Systematics, epidermal defense and bioprospecting of wild orchids

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General introduction

Chapter 1

Introduction

1.1 Indonesia and its biodiversity

Indonesia is a tropical archipelago between Asia and Australia, and the Pacific and Indian Oceans with more than 17,000 islands. Geologically, Indonesia, is situated at the boundaries of three major plates: Eurasia, India-Australia, and the Pacific-Philippine Sea. Indonesia is bordered by tectonically active zones and included in the Circum-Pacific Belt that is also called the Ring of Fire (Hall, 2009). Based on Maryanto and Higashi (2011), Indonesia is classified into seven bio-regions: Sumatra, Java and Bali, Kalimantan, Sulawesi, the Lesser Sunda Islands, Moluccas, and Papua. Indonesia is the second-highest biodiversity hotspot after Brazil for terrestrial flora and fauna and even the highest when combined with marine biodiversity (Widjaja et al., 2014).

Indonesia is known to harbor 25% of all flowering plant species worldwide, of which 40% consists of Indonesian endemics (Ministry of Environment and Forestry of Indonesia, 2015). For orchids, more than 5000 species out of the 25,000 species in the world occur in Indonesia (Banks, 2004). Recent surveys record a total of 7,622 orchid species distributed among the seven bio-regions of Indonesia (see Table 1.1). According to this survey, the highest orchid diversity is found in Kalimantan, but O'Byrne (1994) reported New Guinea is estimated to have more than 3,500 species of orchids and the Lesser Sunda Islands are underexplored as well.

A very popular group of orchids in Indonesia, belonging to subfamily Epidendroideae and subtribe Coelogyninae, are the Necklace orchids. These are common epiphytes found in tropical Asia and the Pacific, occurring from Sri Lanka, through India, Southeast Asia, the Malay Archipelago, Taiwan, Japan, and the tropical Pacific islands east to Samoa (Pridgeon et al., 2005). The term Necklace orchids is based on a characteristic of the most popular species: a long, pendant, multi-flowered inflorescence that resembles a necklace (Figure 1.1). Most species have small (<1 cm in diameter) to medium-sized (<5 cm in diameter) flowers with a sweet scent. The subtribe comprises 21 genera and 755 species in well-studied genera such as *Coelogyne* and *Dendrochilum* and lesser-known genera such as *Glomera*. Many species in this subtribe are used in traditional medicinal practices in China and Himalaya, especially from the genera *Bletilla, Coelogyne, Dendrochilum, Otochilus, Pholidota, Pleione* and *Thunia* (Singh and Duggal, 2009; Subedi et al., 2011; Pant and Raskoti, 2013; Teoh, 2016).

Table 1.1. Number of species distributed among seven bio-regions of Indonesia (Widjaja et al., 2014)

Bioregion	**Number of species**
Java	1604
Kalimantan	2,769
Lesser Sunda Islands	197
Moluccas	755
Papua	2,715
Sulawesi	1,083
Sumatra	2,001

1.2 Bogor Botanic Gardens and their role in species discovery

Bogor Botanic Gardens, formerly known as ‘s Lands Plantentuin, was established in 1817 in the Dutch colonial era by **Caspar Georg Carl Reinwardt**. The garden’s main purpose at that time was to become a productive repository and testing ground for the acclimatization of plants from the Indonesian archipelago and to serve as an important entry point for commercially exploitable plants from Europe and other colonial gardens in Asia and South America (Weber, 2014). The gardens are situated behind the governmental palace and next to the Ciliwung river supplying the plants in the gardens with sufficient water. Reinwardt stressed the economic benefit of the immense fertility and extreme diversity of Indonesia’s biodiversity and the necessity to carry out a meticulous examination of plants, animals, and minerals in their original site to identify and exploit these in a productive way (Reinwardt, 1823).

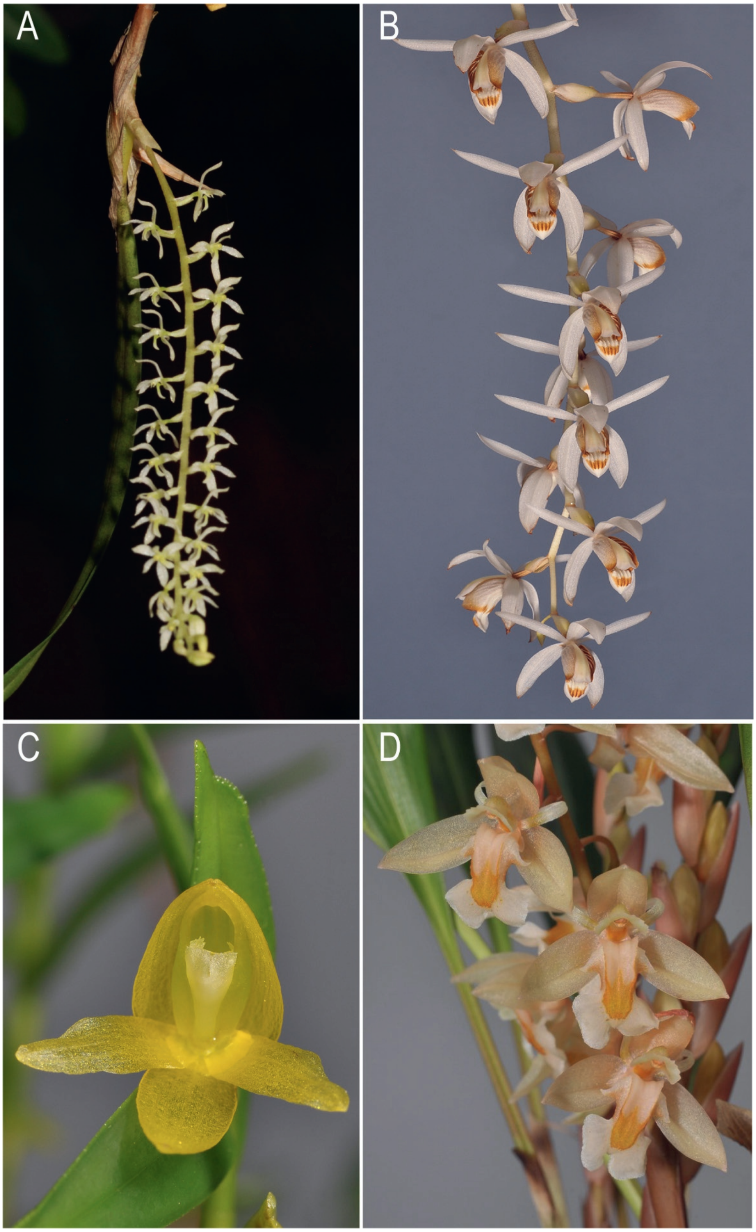

Figure 1.1. Some examples of genera in the Necklace orchids (Coelogyninae) with typically long and pendant inflorescences. A. *Dendrochilum pallidiflavens* Blume, B. *Chelonistele sulphurea* (Blume) Pfitzer, C. *Aglossorhyncha lucida* Schltr., and D. *Coelogyne swaniana* Rolfe. Photograph A by Hendrik A. Pedersen. Photographs B-D by Rogier van Vugt.

Extensive travels and expeditions were undertaken to enrich the botanical gardens with new plant species. **Carl Ludwig Blume** organized expeditions in West and Central Java in 1822 for this purpose. Other expeditions to different islands were carried out by **Alexander Zippelius** in 1828, **Pieter Willem Korthals** in 1823, **Eltio Alegondas Forsten** in 1840, **Heinrich Zollinger** in 1842-1847, **Johannes Elias Teijsmann** in 1853-1877, **Johannes Jacobus Smith** in 1891-1900, **Willem Marius Docters van Leeuwen** in 1913-1929, **Cornelis Gijsbert Gerrit Jan van Steenis** in 1927-1929 and **Reinier Cornelis Bakhuizen van Den Brink** in 1917-1935. In the first catalog of the gardens, published by Blume in 1823, a total of 912 plant species were listed. When Teijsmann was appointed as curator to manage the Bogor Palace and Bogor Botanic Gardens in the period 1830-1868, the plant collection rapidly expanded. **Justus Karl Hasskarl** was appointed to assist Teijsmann with re-arranging the collection according to taxonomic order. These changes made the Bogor Botanic Gardens scientifically more valuable and attractive as a research station for international botanists to conduct research on tropical plants. Consequently, the second catalog from 1844 recorded a total of 150 species of ferns, 25 species of gymnosperms, 510 species of monocots, and 2,200 species of dicots.

Four palm oil seeds (*Elaeis guineensis* Jacq.), brought from Africa, were planted in the gardens in 1848. These oil palm individuals became the ancestors of the oil palms now widely spread throughout plantations in Indonesia and other countries in Southeast Asia (Price et al., 2007). Quinine (*Cinchona calisaya* Wedd.), imported from Bolivia, was planted in the Cibodas Botanic Gardens (*Bergtuin te Cibodas*) in 1852 upon their establishment (Teijsmann, 1861). Teijsmann also undertook many excursions to Japan, China, India, Sri Lanka, Brazil, and Australia to further expand the plant collection. After Teijsmann, **Rudolph Scheffer** was appointed as director of the Bogor Botanic Gardens. He founded the first scientific journal of the gardens called *Annales du Jardin Botanique de Buitenzorg*, in which many new species of tropical plants were described, including orchids. Scheffer was succeeded by **Melchior Treub** in 1880. Under his directorship, taxonomic research at the gardens was expanded with physiology and genetics. Treub also improved the aesthetic value of the gardens by creating beautiful thematic gardens to attract non-scientific visitors (Sukarya and Witono, 2017).

Figure 1.2. The difference between past and current education on biodiversity in Indonesia. A. Class room of **Leendert van der Pijl** in Bandung, where he worked from 1927 until 1947 as biology teacher, in which the jungle was brought to the pupils. B. Excursion let by **Yoga Dwipayana** in the Bogor Botanic Gardens in 2015, where the pupils are brought to the jungle. Photographs kindly provided by Priska Becking and the Bogor Botanic Gardens Flora Tourism Team.

From species discovery to conservation

After the independence of Indonesia in 1956, the Bogor Botanical Gardens, now called Pusat Konservasi Tumbuhan Kebun Raya, were led by **Sudjana Kasan**. Three branches were gradually established: next to the Cibodas Botanical Gardens in West Java (1862), the Purwodadi Botanical Gardens (1941) in East Java, and Eka Karya Botanical Gardens in Bali (1959) were founded. The four Botanic Gardens under the Indonesian Institute of Sciences were called Indonesian Botanic Gardens (IBG). The aim of the gardens gradually evolved from plant collection into plant conservation, research, education (Figure 1.2), ecotourism, and environmental services, the latter especially to curb deforestation (Indonesian Presidential Decree no. 93 on Botanic Gardens, 2011). In this period, **Leendert van der Pijl** and his students discovered many new pollinators of Indonesian plants, especially orchids (van der Pijl and Dodson, 1966). Under the directorship of **Irawati**, the first female director, the orchid research, especially in vitro culture of rare Indonesian orchids, was further developed (Figure 1.3). Since the 19th century, the Bogor Botanic Gardens also became known as a very famous tourist destination. In 2019 the gardens were visited by a total of 1,135,495 local visitors and 15,749 foreign visitors. The gardens have become a national legacy, with an important role in the development and progress of botanical, agricultural, and plantation sciences in Indonesia. Several new research institutions were also initiated by the Bogor Botanic Gardens such as the Bibliotheca Bogoriensis, Museum Zoologicum Bogoriense, Ocean Research Institution, Bogoriense Herbarium, Nature Preservation Institution, Flora Malesiana Foundation, and Microbiological Institution and Academy of Biology. The current generation of researchers of the Bogor Botanic Gardens produced seeds from hand pollination of *Amorphophallus titanum* (Becc.) Becc. (Sudarmono et al., 2016) and successfully developed a protocol for initiating ex situ flowering of *Rafflesia patma* Blume (Mursidawati et al., 2015).

From conservation to education and environmental services

On the international scale, IBG agreed to implement the Global Strategy for Plant Conservation (GSPC) with a total of 16 targets to save plants (Davis, 2008).

According to target 8 of the GSPC, between 2011 and 2020, a minimum of 75% of all endangered plant species should be collected in their original country, and 20% of them should be reintroduced. In the four separate botanic gardens, IBG was only curating a total of 21.5% of all Indonesian threatened plants in four botanic gardens (Purnomo et al., 2015). To create more space and care for all collected plants, Bogor Botanic Gardens, therefore, initiated the establishment of new botanical gardens under a regional government, called the Regional Botanic Gardens (RBG). The target is to establish a total of 47 Botanic Gardens based on the Terrestrial Ecoregion and WWF Ecoregion classification (Olson et al., 2001). In 2012, IBG and RBG managed a total of 24% of Indonesian threatened plants (Purnomo, et al., 2015). So far, a total of 44 additional Botanic Gardens have been established in 23 provinces, 5 Botanic Gardens under LIPI, 37 Botanic Gardens under Regional Governments, and 2 Botanic Gardens under the University of Haluoleo and Institute of Technology Sumatra (ITERA). This initiative will help to conserve the endangered native plants of Indonesia.

1.3 Orchid research in the Bogor Botanic Gardens

Probably the very first study of orchids in the Bogor Botanic Gardens was carried out by Blume, who described a large number of new orchid species from West and Central Java. Gaining advantage from his appointment as inspector of smallpox vaccinations, Blume could observe the flora of many parts of Java. One of the orchid genera published by him was *Glomera*, which was described in 1825 in *Blume's Bijdragen tot de flora van Nederlandsch Indië* with *G. erythrosma* Blume as type, the only species occurring in Java. *Glossorhyncha*, a close relative, now included in *Glomera*, was described in 1891 by **John Ridley** in The Journal of the Linnean Society Botany with *G. amboinensis* as type, the first described species from the Moluccas (Wati, van Vugt and Gravendeel, 2018). Blume's most important contribution to orchidology was constructing a system of affinity for tropical orchids. In his *Catalogus van eenige der merkwaardigste zoo in- als uitheemsche gewassen te vinden in's lands plantentuin te Buitenzorg*, published in 1823, the orchid collection of the Bogor Botanic Gardens at that time was listed. It included species used in traditional medicinal practices such as *Acriopsis javanica* Reinw. ex Blume, of which extracts of the pseudobulbs are used to treat earache and fever by the Sundanese people. Other economically important species included *Cypripedium javanicum* Reinw. ex Lindl., a synonym of *Paphiopedilum javanicum* (Reinw. ex Lindl.) Pfitzer, an Indonesian endemic, nowadays used as the parent of many artificially created orchid hybrids. Other discoveries contributing to commercial exploitation of orchids were made by Teijsmann, who introduced artificial pollination of *Vanilla planifolia* Andrews plants in Java for the production of vanilla pods. Smith and **Rudolf Schlechter** described many new orchid species collected throughout Indonesia.

Around 1900, the Foreigner's Laboratory, later called Treub Laboratorium, was built inside the Bogor Botanic Gardens to attract researchers from all over the world. Treub studied orchid embryology, seeds, and seedlings there and proposed the term protocorm (Treub, 1890) for early-stage orchid seedlings (Yam and Arditti, 2009). He also performed the first histochemical study of the orchid embryo (Treub, 1879). In this same laboratory, **Hans Fittings** studied pollinia of *Phalaenopsis* in 1909 and saw that pollen tubes release a substance that induces post pollination phenomena such as ovule development (Fitting, 1909a, 1909b, 1910). This substance was later discovered to be auxin by **Frits Warmolt Went** (Went, 1926), who worked in the laboratory from 1927 to 1933. **Docters van Leeuwen** studied the seed dispersal of *Epipogium roseum* (D.Don) Lindl., a saprophytic orchid there (Docters van Leeuwen, 1937). He also observed pollination of *Dendrobium hasseltii* (Blume) Lindl. by birds on a mountain summit in Java, autogamy in the genus *Myrmerchis* (van der Pijl and Dodson, 1966) and ant dispersal of orchid seeds with elaiosomes (van Leeuwen, 1929, Wati et al. in prep.). The intimate relationship between orchids and fungi was first studied by **Hans Burgeff** during his visit to the laboratory from 1927 to 1928. Between 1939 and 1955, **Jacoba Ruinen**, the first female orchidologist at the Bogor Botanic Gardens, coined the term epiphytosis for the harmful effects of root-associated fungi of epiphytic orchids to the branches of their host trees, that were revealed by a series of elegant experiments carried out in the laboratory and gardens (Ruinen, 1953).

During 1990-2000 many orchid researchers, amongst others from the Royal Botanic Gardens, Kew, Hortus botanicus Leiden, and Singapore Botanic Gardens visited the gardens to study the plant collections. **Jeffrey James Wood** and **Phillip James Cribb** published their Orchids of Borneo series (1997-2004), and

James Boughtwood Comber his Orchids of Java (1990), and Orchids of Sumatra (2001) books. **Eduard de Vogel** and **André Schuiteman**, next to publishing many scientific articles, produced an innovative CD-ROM series (http://www.orchidsnewguinea.com/). **Peter O'Byrne** published his 'A to Z of South East Asian Orchid Species' guide for orchid novices, and **Barbara Gravendeel** wrote her PhD thesis on *Coelogyne* and allies.

In the 21st century, a tissue culture laboratory was built next to the orchid greenhouse to maintain the collection, supply it with seedlings, and exploit it commercially. Currently, **Djauhar Asikin**, **Dwi Murti Puspitaningtyas**, **Sofi Mursidawati**, **Elizabeth Handini**, **Yupi Isnaini**, **Eka Martha Della Rahayu**, **Vitri Garvita Gandadikusumah**, and myself manage and study this collection. Important discoveries include successful propagation of endangered orchids such as *Phalaenopsis gigantea* J.J.Sm., *P. violacea* H. Witte, *P. celebensis* H.R. Sweet, *P. javanica* J.J.Sm., *Paraphalenopsis serpentilingua* (J.J.Sm.) A.D. Hawkes, and *P. laycockii* (M.R. Hend.) A.D. Hawkes. The regeneration of protocorm and acclimatization of *Cymbidium hartinahianum* J.B. Comber & Nasution, an orchid species that was assumed to be extinct in the wild after its small native area was converted into a potato field, was successfully developed (Handini et al., 2018). Bioprospecting of medicinally used Indonesian orchids was applied to find new sources of bioactive compounds (Wati et al., in press). Collaboration with fellow researchers such as **Aninda Retno Utami Wibowo** from the Eka Karya Botanical Gardens and **Dewi Pramanik** from the Indonesian Agency for Agricultural Research and Development provides new insights in the evolutionary origin of highly specialized orchid floral structures such as the mentum (Pramanik et al., 2020).

1.4 Orchid illustrations: from elite art to commercial products and social media for everyone

Smith and Schlechter illustrated their taxonomic descriptions themselves by making black and white pencil drawings while collecting plants in the field. In the 18th century, these were the only resources available, and their drawings are of high scientific value. They lack important diagnostic characteristics, though, such as plant habit and flower color. Fortunately, Smith and Schlechter were sometimes assisted by professional botanical illustrators, such as **Natadipoera**, a member of the royal family of the Talagamanggung Kingdom in Majalengka, who illustrated nine species of *Glomera* and many species from other orchid genera. **Mas Kromohardjo** illustrated species in the seven volumes of Smith's *Orchideen von Java*, published between 1905 and 1939. Nowadays, ecotourism hugely contributes to science by displaying high-quality photographs of species in the wild on social media such as Facebook, Instagram, Pinterest, Pbase, and Smugmug, in which important details such as flower and leaf color are disclosed (Figure 1.4). A new generation of botanical artists such as **Esmée Winkel** skillfully combines art with science. **Janneke Brinkman** turned her art into a wide array of commercial products (www.jannekebrinkmanshop.com). **Eunike Nugroho** and **Jenny Kartawinata** founded the Indonesian Society of Botanical Artists (IDSBA) in 2017. Its members are botanical artists, illustrators, botanists, researchers, and hobbyists. This organization aims to ensure the continuation of botanical art in Indonesia and increase awareness of Indonesian biodiversity.

1.5 Current challenges for orchid collection management

Management of Indonesian orchid collections ex situ has its challenges. Most of the orchids are obtained from the wild during explorations throughout the archipelago. After freshly collected orchids arrive in one of the branches of the Indonesian Botanic Gardens, the acclimatization process starts. Acclimatization will determine if plants survive in a new environment that is very different from the native habitat. During this process, many orchids die since it is very hard to imitate the macro- and microclimate of the native habitat in a nursery (Trimanto and Rahadiantoro, 2017). Even when the orchids survive, many of them will never flower, making it very difficult to identify the specimens to species level since traditional identification keys often rely on floral characters. The orchid collection in the Bogor Botanic Gardens currently contains 6,004 specimens, consisting of 499 species from 94 genera, of which 1,223 could not yet be identified to the species level (Wati and Mursidawati, 2015). To speed up identification, DNA barcodes from living collections can be linked to data generated from herbarium specimens (Wati et al., in press). Species identification can update information on geographic distributions and answer other questions on the ecology and conservation biology of endangered species, needed to assign priority areas (Guisan and Zimmermann, 2000).

Another challenge in any living orchid collection, but especially in humid tropical regions, is the protection of plants against herbivores and diseases. Especially snails and slugs are major pests of living orchid collections, either in situ or ex situ. After decades of unsustainable chemical spraying, the use of chemical pesticides is now increasingly legally banned, and bio-based alternatives are called for (Subedi et al., 2011; Wati et al. in prep.).

1.6 Future directions for scientific exploration of living orchid collections

Plants have been used as the basis of traditional medicine systems as early as 2600 BC (Cragg and Newman, 2013). Many modern drugs have been developed from plant bioactive compounds. Over the past decade, an increase in drug-resistant microbes urged for the finding of new antimicrobial compounds to replace current antimicrobial agents. It is estimated that only 6% of all plant species have been screened for biological activity so far, and only 15% of these have been tested phytochemically (Verpoorte, 1998). New time-efficient and systematic approaches are needed to unlock the potential of plants in health-care (Ernst et al., 2016). Plants with medicinal properties are usually found more frequently in certain families and are not randomly distributed throughout the Angiosperm Tree of Life (Moerman, 1991; Saslis-Lagoudakis et al., 2011). The orchid family is long known for its medicinal properties (Lawler, 1984; Singh and Singh, 2012). Bioprospecting offers an effective approach, combining a phylogeny with ethnobotanical knowledge to find potential new sources of medicines, but the availability of ethnobotanical data is essential. Most of the ethnobotanical data for orchid uses come from Traditional Chinese Medicine (TCM), and Himalayan sources, but very few orchid uses have yet been recorded for Indonesian species. Exploiting indigenous knowledge is a promising new way of co-financing the maintenance of living orchid collections. Elsewhere in Asia, such as in Bhutan, patent rights of a traditional face cream

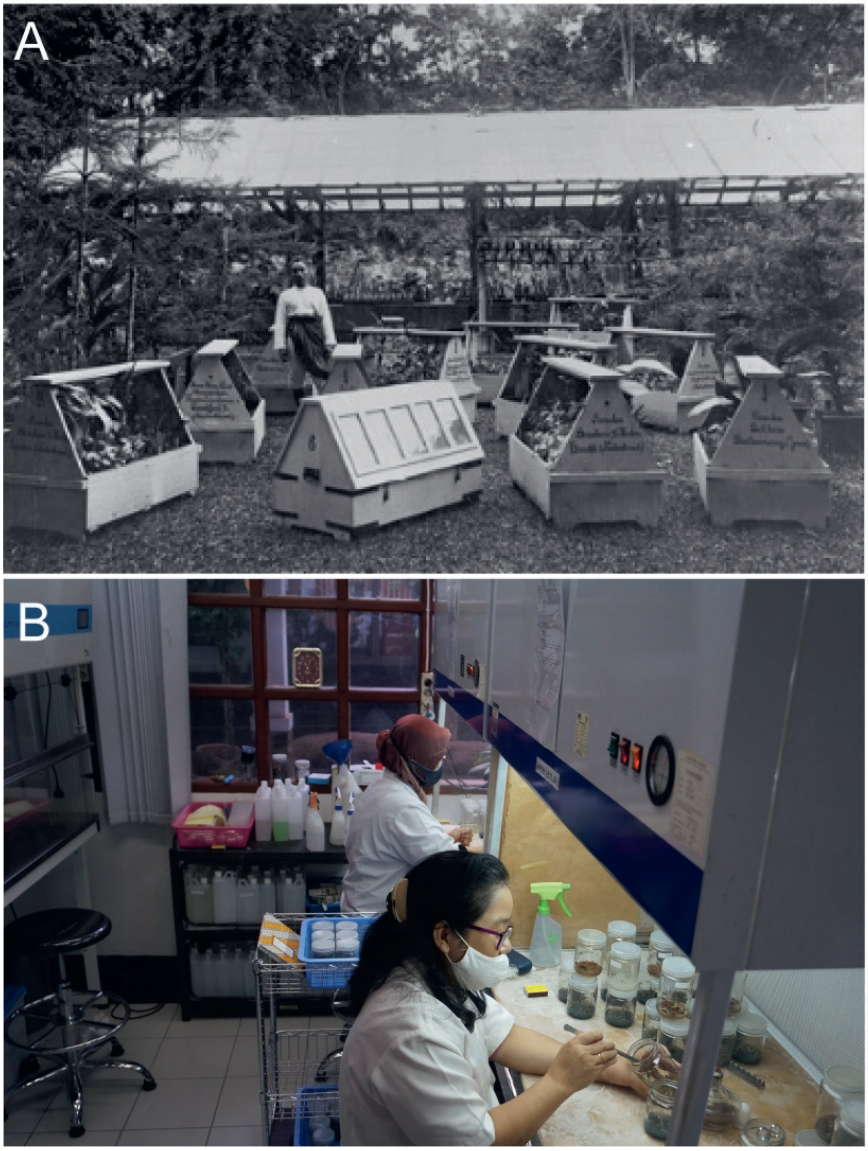

Figure 1.3. The difference between past and current research in the Bogor Botanic Gardens. A. Many new varieties of commercially important plants were developed in the Bogor Botanic Gardens between 1850 and 1890. Cuttings of these new cultivars were sent in large quantities to companies using a *Wardsche box* (Boomgaard and Dijk, 2001). Photograph from the Tropenmuseum Collections. B. Two of the current female researchers producing bottled seedlings of threatened species in the orchid collection. Photograph by Sofi Mursidawati.

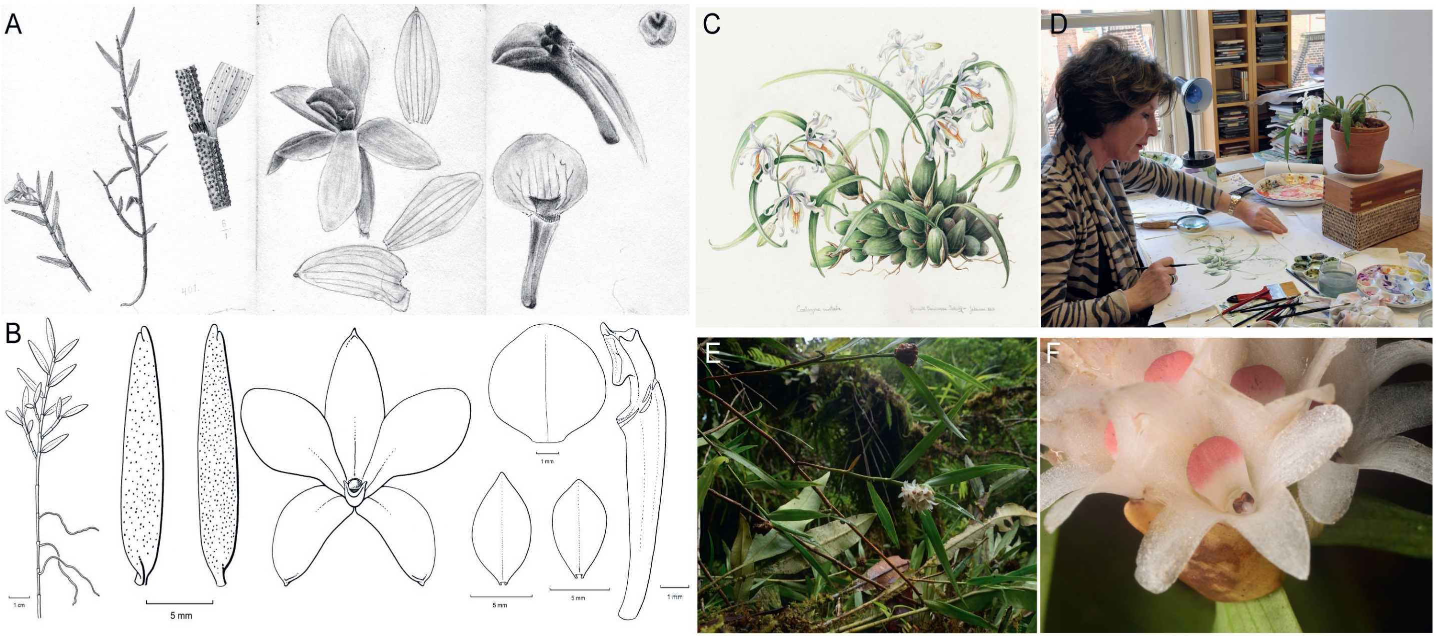

Figure 1.4. An impression of illustrations of Necklace orchids made over the past two centuries. A. Black and white pencil drawing of *Glomera amboinensis* made by Johannes Jacobus Smith to illustrate species descriptions in his publication in the journal *Bulletin du Département de l'Agriculture aux Indes Néerlandaises*. B. Scientific illustration of key diagnostic characters of *G. amboinensis* made by Esmée Winkel for a publication in the journal *Phytokeys*. C. Artistic botanical drawing of *Coelogyne cristata* made by Janneke Brinkman in 2013 for use in commercial products. D. Photograph of Janneke, making the drawing displayed under C, made by Rogier van Vugt. E-F. Photographs of *Glomera montana* in the wild in the Solomon Islands, made by Hsu Tian-Chuan and uploaded in the image hosting platform Flickr.

made from extracts of *Cymbidium erythraeum* Lindl. support economic growth. This ambition was also voiced by the Indonesian Science Fund focus area of Life, Health, and Nutrition to utilize the Biodiversity of Indonesia and develop it into medicine and sources of nutrition.

Aims of the thesis

In this thesis, I targeted the Necklace orchids, with special emphasis on the genus *Glomera*, a highly overlooked and understudied genus with 169 species recorded to date, distributed over Indonesia, Papua New Guinea, and the Philippines. To facilitate an increase in current knowledge on the distribution of all the species of *Glomera*, I made an illustrated, interactive, and bilingual identification key for the entire genus (**Chapter 2**). The online identification key provides easy-access to the detailed information of each species for all users with different knowledge levels. To solve the challenging problem of identification, I developed a DNA barcoding method for the identification of non-flowering specimens in living collections of botanic gardens (**Chapter 3**). Identification is important to update distribution data that could improve the current conservation plan of Indonesian orchids. To improve in situ and ex situ conservation and understanding of orchid defense against herbivory, I investigated the function of the epicuticular properties of orchid leaves by measuring attachment forces of snails (**Chapter 4**). To explore the potential of new plant-based drugs in the light of growing antibiotic resistance worldwide, I carried out bioprospecting of medicinally used Necklace orchids (**Chapter 5**).

Outline of the thesis

The Necklace orchids (Coelogyninae) are an orchid subtribe in subfamily Epidendroideae, comprising 21 genera and over 680 species, that are widely spread throughout Southeast Asia, including Indonesia (Pridgeon et al., 2005). The most overlooked and understudied genus of this subtribe is *Glomera*, which occurs in New Guinea, Papua, the Moluccas, Java, Vanuatu, and the Philippines. The genus is characterized by an elongated, often branching rhizome with many leaves that are enveloped by sheaths at the base. The inflorescences are usually single- or few-flowered. The flowers are mostly white, but some are orange, salmon-pink, or green. Most species are epiphytes in lowland or montane rainforest up to subalpine environments (Wati, van Vugt and Gravendeel, 2018).

To increase awareness for this genus, we present in Chapter 2 a bilingual interactive identification key built in the Linnaeus platform, available online with detailed species descriptions, distribution maps, illustrations, and color photographs of living specimens. The platform encourages citizen scientists to contribute photographs for updating distribution data.

While studying herbarium material from different species of *Glomera* deposited in the collection of Naturalis Biodiversity Center, we were allowed to extract DNA from type specimens to help identify non-flowering specimens in living orchid collections in various botanic gardens. In Chapter 3, we developed DNA barcoding of type specimens and living collections as a method for rapid identification of non-flowering specimens in botanic gardens. We obtained permission to perform destructive DNA extraction of type specimens from the herbarium of Naturalis Biodiversity Center and Herbarium Bogoriense. We managed to fully sequence the nrITS region of these specimens and match these with DNA barcodes obtained from fresh sterile collections. With this method, several sterile specimens in the living collections could be identified to species. However, several other specimens could not yet be matched to any type specimens, indicating that these likely belong to species new to science. Future explorations in their geographical area of origin might retrieve flowering specimens needed for the formal description of these species.

In addition to the difficulties of identification of living collections, keeping them alive is a challenge as well, mainly due to high levels of herbivory, both in situ as well as ex situ. To better understand orchid defenses against snail herbivores, in Chapter 4, we carried out experiments on attachment forces of snails against the epicuticular leaf properties of four different orchid species to understand how different orchids defend themselves against herbivores. For this purpose, a custom-made centrifuge was designed, consisting of a turntable equipped with a synchronized strobe. Two differently shaped snails were used in the experiments. We found that terrestrial and epiphytic orchids have different epicuticular structures to defend themselves against herbivores.

For commercial exploitation of orchid collections, we carried out a bioprospecting analysis of Indonesian orchids in Chapter 5. Ethnobotanical data from orchids used in China and Himalayan were applied to an expanded phylogeny of the Necklace orchids that included Indonesian species to find new potential sources for bioactive compounds with antimicrobial properties. Two different methods are generally usually used for medicinal properties classification, the Economic Botany Data Collection Standard (EBDCS) and the Biological Response method. We discovered that the EBDCS classification is less effective in discovering the potential of bioactive compounds as compared with the biological response method that detected a wider group of potential medicinal Necklace orchid species. In Chapter 6, I discuss further steps to apply the findings presented in my PhD thesis for conservation and utilization of collections of Indonesian orchids in the Bogor Botanic Gardens and beyond.