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You don't have to kill the orchids – good practices in orchid research

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ABSTRACT - Orchids are globally threatened by habitat loss and poaching. In this context, we present suggestions to maximize the gathered scientific information without compromising the continuity of orchid populations: 1. To keep population coordinates in secrecy and avoid unnecessary or careless postings on social media; 2. To acquire and carry valid, working/collecting permits; 3. To make a complete photographic record of the orchid features before pressing the specimens; 4. Plant and insect (pollinators) vouchers are to be kept to the strictly necessary and must be deposited in accredited Herbaria and Entomological collections, respectively; 5. Orchid fruits for propagation purposes should be collected only when they are ripe enough, well-developed, and devoid of insects/illnesses. Recommendations to address all these methodological suggestions are presented. In particular, we advocate that it is completely unnecessary to kill (press) entire orchid specimens and provide instructions to properly voucher epiphytic and terrestrial orchids without pressing whole plants. The advantages of working in association with Living Orchid Collections held in Botanical Gardens or other Institutions are discussed. Only pollinarium-carrying insects are to be considered orchid pollinators and should be sampled in moderate numbers, for their identification by taxonomists. Appropriate techniques to record natural orchid pollination are also discussed.

Keywords: conservation, Orchidaceae, pollination, systematics, taxonomy.

RESUMO – Não precisamos matar orquídeas - boas práticas de pesquisa em Orchidaceae. As orquídeas estão globalmente ameaçadas pela perda de hábitat e pela coleta ilegal. Apresentamos sugestões metodológicas para maximizar a obtenção de informações sem comprometer a existência das populações de orquídeas: 1; Manter as coordenadas das populações sob sigilo e evitar postagens desnecessárias ou pouco cuidadosas nas redes sociais; 2. Ter permissões de coleta atualizadas; 3. Efetuar um registro fotográfico detalhado dos caracteres vegetativos e florais antes da secagem dos espécimes; 4. Materiais-testemunho de plantas e insetos (polinizadores) devem ser restritos ao estritamente necessário e depositados em Herbários e Coleções Entomológicas acreditadas, respectivamente; 5. Para propagação, frutos de orquídeas devem estar suficientemente maduros, bem desenvolvidos e livres de insetos. Apresentamos recomendações metodológicas para todas estas sugestões. Entendemos ser desnecessário prensar orquídeas inteiras e fornecemos instruções para preparar materiais testemunho de espécies terrestres e epífitas apropriadamente. Se discutem as vantagens de trabalhar em associação com Orquidários de Jardins Botânicos ou instituições de ensino. Apenas insetos que carregam polinários são considerados polinizadores das orquídeas e são amostrados em quantidades moderadas, para a identificação por especialistas. Também se discutem técnicas para documentar a polinização das orquídeas na natureza.

Palavras-chave: conservação, Orchidaceae, polinização, sistemática, taxonomia.

INTRODUCTION

Why "Good practices in Orchid research?

Orchidaceae comprises ca. 30.000 described species (Hassler 2004) and is certainly one of the most popular plant groups, with many species being of economic importance, either as ornamentals or for their use in medicine or as food (Vitt *et al*. 2023). Many species, however, are threatened by illegal collection/poaching and habitat loss as well. A recent meta-analysis of the available information pertinent to their conservation has shown that, globally, at least 278 orchid species require immediate conservation actions (Vitt *et al*. 2023). Nevertheless, about 70% of these priority orchids were neither assessed according to the IUCN guidelines nor cultivated and/or propagated through ex-situ conservation programs (Vitt *et al*. 2023). This cogently

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illustrates the urgent need for conservation efforts. All the authors of this contribution have devoted several years to studying different aspects of orchid biology. Collectively, we believe we can thus contribute advice for those currently researching Orchidaceae, as well as those interested in pursuing research in this taxon, as there is much research still required to be conducted. These suggestions are the results of years of experience and will serve to optimize the gathered information and, simultaneously, minimize the impact of the collection on the orchid populations. The question thus guiding the present contribution is: "How can we improve the research of Orchidaceae with the minimum possible impact on their natural populations?"

Avoid exposing the location of orchid populations

In scientific literature, it has become a widely-used practice to avoid indicating the very coordinates of orchid populations. Many journals currently accept that only the coordinates of the Municipality where studies were conducted be indicated (see Singer *et al*. 2018). Such practice difficulties the action of poachers/illegal collectors. Indeed, the websites of many Herbaria block the exact location (Coordinates) of orchids (and other threatened plants as well). The curators of such Herbaria can unlock such data for *bona fide* researchers on request. However, such practices may not be enough. Users of Social Media (whether researchers or not) should responsibly use their postings (Fig. 1). The finding of orchid populations (especially if these plants are threatened by extinction, rare, or of economic importance) should be communicated through Social Media with extreme care. Indeed, users of Social Media should seriously consider if such posts are indeed safe or necessary (Fig. 1). If this is the case, extra care should be taken to avoid involuntarily promoting the further action of illegal collectors. As suggested by Meijaard & Nijman (2014), secrecy is an important conservation tool, and they clearly illustrate that using Social Media to communicate the existence of threatened species without the support of effective conservation policies safeguarding these organisms is dangerous and may have negative consequences. Meijaard & Nijman (2014) discuss the sad fate of Asian rhinoceroses *Dicerorhinus sumatrensis* (J. Fischer, 1814) found (through trap cameras) in a place where they were supposed to be extinct. This finding was widely divulgated through Social Media and, lacking effective local conservation policies, the recently found rhino population was quickly wiped-out by poachers (Meijaard & Nijman 2014). Years ago, one of us (RBS) knew about a similar case, where an orchid reintroduction effort in a protected area in Rio de Janeiro State was substantially difficulted by the very public visiting the area. The park naively publicized the orchid reintroduction and indicated to the visitors the area of reintroduction. Soon after, several of these orchids were stolen. We thus emphatically suggest that readers avoid posting images of wild Orchidaceae on Social Media (Fig. 1), especially if these orchids are threatened by extinction and/or dwell in non-protected areas. We live in an age where posting images for likes is common. Whilst posting on social media can have conservation benefits (Toivonen *et al*. 2019), including leading researchers to track trade (including orchids, see Hinsley *et. al*. (2016)), discover new populations, range expansions (Prendergast 2020), and plant-insect interactions (ElQadi *et al*. 2017), we request that locations should not be shared. This is an issue, as popular apps like iNaturalist encourage the sharing of this kind of information. We instead recommend that when posting images of orchids on social media, the location information should never be shared, and only be shared on the request of qualified specialists. Notice, however, that the cases mentioned above involve formally protected areas, illustrating that even plants (and animals) occurring in Conservation Units are prone to the action of poachers and/or illegal collectors. Then, if in doubt, do not post. If you need taxonomic identification(s), sending the photos exclusively to specialists is the safer way to achieve it without compromising the plants. What is therefore required is a central webpage or directory with the names and contact information of specialists that the public could contact. Currently, such directories are not present or available.

The Importance of scientific collections and scientific working permits

Like any other plant group, scientific research in Orchidaceae must be sustained by vouchers appropriately deposited into scientific collections (especially Herbaria and Living Collections in Botanical Gardens or similar Institutions). Preparing orchid vouchers is essential for research purposes, and in the next section, we will explain the procedures for preparing those specimens. Here we will address the importance of vouchering the studied plants. Plant vouchers (exsiccates) and their labels are essential in multiple ways: 1) they prove a specific orchid was found at a given location and date, 2) they provide vital information (phenology, an idea of abundance, etc.) of a given taxon, 3) in case of incorrect taxonomic identification, they allow orchid taxonomists to correct the identity of the species and 4) for undescribed species or taxa that require revisions, they allow taxonomic research. These vouchers are normally pressed specimens deposited in at least one accredited Herbarium. As for any other plant groups, the specimens should be accompanied by a label (usually, each Herbarium has a standardized one) bearing the appropriate data (plant identification, place and date of collection, etc.). Normally, each plant voucher receives a registration number in the Herbarium where it has been deposited (a.k.a "repository"). Keep in mind that most Journals are currently asking for these registration numbers (as well as other collection data). Thus, having these issues solved is mandatory before attempting publication. If you work with orchid taxonomy, remember that the current Code of Nomenclature does not accept taxonomic descriptions if

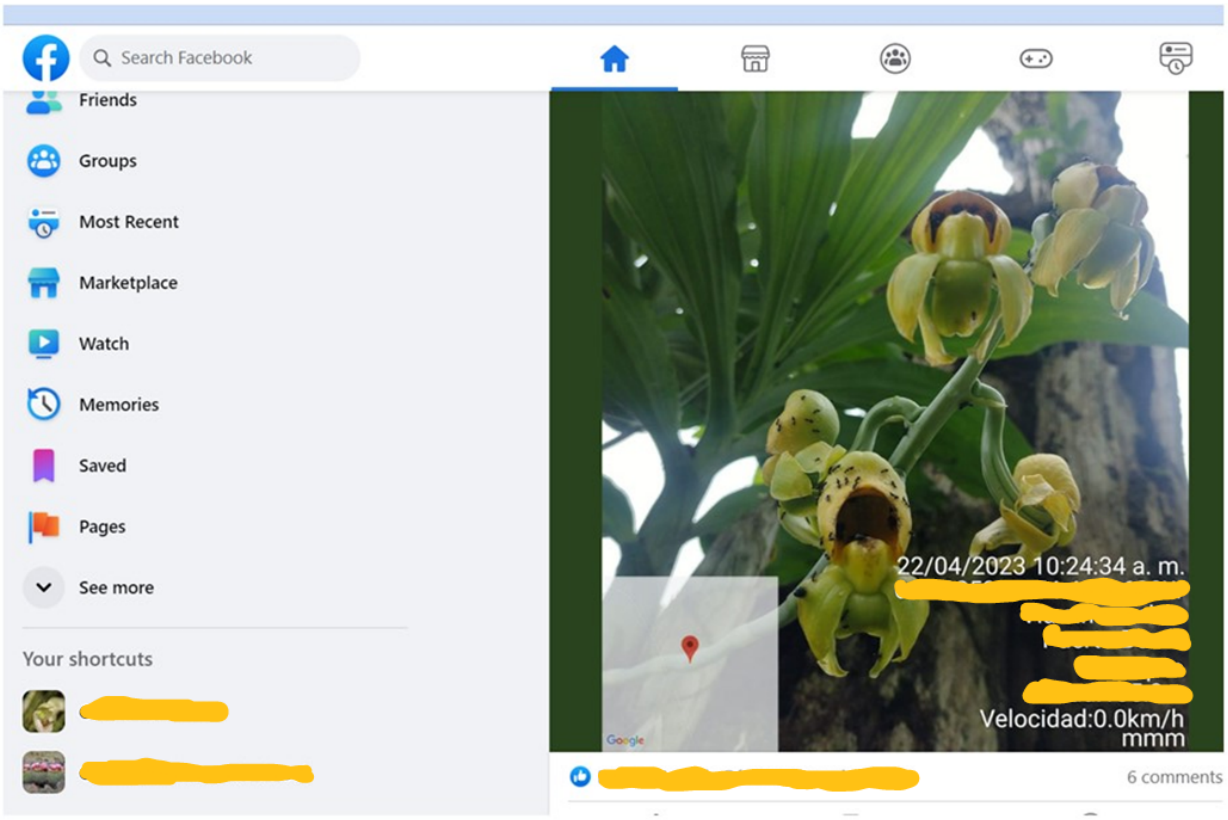

Figure 1. A print screen of a recent post on a Social Web. The original post presented the exact plant coordinates. The post's author asked for the plant's name (in this case, a female plant of Catasetiinae). All names, as well as the plant coordinates, were blurred. To avoid involuntarily promoting poaching, researchers have to avoid doing such posts.

not accompanied by properly prepared voucher specimens functioning as nomenclatural types. In other words, the description of a new taxon is invalid if it doesn't involve a valid typus. Photos alone are not acceptable as types for describing new taxa (see Higgins *et al*. 2007).

Researchers involved with aspects of orchid ecology (conservation, pollination, etc.) should also consider working in association with preexisting Living Orchid Collections (Fig. 2). Such collections are frequent in Botanical Gardens worldwide (sometimes at Universities or similar institutions) and allow several studies that otherwise should be expensive and/or difficult to achieve (see below). It is of great significance that Living Orchid Collections can function as essential germplasm banks for conservation and propagation purposes. Less than 24% of the described orchid species are globally represented in living collections (Vitt *et al*. 2023). Thus, incorporating the still non-represented orchid species is of the utmost importance. Living Orchid Collections normally have a Curator. The researcher(s) and the Curator may establish a covenant between Institutions allowing the research to be done during a given period. Other than for conservation purposes, plants already existing at the collection and/or collected by the researcher can be used in several experiments (ex: the study of breeding systems under controlled conditions, fruit and seed development, fragrance analyses, etc.). Since plants in such collections normally have a good origin record, it can be easily established the number of individuals per species (this is crucial for some experiments, such as breeding systems, since treatments are to be applied to several different individuals) and some other information that may enrich the research. Moreover, Living Orchid Collections normally have the infrastructure and the staff (gardeners to care for the plants) to grant the survival of orchids used in research. Recently, some detailed field studies on the pollination of several Brazilian Orchidaceae were coupled with breeding experiments conducted at the Orchidarium of the Porto Alegre Botanical Garden (Castro *et al*. 2022, Silveira *et al*. 2023) (Fig. 2) and reported in respective papers. In both cases, the studied orchids occur in protected areas. Nevertheless, our field experience has shown that bagged/tagged orchids usually attract the attention of park visitors and native animals, which often destroy the experiments. Using plants cultivated at the Orchidarium of the Porto Alegre Botanical Garden minimized the possibility of such accidents. In sum, researchers interested in orchid biology are encouraged to look for the nearest Living Orchid Collection. If such Institution does not exist in your country or region and funding is available, seriously consider creating one.

Figure 2. Orchid Collections **A.** Living Orchid Collections at the São Paulo Botanical Garden; **B.** Living Orchid Collections at the Porto Alegre Botanical Garden. These extraordinary collections have allowed multiple studies by undergraduate and graduate students.

Countries may be more or less restrictive, but doing research with Orchidaceae normally needs appropriate permits. Journals routinely ask for the number of such permits be published in the articles, either in the Acknowledgements or in an ad-hoc part of the article's structure. So, researchers have to keep their permits up to date, which is particularly important for (but not exclusive to) orchid species threatened by extinction. It is also important to remember that Environmental Agencies responsible for such permits normally limit the number of plants that can be removed and vouchered. Ideally, the number of plants removed for scientific purposes should be kept to the strictly necessary and all possible care to avoid their death should be taken. In some cases, the number of insect pollinators that can be caught can also be limited. All this has to be taken into consideration.

Optimizing orchid sampling for research purposes

First, we would like to make some considerations regarding the need (or not) for sampling Orchidaceae in a given geographic area. If you are working in a place where the existence of the orchid(s) you found is already documented in accredited Herbaria, and you have access to these specimens, consider if vouchering the plants you found is indeed necessary. In such cases, sampling is justified just if the collected material will allow studies (ex: molecular and populational studies, standardized illustrations, etc.) that are not possible with the already available material (Fig. 3). In floristic studies, pressing orchid specimens of species whose presence is already known in that place to increase a particular collector number is nothing but superfluous. Thus, researchers working with Orchidaceae of a given geographic area are suggested to check which orchid species have already been recorded in precedence and the availability of these materials to better plan which plants will be necessary to collect (Fig. 3).

Several (if not all) Botany texts suggest that Herbarium vouchers should include entire plant specimens (the whole plant, including the roots) (see Judd *et al*. 2009, for example), thereby implying that some plant individuals should be killed for scientific purposes. We understand that this situation may be unavoidable in some plant groups (Cyperaceae, for example). However, there is no real need to kill orchid specimens for vouchering. As a rule, an orchid species cannot be identified solely based on vegetative features (Dressler 1993).Those readers familiar with orchid taxonomy are aware that root characters aren't useful or necessary for determination at the species level. On the other hand, floral characters are indispensable for diagnosing orchid species apart (Dressler 1993). Only in rare cases can root features be diagnostic at the generic level. For example, the roots of *Bipinnula* Comm ex Juss. (Chloraeinae) and *Christensonella* Szlach *et al*. (Maxillariinae) species, for example, consistently present annular constrictions which are absent in the remaining Chloraeinae (Buzatto *et al*. 2014, Sanguinetti *et al*. 2015) and Maxillariinae (Koehler *et al*. 2012), respectively. However, pressing entire specimens of these orchids is unnecessary since the species of both genera can only be identified through the use of floral characters (Buzatto *et al*. 2014, Koehler *et al*. 2012, Sanguinetti *et al*. 2015). In theory, the identification

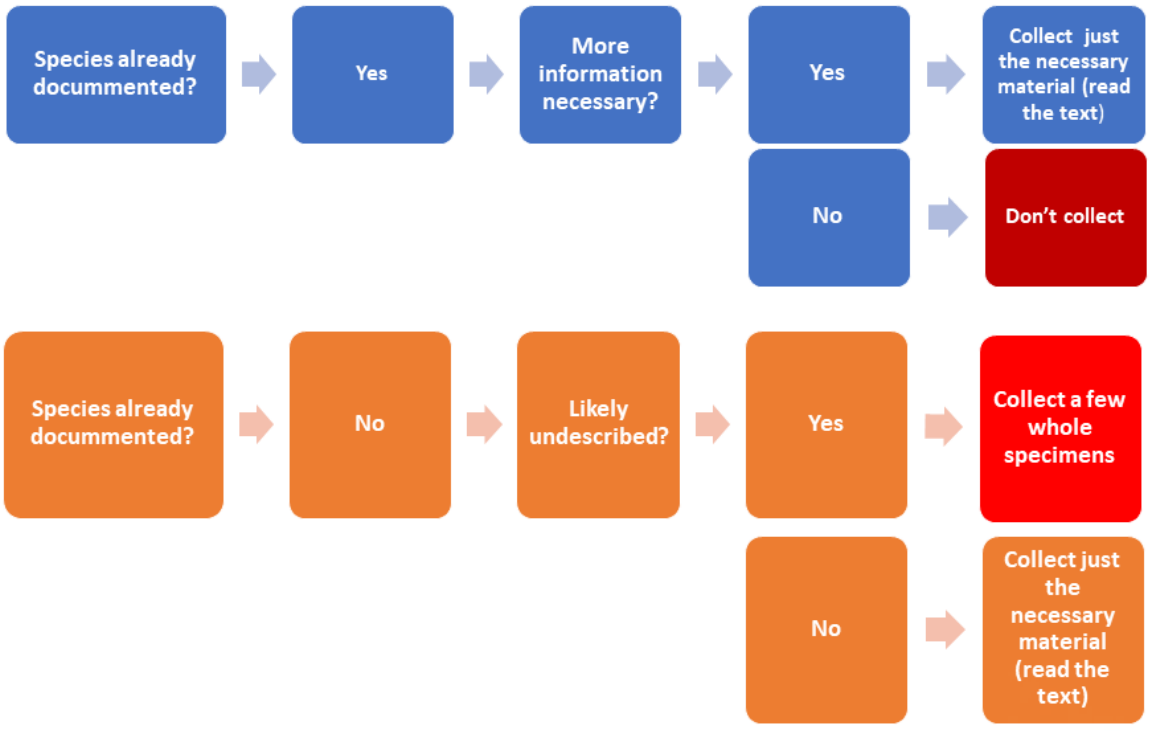

Figure 3. A suggested flowchart for researchers studying Orchidaceae. Notice that the collection of a few whole specimens is only suggested in cases where there are reasons to think that the plants belong to as yet undescribed species.

of sterile (devoid of flowers) orchid specimens could be approached by DNA barcoding (Ghorbani *et al*. 2017) or through detailed anatomic studies but, at the present, such techniques remain expensive and/or impractical for most orchid researchers. Consequently, if roots are poorly or non-informative and flowers are compulsory, there is no need to press the whole orchid specimen. Pressing whole specimens seems only justifiable when there are reasons to believe that the orchid(s) under study may be an undescribed taxon (a). In this case, describing the roots is necessary, and vouchering a few whole specimens is justified (Fig. 3). Otherwise, there is no reason to press whole plants, especially if they belong or likely belong to already known taxa or to threatened species. Thus, we herein propose a simple protocol (Fig. 4) for optimizing the information on collected orchids while minimizing the impact of vouchering on orchid populations.

As a first step (Fig. 4), orchid details should be recorded in the field through photos, with the maximum possible detail. If, for whatever reasons (ex: weather, time schedule, etc.) it is not possible to photograph the plants in the field, you can collect some inflorescences and any other relevant material (see below) and keep it temporarily fresh until you can do the record (preferably digital images, see below). In our experience, inflorescences and vegetative parts can be kept fresh for up to 48 hs if put in a vial with tap water. If necessary, a slice of the basal part of the inflorescence can be cut daily, to grant hydration. However, the sooner you photograph the material, the higher the chances of obtaining better results. If several specimens are available, choose the healthiest (these with the best-preserved vegetative and floral parts) for the photographic record. A scale is to be used in these recordings, and a ruler or pieces of graph paper can be used for this purpose (Fig. 5). In addition to the photos (Fig. 4) and -if possible or convenient – do annotations with the information that you judge relevant. All pertinent information that will be lost during pressing (aroma, ornamentation, etc) could eventually be mentioned on the label of the specimens (Fig. 4). Overall plant shape and size, stem, leaves, pseudobulb (if present), inflorescence, and flower details are to be recorded. Flowers may be photographed in lateral and frontal views (Fig. 6). The perianth should be carefully dissected and photographed. Column and pollinarium details will be photographed as detailed as possible (Fig. 6). If appropriately taken, these photos could be used to assemble illustrations (Figs. 6 and 7) that can be later used in reports and/or publications, hence the importance of using scales during the photographic record.

In terrestrial orchids, a few leaves are cut from their very base and photographed on both (adaxial and abaxial) sides (Figs. 4 and 5). The vouchers are collected in the second step (Fig. 4). It would be enough for terrestrial orchids to cut the inflorescence and a few leaves (ideally, the same used in step 1 to take the photographs) (Fig. 4), allowing the plant to flower during the next seasons. It would be enough for Epidendroideae orchids with pseudobulbs to cut a sample consisting of a few inflorescence-bearing pseudobulbs (Figs. 4 and 5). It may be enough for Epidendroideae orchids devoid of pseudobulbs to press some inflorescence/

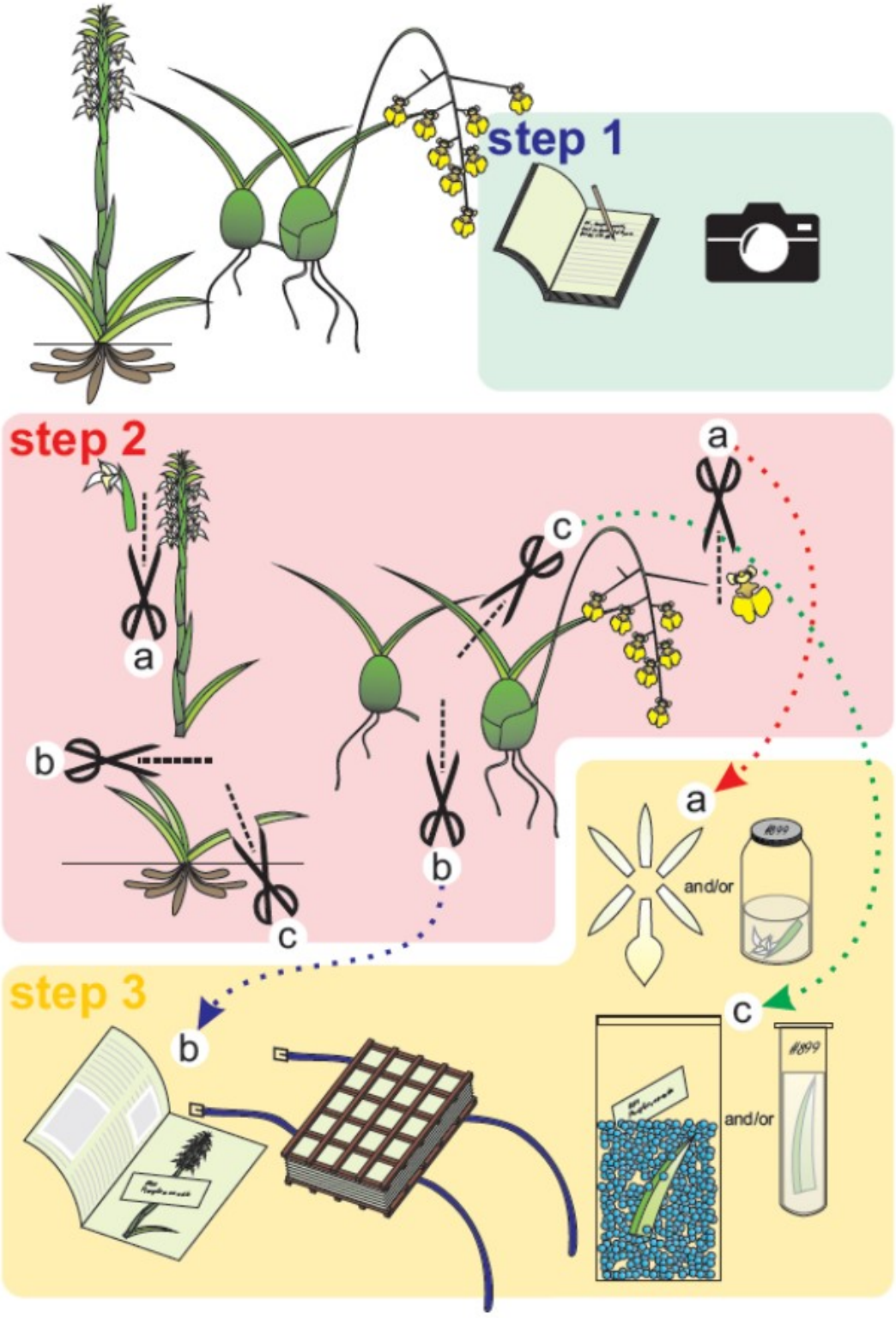

Figure 4. A simple protocol for optimizing orchid collection for research purposes without compromising the survival of the plant individuals or populations. **Step 1:** a complete photographic record in the field, complemented with notes; **Step 2:** take just the necessary samples for vouchering. The inflorescence and a few leaves may be enough in terrestrial orchids. In Epidendroideae orchids, pressing a few inflorescence-bearing pseudobulbs or stems may be enough; **Step 3:** press the vouchers and preserve any additional or necessary samples for further studies (ex: samples for molecular studies, flowers fixed in preservatives for morphological or anatomical studies, etc.).

Figure 5. Applying in the field the protocol explained in Figure 3. **A.** Flowers and a few leaves of *Bipinnula montana* Arechav. were collected for vouchering purposes; **B.** A sample of *Gomesa gomezoides* (Barb. Rodr.) Pabst. consisting of the inflorescence and the two closer pseudobulbs, left the rest of the tussock in the field. In both cases, this collecting methodology avoided the unnecessary death of the plants. The inset in both photos depicts the dissected perianth with a scale.

flower-bearing stems or portions of stems. Finally, in the third step (Fig. 4), vegetative and floral parts are pressed, and tissue samples for molecular analyses (if applicable) are preserved in silica gel or through other methods, allowing further study. In the worst of cases, the whole collection and documenting process would take a few hours. A trained team in a hurry would perform all these steps in an hour or less. Notice that botanists working with trees and/or arborescent cacti (ex: De Groot 2011), succulents, or lianas already use a similar collecting technique since it isn't possible to press whole specimens of these plants. This protocol minimizes the impact of the collection since no plants are killed for vouchering purposes. These vouchered plants have lost -of course- the chance of forming some fruits. However, surviving the collecting process will allow them to reproduce freely in the next years while keeping the genetic diversity of the population. In addition, this collecting protocol allows the production of 'safe" duplicates. Several inflorescences can be picked up, processed, and distributed to several Herbaria (Fig. 5). This approach is particularly interesting for plants that are either threatened by extinction or that are seldom collected due to biological peculiarities (ex: several terrestrial orchids flower and fruit over periods of a few weeks).

Illustrating orchids: drawings, paintings, or photos?

There is a long-lived tradition in Botany of using detailed drawings and/or paintings for illustration purposes. Great orchid taxonomists, such as João Barbosa Rodrigues (1842 – 1909), often illustrated their taxonomic descriptions in great detail. However, researchers working in Botanical Gardens or Universities normally rely on professional illustrators for this task. In the case of Rodrigues, he was a gifted professional illustrator whose orchid descriptions were sided by his detailed pencil drawings or (more frequently) stupendous watercolors. Since Rodrigues's type specimens were likely lost during a deluge in Rio de Janeiro (Buzatto *et al*. 2020), his detailed illustrations are extremely useful as lectotypes (chosen types from the remains of the original descriptions). In such cases, illustrations are extremely important for correctly interpreting these species. However, many (possibly the most) orchid researchers aren't illustrators, and using illustrations in their works normally requires -as commented above- the services of

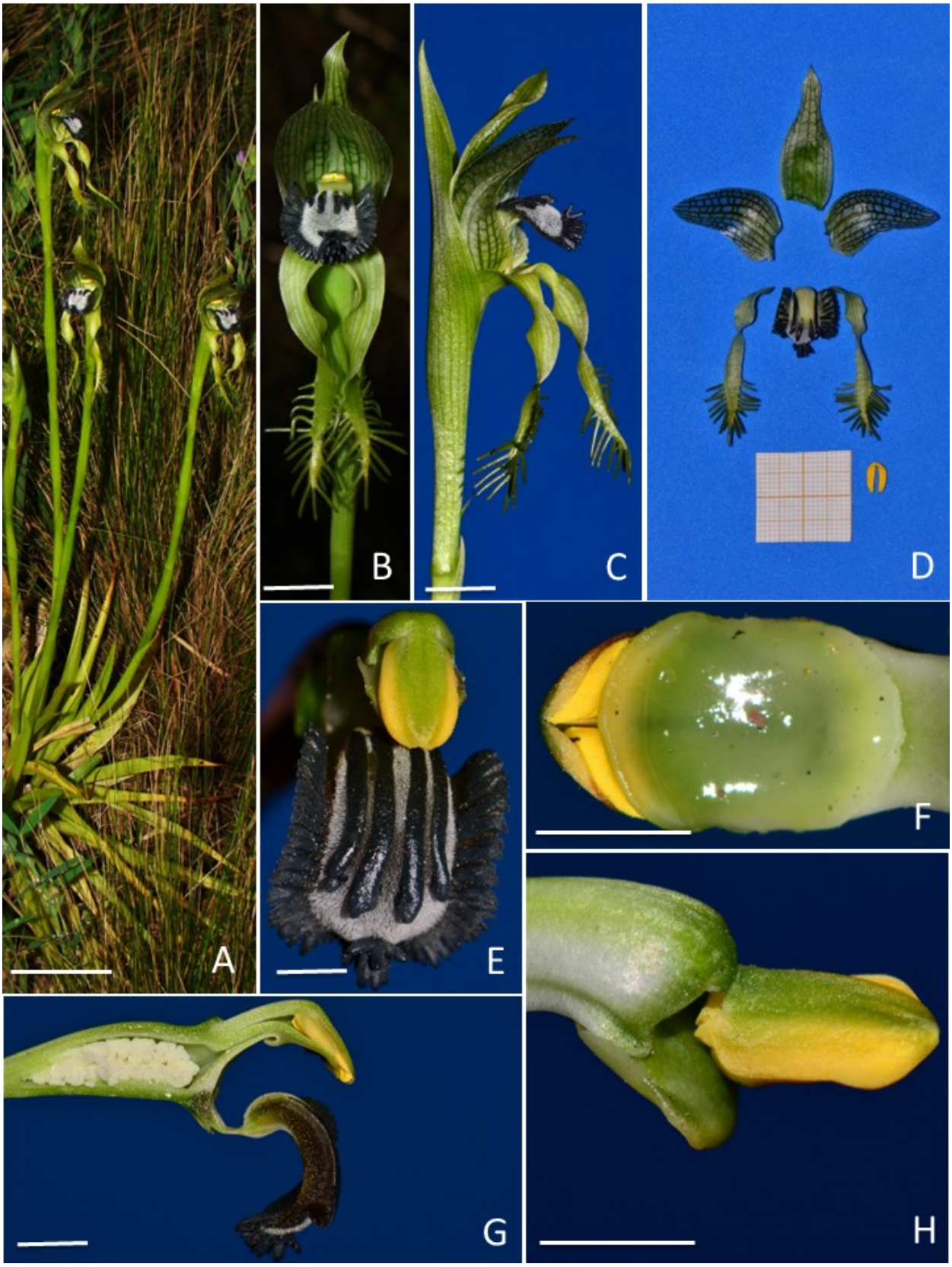

Figure 6. A complete photographic record of *Bipinnula montana* performed in the field. Several floral features will be seriously distorted after the pressing of the voucher. Thus, recording all possible plant features upon living specimens is highly recommended. **A.** Habit; **B-C.** Flower in frontal and lateral view, respectively; **D.** Dissected perianth; **E.** Column and labellum in dorsal view; **F.** Column in ventral view; **G.** Column with labellum articulated at its base and dissected ovarium; **H.** Column in dorsolateral view. Scales A = 50 mm, B-C =10 mm, D = 2 cm, E-H = 5 mm.

other professionals and a considerable amount of time and money. For the standard undergraduate and graduate student, paying for the services of a skilled illustrator is a luxury that simply cannot be afforded.

Digital photographs (Figs. 6 and 7) have democratized the possibilities of illustration. Good-quality photos can be obtained from semi-professional, moderately-expensive digital cameras. Using digital pictures has important advantages: 1) the photographing process is quick, several orchids species can be photographed in detail during a single day; 2) good quality photos can be quickly assembled into fine illustrations (Figs. 5 and 6); and 3) the photos show the natural appearance of orchid plants and their vegetative and floral details. Artistic illustrations can be subject to errors due to biased artists' interpretations. The use of digital photos minimizes the latter possibility. Finally, the current and widespread use of digital pictures allows us to represent orchids (and their features) in all their complexity and beauty. Important features (especially in flowers) are lost or seriously mitigated, or deteriorated in pressed specimens. Indeed, digital illustration can be deposited together with the exsiccates they illustrate, as a way to help future researchers. All in all, we emphatically recommend illustrating orchids from standardized photos. Researchers at the Lankester Botanical Garden, Costa Rica (Pupulin & Bogarín 2004), created a technique for assembling digital photos into a single plate with a black background, hence named LCDP (Acronym for Lankester Composite Digital Plate) (Fig. 7) that is becoming increasingly popular. Such illustrations are extremely useful and feasible, and we strongly recommend this technique to illustrate Orchidaceae.

Studying orchid pollination

Most orchids present their pollen agglutinated into well-developed pollinia. The collective name for the pollinia and any secretion or additional floral part aiding in pollen removal is pollinarium (plural: pollinaria). Thus, orchids are especially interesting for pollination studies since they are one of the rare plant groups where pollination can be most easily tracked and quantified, meaning that the whole pollen packet is withdrawn from the flowers as discrete macroscopic structures that can be visualized onto the body surface of their pollinators. Thus, identifying animals as orchid "pollinators" requires convincing evidence supporting that these animals dislodge and deposit the pollinaria (Adams & Lawson 1992). In other words, only animal pollen vectors for whom there is conclusive evidence of the above should be considered pollinators (Fig. 8). The presence of pollinaria on these animals is then a *sine qua non* precondition, and the use of observation techniques that do not grant that the observed animals do transfer pollinia should be avoided (see Cabrera-Reyes *et al*. 2021). Researchers working with orchid pollination should be prepared to spare considerable time to record orchid pollinators through photographs and -whenever possible- by film. The use of film isn't mandatory but is especially useful to illustrate and quantify (number of flower visits, time of permanency per flower, etc.) pollinator behavior. In terms of didacticism, the film is particularly useful in illustrating and teaching uncommon pollination strategies such as sexual mimicry or others.

It is important to highlight that a few insect pollinators vouchers are necessary and should be deposited at a widely-known Institution (Museum, Collection, etc.) (Prendergast & Hogendoorn 2021). For vertebrate pollinators, there is no need to capture such vouchers, and most journals accept a good photographic and/or film record. Some institutions prefer that you deposit the insects already prepared (ex: appropriately mounted on entomological pins and labeled or preserved in liquid). Others have a very rigorous preparation protocol and prefer that the vouchers be prepared by their staff; this will depend on their taxonomic group and on the rules of the Institution that will serve as a repository. Consult the collection Curator in this regard before sending the insect vouchers. As for the number of insect pollinators to be collected, we strongly suggest keeping it to a minimum. In our experience only a few specimens are enough for a proper identification. Unless there are reasons to believe that more insects are necessary, 1-2 specimens per pollinator species would be enough. Researchers should keep in mind that: 1) some insects are rare (either intrinsically rare or threatened by extinction, like some bumblebee species; for example), 2) collections may eventually impact insect pollinator populations if they aren't abundant (Prendergast *et al*. 2020). The latter situation is particularly peremptory when pollinators are females. So, a common sense approach is desirable: avoid over-collecting. Pollinarium-bearing insects are to be euthanized for further preparation. Bee collectors often use ethyl acetate for this purpose, but in our experience, the pollinaria may easily take off using this reagent. So, we do suggest putting the insects into a killing jar with cyanide or putting the vial with the insect(s) into a freezer overnight. Remember that small orchid pollinaria, even with the utmost care, should unglue or fall during the preparation process, hence the importance of a good photographic record, preferably, before the capture of the insects (Figure 8).

Some techniques to capture insects, such as pan-traps and some traps used to census Euglossine bees, successfully capture large quantities of insects (Prendergast *et al*. 2020). However, not only do passive methods have issues when looking at pollinator associations (Prendergast & Hogendoorn 2021), such techniques are completely inadvisable for studying orchid pollination for two main reasons: 1) these methods capture large quantities of insects, only a fraction of which may be pollinators (pollinarium-bearing specimens), and/or 2) these capture techniques use liquids (ex: detergents, in pan-traps) that may unglue the pollinaria from the pollinators, making uncertain which insects carried them. Techniques such as the above are

Figure 7. A plate of *Bipinnula montana* (Chloraeinae) in the LCDP style, assembled with photos made in Figure 4. **A.** Habit (in the background); **B-C.** Flower in frontal and lateral view, respectively; **D.** Dissected perianth; **E.** Column with labellum articulated at its base and dissected ovarium; **F.** Column and labellum in dorsal view; **G.** Column in dorsolateral view; **H.** Column in ventral view; **I.** Anther elevated to expose the pollinia. Scales A = 50 mm, B-C = 10 mm, E-I = 5 mm.

Figure 8. Orchid pollinators. Only animals able to dislodge and deposit pollinia are to be considered pollinators. Insect pollinators are to be collected (vouchered) in moderate numbers. **A.** Syrphid fly with pollinaria of *Prescottia densiflora* (Brongn.) Lindl. onto its proboscis; **B-C.** Augochlorini Halictidae bees with pollinaria of *Cyclopogon elegans* Hoehne glued below their labri; **D.** Female of *Xylocopa augustii* (Lepeletier, 1841) leaving a *Cattleya tigrina* A. rich. flower with a pollinarium on its dorsum; **E.** Female of *Tetrapedia* sp. on flowers of *Trichocentrum pumilum* (Lindl.) M.W.Chase & N.H.Williams. The bee presents a pollinarium glued on its labrum.

useful for tracking insect populations but are completely unsuitable for pollination studies, whether of orchids or not.

Camera traps have become widely used to track animal presence and behavior. Such devices have important advantages: 1) minimal interference in animal behavior, 2) endurance, and 3) stability. Whereas camera traps could be used to monitor pollinator behavior in Orchidaceae, there are a few shortcomings in insect-pollinated taxa: 1) some camera models have a Macro function, but they are comparatively expensive, and 2) the identification of the pollinator species may be difficult without a good image resolution, and, depending on the insect group, impossible without a few insect vouchers (for example, species identification in some cases requires the examination of the genitalia in some insect groups, as in some groups of Diptera). Thus, camera traps alone don't seem advisable for orchids pollinated by insects, unless pollinators have already been identified and cameras are used complementarily (ex: to film and quantify orchid visits, behavior, permanency at flowers, etc.) (Houlihan *et al*. 2019). On the other hand, camera traps may be extremely useful for studying Orchidaceae pollinated by birds (Johnson & Van Der Niet 2019). The image resolution of standard camera traps is normally good enough to identify birds at the species level. At the moment of writing these lines, we are obtaining promising results in Hummingbird-pollinated Orchidaceae through camera traps, and we believe this tool may prove useful in bird-pollinated orchids outside the Americas as well. Yet, remember that even in the case of vertebrates is necessary to obtain clear evidence that these animals transfer pollinaria. We strongly recommend programming the cameras to film (instead of photographing) with the highest possible resolution to maximize the chances of a good record. Programming the camera to photograph alone could lead to disappointing results. By programming to film, you grant that orchid pollinaria can be noticed on the birds for at least some moments, thus obtaining the necessary evidence.

Collecting orchid fruits, seeds, and pollinaria for propagation and conservation purposes

Collecting fruits, seeds, and pollinaria has become an extremely useful tool for conserving threatened orchid species. Orchid seeds may be propagated in appropriate media and used for ex-situ conservation programs or reintroduced when appropriated. Orchid fruits should be clean, contain no signs of illness, and be mature enough for propagation. If these fruits are being tracked or followed, an appropriate moment for collection can be chosen. At maturity, fruits of terrestrial orchids tend to present a cardboard-like texture and color, often showing longitudinal slits (Fig. 9). In large fruits of Epidendroid orchids, yellowing of the apical part of the fruit may be a sign of ripening (Fig. 9). However, it is important to note that fruit maturation time is quite variable: in terrestrial Orchidoideae, maturing in a month or less, whereas maturation can take several months in Epidendroideae.

Figure 9. Examples of orchid fruits. **A-B.** Terrestrial orchids (Orchidoideae). **A.** *Gavilea odoratissima* Poepp. (Chloraeinae); **B.** *Bipinnula montana* (Chloraeinae); **C-D.** Epidendroideae orchids; **C.** *Cattleya intermedia* Graham. (Laeliinae) fruit in early development, notice the swollen column; **D.** Dehiscent fruit of *Gomesa flexuosa* (Lodd.) M. W. Chase & N. H. Williams (Oncidiinae), notice the apical yellowing that normally precedes dehiscence in fruits of Epidendroideae; **E-F.** *Cattleya tigrina*; **D.** fruit with petals; **E.** fruit without petals, sepals and peduncle.

Fruits too immature or, on the contrary, too old may lead to unsuccessful germination or storage attempts. As for the number of fruits to be collected, it will depend on some factors: 1) how many fruits are indeed available and how many of these fruits are in an appropriate state of maturation; 2) the orchid taxonomic group involved (see below) and; 3) if the fruits to be collected belong to a threatened species. In accordance, try to collect fruits exclusively in an appropriate state of maturity. Otherwise, the results may be uncertain. Globally, orchid fruiting success in natural conditions rarely exceeds a mean of 17% (Tremblay *et al*. 2005). As a rule, orchids that reward their pollinators with nectar or other resources yield more fruit than their rewardless, deceptive species (Tremblay *et al*. 2005). Nectariferous Orchidoideae orchids produce more fruits than their Epidendroideae counterparts (Tremblay *et al*. 2005). Indeed, we have recently documented (Buzatto *et al*. 2022, Pedron *et al*. 2012) excellent fruiting successes (≥ 65%) in several Neotropical terrestrial orchids. However, fruits of Epidendroid orchids tend to be bigger (Fig. 8), holding a considerably higher number of seeds (Dressler, 1993). In other words, terrestrial orchids can produce more fruits but normally hold fewer seeds than these of Epidendroideae. Thus, collecting more fruits of Orchidoideae orchids could be necessary to obtain as many seeds as in Epidendroideae. It is important, in addition, to consider if the fruits to be collected belong to a threatened species. If so, a common sense approach is again invoked: collect the necessary number of fruits, avoid overcollection, and spare some fruits for natural dispersal. If you are working for a Conservation Program, remember to collect fruits from different individuals and, if possible, from different populations, as a way to grant as much genetic diversity as possible. In plants with large vegetative propagation the extent of a single individual may be elusive. In cases like this, it could be useful to establish a minimum distance in meters (5-10 m) between non-connected collecting points. In the field, fruits can be harvested into paper bags. The name of the species and other collected data is to be written with a pen on the outside of the bags. Collected fruits can be kept in this way for a brief time. Ideally, the seeds should be cleaned and stored as soon as possible.

Orchid pollinaria collection and storage

Orchid pollinaria can be collected and stored for future use in pollination and propagation programs (Custodio *et al*. 2020). However, it is important to remember that the pollen's viability in these pollinaria will be affected by factors such as moisture content, temperature of storage, and lipid content (Custodio *et al*. 2020). Ideally, the pollinaria should belong to really fresh flowers, and it has been suggested that pollen must be collected from almost-opening buds (Custodio *et al*. 2020). Fruits, seeds, and orchid pollinaria can be collected and temporarily stored in paper bags (Fig. 10). Each bag has to be labeled with

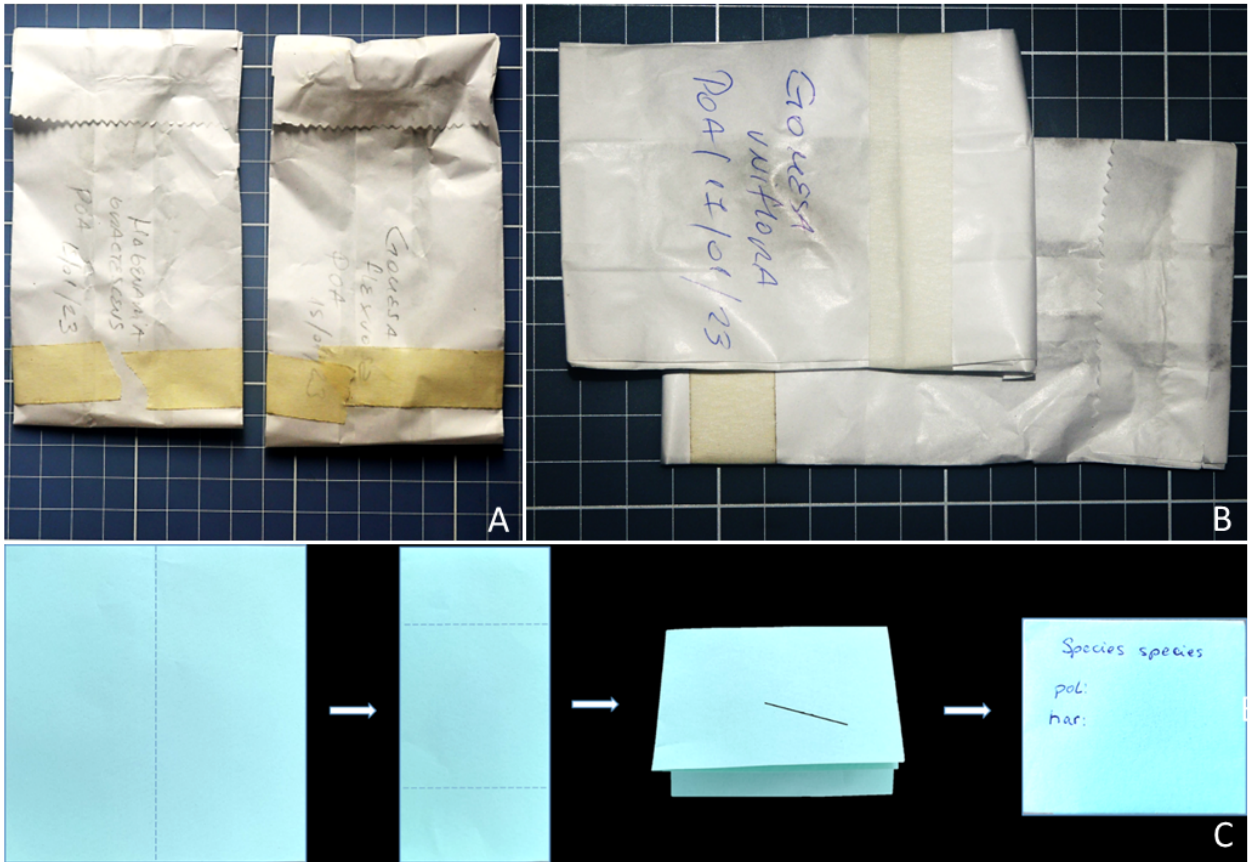

Figure 10. Sampling orchid fruits and seeds. **A-B.** Examples of a simple methodology for sampling small fruits and seeds of Orchidaceae. Ripe fruits and seeds were collected in small paper bags and the pertinent data (Species, place of collection and date) are written onto the bag. These bags can be stored for further use in propagation; **C.** Making a thin paper envelope to store fruit or seeds for equilibrating them for storage. The final envelope may contain the species identification, pollination date if possible and harvesting date. This kind of Envelope avoid the loss of seeds in the foldings.

the name of the orchid species and the collecting date and location (Fig. 10). These bags can be temporarily stored in a fridge until their further use.

Seed Collection and Storage

Proper seed collection must observe the age of the fruit. In field collections, if there will be a capsule to be harvested and this is not exhibiting any signs of maturation, we have two choices: 1) to collect the plant with the capsule and allow the maturation to happen in the plant, and then relocate the plant in a living collection or 2) leave the capsule in the plant and return later to collect it, in a period closer to the final maturation, this second option is valid for plants that remain with capsules for several months. When the plants are in a living collection, using two different individuals of the same species is recommended to make a cross, guaranteeing a wider genetic diversity. Avoid making so many capsules for good seed filling and quality, even in plants with multiple flowers. The inflorescences must be marked with a tag containing the species and date of pollination.

In the case of mature or near-mature capsules, they must be trimmed of any perianth (Fig. 9 D and E) and put in a paper envelope (Fig. 10) containing the identification and collection date. Ensure the paper involving the fruit is thin enough to allow moisture exchange between the fruit and the environment. Plastic or impermeable paper must be avoided. Annotations must be done before enclosing the fruit. Leave the envelope containing the fruit in a desiccator containing silica gel or even oven-dried rice (Seaton *et al*. 2018) and, if possible, with orange silica gel to monitor the amount of moisture in the flask.

After ripening, the seeds must be dehydrated properly. They must not have more than 6% moisture content (Hengling *et al*., 2020, Fileti *et al*. 2021). Otherwise, the deterioration events will occur faster in the seeds during storage, and they did not store properly. Seed might be conditioned in polypropylene or glass vials (Fig. 11) since they have a rubber or silicon stopper and then equilibrated over freshly regenerated silica gel or saturated lithium chloride solutions (Hay *et al*. 2008) for at least three days, then the vials must be tightly closed and kept in larger flasks containing freshly regenerated silica gel and stored. Our experience showed that seeds, conditioned in this way, may conserve for long periods at -18 ºC. Alternatively, they may be stored at lower temperatures at -80 ºC. Orchid seeds are rich in fatty acids, mainly linoleic acid (Hengling *et al*. 2020; Fileti *et al*., 2021; Diantina *et al*. 2022), but

Figure 11. Examples of flasks for storing seeds. **A.** Flasks with silicon seals in the caps for seeds; **B.** Flasks for store few containing seeds, when possible a sachet of silica must be included in this larger flask; **C.** Large flask to storage of large amount of flasks, they must contain 2 cm of silica gel to maintain low relative humidity; **D.** Detail of the flask cover with the silicon seal.

seeds rich in other acids as oleic or even saturated acids, palmitic or stearic acids, may occur. The way they will be taken out of the storage may have this into account, and at least 24h at room temperature may help to recover the seed viability/germinability (Hengling *et al*. 2020; Fileti *et al.*, 2021; Diantina *et al.* 2022).

CONCLUSIONS

Studying orchids in the twenty-first century

Like all other scientific disciplines, Botany has evolved following significant technological changes and the way Science is thought and performed. Drawings or paintings were the only way to illustrate Orchidaceae until the beginning of the twentieth century, with the advent of photography. However, the real change came with digital photography in the early 2000s, which allowed a more accessible use of illustrations in ways that were thoughtless decades earlier. Nonetheless, the world has also changed: natural areas are shrinking, and many species are threatened, including many Orchidaceae. The time comes to reconsider the methodologies we use: how many specimens do I really need? Am I using the best possible approach for sampling? How can I minimize the impact of my studies on the orchid populations I study? Orchid researchers should ask themselves questions like these. Orchids are a diverse, ecologically-fascinating group. Yet they are also threatened. To conduct the necessary research to advance our understanding, we need to adopt best practices so that the research is of high-quality, and doesn't inadvertently harm the populations we aim to conserve. Until the end of the twentieth century, orchid research was primarily performed by researchers of the Northern Hemisphere, especially from Europe and the United States. This situation has changed. Now we have highly skilled and qualified orchid researchers worldwide. Promoting the development of local science and scientists is a fundamental goal for all countries. Ideally, researchers should focus on the orchid floras of their native regions. On the other hand, research should no longer be performed like it was until the end of the nineteenth century. Anybody interested in orchid biology should know the plants in their natural habitat and in all their diversity. Today we can study Orchidaceae in all their complexity, as living beings. In the twentieth-first century, studying orchids from Herbarium vouchers alone is anachronic and well below our current possibilities.

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