Systematics, epidermal defense and bioprospecting of wild orchids

Kusuma Wati, R.

Author: Kusuma Wati, R.
Title: Systematics, epidermal defense and bioprospecting of wild orchids
Issue Date: 2021-03-25

General discussion and conclusions

Chapter 6

General discussion and conclusions

In this chapter, I discuss the further steps needed to compliment the findings of this thesis and the work that must be continued to understand the (i) systematics, (ii) orchid-snail herbivory interactions, and (iii) bioprospecting of wild species of Necklace orchids in more detail.

6.1 Systematics

In the past two decades, orchid systematic studies have significantly advanced our understanding of the identification, diversification, and classification of the native species of Indonesia. Necklace orchids (Coelogyninae) have been intensively studied, especially the genera *Coelogyne* and *Dendrochilum* (Gravendeel et al., 2001; Clayton, 2002; George and George, 2011; Pedersen et al., 2019). However, it remained challenging to identify species of the overlooked genus *Glomera* because species description and distribution data were scattered throughout literature and natural history collections. In Chapter 2, I present a complete overview of the genus and species descriptions, distribution data, and illustrated interactive keys in a digital platform, Linnaeus, with a bilingual option (English-Bahasa). This method of presenting data is a major step forward, especially in the digital era, where it is essential to provide data that could be accessed anywhere and anytime by anyone. This platform provides easy access to hobbyists, naturalists, students, and researchers. The dual-language option makes it possible for Indonesian plant enthusiasts to read the species descriptions and associated information and increase knowledge about flower color and actual distribution patterns in the wild on popular social media such as Facebook, Observation.org, SmugMug, and WhatsApp.

During the research carried out for *Glomera*, I noticed that not all associated information of the specimens studied had been digitalized. Most herbaria only digitalize a herbarium sheet and the information written on the label, but not any of the associated data hidden in exploration journals from the collector. This exploration journal could be in the archives of herbaria, other musea, or descendants of the researcher. In an exploration journal, a scientist writes more detailed information, like the color of flowers, altitude, local name, and possible uses, collecting dates, or even sketches or photographs of fresh plants and their natural surroundings. For my research, I could work with the diary of the Dutch explorer Gerard Marinus Versteeg (1876 - 1943), kindly made available by his grandson Anton Versteeg, that revealed important additional details about collecting dates and localities in New Guinea. Similar initiatives disclosing colonial cultural heritage such as '*Het geheugen van Nederland'* (geheugen.delpher.nl/nl) and '*Vele handen*' https://velehanden.nl/) will reveal many more metadata of natural history specimens and should be explored for this purpose much more often. Scientific explorations are time-consuming and costly, especially in the Indonesian archipelago due to scattered infrastructure. The involvement of the public in science, known as citizen science, is a solution where a collaboration between scientists and, in this case, amateur historians can help preparing botanical expeditions and increase the chances of finding rare orchid species flowering in the wild.

A major challenge for orchid identification is identifying non-flowering specimens as most identification keys are based on floral characters. Most orchid species require a very specific microclimate to produce flowers that is very hard to create in cultivation. Many wildly collected orchids, therefore, remain sterile and unidentified in cultivation. An accurate identification of species is essential for orchid conservation. DNA barcoding has been developed as an established tool for a rapid identification of species with short standard DNA sequences. This method is increasingly used to identify non-flowering orchids. However, DNA barcoding is usually carried out with fresh material, which is hardly available for rare and overlooked genera. In Chapter 3, I present a new approach: DNA barcoding of type specimens to identify non-flowering specimens of *Glomera*. Types usually remain untouched because the general view is that they should be left intact. However, sacrificing a small portion of a few leaves of types that have many can provide an essential DNA barcode for the identification of many sterile specimens. From a total of 84 specimens studied, we could identify 6 sterile living collections with DNA barcodes generated from 32 types and 11 non-type specimens. To identify more sterile specimens, we urge for a more flexible policy regarding permission to generate DNA barcodes from additional type specimens.

A recent publication by Cámara-Leret et al. (2020) presented New Guinea as the world's island with the richest flora. Orchidaceae were listed as the plant family with the highest number of endemic species, 2,464, of which 144 are species from the genus that I studied in this thesis, *Glomera*. However, New Guinea lags behind other tropical regions, especially in taxonomic effort and explored area. More international collaboration with experts is needed to do joint scientific explorations to underexplored regions in New Guinea. This kind of collaboration is very important for young researchers to receive training and create a next generation of taxonomists and other local experts to safeguard the rapidly disappearing biodiversity of the Indonesian archipelago and beyond in Southeast Asia.

6.2 Orchid-snail herbivory interactions

Plant and herbivore interactions have been the subject of interest for many decades. Plants evolved various defenses to avoid consumption by herbivores, most notably biotic, physical, and chemical features. In contrast to the well-studied field of orchid pollination, only a few studies have been published on orchid-herbivory interactions, and most focus on insect herbivores (van Leeuwen, 1929; Subedi et al., 2011; Lev-Yadun and Ne'eman, 2012; Light and Macconaill, 2014) and myrmecochory, a mutualistic interaction with ants. The ants are attracted to elaiosomes, a lipid-rich part of orchid seeds. As a consequence, the ants transport the seeds to their nest, where the orchid can safely germinate and further develop under their protection (Dutta and Wetterer, 2008; Gegenbauer et al., 2012; Fisher, 2014).

In contrast, orchid-snail herbivores had not yet been studied. In Chapter 4, I investigated the epicuticular structures of four orchid species and their effect on snail attachment by measuring attachment forces using a centrifuge. I discovered that non-glandular and glandular trichomes and wax layers significantly reduce snail attachment. Cryo-Scanning Electron Microscopy, using liquid nitrogen rather than dehydration by ethanol and subsequent carbon dioxide fixation, could improve the study of wet epicuticular structures. A better understanding of the movements of epiphytic and terrestrial orchids, caused by rain and wind, is also needed. Such knowledge could be obtained by using camera traps and measuring devices outside to record plant movements. The field data obtained can then be correlated with forces calculated in the laboratory for this thesis. In addition, cafeteria experiments should be carried out to further investigate the preference of snails for species of orchids with different protective structures next to trichomes and wax layers such as for instance, the membranous sheaths that subtend the flowers of *Glomera*. Knowledge obtained can be used to develop more eco-friendly protection of ex situ conserved orchids than the slug pesticides and other environmentally damaging controls currently employed.

6.3 Bioprospecting of wild orchids

Many Necklace orchid genera have traditional medicinal properties, such as *Coelogyne*, *Dendrochilum*, *Otochilus*, *Pholidota* and *Thunia* (Majumder et al., 2001; Wang et al., 2006; Moin et al., 2012; Marasini and Joshi, 2013; Shibu et al., 2013; Pant, 2014). However, none of these genera had been investigated ethnobotanically in a phylogenetic context yet. Bioprospecting has emerged as a time-efficient and systematic approach to discover potentially new medicinal plant species. To standardize the classification of ethnobotanical data from all countries, a system called Economic Botany Data Collection Standard (EBDCS) was established (Cook, 1995). In Chapter 5, I show that the organ-targeted EBDCS method is less evolutionary informative compared with the biological response method. A total of eight hot nodes were revealed using the biological responses method as compared to only three hot nodes with the EBDCS method. There is currently a lack of ethnobotanical information of Indonesian orchids. Bioprospecting with the biological response method might find additional clades containing medicinal Indonesian species when applied to other orchids used in traditional medicine in countries like China and India that have more established ethnobotanical practices than Indonesia. Possible examples are the Indonesian relatives of *Flickingeria macraei* (Lindl) Seidenf. and *Habenaria pectinata* D. Don. Compounds of these two orchid species are used to treat snakebites in India (Joshi et al., 2009). Both genera also occur in Indonesia, and phylogenetic prospecting could help detect Indonesian species with compounds that are useful for treating snake bites, also commonly occurring in Indonesia, that harbors ca. 450 species of snakes. An international Snakebite program was recently initiated by Naturalis Biodiversity Center and Leiden University to stimulate scientific knowledge exchange between science, government, industry, and societal and humanitarian aid organizations to improve snakebite prevention and treatment. This initiative is supported by the World Health Organization and many countries like the Netherlands, Singapore, Costa Rica, Nigeria, the United Kingdom, and Denmark.

Nuclear Magnetic Resonance (NMR) could be applied to identify the bioactive compounds present in species from hot node clades. With the results obtained, public-private collaborations can be stimulated between botanical gardens, local communities, and companies like Martina Berto Tbk, producing cosmetics and herbal medicine that are committed to use local natural bioactive compounds in their products. Such initiatives are urgently needed to combat antibiotic-resistant microbes and new respiratory viruses like SARS-CoV-2 that cause COVID-19. *Eulophia nuda* Lindl and *Vanda tessellata* (Roxb.) Hook. ex G.Don have been used in India and Nepal to treat bronchitis (Vaidya et al., 2000; Singh and Duggal, 2009). These orchid species might contain a bioactive compound that could help relieve COVID-19 symptoms.

Finally, I discovered during the research carried out for this thesis that bioactive compounds are also produced when medicinal orchids are grown in cultivation. This discovery discards the persistent common belief that medicinal plants can only be collected from the wild, resulting in over-collecting and local extinction of many plant species. When exposed to more UV light and herbivores, the compounds of plants of *Coelogyne cristata* Lindl. and *C. fimbriata* Lindl. that I investigated showed an increased antimicrobial activity. Experiments conducted with more ecological variables should be conducted to develop sustainable and profitable cultivation of medicinal orchids.