New species, pollinator interactions and pharmaceutical potential of Himalayan orchids

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Chapter 6

Summary and Conclusions

New species of Coelogyninae in the Himalayan region

Orchids of the Himalayan region have been studied for more than two centuries. These studies largely concentrated on relatively accessible geographical areas such as India, Nepal, and Bhutan. However, the orchid flora of inaccessible areas of the Himalayan region such as the southern part of the Yunnan province of China, northern part of Myanmar and higher altitude regions of Nepal are still relatively unknown. These areas used to be closed for foreign explorers but are now gradually being opened up for biodiversity studies by local taxonomists. The present study on Coelogyninae of the Himalayan region revealed 3 species and 4 subspecies of *Coelogyne* from India, Myanmar and Yunnan and one species of *Panisea* from Nepal new to science.

The systematics of Nepalese orchids is not well understood and the exact boundaries of many species are still unclear. Prime examples are the horticulturally popular species *Coelogyne corymbosa, C. nitida* and *C. punctulata*. We taxonomically revised *Coelogyne* sect. *ocellatae* Pfitz. and Kraenzl. and discovered that the main confusion between the species mentioned above can be traced back to the fact that the type specimens of these species were mixed on a single herbarium sheet. 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 These incorrect identifications are based on outdated literature. A hysteranthous inflorescence with a sterile lowermost bract is the key diagnostic feature for *C. punctulata*. *Coelogyne nitida* and *C. corymbosa* have proteranthous to synanthous inflorescences of which the lowermost bract always bears a flower. The latter two species are easily distinguished by their unique ornamentation of the keels on the lip of the flower.

Pollination ecology and herbivory of Nepalese Coelogyninae

The existing floral syndrom hypotheses predict that flowers of most *Coelogyne* species are pollinated by bees because of their zygomorphic shape, yellow/white colours, prominent landing platform, sweet scent, and presence of nectar guides. Pollination studies carried out by us in central Nepal with *Coelogyne flaccida*, *C. nitida* and *Otochilus albus* provided support for these hypotheses. We found that *Coelogyne flaccida* and *C. nitida* are pollinated by wild bees identified as *Apis cerana* and *Otochilus albus* is pollinated by bumblebees identified as *Bombus kashmirensis.* Floral odours seem to be the primary attractant for the pollinators observed since *C. flaccida*, *C. nitida* and *O. albus* have heavily scented flowers but offer no reward. Additional visual signals might be provided by the bright yellow coloured patches on the white lip of these species. Because none of the bagged flowers of *C. flaccida* and *C. nitida* set fruit in our pollination exclusion experiment, we concluded that these species need pollinators for successful fruit set.

Exudation of extrafloral nectar in orchids was observed earlier to occur through nectary-modified stomata in epiphytic species belonging to *Catasetum* and *Epidendrum*.

In the LM, SEM and TEM studies carried out by us, this nectar was found to be exudated from the phloem to the stomata through intercellular spaces in the outer parenchymatous layer underlying the stomata in various Nepalese Coelogyninae. The nectary-modified stomata were found to be located in a very limited area on the inflorescence just below the pedicels. Experiments with Fehlings' reagent showed that these exudates contained high amounts of sugars. A number of earlier experimental studies on plants with extrafloral nectaries already showed that these species are protected by nectar-foraging ants against flower and leaf herbivores and seed predators. The results of ant survey and ant-exclusion experiments carried out by us clearly showed a significant difference between *C. nitida* plants living in trees with ant nests vs. ant-free trees in damage by flower and leaf-eating beetles.

Ethnobotany and illegal trade of Nepalese orchids

Orchids are long known for their medicinal value especially in the traditional systems of pharmacopeia of China and '*Ayurveda*' of the Indian subcontinental region. In Nepal, over 590 studies related to ethnobotany have been published. Despite this impressive number of publications, traditional uses of wild orchids in Nepal are still poorly documented. We discovered that a total of 59 species of orchids are being used to cure at least 38 different ailments in Nepal. *Coelogyne*, *Dendrobium*, *Cymbidium*, *Bulbophyllum*, *Habenaria*, *Malaxis* and *Pholidota* are most popular for the production of traditional medicines. Interviews with key informants revealed that wild orchids are widely used for the preparation of aphrodisiacs, energizers, and treatments of burnt skin, fractured or dislocated bones, headaches, fever, and wounds. An addiontal 25 species of orchids were reported as fodder for livestock and another 6 species were consumed as vegetables and used in different ritual ceremonies.

An antibacterial screening carried out by us on Nepalese orchids showed that 94% of the species sampled could inhibit growth of one or more test bacteria. *Aerides multiflora, Calanthe puberula, Coelogyne flaccida*, *Coelogyne nitida, Coelogyne punctulata, Coelogyne stricta, Cymbidium iridioides, Dendrobium erriiflorum, Flickingeria fugax, Luisia trichorhiza* and *Pholidota imbricata* demonstrated a broad spectrum of antibacterial activity. These results showed that traditional knowledge of local communities is not based on folk belief only but can be used for the development of alternative drugs.

A total of 61 wild orchid species were found to be involved in illegal trade at the four study sites investigated by us in central Nepal. Circa 10% of the confiscated orchids remained sterile but could be identified to species level by applying DNA barcoding and chemical profiling. We estimated that 28 tons of wild orchids were illegally traded during 2008-2009 and exported to China and India generating a substantial income to local orchid collectors. Predominantly local youths, women and children were involved in orchid collecting whereas local Nepalese or foreigners were involved in exporting the orchids. January to March and July to October were the peak seasons for collecting and trading. For medicinal purposes, species belonging to *Acampe, Aerides, Coelogyne, Crepidium, Dactylorhiza, Dendrobium, Gastrodia, Eulophia, Flickingeria, Otochilus, Pholidota, Satyrium* and *Vanda* were the most exploited. Similarly, for floricultural purposes, *Coelogyne*, *Cymbidium*, *Dendrobium*, *Pholidota* and *Vanda* were most commonly sold. Only a few private companies within Nepal have tissue culture facilities but none of them are yet involved in indigenous orchid species propagation as they all focus on hybrid orchid cut flower production.

Conclusions

Coelogyne section *Ocellatae* was found to be monophyletic for at least the species of which DNA sequences could be analyzed. The presence of an eye-shaped patch on the lip was identified as the main diagnostic character for this particular section. Main characters to distinguish the species in this section from each other are the type of inflorescence (hysteranthous, proteranthous or synanthous), presence or absence of sterile bracts on the inflorescence, and shape and ornamentation of the keels on the lip. Many species in this section are endangered due to overcollection for medicinal and/or floricultural purposes. The illustrated keys provided in this thesis will enable a better identification of confiscated orchids and improve control of illegal trade.

Extrafloral nectar produced by Nepalese Coelogyninae was found to play a key role in attracting aggressive ants protecting flowers and leaves against herbivorous beetles. This nectar was found to be exudated by nectary-modified stomata positioned just below the pedicels on the inflorescence. Extensive observations of flowering *C. flaccida* and *C. nitida* and *O. albus* identified *Apis cerana* and *Bombus kashmirensis* as the natural pollinators. Identification of the natural pollinators and other insects with mutualistic interactions is crucial for protection of endangered orchid species in Nepal.

Our market surveys in central Nepal showed that many wild orchid species are involved in illegal trade and exported to China and India for preparation of herbal products and traditional chinese medicines. We estimated that 28 tons of wild orchids were illegally traded from the study sites investigated generating a substantial income loss to Nepal. Our ethnobotanical survey showed that an exceptionally high number of species of wild orchids are being used in traditional medicinal practices by the local people in Nepal. The antibacterial screening carried out by us indicated that the majority of the medicinal orchid species used by local people in Nepal showed biological activity.

We suggest initiation of sustainable orchid enterprises focusing on medicinal orchid species as alternative to hybrid cut flower industries to reduce extinction in the wild and promote sustainable harvesting of wild orchids by local communities. We have shown that many wildly collected orchids display antibacterial activities comparable with widely used antibiotics. Furthermore, the few clinical studies carried out so far with compounds extracted from orchids indicate that wildly collected species have a huge pharmaceutical potential. This relatively undeveloped niche in the international orchid market deserves further attention. A total of 10% of confiscated orchids remained sterile but could be identified to species level using DNA barcoding and chemical profiling. We suggest a wider application of DNA barcoding and chemical profiling to provide proof for illegal export of wild orchids.