New species, pollinator interactions and pharmaceutical potential of Himalayan orchids

Subedi, A.

Chapter 1

General Introduction

Systematics of Nepalese orchids

Wild orchids in Nepal are popularly known by the vernacular name *'Sungava'* or *'Sunakhari'* which refers to their shiny yellow ('golden') coloured pseudobulbs. Between the first known orchid collection made by Francis Buchanan Hamilton in 1802-1803 and the most recent publication by Raskoti (2010) a total of 377 species of orchids belonging to 100 genera including 12 endemic species were described for Nepal. Nepalese orchids are part of a flora encompassing the Himalayas, Myanmar, Thailand and China (Press et al., 2000). Orchids in these regions are facing serious threats caused by habitat fragmentation and illegal trade (Rajbhandari and Bhattarai, 2001; Chaudhary et al., 2002; Subedi, 2005). Because of these threats, a total of 53 species of wild orchids of Nepal are curently threatened or endangered. The endemic *Liparis olivacea* went extinct recently (Subedi, 2002). For a good grasp of biodiversity losses, up-to-date information is required of the systematics of the species involved.

Systematic studies on Nepalese orchids can be grouped into several categories. The first encompasses local inventories, sometimes accompanied by descriptions of selected species or flora's (Banerji and Thapa, 1969; Rajbhandari and Bhattarai, 2001; Chaudhary et al., 2002; Subedi, 2002). The second category consists of taxonomic revisions of selected genera based on morphological characters of species-rich genera such as *Eria* and *Oberonia*, and reports on newly discovered species (Shakya and Chaudhary, 1999). The third category concerns orchid conservation (Bailes, 1985; Bajracharya et al., 1994; Shakya et al., 1994; Subedi, 2005). The final category is made up of popular (White and Sharma, 2000; Raskoti, 2010) and more professional orchid guides. In the latter category falls Orchids of Nepal by Banerji and Pradhan (1984) which provides descriptions of 247 species. The Orchids of Bhutan (Pearce and Cribb, 2002) and Orchids of Sikkim (Lucksom, 2007) give more realistic floristic accounts. These flora's can be used for identification of Nepalese orchids but not all species found in Nepal are covered by these publications. Molecular phylogenetic studies are not included either. Nepal is therefore still lacking a comprehensive orchid flora also encompassing phylogenetically based classifications.

Next to phylogenetic studies, nomenclatural studies of many Nepalese orchid species also need to be undertaken as the exact identity of type specimens of many species is unclear. Prime examples are *Coelogyne corymbosa, C. nitida* and *C. punctulata* of which the type specimens are mixed on a single herbarium sheet which causes a lot of misunderstanding (Fig. 1.1; Hunt and Summerhayes, 1966; Seidenfaden, 1975; Seidenfaden and Wood, 1992). These species are horticulturally very popular orchids so a clear nomenclature is important to orchid breeders, conservationists and hobbyists. Many orchid breeders still label their plants incorrectly as *C. ochracea* (which is a synonym of *C. nitida*) or *C. nitida* instead of *C. punctulata*. These incorrect identifications are based on outdated literature (Lindley, 1854; Reichenbach, 1861; Hooker, 1890; Pfitzer and Kraenzlin, 1907; Banerji and Thapa, 1969; Hara et al., 1978). We have carried out a taxonomic revision of *Coelogyne* sect. *Ocellatae* based on a morphological study, field surveys carried out in Nepal and Northeastern India and phylogenetic analyses of DNA sequences, and we have sorted out the confusing nomenclature of the species mentioned above by a detailed analysis of the type specimen sheets and accompanying original literature.

Fig 1.1. Herbarium sheet Wallich Cat. no. 1954 (deposited at BM) containing specimens of *Coelogyne corymbosa* (left), *C. cristata* (lower right corner), *C. nitida* (upper right corner - lectotype) and C. *punctulata* (in the middle on lowest row - holotype).

Research Questions

The following main research questions are addressed in this thesis:

1. Is *Coelogyne* section *Ocellatae* monophyletic?
2. Which species can be distinguished within *Coelogyne* section *Ocellatae*, and how can they be identified morphologically?
3. What are the main characters delimitating the horticulturally popular species *C. nitida, C. punctulata* and *C. corymbosa?*
4. What is the function of extrafloral nectar of Coelogyninae?
5. Which structures function as extrafloral nectaries in Coelogyninae?
6. What are the natural pollinators of Nepalese Coelogyninae?
7. Which Nepalese orchid species are involved in illegal trade and why?
8. Do medicinally used Nepalese orchids contain biologically active compounds or is their use based on folk belief only?
9. Can illegally collected nonflowering orchids be identified up to species level by DNA barcoding and chemical profiling?

New species of Coelogyninae from the Himalayan region

We described a total of 4 species and 4 subspecies of *Coelogyne* and one species of *Panisea* from the Himalayan region based on analysis of morphological characters and DNA sequences. The orchids of this region have been studied for over two centuries. These studies largely focused on relatively accessible geographic areas only such as the northwestern part of the Himalaya (Deva and Naithani, 1986), central and east Nepal (Banerji and Pradhan,1984), the northeastern part of the Himalaya (King and Pantling, 1898; Chowdhery, 1998; Pradhan, 2005; Lucksom, 2007) and Bhutan (Pearce and Cribb, 2002). Pillon and Chase (2007) argued that in European orchids an extremely narrow species concept is applied: many more species are recognized than actually exist. They warned that this affects orchid conservation negatively. In contrast to Europe, the orchids of the Himalayan region are still poorly understood due to the inaccessibility of many areas. Many new species therefore still remain to be discovered in areas such as the southern part of Yunnan and northern part of Myanmar. These areas used to be closed for foreign explorers but are now gradually being opened up for biodiversity studies by indigenous taxonomists.

Ecology of Nepalese Coelogyninae

Species of Coelogyninae found in Nepal are epiphytes or lithophytes. Their distribution patterns show great similarity with altitude and rainfall patterns (Dahal and Shakya, 1989; Sparrow, 1996). The areas of eastern and central Nepal which receive the highest monsoon rainfall host 90% of the total number of species of Coelogyninae (Dahal and Shakya, 1989; Subedi, 2005). Major forest types providing the most suitable habitats for Coelogyninae are subtropical *Schima wallichi* and *Castonopsis indica* mixed forest and lower temperate *Quercus semicarpifolia* and *Rhododendron arboreum* mixed forest (Rajbhandari et al., 1999; Chaudhary et al., 2002; Subedi, 2002). Sparrow (1996) argued that local patterns of orchid species diversity are determined by light conditions and the bark characteristics of the host trees.

In contrast to the general ecological factors described above, the pollination ecology of Himalayan orchids is still poorly understood. Cheng et al. (2009) found *Coelogyne fimbriata* to be pollinated by a single species of wasp belonging to the genus *Vespa*. We observed *C. flaccida* and *C. nitida* to be pollinated by a species of wild honey bee (*Apis cerena*). We also recorded *Otochilus albus* being pollinated by bumblebees (*Bombus kashmirensis*) which is the first pollination record for this genus. Observing pollinators is very time consuming since pollination events are rare in Coelogyninae. Their pollination is based on deception: the flowers do not offer a reward, and pollinators quickly learn to avoid them. This means that many hours have to be spent in the field to catch rare pollination events on camera. Obtaining good identifications of the pollinators proved to be difficult as well, because only few entomologists specialize in the Himalayan region. Nonetheless, we managed to obtain identifications up to species level by contacting specialists all over the world. Information on the pollination of species is important because locally threatened orchid species can only be conserved if their natural pollinators survive and their ecology is understood. An illustrative example is the endemic orchid *Cymbidium whiteae* that vanished from the type locality in Sikkim in India due to local extinction of its pollinator (Lucksom, 2007).

Next to pollinators, plants also interact with other insects, mainly herbivores. We recorded and identified over nine different beetle species eating both leaves and flowers of Nepalese Coelogyninae. Protection against herbivory by ants has been described already for both orchids (Rico-Gray and Thien, 1989) and other plant species (Oliveria, 1997; Wackers et al., 2001; Palmer et al., 2008; Goheen and Palmer, 2010). We show that *Coelogyne nitida* is protected by at least four different species of ants against beetle induced herbivory. The ants are attracted by nectar exudated by stomata situated in a small nectiferous area on the rhachis just below the pedicel of each individual flower. We carried out detailed LM, SEM and TEM analyses and showed for the first time that this extrafloral nectar flows from the phloem to nectary-modified stomata by intercellular spaces in the outer parenchymatous layer of the inflorescence.

Medicinal use and trade of wild orchids in Nepal

We documented the use of 53 species of wild orchids in Nepal (i.e 14% of the total orchid flora) for traditional therapeutic purposes. Traditional knowledge of local people is widely exploited by illegal trade through unsustainable harvesting. We estimated that at least 58 tons of wild orchids are illegally traded at four different localities in central Nepal every year. This amount is probably higher for the whole of Nepal since we did not study all parts of Nepal for which illegal trade was reported earlier (Shakya et al., 1994). Species are not only used for medicinal purposes. Horticulturally popular species belonging to the genera *Calanthe*, *Coelogyne*, *Cymbidium* and *Dendrobium* are traded as well. The results of a survey carried out by us in 2008 and 2009 confirmed previous studies which showed that illegal trade of wild orchids in Nepal is increasing (Bailes, 1985; Bajracharya et al., 1994; Shakya et al., 1994; WCN, 2003). The orchids are mostly collected locally by rural youths, women and children. In most cases whole plants were purchased by middlemen or traders at local markets and illegally exported to India and China where they are further processed into herbal products and sold for at least fifty times the original price. This illegal trade causes severe national income losses to Nepal and has a large impact on conservation of wild orchids in Nepal.

Not many medicinally used orchids have been subjected to detailed pharmacological studies yet, despite their immense potential in the treatment of conditions such as neurodegenerative disorders. Anticonvulsive, anticancer and antidiabetic properties have also been proved (Singh and Duggal, 2009; Perez Gutierrez, 2010). We have carried out a bio-screening of wild-collected orchids used by local communities in central Nepal. The results show that 94% of the species sampled contained antibacterial properties. These results clearly show that traditional knowledge of local communities is based on experience, and not solely on folk belief. They indicate that the plants can be used for the development of alternative drugs. Production of these alternative drugs could help initiate sustainable local orchid enterprises where orchids with medicinal properties are produced from seeds and cuttings of stock plants. In this way, production of herbal products would be sustainable and generate additional income to poor rural communities who are now involved in hardly profitable and very unsustainable collecting of wild orchids only.

Curbing cross border crime in illegally traded orchids used to be problematic since material is usually confiscated without flowers which provide most of the characters in traditional identification keys. We applied DNA barcoding and chemical profiling as modern identification tools and were able to identify an additional 10% of confiscated orchid material up to species level. All orchids are listed on CITES Appendix II and are therefore legally protected. To more effectively monitor the trade in critically endangered species, which would be better protected by an Appendix I status, identification up to the species level is often required. DNA barcoding and chemical profiling are therefore now increasingly used to control trade in species placed on either CITES Appendix I or II (Eurlings and Gravendeel, 2005; Eurlings et al., 2010; Ogden et al., 2009). These methods are also increasingly applied to proof cross-border wildlife crime (Dawnay et al., 2006) and will enable authorities to enforce CITES regulations more effectively.

Thesis Goal and Outline

The main goal of this PhD project was to study a selected group of species of Coelogyninae occurring in the Himalayan region taxonomically, phylogenetically, ecologically, anatomically and ethnobotanically, to generate scientific evidence contributing to a better conservation and more sustainable use of wild orchids in Nepal.

In chapter 2, the results of a taxonomic revision of *Coelogyne* sect. *Ocellatae* are presented in which we showed that the section is monophyletic based on a phylogenetic study using nuclear and plastid DNA sequences. We provided detailed descriptions of a total 15 taxa out of which 8 taxa were described new to science. An identification key including the very similarly looking *C. nitida, C. punctulata* and *C. corymbosa* is also provided.

In chapter 3, we describe a new species of *Panisea* from Nepal based on morphological data and a genetic comparison with the only other *Panisea* species for DNA sequence data are currently available. An updated identification key for all species of *Panisea* currently recognized is also provided.

In chapter 4, a pollinator study and ant exclusion experiments were carried out in central Nepal. Ants were found to protect *Coelogyne nitida* from beetle induced herbivory.

Ants were attracted by extrafloral nectar exudated by stomata. New pollination information was provided for three species whose pollination depends on deception of the pollinator: *Coelogyne nitida*, *C. flaccida* and *Otochilus albus*.

In chapter 5, we studied illegal trade of wild orchids in central Nepal. For the species involved, estimations of total volumes traded and incomes generated are provided. Bio-screening of wild-collected medicinal orchids were carried out and DNA barcoding and chemical profiling were applied to identify confiscated nonflowering material up to species level.

In chapter 6, we discuss how our findings on the taxonomy, phylogeny, pollination ecology, and ethnobotany can be used to improve conservation and sustainable use of wild orchids in Nepal.