Systematic, phylogenetic and pollination studies of Specklinia (Orchidaceae)

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Chapter 6

Genetic similarity versus morphological divergence: phylogenetics of *Specklinia* (Orchidaceae)

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The phylogenetic relationships within *Specklinia*, a recently re-established genus of the Orchidaceae (Pleurothallidinae), and related genera are re-evaluated using Bayesian analyses of nuclear ITS and chloroplast *matK* sequence data of a wide sampling of species. *Specklinia* is found basically biphyletic in the DNA based trees, with species alternatively assigned to *Muscarella* proven distinct, monophyletic and well recognizable. *Muscarella* is therefore recognized as distinct. *Specklinia* as such includes about 95 morphologically highly variable species. Their phenotypic differences had prompted the creation of up to eleven generic names within this relatively small group. Here we show not only that these morphologically divergent species are closely related, but also that they can still be recognized by certain conserved morphological traits. The genera *Acostaea*, *Areldia*, *Empusella*, *Cucumeria*, *Gerardoa*, *Pseudoctomeria*, *Sarcinula*, *Sylphia*, *Tribulago* and *Tridelta* are found imbedded within Specklinia, and therefore placed in synonymy. *Specklinia* is confirmed sister to a clade that includes *Platystele*, *Scaphosepalum* and *Teagueia*. Five well supported subgenera are proposed for *Specklinia* and are characterized both geographically and morphologically. The species belonging to each subgenus are listed. *Incaea* is synonymized with *Dryadella*, and *Rubellia* is reduced under *Platystele*. New combinations for several species of *Dryadella*, *Muscarella*, *Platystele* and *Specklinia* are proposed.

Keywords: molecular phylogeny; morphology; Pleurothallidinae; *Specklinia*; systematics; taxonomy

Introduction

How to adequately circumscribe a genus is still highly debatable. Genera, as well as other above species-level groupings, are frequently considered arbitrary groups of species. Arbitrariness is reduced by the implementation of objective methodologies that result in the establishment of biologically significant groups. Recent systematic work, especially such that include molecular data, tends to result in more inclusive generic delimitations, whereas work based on morphological data tends to result in narrower generic delimitations. Humphreys and Linder (2009) suggested that "good genera are predictive and stable", which can be attained assessing, for example, their morphological recognisability, monophyly and reproductive isolation (Scopece *et al*. 2010).

Traditionally, *Specklinia* Lindl. (Orchidaceae: Pleurothallidinae) had been considered a synonym of *Pleurothallis* R.Br. (Luer 1986). However, the generic limits of the mammoth genus *Pleurothallis* were recircumscribed (Pridgeon & 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. Both in the bootstrap consensus trees of the *matK*/*trnL-F* dataset and the most parsimonious tree from the combined *matK*/*trnL-F*/ITS DNA dataset a morphologically highly heterogeneous set of taxa, including *Dryadella simula* (Rchb.f.) Luer, *Pleurothallis costaricensis* Rolfe, *P. lentiginosa* Lehmann

& Kraezlin, *P. endotrachys* Rchb.f., *Acostaea costaricensis* Schltr., and species of the genera *Platystele* Schltr. and *Scaphosepalum* Pfitzer, are found together in a clade. In the tree obtained from the nrITS DNA matrix, based on a larger sampling, *P. lanceola* (Sw.) Sprengel —the type species of the genus *Specklinia*— was found together with *P. endotrachys*, *P. fulgens* Rchb.f., *P. lateritia* Endrés ex Rchb.f., *P. lentiginosa*, and *P. tribuloide*s (Sw.) Lindl., forming a distinct subclade treated by the authors as the "core" *Specklinia*.

The recircumscribed *Specklinia* included species of Pleurothallis subgen. *Specklinia* [*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). Among the morphological features useful to define *Specklinia*, the authors indicated the, usually, small plants with a short stem with an annulus, the variously connate sepals, and the hinged lip; the sepals and petals of *Specklinia* mostly membranous; the column with a toothed apex; and ventral anther and stigma. However, even with the removal of the basal *Dryadella* Luer and the derived *Platystele* and *Scaphosepalum*, the resulting circumscription of *Specklinia* is variable both in terms of vegetative and floral morphology (Luer 2006; Pupulin *et al*. 2012; Bogarín *et al.* 2013b, Chapter 4; Karremans *et al.* 2013b).

Specklinia is thus difficult to characterize on the basis of a particular set of distinguishing morphological features (Karremans 2014, Chapter 7), promoting the creation of several new genera, expressly designed to fit one or more morphologically aberrant species of *Specklinia* (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 some 40 species. Most recently Barros & Trettel Rodrigues (2009) accounted for 420 binomials, about five times the original number transferred by Pridgeon & Chase (2001).

Here we have chosen to re-evaluate phylogenetic relationships among the species with Specklinia affinity using a wide range of evidence. Our approach is to combine a molecular phylogeny covering about half of the species that belong to the genus, with a morphological and geographical characterization, as well as the establishment of a subgeneric classification. Our main goal is to understand relationships among species of all the proposed genera within this species group: *Acostaea*, *Areldia* Luer, *Cucumeria* Luer, *Dryadella*, *Gerardoa* Luer, *Incaea* Luer, *Muscarella* Luer, *Platystele*, *Pseudoctomeria* Kraenzl., *Rubellia* (Luer) Luer, *Sarcinula* Luer, *Scaphosepalum*, *Specklinia*, *Sylphia* Luer, *Teagueia* (Luer) Luer, *Tribulago* Luer, *Tridelta* Luer, *Trigonanthe* (Schltr.) Brieger and *Verapazia* Archila.

Materials and methods

Specimens were field-collected or obtained from the living collections at Lankester Botanical Garden (JBL), University of Costa Rica, the Hortus botanicus in Leiden (L), or from the private collections of G. Villalobos in Costa Rica, G. Vierling in Germany, and W. Driessen, P. Dubbeldam, T. Sijm and J. Wubben in the Netherlands. Selection of material was done on the basis of availability and interspecific variation. At least one representative of the genera, subgenera, or other groupings accepted in the alternative classification systems was included in the sampling when available. Many of the species included are Costa Rican in distribution, reflecting the prevailing nature of the JBL collections; however, specimens from a wide geographical range have been included as well. Putative species are represented by more than a single accession whenever possible, in order to assure better species delimitation, reducing risks of laboratory mix-ups and in accounting for sequencing error. Vouchers of specimens used are kept in the liquid collections at JBL or L, unless specified otherwise (Table 7).

DNA sequences of *Masdevallia hornii* Königer (= *Phloeophila yupanki* (Luer & R.Váquez) Pridgeon & M.W.Chase), *Platystele catiensis* Karremans & Bogarín, *Platystele tica* Karremans & Bogarín, *Specklinia absurda* Bogarín, Karremans & R.Rincón, *Specklinia acoana* Bogarín, *Specklinia berolinensis* Bogarín, *Specklinia remotiflora* Pupulin & Karremans and *Specklinia succulenta* Bellone & Archila were obtained from the plants that served as type material (Chiron *et al.* 2012; Pupulin *et al.* 2012, Chapter 1; Bogarín *et al.* 2013b, Chapter 4; Bogarín *et al.* 2014; Fernández *et al.* 2014).

Table 7. List of vouchers and GenBank number used in the phylogenetic analyses. Scientific names mostly follow Pridgeon (2005).

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Anathallis grayumii (Luer) Luer (1)	*Karremans 2747*	KC425730	-	Karremans 2014
Anathallis grayumii (Luer) Luer (2)	*Pupulin 3794*	KC425731	KP012494	Karremans 2014
Anathallis lewisiae (Ames) Solano & Soto Arenas	Bogarín 1056	KC425733	KC425858	Karremans 2014
Anathallis pabstii (Garay) Pridgeon & M.W.Chase	*Karremans 4821*	KC425737	KC425859	Karremans 2014
Anathallis rabei (Foldats) Luer	*Karremans 4794*	KC425738	KC425860	Karremans 2014
Dryadella albicans (Luer) Luer	*Karremans 4861*	KC425742	KC425863	This Study
Dryadella aviceps (Rchb. f.) Luer	*van den Berg 1989*	JQ306381	-	GenBank
Dryadella edwallii (Cogn.) Luer	*Chase 305*	AF262824	AF265454	Pridgeon *et al*. 2001
Dryadella guatemalensis (Schltr.) Luer	*Karremans 3642*	KC425743	-	This Study
Dryadella hirtzii Luer	*BGH-123364*	EF079367	EF079327	GenBank
Dryadella kautskyi (Pabst) Luer	*van den Berg 1997*	JQ306380	-	GenBank
Dryadella simula (Rchb. f.) Luer	*Chase 1095*	AF262825	AF265453	Pridgeon *et al*. 2001
Dryadella susanae (Pabst) Luer	*Chiron 11240*	JQ306486	-	GenBank
Echinosepala aspasicensis (Rchb.f.) Pridgeon & M.W.Chase	*Chase 971*	AF262905	-	Pridgeon *et al*. 2001
Echinosepala aspasicensis (Rchb.f.) Pridgeon & M.W.Chase	*Bogarín 1945*	-	EU214340	GenBank
Lankesteriana barbulata (Lindl.) Pridgeon & M.W. Chase	*Bogarín 8606*	KC425726	KC425856	Karremans 2014
Lepanthopsis apoda (Garay & Dunst.) Luer	*Pridgeon 126*	KF747841	-	This Study
Pabstiella parvifolia (Lindl.) Luer (1)	*Karremans 2680*	KC425812	KP012497	This Study
Pabstiella parvifolia (Lindl.) Luer (2)	*Karremans 2680*	KC425813	-	This Study
Phloeophila nummularia (Rchb. f.) Garay (1)	*Karremans 5959*	KF747839	KP012380	This Study
Phloeophila nummularia (Rchb. f.) Garay (2)	*Karremans 5982*	-	KP012381	This Study
Phloeophila nummularia (Rchb. f.) Garay (3)	*Stenzel 896*	KC425841	-	Stenzel 2004
Phloeophila pelecaniceps (Luer) Pridgeon & M.W. Chase	*Chase 1128*	AF262810	AF265450	Pridgeon *et al*. 2001
Phloeophila peperomioides (Ames) Garay (1)	*None*	AF275690	AF291103	Pridgeon *et al*. 2001
Phloeophila peperomioides (Ames) Garay (2)	*Bogarín 7112*	KC425745	-	This Study
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (1)	*Chase 978*	AF262812	-	Pridgeon *et al*. 2001
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (2)	*Chase 5638*	AF262811	AF265451	Pridgeon *et al*. 2001
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (3)	*Karremans 4818*	KC425746	KP012495	This Study
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W. Chase (4)	*Karremans 4856*	KC425747	KP012496	This Study
Phloeophila yupanki (Luer & R.Vásquez) Pridgeon & M.W.Chase (1)	*Karremans 4858*	KC425748	KP012498	This Study
Phloeophila yupanki (Luer & R.Vásquez) Pridgeon & M.W.Chase (2)	Karremans 5706a	KF747776	KP012382	This Study
Phloeophila yupanki (Luer & R.Vásquez) Pridgeon & M.W.Chase (3)	*Karremans 5706b*	KF747777	-	This Study
Platystele acicularis Luer & Hirtz	*Karremans 5785*	KF747778	KP012383	This Study
Platystele aurea Garay (1)	*Karremans 4807*	KC425762	-	This Study
Platystele aurea Garay (2)	*Karremans 5707b*	-	-	This Study
Platystele aurea Garay (3)	*Karremans 5707a*	KF747779	-	This Study
Platystele beatricis P. Ortiz	*Karremans 4801*	KC425749	KP012499	This Study
Platystele catiensis Karremans & Bogarín	*Bogarín 9661*	-	KP012384	This Study
Platystele caudatisepala (C.Schweinf.) Garay	*Bogarín 10230*	-	KP012385	This Study
Platystele compacta (Ames) Ames	*Karremans 4088*	KC425750	-	This Study
Platystele consobrina Luer	*Karremans 4835*	KC425751	-	This Study
Platystele gyroglossa Luer	*Karremans 4834*	KC425752	-	This Study
Platystele hirtzii Luer	*Karremans 5755*	KF747780	-	This Study
Platystele lancilabris (Rchb.f.) Schltr.	*Bogarín 10593*	-	KP012386	This Study
Platystele microtatantha (Schltr.) Garay	*Bogarín 8022*	KF747781	-	This Study
Platystele minimiflora (Schltr.) Garay	*Karremans 5980*	KF747782	KP012387	This Study
Platystele misasiana P. Ortiz	*Karremans 5768*	KF747783	KP012388	This Study
Platystele misera (Lindl.) Garay (1)	*Karremans 5749*	KF747784	KP012389	This Study
Platystele misera (Lindl.) Garay (2)	*Chase 5625*	AF262823	AF265470	Pridgeon *et al*. 2001
Platystele ovatilabia (Ames & C. Schweinf.) Garay	*Bogarín 3941*	KC425753	-	This Study
Platystele oxyglossa (Schltr.) Garay	*Karremans 4253*	KC425754	KP012500	This Study
Platystele oxyglossa (Schltr.) Garay aff.	*Karremans 5407*	KC425755	-	This Study
Platystele propinqua (Ames) Garay	*C.M. Smith 500*	KF747785	KP012390	This Study
Platystele reflexa Luer aff.	*Karremans 5733*	KC425756	-	This Study
Platystele schmidtchenii Schltr.	*Karremans 5995*	KF747786	-	This Study
Platystele stenostachya (Rchb.f.) Garay (1)	*Bogarín 5806*	KF747787	-	This Study
Platystele stenostachya (Rchb.f.) Garay (2)	*Pupulin 7919*	KC425759	KP012501	This Study
Platystele stenostachya (Rchb.f.) Garay (3)	*Chase 5618*	AF262821	-	Pridgeon *et al*. 2001

TABLE 7. Continued.

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Platystele tica Karremans & Bogarín	*Karremans 5829A*	KP012458	KP012391	This Study
Platystele ximenae Luer & Hirtz	*Karremans 4865*	KC425760	KP012502	This Study
Scaphosepalum anchoriferum (Rchb.f.) Rolfe	*Bogarín 5418*	KP012459	KP012392	This Study
Scaphosepalum gibberosum (Rchb.f.) Rolfe	Chase 968	AF262817	AF265458	Pridgeon *et al.* 2001
Scaphosepalum grande Kraenzl.	*Chase 1107*	AF262819	-	Pridgeon *et al.* 2001
Scaphosepalum medinae Luer & J. Portilla (1)	*Karremans 4810a*	KC425763	-	This Study
Scaphosepalum medinae Luer & J. Portilla (2)	*Karremans 4810b*	KF747788	-	This Study
Scaphosepalum microdactylum Rolfe	*Pupulin 7897*	KP012460	KP012393	This Study
Scaphosepalum ovulare Luer	*Karremans 4809*	KC425764	KP012503	This Study
Scaphosepalum swertiifolium (Rchb.f.) Rolfe	*Chase 1383*	AF262818	-	Pridgeon *et al.* 2001
Scaphosepalum swertiifolium (Rchb.f.) Rolfe aff.	*Karremans 4811*	KC425765	KP012504	This Study
Scaphosepalum ursinum Luer (1)	*Karremans 4817*	KC425766	-	This Study
Scaphosepalum ursinum Luer (2)	*BGH-124283*	EF079365	-	GenBank
Scaphosepalum verrucosum (Rchb.f.) Pfitzer (1)	*Karremans 4812*	KC425767	KP012505	This Study
Scaphosepalum verrucosum (Rchb.f.) Pfitzer (2)	*Chase 1331*	AF262820	-	Pridgeon *et al.* 2001
Specklinia absurda Bogarín, Karremans & Rincón (1)	*Bogarín 9772*	KC425826	-	This Study
Specklinia absurda Bogarín, Karremans & Rincón (2)	*Bogarín 8711*	KC425827	KP012506	This Study
Specklinia acanthodes (Luer) Pridgeon & M.W.Chase	*Pridgeon 232*	KF747842	-	This Study
Specklinia acicularis (Ames & C.Schweinf.) Pridgeon & M.W.Chase	*Pupulin 5232*	KF747789	-	This Study
Specklinia acoana Bogarín	*A. Rojas 7718*	KF747800	-	This Study
Specklinia acrisepala (Ames & C.Schweinf.) Pridgeon & M.W.Chase (1)	*Karremans 3770*	KC425768	-	This Study
Specklinia acrisepala (Ames & C.Schweinf.) Pridgeon & M.W.Chase (2)	*M. Fernández 604*	KF747790	-	This Study
Specklinia alajuelensis Karremans & Pupulin (1)	*Karremans 5501*	KC425792	-	This Study
Specklinia alajuelensis Karremans & Pupulin (2)	*Karremans 3268*	KP012455	KP012411	This Study
Specklinia alajuelensis Karremans & Pupulin (3)	*Bogarín 2895*	KP012454	KP012412	This Study
Specklinia alajuelensis Karremans & Pupulin (4)	*Karremans 3265*	KC425791	-	This Study
Specklinia alata (A.Rich. & Galeotti) Solano & Soto Arenas	*Karremans 4840*	KC425806	-	This Study
Specklinia alta (Luer) Luer	*Karremans 5721*	KF747791	KP012394	This Study
Specklinia aristata (Hook.) Luer	*Stenzel 996*	KC425842	-	Stenzel 2004
Specklinia barbae (Schltr.) Luer (1)	*Karremans 5396*	KC425770	-	This Study
Specklinia barbae (Schltr.) Luer (2)	*Karremans 4853*	KC425771	-	This Study
Specklinia barbae (Schltr.) Luer (3)	Karremans 3928	KC425769	-	This Study
Specklinia barbae (Schltr.) Luer (4)	*M. Fernández 646*	KP012461	KP012395	This Study
Specklinia blancoi (Pupulin) Soto Arenas & Solano ano	*Karremans 5701*	KC425772	-	This Study
Specklinia brighamii (S.Watson) Pridgeon & M.W.Chase (1)	*Karremans 4799*	KC425773	-	This Study
Specklinia brighamii (S.Watson) Pridgeon & M.W.Chase (2)	*JBL-00887*	KC425774	-	This Study
Specklinia cabellensis (Rchb.f.) Karremans (1)	*Karremans 5712*	KF747792	KP012396	This Study
Specklinia cabellensis (Rchb.f.) Karremans (2)	*Karremans 5712*	KF747793		This Study
Specklinia cabellensis (Rchb.f.) Karremans (3)	*Karremans 5712*	KF747794		This Study
Specklinia cactantha (Luer) Pridgeon & M.W.Chase (1)	*Karremans 5965*	KF747795	KP012397	This Study
Specklinia cactantha (Luer) Pridgeon & M.W.Chase (2)	*Karremans 5979*	KF747796	-	This Study
Specklinia calyptrostele (Schltr.) Pridgeon & M.W.Chase (1)	*Pupulin 7060*	KC425775	KP012507	This Study
Specklinia calyptrostele (Schltr.) Pridgeon & M.W.Chase (2)	*Pupulin 7724*	KF747798	KP012398	This Study
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (1)	*Pupulin 6543*	KC425776	-	This Study
Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer (2)	*Pupulin 6543*	KF747799	KP012399	This Study
Specklinia claviculata (Luer & Hirtz) Luer	*Karremans 4827*	KC425777	-	This Study
Specklinia colombiana (Garay) Pridgeon & M.W.Chase aff.	*Karremans 4942*	KC425825	-	This Study
Specklinia colombiana (Garay) Pridgeon & M.W.Chase (1)	*Karremans 3235*	KC425809	-	This Study
Specklinia colombiana (Garay) Pridgeon & M.W.Chase (2)	*M. Fernández 481*	KC425810	-	This Study
Specklinia condylata (Luer) Pridgeon & M.W.Chase (1)	*Bogarín 7855*	KP012462	-	This Study
Specklinia condylata (Luer) Pridgeon & M.W.Chase (2)	*M. Fernández 170*	KP012463	-	This Study
Specklinia condylata (Luer) Pridgeon & M.W.Chase aff.	*Chase 6808*	AF262873	-	Pridgeon *et al.* 2001
Specklinia corniculata (Sw.) Steud. (4)	*Karremans 5180*	KF747801	KP012400	This Study
Specklinia corniculata (Sw.) Steud. (5)	*JBL-02240a*	KF747802	KP012401	This Study
Specklinia corniculata (Sw.) Steud. (6)	*JBL-02240b*	KF747803	KP012402	This Study
Specklinia corniculata (Sw.) Steud. (1)	*JBL-02227*	KC425781	-	This Study
Specklinia corniculata (Sw.) Steud. (2)	*Karremans 4782*	KC425782	-	This Study
Specklinia corniculata (Sw.) Steud. (3)	*Stenzel 889*	KC425844	-	Stenzel 2004

Table 7. Continued.

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase (1)	*Chase 5636*	AF262863	-	Pridgeon *et al*. 2001
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase (2)	*Bogarín 5643*	KC425783	-	This Study
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase (3)	*Chase 5612*	AF262862	AF265459	Pridgeon *et al*. 2001
Specklinia cucumeris (Luer) Karremans (1)	Karremans 5757a	KF747804	KP012403	This Study
Specklinia cucumeris (Luer) Karremans (2)	*Karremans 5757b*	KF747805	-	This Study
Specklinia digitalis (Luer) Pridgeon & M.W.Chase	*Karremans 5737*	KF747806	KP012404	This Study
Specklinia displosa (Luer) Pridgeon & M.W.Chase (1)	*Karremans 5713b*	KF747807	KP012405	This Study
Specklinia displosa (Luer) Pridgeon & M.W.Chase (2)	*Karremans 5713c*	KF747808	-	This Study
Specklinia dodii (Garay) Luer	*Karremans 5963*	KF747809	KP012406	This Study
Specklinia dunstervillei Karremans, Pupulin & Gravend. (1)	*Karremans 5966*	KP012456	-	This Study
Specklinia dunstervillei Karremans, Pupulin & Gravend. (2)	*Karremans 5899*	-	KP012423	This Study
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (1)	*Blanco 961a*	KC425784	KP012508	This Study
Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase (2)	*Blanco 961b*	KF747810	KP012407	This Study
Specklinia fimbriata (Ames & C. Schweinf.) Luer	*Karremans 3718*	KC425785	-	This Study
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas (1)	*Karremans 5600*	KC425786	KP012408	This Study
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas (2)	*Karremans 5600*	KF747811	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (1)	*Chase 5630*	AF262872	-	Pridgeon *et al*. 2001
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (2)	*Karremans 3284*	KC425800	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (3)	*JBL-001675*	KC425790	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (4)	*Karremans 4843*	KC425788	-	This Study
Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase (5)	*Karremans 3593*	KC425787	KP012409	This Study
Specklinia gersonii Bogarín & Karremans	*Karremans 6025*	KP012457	KP012424	This Study
Specklinia gracillima (Lindl.) Pridgeon & M.W.Chase (1)	*Karremans 4831*	KC425793	-	This Study
Specklinia gracillima (Lindl.) Pridgeon & M.W.Chase (2)	*Karremans 5999*	KF747812	-	This Study
Specklinia grisebachiana (Cogn.) Luer	*Stenzel 619*	KC425846	-	Stenzel 2004
Specklinia grobyi (Bateman ex Lindl.) F.Barros (1)	*Karremans 5463*	KF747813	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (2)	*JBL-10285*	KF747814	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (3)	*Pupulin 8187*	KC425799	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (4)	*Chiron 09357*	JQ306388	-	GenBank
Specklinia grobyi (Bateman ex Lindl.) F.Barros (5)	*Chase 1093*	AF262860	-	Pridgeon *et al*. 2001
Specklinia grobyi (Bateman ex Lindl.) F.Barros (6)	*Karremans 4220*	KC425794	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros (7)	Karremans 3759	KC425796	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros aff. (1)	*Karremans 4833*	KC425798	-	This Study
Specklinia grobyi (Bateman ex Lindl.) F.Barros aff. (2)	*Chiron 04524*	JQ306485	-	GenBank
Specklinia grobyi (Bateman ex Lindl.) F.Barros aff. (3)	*Karremans 5958*	KF747829	KP012413	This Study
Specklinia guanacastensis (Ames & C.Schweinf.) Pridgeon & M.W. Chase	*Karremans 6018*	KP012464	KP012414	This Study
Specklinia hastata (Ames) Pridgeon & M.W.Chase	*Bogarín 4910*	KF747773	-	This Study
Specklinia helenae (Fawc. & Rendle) Luer	*Stenzel 766*	KC425847	-	Stenzel 2004
Specklinia herpestes (Luer) Luer (1)	*Karremans 4082a*	KC425801	-	This Study
Specklinia herpestes (Luer) Luer (2)	*Karremans 4082b*	KC425802	-	This Study
Specklinia icterina Bogarín	*Bogarín 8767*	KC425778	-	This Study
Specklinia lanceola (Sw.) Lindl. (1)	*Karremans 5503*	KC425803	-	This Study
Specklinia lanceola (Sw.) Lindl. (2)	*Pridgeon s.n.*	KC425838	-	Pridgeon & Chase 2002
Specklinia lanceola (Sw.) Lindl. (3)	*Chase 1433*	AF262861	-	Pridgeon *et al*. 2001
Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase (1)	*None*	AF275692	-	Pridgeon *et al*. 2001
Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase (2)	*Karremans 3011*	KC425804	-	This Study
Specklinia lichenicola (Griseb.) Pridgeon & M.W.Chase	*Stenzel 452*	KC425845	-	Stenzel 2004
Specklinia llamachoi (Luer) Luer	*Stenzel 545*	KC425848	-	Stenzel 2004
Specklinia longilabris (Lindl.) Luer	*Stenzel 895*	KC425849	-	Stenzel 2004
Specklinia lugduno-batavae Karremans, Bogarín & Gravend. (1)	*Pupulin 7709*	KC425824	-	This Study
Specklinia luis-diegoi (Luer) Luer (1)	*Karremans 5500*	KC425835	-	This Study
Specklinia luis-diegoi (Luer) Luer (2)	*Karremans 5500*	KF747815	-	This Study
Specklinia macroblepharis (Rchb. f.) Luer	*Karremans 4860*	KC425805	-	This Study
Specklinia megalops (Luer) Luer	*Karremans 4792*	KC425807	-	This Study
Specklinia microphylla (A.Rich. & Galeotti) Pridgeon & M.W.Chase (1)	*Bogarín 9394*	KC425808	-	This Study
Specklinia microphylla (A.Rich. & Galeotti) Pridgeon & M.W.Chase (2)	*JBL-00968*	KP012465	-	This Study
Specklinia montezumae (Luer) Luer (1)	*Karremans 229*	KC425811	KP012509	This Study

Table 7. Continued.

Taxon	Sequence Voucher	GenBank Accession Number ITS	GenBank Accession Number *matK*	Sequence Source
Specklinia montezumae (Luer) Luer (2)	*Karremans 5751*	KF747816	-	This Study
Specklinia morganii (Luer) Luer (1)	*Karremans 5728a*	KF747817	KP012415	This Study
Specklinia morganii (Luer) Luer (2)	*Karremans 5728b*	KF747818	-	This Study
Specklinia mucronata (Lindl. ex Cogn.) Karremans	*Stenzel 478*	KC425850	-	Stenzel 2004
Specklinia obliquipetala (Acuña & C.Schweinf.) Karremans	*Stenzel 789*	KC425851	-	Stenzel 2004
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (1)	Karremans 4825	KC425814	KP012510	This Study
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (2)	*Karremans 3656*	KF747819	-	This Study
Specklinia pfavii (Rchb.f.) Pupulin & Karremans (3)	*JBL-11086*	KF747820	-	This Study
Specklinia picta (Lindl.) Pridgeon & M.W.Chase (1)	*van den Berg 2146*	JQ306384	-	GenBank
Specklinia picta (Lindl.) Pridgeon & M.W.Chase (2)	*Karremans 4836*	KC425815	-	This Study
Specklinia picta (Lindl.) Pridgeon & M.W.Chase aff.	*Chiron 06131*	JQ306385	-	GenBank
Specklinia pisinna (Lindl.) Solano & Soto Arenas (1)	*Karremans 4797*	KC425795	-	This Study
Specklinia pisinna (Lindl.) Solano & Soto Arenas (2)	*Karremans 4839*	KC425797	-	This Study
Specklinia psichion (Luer) Luer (1)	*Bogarín 8299*	KC425816	-	This Study
Specklinia psichion (Luer) Luer (2)	*Karremans 5955*	KF747821	-	This Study
Specklinia quinqueseta (Ames) Luer	*Karremans 3940*	KC425817	-	This Study
Specklinia recula (Luer) Luer (1)	*Karremans 5300a*	KF747822	KP012416	This Study
Specklinia recula (Luer) Luer (2)	*Karremans 5300b*	KF747823	KP012417	This Study
Specklinia recula (Luer) Luer (3)	*Karremans 5832*	KF747824	KP012418	This Study
Specklinia recula (Luer) Luer (4)	*Karremans 5823*	KP012466	-	This Study
Specklinia remotiflora Pupulin & Karremans (1)	*Karremans 4798a*	KC425818	KP012511	This Study
Specklinia remotiflora Pupulin & Karremans (2)	*Karremans 4798b*	KC425819	-	This Study
Specklinia remotiflora Pupulin & Karremans (3)	*Karremans 4854*	KC425820	-	This Study
Specklinia remotiflora Pupulin & Karremans aff.	*Chase 1303*	AF262859	AF265456	Pridgeon *et al*. 2001
Specklinia schaferi (Ames) Luer	*Stenzel 453*	KC425852	-	Stenzel 2004
Specklinia scolopax (Luer & R.Escobar) Pridgeon & M.W.Chase	*Karremans 4820*	KC425821	KP012512	This Study
Specklinia segregatifolia (Ames & C.Schweinf.) Solano & Soto-Arenas	*Bogarín 7990*	KC425822	-	This Study
Specklinia simmleriana (Rendle) Luer	*Karremans 4205*	KC425823	-	This Study
Specklinia sp. (1)	*Karremans 5988*	KF747774	KP012419	This Study
Specklinia sp. (2)	*Karremans 5989*	KF747775	KP012420	This Study
Specklinia sp. (3)	*Bogarín 9668*	KF747832	-	This Study
Specklinia sp. (4)	*Karremans 5962*	KF747828	KP012421	This Study
Specklinia sp. (5)	*Karremans 5997a*	KF747825	-	This Study
Specklinia sp. (6)	Karremans 5997b	KF747826	-	This Study
Specklinia sp. (7)	*Karremans 5996*	KF747827	KP012422	This Study
Specklinia sp. (8)	*Karremans 4823*	KC425779	KP012513	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (1)	*Karremans 5250*	KC425829	-	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (2)	*Bogarín 7401*	KC425830	-	This Study
Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans (3)	*Karremans 5699*	KC425828	-	This Study
Specklinia strumosa (Ames) Luer	*Karremans 4359*	KC425831	-	This Study
Specklinia subpicta (Schltr.) F.Barros	*Chiron 11046*	JQ306389	-	GenBank
Specklinia succulenta Bellone & Archila	*Bellone 680*	JQ306383	-	GenBank
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (1)	*Chase 5615*	AF262867	-	Pridgeon *et al*. 2001
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (2)	*Stenzel 634*	KC425853	-	Stenzel 2004
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (3)	*Karremans 3276*	KC425834	-	This Study
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (4)	*Karremans 4804a*	KC425832	-	This Study
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase (5)	*Karremans 4804b*	KC425833	-	This Study
Specklinia trichyphis (Rchb.f.) Luer	*Stenzel 620*	KC425854	-	Stenzel 2004
Specklinia trilobata (Luer) Pridgeon & M.W.Chase	*Pridgeon 112*	KF747843	-	This Study
Specklinia truncicola (Rchb.f.) F.Barros & L.R.S.Guim.	*JG 4131*	JQ306391	-	GenBank
Specklinia turrialbae (Luer) Luer (1)	*Karremans 5635*	KF747830	KP012425	This Study
Specklinia turrialbae (Luer) Luer (2)	*Karremans 5601*	KF747831	-	This Study
Specklinia vierlingii Baumbach	*Pupulin 2894*	KC425780	-	This Study
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase (1)	*Karremans 2945*	KP012452	KP012410	This Study
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase (2)	*Karremans 5944*	KP012453	-	This Study
Specklinia wrightii (Rchb.f.) Luer	*Stenzel 733*	KC425855	-	Stenzel 2004
Teagueia tentaculata Luer & Hirtz	*Pridgeon 142*	KF747844	-	This Study
Trichosalpinx notosibirica (T. Hashim.) Luer	*Pridgeon 225*	KF747845	-	This Study

DNA extraction and sequencing:—Fresh leaf and flower cuttings of about 1 cm² were obtained from all the selected individuals of each species. Each individual sample was put into a polypropylene bag with silica gel to dry for about a week after which the silica was removed and new dry silica was added. Twenty mg of every individual sample was pulverized in liquid nitrogen with a Retsch MM 300 shaker for 5 min using three bullets/glass beads. Extraction was performed following the DNEasy Plant Mini Kit extraction protocol (QIAGEN). DNA concentration for each sample was adjusted to 10 µmol/l using a NanoDrop Spectrophotometer (ND 1000).

The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using the methods and primers 17SE (ACGAATTCATGGTCCGGTGAAGTGTTCG) and 26SE (TAGAATTCCCCGGTTCGCTCGCCGTTAC) for sequencing and amplification, described by Sun *et al.* (1994). The chloroplast gene matK was amplified and sequenced using the Kew *matK* primers 2.1aF (ATCCATCTGGAAATCTTAGTTC) and 5R (GTTCTAGCACAAGAAAGTCG). Amplification was done by preparing each sample with a PCR mix composed of genomic DNA, Dream Taq Buffer, dNTPs, both primers, Dream Taq, water, and the extracted DNA. Samples were amplified in a MJ Research PTC-200 Pelthier Thermal Cycler, using a temperature profile of 94°C/5 min, followed by 34 cycles of 94°C/30 s, 55°C/30 s, and 72°C/2 min, and finally 72°C/10 min. Sanger sequencing was performed by Macrogen (http://www.macrogen.com) or BaseClear (http://www.baseclear.com) on an ABI 3730xl (Applied Biosystems).

Building the data sets:—The STADEN (Staden *et al.* 2003) package was used for editing the sequences. Where more than one base pair was equally probable among the Sanger tracers, the Unicode nomenclature (IUPAC) was used. In a few cases the two reads for one sample were too short and there was no overlap, so Pregap was unable to build a contig. In these cases, the forward and reverse sequences were merged by filling in missing positions with Ns. Sequences were aligned manually in Mesquite v2.72 (Maddison & Maddison 2007). The ends of each data set were trimmed and sequences were edited manually.

After the alignments had been edited, additional sequences were obtained from Hagen Stenzel (Stenzel 2004), and from NCBI GenBank, the latter using nBLAST. *Echinosepala aspasicensis* (Rchb.f.) Pridgeon & M.W.Chase was used as outgroup in all cases, as this taxon has been suggested to be the most earliest-branching lineage of all included species (Pridgeon *et al.* 2001).

Phylogenetic analysis:—The nrITS, *matK* and nrITS+*matK* data sets were analyzed using the Find Model web server (available at http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) which uses MODELTEST [a program designed to compare different nested models of DNA substitution in a hierarchical hypothesis-testing framework (Posada & Crandall 1998)] to calculate the model scores, based on the AIC criterion. Gaps were small and scarce and therefore treated as missing data or eliminated from the data set. Phylogenetic inference with the maximum likelihood method was done using the randomized accelerated maximum likelihood (RAxML; Stamatakis, 2006). The nrITS+*matK* data set was analyzed using RAxML v8.1.11 (Stamatakis 2014), available on the CIPRES Science Gateway (Miller *et al.* 2010), with the GTR + CAT model. The program Bayesian Evolutionary Analysis and Sampling of Trees (BEAST; Drummond & Rambaut 2007) was used to analyze nrITS (results not shown), *matK* (results not shown), and nrITS + *matK* combined matrices. BEAST estimates rooted, time-measured phylogenies inferred using strict or relaxed molecular clock models, and was therefore preferred over Bayesian analyses methods. It is also a framework for testing evolutionary hypotheses without relying on a single tree topology. Substitution and clock models were set as unlinked. The GTR + Γ model included estimated frequencies, and 10 rate categories were used to model Γ distribution for both nrITS + *matK*. A relaxed clock model was used for both partitions; however, the model used for nrITS was Lognormal, while for *matK* it was set to Exponential, a better fit for the data. The used tree prior was speciation - yule birth, and the number of generations of the Markov Chain was set to 30,000,000.

Concatenating gene sequences for phylogenetic analysis can lead to artifacts, especially when discrepancies are found between the individual gene trees (Edwards *et al.* 2007; Kubatko & Degnan 2007). Therefore we tested whether strongly supported incongruence existed between our nrITS and *matK*-based trees. In the concatenated data set, nrITS sequences are directly followed by the *matK* sequence. In some cases one of the two sequences was not available but these were then equally analyzed as missing data. This was proven not to interfere with the final results when sampling size is large enough (Wiens 2006; Karremans 2010; Karremans *et al.* 2013a). Trees were visualized

in FigTree v.1.3.1 (Rambaut 2009). Posterior probability (PP) values and bootstraps were added to the branches of the trees using the labeling option. Branches were re-ordered decreasingly.

Morphological characterization:—The morphological dissimilarities among species of *Specklinia* has led to a proliferation of generic concepts, proposing the segregation of several small species groups from the genus. With 95 species in a broad sense, *Specklinia* includes at least the type species of the genera *Acostaea* Schltr., *Cucumeria* Luer, *Empusella* Luer, *Pseudoctomeria* Kraenzl., *Sarcinula* Luer, *Sylphia* Luer, and *Tribulago* Luer (Pridgeon 2005). The type of the monotypic genus *Gerardoa* Luer was also transferred to *Specklinia* (Luer 2004), and morphological similarity would suggest that the monotypic *Areldia* Luer and *Tridelta* Luer might also belong in a broad concept of *Specklinia*. Lastly, Luer (2006) segregated species of *Pleurothallis* R.Br. subgen. *Specklinia* (Lindl.) Garay sect. *Muscariae* Luer into *Muscarella* Luer, a genus that has been mostly considered a synonym of *Specklinia*. For discussion and characterization purposes the most frequently taxonomically used morphological characters were manually added to a "per clade" summarized tree. This was done by collapsing the node subtending each clade in the consensus tree obtained from the combined nrITS+*matK* dataset in the BEAST analysis, using FigTree v.1.3.1.

Scanning Electron Microscopy (SEM):—Tissue samples of floral parts were prepared for SEM observation by harvesting tissue from flowers up to 48 h after the beginning of anthesis, fixing in FAA (ethanol 50%, acetic acid, formalin at a proportion of 18:1:1 v/v), and dehydration through a series of ethanol steps and critical-point drying using liquid CO_2. Dried samples were mounted and sputter-coated with gold and observed with a JEOL JSM-5300 scanning electron microscope, at an accelerating voltage of 10 kV. All images were processed digitally.

Macrophotography:—Color illustrations of whole flowers and pollinaria were made using a Nikon® D5100, D5300 or D7100 digital camera, a DFC295 Leica® digital microscope color camera with Leica FireCam version 3.4.1 software, and an Epson® V370 Photo Scanner. Adobe Photoshop® was used for editing images and stacking whenever necessary.

Geographical distribution patterns:—For discussion and characterization purposes, geographical labels were manually added to a "per clade" summarized tree. This was done by collapsing the node subtending each clade in the consensus tree obtained from the combined nrITS+*matK* dataset in the BEAST analysis, using FigTree v.1.3.1. Geographical (Table 8) data were taken from known distributions reported in the literature, especially by Luer (1988; 1990; 1991; 2005; 2006). Only the two countries where the species of each clade were most represented are shown. A comparison of clade composition in four distant and well-botanized regions —Antilles, Brazil, Ecuador, and Mexico— is given for comparative purposes.

Results

Nomenclature:—Taxon names follow Pridgeon (2005) unless indicated otherwise. Clades have been coded from A to J to simplify description of some species groups (Fig. 56).

The diverse analyses:—Six different analyses are presented here. Bayesian and likelihood methods are used to analyze the nrITS, *matK* and combined nrITS+*matK* matrices. The resulting consensus tree of the Bayesian and Likelihood analysis of the combined nrITS and *matK* matrices have been used to establish the clades (Fig. 56); those clades were not found back in all the tree topologies retrieved. The two support values from those analyses are given for each clade discussed here-forth. The resulting trees from the individual datasets can be found as supplementary files, their results are not presented here in detail. A summary of all the support values is given (Table 9). Differences between the separate analyses of the plastid *matK* and nuclear ITS matrices were found. Nevertheless, this is mostly due to the low resolution of the *matK* analyses and do not represent "hard" incongruences. The combined matrix mostly resulted in higher clade support and more consistent results and is thus preferred for the discussion. Concatenation of sequences was not always possible as fewer *matK* sequences were available.

Table 8. Absolute numbers of species belonging to each clade reported per country. The figures are based largely on Luer (1988; 1990; 1991; 2005; 2006).

Country	*Clade A*	*Clade B*	*Clade C*	*Clade D*	*Clade E*	*Clades A to E (Speck.)*	*Clade F (Platy.)*	*Clade G (Scaph.)*	*Clade H (Teag.)*	*Clades F to H*	*Clade I (Musca.)*	*Clades A to I*	*Clade J (Dryad.)*	*Clades A to J (Total)*
Belize	2	0	2	0	1	5	6	0	0	6	1	12	1	13
Bolivia	0	0	2	0	1	3	6	2	0	8	6	17	5	22
Brazil	0	0	2	0	0	2	4	0	0	4	2	8	17	25
Colombia	2	0	4	3	8	17	35	20	3	58	12	87	15	102
Costa Rica	16	2	6	2	6	32	16	4	0	20	7	59	5	64
Cuba	2	0	5	0	1	8	2	0	0	2	5	15	0	15
Dominican Rep.	0	0	7	0	0	7	0	0	0	0	2	9	0	9
Ecuador	1	0	10	2	4	17	56	35	10	101	29	147	18	165
Guatemala	3	1	3	0	2	9	14	1	0	15	5	29	3	32
Guyana	3	0	3	0	1	7	2	1	0	3	2	12	0	12
Haiti	2	0	6	0	1	9	0	0	0	0	2	11	0	11
Honduras	3	1	2	0	2	8	5	1	0	6	2	16	2	18
Jamaica	3	0	1	0	1	5	0	0	0	0	2	7	0	7
Mexico	4	1	4	0	1	10	8	1	0	9	2	21	3	24
Nicaragua	4	1	2	0	1	8	4	1	0	5	2	15	2	17
Panama	11	2	6	2	7	28	18	4	0	22	0	50	6	56
Peru	0	0	1	0	0	1	7	2	1	10	5	16	9	25
Puerto Rico	0	0	0	0	0	0	0	0	0	0	1	1	0	1
Venezuela	2	0	1	0	1	4	9	5	0	14	6	24	1	25

The *Specklinia* clade (Fig. 2 & 3; P.P.=61; Bp=56) is sister to a clade that includes accessions of the genera *Platystele*, *Scaphosepalum* and *Teagueia*. It can be subdivided into several subclades:

Clade A (Fig. 1 & 2; *Specklinia* subgen. *Specklinia*; P.P.=1; Bp=84) includes all species of *Specklinia* with reddish orange to greenish orange stained flowers. It includes the accessions of *Specklinia alajuelensis*, *S. barbae*, *S. blancoi*, *S. chontalensis*, *S. corniculata*, *S. displosa*, *S. dunstervillei*, *S. endotrachys* (type species of *Empusella*), *S. fulgens*, *S. gersonii*, *S. guanacastensis*, *S. lanceola* (type species of *Specklinia*), *S. lentiginosa* (type species of *Pseudoctomeria*) *S. montezumae* (type species of *Gerardoa*), *S. pfavii*, *S. psichion*, *S. remotiflora*, *S. spectabilis*, *S. tribuloides* (type species of *Tribulago*) and *S. vittariifolia*.

Clade B (Fig. 2 & 3; *Specklinia* subgen. *Sylphia*; P.P.=1; Bp=99) is sister to Clade A and contains the accessions of *Specklinia absurda*, *S. cucumeris* (type species of *Cucumeria*), *S. fuegi* (type species of *Sylphia*), and *S. turrialbae*.

Clade C (Fig. 2 & 3; *Specklinia* subgen. *Hymenodanthae*; P.P.=1; Bp=100) is sister to a clade including Clade A and Clade B (P.P.=0.97; Bp=39). It includes all species of *Specklinia* related to *S. grobyi*. The flowers of this group are characteristically whitish to yellowish, never stained orange. This includes *S. alta, S. calyptrostele, S. costaricensis, S. digitalis, S. dodii, S. gracillima, S. grobyi, S. grisebachiana, S. lichenicola, S. lugduno-batavae, S. microphylla, S. morganii, S. picta, S. pisinna, S. schaferi, S. subpicta, S. succulenta, S. trichyphis, S. truncicola* and *S. wrightii*.

Clade D (Fig. 2 & 3; *Specklinia* subgen. *Acostaea*; P.P.=1; Bp=81) contains *Specklinia cactantha*, *S. luis-diegoi*, *S. colombiana* (type species of *Acostaea*), *S. recula* and *S. trilobata*.

Clade E (Fig. 2 & 3; *Specklinia* subgen. *Sarcinula*; P.P.=1; Bp=100) includes the accessions of *Specklinia acoana, S. acrisepala, S. berolinensis, S. brighamii, S. condylata, S. scolopax, S. simmleriana* and *S. vierlingii*.

Clade F (Fig. 2 & 3; *Platystele*; P.P.=0.72; Bp=54) includes the accessions of *Platystele aurea* (type species of genus *Rubellia*), which are sister to the type clade (P.P.=0.91; Bp=72), which includes the accession of *Platystele beatricis, P. catiensis, P. caudatisepala, P. compacta* (type species of *Platystele*), *P. consobrina, P. gyroglossa, P. hirtzii, P. microtatantha, P. minimiflora, P. misasina, P. misera, P. ovatilabia, P. oxyglossa, P. propinqua, P. schmidtchenii, P. stenostachya, P. tica* and *P. ximenae*.

Clade G (Fig. 2 & 3; *Scaphosepalum*; P.P.=0.87; Bp=52) includes the accessions of *Scaphosepalum anchoriferum, S. clavellatum, S. gibberosum, S. grande, S. microdactylum, S. ovulare, S. swertiifolium, S. ursinum* and *S. verrucosum* (type species of genus *Scaphosepalum*).

TABLE 9. Support values for selected clades obtained in the six different phylogenetic reconstructions made from the nrITS, *matK* and combined (nrITS+*matK*) matrices. Each matrix was analyzed by using Bayesian (BEAST) and Likelihood (RAxML) methods. Values are presented in the for of posterior probabilities (P.P.) in case of the BEAST analyses and bootstrap values (Bp) in the case of the RAxML analyses. Not Applicable (NA) is indicated when a clade consists of a single sequence. Unsupported (UN) is indicated when a clade is not found back.

	nrITS BEAST	***nrITS RAxML***	***matK BEAST***	***matK RAxML***	***Combined BEAST***	***Combined RAxML***
Clade A	1	75	UN	UN	1	84
Clade B	0.99	78	0.98	58	1	98
Clade C	1	100	1	94	1	100
Clade D	1	86	NA	NA	1	81
Clade E	1	100	0.99	50	1	100
Specklinia (A-E)	0.98	81	UN	UN	0.65	56
Clade F	0.88	52	0.92	56	0.88	54
Clade G	0.88	62	0.61	UN	0.87	52
Clade H	NA	NA	NA	NA	NA	NA
Clade I	0.48	UN	0.98	63	0.98	89
Clade J	1	100	0.99	69	1	98
Phloeophila	1	91	UN	UN	1	88

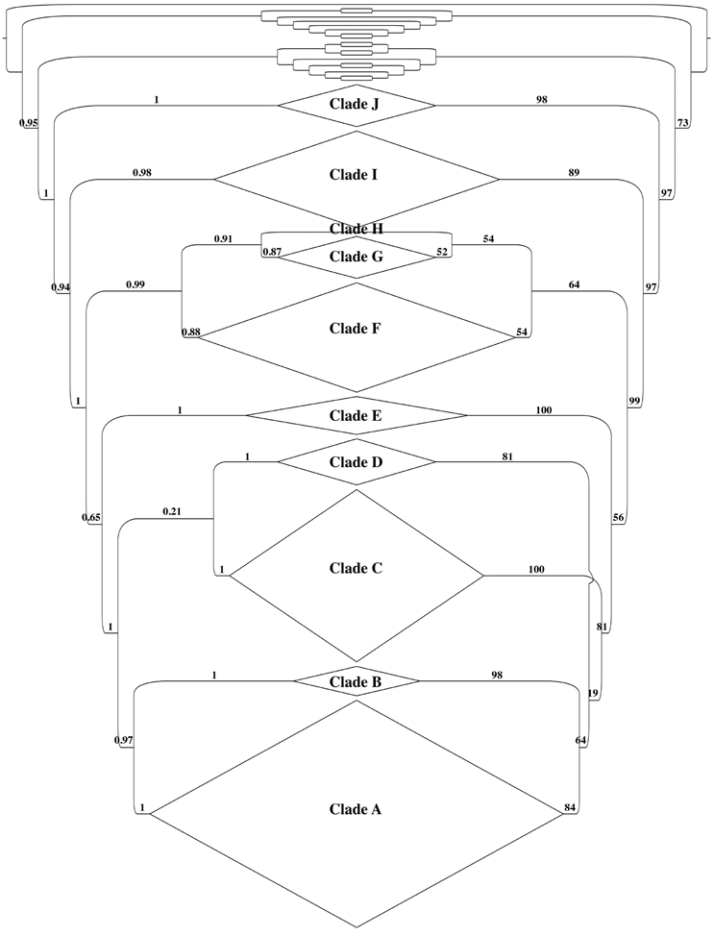

FIGURE 56. Phylogenetic relationship amongst the species of *Specklinia* and relatives inferred from the combined nrITS+*matK* dataset, summarized by clades. A. Using BEAST v1.6.0., where node values are posterior probabilities. B. using RAxML v8.1.11., where node values are bootstraps.

Clade H (Fig. 2 & 3; *Teagueia*) includes only the accession of *Teagueia tentaculata*. It is found sister to Clade *Scaphosepalum* (P.P.=0.74; Bp=54).

Clade I (Fig. 2 & 3; *Muscarella*; P.P.=0.98; Bp=89). It is sister to a highly supported clade (P.P.=1; Bp=99), which includes *Platystele*, *Scaphosepalum*, *Specklinia* and *Teagueia*. *Muscarella* includes the accessions of *Pabstiella parvifolia*, which are sister to the highly supported type clade (P.P.=1; Bp=99), that including *Specklinia alata, S. aristata* (type species of *Muscarella*), *S. cabellensis, S. claviculata, S. fimbriata, S. hastata, S. helenae, S. herpestes, S. llamachoi, S. longilabris, S. macroblepharis, S. marginata, S. megalops, S. mucronata, S. obliquipetala, S. quinqueseta, S. segregatifolia*, and *S. strumosa*.

Clade J (Fig. 2 & 3; *Dryadella*; P.P.=1; Bp=98) is sister to a highly supported clade (P.P.=0.94; Bp=97) including *Muscarella*, *Platystele*, *Scaphosepalum*, *Specklinia* and *Teagueia*. *Dryadella* includes the accessions of *Dryadella albicans, D. aviceps, D. edwallii, D. guatemalensis, D. hirtzii, D. kautskyi, D. simula, D. susanae* and *Phloeophila yupanki* (type species of *Incaea*).

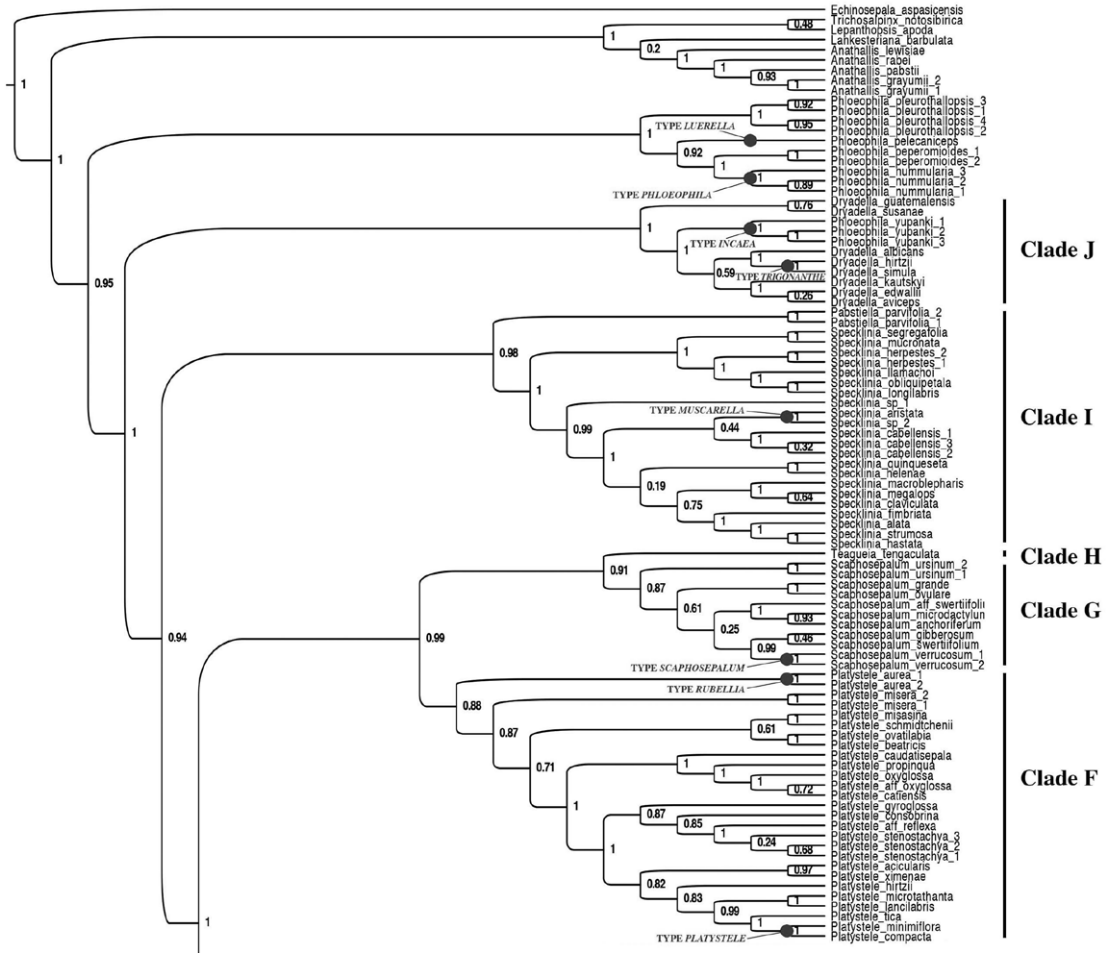

Figure 57. Phylogenetic relationship amongst the species of *Specklinia* based on a combined nrITS + *matK* dataset, using BEAST v1.6.0. Node values are posterior probabilities. A. Tree with branches transformed to be of equal length. B. Branch lengths relate to the relative number of changes.

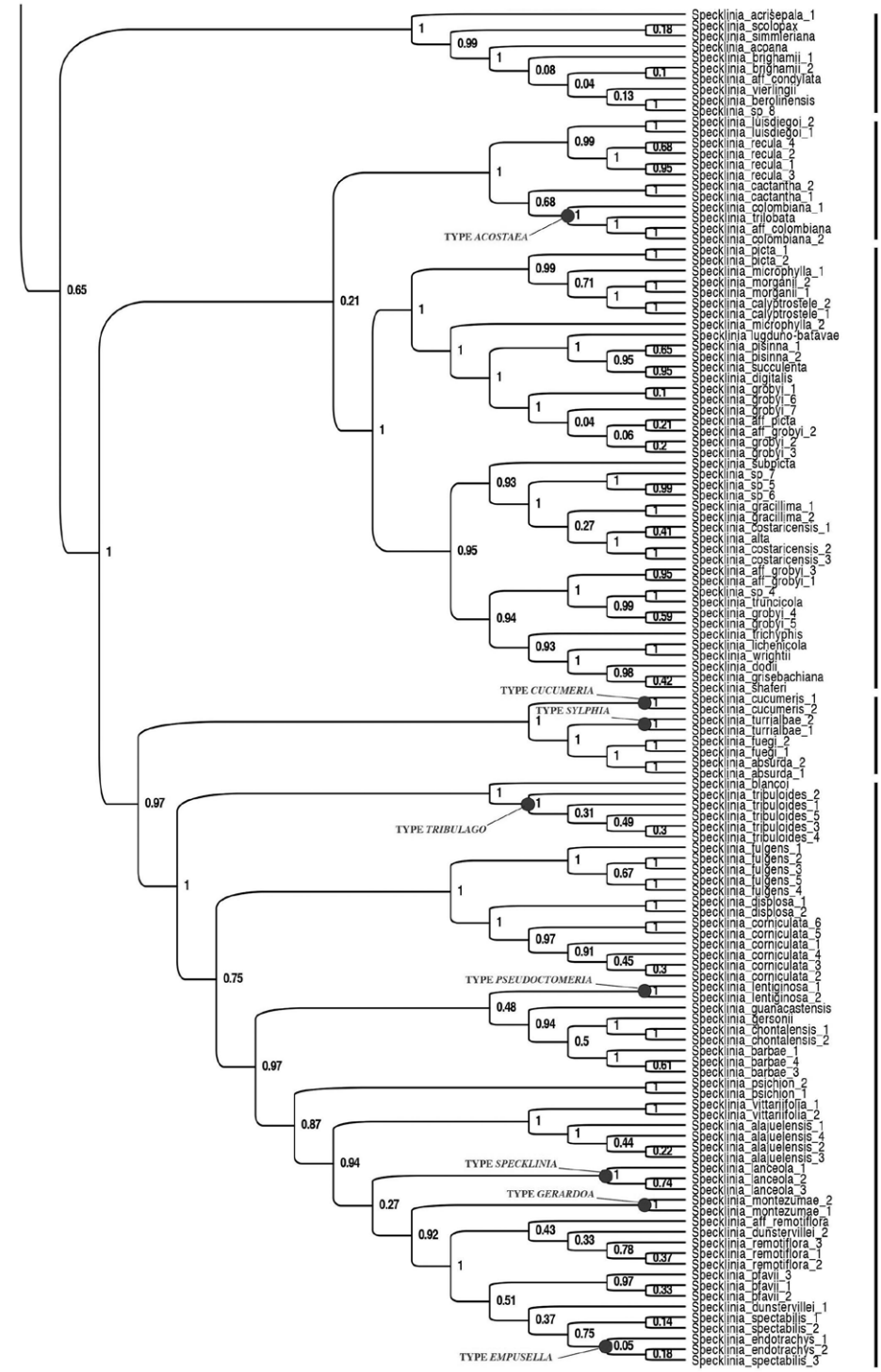

FIGURE 57. Continued

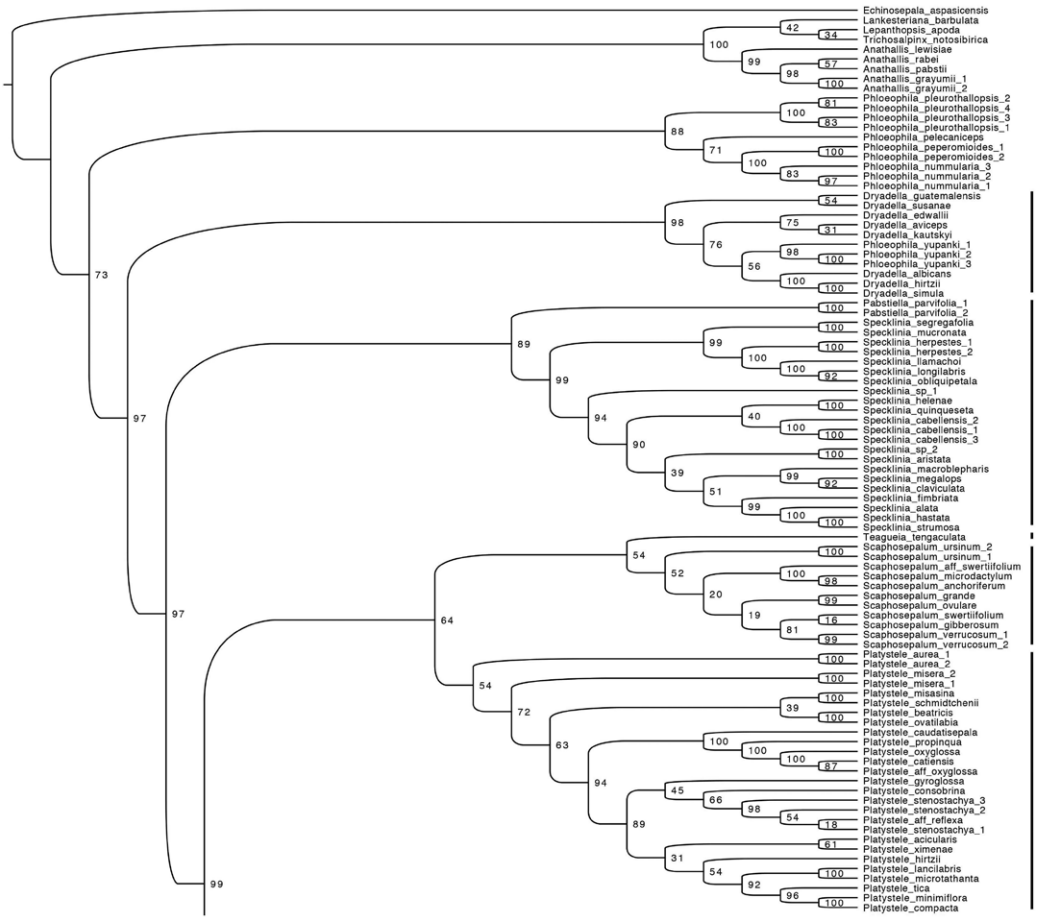

Figure 58. Phylogenetic relationship amongst the species of *Specklinia*. The tree was produced with an analysis of a combined nrITS + *matK* dataset using RAxML v8.1.11. Node values are bootstraps.

Table 10. Morphological recognition of the diverse clades within the *Specklinia* group.

Taxon	*Inflorescence*	*Flower Color*	*Pollinaria*
Dryadella (Clade J)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, streaks of stains.	Pollinia + Caudicles
Muscarella (Clade I)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, streaks of stains.	Pollinia + Caudicles
Teagueia (Clade H)	Simultaneous, several flowers open at once.	Monochrome, color varying.	Pollinia + Viscidium
Scaphosepalum (Clade G)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, streaks of stains.	Pollinia (naked)
Platystele (Clade F)	Varying from successively single-flowered to simultaneous.	Monochrome, color varying.	Pollinia + Viscidium
Specklinia subgen. *Specklinia* (Clade A)	Successive, a single flower open at the same time.	Monochrome reddish orange or yellowish orange.	Pollinia (naked)
Specklinia subgen. *Sylphia* (Clade B)	Successive, a single flower open at the same time.	Mostly whitish with some purple streaks.	Pollinia (naked)
Specklinia subgen. *Hymenanthae* (Clade C)	Simultaneous, several flowers open at once.	Mostly monochrome purple, yellow, green or whitish.	Pollinia (naked)
Specklinia subgen. *Acostaea* (Clade D)	Successive, a single flower open at the same time.	Color varying.	Pollinia (naked)
Specklinia subgen. *Sarcinula* (Clade E)	Successive, a single flower open at the same time.	Greenish yellow with purple spots, steaks of stains.	Pollinia (naked)

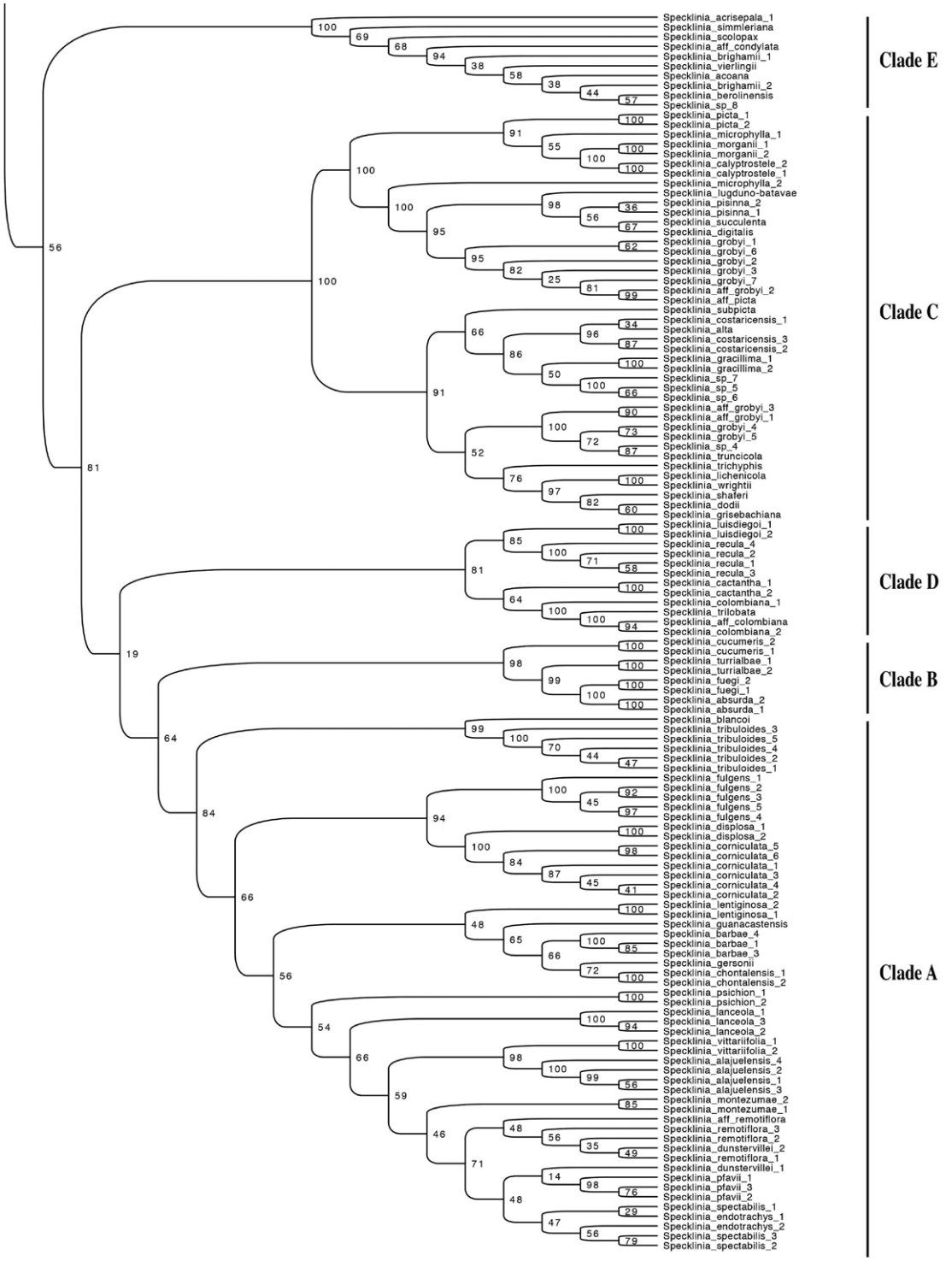

FIGURE 58. Continued

Morphology:—Morphological characterization of clades (Fig. 59; Table 10) was achieved by evaluating the available plant material or, when no entire voucher was available, by relying on the cited literature, mostly Luer (2006). Most species of *Specklinia* (Clades A though E) do share a short stem (much shorter than the leaves), obtuse petals and a ligulate-oblong lip; however, a single synapomorphy is shared by all species —the pollinia are nude. The lack of a caudicle and viscidium in *Specklinia* and *Scaphosepalum* allows for each pollinium to be free, albeit adjacent (Fig. 60). In species of *Dryadella* and *Muscarella*, pollinia are linked by a flat, granular, bilobed caudicle (whale-tail type pollinarium). In *Platystele* and *Teagueia*, pollinia lack caudicles but are linked by a drop-like viscidium (bubble-like pollinarium). The latter is associated with the apical disposition of the anther and stigma in the column (Fig. 61).

Other characters that proved most consistently distinct among the clades were inflorescence type (Fig. 62), flower coloration patterns and lip and column features. Characters such as resupination (=orientation of the flowers in such a way that the labellum is in abaxial position), so-called fasciculate inflorescences associated with a reduction in the length of the rachis, long-apiculate sepals, and prominently winged columns seem to have evolved several times independently. A sensitive lip evolved several times independently in Pleurothallidinae, but in the *Specklinia* clade it evolved only once (subgen. *Acostaea*).

Inflorescence (Fig. 62). Successively developing inflorescences, with one or few flowers open at once, are found in clades A, B, D, E, F, G and I. Simultaneously developing inflorescences, typically with several flowers open at the same time, are found in clades C, F and H. An extremely reduced rachis on which the pedicels are clustered (so-called fascicled inflorescences) is found in clades A, E, F and I.

Resupination (Fig. 62). In general species of this group have resupinate flowers, with a few exceptions per clade. Notably, for clade G non-resupination is typical.

Flower color (Fig. 63 & 64). Species of most clades have white to green flowers diversely spotted, striped or suffused with purple. Exceptions are found in clades A, C, F and H, of which the flowers are diversely colored, but mostly monochrome. Reddish orange to yellowish orange flowers are characteristic of clade A.

Lateral sepals (Fig. 63 & 64). Lateral sepals are generally convergent, forming an obtuse to acute synsepal; exceptions are found in clades B, F, H and I where the lateral sepals are free and divergent, and frequently long-apiculate. In clade G, the lateral sepals form a basally concave synsepal and are apically narrowed and thickened, usually with thickened calli on the distal portion.

Petals (Fig. 63 & 64). Simple, obtuse to acute petals are found throughout all clades except for clade I, where the petals are characteristically fimbriate and acute to caudate.

Lip. The lip of species in clades A and C is simple, ligulate-oblong. The lip of species of clade E is similar but provided with a pair of basal lobules. The lip of species in clade B is unguiculate. The lip of species of clade D has a series of complex lobes and calli, in several species it is extremely sensitive to touch. In clades F and H the lip is ovate-cordate, and in the latter it embraces the column.

Column (Fig. 61). The column of the species belonging to clades A, B, C, D, E, G, I and J is elongate and slender, with an incumbent anther and a ventral stigma. The column of species of clade F and H is short and stout, and the anther and stigma are apical. The column of species of clade C and D have a pair of prominent, rounded wings near the apex and a pair of orbicular glands at the base. In clade I the column is characteristically inornate.

Pollinia (Fig. 60 & 61). The "whale-tail" type pollinia, connected by a dry, granulose, bilobate caudicle, are only found in clades I and J. In clades F and H the pollinia are minuscule, lack caudicles and are provided with a drop-like viscidium at the base. In clade A, B, C, D, E and G the pollinia lack caudicles and a viscidium.

Geographical distribution:—The genus *Specklinia* is widespread, extending from Mexico to Bolivia and Brazil, through Central America and the Antilles. Nevertheless, geographical patterns of clade diversity can be seen in the resulting phylogenetic trees (Table 8; Fig. 65). Clades A and B are predominantly Costa Rican and Panamanian in distribution. Clade C has two disjunct centers of diversity, one in Hispaniola (Haiti and Dominican Republic) and another in Ecuador. Clade D is best represented in Colombia, while Clade E has the highest species diversity in Costa Rica and Panama. In general terms, *Specklinia* (Clades A to E) is most diverse in Costa Rica and Panama, followed by Ecuador and Colombia with about half the species. The sister genera, in clades F, G, H and I are mostly Andean in distribution, all with the highest diversity in Ecuador and Colombia. Finally, Clade J has two disjunct centers of diversity, one in Ecuador/Colombia (Andes) and another in Brazil.

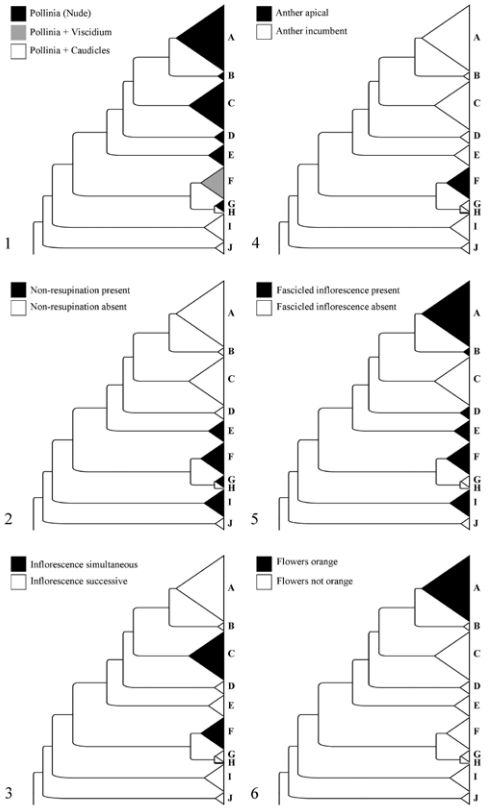

FIGURE 59. The combined nrITS + *matK* based phylogeny with the clades collapsed showing. 1: Pollinarium type. 2: Non-resupination. 3: Multi-flowered inflorescence 4: Apical anther. 5: Fascicled inflorescence. 6: Orange flowers.

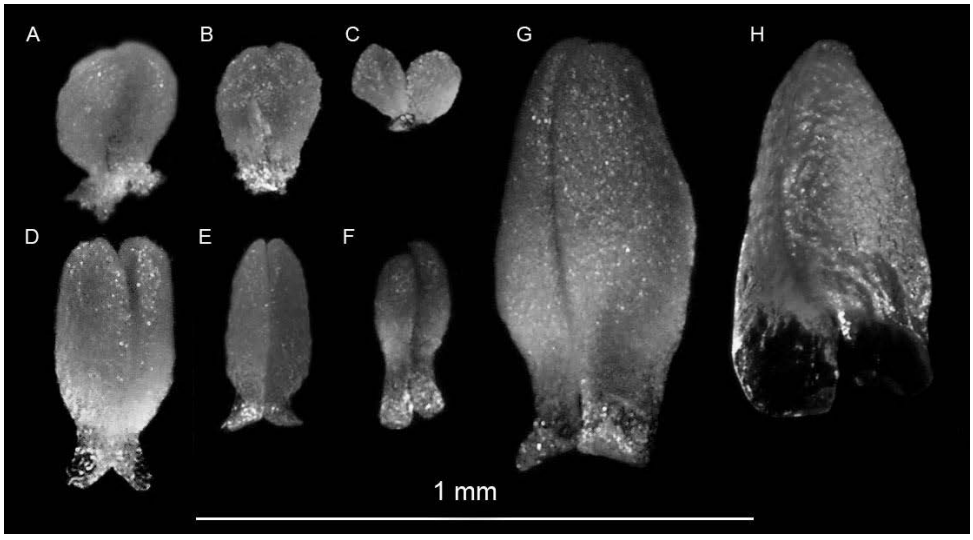

FIGURE 60. Pollinarium variation within the *Specklinia* group. Whale-tail pollinia linked by a caudicle (A-B), Bubble-like pollinia, brought together by a drop-like liquid viscidium (C), naked pollinia, adjacent but free (D-H). A: *Dryadella* (*AK6180*). B: *Muscarella strumosa* (*AK6450*). C: *Platystele* aff. *oxyglossa* (*MF789*). D: *Scaphosepalum microdactylum* (*DB10529*). E: *Scaphosepalum clavellatum* (*DB9218*). F: *Specklinia colombiana* (*DB8826*). G: *Specklinia condylata* (*MF173*). H: *Specklinia* aff. *endotrachys* (*AK5899*). Photographs by A.P. Karremans.

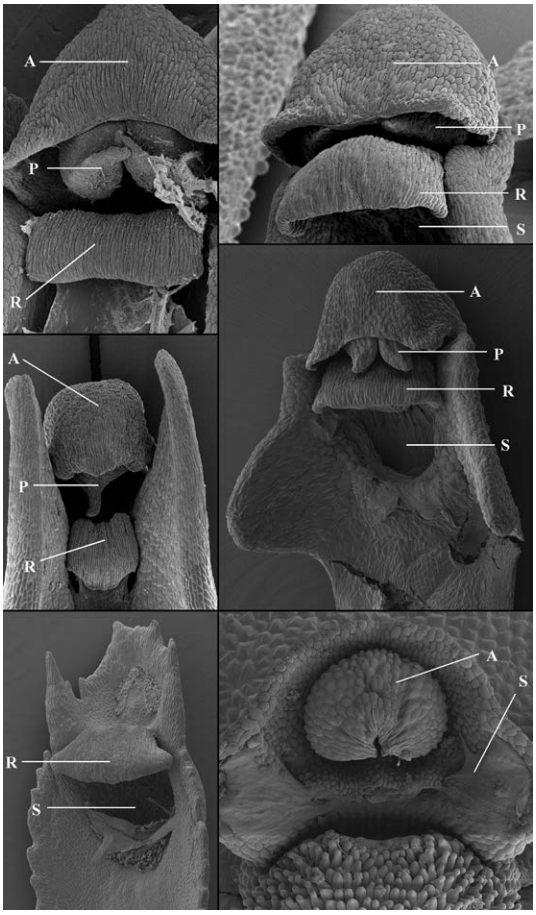

Figure 61. Column variation within the *Specklinia* group. Incumbent anther, ventral stigma covered by a large bubble-shaped rostellum, pollinia free (A-E), apical anther and stigma, rostellum reduced (F). A: *Specklinia barbae* (Clade A; *DB6483*). B: *Specklinia absurda* (Clade B; *DB9772*). C: *Specklinia grobyi* (Clade C; *AK4217*). D: *Specklinia recula* (Clade D; *AK5300*). E: *Specklinia berolinensis* (Clade E; *AK5806*). F: *Platystele* aff. *reflexa* (*AKsn*). Figure nomenclature is: A - anther cap, P - pollinia, R - rostellum, S - stigma. Photographs by A.P. Karremans.

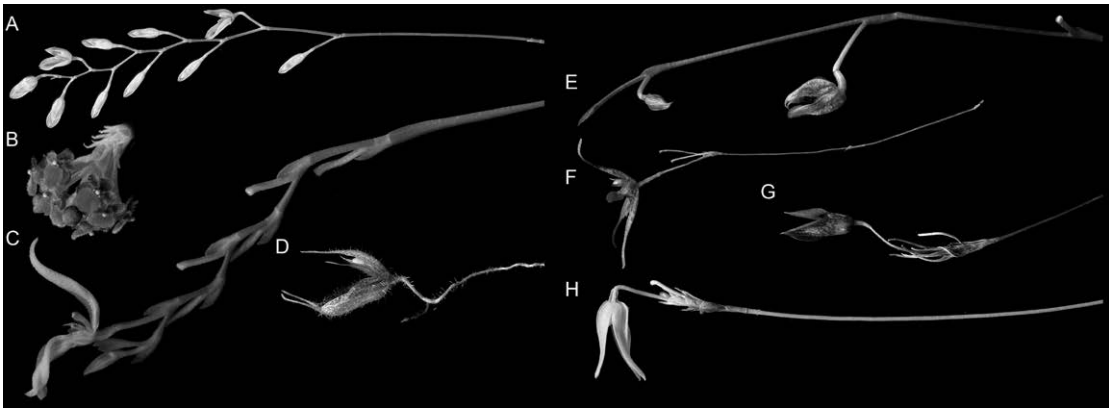

Figure 62. Inflorescence variation within the *Specklinia* group. Inflorescence simultaneous and elongate (A), simultaneous and fasciculate (B), successive and elongate (C-E), successive and fasciculate (F-H). A: *Specklinia grobyi*. B: *Platystele umbellata.* C: *Specklinia pfavii*. D: *Muscarella fimbriata*. E. *Scaphosepalum microdactylum*. F. *Muscarella strumosa*. G: *Specklinia acrisepala*. H: *Specklinia fulgens*. Photographs by A.P. Karremans, except for B, which was made by W. Driessen.

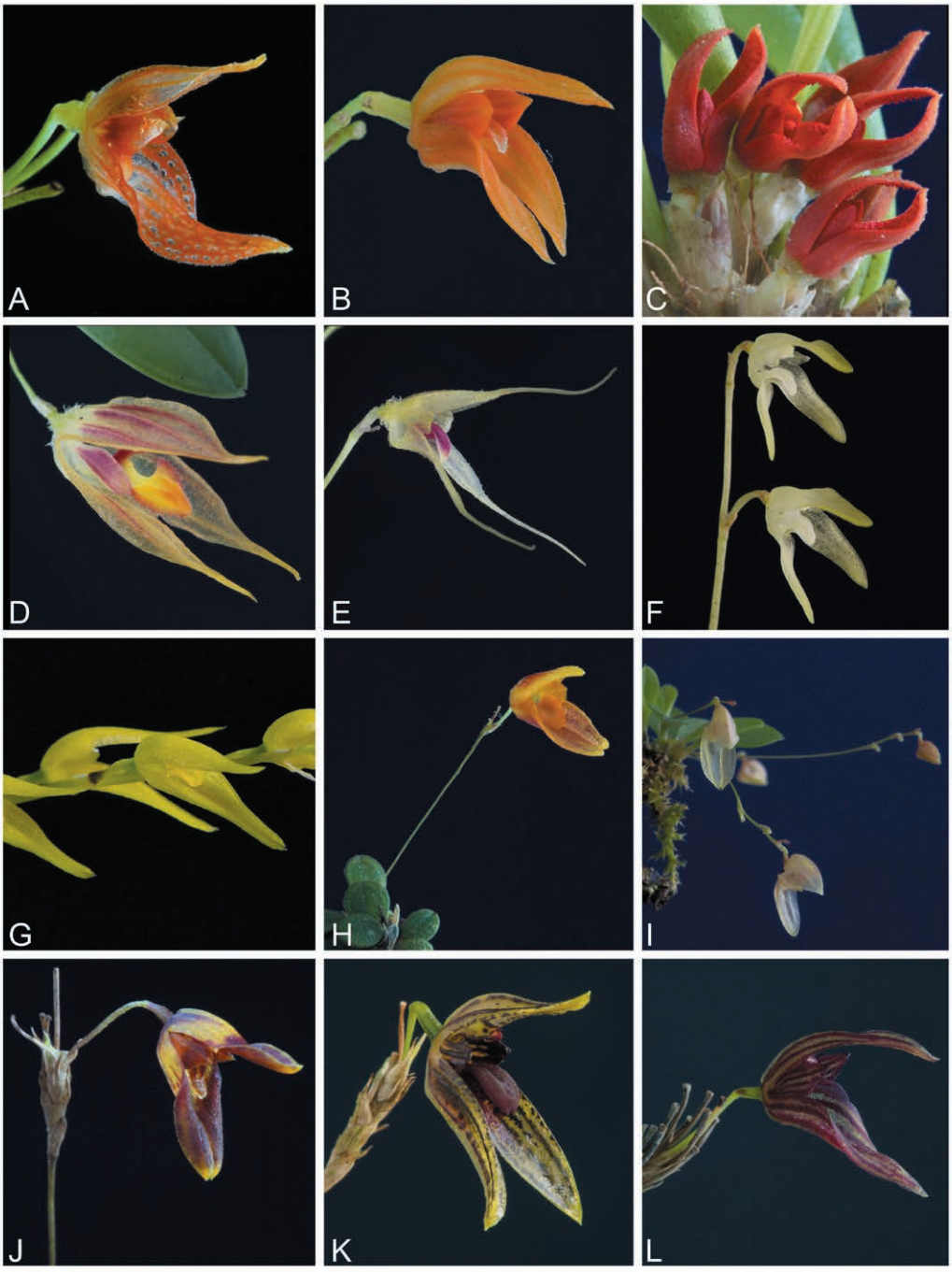

Figure 63. Representative species of each of the five clades of *Specklinia*. A-C: *Specklinia* subgen. *Specklinia* (Clade A). D-E: *S.* subgen. *Sylphia* (Clade B). F-G: *S.* subgen. *Hymenodanthae* (Clade C). H-I: *S.* subgen. *Acostaea* (Clade D). J-L: *S.* subgen. *Sarcinula* (Clade E). Photographs by A.P. Karremans.

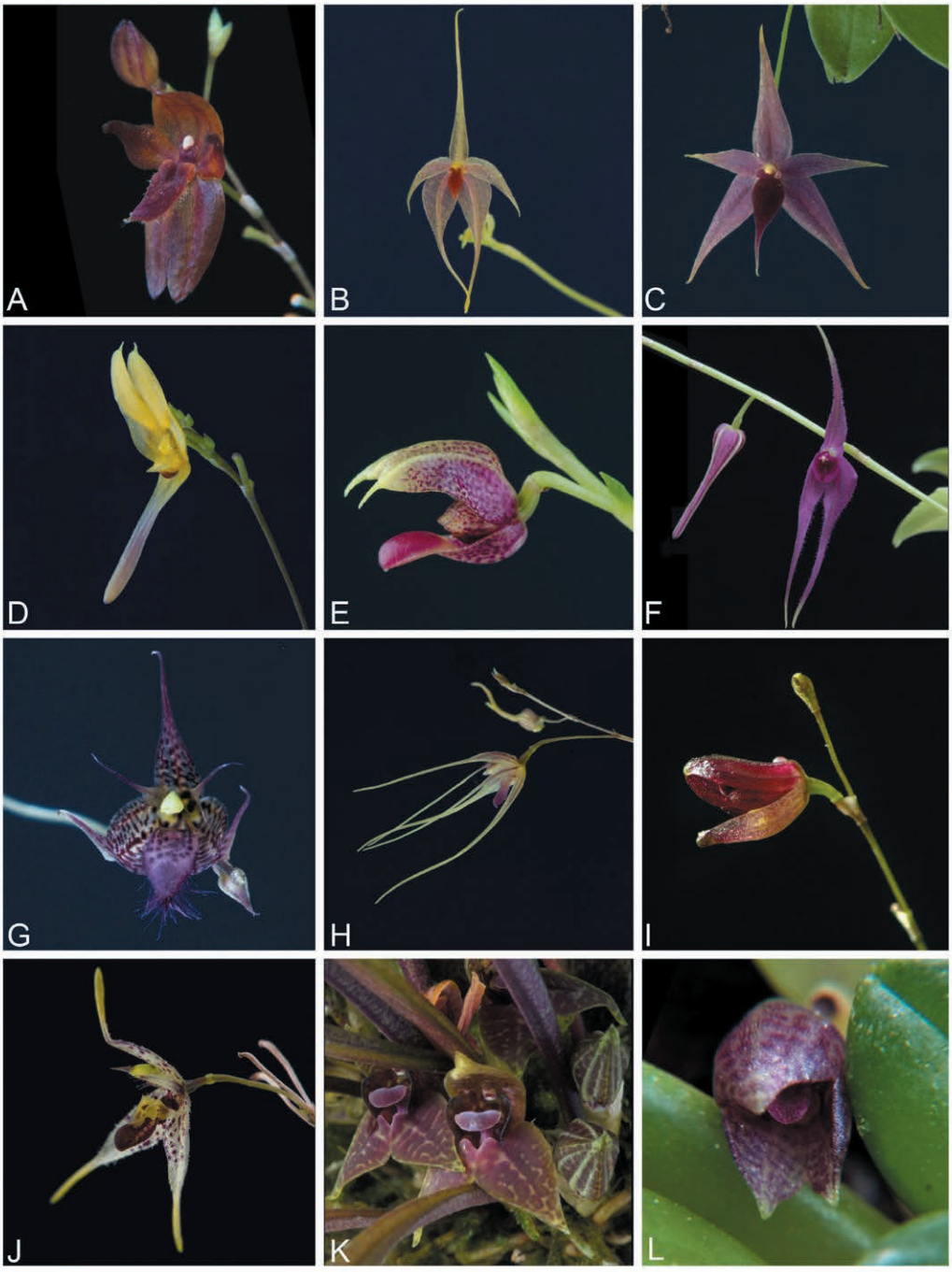

Figure 64. Representative species of each of the genera sister to *Specklinia*. A-C: *Platystele* (Clade F). D-E: *Scaphosepalum* (Clade G). F: *Teagueia* (Clade H). G-J: *Muscarella* (Clade I). K-L: *Dryadella* (Clade J). All photographs were made by A.P. Karremans, except for A, G & L, which were made by W. Driessen.

Overall distinct presence and absence patterns of species of each clade are also evident (Fig. 66). Clades A and B are absent from Brazil and Ecuador, whereas only B is absent from the Antilles. Clade C is present in all the evaluated areas, the Antilles, Brazil, Ecuador and Mexico. Clade D is absent from all except Ecuador. Clade E is absent from Brazil, rare in the Antilles and Mexico, but present in Ecuador. Clade F is rare in the Antilles and Brazil. Clade G is absent in the Antilles and rare in Brazil and Mexico. Clade H is absent from all areas except Ecuador. Clade I is present in all, but rare in Brazil. Clade J is absent from the Antilles, and present in all others. No distribution is given for Costa Rica and Panama because all clades are present except for clade H, which is endemic to the Andes.

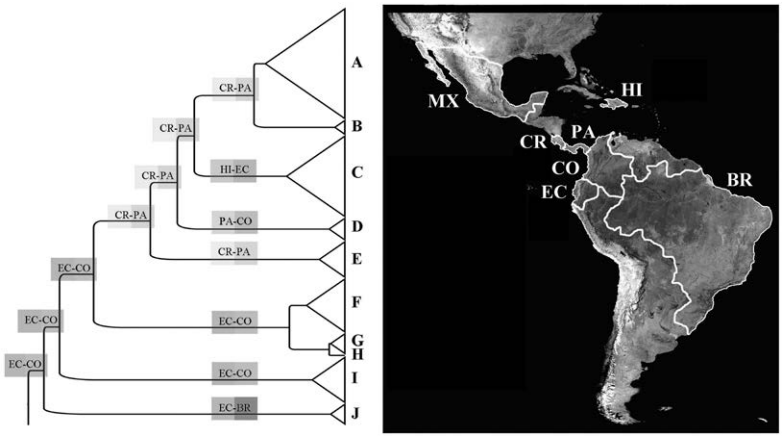

FIGURE 65. The combined ITS+*matK* based phylogeny with the clades collapsed showing the number one and two countries with most species of each clade, respectively. BR = Brazil, CO = Colombia, CR = Costa Rica, EC = Ecuador, HI = Hispaniola (Dominican Republic + Haiti), MX = Mexico and PA = Panama.

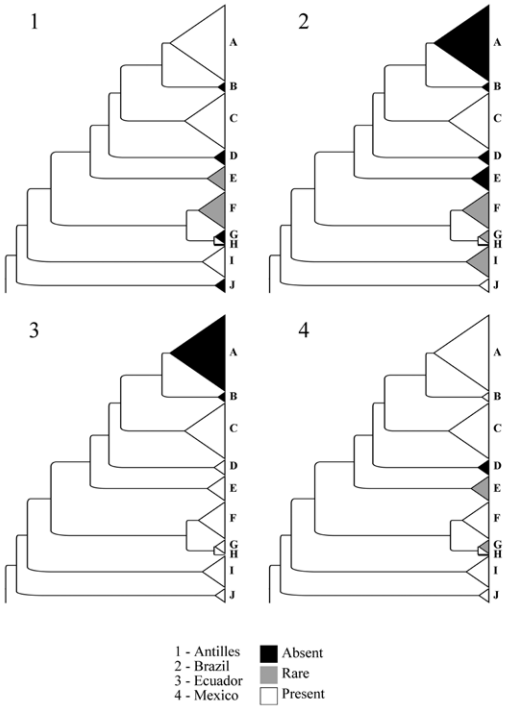

FIGURE 66.The combined nrITS+*matK* based phylogeny with the clades collapsed showing the presence/absence of species of each clade in reference regions, the Antilles, Brazil, Ecuador and Mexico. A clade is considered rare if 5% or less of its species are present.

Discussion

Our analysis with a broad sampling of *Specklinia* species proves that the genus by any current definition (Pridgeon & Chase 2001; Pridgeon 2005; Luer 2006; Barros & Trettel Rodrigues 2009) is not monophyletic, and is in need of re-circumscription. Similar issues have been encountered in most analyses of individual genera in the Pleurothallidinae (Karremans 2010; Chiron *et al*. 2012; Karremans *et al*. 2013a). The morphological dissimilarities among species of *Specklinia* led to a proliferation of generic concepts, and to the proposal of segregating several small species groups from the genus. Clade A, which includes the type species of *Specklinia*, together with clades B, C, and D forms a highly supported monophyletic group in all our analyses (P.P.=1; Bp=81). The of clades A, B, C, D and E (here *Specklinia* clade) received much higher support in the nrITS only analyses (P.P.=0.98; Bp=81) vs the combined analyses (P.P.=0.65; Bp=56), this is due to the fact that the clade is not supported by the *matK* data. Conservatively, we have chosen also to include clade E within our concept of *Specklinia*, because even though that received low support, species belonging to that clade are hardly distinguishable morphologically from other *Specklinia*. Recognizing them as a separate genus is not only not supported by our data, but would also make *Specklinia* almost undiagnosable.

In this sense, *Specklinia* includes 95 species, amongst which are the type species of the genera *Acostaea*, *Areldia*, *Cucumeria*, *Empusella*, *Gerardoa*, *Pseudoctomeria*, *Sarcinula*, *Sylphia*, *Tribulago* and *Tridelta*. Recognizing these genera reduces *Specklinia* to just a few species and requires the recognition of quite a large number of additional generic names. As *Specklinia* in a broad sense has a manageable number of species and can be easily recognized morphologically we feel it unnecessary to recognize additional segregate generic concepts. Nevertheless, we believe the five clades here included within *Specklinia* (A, B, C, D and E) are distinct enough to warrant subgeneric recognition. They all form highly supported clades (P.P.≥95; Bp≥80) and are placed on well separated branches. They are composed by morphologically similar species with unique distribution patterns, and have been mostly recognized at one time or another as distinct units (4 out of 5 have been given at least one generic name).

Clades F, G and H include the type species of the genera *Platystele*, *Scaphosepalum* and *Teagueia* respectively (Fig. 1 & 2). The three are always found together in a well supported clade (P.P.=0.99; Bp=64) that is sister to *Specklinia*. The type species of genus *Rubellia*, *R. rubella* (=*Platystele aurea*), was found sister with moderate support (P.P.=0.88; Bp=54) to a well supported clade (P.P.=0.87; Bp=72) which includes all other species of *Platystele*. Morphologically *Platystele aurea* is quite similar to other species of the genus, the plant habit being indistinguishable from other *Platystele* species, and it also share the typical apical anther and stigma. We therefore believe it best not to recognize this monotypic genus as separate. *Rubellia*, which was previously unplaced (Pridgeon 2005), is therefore placed under synonymy of *Platystele*. Genus *Teagueia*, which had been previously associated with *Platystele* (Luer 1990), was found sister to *Scaphosepalum* instead (P.P.=0.91; Bp=54). Flower morphology of *Teagueia* species is similar to some *Platystele*. Nevertheless the plant habit, which is not under the pollinator's selective pressure, is indeed more similar to *Scaphosepalum*. A broader sampling of *Teagueia* species might clear up their phylogenetic relationships in the future. The *Scaphosepalum* clade had moderate support (P.P.=0.87; Bp=52), it includes of the accessions of species of the genus, including its type.

From *Specklinia* we do exclude the species found in clade I. The clade, which includes the type species of genus *Muscarella*, was found well supported in our analyses (P.P.=0.98; Bp=89). *Muscarella* was always found sister to a clade that includes *Platystele*, *Scaphosepalum*, *Specklinia* and *Teagueia*, and thus its inclusion within *Specklinia*, as proposed by Pridgeon & Chase (2001) and Pridgeon (2005) would make the genus paraphyletic.

Clade J includes the type species of genus *Dryadella*, in a highly support (P.P.=1; Bp=98) which includes all other species ascribed to the genus. The type species of *Incaea*, a monospecific genus that was previously unplaced, is here found embedded within *Dryadella*. The two are therefore synonymized, with *Dryadella* having priority.

Incongruences between nrITS and *matK*:—The nuclear ITS and plastid *matK* are the most commonly used genetic regions for phylogenetic reconstruction in Pleurothallidinae (Pridgeon *et al.* 2001; Chiron *et al.* 2012; Karremans *et al.* 2013a; Karremans 2014, Chapter 7). Nevertheless, those studies clearly show that the faster evolving nrITS has much higher resolution than the more conserved *matK*, especially at generic level or below. In the particular case of our study, the phylogenetic reconstruction based solely on *matK* suffered from the low sequence variation

and therefore had little resolution. *Specklinia* was not retrieved as monophyletic, and within Specklinia, clade A was also not retrieved. Nevertheless, all the other clades evaluated here (B through J) were diversely supported. One noteworthy difference is that clade E was found sister to clade G (P.P.=0.62; Bp 19) instead of it being sister to the rest of *Specklinia* (clades A, B, C and D) as was found in all nrITS and combined analyses. Even though the relationship between clade E and G is not highly supported, it also not very highly supported as a member of *Specklinia*, and it should be considered in future studies if the inclusion of clade E within *Specklinia* is adequate. Morphologically the species belonging to clade E are very difficult to set apart from other Specklinia, and it would not be advantageous to segregate them for the time being.

The phylogenetic reconstructions based solely on nrITS were very similar in structure to the combined analyses. The most noteworthy difference between the nrITS and combined analyses is that the *Specklinia* clade (sum of clades A, B, C, D and E) has a much higher support when *matK* is excluded (Bp=81 vs. Bp 56). This would be expected as it was mentioned previously that the *matK* data finds affinity of clade E with clade G instead of with the *Specklinia* clades. There are other seldom incongruences between nrITS and *matK*, but they can be considered "soft", as none have high support (most nodes collapse using a threshold 50 for the bootstrap support).

Differences between Bayesian and ML:—Between the Bayesian and ML analyses it is more accurate to talk about differences rather than incongruences. Although not directly comparable, support was overall lower in the RAxML (presented as bootstrap values) vs BEAST (presented as posterior probabilities) analyses. The main nodes discussed here, clades A through J, and the *Specklinia* clade (A through E), were all retrieved with the same species composition in both analyses. One slight difference is that sister to clades A and B in the RAxML analysis is clade D (Bp=19), while in the BEAST analysis it is clade C (P.P.=0.49); both with very low support. Some differences are found amongst species groups within each of the main clades. However, these too are not highly supported (P.P.≤0.8; Bp≤60), and have no impact on the discussion here.

Recognition of groups at generic and subgeneric level:—A common misconception amongst modern authors is that DNA data will in itself resolve taxonomic issues. DNA data albeit less subjective, is also subject to the correct application of names, data reading mistakes, and adequate interpretation of the observed variation (Karremans *et al*. 2015b, Chapter 2). In our view genera should be monophyletic, but also diagnosable and informative, and at the same time should both reflect past proposals in order to keep a stable classification.

Genetically it is difficult to establish a cut off value to recognize genera. Nevertheless, genetic distance, measured by the length of branches in the phylogenetic reconstructions can be a good point of comparison. Branch lengths in other genera presented here, for instance *Dryadella*, *Muscarella*, *Platystele* and *Phloeophila*, are similar or even longer than those observed within *Specklinia*, and only those of *Scaphosepalum* are significantly shorter (Fig. 57). It is also possible to compare sequence diversity as a measure of relative number of variable sites in the sequences belonging to each clade (Table 11). The combined nrITS + *matK* matrix includes 1576 characters. After excluding the outgroups (*Echinosepala* Pridgeon & M.W.Chase, *Anathallis* Barb.Rodr., *Lankesteriana* Karremans and *Trichosalpinx* Luer) the combined matrix shows variation in 637 characters corresponding to about 40% of the total characters analyzed. *Specklinia* by itself, which includes 57 of the 95 species attributable to the genus, shows variation in 28% of the total characters analyzed. *Platystele*, of which we analyzed less than a fifth of the known species, shows variation in 20% of the characters. *Muscarella*, with about one third of the species included, shows variation in 18% of all its characters. It is likely that, with a larger sampling of *Muscarella* and *Platystele* species, both genera will have similar sequence variations as those observed in *Specklinia* or even more.

Within *Specklinia*, the lowest number of variable sites was found in clade E, with only 3%, while the highest is found in clade A, with 15%. This is undoubtably in part explained by the total and relative number of species analyzed, for clade E we analyzed only 8 species (44% of the total species that belong to the clade) while for clade A we analyzed 20 species (77% of the total). Nevertheless, not all the variation is explained by species number. In clade A for example, the ITS sequences of sister species can differ from 2% to up to 6% (Karremans *et al.* 2015b, Chapter 2; Karremans *et al.* 2015c, Chapter 3).

Table 11. Species belonging to each representative clade; comparison of the here analyzed species, the total species known to belong the that particular clade, and the percentage of analyzed species as to the total. Variable sites in the combined nrITS+*matK* dataset; variable sites amongst all sequences of specimens within each clade, the variable sites in relation to the total number of sites (base pairs in the combined matrix = 1576). Not Applicable (NA) indicates clades with a single sequence.

	Analyzed Species	*Total Species*	*Analyzed vs Total (%)*	*Variable Sites*	*Variable vs Total (%)*
Clade A	20	26	77	236	15
Clade B	4	5	80	109	7
Clade C	20	32	63	212	13
Clade D	5	12	42	169	11
Clade E	8	18	44	51	3
Specklinia (A to E)	57	95	60	445	28
Clade F	19	100	19	311	20
Clade G	9	52	17	96	6
Clade H	1	14	7	NA	NA
Clade I	19	53	36	278	18
Clade J	9	55	16	135	9
Phloeophila	4	9	44	134	9

Geographical patterns:—As defined here *Specklinia* includes 95 species found growing from Mexico to Bolivia and Brazil, through Central America and the Antilles (Fig. 65). The highest species diversity can be found in Costa Rica and Panama, and it is also there where most clade diversity is found. Species of *Specklinia* are commonly found in Ecuador, but species from subgen. *Specklinia* (clade A) and subgen. *Sylphia* (clade B) are absent or rare. Several *Specklinia* species are known from the Antilles, with the notable exception of species from subgen. *Acostaea* (clade D) and subgen. *Sylphia* (clade B). The combination of geographical and genetic data allows for the interpretation that *Specklinia* has a north-Andean (Ecuador and Colombia) ancestry and that it diversified in southern Central America (Costa Rica and Panama) and the Antilles later on. Based on the similarity of species groups, the radiation into the Antilles most likely occurred through the North of Middle America (Mexico and Guatemala) rather than through South America (Venezuela).

Platystele, *Teagueia* and *Scaphosepalum*, the sister taxa of *Specklinia* (Fig. 65), are all of north-Andean ancestry. The Andes is also the center of diversity of these three genera (*Teagueia* being endemic); only a few species venturing into Central America. Those genera are, not surprisingly, almost absent from the Antilles. The whole clade is not well represented in Brazil either, strengthening the north-Andes to south-Central America speciation pattern of this group.

Species of other genera that have been placed in *Specklinia*:—Many Brazilian endemics have been treated as *Specklinia* (Luer 2004; Barros & Trettel Rodrigues 2009), but most of those actually belong to the genera *Anathallis* and *Pabstiella* (Luer 2007; 2009; Chiron *et al.* 2012). *Specklinia* species although uncommon do occur in Brazil, but it is only members of subgen. *Specklinia* that are found there. Those species can be recognized by multi-flowered inflorescences with whitish to yellowish flowers, a linear lip (vs. trilobate in *Pabstiella*), obtuse petals (vs. acute in *Anathallis*), a prominently winged column (vs. wingless in *Pabstiella*) with a toothed apex (vs. prominently fringed in *Anathallis*) and naked pollinaria (vs. pollinaria with granular caudicles in both *Anathallis* and *Pabstiella*).

Species of *Lankesteriana* Karremans have also been treated as *Specklinia* (Luer 2004). Nevertheless, Karremans (2014, Chapter 7) showed that these species are relatives of *Trichosalpinx* and *Zootrophion* instead, and are therefore only distant relatives of *Specklinia*. *Lankesteriana* species have linear to lanceolate petals (vs. elliptic in *Specklinia*) and the androclinium is conspicuously fimbriate (vs. androclinium erose or inornate), and pollinia with caudicles (vs. without caudicles in *Specklinia*).

A few dozen species previously placed in *Pleurothallis* subgen. *Acuminatia* Luer and *Pleurothallis* subgen. *Effusia* Luer were transferred to *Specklinia* by Luer (2004). Nevertheless these species are morphologically quite different from *Specklinia* species, and DNA data shows that they belong in *Stelis* (Karremans *et al.* 2013a).

Taxonomic consequences

***Specklinia* Lindl., Gen. Sp. Orch. Pl., 8. 1830**:—Lectotype: *Epidendrum lanceola* Sw., Nov. Gen. Sp. Prodr., 123. 1788 (selected by Garay & Sweet, J. Arnold Arb. 53: 528. 1972).

Synonyms:

Acostaea Schltr., Repert. Spec. Nov. Regni Veg., Beih. 19: 283. 1923.
Areldia Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 255. 2004.
Cucumeria Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 257. 2004.
Empusella Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004.
Gerardoa Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 86. 2006.
Pseudoctomeria Kraenzl., Bull. Misc. Inform. Kew 1925(3): 116. 1925.
Sarcinula Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 201. 2006.
Sylphia Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 227. 2006.
Tribulago Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 265. 2004.
Tridelta Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 232. 2006.

Species of *Specklinia* can be recognized by having ramicauls shorter than the leaves, an abbreviated stem with an annulus, sepals and petals mostly membranaceous, lateral sepals connate for at least half their length and convergent, petals mostly obtuse and entire (never acuminate or lanceolate), wider above the middle, and a linear to sub-rectangular lip hinged to the column foot. The column has a toothed androclinium, a pair of prominent rounded wings near the apex, ventral anther and stigma. The most unique feature shared between all members of *Specklinia* are the pollinaria that are flattened towards the base and that lack both caudicles and a viscidium.

Specklinia subgen. ***Acostaea*** (Schltr.) Karremans.

Bas. *Acostaea* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 22, 102, 283. 1923. Type: *Acostaea costaricensis* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 22, 102, 284. 1923. Lectotype designated by Summerhayes (1967).

Syn. *Areldia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 255. 2004. Bas. *Pleurothallis* subgen. *Dresslera* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 38. 1986. Type: *Pleurothallis dressleri* Luer, Selbyana 3(1-2): 98-100, f. 152. 1976.

Specklinia subgen. *Acostaea* (Clade D) was highly supported and contains the species assigned to *Acostaea*, plus a few species of *Specklinia* and of *Sylphia*. The species are rare and regional, with the notable exceptions of *Specklinia* colombiana and *Specklinia recula*. They all share a tiny plant size, frequently creeping habit, elongate inflorescences and a column with prominent wings at the apex and a pair of glands on the column foot. It includes 12 species endemic to Costa Rica, Panama, Colombia and Ecuador, with a peak of diversity in Panama and Colombia.

Specklinia bicornis (Luer) Pridgeon & M.W.Chase

Bas. *Acostaea bicornis* Luer, Phytologia 54: 379. 1983.

Specklinia campylotyle (P.Ortiz) Pridgeon & M.W.Chase

Bas. *Acostaea campylotyle* P.Ortiz, Orquideología 13: 240. 1979.

Specklinia colombiana (Garay) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.

Bas. *Acostaea colombiana* Garay, Orquideología 9: 112. 1974.

Syn. *Specklinia mirifica* Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.

Bas. *Acostaea costaricensis* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 284. 1923.

The best-known species of *Acostaea*, *A. costaricensis*, was renamed *Specklinia mirifica* by Pridgeon and Chase (2001) when *Acostaea* was placed under the synonymy of *Specklinia*. Nevertheless if *Specklinia colombiana* is considered a synonym then it would have priority. If they are considered different then the next name to be applicable to this concept would be *Acostaea glandulata* P.Ortiz and not *S. mirifica*.

Specklinia coronula (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.

Bas. *Pleurothallis coronula* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 171. 1999.

Specklinia cactantha (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis cactantha* Luer, Selbyana 3: 72. 1976.
Specklinia cycesis (Luer & R.Escobar) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
Bas. *Pleurothallis cycensis* Luer & R.Escobar, Orquideología 20: 49. 1996.
Specklinia dressleri (Luer) Bogarín & Karremans, Lankesteriana 14(3): 262. 2014.
Bas. *Pleurothallis dressleri* Luer, Selbyana 3: 98. 1976.
No DNA data were available for *S. dressleri*, the type species of the monotypic genus *Areldia*, for this study. Nevertheless, plant and flower morphology suggest affinity with subgen. *Acostaea*. A creeping plant with a relatively long inflorescence with a single flower open at once is reminiscent of *S. luis-diegoi*, whereas the broad column wings and callus of the lip suggest affinity with *S. colombiana*.
Specklinia luis-diegoi (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis luis-diegoi* Luer, Revista Soc. Boliv. Bot. 3: 55. 2001.
Specklinia recula (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.
Bas. *Pleurothallis recula* Luer, Lindleyana 11: 92. 1996.
Specklinia tenax (Luer & R.Escobar) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Acostaea tenax* Luer& R.Escobar, Orquideologia 15: 123. 1982.
Specklinia trilobata (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Acostaea trilobata* Luer, Selbyana 1(3): 216. 1975.
Specklinia unicornis (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Acostaea unicornis* Luer, Phytologia 54: 379. 1983.

Specklinia subgen. ***Hymenodanthae*** (Barb.Rodr.) Karremans.

Bas. *Pleurothallis* sect. *Hymenodanthae* Barb.Rodr., Gen. Sp. Orchid. 2: 9. 1882. Lectotype: *Pleurothallis grobyi* Bateman ex Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835. Lectotype designated by Luer (1986).

Syn. *Lepanthes* sect. *Longicaulae* Barb.Rodr., Gen. Sp. Orchid. 2: 40. 1882. Type: *Pleurothallis trilineata* Barb. Rodr., Gen. Sp. Orchid. 1: 6--7. 1877. Lectotype designated by Luer (1986).

Syn. *Pleurothallis* subsect. *Longicaulae* (Barb.Rodr.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 86. 1986. Bas. *Lepanthes* sect. *Longicaulae* Barb.Rodr., Gen. Sp. Orchid. 2: 40. 1882. Type: *Pleurothallis trilineata* Barb.Rodr., Gen. Sp. Orchid. 1: 6--7. 1877. Lectotype designated by Luer (1986).

Specklinia subgen. *Hymenanthae* (Clade C) is a highly supported clade that includes the species of the *Specklinia* grobyi-picta complex. Species belonging to this clade can be recognized as species of *Specklinia s.l.* by their convergent lateral sepals, the obtuse petals, ligulate lip and pollinaria without caudicles or viscidium, and within *Specklinia* by the inflorescence that is frequently elongate, exceeding the leaves, racemose, multi-flowered, with several flowers open at once, the flowers mostly monochrome purple, yellow, green or whitish, never orange, a column with a pair of prominent, rounded wings near the apex and a pair or orbicular glands at the base, and a linear-ligulate lip. This subgenus of 32 species has the widest distribution in the genus. It is the only clade of *Specklinia* found in all areas from Mexico, through Central America and the Antilles, south to Bolivia and Brazil. The most variable and widespread of all species of the genus, *S. grobyi*, belongs to this group. All species of *Specklinia* from Brazil, as well as most species of *Specklinia* from the Antilles, Ecuador and Mexico belong to this subgenus.

Specklinia acutiflora (Ruiz & Pav.) Pupulin, Anales Jard. Bot. Madrid 69(2): 167. 2012.
Bas. *Humboldtia acutiflora* Ruiz & Pav., Syst. Veg. Fl. Peruv. Chil. 1: 236. 1798.
Specklinia alta (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004.
Bas. *Pleurothallis alta* Luer, Lindleyana 11(3): 143-144, f. 4. 1996.
Specklinia acutidentata (Cogn.) Luer = *Specklinia grobyi*
Specklinia barbosana (De Wild.) Campacci, Bol. CAOB 69-70: 27. 2008.
Bas. *Pleurothallis barbosana* De Wild, Gard. Chron. 39. 244. 1906.
Specklinia biglandulosa (Schltr.) Pridgeon & M.W.Chase = ***Specklinia grobyi***
Specklinia bipapularis (Dod) Luer = ***Specklinia schaferi***

Specklinia blepharoglossa (Luer) Luer = ***Specklinia grisebachiana***
Specklinia calyptrostele (Schltr.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis calyptrostele* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 23. 1923.
Specklinia costaricensis (Rolfe) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis costaricensis* Rolfe, Bull. Misc. Inform. Kew 1917(2): 80. 1917.
Specklinia curtisii (Dod) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis curtisii* Dod, Moscosoa 3: 111. 1984.
Specklinia digitalis (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis digitalis* Luer, Orquídea (Mexico City), n.s. 6(1): 3-4. 1976.
Specklinia dodii (Garay) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
Bas. *Pleurothallis dodii* Garay, J. Arnold Arbor. 50: 463. 1969.
Specklinia feuilletii Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 103: 311. 2005.
Specklinia florulenta (Linden & Rchb.f.) Pridgeon & M.W.Chase = ***Specklinia picta***
Specklinia flosculifera (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
Bas. *Pleurothallis flosculifera* Luer, Lindleyana 14: 113. 1999.
Specklinia formondii (Dod) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis formondii* Dod, Moscosoa 3: 116. 1984.
Specklinia gracillima (Lindl.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis gracillima* Lindl., Fol. Orchid. 9: 35. 1859.
Specklinia grisebachiana (Cogn.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
Bas. *Pleurothallis grisebachiana* Cogn. Symb. Antill. 6: 409. 1909.
Specklinia grobyi (Bateman ex Lindl.) F.Barros, Hoehnea 10: 110. 1983 (1984).
Bas. *Pleurothallis grobyi* Bateman ex Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835.
Specklinia jesupii (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 261. 2004.
Bas. *Pleurothallis jesupii* Luer, Lindleyana 14: 116. 1999.
Specklinia lichenicola (Griseb.) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.
Bas. *Pleurothallis lichenicola* Griseb., Cat. Pl. Cub.: 259. 1866.
Specklinia lugduno-batavae Karremans, Bogarín & Gravend., Blumea 59: 180. 2015.
Specklinia marginalis (Rchb.f.) F.Barros, Hoehnea 10: 110. 1983 [1984].
Bas. *Pleurothallis marginalis* Rchb.f., Bonplandia (Hannover) 3(15-16): 224-225. 1855.
Specklinia microphylla (A.Rich. & Galeotti) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.
Bas. *Pleurothallis microphylla* A.Rich. & Galeotti, Ann. Sci. Nat., Bot., sér. 3, 3: 17. 1845.
Specklinia mitchellii (Dod) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis mitchellii* Dod, Moscosoa 3: 109. 1984.
Specklinia morganii (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis morganii* Luer, Lindleyana 11: 171. 1996.
Specklinia mornicola (Mansf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis mornicola* Mansf., Ark. Bot. 22A(8): 13. 1929.
Specklinia pectinifera Luer & Hirtz, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 61. 2006.
Specklinia picta (Lindl.) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Pleurothallis picta* Lindl., Edwards's Bot. Reg. 21: t. 1797. 1835.
Specklinia pisinna (Luer) Solano & Soto Arenas, Icon. Orchid. 5--6: xi. 2002 (2003).
Bas. *Pleurothallis pisinna* Luer, Lindleyana 6(2): 105, f. 1991.
Specklinia producta (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Pleurothallis producta* Luer, Selvyana 3: 176. 1976.
Specklinia schaferi (Ames) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.
Bas. *Pleurothallis schaferi* Ames, Orchidaceae 7: 119. 1922.
Specklinia stillsonii (Dod) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Pleurothallis stillsonii* Dod, Moscosoa 3: 107. 1984.

Specklinia subpicta (Schltr.) F.Barros, Orchid Memories: 19. 2004.
Bas. *Pleurothallis subpicta* Schltr., Anexos Mem. Inst. Butantan, Secc. Bot. 1(4): 42. 1922.
Specklinia trichyphis (Rchb.f.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 264. 2004.
Bas. *Pleurothallis trichyphis* Rchb.f., Flora 48: 276. 1865.
Specklinia viridiflora (Seehawer) F.J. de Jesus, R.Miranda & Chiron, Richardiana 14: 284-285.
Bas. *Pleurothallis viridiflora* Seehawer, Die Orchidee 50: 637. 1999.
Specklinia wrightii (Rchb.f.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 265. 2004.
Bas. *Pleurothallis wrightii* Rchb.f., Flora 48: 276. 1865.

Specklinia subgen. ***Sarcinula*** Karremans.
Type: *Pleurothallis condylata* Luer, Selbyana 3:80. 1976.
Syn. *Sarcinula* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 201. 2006. Bas. *Pleurothallis acicularis* Ames & C.Schweinf., Sched. Orch. 10: 21-23. 1930.

Specklinia subgen. *Sarcinula* (Clade E) was found to be a highly supported clade, basically including the non-orange-flowered species of Luer's *Sarcinula*. The exact phylogenetic position of *Specklinia acicularis*, the type species of *Sarcinula*, remains unclear. With its narrow leaves it is an outlier amongst the other members of *Sarcinula*. However, floral coloration pattern also do not suggest affinity with subgen. *Specklinia*. Because of this uncertainty we prefer to describe subgenus *Sarcinula* with a different type species, one that is also "typical" for the group but ending up consistently in the same clade in all analyses.

Leaves are linear to narrowly obovate, the inflorescence is longer than the leaf, successive, with a single flower open at once, the rachis is reduced making the pedicels appear fasciculate, the flowers are yellowish to greenish diversely suffused, dotted or striped with purple or brown, and the lip has a pair of basal lobules. Eighteen species are distributed across Central America, Colombia and Ecuador, with the highest diversity in Costa Rica and Panama. A single species extends into Mexico and the Antilles, and one species is reported from Bolivia and another from the Guyanas. No species are known from Peru and Brazil.

Specklinia acanthodes (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis acanthodes* Luer, Selbyana 1(3): 222, f. 46. 1975.
Specklinia acicularis (Ames & C.Schweinf.) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis acicularis* Ames & C.Schweinf., Sched. Orch. 10: 21-23. 1930.
Specklinia acoana Bogarín, Lankesteriana 13(3). 2013.
Specklinia acrisepala (Ames & C.Schweinf.) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis acrisepala* Ames & C.Schweinf., Sched. Orch. 8: 22-23. 1925.
Specklinia alexii (A.H.Heller) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis alexii* A.H.Heller, Phytologia 14(1): 8-9, t. 4. 1966.
Specklinia areldii (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis areldii* Luer, Selbyana 2(4): 383-384. 1978.
Specklinia berolinensis Bogarín, Lankesteriana 13(3). 2013.
Specklinia brighamella (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis brighamella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 171, f. 22a. 1999.
Specklinia brighamii (S.Watson) Pridgeon & M.W.Chase, Lindleyana 16: 256. 2001.
Bas. *Pleurothallis brighamii* S.Watson, Proc. Amer. Acad. Arts 23(2): 285-286. 1888.
Specklinia calderae (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.
Bas. *Pleurothallis calderae* Luer, Orquideología 22(1): 53-56. 2001.
Specklinia condylata (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis condylata* Luer, Selbyana 3:80. 1976.
Specklinia icterina Bogarín, Lankesteriana 13(3). 2013.
Specklinia purpurella (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Pleurothallis purpurella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 176, f. 31a. 1999.

Specklinia rinkei (Luer) J.M.H.Shaw, Orchid Rev. 122(1308): 77. 2014.

Bas. *Sarcinula rinkei* Luer, Selbyana 30: 18, f. 35. 2009.

Specklinia scolopax (Luer & R.Escobar) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.

Bas. *Pleurothallis scolopax* Luer, Orquideología 14(2): 172. 1981.

Specklinia simmleriana (Rendle) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.

Bas. *Pleurothallis simmleriana* Rendle, J. Bot. 38(451): 274-275. 1900.

Specklinia striata (H.Focke) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 264. 2004.

Bas. *Pleurothallis striata* H.Focke, Tijdschr. Wis-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensh. 4: 63-64. 1851.

Specklinia vierlingii Baumbach, Orchideen (Hamburg) 63(5): 405-406. 2012.

Specklinia subgen. ***Specklinia***.

Type: *Epidendrum lanceola* Sw., Prodr. 123. 1788.

Syn. *Empusella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004. Bas. *Pleurothallis* subgen. *Empusella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 41. 1986. Type: *Pleurothallis endotrachys* Rchb.f., Linnea 41: 95. 1876.

Syn. *Gerardoa* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 86. 2006. Bas. *Pleurothallis montezumae* Luer, Lindleyana 11(2): 83, f. 20. 1996.

Syn. *Pleurothallis* sect. *Apodae-caespitosae* Lindl., Fol. Orchid. ~*Pleurothallis*~ 35. 1859. Type: *Epidendrum corniculatum* Sw., Prodr. 123. 1788. Lectotype designated by Luer (1986).

Syn. *Pleurothallis* subsect. *Apodae-caespitosae* (Lindl.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 84. 1986. Type: *Epidendrum corniculatum* Sw., Prodr. 123. 1788. Lectotype designated by Luer (1986).

Syn. *Pleurothallis* subgen. *Empusella* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 41. 1986. Type: *Pleurothallis endotrachys* Rchb.f., Linnea 41: 95. 1876.

Syn. *Pleurothallis* subgen. *Pseudoctomeria* (Kraenzl.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 67. 1986. Bas. *Pseudoctomeria* Kraenzl., Bull. Misc. Inform. Kew 1925(3): 116. 1925. Type. *Pleurothallis lentiginosa* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26(3--4): 446. 1899.

Syn. *Pleurothallis* sect. *Tribuloides* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 91. 1986. Bas. *Epidendrum tribuloides* Sw. Prodr. 123. 1788.

Syn. *Pseudoctomeria* Kraenzl., Bull. Misc. Inform. Kew 1925(3): 116. 1925. Bas. *Pleurothallis lentiginosa* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26(3--4): 446. 1899.

Syn. *Tribulago* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 265. 2004. Bas. *Pleurothallis* sect. *Tribuloides* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 91. 1986. Type. *Epidendrum tribuloides* Sw. Prodr. 123. 1788.

Specklinia subgen. *Specklinia* (Clade A) includes morphologically highly diverse species, which is reflected in the number of generic names proposed for such a relatively low number of species. Nonetheless they can be recognized as species of the *Specklinia s.l.* clade by their convergent lateral sepals, obtuse petals, ligulate lip and pollinaria lacking caudicles and a viscidium, and within *Specklinia* particularly for their reddish-orange stained flowers. Orange-stained flowers are rare in the other clades of *Specklinia s.l.* The inflorescence is successive, rarely with more than one flower per inflorescence open at once. Such an inflorescence is also found in species assigned to subgen. *Sarcinula* (Clade E), but the pedicels of the flowers of species in subgen. *Specklinia* remain green (vs. papery) and can further be distinguished by the lack of a pair of basal lobes at the base of the lip.

This clade consists of 27 species distributed in Central America, Colombia, Venezuela, the Guyanas and the Antilles. The highest diversity is found in Costa Rica and Panama, which together account for 23 reported species. Two species are known from Mexico, and two from the Antilles. No species of this group seem to be present in Ecuador, Peru, Bolivia and Brazil.

Specklinia alajuelensis Karremans & Pupulin, Phytotaxa 218(2): 108. 2015.

Specklinia barbae (Schltr.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.

Bas. *Pleurothallis barbae* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 104. 1923.

Specklinia barboselloides (Schltr.) Pridgeon & M.W.Chase = ***Specklinia corniculata***

Specklinia blancoi (Pupulin) Soto Arenas & Solano, Icon. Orchid. 5--6: t. 669. 2002 (2003).
Bas. *Pleurothallis blancoi* Pupulin, Caesiana 15: 1-4, f. 1-2. 2000.

Specklinia chontalensis (A.H.Heller & A.D.Hawkes) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 259. 2004.
Bas. *Pleurothallis chontalensis* A.H.Heller & A.D.Hawkes, Phytologia 14(1): 10-11. 1966.

Specklinia corniculata (Sw.) Steud., Nomencl. Bot., ed. 2, 2: 489. 1841.
Bas. *Epidendrum corniculatum* Sw., Prodr. 123. 1788.

Specklinia displosa (Luer) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis displosa* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 172, f. 24a. 1999.

Specklinia emarginata Lindl., Gen. Sp. Orchid. Pl. 8-9. 1830. = ***Specklinia corniculata***

Specklinia dunstervillei Karremans, Pupulin & Gravend., PLoS ONE 10(7): e131971(5). 2015.

Specklinia endotrachys (Rchb.f.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis endotrachys* Rchb.f., Linnea 41: 95. 1876.

Specklinia exilis (C.Schweinf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 260. 2004.
Bas. *Pleurothallis exilis* C.Schweinf., Fieldiana, Bot. 28(1): 1951.

Specklinia fulgens (Rchb.f.) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis fulgens* Rchb.f., Gard. Chron., n.s. 4(95): 516. 1875.

Specklinia gersonii Bogarín & Karremans, Phytotaxa 218(2): 112. 2015.

Specklinia glandulosa (Ames) Pridgeon & M.W.Chase, Lindleyana 16: 257. 2001.
Bas. *Pleurothallis glandulosa* Ames, Sched. Orch. 6: 60-61. 1923.

Specklinia guanacastensis (Ames & C.Schweinf.) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.
Bas. *Pleurothallis guanacastensis* Ames & C.Schweinf., Sched. Orch. 10: 27-29. 1930.

Specklinia juddii (Archila) Pupulin & Karremans, Orchidee (Hamburg) 64(6): 480. 2013.
Bas. *Empusella judii* Archila, Revista Guatemal. 15(1): 99. 2012.

Specklinia lanceola (Sw.) Lindl., Gen. Sp. Orchid. Pl.: 8. 1830.
Bas. *Epidendrum lanceola* Sw., Prodr. 123. 1788.

Specklinia lentiginosa (F.Lehm. & Kraenzl.) Pridgeon & M.W.Chase, Lindleyana 16: 258. 2001.
Bas. *Pleurothallis lentiginosa* F.Lehm. & Kraenzl., Bot. Jahrb. Syst. 26(3--4): 446. 1899.

Specklinia leptantha (Schltr.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 261. 2004.
Bas. *Pleurothallis leptantha* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 7: 107. 1920.

Specklinia minuta (Ames & C.Schweinf.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis minuta* Ames & C.Schweinf., Sched. Orch. 10: 30-32. 1930.

Specklinia montezumae (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis montezumae* Luer, Lindleyana 11(2): 83, f. 20. 1996.
Syn. Nov.: *Kraenzlinella rinkei* Luer, Harvard Pap. Bot. 16(2): 326. 2011.

We were originally going to transfer *K. rinkei* to *Specklinia* based on the description and illustration. The short stem, long, petiolate leaves, short, successive inflorescences, lamellate ovaries, orange flowers, a pair of lobes at the base of the column foot, the lip with an apiculum beneath the tip, the disc with a pair of low, serrated calli and a conspicuous, acute anther, all suggested affinity with both *S. montezumae* and *S. fulgens*. The main difference being that the flowers of *K. rinkei* are non-resupinate. In the meantime we were able to obtain photographs of the specimen from which the type material was prepared from Bryon Rinke, and those show resupinate flowers of something which we believe is conspecific with *S. montezumae*.

Specklinia pertenuis (C.Schweinf.) Karremans & Gravend., Phytotaxa 218(2): 116. 2015.
Bas. *Pleurothallis pertenuis* C.Schweinf. Bot. Mus. Leafl. 8: 83. 1935.

Specklinia pfavii (Rchb.f.) Pupulin & Karremans, Phytotaxa 63: 8. 2012.
Bas. *Pleurothallis pfavii* Rchb.f., Flora 69(34): 555. 1886.

Specklinia psichion (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 263. 2004.
Bas. *Pleurothallis psichion* Luer, Lindleyana 11(2): 89, f. 24. 1996.

Specklinia remotiflora Pupulin & Karremans, Phytotaxa 63: 11. 2012.

Specklinia spectabilis (Ames & C.Schweinf.) Pupulin & Karremans, Phytotaxa 63: 15. 2012).
Bas. *Pleurothallis spectabilis* Ames & C.Schweinf., Sched. Orch. 8: 34-35. 1925.
Specklinia tribuloides (Sw.) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Epidendrum tribuloides* Sw., Prodr. 123. 1788.
Specklinia vittariifolia (Schltr.) Pridgeon & M.W.Chase, Lindleyana 16: 259. 2001.
Bas. *Pleurothallis vittariifolia* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 26. 1923.

Specklinia subgen. ***Sylphia*** (Luer) Karremans.
Bas. *Sylphia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 227. 2006. Type: *Pleurothallis turrialbae* Luer, Lindleyana 6(2): 105, 106--108, f. 1991.
Syn. *Cucumeria* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 257. 2004. Bas. *Pleurothallis* sect. *Cucumeres* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 81. 1986. Type. *Pleurothallis cucumeris* Luer, Selbyana 5(2): 162--163. 1979.

Specklinia subgen. *Sylphia* (Clade B). The inflorescence is successive, with one flower per inflorescence open at once. Flowers are resupinate, transparent whitish to greenish, diversely suffused with purple. The lateral sepals are divergent, free, and long-apiculate. Petals are obtuse. The lip is unguiculate. Pollinia lack caudicles and a viscidium.

This little group contains five species found in Costa Rica and Panama. A single species extends northward into Guatemala and Mexico. The type species of the polyphyletic *Sylphia*, *S. turrialbae*, is included in this clade. Together with the morphologically similar *S. absurda*, *S. echinata* and *S. fuegi* they form a natural group. The type of the monotypic *Cucumeria*, *S. cucumeris*, is included in this subgenus based on DNA data. However it is different morphologically from all other members. Future studies might reveal it does not belong here. Nevertheless, all of these species are morphologically "typical" within *Specklinia*, even *S. cucumeria*, which resembles *S. lentiginosa*.

Specklinia absurda Bogarín, Karremans & Rincón, Phytotaxa 115(2): 34. 2013.
Specklinia cucumeris (Luer) Bogarín & Karremans, Lankesteriana 14(3): 261. 2014.
Bas. *Pleurothallis cucumeris* Luer, Selbyana 5(2): 162-163. 1979.
Specklinia echinata (L.O.Williams) Soto Arenas & Solano, Icon. Orchid. (Mexico) 5-6: t. 670. 2002 (2003).
Bas. *Pleurothallis fuegii* var. *echinata* L.O.Williams, Ann. Missouri Bot. Gard. 33(1): 120. 1946.
Specklinia fuegi (Rchb.f.) Solano & Soto Arenas, Icon. Orchid. 5-6: x. 2002 (2003).
Bas. *Pleurothallis fuegi* Rchb.f., Beitr. Orchid.-K.C.Amer. 97-98, t. 10. f. 11-15. 1866.
Specklinia turrialbae (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 264. 2004.
Bas. *Pleurothallis turrialbae* Luer, Lindleyana 6(2): 105, 106-108, f. 1991.

Unplaced names:

Specklinia mazei (Urb.) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 262. 2004.
Bas. *Pleurothallis mazei* Urb., Repert. Spec. Nov. Regni Veg. 15: 1004. 1917.
This is another morphologically aberrant species. We have been unable to study any living material or obtain DNA sequences of this species. There are several morphological features that would indicate an affinity to *Specklinia* rather than to *Anathallis*, including the short stem, the non-apiculate, short petals, the ligulate, hairless lip, and the pollinia lacing caudicles and a viscidium. Without further information we cannot place it more specifically.

Specklinia aurantiaca (Dod) Karremans, comb. nov.
Bas. *Cryptophoranthus aurantiacus* Dod, Moscosoa 1(1): 50. 1976.
We have been unable to study any living material or obtain a DNA sequence of this aberrant species. It was designated as type species of the monospecific genus *Tridelta* Luer. Its phylogenetic placement is currently unknown. In the drawing and description we find some similarities with other species of *Specklinia* such as the broad column wings, almost linear lip and orange-colored flowers, and without further information we cannot place it more specifically.

Excluded names:

Pabstiella integripetala (E.Pessoa & F.Barros) Karremans.

Bas. *Specklinia integripetala* E.Pessoa & F.Barros, Nordic J. Bot. 32(2): 129, 131, f.1A-E. 2014.

The authors of this species compared it to *Muscarella semperflorens* (Lindl.) Luer [as *Specklinia semperflorens* (Lindl.) Pridgeon & M.W.Chase], and distinguished it by the "acute sepals, petals with entire margin and column with a clinandrium with an entire margin". Those characters, although rare in *Muscarella* are standard within *Pabstiella*, where this species clearly belongs.

Pabstiella brasilica Luer & Toscano, Harvard Pap. Bot. 17(2): 310, 312, f.5. 2011.

Syn.: *Specklinia ianthina* E.Pessoa & F.Barros, Nordic J. Bot. 32(2): 131, 132, f.1F-J. 2014.

The illustrations of *S. ianthina* and *P. brasilica* are extremely similar and the types come from neighboring localities. No explanation as to how these species can be distinguished from each other was provided by the authors, and therefore the names are here considered synonyms. The exact phylogenetic position of *Pabstiella brasilica* and its close relative *Anathallis spiculifera* (Lind.) Luer is still not resolved (to our knowledge). We believe both are related to *Madisonia kerrii* (Braga) Luer, a monospecific genus that is yet unplaced. Despite all these uncertainties, they certainly do not belong in *Specklinia*.

Specklinia alata (A.Rich. & Galeotti) Solano & Soto Arenas = ***Muscarella marginata***

Bas. *Pleurothallis alata* A.Rich. & Galeotti, Ann. Sci. Nat., Bot., sér. 3, 3: 17. 1845.

Specklinia bulbophylloides (Schltr.) Luer = ***Muscarella zephyrina***

Bas. *Pleurothallis bulbophylloides* Schltr., Repert. Spec. Nov. Regni Veg. 27: 50. 1929.

Specklinia discalis (Luer & J.Portilla) Luer = ***Muscarella trullifera***

Bas. *Pleurothallis discalis* Luer & J.Portilla, Selbyana 23: 35. 2002.

Dryadella **Luer, Selbyana 2(2-3): 207. 1978.**:— Type: Masdevallia elata Luer, Phytologia 39(4): 199. 1978.

Synonym:

Incaea Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 87. 2006. Type: *Pleurothallis yupanki* Luer & R.Vásquez, Phytologia 55(3): 203. 1984.

Dryadella as defined by Luer (2005) and Pridgeon (2005) is accepted. As such it includes 55 species, distributed from Mexico to Bolivia and Brazil, through Central America. They are absent from the Antilles. Vegetatively they are tufted little plants with narrow fleshy leaves. The flowers are frequently yellowish spotted with brown or purple. The sepals are caudate, and connate basally. The lip is bicallous, and hinged to the column foot by a slender claw. The column is broadly winged, with a ventral anther and stigma. The pollinia are "whale-tail" type, with a pair of flat caudicles. The genus is here modified only by the inclusion of the following species:

Dryadella yupanki (Luer & Vasquez) Karremans.

Bas. *Pleurothallis yupanki* Luer & R.Vásquez, Phytologia 55: 203. 1984.

The monospecific genus *Incaea* was previously unplaced in the Pleurothallidinae. In the analyses presented here its type species is placed amongst members of *Dryadella*. Morphologically *D. yupanki* is in fact similar to other species of this genus.

Muscarella **Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 105: 94. 2006.**:— Type: *Pleurothallis aristata* Hook. Ann. Nat. Hist. 2(1): 329--330, pl. 15. 1839.

Synonyms:

Verapazia Archila, Rev. Guatemalensis 2(3): 32--33, f. 1. 1999. This name is invalid for lack of indication of the type species under articles 9 and 10.

Pleurothallis R.Br. subgen. *Specklinia* (Lindl.) Garay sect. *Muscariae* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 89. 1986.

Species of *Specklinia* sect. *Muscariae* (Luer 1986), which later formed the genus *Muscarella* (Luer, 2006), have been mostly accepted as part of *Specklinia* (Pridgeon & Chase 2001; Pridgeon 2005). However, the genus forms a well-defined clade, which cannot be included within *Specklinia*. Species of *Muscarella* can be recognized by having a stem shorter than the leaves, inflorescences that are frequently lax-flexuous but can vary from elongate to fasciculate, always develop successively, and have one or rarely a few flowers open at the same time. Flowers are resupinate. Sepals are usually caudate, the petals fimbriate and acute to caudate. The column is elongate, without prominent wings or ornamentation. The pollinia are of the "whale-tail" type, with a dry, granulose, bilobate caudicle. *Muscarella* as defined by Luer (2006) is accepted. It then included 48 species, five species are added here to bring the total number up to 53.

Muscarella cabellensis (Rchb.f.) Karremans, comb. nov.
Bas. *Pleurothallis cabellensis* Rchb.f., Linnaea 22: 832 (1850).
Muscarella hastata (Ames) Karremans, comb. nov.
Bas. *Pleurothallis hastata* Ames, Orchidaceae 2: 268. 1908.
Muscarella mucronata (Lindl. ex Cogn.) Karremans, comb. nov.
Bas. *Pleurothallis mucronata* Lindl. ex Cogn. in I.Urban, Symb. Antill. 6: 424. 1909.
Muscarella obliquipetala (Acuña & C.Schweinf.) Karremans, comb. nov.
Bas. *Pleurothallis obliquipetala* Acuña & C.Schweinf., Bot. Mus. Leafl. 6: 3. 1938.
Muscarella segregatifolia (Ames & C.Schweinf.) Karremans, comb. nov.
Bas. *Pleurothallis segregatifolia* Ames & C.Schweinf., Sched. Orchid. 8: 33. 1925.

The accessions of *Pabstiella parvifolia* Lindl. that were included here showed affinities with *Muscarella* rather than *Pabstiella*. However, the type specimen of *P. parvifolia* is Brazilian and morphologically different from Costa Rican material. We do not venture into making a combination in *Muscarella* because it might well be that the type of *P. parvifolia* is a true *Pabstiella*, whereas what we are calling by that name might be another species.

Platystele **Schltr., Repert. Spec. Nov. Regni Veg. 8: 565. 1910.**:— Type: *Platystele bulbinella* Schltr., Repert. Spec. Nov. Regni Veg. 8(191-195): 565. 1910.

Synonym:
Rubellia Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004. Bas. *Pleurothallis* subgen. *Rubellia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 20: 73. 1986. Type: *Pleurothallis rubella* Luer, Selbyana 3(3-4): 378-379, f. 289. 1977.

Platystele as defined by Luer (1990) and Pridgeon (2005) is accepted. As such *Platystele* includes 100 species that are found distributed from Mexico to Brazil and Bolivia, through Central America and the Antilles. Most species diversity is found in the northern Andes, especially Ecuador. Platystele species can be recognized by the small plants, the tiny flowers which are frequently flat with free and spreading segments, a simple lip, a short column with an apical anther and stigma. The genus is here modified only by the inclusion of the following species:

Platystele aurea Garay, Orquideología 8(3): 182. 1973.
Syn. *Pleurothallis rubella* Luer, Selbyana 3(3-4): 378-379, f. 289. 1977.
Syn. *Rubellia rubella* (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 258. 2004.

The monospecific genus *Rubellia* was previously unplaced in Pleurothallidinae. In the analyses presented here, its type species is placed sister to *Platystele* (Fig. 1 & 2). Its morphological similarities with species of *Platystele* had already been noted by Garay (1973) when he described *Platystele aurea*, a name frequently placed in synonymy of *Pleurothallis rubellia*. In our view, *Platystele aurea* and *Pleurothallis rubella* might represent two closely related yet different species. However, if considered synonyms, Garay's name has priority.

The genus *Rubellia* could have been kept separate from *Platystele* using the evidence presented here. However, the plants are similar to other members of the genus and the flowers share the apical anther and stigma and the presence of a glenion. Keeping *Rubellia* separate would not present any advantages.

***Scaphosepalum* Pfitzer, Nat. Pflanzenfam. 2(6): 136, 139. 1889[1888].**:— Type: *Masdevallia ochthodes* Rchb.f., Bonplandia 3: 70. 1855.

Scaphosepalum as defined by Luer (1988), Pridgeon (2005) and Endara (2011) is accepted. We are able to account for 52 species in the genus, with a distribution from Costa Rica to Bolivia and the Guyana Shield, and the highest diversity in the northern Andes of Colombia and Ecuador. They are distinguished especially by the non-resupinate flowers and the lateral sepals forming a basally concave synsepal and that are apically narrowed and thickened, usually with thickened calli on the distal portion