Systematics, epidermal defense and bioprospecting of wild orchids

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Summaries

Summary

Indonesia is a tropical archipelago between Asia and Australia, and the Pacific and Indian Oceans with more than 17,000 islands. It is the second-highest biodiversity hotspot after Brazil for terrestrial flora and fauna and even the highest when combined with marine biodiversity. Indonesia is known to harbor 25% of all flowering plant species worldwide, of which 40% consists of Indonesian endemics. Recent surveys record a total of 7,622 orchid species distributed among the seven bio-regions of Indonesia. With approximately 7,622 species of orchids being present in Indonesia, more than 30% of all orchid species worldwide belong to the Indonesian flora. Over the past two centuries, research on Indonesian orchids gradually shifted from the discovery and description of new species to conservation and environmental studies.

This thesis reflects the gradual change in focus of research on Indonesian orchids as described in the previous paragraph. In Chapter 2, I present a multilingual interactive key, available online (http://glomera.linnaeus.naturalis.nl), that can be used on any web browser without the need for installing additional software. This key includes characters of 169 species of *Glomera*, a genus within the necklace orchids (Coelogyninae - Epidendroideae) not yet comprehensively treated in any recent field guide or web-based survey. With this key, species can be identified using a combination of vegetative and floristic characters in addition to distribution and ecology as a first step to further taxonomic revisions. In this chapter, I urge anyone with an interest in wild orchids in Southeast Asia to contribute new observations to update current information on the distribution of overlooked species to gain more insight into their conservation status.

In Chapter 3, I tackle a common challenge of ex situ orchid collections in botanic gardens. Most orchid species require very specific temperature, humidity, light levels and nutrient concentrations for flower induction and survival and therefore often remain sterile or die shortly after collection from the wild. This severely hampers the identification of such collections to species level as most identification keys require floral characters. DNA barcodes obtained from fertile type specimens in herbaria are a potential tool for fast species identification of sterile living collections, but only a few curators of herbaria allow destructive sampling of type specimens. I obtained permission to perform destructive DNA

extraction of a small number of the numerous leaves from type and non-type specimens of the poorly known necklace orchid genus *Glomera* preserved in the herbarium of Naturalis Biodiversity Center and Herbarium Bogoriense that were collected up to 194 years ago. I used four primer combinations to fully sequence the nrITS region of these specimens and fresh sterile living collections and obtained Sanger sequences for dried specimens and living collections. With the short sequences obtained, several sterile living collections could be identified to species level. Many of the living collections analyzed remained unnamed and are possibly new to science. My results show that DNA barcodes obtained from type material can provide reliable taxonomic information of sterile living collections. I propose a less rigorous policy regarding permission to generate DNA barcodes from additional type specimens to improve the identification of sterile specimens in living collections for better protection of poorly known orchid genera.

Once an orchid specimen with a known geographical origin in a living collection is identified to species level, it can for instance be used for the production of seeds and seedlings for reintroduction of a species into the wild if it is locally extinct. For such conservation efforts, it is important that plants in living collections remain healthy and alive for several years. In Chapter 4, I focus on one of the other challenges of a living orchid collection: suppressing herbivory. Intriguingly, some orchid species are less prone to herbivory than other species. Studies analysing protection of orchids against herbivores require an integrative approach, combining anatomical studies of epicuticular trichomes and waxes with behavioural experiments of the herbivores. In this study, I show for the first time that the terrestrial orchids *Orchis mascula* and *Calanthe triplicata*, the first species deciduous and the second evergreen, protect themselves very differently against attachment of herbivorous land snails than two evergreen epiphytic orchids *Dendrochilum pallidiflavens* and *Trichotosia ferox*, using histochemistry, 'cafetaria' and centrifuge experiments. Size and ornamentation of wax layers and density and histochemistry of epicuticular glandular and non-glandular trichomes on the orchid leaves were assessed with Light Microscopy, Scanning Electron Microscopy and Transmission Electron Microscopy. Total forces needed to detach two differently shaped snail species, *Subulina octona* and *Pleurodonte isabella*, were measured using a turntable equipped with a synchronized strobe. Snails were placed in two positions, either perpendicular or parallel to the main veins on the orchid leaves and on the adaxial (=upper) or abaxial (=lower) side. *Subulina octona* snails were fed with young leaves of *Calanthe triplicata* leaves, of which the hairs had been removed with a lighter in half of the leaves beforehand. The percentage of leaves consumed by the snails was significantly higher for the leaves without hairs. The results obtained provide two other insights. First of all, a perpendicular or parallel position of the snails to the main veins significantly affects the performance of the smaller species tested. Secondly, snails come off significantly faster on sides covered by a thick wax layer or high density of lignin filled non-glandular epicuticular trichomes. This study highlights the importance of histology in combination with behaviour and attachment force experiments for obtaining a better understanding of the defense mechanisms employed by different species of epiphytic and terrestrial orchids to deter herbivorous snails. With this knowledge, orchid individuals with an optimal combination of protective traits, such as a thick wax layer or long non-glandular lignified trichomes, can be selected for future breeding and reintroduction programs.

Another challenge of maintaining collections of living specimens of orchids is the many hours of manual labor required to keep the plants alive. It is a lot of work to daily water, fertilize and/or shade each individual plant according to its own specific needs and keep it protected from herbivores. This work cannot be robotized. In Chapter 5, I focus on a potential extra source of income that could be generated for paying sufficient staff to maintain labor intensive living orchid collections. Extra revenues could be generated by setting up innovative public-private collaborations that are making use of bio-active compounds harvested from orchids. Necklace orchids have been used for traditional medicine practices for centuries. Previously carried out bioassays on a subset of unrelated species showed promising antimicrobial, anti-inflammatory, and anti-oxidant bioactivity, providing experimental proof for medicinal properties as recorded in traditional uses. However, none of these species had been investigated ethnobotanically in a phylogenetic context, yet, at the onset of my PhD project. For my thesis, I carried out comparative bioprospecting of a group of wild orchids using EBDCS (the Economic Botany Data Collection Standards) organ targeted and biological response methods. Traditional medicinal use was recorded from books and journals. Bioassays with a selection of human pathogen microbes were carried out in triplicate on various extractants of leaves and pseudobulbs of *Coelogyne*

cristata and *C. fimbriata* plants cultivated indoors or outdoors. A molecular phylogeny of Coelogyninae based on nuclear ribosomal ITS and plastid matK DNA sequences obtained from a total of 148 species was reconstructed with Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference using MrBayes. Bioprospecting comparison of EBDCS and biological response was carried out using customized R scripts. For a total of 28 necklace orchid species, traditional uses could be compiled, encompassing 19 organ-targeted categories and one biological response category with three different character states. Ethanolic extracts obtained from leaves of *C. fimbriata* were found to inhibit growth of *Bacillus cereus*, *Staphylococcus aureus*, and *Yersenia enterocollitica* and confirmed traditional antimicrobial uses recorded in the literature. Leaf extracts were found to have similiar antimicrobial properties for plants cultivated outdoors and indoors. Three hot nodes with high potency for antimicrobial activities were detected for the EBDCS organ targeted classification method whereas eight hot nodes were detected for the biological response classification method. I conclude that the biological response classification method is more effective in uncovering hot nodes leading to clades of species with high medicinal potential as compared with the EBDCS classification method. I recommend to apply this method to other subtribes to further explore the potential economic uses of bio-active compounds of Indonesian orchid species.