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Systematic, phylogenetic and pollination studies of *Specklinia* (Orchidaceae)

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Chapter 4

A new *Specklinia* (Orchidaceae: Pleurothallidinae) from Costa Rica and Panama

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A new species of *Specklinia* from the Cordillera de Talamanca in southern Costa Rica and western Panama is described and illustrated. *Specklinia absurda* most closely resembles *Specklinia fuegi*. It differs from that species in the pendent, single flowered inflorescence, whitish yellow sepals with red-pink veins and yellow apex, red petals with translucent margins, pandurate-trilobed, unguiculate lip with a distinct papillose isthmus below the anterior lobe, and reniform, erose, erect lateral lobes, with a Y-shaped thickened, hirsutulous apical callus. The affinities of this species to other *Specklinia* species are discussed.

Keywords: *Specklinia absurda*, *Sylphia*, Talamanca, taxonomy, *Trichosalpinx*, systematics.

Introduction

The orchid genus *Specklinia* Lindl. was redefined by Pridgeon and Chase (2001) to group the species of several infrageneric concepts of polyphyletic *Pleurothallis*, including sects. *Hymenodanthe* Barb.Rodr., *Tribuloides* Luer, *Muscariae* Luer, *P.* subgenus *Empusella* Luer, *P.* subgenus *Pseudoctomeria* Kraenzl., and genus *Acostaea* Schltr. In Pridgeon (2005), *Specklinia* was defined as a genus of around 200 species, ranging from Mexico and West Indies to Brazil and Bolivia. A later account of the genus by Barros and Trettel Rodrigues (2009) yielded about 420 binomials. However, a phylogenetic study by Karremans *et al.* (Chapter 6) suggests that only around 95 species can be included in the redefined concept of the genus to obtain monophyly. As such, *Specklinia* is still variable both in terms of vegetative and floral morphology, but can be recognized by the frequently small plants with ramicauls shorter than the leaves provided with an abbreviated stem with an annulus, the sepals and petals mostly membranaceous, the 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 is provided with a toothed androclinium, and a pair of prominent rounded wings near the apex, the ventral anther and stigma, and the nude pollinaria, which are flattened towards the base and have no caudicles or viscidium.

Luer (2006) regarded the new circumscription of Pridgeon and Chase (2001) as a polyphyletic aggregation of many taxa. Instead, he proposed to split *Specklinia* in ten genera with five major groups: *Muscarella* Luer, *Pabstiella* Brieger & Senghas, *Panmorphia* Luer, *Sarcinula* Luer and *Specklinia*, and four other monospecific genera created for the rest of the morphologically “aberrant” species in addition to *Sylphia* Luer, a genus of four species with long tailed sepals and crested or spiculate ovaries. Few authors have used these segregate genera, and circumscription of most has still to be evaluated with DNA analyses.

Materials and methods

This study was conducted at Jardín Botánico Lankester (JBL) of the Universidad de Costa Rica, Naturalis Biodiversity Center, Leiden, The Netherlands and Herbario UCH of Universidad Autónoma de Chiriquí, Panamá between April 2011 and January 2013. Specimens at JBL (from living and spirit collections), and dried and spirit material available at CR, INB, JBL, L, UCH and USJ were revised. Phenological data were recorded in the field and from cultivated specimens.

Herbarium specimens were deposited at CR, JBL, PMA, UCH and USJ. The map and georeferences for specimens were obtained using a Garmin eTrex Vista GPS and Google Earth 6.1.0 ©. Ecological zones were estimated by using the Holdridge Life Zone System (Holdridge, 1987) and the Mapa Ecológico de Costa Rica by Bolaños *et al.* (2005). Sketches of specimens were drawn with a Leica MZ 9.5 stereomicroscope with a drawing tube. Color illustrations were made using an Epson Perfection 4490 Photo Scanner, a Nikon D5100 digital camera and a DFC295 Leica digital microscope color camera with Leica FireCam version 3.4.1 software. The new species was illustrated and described from living specimens. Scanning electron microscope (SEM) micrographs were taken from flowers fixed in FAPA (Ethanol 50%, Acetic Acid, Formalin at a proportion of 18:1:1). The samples were then dehydrated through a series of ethanol steps and were subjected to critical-point drying using liquid CO₂. Dried samples were mounted and sputter-coated with gold and observed with a JEOL JSM-5300 scanning electron microscope, at an accelerating voltage of 10kV.

Phylogenetic analysis

Of the 35 sequences used here, 31 were downloaded from NCBI GenBank, where they were deposited by Pridgeon *et al.* (2001) and Chiron *et al.* (2012). Those sequences were used to place four sequences, which belong to an unpublished analysis of *Specklinia* (Chapter 6) (Table 5). Plants were obtained from the living collections at JBL, and their vouchers are kept in the spirit collections at JBL. Fresh leaf and flower cuttings of approximately 1 cm² were dried with silica gel. Samples (20 mg) were pulverized and extraction was performed by following the DNEasy extraction procedure (Qiagen). The nuclear ribosomal internal transcribed spacer (nrITS) region was amplified using the methods and primers for sequencing and amplification described by Sun *et al.* (1994), while Sanger sequencing was done commercially by Macrogen on a 96-capillary 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Inc.) using standard dye-terminator chemistry (Macrogen, Inc.).

The Staden *et al.* (2003) package was used for editing of the sequences. Contigs were exported as .fas files and opened in Mesquite v2.72 (Maddison & Maddison 2007), where they were checked for misalignments and adjusted manually. The ends of each data set were trimmed to eliminate possible erroneous data, and gaps were regarded as missing data (filled with Ns). *Phloeophila peperomioides* AF275690 was used as outgroup, as it was found to be the most distantly related of all included species (Pridgeon *et al.* 2001). The trees were produced with an analysis of the nrITS dataset of 35 sequences using BEAST v1.6.0. (Drummond & Rambaut 2007). Parameters were set to preset, except for substitution model GTR with 8 categories, clock model uncorrelated lognormal, tree prior Yule process, and number of generations 20,000,000. The resulting trees were combined using TreeAnnotator v1.6.0., where the first 100 trees were used as burn-in. FigTree v1.3.1. (Rambaut 2009) was used to edit the resulting tree. Branch fading is correlated to posterior probabilities of those branches.

12. *Specklinia absurda* Bogarín, Karremans & Rincón, Phytotaxa 115(2): 34. 2013.

The species is similar to Specklinia fuegi (Rchb.f.) Solano & Soto Arenas; however, it differs in the pendent plant, single-flowered inflorescence, whitish-yellowish sepals with red-pink veins and yellow apex, red petals with translucent margins and the pandurate-trilobed, unguiculate lip with a distinct papillose isthmus, and erect, reniform, erose lateral lobes, with a Y-shaped thickened, hirsute callus. It is also similar to Specklinia cactantha Pridgeon & M.W.Chase but differs in the erect, glabrous inflorescence, not spiculate sepals and pandurate-trilobed lip.

Type:—COSTA RICA-PANAMÁ. Puntarenas-Chiriquí: Coto Brus-Renacimiento, línea fronteriza hacia el Cerro Pando, después del mojón N.338, 8°55'11.22" N 82°43'18.18" W, 2446 m, bosque muy húmedo montano bajo, epífita en bosque primario, “*in sylvis virginis versus montium Pando in itinere ad summum Costa Rica austro-orientalis in finibus utrimque Costa Rica et Panama*”, 19 abril 2011, D. Bogarín 8711, Jiménez & Karremans (holotype, CR!; isotypes, JBL!, PMA!, UCH!, USJ!; Fig. 45 & 46).

TABLE 5. List of the 35 taxa used in the phylogenetic analysis. The vouchers, NCBI GenBank accession number and source are given. Scientific names follow Pridgeon 2005.

Taxon	Sequence Voucher	GenBank Accession Number	Sequence Source
<i>Dryadella edwallii</i> (Cogn.) Luer	MWC305	AF262824	GenBank
<i>Dryadella hirtzii</i> Luer	BGH123364	EF079367	GenBank
<i>Dryadella kautskyi</i> (Pabst) Luer	CVDB1997	JQ306380	GenBank
<i>Dryadella simula</i> (Rchb.f.) Luer	MWC1095	AF262825	GenBank
<i>Dryadella susanae</i> (Pabst) Luer	GC11240	JQ306486	GenBank
<i>Phloeophila peperomioides</i> (Ames) Garay	None	AF275690	GenBank
<i>Platystele compacta</i> (Ames) Ames	MWC5637	AF262822	GenBank
<i>Platystele misera</i> (Lindl.) Garay	MWC5625	AF262823	GenBank
<i>Platystele stenostachya</i> (Rchb.f.) Garay	MWC5618	AF262821	GenBank
<i>Scaphosepalum gibberosum</i> (Rchb.f.) Rolfe	MWC968	AF262817	GenBank
<i>Scaphosepalum grande</i> Kraenzl.	MWC1107	AF262819	GenBank
<i>Scaphosepalum swertiifolium</i> (Rchb.f.) Rolfe	MWC1383	AF262818	GenBank
<i>Scaphosepalum ursinum</i> Luer	BGH124283	EF079365	GenBank
<i>Scaphosepalum verrucosum</i> (Rchb.f.) Pfitzer	MWC1331	AF262820	GenBank
<i>Specklinia absurda</i> Bogarín, Karremans & R.Rincón	DB9772 (JBL-Spirit)	KC425826	Karremans <i>et al.</i> (unp.)
<i>Specklinia absurda</i> Bogarín, Karremans & R.Rincón	DB8711 (JBL-Spirit)	KC425827	Karremans <i>et al.</i> (unp.)
<i>Specklinia brighamii</i> (S. Watson) Pridgeon & M.W.Chase	SOL761	AF262925	GenBank
<i>Specklinia condylata</i> (Luer) Pridgeon & M.W.Chase	MWC6808	AF262873	GenBank
<i>Specklinia costaricensis</i> (Rolfe) Pridgeon & M.W.Chase	MWC5612	AF262862	GenBank
<i>Specklinia costaricensis</i> (Rolfe) Pridgeon & M.W.Chase	MWC5636	AF262863	GenBank
<i>Specklinia fimbriata</i> (Ames & C.Schweinf.) Solano	SOL769	AF262924	GenBank
<i>Specklinia fuegi</i> (Rchb.f.) Luer	AK 5600 (JBL-Spirit)	KC425786	Karremans <i>et al.</i> (unp.)
<i>Specklinia fulgens</i> (Rchb.f.) Pridgeon & M.W.Chase	MWC5630	AF262872	GenBank
<i>Specklinia grobyi</i> (Bateman ex Lindl.) F.Barros	GC09357	JQ306388	GenBank
<i>Specklinia grobyi</i> (Bateman ex Lindl.) F.Barros	MWC1093	AF262860	GenBank
<i>Specklinia grobyi</i> (Bateman ex Lindl.) F.Barros	GC04524	JQ306485	GenBank
<i>Specklinia lanceola</i> (Sw.) Lindl.	MWC1433	AF262861	GenBank
<i>Specklinia lanceola</i> (Sw.) Lindl.	AP <i>s.n.</i>	KC425838	Pridgeon & Chase 2002
<i>Specklinia mirifica</i> Pridgeon & M.W.Chase	MWC6800	AF262865	GenBank
<i>Specklinia montezumae</i> Luer (Luer)	AK229 (JBL-Spirit)	KC425811	Karremans <i>et al.</i> (unp.)
<i>Specklinia picta</i> (Lindl.) Pridgeon & M.W.Chase	CVDB2146	JQ306384	GenBank
<i>Specklinia picta</i> (Lindl.) Pridgeon & M.W.Chase	GC06131	JQ306385	GenBank
<i>Specklinia remotiflora</i> Pupulin & Karremans	MWC1303	AF262859	GenBank
<i>Specklinia subpicta</i> (Schltr.) F.Barros	GC11046	JQ306389	GenBank
<i>Specklinia tribuloides</i> (Sw.) Pridgeon & M.W.Chase	MWC5615	AF262867	GenBank

Epiphytic, caespitose herb, up to 2.5 cm long. Roots flexuous, to 1 mm in diameter. Ramicauls erect, up to 1 cm long, enclosed by 2 tubular sheaths up to 5 mm long. Leaves suberect, elliptic, obtuse, emarginate, with an apicule, lenticular, 1.0–2.2 × 0.5–0.8 cm, cuneate at the base, narrowing into an indistinct petiole less than 3 mm long. Inflorescence racemose, single-flowered, pendent, glabrous, shorter or as long as the leaves, 1.0–1.5 cm long; peduncle, up to 1.1 cm long, rachis 1 mm long. Floral bracts ovate, acute, conduplicate, membranaceous, up to 1.0 mm long. Pedicels 8 mm long, persistent. Ovary to 1.5 mm long, carinate, echinate. Flowers with the sepals whitish-yellowish with red-purple veins, apex yellow, petals red with translucent margins, lip with the lateral lobes reddish, midlobe yellow with margin pinkish white and column whitish-greenish. Dorsal sepal ovate to elliptic, acuminate, entire, dorsally with three keels, concave, 9.2 × 3.5 mm. Lateral sepals connate up to 4.5 mm into an ovate, entire, dorsally keeled lamina 11.3 × 5.7 mm, the acuminate, filiform apices to 2.5 mm long. Petals spatulate-lanceolate, acute, entire, 4.3 × 1.6 mm. Lip unguiculate, pandurate-trilobed, 4.8 × 4.0 mm, with a distinct isthmus below the midlobe, lateral lobes reniform, erose, erect in natural position, 1.4 × 1.8 mm, isthmus quadrate, papillose, 0.9 × 0.9 mm, midlobe triangular, puberulent, erose, 2.8 × 2.1 mm, with a Y-shaped, thickened, hirsute callus from the middle towards the apex, attached to the column foot. Column cylindrical, footed, arcuate, entire apically, with a pair of minute calli at base, anther apical and stigma ventral. Pollinia two, ovoid, without caudicles or viscidium. Anther cap rounded, cucullate. (Fig. 47A–E)

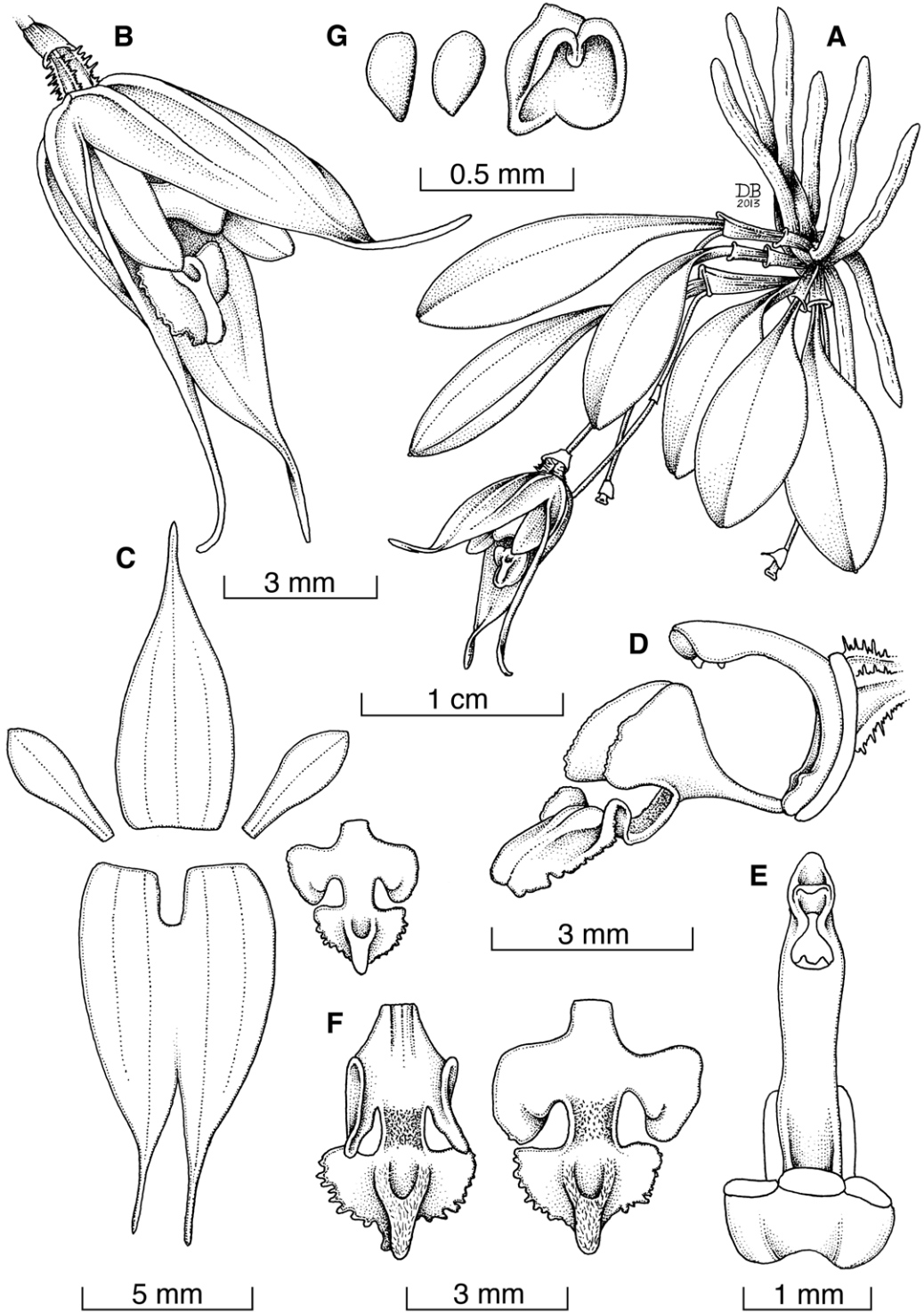


FIGURE 45. *Specklinia absurda*. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Column, front view. F. Lip, natural position and spread. G. Pollinarium and anther cap. Drawn from the holotype by D. Bogarin.

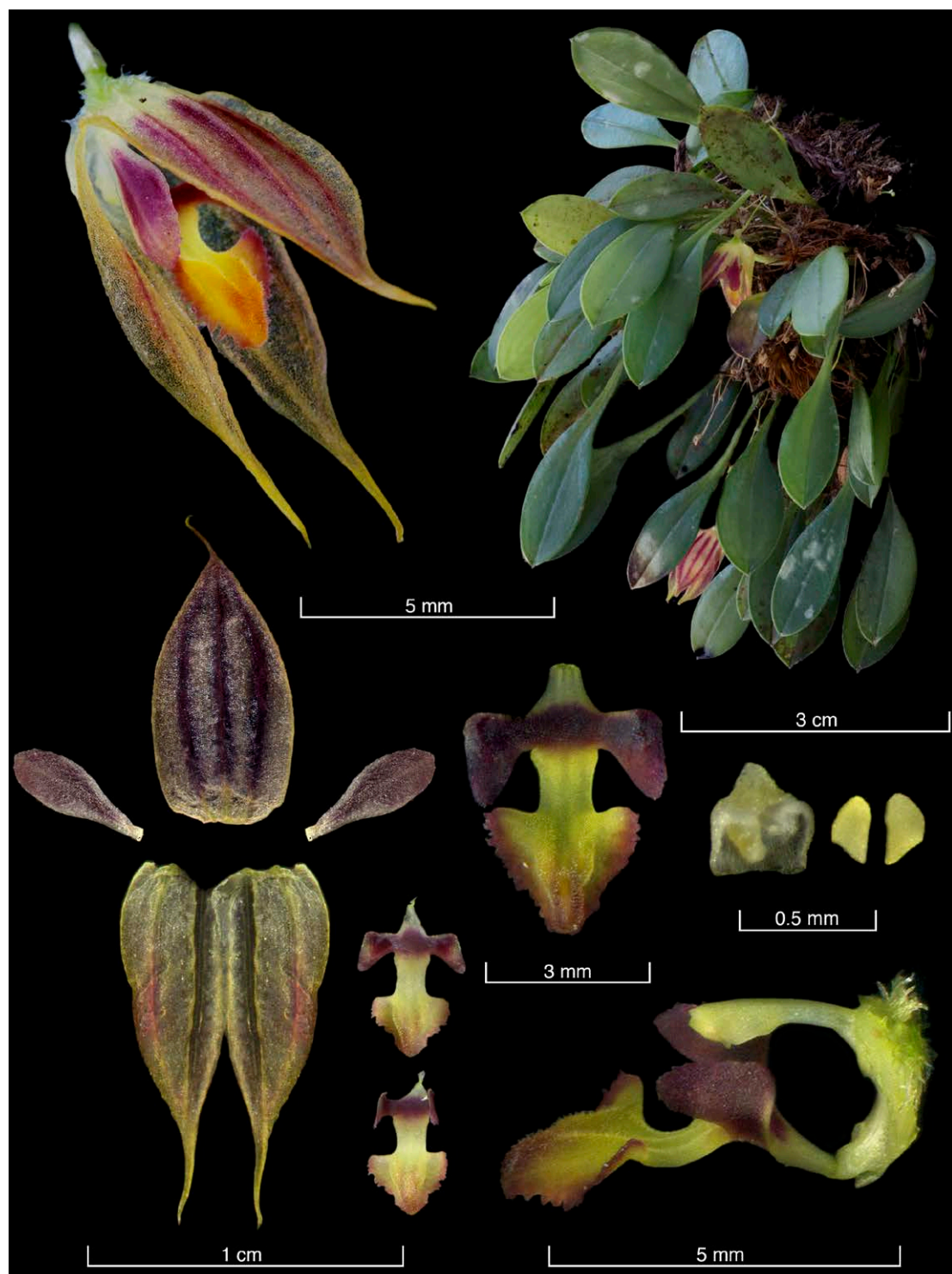


FIGURE 46. Lankester Composite Dissection Plate (LCDP) of *Specklinia absurda*. A. Habit. B. Flower. C. Dissected perianth. D. Column and lip, lateral view. E. Lip, spread. F. Pollinarium and anther cap. Based on photographs of Bogarin *et al.* 9772 (JBL) by A.P. Karremans and D. Bogarin.

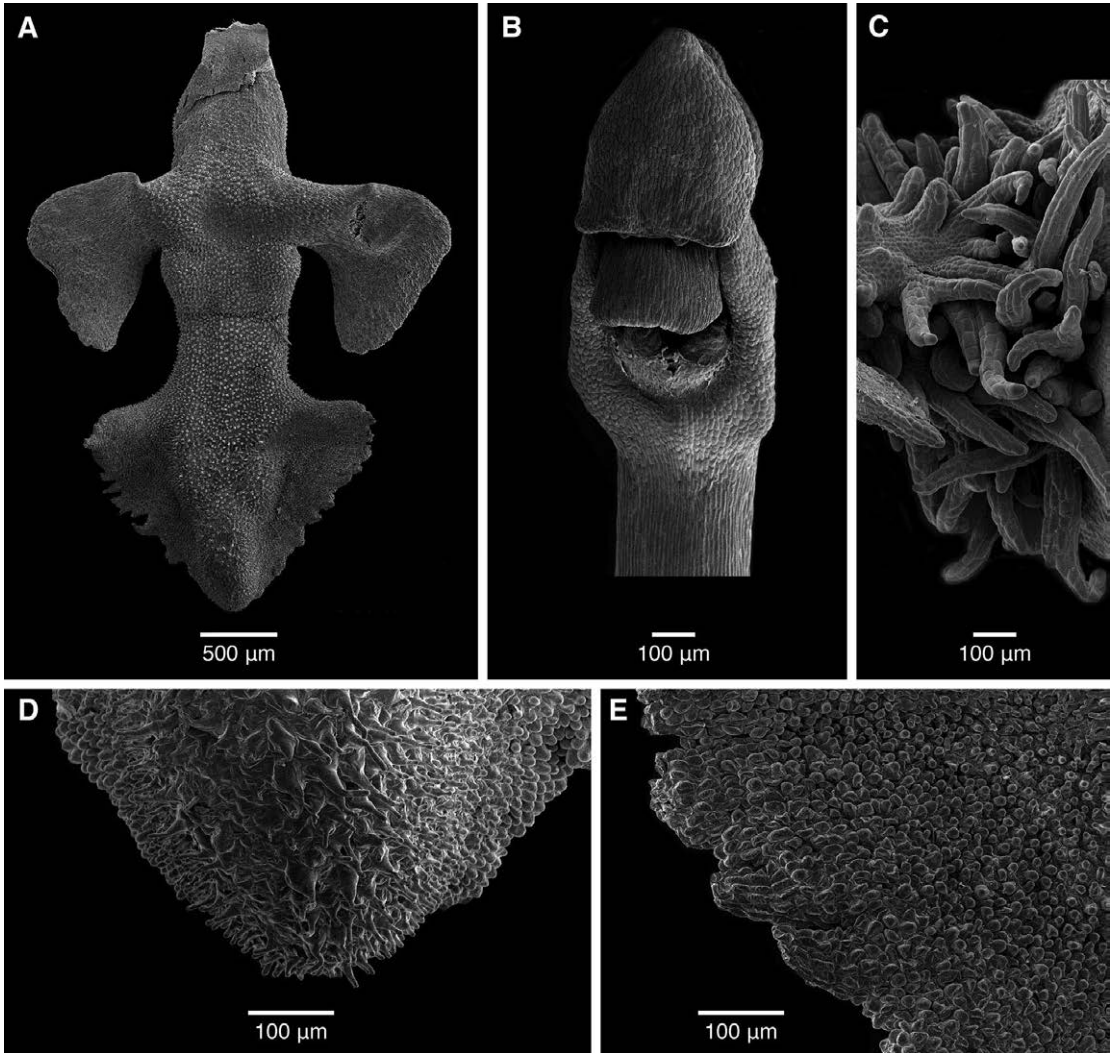


FIGURE 47. SEM images of micromorphology of *Specklinia absurda*. A. Lip, flattened. B. Column front view showing the anther cap, rostellum and stigma. C. Surface of the echinate ovary showing the cellular projections. D. Lip apex showing the epidermal cells of the apex of the callus. E. Basal lip lobe margin showing the shape of the papillate epidermal cells. Voucher specimen: *Bogarín et al. 9772* (JBL). By A.P. Karremans.

Distribution:—Endemic to the Cordillera de Talamanca in southern Costa Rica and western Panama (Fig. 48).

Habitat and ecology:—Epiphytes forming large colonies on main trunks in oak forest mostly in shaded spots in wet forest (Holdridge 1987) at around 2400–2550 m elevation.

Etymology:—From the Latin *absurdum*, “absurd, illogical, out of tune, contrary to common sense” in reference to the flower morphology, especially the lip, which seems exceptional when compared to its closest relatives.

Other material examined:—COSTA RICA. Limón: Talamanca, Bratsi, Parque Internacional La Amistad, Valle del Silencio, orillas del Río Terbi cerca del andarivel, 9°06'41.81" N 82°57'42.44" W, 2462 m, bosque pluvial montano, 16 agosto 2012, *Bogarín 9864, Fernández, Godínez, Karremans, Kruizinga & C. M. Smith* (JBL-spirit). Límite entre

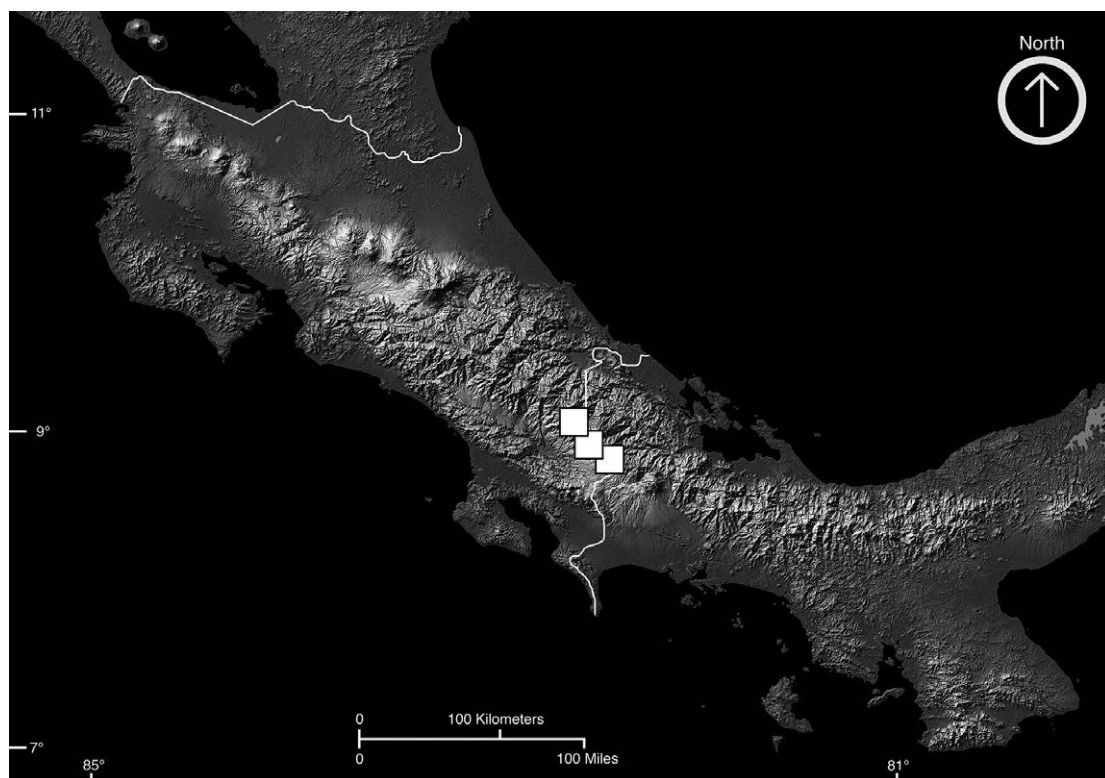


FIGURE 48. Distribution of *S. absurda* in Costa Rica and Panama, by D. Bogarín.

Limón y Puntarenas: Talamanca-Buenos Aires, Bratsi-Potrero Grande, Parque Internacional La Amistad, Sector Altamira, sendero al Valle del Silencio, Cerro Hoffman, sobre la divisoria de aguas, $9^{\circ}05'38.2''$ N $82^{\circ}58'37.73''$ W, 2553 m, bosque pluvial montano, 14 agosto 2012, *Bogarín 9772, Fernández, Godínez, Karremans, Kruizinga & Smith* (JBL-spirit).

Notes:—This species is similar to *Specklinia fuegi* (Rchb.f.) Solano & Soto Arenas. It differs mainly in the pendent, single-flowered inflorescence (rather than erect, few-flowered), whitish-yellowish sepals with red-pink veins and yellow apex, red petals with translucent margins (rather than white-cream) and pandurate-trilobed, unguiculate lip with a distinct papillose isthmus below the anterior lobe, and reniform, erose lateral lobes, erect in natural position (Fig. 47A), with a Y-shaped thickened, hirsute, papillose apical callus (Fig. 47D, E; rather than a simple, smooth lip without an isthmus). *Specklinia absurda* is also similar to *Specklinia cactantha* (Luer) Pridgeon & M.W.Chase and *S. turrialbae* (Luer) Luer. The main differences among the species related to *S. absurda* are summarized in Table 6 and can be observed in Fig. 49.

TABLE 6. Comparison of the species of *Specklinia* related to *S. absurda*.

Character	<i>S. absurda</i>	<i>S. cactantha</i>	<i>S. fuegi</i>	<i>S. turrialbae</i>
Inflorescence	creeping or pendent, single-flowered, glabrous	erect, single-flowered, spiculate	erect, successively flowered, glabrous	erect, single-flowered, glabrous
Sepals	entire, acuminate, white suffused with purple	spiculate externally, long acuminate, white-rose	entire, acuminate, white-cream	entire, filiform, white
Synsepal size	11.3 x 5.7 mm	11.0 x 3.5 mm	7.5 x 3.0 mm	10 x 3 mm
Petals	acute, red-purple	obtuse or truncate-retuse, pale green with rose	obtuse, greenish-white	obtuse to acute, white-yellowish
Lip	pandurate-trilobed, with a distinct isthmus, erose	oblong-ovate, without isthmus, entire	subtrilobed, without isthmus, entire	subtrilobed or elliptic, without isthmus, entire
Lip callus	Y-shaped, thickened, pilose	a pair of low carinae, entire	a pair of marginal carinae, entire	a pair of marginal carinae, entire

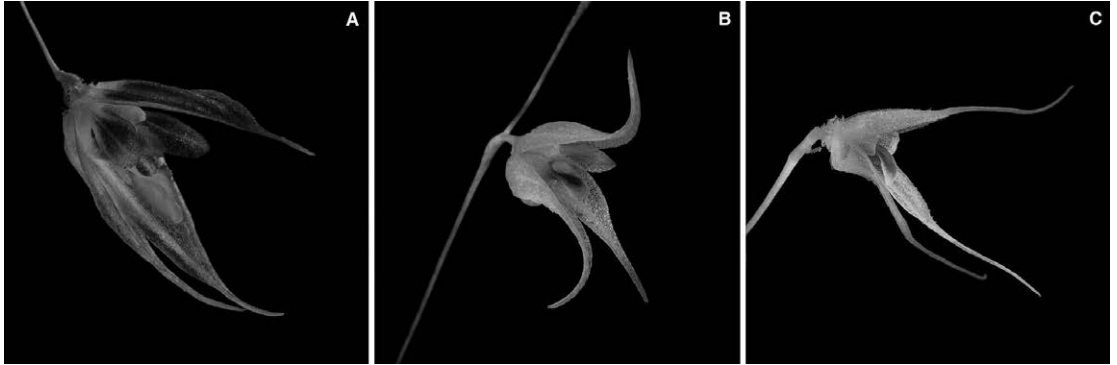


FIGURE 49. Flower morphology: A. *Specklinia absurda* (Bogarín 9772, JBL). B. *Specklinia fuegii* (Karremans 5600, JBL). C. *Specklinia turrialbae* (Karremans 5635, JBL). A-B by D. Bogarín. C by A.P. Karremans.

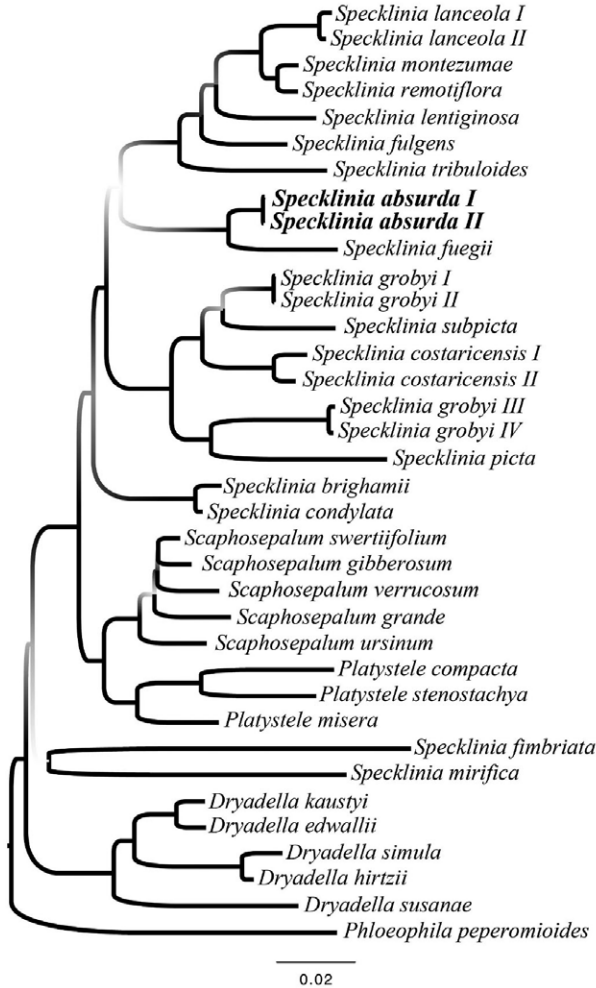


FIGURE 50. Phylogenetic placement of *S. absurda*. The trees were produced with an analysis of the ITS dataset of 35 sequences using BEAST v1.6.0. High posterior probabilities are viewed as “hard” branches (above 0.9), while low support can be seen as disappearing branches (below 0.5). Tree edited by A.P. Karremans using FigTree v.1.3.1.

Phylogenetic Placement:—Placing *S. absurda* among its closest relatives based on morphology proved to be a difficult task. The plant habit resembles species of the *S. grobyi* (Bateman ex Lindl.) Barros complex with elliptic-ovate or suborbicular thick leaves, whereas the pendent, single-flowered inflorescence and acuminate sepals resemble those of *S. fimbriata* (Ames & C.Schweinf.) Solano and other species placed in *Muscarella*. However, *S. absurda* lacks the erect multi-flowered inflorescence, linear lip and glabrous ovary of the *S. grobyi* complex, and the loose raceme, denticulate, fringed or fimbriate petals, and pollinaria with caudicles, which characterize species of *Muscarella*. Superficially *Trichosalpinx webbiae* Luer & Escobar is also similar, sharing the elliptic, coriaceous thick leaves, acuminate, dorsally carinate sepals, obovate petals and a trilobed lip with the lateral lobes erect in natural position; however, that species has obscurely lepanthiform bracts (rather than tubular), prostrate leaves mottled with purple beneath (rather than erect, immaculate), erect inflorescences (rather than pendent), a glabrous ovary (rather than echinate) and a simple, trilobulate lip with entire margins (rather than a pandurate-trilobed, erose lip with a distinct papillose isthmus below the anterior lobe, and a Y-shaped thickened apical callus in *S. absurda*). Further morphological and molecular study of *T. webbiae* will be useful to confirm its phylogenetic affinities. An unpublished molecular phylogenetic analysis of *Specklinia* carried out by Karremans *et al.* indicates that *S. absurda* is related to some species placed in *Sylphia* by Luer (2006), all found embedded within *Specklinia*, sister to a clade that includes species of the *S. grobyi* complex and all the orange-flowered species of *Specklinia*, including the type of the genus, *Specklinia lanceola* (Sw.) Lindl. (Fig. 50, Table 5). The new species is similar to *Specklinia cactantha*, *S. fuegi* and *S. turrialbae* in habit, ramicauls and petiolate leaves, inflorescence, echinate ovary (in the material we studied; Fig. 47C), acuminate sepals and rounded to apiculate, obovate petals, and column thickened towards the apex, and pollinaria without caudicles or viscidium. Nevertheless, the combination of unusual characters observed in the new species, especially the complex lip morphology (Fig. 47A), is not found in any of those species groups, and we have yet to find a species that we can say is sister to this species of *Specklinia*.

