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Phylogeny of the sundews, *Drosera* (Droseraceae) based on chloroplast *rbcL* and nuclear 18S ribosomal DNA Sequences

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Abstract. *Drosera* 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, *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 5 of 11 sections including 3 monotypic sections are polyphyletic. Combined *rbcL* and 18S rDNA sequence data revealed that all *Drosera* species form a clade sister to a clade including *Dionaea* and *Aldrovanda*. MacClade reconstructions indicated that aneuploidy occurred several times in a Australian clade, while the chromosome numbers in the other clades are mostly stable. *D. regia* and most Australian species 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 North Hemisphere from the South Hemispere occurred in a few different lineages.

Introduction

The genus *Drosera* includes nearly 150, mostly perennial, species (Juniper *et al.*, 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. *A. vesiculosa* and *Di. muscipula* share a similar trapping mechanism, called a snap trap, exclusive to these two taxa (Juniper *et al.*, 1989). *A. vesiculosa* is a floating aquatic species that is found throughout the Old World and Northern and Eastern Australia, while *Di. muscipula* is a terrestrial plant that is endemic to marshy habitats on the coastal plains of North and South Carolina (Juniper *et al.*, 1989). Relationships among *Drosera*, *Aldrovanda*, and *Dionaea* 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 *et al.*, 1994; Meimberg *et al.*, 2000).

New systems on *Drosera* have been proposed recently (Marchant *et al.*, 1982; Seine and Barthlott, 1994; Schlauer, 1996), the delimitations of the subgenera and sections of *Drosera* are controversial. Williams *et al.* (1994) inferred the phylogenetic relationship of 12 *Drosera* species covering most sections *sensu* Sein and Barthlott (1994), and further analyses with more taxa are obviously necessary to overview the phylogeny of *Drosera*, which is morphologically divergent and includes more than 150 species.

In this presentation, we inferred the intergeneric relationship among *Drosera*, *Aldrovanda*, and *Dionaea*, and Interspecific relationships among the genus *Drosera* using *rbcL* and 18S rDNA. Base d on the inferred phylogenetic tree, evolution of chromosome number and biogeography of *Drosera* was discussed.

Materials and Methods

All subgenera and sections of *Drosera sensu* Seine and Barthlott (1994) except sect. *Meristocaulis*, *Dionaea*, and *Aldrovanda* were used in this study. Total DNA extraction, sequencing, and phylogenetic analyses generally followed Hasebe *et al.* (1994).

Results and Discussion

Parsimony analysis produced the 4608 most parsimonious (MP) trees of 1087 steps in 12 islands (Maddison 1991) using the data matrix of 1227 bp *rbcL* for the 75 taxa including 16 outgroup. A strict consensus tree of the 4620 MP trees is shown in Fig. 1 with bootstrap values. The 1648 bp region of 18S rDNAs and the 1227 bp *rbcL* of *Dionaea, Aldrovanda,* and some representative species of *Drosera* were used to infer their phylogenetic relationship. Parsimony analysis produced a single MP tree of 589 steps (Fig. 2)

The MP tree for the combined dataset in Fig. 2 showed that *Dionaea* and *Aldrovanda* form a sister group with 80% BP. This 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, but 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 non-carnivorous 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.

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.

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.

D. regia is basal, while the clade including all the other African species except *D. indica* clustered at the terminal position. *D. 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. 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. Dispersal from Australia to South America also likely occurred in the clade that includes *D. brumannii* and *D. sessilifolia*. 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. capillaries*, *D. brevifolia*, *D. indica*, *D. burmannii*, and *D.*

peltata) expanded their distributions from the Southern Hemisphere.

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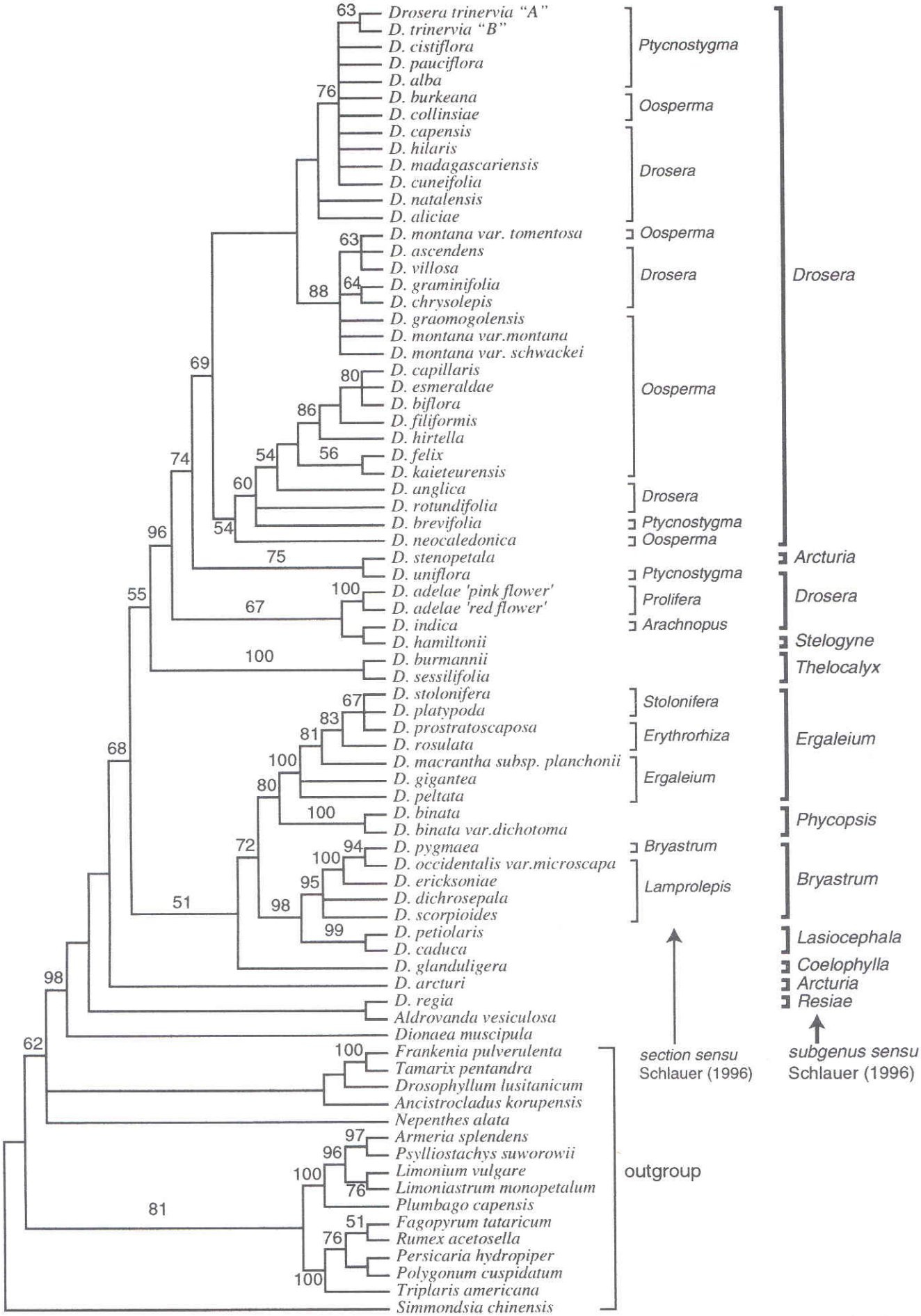
Figure legends

Figure 1. A strict consensus tree of the 4620 most parsimonious trees of *rbcL* sequences. Bootstrap values are indicated above the branches occurring in more than 50% of 10,000 bootstrap replicates. The higher classifications *sensu* Schlauer (1996) and Seine and Barthlott (1994) are shown on the right.

Figure 2. The most parsimonious tree resulting from parsimony analysis of the combined *rbcL* and 18S rDNA sequences. The numbers above the branches are the bootstrap values greater than 50% for 10,000 bootstrap replicates.



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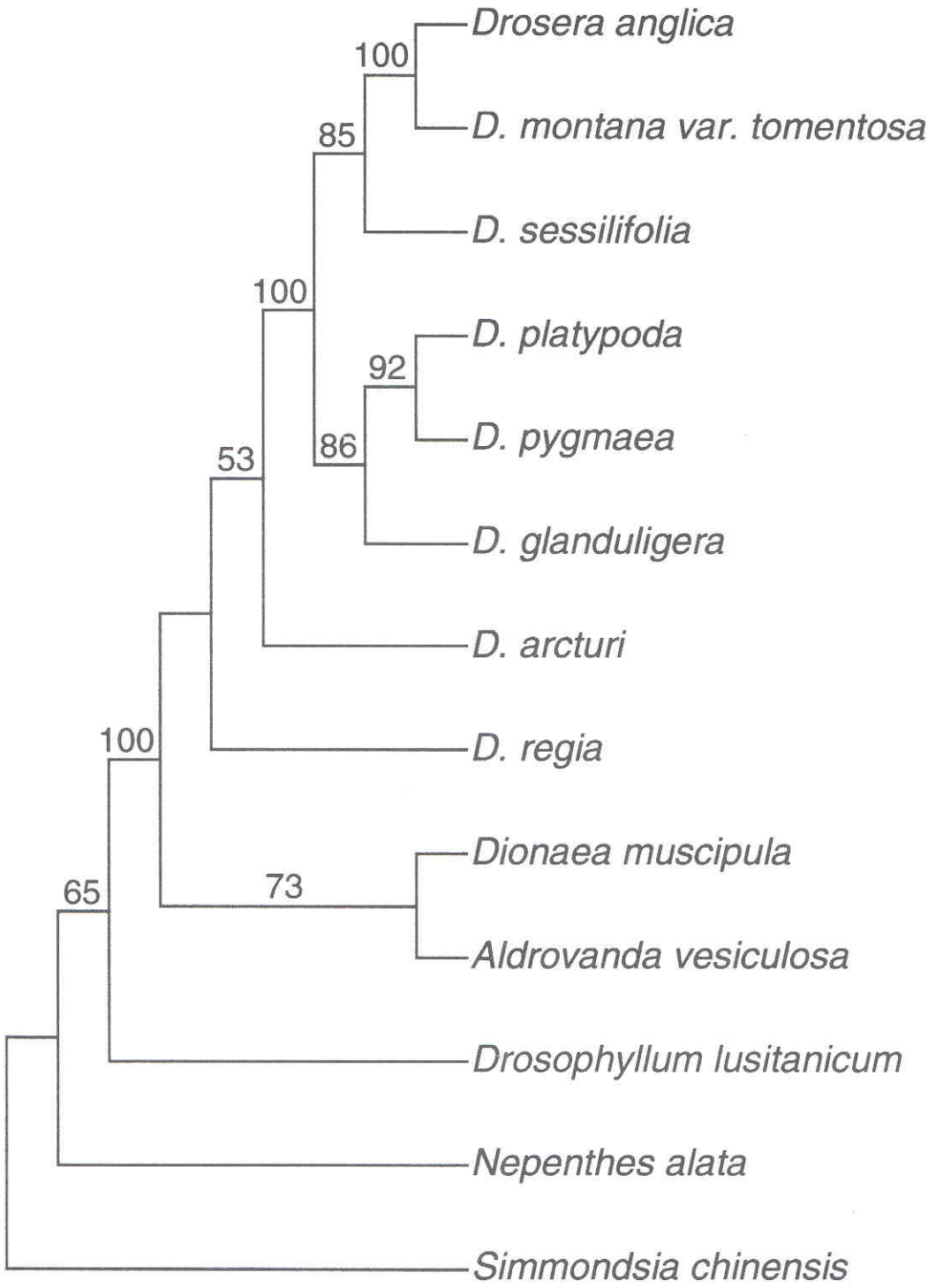

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