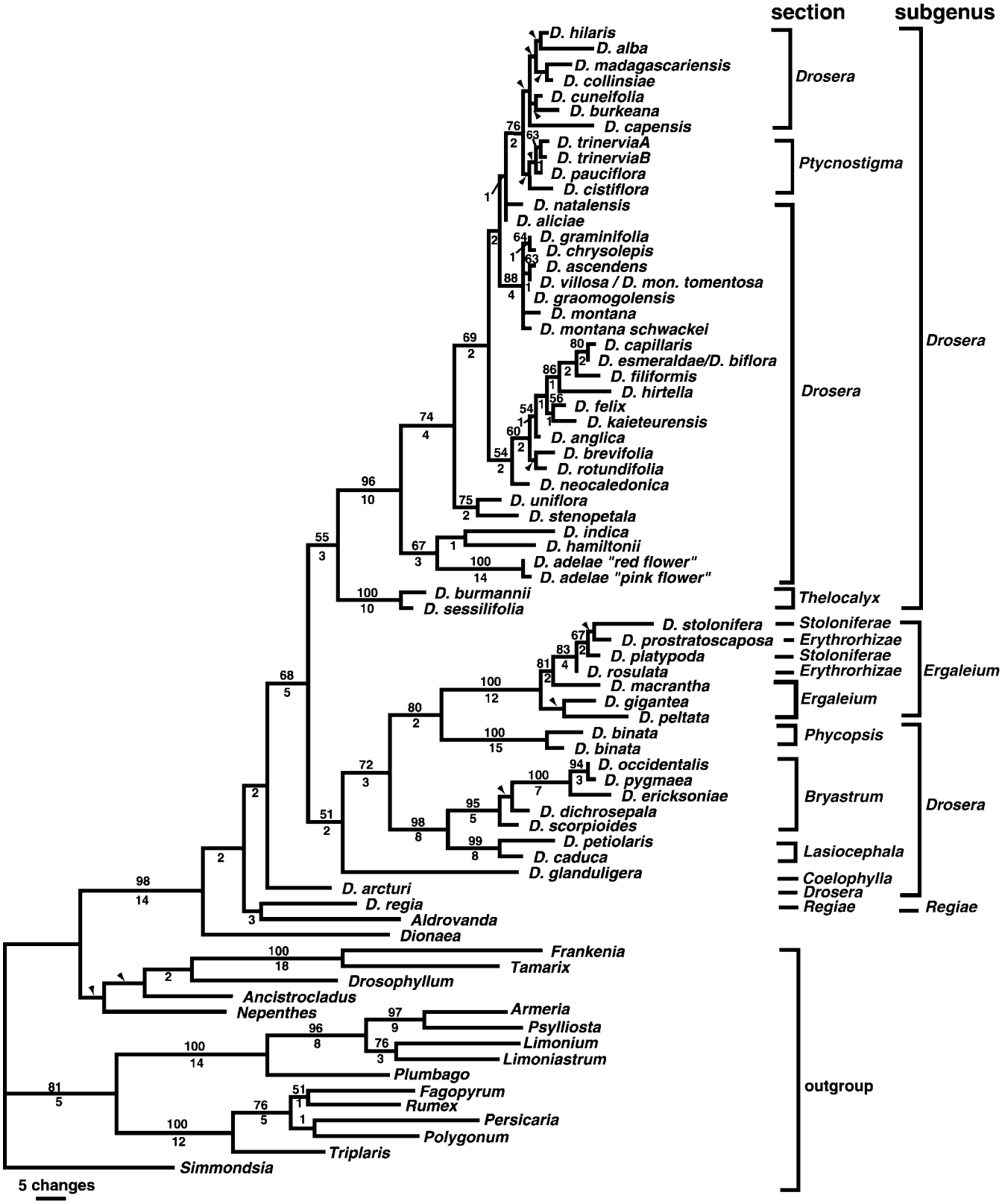

Fig. 1. One of the 4608 most-parsimonious trees resulting from the parsimony analysis of *rbcL* sequences. The branch lengths correspond to the number of nucleotide substitutions (ACCTRAN optimization). The numbers above the branches are the bootstrap values greater than 50% for 10 000 bootstrap replicates, and the numbers below branches are the decay indices (Bremer, 1988). Arrows indicate branches not found in all of the shortest trees. The infrageneric classification sensu Seine and Barthlott (1994) is shown on the right.

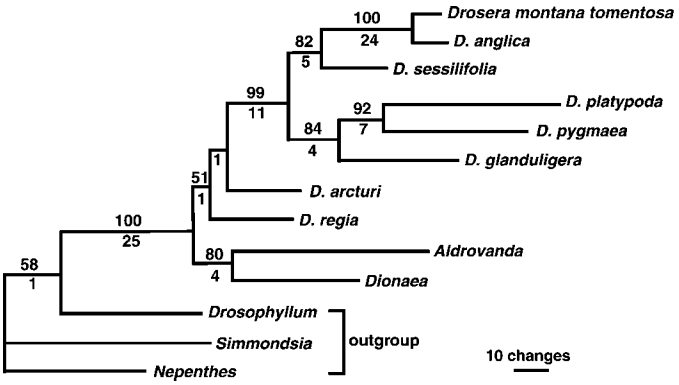

Fig. 2. The most-parsimonious tree resulting from parsimony analysis of the combined *rbcL* and 18S rDNA sequences. The branch lengths correspond to the number of nucleotide substitutions (ACCTRAN optimization). The numbers above the branches are the bootstrap values greater than 50% for 10 000 bootstrap replicates, and the numbers below the branches are decay indices (Bremer, 1988).

18S rDNA between base pairs 49 and 1707, numbered from the first nucleotide of *Glycine max* 18S rDNA (Eckenrode, Arnold, and Meagher, 1985), were aligned, and the 1648-bp region excluding insertions and deletions was combined with the 1227-bp *rbcL* nucleotide sequence. The 2875-bp data matrix for the 13 taxa, including two outgroup taxa, contained 357 variable sites, of which 181 were informative. Parsimony analysis produced a single MP tree of 589 steps in a single island with a consistency index of 0.727 (0.600 excluding uninformative sites), a retention index of 0.574, and a rescaled consistency index of 0.417 (Fig. 2). The ML analysis yielded a single ML tree ($-\ln = 7748.00$) identical to the MP tree in a single island (data not shown).

DISCUSSION

Phylogeny of Droseraceae—The monophyly of the Droseraceae, including *Drosera*, *Dionaea*, and *Aldrovanda* but excluding *Drosophyllum*, was supported with high statistical confidence in the trees based on the *rbcL* (Fig. 1) and combined *rbcL* and 18S rDNA data sets (Fig. 2). *Drosera*, except *D. arcturi* and *D. regia* (core *Drosera*), formed a clade with 68% bootstrap support (BP) in the *rbcL* tree and with 99% BP in the combined data set tree. The phylogenetic relationships among *D. arcturi*, *D. regia*, *Dionaea*, and *Aldrovanda* were not well resolved in the *rbcL* tree with high BP, while the MP tree for the combined data set showed that *Dionaea* and *Aldrovanda* form a sister group with 80% BP. Although the morphology of the flypaper trap system of *Drosera* differs markedly from that of the snap trap system of *Dionaea* and *Aldrovanda*, some structures have been proposed to be homologous between the two systems (Williams, 1976; Juniper, Robins, and Joel, 1989). Both systems have sessile glands for absorbing digested prey, and the cellular anatomy of these glands is similar in the two systems. Comparative studies of cellular organization of the stalked glands of *Drosera* and the trigger hairs of *Dionaea* and *Aldrovanda* indicate that these multicellular hairs have a close relationship. The origin of these glandular hairs, trigger hairs, and sessile glands is likely traced to adhesive glands observed in the Plumbaginaceae and other families, which are outgroups of the Droseraceae clade (*Drosera*, *Dionaea*, and *Aldrovanda*) and the Dioncophyllaceae clade, which includes two flypaper carnivorous plants, *Drosophyllum* and *Triphyophyllum* (Meimberg et al., 2000). Our result indicates that the flypaper system of *Drosera* and the snap trap system of *Dionaea* and *Aldrovanda* were established early in the evolution of these carnivorous plant taxa. As the *rbcL* tree does not show high bootstrap support for particular relationships to any of the outgroup taxa, it was not possible to elucidate which trap system the common ancestor of these two lineages had or whether these two systems evolved independently from noncarnivorous plants. The sister relationship of *Dionaea* and *Aldrovanda* indicates a single evolutionary origin of the elaborate snap trap system in plants, although terrestrial *Dionaea* and aquatic *Aldrovanda* have different habitats.

Interspecific relationships of Drosera—The basal relationships of *Drosera* were ambiguous in both the *rbcL* and combined trees, although *D. regia* and *D. arcturi* clustered more basally than the other *Drosera* species in both trees. *Drosera regia*, which occurs in a single mountain valley in South Africa (Obermeyer, 1970), has traditionally been treated as a different group from the other *Drosera*, because it has several plesiomorphic characters, such as operculate pollen (Takahashi and Sohma, 1982) and a lack of stipules (Williams, Albert, and Chase, 1994), both of which are similar to characters found in *Dionaea*. *Drosera regia* also has some autapomorphic characters, which are not observed in other *Drosera* species, such as woody rhizome, the longest leaves and inflorescences in the genus, and somewhat zygomorphic flowers resulting from the odd position of the three exceptionally long and undivided styles except at the very apicies (Stephens, 1926).

The basal clustering of *D. arcturi* is unexpected, because this species does not have plesiomorphic characters as observed in *D. regia*. *Drosera arcturi* is native to New Zealand and southeastern Australia, including Tasmania (Allan, 1961; Lowrie, 1998), and is thought to be closely related to *D. stenopetala* and *D. uniflora* because of their shared characters, such as solitary white flowers on relatively short scapes and reduced or absent stipules (Diels, 1906, 1936; Schlauer, 1996; Lowrie, 1998). On the *rbcL* tree, *D. arcturi* was not closely related to *D. stenopetala* and *D. uniflora*. Further analyses of morphological characters, such as pollen morphology, which is divergent among major groups of *Drosera* (Takahashi and Sohma, 1982), may produce further evidence that supports the basal relationship of *D. arcturi* in *Drosera*.

Australian species—We will follow the system of Seine and Barthlott (1994) in the following discussion (http://ajbsupp.botany.org/v90/, Fig. 1). The clade from *D. stolonifera* to *D. glanduligera* in Fig. 1 includes species distributed in Australia. Section *Coelophylla* is basal to sect. *Bryastrum*, sect. *Lasiocephala*, sect. *Phycopsis*, and subgen. *Ergaleium* including sect. *Ergaleium*, *Erythrorhizae*, and *Stoloniferae*. A clade composed of these sections was found in a smaller scale *rbcL* analysis (Albert et al., 1992; Williams, Albert, and Chase, 1994) and was further confirmed in this study, which included several more species. Species in this clade are well adapted to dry environments and have tubers (subgen. *Ergaleium*), stout roots (sect. *Phycopsis*), or an annual habit (sect. *Coelophylla*) (Lowrie, 1987, 1989, 1998), and species with each adaptive character form a different clade. Species with gemmae for vegetative propagation form a monophyletic group (sect. *Bryastrum* and *Lasiocephala*). The monophyly of sect. *Phycopsis*

and subgen. *Ergaleium* proposed based on pollen morphology characterized by poorly developed radial plates (Takahashi and Sohma, 1982) was supported with a high bootstrap probability (BP) (80%).

All species in sect. *Bryastrum*, except *D. pygmaea*, have pentamerous flowers and are restricted to southwestern Australia. *Drosera pygmaea* has tetramerous flowers and occurs in New Zealand (Allan, 1961) and both eastern and western Australia (Lowrie, 1989). Due to its tetramerous flowers and unique distribution, *D. pygmaea* has been placed in a different section from all other pygmy sundews (Planchon, 1848; Diels, 1906, 1936; Marchant, Ashton, and George, 1982; Schlauer, 1996). The *rbcL* tree indicates that *D. pygmaea* forms a clade with other pygmy sundews and that tetramerous flowers are an autapomorphic character that evolved in this species from the pentamerous flowers shared by other pygmy sundews. This result supports the system of Seine and Barthlott (1994) in which *D. pygmaea* is included in sect. *Bryastrum* with other pygmy sundews.

The three sections of subgen. *Ergaleium* are distinguished by their leaf shape and the presence or absence of erect stems (Marchant, Ashton, and George, 1982). Section *Ergaleium* is characterized by erect or scrambling stems. Section *Erythrorhizae* includes species with non-erect short stems with leaves in rosettes. Section *Stoloniferae* comprises a few species that form two growth phases: a short stem with rosette leaves in early development and short erect stems at a later stage. The *rbcL* tree suggests that the species with an erect stem without rosette leaves in sect. *Ergaleium* are more basal than those with rosette leaves in sect. *Erythrorhizae* and *Stoloniferae*. The latter two sections are paraphyletic in the *rbcL* tree, although their bootstrap values are not so high. Further analyses using more taxa are necessary for systematic revision of these sections.

Drosera burmannii and *D. sessilifolia* of sect. *Thelocalyx* have plesiomorphic pollen with simple cohesion, like that observed in *Aldrovanda* and *Dionaea*, instead of cross wall cohesion as observed in other *Drosera* species, except *D. glanduligera*. Their pentamerous gynoecium is also observed in the outgroup taxa, but not in other *Drosera* species (Diels, 1906, 1936). A sister relationship of both species was strongly supported with 100% BP in the *rbcL* tree, although these species are distributed in disjunct areas: Australia, India, China, Japan throughout Southeastern Asia for *D. burmannii* and endemic in South America for *D. sessilifolia* (Lowrie, 1998; de Stefano and Culham, 1998).

Polyphyly of section Drosera—Section *Drosera* is not monophyletic (Fig. 1). The most basal clade includes *D. hamiltonii*, which is native to southwestern Australia (Lowrie, 1989) and has a fused style, a characteristic not observed in any other *Droseraceae*, except *Dionaea* (Diels, 1906, 1936; Lowrie, 1989). Although *D. hamiltonii* has been classified in a section separate from other species of sect. *Drosera* (Diels, 1906, 1936; Marchant, Ashton, and George, 1982; Schlauer, 1996), it formed a clade with two species of sect. *Drosera*: *D. adelae* and *D. indica*. Although the close relationship between *D. adelae* and *D. indica* has been suggested by previous studies (Planchon, 1848; Diels, 1906, 1936; Marchant, Ashton, and George, 1982), neither of these species has ever been closely associated with *D. hamiltonii*. One character supporting the monophyly of this clade is the chromosome number of these species, $2n = 28$ or 30, which is rare in other *Drosera* species (Fig. 3A; Venkatasubban, 1950; Kondo, 1976; Kondo and Olivier, 1979; Kondo and Lavarack, 1984).

Drosera uniflora and *D. stenopetala* formed a sister group, which is also supported by their similar morphological characters mentioned above. The clade from *D. capillaris* to *D. neocaledonica* in Fig. 1 includes species distributed in both Eurasia and America, plus *D. neocaledonica*, which is endemic to New Caledonia. *Drosera rotundifolia* and *D. anglica* are widely distributed in both Eurasia and North America, while the other species studied are native to North and South America. It has been suggested that *D. anglica* is a hybrid between *D. rotundifolia* and *D. linearis* (Wood, 1955). The *rbcL* nucleotide sequences of *D. rotundifolia* and *D. anglica* differed by a single nucleotide, suggesting that *D. rotundifolia* is the maternal parent of *D. anglica*, if chloroplasts are maternally inherited in *Drosera*, as occurs in other angiosperm species (Sears, 1980).

The clade from *D. graminifolia* to *D. montana* var. *schwackei* in Fig. 1 is distributed in South America, mainly in central and eastern Brazil. *Drosera montana* is one of the most taxonomically confusing species in the genus *Drosera*. Saint-Hilaire (1824) described *D. tomentosa*, *D. hirtella*, and *D. montana*, but Diels (1906, 1936) eliminated the former two taxa as varieties of *D. montana* and further described *D. montana* var. *schwackei*. In the *rbcL* tree, *D. hirtella* belongs to a different clade from other varieties of *D. montana*, supporting the designation of Saint-Hilaire (1824). The three varieties of *D. montana* examined in this study did not form a clade, which suggests that these varieties should be treated as different species rather than as varieties of *D. montana*.

The clade from *D. hilaris* to *D. aliciae* is composed of African species. The species in sect. *Ptycnostigma* are characterized by their thickened roots. Some authors (Diels, 1906, 1936; Seine and Barthlott, 1994) placed these species in an independent section, while Marchant, Ashton, and George (1982) merged this section with sect. *Drosera*. The *rbcL* tree supports the classification treating sect. *Ptycnostigma* as different from other sections.

The *rbcL* tree is not concordant with any intrageneric classification of *Drosera*, although some clades characterized by morphological characters, chromosome number, and geographic distribution were detected in the *rbcL* tree. It is necessary to revise the classification of *Drosera* by incorporating the *rbcL* tree data and further analyses of morphological characters.

Evolution of chromosome number—Our analysis showed that conspicuous chromosome diversity caused by both aneuploidization and polyploidization is observed extensively in the clade from *D. stolonifera* to *D. glanduligera*, which is almost exclusively Australian, while chromosome number is moderately conserved in the other clades (Fig. 3A). Aneuploidy in *Drosera* is likely caused by the diffused centromeres, which should be stably transmitted after cell division, even if some chromosomes are fragmented (Kondo, Segawa, and Nehira, 1976). Distinct primary constrictions or centromeres and clear gaps between sister chromatids have not been observed in *Drosera* (Kondo, Segawa, and Nehira, 1976; Kondo and Segawa, 1988), which supports this hypothesis. It has been shown that chromosomes artificially fragmented by gamma radiation are mitotically functional, which also supports this hypothesis (Sheikh, Kondo, and Hoshi, 1995). The reason why aneuploidy is more popular in Australia than in other areas is

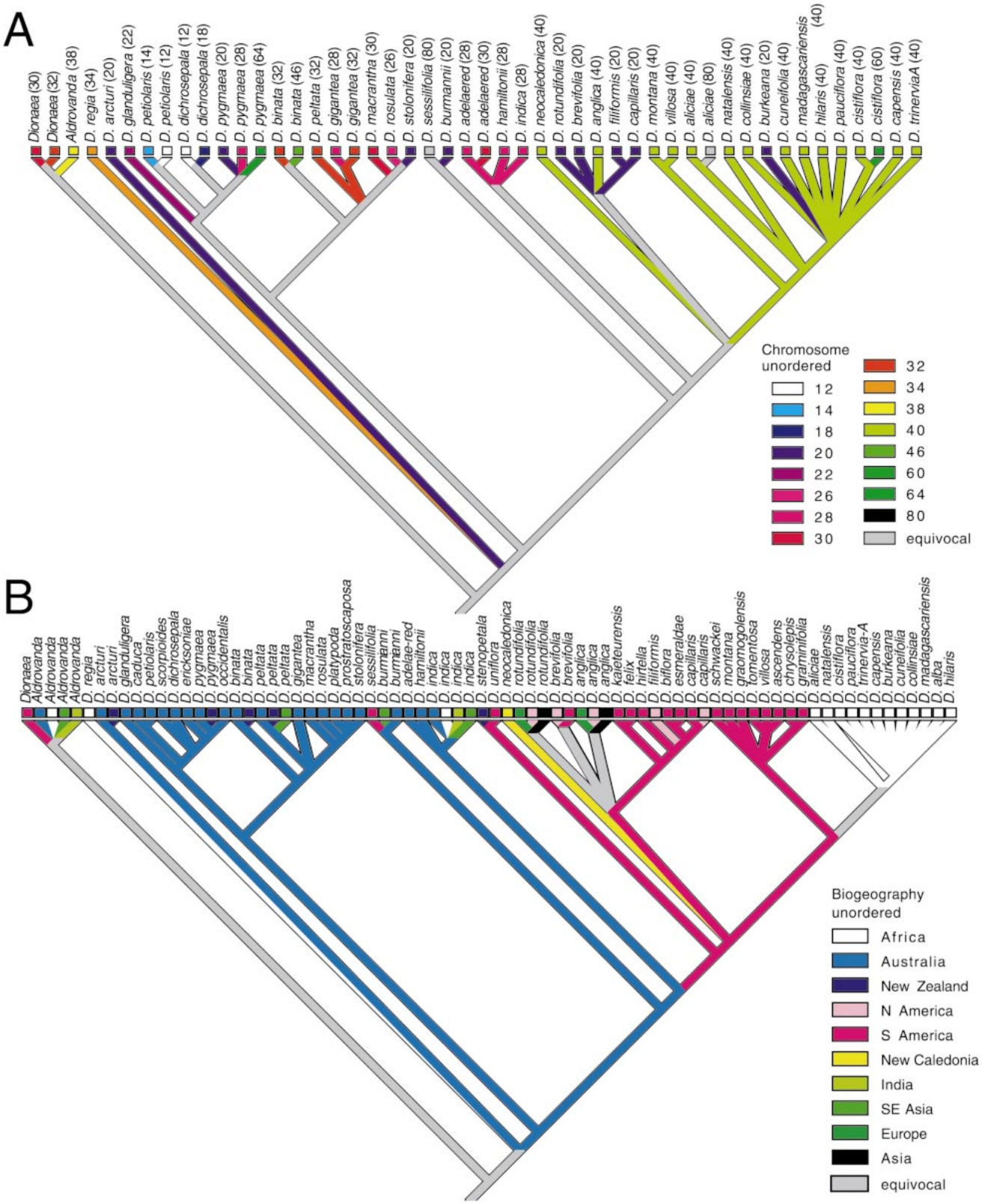

Fig. 3. Strict consensus of the shortest trees obtained for *Drosera* using the *rbcL* sequence data, onto which chromosome numbers (A) and distributions (B) have been mapped using MacClade (Maddison and Maddison, 1992). (A) Operational taxonomic units (OTUs) whose chromosome numbers were not reported are excluded from the tree. The chromosome numbers are from the references listed in http://ajbsupp.botany.org/v90/. For those species for which more than one chromosome number has been reported, each chromosome number corresponds to a different OTU forming a clade. The chromosome number is given in parentheses after the species name. (B) The distributions are from the Carnivorous Plant Database (http://www2.labs.agilent.com/bot/cp_home). Those species distributed in different geographic areas were treated as a clade, including OTUs with different distributions. Species distributed in different geographic areas were treated as a clade, including OTUs with different distributions.

unknown, and further studies on polymorphisms of chromosome numbers and karyotypes in Australian *Drosera* species are necessary.

The chromosome number of *D. regia* is $2n = 34$, which is unique among *Drosera* species. This number is similar to those of *Dionaea* ($2n = 30$ or 32) and *Aldrovanda* ($2n = 38$), which is concordant with the basal position of *D. regia* in the *Drosera rbcL* tree. On the other hand, the chromosome number of the other basal species, *D. arcturi*, is $2n = 20$, which is also found in other *Drosera* species (Fig. 3A). This suggests that *D. regia* is more basal than *D. arcturi*.

Most of the species in the terminal clades from *D. hilaris* to *D. montana* var. *schwackei* have a chromosome number of $2n = 40$, which is probably related to $2n = 20$. A decrease in chromosome number from 40 to 20 occurred in *D. burkeana*.

Phytogeography of Drosera—*Drosera* is widely distributed in both hemispheres (Juniper, Robins, and Joel, 1989; Schlauer, 1996), and the center of diversity of *Drosera* appears to be Australia, where over 80 species are found (Lowrie, 1987, 1989, 1998). Africa contains over 30 species, although species distributed in northern Africa are limited and half of the species are distributed in South Africa. South America also has about 30 species, some of which have expanded their range into North America. Less than ten species occur in Eurasia and North America, although some of these species are recorded over a wide range. Our analysis showed that the species endemic to Africa are divided into three groups: *D. regia*, *D. indica*, and the other African species (Fig. 3B). *Drosera regia* is basal, while the clade including all the other African species except *D. indica* clustered at the terminal position. *Drosera arcturi*, which is native to Australia and New Zealand, is also basal, and all the other Australian species clustered next to *D. regia* and *D. arcturi*, indicating that the origin of *Drosera* was in Africa or Australia. *Aldrovanda* is widely distributed in both hemispheres, including Australia and Africa, while *Dionaea* is endemic to North America. The distributions of the other outgroup species used in this study (http://www.ajbsupp.botany.org/v90/) varied, and it was impossible to specify whether *Drosera* originated in Africa or Australia.

The *rbcL* tree shows that the South American species arose by dispersal from Australia and that the African species other than *D. regia* and *D. indica* arose from South America. This pattern of distribution recalls the continental drift of Gondwanaland. This hypothesis is not plausible, because the Droseraceae are located close to the tip of the angiosperm phylogenetic tree, while this family would have to have split from the other angiosperm families at a very distant time for the Gondwanaland break-up to explain the divergence of *Drosera* species. Although some extinct angiosperm taxa related to extant families were reported (reviewed in Crepet, 2000), it is hard to extrapolate that ancestors of extant species in an extant genus were diverged at the age of Gondwana breakage. The hypothesized Gondwana origins of the Coriariaceae and Melastomataceae were rejected by the molecular clock (Yokoyama et al., 2000; Renner, Clausing, and Meyer, 2001), and this is also the case for the *Drosera*, whose disjunct distribution likely resulted from long-distance dispersal. Even though a molecular clock of *rbcL* is episodic, divergence of extant genera does not ascend to the Cretaceous (Sanderson and Doyle, 2001).

Dispersal from Australia to South America also likely occurred in the clade that includes *D. burmannii* and *D. sessilifolia*. Two closely related species, *D. uniflora* and *D. stenopetala*, have disjunct distributions in South America and New Zealand. Similar close relationships between New Zealand and South American species have been reported in *Coriaria* (Yokoyama et al., 2000), and there might be some unknown mechanism for long-distance dispersal between these two continents. The species endemic to New Caledonia, *D. neocaledonica*, likely originated from a South American ancestor via long-distance dispersal.

Dispersal from Australia to Asia via Southeast Asia occurred in *D. burmannii*, *D. indica*, and *D. peltata*, although it is not known why these species were the only members of their respective clades to expand their distributions in such a manner. Smaller numbers of Drosera species are distributed in the Northern Hemisphere than in the Southern Hemisphere, as mentioned above. Our analysis suggests that all the Northern Hemisphere species examined (*D. rotundifolia*, *D. anglica*, *D. filiformis*, *D. capillaris*, *D. brevifolia*, *D. indica*, *D. burmannii*, and *D. peltata*) expanded their distributions from the Southern Hemisphere, although further analyses with more taxa will be necessary to confirm this inference. *Drosera anglica* and *D. rotundifolia* are distributed in both Eurasia and North America, and it is likely that these species expanded their range by dispersal from South America to Eurasia via North America.

LITERATURE CITED

ALBERT, V. A., S. E. WILLIAMS, AND M. W. CHASE. 1992. Carnivorous plants: phylogeny and structural evolution. *Science* 257: 1491–1495.

ALLAN, H. H. 1961. Droseraceae. *In* H. H. Allan [ed.], Flora of New Zealand, vol. 1, 200–203. Government Printer, Wellington, New Zealand.

BEHRE, K. 1929. Physiologische und zytologische Untersuchungen über *Drosera*. *Planta* 7: 208–306.

BENNETT, S. T., AND M. CHEEK. 1990. The cytology and morphology of *Drosera slackii* and its relatives in South Africa. *Kew Bulletin* 45: 375–381.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BULT, C., M. KÄLLERSJÖ, AND Y. SUH. 1992. Amplification and sequencing of 16/18S rDNA from gel-purified total plant DNA. *Plant Molecular Biology Reporter* 10: 273–284.

CANDOLLE, A. P. DE. 1824. Droseraceae. *Prodomus systematis naturalis regni vegetabilis* 1: 317–320.

CONRAN, J. G., V. G. JAUDZEMS, AND N. D. HALLAM. 1997. Droseraceae germination patterns and their taxonomic significance. *Botanical Journal of the Linnean Society* 123: 211–223.

CREPET, W. L. 2000. Progress in understanding angiosperm history, success, and relationships: Darwin's abominably ''perplexing phenomenon.'' *Proceedings of National Academy of Science USA* 97: 12 939–12 941.

CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Cambridge University Press, New York, New York, USA.

CULHAM, A., AND R. J. GORNALL. 1994. The taxonomic significance of naphtoquinones in the Droseraceae. *Biochemical Systematics and Ecology* 22: 507–515.

DE STEFANO, R. D., AND A. CULHAM. 1998. Droseraceae. *In* J. A. Steyermark, P. E. Berry, and B. K. Holst [eds.], Flora of the Venezuelan Guayana, 697–703. Missouri Botanical Garden Press, St. Louis, Missouri, USA.

DIELS, L. 1906. Droseraceae. *In* A. Engler [ed.], Das Pflanzenreich, vol. 4, 112. (Heft 26), 1–128. W. Engelman, Weinheim, Germany.

DIELS, L. 1936. Droseraceae. *In* A. Engler [ed.], Die natürlichen Pflanzenfamilien, vol. 17b, 766–787. W. Engelmann, Leipzig, Germany.

DONOGHUE, M. J., R. G. OLMSTEAD, J. F. SMITH, AND J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on *rbcL* sequences. *Annals of Missouri Botanical Garden* 79: 333–345.

ECKENRODE, V., J. ARNOLD, AND R. MEAGHER. 1985. Comparison of the nucleotide sequence of soybean 18S rRNA with the sequences of other small subunit rRNAs. *Journal of Molecular Evolution* 21: 259–269.

FAY, M. F., K. M. CAMERON, G. T. PRANCE, M. D. LLEDÓ, AND M. W. CHASE. 1997. Familial relationships of *Rhabdodendron* (*Rhabdodendraceae*): plastid *rbc*L sequences indicate a caryophyllid placement. *Kew Bulletin* 52: 923–932.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

HASEBE, M., T. OMORI, M. NAKAZAWA, T. SANO, M. KATO, AND K. IWATSUKI. 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. *Proceedings of the National Academy of Sciences USA* 91: 5730–5734.

HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 21: 160–174.

JUNIPER, B. E., R. J. ROBINS, AND D. M. JOEL. 1989. The carnivorous plants. Academic Press, London, UK.

KONDO, K. 1976. A cytotaxonomic study in some species of *Drosera*. *Rhodora* 78: 532–541.

KONDO, K., AND P. S. LAVARACK. 1984. Study of some Australian species of *Drosera* L. (Droseraceae). *Botanical Journal of the Linnean Society* 88: 317–333.

KONDO, K., AND M. C. OLIVIER. 1979. Chromosome numbers of four species of *Drosera* (Droseraceae). *Annals of the Missouri Botanical Garden* 66: 584–587.

KONDO, K., AND M. SEGAWA. 1988. A cytotaxonomic study in artificial hybrids between *Drosera anglica* Huds. and its certain closely related species in series *Drosera*, section *Drosera*, subgenus *Drosera*, *Drosera*. *La Kromosoma* II-51-52: 1697–1709.

KONDO, K., M. SEGAWA, AND K. NEHIRA. 1976. A cytotaxonomic study in four species of *Drosera*. *Memoirs of the Faculty of Integrated Arts and Science Hiroshima University Ser. 4* 2: 27–36.

LOWRIE, A. 1987. Carnivorous plants of Australia, vol. 1. University of Western Australia Press, Western Australia, Australia.

LOWRIE, A. 1989. Carnivorous plants of Australia, vol. 2. University of Western Australia Press, Western Australia, Australia.

LOWRIE, A. 1998. Carnivorous plants of Australia, vol. 3. University of Western Australia Press, Western Australia, Australia.

MADDISON, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology* 40: 315–328.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MARCHANT, N. G., H. I. ASHTON, AND A. S. GEORGE. 1982. Droseraceae. *In* A. S. George [ed.], Flora of Australia, Lecythidales to Batales, vol. 8, 9–66. Australian Government Printing Service, Canberra, Australia.

MEIMBERG, H., P. DITTRICH, G. BRINGMANN, J. SCHLAUER, AND G. HEUBL. 2000. Molecular phylogeny of caryophyllidae *s. l.* based on *matK* sequences with special emphasis on carnivorous taxa. *Plant Biology* 2: 218–228.

OBERMEYER, A. A. 1970. Droseraceae. *In* L. E. Codd, B. Winter, D. J. B. Killick, and H. B. Rycroft [eds.], Flora of South Africa, vol. 13, 187–201. Department of Agricultural Technical Services, Pretoria, South Africa.

PLANCHON, M. J. E. 1848. Sur la famille des Droseracees—revisio systematica Droseracearum. *Annales des Sciences Naturelles Botanique* 9: 185–207.

RENNER, S. S., G. CLAUSING, AND K. MEYER. 2001. Historical biogeography of Melastomataceae: a roles of Tertiary migration and long-distance dispersal. *American Journal of Botany* 88: 1290–1300.

SAINT-HILAIRE, A. 1824. Histoire des Plantes les Plus Remarquables du Brasil et Paraguai 1: 261. A. Berlin, Paris, France.

SANDERSON, M. J., AND J. A. DOYLE. 2001. Sources of error and confidence intervals in estimating the age of angiosperms from *rbcL* and 18S rDNA data. *American Journal of Botany* 88: 1499–1516.

SCHLAUER, J. 1996. A dichotomous key to the genus *Drosera* L. (Droseraceae). *Carnivorous Plant Newsletter* 25: 67–88.

SEARS, B. B. 1980. Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. *Plasmid* 4: 233–255.

SEINE, R., AND W. BARTHLOTT. 1994. Some proposals on the infrageneric classification of *Drosera* L. *Taxon* 43: 583–589.

SHEIKH, S. A., K. KONDO, AND Y. HOSHI. 1995. Study on diffused centromeric nature of *Drosera* chromosomes. *Cytologia* 60: 43–47.

SHINOZAKI, K., ET AL. 1986. The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. *EMBO Journal* 5: 2043–2049.

STEPHENS, E. L. 1926. A new sundew, *Drosera regia* Stephens, from the Cape Province. *Transactions of the Royal Society of South Africa* 8: 309–312.

SWOFFORD, D. L. 2000. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0 b4a. Sinauer, Sunderland, Massachusetts, USA.

TAKAHASHI, H., AND K. SOHMA. 1982. Pollen morphology of the Droseraceae and its related taxa. *Science Reports of the Research Institutes Tohoku University, 4th Series, Biology* 38: 81–156.

TAKHTAJAN, A. 1997. Diversity and classification of flowering plants. Cambridge University Press, New York, New York, USA.

THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acid Research* 22: 4673–4680.

VENKATASUBBAN, K. R. 1950. Studies in Droseraceae I. The cytology of *D. indica* L., *D. burmanni* Vahl, and *D. peltata* Sm. with special reference to pollen mitosis. *Proceedings of the Indian Academy of Sciences, Section B* 31: 308–330.

WILLIAMS, S. E. 1976. Comparative sensory physiology of the Droseraceae—the evolution of a plant sensory system. *Proceedings of the American Philosophical Society* 120: 187–204.

WILLIAMS, S. E., V. A. ALBERT, AND M. W. CHASE. 1994. Relationships of Droseraceae: a cladistic analysis of *rbcL* sequence and morphological data. *American Journal of Botany* 81: 1027–1037.

WOOD, C. E. 1995. Evidence for the hybrid origin of *Drosera anglica*. *Rhodora* 57: 105–130.

YOKOYAMA, J., M. SUZUKI, K. IWATSUKI, AND M. HASEBE. 2000. Molecular phylogeny of *Coriaria*, with special emphasis on the disjunct distribution. *Molecular Phylogeny and Evolution* 14: 11–19.