Interspecies Boundaries in Orchids

Orchids are a fascinating world unto themselves, featuring incredible diversity of species, myriad forms and dazzling colors. At the same time, orchids are among the least explored groups of plants, with much work left to be done on their careful classification and study. How should different orchid species be distinguished, and how should the boundaries between different genera be delineated?

Marta Kolanowska, PhD, DSc

is a botanist employed as an Associate Professor at the University of Łódź. She also works at the Global Change Research Institute (Czech Academy of Sciences). She is president of the Biodiversitatis Foundation. Her main research interest lies in the taxonomy and biogeography of orchids. She is active in tropical ecosystem conservation.

marta.a.kolanowska @gmail.com

M a r t a   K o l a n o w s k a

Faculty of Biology and Environmental Protection, University of Łódź, Poland
Department of Biodiversity Research, Global Change Research Institute AS CR, Brno, Czechia

Orchidaceae are one of the largest families of flowering plants, including species found all over the globe (apart from polar and desert regions). Theophrastus wrote about them around 300 BC, and the Orchidaceae family was first formally described by Antoine Laurent de Jussieu in 1789.

What's in a name?

Carl Linnaeus, the father of contemporary taxonomy, famously wrote, "If you do not know the names of things, the knowledge of them is lost, too." How much have we learned about the diversity of orchids since the first formal description of the nominal genus *Orchis*? While Linnaeus' 1753 *Species Plantarum* lists just 62 species, by the end of 2020 over 80,000 different binomial names of Orchidaceae had been published!

According to our current knowledge, there are over 27,000 recognized orchid species worldwide, with the greatest diversity found in tropical regions. The appearance of the flowers remains the most important morphological feature used to distinguish between orchid species and marking boundaries between lower-order taxa. In 1862, Charles Darwin noted the significance of cross-pollination in the process of natural selection, and it is likely that the extremely varied and complex structures of orchid flowers are the result of adaptations to very specific, limited groups of pollinators. An example of two superficially similar species adapted to different pollinators can be found in the lesser butterfly-orchid (*Platanthera bifolia*) and the greater butterfly-orchid (*P. chlorantha*). In the former, pollinated by hawk moths, pollinia are arranged in parallel and have a narrow connective. Pollinia of the lesser butterfly-orchid settle on the insect's proboscis. In the greater butterfly-orchid, the connective is wide, pollinia are spaced wide apart in the lower section and

*Platanthera chlorantha
– a wild orchid*

they lean towards one another in the upper section. They are pollinated by owlet moths, and the pollen grains attach themselves near the insects' eyes.

Diversity – more than just color

Until the mid-19th century, morphological descriptions of newly discovered orchid species were rather poor, and the authors of new names frequently only described the size and color of their flowers. A major challenge facing today's researchers into orchids is the correct identification and re-characterization of "old" species, using materials found in herbariums (collections held at institutions as documentation for scientific research) to present complete descriptions of these taxa. We now know that even seemingly minor morphological differences between flowers can have a major influence on a given species' pollinators and thus serve as a mechanism of reproductive isolation, preventing cross-pollination between different species.

Insufficient understanding of the morphology and diversity of different species can lead to different names being assigned to plants which in fact represent the same species. An example is the pan-tropical greater yellowspike orchid (*Polystachya concreta*) which has been given over 20 synonymous names.

Apart from flower appearance, another important barrier to the number and type of pollinators that service specific orchids is their scent. Many observations confirm the importance of this feature as a boundary between orchids pollinated through a phenomenon known as "pseudocopulation," seen for example in the bee orchids (*Ophrys*). Their flowers attract male pollen-carrying insects by the color and ornamentation of the inner petals which resemble females of certain species of bees, and by their scent resembling insect pheromones. As amorous males mate with the "false females," they collect pollen grains. Each *Ophrys* is adapted to a specific limited group of pollinators, therefore their appearance and relationships with insects are on the species level.

In many orchids which do not have such highly specialized mechanisms of pollination as *Ophrys*, for example *Gymnadenia*, additional boundaries are set by the different flowering times of individual species and diverse preferences for growing conditions. In mountainous regions, in particular in the tropics, rel-

Dendrobium christyanum – a wild orchid from Thailand

atively small areas are home to vast numbers of micro-habitats which vary in terms of insolation, humidity and temperature, and as such are inhabited by different species of orchids and their pollinators.

New boundaries

A spectacular outcome of carefully studying morphological diversity and delineating boundaries between individual orchid species can be noted for the *Microchilus* genus in America. Until 1852, the genus was believed to number just 14 species. However, research by the Australian botanist Paul Ormerod, who analyzed the small *Microchilus* flowers, led to the description of over 100 new species within the genus. Boundaries between individual taxa describe flower size and leaf shape as well as the appearance of the spur (a tubular-like projection that produces and retains nectar), shape and enervation of individual elements of the flower and details such as the ornamentation and shape of individual petals.

Subjective boundaries

It is notable that there is still no single system for separating orchid species from one another. This is partly due to the extraordinary diversity of orchids, which is frequently the result of unusually fast speciation. One of the most problematic Orchidaceae taxa is *Ophrys* (bee orchids), which evolved around 4.9 million years ago and is now found across Europe, North Africa and the Middle East. The huge variety observed between the populations of these bee orchids

has resulted in various classification systems based on morphological features. While Hans Sundermann listed just 16 *Ophrys* species in 1980, by 1982 Helmut Baumann and Siegfried Künkele recognized 49; the number rose to 150 in 1994 (Pierre Devillers and Jean Devillers-Terschuren) and 252 in 2006 (Pierre Delforge). New species of bee orchids are still being discovered today, with the latest, *O. querciphila*, being formally described in 2017. The absence of a uniform, consistent system defining morphological boundaries between individual orchid species is in part due to the high diversity of individual groups of orchids which evolved at different rates and under differing conditions. Families can include relatively uniform genera of a few or just a few species (e.g. *Thecostele*, *Trizeuxis*) as well as highly diversified taxa including over a thousand species (e.g. *Epidendrum*).

The development of multivariate morphometrics based on specimen measurements is an important scientific achievement, making it possible to make more objective assessments of the differences between individual species. However, statistical methods are still rarely used for delineating taxonomic boundaries in orchids, and they are generally applied in studies dealing with relatively small genera or complexes of species, e.g. *Stenoglottis*, *Brachycorythis helferi*, *Pomatocalpa maculosum* and Greek species of *Himantoglossum*.

Population genetics has revealed classification problems with many interspecies hybrids from the *Dactylorhiza* genus (marsh orchids). Researchers have discovered that the observed diversity of these orchids may be due to the fact that many recent species have

yet to develop their own discriminatory features. Additionally, the high diversity of the appearance of *Dactylorhiza* flowers is in part due to a high likelihood of hybridization.

Unfortunately, limited access to genetic material of tropical orchids means that new species are rarely described based on DNA differences. Obtaining comparative material from species originally described over a century ago can also be difficult, since the only specimens are oftentimes those preserved in herbariums.

Phylogenetic research, which aims to recreate the evolutionary development of organisms by describing their kinship, is mainly employed in taxonomy to define the boundaries between taxa above the species level (genus, family). Unfortunately, describing "good" genera appears to be just as difficult as defining boundaries between species.

Let's look at the genus *Pleurothallis* (bonnet orchids), which has been extensively studied by scientists since 1896. The taxon, originally described in 1813, once included over 2300 named species; however, subsequent studies led to many of them being assigned to new genera (e.g. *Acianthera*, *Acronia*, *Anathallis*, *Colombiana*, *Crocodeilanthe*, *Pabstiella* and *Specklinia*). Moreover, molecular studies have encouraged researchers to propose differing classification systems of *Pleurothallis*, and there appears to be no sign of consensus.

Most molecular taxonomists agree that the term "genus" can only be applied to a monophyletic group of organisms (including all descendants of a last common ancestor); however, the issue of assigning paraphyletic genera (including just some of the descendants of the last common ancestor) still remains highly controversial. Monophyletic Orchidaceae genera are frequently undefinable in terms of morphology, due to the vast diversity of species they embrace. Inconsistencies between results of morphological and genetic analyses are common and have been observed in *Erycina*, *Gomesa*, *Oncidium*, *Otoglossum* and *Stelis*. Another complication with phylogenetic studies is that inconsistencies often arise between the findings of studies using different markers. Researchers are still trying to identify DNA fragments that reveal sufficiently high variability on different taxonomic levels.

It would appear that many questions could be answered by the increasing use of next-generation sequencing techniques – massively parallel sequencing of thousands of overlapping nucleotide fragments which can be arranged into a single sequence during bioinformatic analysis.

Why is defining boundaries and naming species so important?

Delineating correct boundaries between species and assigning names is important not only for the obvious scientific and academic reasons, but also for various practical reasons.

In environmental conservation, for instance, it is extremely important to have consistent lists of species and subspecies so that protected regions can be designated on the basis of comparable information on biodiversity. A certain species of rare marsh orchid with an appearance deceptively similar to a common species has only been protected in Poland since 1983. Previously, it had been mentioned in legislation only as "the most common red-flowering meadow species" (Ordinance of the Minister of Education from 29 August 1946 on the introduction of plant species protection, *Journal of Laws* no. 70 item 384). Such failures to include particular species under conservation regulations has often been due to difficulties in identifying individual species, and this has had a negative impact on the survival of rarer taxa such as *Dactylorhiza incarnata*, which were confused with the common *D. majalis*.

Additionally, increasing numbers of research institutions are exploring the medicinal properties of orchids. Certain therapeutic properties were already known back in ancient China, and they are used as natural remedies in Asia and South America. However, if we are to make the most of such therapeutic properties, we must first be able to precisely identify the correct orchid species. Superficially similar orchids may be found to have very different active substances with very different mechanisms of action. ■

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Ophrys Minoa Var Candica with a bee