Chapter 1

GENERAL INTRODUCTION

GOAL OF SYSTEMATICS

Systematics has two fundamental aims:

1) to discover, describe and name all species – the tips of the branches of the tree of life, and
2) to document the changes on the branches that have occurred during evolution and transform these into a predictive classification system that reflects evolution (Systematics Agenda 2000). Systematics is therefore the study of the biological diversity that exists on earth today and its evolutionary history (Judd et al., 1999).

In the first half of this introduction the main aspects of the practice of systematics are briefly discussed. An overview of these aspects and the sequence in which they are performed is also presented as a flow-chart (Fig. 1.1). In the second half the main subject of this study (the orchid genus *Coelogyne*) is introduced and the aims and outlines of this thesis are explained.

Recognition of species

To describe the tips of the branches of the tree, for practical reasons it is necessary to have a clear idea of the species concept taken as a starting point. In this thesis, the morphological species concept of Van Steenis (1957) is used. Distinct species are recognised when at least two morphological character (states) indicate substantial differences. Specimens, without two clearly fixed morphological differences are considered to belong to the same species.

Recognition of two morphological characters defining a species is a personal choice: what one taxonomist considers as a good delimitation character can be dismissed as irrelevant by a colleague, who studied more material. Ideally, molecular data should be collected to provide more information about permanent decreasing gene flow between different populations in the process of speciation. However, only few living collections were available for most of the species studied in this thesis, and DNA extracted from herbarium specimens turned out to be too degraded in most of the cases.

Why is this particular species concept used? The morphological species concept has some advantages over other concepts:

1) selfing individuals (quite common in plants and also present in *Coelogyne*) do not need to be called new species, as they should according to the biological (Mayr, 1942) and recognition (Patterson, 1985) species concept;
2) evolutionary lineages can be identified by a specific morphologically based criterion, instead of only assumed to be there, like in the evolutionary species concept (Simpson, 1951);
3) the fixation of two morphological characters is easier to recognise than monophyly, the criterion of the phylogenetic species concept (Cracraft, 1983);

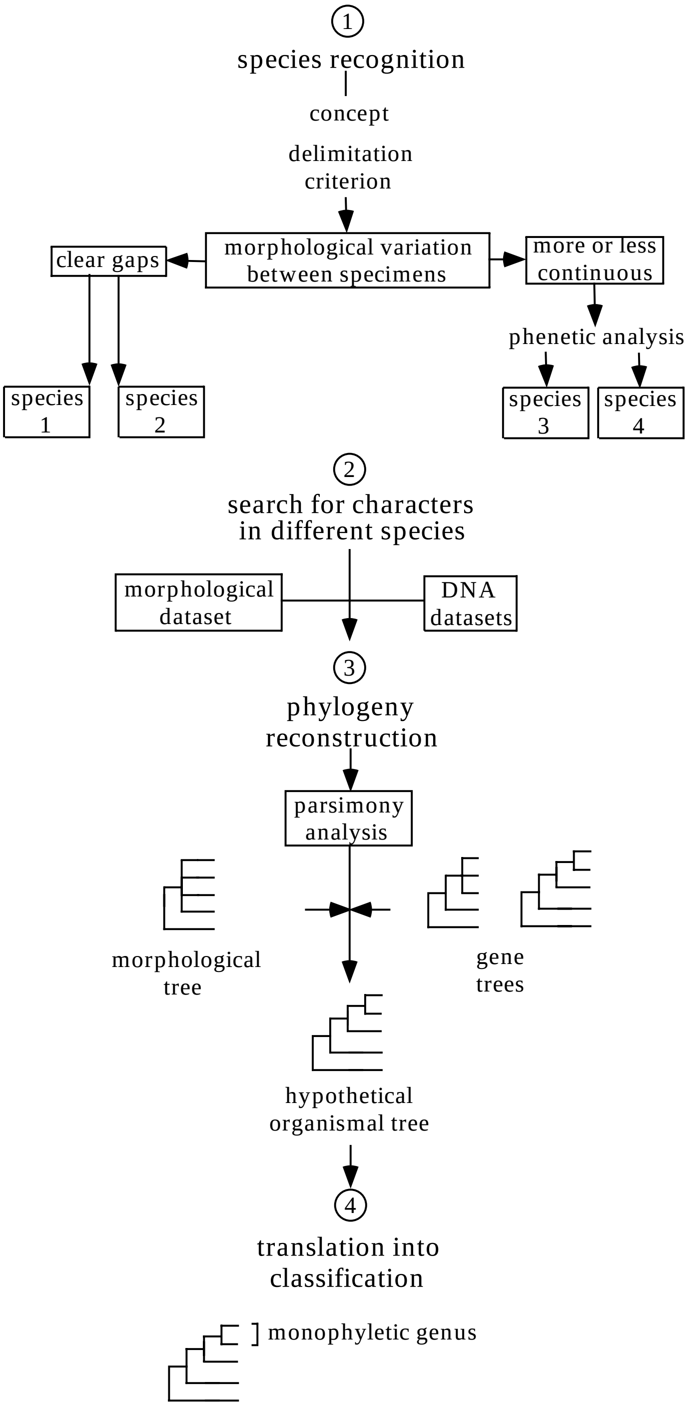

Fig. 1.1. Flow-chart of the main aspects of the practice of systematics.

4) as species are used as terminal units in phylogeny recontruction, the historical aspect should be more fundamental to a species than its morphological distinctness. However, permanence of character fixation, as required for the recognition of phylogenetic (Cracraft, 1983) or composite species (Kornet, 1993) could not be assessed in the *Coelogyne* specimens studied for this thesis, as they were all collected in the same time-slice. Moreover, these concepts require information about the permanency of splits between populations, which is difficult to assume without extra information, as many species of this study show overlaps in distribution areas.

For most of the species in this study clearly fixed morphological differences were present and species delimitation was not problematic. However, in two groups of closely related taxa, variation of most morphological characters studied appeared to be more or less continuous. Phenetic methods were used to find gaps in multivariate morphometric space and search for a good combination of delimiting characters. These methods are suitable for solving difficult species complexes, as they do not impose a rigidly hierarchical pattern on the data when none is to be expected (Crisp & Weston, 1993).

In this thesis, a species can be paraphyletic (consisting of an ancestor with only part of all its descendants). To put it more precisely, the species is not paraphyletic, but it can be a group of paraphyletic populations. Only part of the populations of one species is active in forming a new species, thus the remaining populations, which are consequently paraphyletic, remain as ancestral species. On the species level therefore, taxon names in this thesis do not solely refer to monophyletic groups (consisting of an ancestor with all its descendants), in contrast with Pleijel (1999).

Search for characters

In this thesis sequence data are the main information used for the reconstruction of evolutionary histories. This is not based on the belief that morphological information is worthless, but rather that it is extremely difficult to interpret morphological variation accurately in Orchidaceae (Chase, 1999). If sequence divergence stays below 15%, alignment is usually straightforward and the homology of a change is easily assessed (Patterson, 1988). Homology of morphological characters is often much more difficult to interpret accurately without time-consuming ontogenetic and anatomical studies. Moreover, generic delimitation in the Orchidaceae has long been based mainly on floral traits which are associated with pollinator attraction. Several recent studies show that floral characters mapped on molecular cladograms can be very homoplasious (Dressler, 1981; Chase & Palmer, 1992; Hapeman & Inoue, 1997).

Reconstructions of evolutionary histories based on sequence data are called gene trees. Caution must be exercised to directly translate these gene trees into organismal trees, as processes of introgression, gene duplication, loss and lineage sorting can cause incongruence (Page & Charleston, 1997). Congruence between different gene trees is often assumed to be strong evidence for an accurate estimate of the organismal tree (Slowinski & Page, 1999). That is why in this study information was collected from multiple DNA regions, representing both coding and non-coding as well as slowly evolving plastid and more divergent nuclear regions.

Reconstruction of the phylogeny

To reconstruct the evolutionary history of a group of organisms, a hypothesis on the genealogical relationships (a so-called phylogeny) has to be made. A phylogeny is an evolutionary chronicle. Evolutionary relationships are inferred by using various kinds of evidence: in this thesis molecular, morphological and, to a lesser extent, anatomical characters are used. A group of organisms that shares many identical states in these characters (for instance six shared mutations in the *matK* gene of the plastid genome, hairy ovaries and trichomes with acute top on the leaves) is considered to be closely related and are assumed to be derived from a common ancestor. This ancestor, together with all its descendants, forms a monophyletic group: a group that exists in nature as a result of the historical process of evolution. By comparison with outgroups (taxa assumed to be closely related with the organisms under study based on earlier collected evidence) characters are polarised: the states also occurring in the outgroup are considered to be plesiomorphic, the states in (part of) the ingroup (the group of interest) apomorphic. Presumed synapomorphies are used to investigate the relationships between taxa in the ingroup.

A phylogeny can be represented as a branching diagram, a so-called cladogram. Most optimal cladograms in this thesis are reconstructed using the parsimony criterion, in which the character transformations on the branches of a cladogram are minimised. Most current methods of phylogeny reconstruction impose hierarchical patterns, which are incompatible with reticulate patterns caused by hybridisation. However, hybridisation between both closely and more distantly related taxa frequently produces new plant species. Divergently branching phylogenetic hypotheses cannot be used to detect hybrids, as their behaviour can be identical to that of nonhybrid taxa (McDade, 1990). Few natural hybrids are known to exist among the *Coelogyne* species studied for this thesis. However, for one species incongruency was found between the phylogeny based on the uniparentally inherited plastid genome and the phylogeny based on recombined nuclear data. Incongruencies between nuclear and organellar phylogenetic trees are often attributed to introgression of a cytoplasmic genome from one species into the nuclear background of another species (Wendel & Doyle, 1998). The nuclear DNA is assumed to be eliminated through backcrossing to the other parental species, whereas the plastid DNA was retained, and is now coupled with the nuclear genome of the other species. However, introgression is not the only process that could produce incongruencies. A second cause might be coalescence of alleles antedating species divergence (lineage sorting). It is difficult to distinguish between introgression and lineage sorting, because they both may produce similar phylogenetic patterns (Hardig et al., 2000). However, there are relatively few examples of plastid DNA polymorphisms that transcend species boundaries, probably because of the generally slow rate of plastid DNA evolution (Wendel & Doyle, 1998). Therefore, hybridisation due to introgression appears to be the most probable explanation for the incongruence found in this study.

Translation into a classification

Once the phylogeny is reconstructed, the knowlegde of this part of the tree of life – of the tips and terminal branches and all their phylogenetic relationships to one another – can be translated into an unambiguous system of classification. The main aim of classifications is enabling communication by naming supra-specific categories. This has nothing to do with evolution. Naming is entirely an abstraction and can be made to fit whatever criteria we wish.

Different types of classifications exist. The traditional system is the Linnaean classification. In this system, the names of organisms are anchored by reference to a rank (species, genus, family, order). The stability of this system depends largely on taxonomists choosing to agree on the general membership of named groups (Baum et al., 1998). In this thesis, the phylogenetic classification system is used, in which only monophyletic groups are recognised: taxa are not anchored by rank but by reference to phylogenetic relationships with other taxa (De Queiroz & Gauthier, 1990). Using only monophyletic groups is impossible in the Linnaean classification system, because of its mandatory ranks: at one level all groups in this system would cause an enormous proliferation of ranks and ancestral species cannot be included (Brummitt, 1997; Van Welzen, 1997). Maintaining the Linnaean classification system therefore inevitably leads to acceptance of paraphyletic taxa (Sosef, 1997).

In this thesis, classifications are strictly based on monophyletic groups. This is done because the criterium of common descent is objective and makes the system defendable instead of intuitive (Líden et al., 1997; Van Welzen, 1998), in contrast with traditional classifications, which are guided by authority and convention (Baum et al., 1998). Moreover, evolution is now the unifying theory of biology, so modern biology requires taxonomy reflecting evolution (De Queiroz & Gauthier, 1994). According to Sosef (1997) reticulate patterns make the monophyletic hierarchical model unfit for classification of the world around us. On the species level, paraphyly is indeed accepted in this thesis, as hybridisation between different populations is assumed to produce new species. On higher taxonomic levels, however, hybridisation is assumed to be nearly absent and paraphyly is therefore considered unacceptable.

THE GENUS COELOGYNE

Lindley described the orchid genus *Coelogyne* in 1821, naming it *Caelogyne* (from the greek 'koilos ' = 'hollow' and 'gyne ' = 'female') because of the concave stigma. Soon after he corrected this spelling to *Coelogyne* (Lindley, 1825). *Coelogyne* is characterised by a free, never-saccate lip with high lateral lobes over the entire length of the hypochile and smooth, papillose, toothed or warty keels (Seidenfaden & Wood, 1992). The genus comprises over 200 species distributed throughout southeast Asia with main centers of diversity in Borneo, Sumatra and the Himalayas (Butzin, 1992a).

Most *Coelogyne* species are epiphytes and occur in primary forest, from sea level up to c. 3000 m elevation. For example, in the lowland Dipterocarp-dominated rainforest of Peninsular Malaysia, Sumatra and Borneo, *C. asperata*, *C. septemcostata* and *C. xyrekes* are quite common on the trunks and main branches of trees along the river banks, where the light regime is more favourable compared with the shaded forest interior (Chan et al., 1994). In the lower montane rainforest of Java, *C. flexuosa* and *C. miniata* are growing in dense clumps on mossy rocks in high light levels (Comber, 1990). In montane cloud forest of the Himalayan range, where the climate is seasonally dry and temperatures are relatively low, *C. cristata*, *C. fimbriata* and *C. flaccida* occur on trees, covered with thick coats of mosses (Sparrow, 1995). In the alpine scrub vegetations of Mount Kinabalu, *C. papillosa* can be found, growing as a lithophyte or even as a terrestrial (Wood et al., 1993). In New Guinea *C. fragrans* often grows low down on the trunks of small trees in rather open montane forest, where the plants catch large amounts of leaf-litter. At somewhat lower elevations *C. beccarii* occupies similar niches in Castanopsis-dominated forest and in forest on the ridges of limestone hills (Schuiteman, pers. comm.).

Most species are characterised by a fairly large number of medium-sized to large flowers with delicate colours and a sweet scent and are pollinated by bees (Van der Pijl & Dodson, 1966), beetles (O'Byrne, 1994) or wasps (Carr, 1928; Dressler, 1981). A selection of species with long, pendulous, multiflowered inflorescences is widely cultivated and known as the necklace orchids (De Vogel, 1992). The number of recent artifical hybrids published indicates the growing commercial interest in this group (Erfkamp & Gruß, 1996). Concerning chromosome numbers, two polyploid series are present in the genus, with n = 19 (2n = 38; 4n = 76) in several species and n = 20 (2n = 40; 4n = 80) in the majority of the species studied according to Mehra & Kashyap (1989) and Brandham (1999).

Generic and sectional delimitations of Coelogyne

Coelogyne is placed in subtribe Coelogyninae (tribe Coelogyneae, subfamily Epidendroideae) with a total of approximately 550 species (Pedersen et al., 1997). Synapomorphies of the subtribe are sympodial growth, pseudobulbs of one internode, terminal inflorescences, a winged column and massive caudicles (Dressler, 1981; De Vogel, 1986; Butzin, 1992b). Within this subtribe, 16 genera are currently recognised (Pedersen et al., 1997). However, numerous taxonomists have proposed different subdivisions. A summary of the most important opinions on the classification of subtribe Coelogyninae is given in Chapter 3. In phylogenetic analyses using morphological data (Burns-Balogh & Funk, 1986), *ndhF* (Neyland & Urbatsch, 1996), *rbcL* (Cameron et al., 1999), *nad1* b–c (Freudenstein et al., 2000) and *matK* evidence (Chase et al., unpubl.) *Thunia alba* (Lindl.) Rchb.f. is placed as sister taxon to Coelogyninae.

Lindley subdivided *Coelogyne* into five sections in 1854, when only few species of large and diverse groups were known for comparison. As more and more new species were described, which could not be assigned to one of those sections, Pfitzer & Kraenzlin (1907d) published an entirely new classification of 14 sections. Many later authors used this classification and the same key characters with minor changes until De Vogel (1994) and Clayton (in press) came up with 23 subdivisions, of which 12 are identical with those of Pfitzer & Kraenzlin. Differences of opinion are mainly due to the relative lack of morphological characters available to define groups of species. For instance, both sect. *Coelogyne* and *Ocellatae* are defined by white flowers with yellow keels. Many characters are known to intergrade among the species of different sections, too. For example, the presence of hairs on the ovary has been used to define sect. *Tomentosae* (De Vogel, 1992). However, this character is likely to have evolved convergently in section / subgenus *Coelogyne*, *Cyathogyne*, *Rigidiformes*, *Veitchiae* and *Verrucosae*. Conflicts in the assignment of species to different sections in *Coelogyne* have been present in the literature for years (see Chapter 3 for a summary of the most important opinions on infrageneric classifications in *Coelogyne*), but there was an impasse about how to proceed. The phylogenetic analyses performed with the molecular and morphological data collected for this research have provided new insights.

Aims and outlines of this thesis

The aims of this thesis are:

1) the reconstruction of a skeleton phylogeny of the orchid genus *Coelogyne* and allied genera;
2) the incorporation of this phylogeny into a phylogenetic classification and
3) a taxonomic revision of a selection of monophyletic groups of *Coelogyne* species.

Chapter 2 provides a general framework of *Coelogyne* and allied genera in the Coelogyninae based on plastid PCR RFLPs and plastid and nuclear sequences. The results of this analysis show that *Coelogyne* as currently defined is not a monophyletic group because it is composed of two unrelated groups of species. Several of the floral traits that previous authors used to recognise this concept of the genus (the 'key' characters) appear not to be phylogenetically informative. Possible taxonomic solutions are discussed and a new classification of the genus is proposed.

Chapter 3 deals with the integration of this molecular phylogeny with results of a morphological analysis. More insight is gained in the evolution of specific morphological traits by reconstructing their character state evolution on a total evidence tree. Some clades in Coelogyninae remain unresolved in the total evidence phylogeny. Others cannot be easily recognised by morphological characters yet. These are the drawbacks that often accompany new phylogenetic classifications. Still, these disadvantages are to be preferred above the traditional classification, because we now have an empirically based taxonomy in which taxa are assigned a position in a phylogenetic system. Possibilities for bringing more resolution in the unresolved groups are briefly discussed at the end of Chapter 3.

With the phylogenetic boundaries of Chapter 3 as a reference, a start with a taxonomic treatment of the whole genus is made in Chapters 4, 5 and 6, which focus on three differrent monophyletic groups of species. An integrated phylogenetic analysis of morphological and molecular characters is performed for the species of sect. *Speciosae* (Chapter 4) and sect. *Verrucosae* (Chapter 6), whereas a complex of the closely related species of sect. *Fuliginosae* is resolved with a phenetic analysis using morphological characters (Chapter 5). For the three sections, a taxonomic treatment of all species is provided, with descriptions, colour photos, drawings, distribution maps and identification keys. Three new species are described and several others are reduced to synonymy. Revisions of other monophyletic groups within the new boundaries of *Coelogyne* are planned for the near future.