Chapter 5. Synthesis of phenetic analysis, molecular data analysis and reproductive biology of the *Drosera peltata* complex (Droseraceae): a case of cryptic species?

5.1 Introduction

The taxonomy and classification of the *Drosera peltata* complex has been a problem of over 200 years in the making (see Chapter 1). Various members of the complex have been described from many locations on the western Pacific Ocean margin, southern Asia and central Africa (Thunberg 1797; Planchon 1848; Diels and Pritzel 1905; Diels 1906; Taton 1951; Schlauer 1996) (Figure 2). The circumscriptions of the 18th and 19th centuries were often scant in location details and the associated type specimens were often sterile, and many have subsequently become damaged. Conn (1981) provided a valuable summary of the nomenclatural history of the complex up to that time, except for taxa from eastern Asia. Since Conn's paper (1981) a further three taxa in the complex have been described (*D. bicolor*, *D. peltata* var. *glabra* and *D. peltata* var. *multisepala*) (Figures 4 and 6). This chapter links the results of phenetic analysis (Chapter 2), molecular data analysis (Chapter 3) and experimental pollinations (Chapter 4) to provide an update on the taxonomy of this group of plants and to consider whether the complex includes cryptic species.

Cryptic species present problems for taxonomy and classification (Wiley 1981; Whittall *et al.* 2004). According to Paris *et al.* (1989), they have the following characteristics:

- Cryptic species are poorly differentiated morphologically.
- Cryptic species represent distinct evolutionary lineages because they are reproductively isolated.
- Cryptic species have historically been misinterpreted as members of a single species.

The recognition of cryptic species is often difficult due to the widespread application of the morphological species concept in biological science (Chapter 1; McDade 1985). However, with the increasingly widespread use of molecular techniques (Soltis and Soltis 1998; Judd *et al.* 2002), many cryptic species have been

discovered e.g. Chan *et al.* (2002). Once identified, hitherto unrecognized morphological characters between species may be discovered that then enables previously cryptic species to be more easily recognized Paris *et al.* (1989).

Quantitative, or numerical, taxonomy is ideally suited to objectively investigating the composition of species complexes (Sokal and Rohlf 1969; Sneath and Sokal 1973; Sokal 1986; Stuessy 1990; Quicke 1993; Hodgon *et al.*2006). In many cases, phenetic analysis is able to elegantly establish that members of a complex are in fact closely allied congeners, often distinguished by a suite of newly-recognized characters (Kottek *et al.* 1990; Crisp and Weston 1993; Wills *et al.* 2000; Susandarini *et al.* 2002). In some cases, e.g. Brown and Wiecek (1996), Conn (1984), Burke and Adams (2002), and van den Berg *et al.* (1998) phenetic analysis may not resolve a complex into distinctly different entities. This may be due to intrapopulational and interpopulational variation so that entities within the complex of study do not have unique characters. In the case of the *Drosera peltata* complex, the narrow endemic *D. bicolor* had many unique characters (apomorphies) which distinguished it from the otherwise widespread and variable *D. peltata* (Chapter 2). However, what had been assumed to have been taxonomically useful characters to identify species in the complex (Marchant and George 1982) did not prove to be the case upon analysis (Chapter 2). This may be due to the variable expression of some characters (such as basal rosette development) and the discovery of intermediate character states between what were previously suspected to be discrete states, which are better treated as continuous (such as sepal surface hair development, and sepal hair characters).

Molecular studies have been undertaken on species complexes to explore evolutionary links between entities and populations (see Section 3.1). The results of some investigations have revealed relatively large genetic differences between members of the group studied, and this has led to the recognition of formally cryptic species; e.g. *Potamogeton* species: Potamogetonaceae (Whittall *et al.* 2004) and *Botrychium* species: Ophioglossaceae (Hauk 1995). The degree of divergence of molecular markers between taxa reflects the degree of reproductive isolation between them. It can also help to identify the origin of hybrid taxa and clonally reproducing taxa, which have implications for the appropriate taxonomic rank to apply to once cryptic species (Paris *et al.* 1989). The molecular analysis of the cpDNA molecule

trnL and ITS1 and ITS2 molecules of nrDNA from the *D. peltata* complex indicated that the complex was paraphyletic (Chapter 3). The *D. peltata* complex was found to be monophyletic save for the entity *D. peltata* 'Western Australian Form'. This was the case for both molecular markers examined, and all three samples of this entity formed a monophyletic group that was well separated from the remainder of the complex. *Drosera peltata* 'Western Australian Form' grows well apart from other members of the complex; occurs on the margins of granite outcrops in inland southern Western Australia, and appears to have more highly divided style segments than other members of the complex (Figure 33). Whilst OTUs of this entity of the complex often clustered together on the phenograms, they did not occur as a clearly separated discrete group in ordination space. Therefore this entity may qualify as a cryptic species according to the three criteria listed above, and may also qualify as a distinct species based on the phylogenetic species concept.

A study of the breeding system of a group also provides information about the degree of reproductive isolation between its members. In this study, a subset of the complex from south eastern Australia was used and all entities were found to be highly inter-fertile (Chapter 4). *Drosera peltata* 'Western Australian Form' was not included in this study due to the limited number of flowering plants in cultivation when experimental pollinations were conducted. Therefore it was not possible to determine if the divergence indicated by molecular markers between *D. peltata* 'Western Australian Form' and the rest of the complex also included reproductive incompatibility. If this had been found then it could be argued that *D. peltata* 'Western Australian Form' is a cryptic species, for which the degree of style division may have been used to distinguish it from the rest of the complex (Figures 31 and 33). The high interfertility of members of the *D. peltata* complex in south eastern Australia does not support the hypothesis that the proposed entities qualify as cryptic species. The molecular data (Chapter 3) does, however, suggest that the different entities have commenced divergence. This situation has been elegantly summed up by McDade (1995; p. 607) "[w]hen divergence is recent, incomplete, unmarked by detectable characters, or of uncertain fate, it is bound to be difficult to resolve species limits".

5.2 The names in light of new data

Drosera insolita Taton was described from a single specimen collected by Homblé (specimen number 169) in April 1911, allegedly from Katanga Province in southern Democratic Republic of Congo (Figure 3). Its resemblance to *Drosera peltata* was noted but it was distinguished by this species by its provenance, and the specimen characters of scarcely developed to absent axillary leaves, having a bifid bracteole, and scapes with three or four flowers (Taton 1951). Conn (1981) did not examine this species with his review of *Drosera peltata*. Schlauer (1996), in his revised dichotomous key to the genus *Drosera*, retained *D. insolita* based on its provenance and bifid bracteole. No further collections have been made of this species since Homblé almost a century ago, and so this species is based only on the one specimen.

The type specimen of *D. insolita* is in Brussels (BRU). The specimen is multi-branched, lacks a basal rosette (although this is described in the circumscription and thus may have disintegrated over time), and has few bracteoles present on any of the scapes (Figure 3). Whilst studying this specimen I was informed by the director of the herbarium, Dr. Elmar Robbrecht (pers. comm. 2002) that there had been a curatorial error regarding Homblé's collections. Homblé began his collecting career is southern China before he went to Africa, where he did most of his collecting. His first two hundred or so specimens had been incorrectly ascribed to coming from Africa. *Drosera insolita* was thus collected in southern China where Ruan (1981) has documented much variation in sepal morphology (see Figure 4). Therefore *D. insolita* is not distinct from *D. peltata* and should be appropriately reduced to synonymy.

Whilst studying *D. bicolor* specimens for this project it became apparent that the cauline leaves vary systematically in more than just petiole length and lamina size up the stem; the leaves also change in shape, from ovate to crescentic and the petiole varies in its attachment from the lower leaf margin to the centre of upper leaves (Figure 8 c, d, e). This was not documented by Lowrie and Carlquist (1992), and adds more autapomorphies to this entity. This systematic variation in cauline leaves was not seen in other entities in the *D. peltata* complex which instead exhibit polymorphism in leaf shape and size (Chapter 2). Therefore, I argue that *D. bicolor* should continue to be recognized at the species level.

5.3 Conclusions: a case for cryptic species within the *D. peltata* complex?

Drosera peltata has long been recognized as being a morphologically variable and widespread species (Planchon 1848; Diels 1906; van Steenis 1953; Ruan 1981).

Drosera insolita arose through a clerical error in the curation of herbarium specimens. The sole specimen on which the species was based was collected in southern China, as opposed to southern Democratic Republic of Congo (Figure 2). Thus *Drosera* subgenus *Ergaleium* does not occur in Africa. Based on variation of *D. peltata* from southern China, the reported autapomorphies of *D. insolita* appear to be part of the variation in the species and its recognition as a separate species is rejected.

Drosera bicolor has many additional autapomorphies than its circumscription suggested and from phenetic analysis it does indeed occur as a separate cluster of OTUs to the OTUs of the remainder of the *D. peltata* complex (Chapter 2). Whilst superficially similar to *D. peltata*, it should be retained as a separate species.

Phylogenetic analysis suggests that *Drosera peltata* 'Western Australian Form' is a cryptic species. In phenetic analysis (Chapter 2), OTUs of this entity grouped together in phenograms but did not form a clear and discrete group in ordination space from the remainder of OTUs in the *D. peltata* complex, despite differences in style morphology (Figures 31 and 33). However, all three samples of this entity occurred together and separate from the remainder of the complex during phylogenetic analysis (Chapter 3). The clearly distinct molecular sequences, and great isolation from other members of the complex indicate that the populations sampled are isolated and diverging lineages, and thus *D. peltata* 'Western Australian Form' also qualifies as a species based on the phylogenetic species concept.

The present study suggests that Conn (1981) did not go far enough in reducing *D. auriculata* to a subspecies of *D. peltata*. This is true even when members of this complex from eastern Asia and the enigmatic *D. insolita* are taken into account. A key finding of this study is that *D. peltata* is one wide-ranging and morphologically variable species (Table 25; Chapter 6).