Systematic, phylogenetic and pollination studies of Specklinia (Orchidaceae)

Karremans, Adam Philip

General Introduction

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The rich biodiversity of Costa Rica

Uniting the large territories of North and South America there is a thin strip of land known as Central America. Central America serves both as a bridge for the northern and southern flora and fauna as well as a barrier for marine life trying to cross from east to west, and vice-versa (Fig. 1). However this bridge and barrier has not always been there. In the Miocene, Central America was an archipelago without continental connection, and it is estimated that the land finally closed between 3 and 15 million years ago (Montes *et al.* 2015).

The Costa Rican landscape (Fig. 2) is quite mountainous, with elevations that go from sea-level to above 3800 m on Cerro Chirripó, and with several peaks over 3000 m. The climate in the area is quite variable, but there are two basic water regimes in Central America, the Atlantic (Caribbean) is more rainy in November to January, and the Pacific one in which rain is almost absent from December to April. In between, a mix of both regimes is found, with the highest rainfall found at mid-elevations. Rainfall increases with elevation until a certain point and then it decreases again. In Costa Rica this turning point was calculated at about 1000 m. The large valleys have the lowest rainfall, while the highest rainfall can be found at some mountain bases. The areas around Tapantí National Park can have more than 315 days of rain. Costa Rica, being close to the Equator, has a very stable temperature. The average temperature of the warmest month does not exceed the average temperature of the coldest month by 5 degrees. With higher elevations the temperatures become lower, and the difference of day vs. night temperature also decreases. Northern winds are frequent from January to March, and can be up to 90 km/hr in some areas. On the Caribbean coast, winds are more constant and have an average speed of 7 km/hr (Janzen 1991).

The effects of climate on the biology of plants and animals in Costa Rica is poorly known, Janzen (1976) suggests that in tropical conditions, where the climate fluctuates very little, the high mountain peaks are probably a much greater barrier than in temperate regions, where the organisms are more used to seasonal changes. There are about 20 vegetation types that can be recognized in Costa Rica using the Life Zone system of Holdridge (1987), ranging from tropical dry forest, to sub-alpine rain paramo, with many transitional zones (Dressler 1993a). This complex landscape combined with different environmental conditions allow for a plethora of micro-climates to which epiphytic orchids are especially sensitive, and explain the high variety of species found in such a small country.

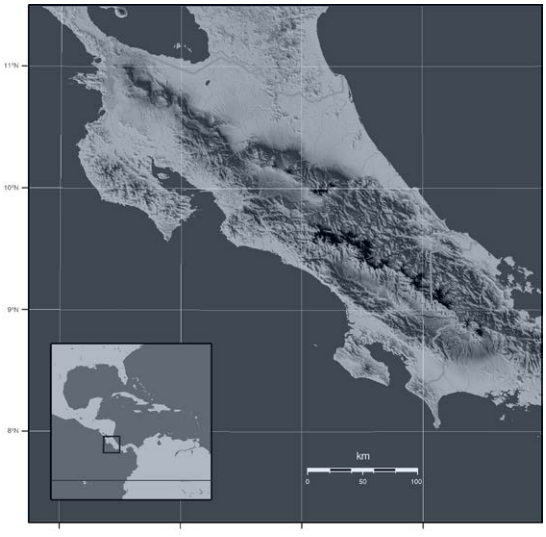

Figure 1. Map of Costa Rica and its position in Central America. Courtesy of Franco Pupulin.

Figure 2. View of the Talamanca mountain range in Costa Rica. Photograph by A.P. Karremans.

Orchidology in Costa Rica

With more than 1,600 reported species, Costa Rica has one of the richest orchid floras in tropical America, currently surpassed in total species number only by Brazil, Colombia, Ecuador and Peru. However, with an area of only 51,000 km^2, Costa Rica is five to 150 times smaller than each of those countries. As such, with 1 species per each 32 km^2, Costa Rica has the highest orchid species/area ratio in the world. The large number of reported orchid species is clearly a direct result of the combination of high diversity, intense botanical exploration and the presence of active research groups and institutions (Karremans & Bogarín 2013).

During the colonial period botanical exploration in Central America was almost non-existent, and it is not until their independence that interest in the local flora begins. The collections of George W. **Barclay** are likely to be the first documented orchid specimens from Costa Rica. He travelled under the command of the British Royal Navy admiral Sir Edward Belcher (Fig. 3) in the voyage of the HMS Sulphur off the Pacific Coast of Central America. *Rossioglossum ampliatum* (Lindl.) M.W.Chase & N.H.Williams, collected in 1839, was among the first species recorded from the country. The visit of Anders Sandoe **Oersted** to Costa Rica in 1846 is the first of a long list of naturalists who would visit the country in the following decades. He was followed by Józef **Warszewicz** Ritter von Rawicz and Hermann A. **Wendland**, among others. Their orchid collections in Costa Rica were studied and described by Heinrich Gustav **Reichenbach**, the most prominent authority on orchidology in the nineteenth century after the death of John **Lindley** (Ossenbach 2002; Bogarín *et al.* 2013a). Reichenbach *filius* also worked extensively with the collections of Auguste (Augustus) **Endrés**. Endrés' specimens, plants descriptions and drawings were extremely carefully and accurately prepared, very unlike his time. Endrés, an Alsace native of German origin, would initially start collecting orchids in Costa Rica employed by George Ure **Skinner** and James **Bateman** in 1866. Unfortunately his very early and impressive orchid flora of Costa Rica remained unpublished after his untimely death in Colombia, and it would be kept mostly "hidden" in Reichenbach's herbarium at the Natural History Museum in Vienna (Pupulin *et al.* 2014). On his drawing of «Pleurothallis mellifera» (which was later named *Specklinia endotrachys*) Endrés wrote "inner surface of sepals slightly viscous? / much visited by a small fly" and in his description again "sepals scabrous in inner surface (exuding honey)" in what is likely to be the first ever published observation on pollination in Pleurothallidinae [cited by Pupulin *et al.* 2012 (Chapter 1)].

Anastasio **Alfaro**, Paul **Biolley**, Alexander Curt and Alfred **Brade**, Alberto Manuel **Brenes**, Charles Herbert **Lankester**, Richard **Pfau**, Henry François **Pittier**, Paul C. **Standley**, Jean François Adolphe **Tonduz** and Karl **Wercklé** contributed extensively to collecting Costa Rican orchids by the end of the nineteenth and beginning of the twentieth centuries. Their collections were the basis for the creation of the Herbario Nacional, and allowed for the study of Costa Rican orchids by some of the worlds most renowned scientists of the time. Oakes **Ames**, Robert Allen **Rolfe**, Rudolf **Schlechter** and Charles **Schwienfurth** described hundreds of new orchid species on the basis of Costa Rican material collected by the first, and contributed significantly to our knowledge of the country's flora (Ossenbach 2002; Bogarín *et al.* 2013a).

In the second half of the twentieth century, a new cohort of students of Costa Rican orchids would appear. In contrast with their predecessors these would do both the "field" and "desk" work of more specific groups of orchids, and therefore contribute immensely to our complete understanding of the whole orchid flora. Including, Paul H. **Allen**, John **Atwood**, Calaway H. **Dodson**, Robert L. **Dressler**, James A. **Fowlie**, Leslie A. **Garay**, Eric **Hágsater**, Clarence K. **Horich**, Alex D. **Hawkes**, Carlyle **Luer**, Dora Emilia **Mora**, Rafael Lucas **Rodríguez Caballero** and Carl **Withner** (Ossenbach 2002; Bogarín *et al.* 2013a).

Figure 3. Portrait of British Royal Navy admiral Sir Edward Belcher by Stephen Pearce.

The turn of the century was marked by the appearance of the "Catálogo anotado de orquídeas de Costa Rica" in 2002, and the "Manual de Plantas de Costa Rica", in 2003. Both included more than 1,400 species of the Orchidaceae family, neatly summarizing the activities of the mentioned naturalists and scientists in almost two centuries of botanical exploration in the country. It also coincided with the creation of a research department at Lankester Botanical Garden (JBL), of the University of Costa Rica. JBL is dedicated exclusively to the study of orchids at the hand especially of Franco **Pupulin** and Jorge **Warner**. A healthy and young group of researchers, including Mario A. **Blanco**, Diego **Bogarín**, Melania **Fernández** and myself, and an upcoming group of students are now part of the orchidology team at JBL. In an effort to produce a comprehensive flora of the Orchidaceae for Costa Rica, dozens of scientific publications in the last decade have added more than 200 species of orchids to the Costa Rican flora (Karremans *et al.* 2012; Karremans & Bogarín 2013; Fernández *et al.* 2014).

The Pleurothallidinae, a major challenge for systematic research

Pleurothallis R.Br., type genus of subtribe Pleurothallidinae Lindl., was described already more than two centuries ago. Historically, *Pleurothallis* and Pleurothallidinae have been treated almost as synonyms, with the exclusion from *Pleurothallis* of only a few morphologically well-recognizable genera throughout the years. The first systematic classification of the members of Pleurothallidinae is possibly that of Lindley (1836; 1859). Several authors followed with additional proposals to tackle *Pleurothallis* and its segregate genera (i.e. Reichenbach f., Barbosa Rodrigues, Cogniaux, Schlechter, Garay, Dressler, and others). Members of the genus had however not suffered as many changes as they have in the last 30 years. Luer's first monograph of the group in 1986 triggered a proliferation of systematic studies that would have been impossible before. He published dozens of monographs thereafter. The first molecular phylogeny of the subtribe was published by Pridgeon *et al.* (2001), and was followed by a proposal to redefine it completely (Pridgeon & Chase 2001; Pridgeon 2005). However, the significant systematic and taxonomic changes proposed after that, in addition to the rapid increase in species numbers within Pleurothallidinae, has more than ever fueled the need to have a comprehensive picture of phylogenetic relationships within the subtribe.

The issue with the classification of the megadiverse *Pleurothallis* (in a traditional sense) has historically been the same one: the realization that it is not monophyletic, but the impossibility of resolving its systematics with the available data. In 1859, Lindley said about *Pleurothallis* that "I think it necessary to preserve this great and difficult genus without dismemberment. Not that I regard it as a really single aggregation of species...". A century later Luer would say that "*Pleurothallis* is indeed capable of being divided, but because of the various interrelationships, most divisions at the subgeneric and sectional levels seem more practical" and added "A *Pleurothallis* might be described as any pleurothallid that does not fit into any of the other genera" (Luer 1986). After their morphologically based cladistic study of the group, Neyland *et al.* (1995) wrote "the large genus *Pleurothallis* is polyphyletic and, therefore, may be divided into several genera", something that Garay had already noticed two decades before, "It is quite possible that *Pleurothallis* may be drastically segmented in the future; the most likely candidate is the former genus *Specklinia*" (Garay 1974). But, it was not until Pridgeon and Chase (2001), relying on the molecular-based studies by Pridgeon *et al.* (2001), that *Pleurothallis* was finally dismembered. They pleaded that it "has been nothing but a polymorphic assemblage for almost two centuries" and that "many taxa with conspicuous autapomorphies were segregated from it, gradually leaving the genus itself with no defining synapomorphies".

In their phylogenetic study of the group, Pridgeon and Chase (2001) found that "many characters are difficult to score in cladistic analyses because they are either continuous or probably not homologous. These same characters show up repeatedly in his [Luer's] artificial key to the subgenera". Nevertheless, the complexity of the group and their limited sampling size forced them to admit that "for nomenclatural transfers we extrapolated from the study taxa to morphologically similar taxa as recognized by Luer". Not surprisingly, subsequent phylogenetic studies within the Pleurothallidinae have been clear evidence that the generic, subgeneric and sectional systematics of the subtribe were far from fully resolved. Re-circumscriptions and emends were either made or at least suggested by several authors that used novel analytical methods and/or included a broader sampling of species (Stenzel 2004; Abele 2008; Karremans

2010; Chiron *et al.* 2012; Karremans *et al.* 2013a; Karremans 2014, Chapter 7; Karremans *et al.* unp., Chapter 6). Meanwhile, hundreds of species' names, be it new species or combinations, and dozens of new genera have since then been proposed by Luer (2002; 2004; 2005; 2006; 2007; 2009) and others, mostly on the basis of morphology. There is a pressing necessity of reviewing the phylogenetic relationships of many groups within the pleurothallids.

The genus Specklinia

The first species attributable to *Specklinia* ever to be described were *Epidendrum corniculatum* Sw., *E. lanceola* Sw. and *E. tribuloides* Sw. (Fig. 4) from Jamaica. They were described simultaneously by Olof Swartz in his *Nova genera & species plantarum; seu, Prodromus descriptionum vegetabilium, maximam partem incognitorum quæ sub itinere in Indiam Occidentalem annis 1783-87 digessit* in 1788. The original description included not more than a dozen words (Fig. 5), typical of the epoch and which has made their interpretation quite hazardous.

The generic name *Specklinia* appeared for the first time in 1830 in John Lindley's The Genera and Species of Orchidaceous Plants. Lindley honored Rudolph Specklin with the generic name, pleading that he had been an "outstanding sculptor in wood, whose grandfather's exceptional illustrations of plants well-deserved to be included in Fuchs' *Historia stirpium*". Little is known about Rudolph Specklin, Luer (2006) writes that he was an early nineteenth century English engraver, nevertheless this was quite unlikely. Based on Lindley's original statement in Latin, Veit Rudolph Specklin (Fig. 6) a renowned woodcutter, who famously illustrated the plants presented in *De Historia Stirpium Commentarii Insignes* of Leonhart Fuchs (Fig. 7), was the grandfather of his honoree Rudolph Specklin. Veit Rudolph Specklin, who lived in Straßburg and was of Alsatian decent, passed away in 1550. He left five children, of which the last would pass away in 1600. His grandson, Rudolph Specklin therefore likely lived from around the end of the sixteenth century to the beginning of the seventeenth century. Lindley was born two hundred years after, and surely did not know any of the Specklins personally.

In the original publication, Lindley included only five names in *Specklinia*. Swartz's *Epidendrum corniculatum* and *E. lanceola*, and three other species that are now generally placed in the genera *Anathallis* and *Acianthera*. He did not designate a type species for the genus. Garay and Sweet (1972) lectotypified the genus using *Specklinia lanceola* (Sw.) Lindl. It was chosen as type species by the authors because it "is the one which most approximates the generic characters given by Lindley". Lindley's generic description

Figure 4. *Epidendrum corniculatum* Sw., *E. lanceola* Sw. and *E. tribuloides* Sw., now better known as *Specklinia corniculata, S. lanceola and S. tribuloides* respectively. Photographs by A.P. Karremans.

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tribuloides. E. foliis pedicellatis lanceolatis obtusis emargi-natis, pedunculis brevissimis, capsulis glo-bosis echinatis.
Jamaica. ɣ.

corniculatum. E. foliis pedicellatis cuneatis oblongis, pedun-culis radicalibus uniflοris, corollis acumi-natis curvis.
Jamaica. ɣ.

lanceola. E. foliis pedicellatis lanceolatis acutis, pedun-culis ex vaginis radicalibus bifloris.
Jamaica. ɣ.

Figure 5. Original publication of the first three species of *Specklinia* to be described. Taken from *Nova genera & species plantarum* by Olof Swartz.

Figure 6. Self-portraits of the illustrators of *De Historia Stirpium Commentarii Insignes* by Leonhart Fuchs, as found within the book itself.

Figure 7. Woodcutting of Leonhart Fuchs by Veit Rudolph Specklin as found in *De Historia Stirpium Commentarii Insignes* itself.

seems to be easily applicable to all of the other cited species, but in the sake of nomenclatural stability it is best to adhere to the proposed lectotype.

Traditionally, *Specklinia* Lindl. (Orchidaceae: Pleurothallidinae) had been considered a synonym of *Pleurothallis* R.Br. (Luer 1986). However, the generic limits of the large genus *Pleurothallis* were recircumscribed by Pridgeon and Chase (2001) on the basis of molecular studies by Pridgeon *et al*. (2001). The authors presented new evidence to re-establish *Specklinia*, recognizing 86 species. The recircumscribed *Specklinia* included species of *Pleurothallis* subgen. *Specklinia* (Lindl.) Garay [*P*. sects. *Hymenodanthae* Barb.Rodr., *Tribuloides* Luer, Muscariae Luer], subgen. *Empusella*, subgen. *Pseudoctomeria* and *Acostaea* Schltr., showing low levels of sequence divergence (Pridgeon & Chase 2001). *Specklinia* was difficult to characterize on the basis of a particular set of distinguishing morphological features (Karremans 2014, Chapter 7; Karremans *et al.* unp., Chapter 6), promoting the creation of several new genera, expressly designed to fit one or more morphologically aberrant species of the genus (Luer 2004; 2006). Due to the different interpretations of the circumscription of *Specklinia*, it had been difficult to estimate the actual number of species belonging to the genus. Pridgeon (2005) accounted for 200 species, but one year later Luer (2006) reduced the genus to no more than 40 species. Most recently Barros & Trettel Rodrigues (2009) accounted for 420 binomials, about five times the original number transferred by Pridgeon & Chase (2001). Finally the broadly sampled molecular phylogeny combined with morphological and geographical data presented here (Chapter 6) shows that 95 specific epithets are attributable to *Specklinia* at this time.

Specklinia has not been accepted by everyone yet. Some authors continue to place it under the synonymy of *Pleurothallis* (Ackerman 2014), this is especially true among orchids growers. Nevertheless, it has been amply proven that *Specklinia* species are not closely related to *Pleurothallis*, in fact they are closely related to other, generally accepted and traditionally used genera such as *Dryadella* Luer, *Platystele* Schltr. and *Scaphosepalum* Pfitzer (Pridgeon *et al*. 2001; Chapter 6).

Outline of this PhD thesis

The present work brings together the results of systematic, phylogenetic and pollination studies of species belonging to the genus *Specklinia*, with special emphasis on Costa Rican species. It is organized in three distinct sections that contain manuscripts of similar topics for ease of the reader.

Contributions towards our systematic knowledge of *Specklinia*:—Almost without exception any biological study on a particular species or group of species would be seriously flawed without the definition, clarification and understanding of the subject itself and the taxonomic name that should be applied to it. If, like in the case of the species studied here, our initial concept of a specific taxon is mistaken then we risk either not being able to answer our biological questions or doing it wrongly. In molecular phylogenies, the initial determination of a sampled taxon is key in the assessment of the resulting trees. Having misidentified terminals can lead to (1) the allocation of species to wrong genera, (2) the assumption that species or genera are non-monophyletic, (3) and the grouping of unrelated species while displacing close relatives. In ecological studies, the use of broad species' concepts, which actually include more than one species can have a detrimental effect. Mixing ecological preferences and interactions of different species will lead to missing the actual patterns of each species. Similarly, but possibly not as grim, over-splitting a species will lead to having several species with the same ecological preferences and interactions.

Applying the correct taxonomical name is essential. It is important to remember at this point that for any species only type specimens can be determined with complete certainty, everything else is our own interpretation. As such our conclusions based on non-type material should be handled with care. The chapters in this section attempt to clarify the taxonomic status of a series of *Specklinia* species. The species of the *Specklinia endotrachys* complex are disentangled in chapters 1 and 2, while the species of the *Specklinia glandulosa* complex are treated in chapter 3. Additional taxonomic novelties encountered during the different stages of this study are presented in chapters 4 and 5. In total thirteen *Specklinia* species are being characterized and illustrated, of which six are new to science.

Phylogenetic reassessment of *Specklinia* and its allied genera:—One of the initial challenges of proposing a study on *Specklinia* was the difficulty in trying to circumscribe the genus. The lack of consistency among authors as to how many and which species belonged to the genus in recent literature was a mayor issue. A broadly sampled phylogenetic analysis of *Specklinia* and its closest relatives was necessary to establish not only the below genus-level relationships, but also how the genus relates to other genera.

One of our main goals was to understand the relationships among species from all the proposed genera within this species' group, which includes the generic concepts of *Acostaea* Schltr., *Areldia* Luer, *Cucumeria* Luer, *Dryadella* Luer, *Gerardoa* Luer, *Incaea* Luer, *Muscarella* Luer, *Platystele* Schltr., *Pseudoctomeria* Kranezl., *Rubellia* (Luer) Luer, *Sarcinula* Luer, *Scaphosepalum* Pfitzer, *Specklinia*, *Sylphia* Luer, *Teagueia* (Luer) Luer, *Tribulago* Luer, *Tridelta* Luer, *Trigonanthe* (Schltr.) Brieger and *Verapazia* Archila.

Assessing the pollination mechanisms of *Specklinia*:—Chapter 8 is dedicated to a multi-disciplinary study of the pollination syndrome of *Specklinia* species. The study focuses on the *Specklinia endotrachys* complex, a group of orange-flowered *Specklinia* species that are visited and pollinated by *Drosophila* species. Our main questions were: 1) how are the *Specklinia* attracting their pollinators?, 2) are the *Specklinia* deceitful or rewarding?, 3) which and how many species of *Drosophila* are pollinating the *Specklinia*?, 4) what behaviors do the *Drosophila* show whilst visiting the flowers? 5) how do these species of *Specklinia* prevent hybridization?

General discussion:—The final chapter of the thesis brings together the most relevant results of all the previous chapters. An integrative reflection is given with thoughts about the findings and possible complementary future studies.