A New Ecuadorian species of *Dracula:* Pleurothallidinae (Orchidaceae)

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Abstract: *Dracula trigonopetala,* a proposed new Ecuadorian species in the Pleurothallidinae, is described. Affinities and taxonomic notes on this new species are presented.

Key Words: *Dracula trigonopetala*, Pleurothallidinae, Orchidaceae, endangered species, Ecuador.

Introduction: An uncommon cultivated Dracula questionably identified as *Dracula radiosa* (Rchb.f.) Luer has recently been found in the wild in extreme northern Ecuador. This naturally occurring plant bears unique morphological and genetic features that warrant its consideration as a distinct species, *Dracula trigonopetala* (Figures 1-3).

Dracula trigonopetala Gary Mey. and Baquero sp. nov.

Similar to *Dracula radiosa* (Rchb.f.) Luer, but distinguished by a smaller, shorter-tailed flower bearing a lip with a hypochile with prominent lateral and midline paired projections.

Holotype: ECUADOR, Province of Carchi, between Maldonado and El Chical, collected by L. Baquero, 1800 m elevation. Flowered in cultivation by L. Baquero, Quito, Ecuador, April 2011. *FLOARE 1887*(QCNE)

Plant epiphytic, small to medium in size, densely caespitose, roots coarse. Ramicauls slender, erect, enclosed by 2-3 loose consecutive tubular sheaths, 1.6-2 cm long. **Leaf** erect, carinate, thinly coriaceous, elliptical, subplicate, apex acute, with a distinctly narrowed conduplicate petiole, 10-20 cm long including petiole, 4-6 cm wide. **Inflorescence** successively few flowered, from the base of the ramicaul, breaking through the basal bract, usually horizontal or slightly descending; peduncles green

speckled in brown, subverrucose, with several brown tubular bracts up to 12 cm long, each bract 2-4 mm long; pedicel green to brown, verrucose, 3-5 mm long. Ovary green, heavily overlaid with fine brown spotting, smooth, with 3 furrows running the length of the ovary, 7 mm long x 3 mm in diameter. Sepals golden brown externally and internally, speckled internally with fine spots of dark brown or charcoal in varying densities, indumentum consisting of fairly sparse short papilla, suborbicular, sometimes involute, forming a deep cup. Dorsal sepal 15 mm long (excluding tail) x 18 mm wide, connate to the lateral sepals for 7-10 mm, with the dark brown or charcoal-colored dorsal tail of 1-3 cm in length. Lateral sepals 16 mm long (excluding tail) x 15 mm wide, connate to each other at the base for 12 mm and forming a shallow, whitish mentum with sparse coarse charcoal or blackish spotting, a 1-3 cm charcoal-black tail abruptly continuing from the midline of each lateral sepal. Petals yellow towards the base, nearly black at the apex, cartilaginous, oblong, bivalvate, apex distinguished by a sharp angle laterally and two sharp angles medially (giving the apex a triangular appearance), in close approximation to the column, 4 mm long x 3 mm wide at the apex. Lip articulated with the column, divided into a hypochile and epichile; hypochile light yellow, ovate, each lateral margin elaborated into a smooth rounded upwardly projecting ala, central cleft bordered by a second pair of prominent alae tapering into forward-pointing projections that turn medially and nearly touch at the midline, pseudonectary channel running medially along the superior surface of the hypochile and ending abruptly at the junction with the epichile, 3 mm long x 3 mm wide; epichile light yellow, concave, semiorbicular, margins smooth and non-involute, joined to the hypochile at a 90 degree angle along a base 3 mm wide, from which originate seven continuous, unbranched, pink-tinted lamellae that project radially almost to the outer margin of the epichile, at which point they dissipate, leaving the epichile margin entire, 3 mm long x 6 mm wide x 2 mm deep. **Column** vellow basally, white on the inferior aspect, smudged heavily with dark brown or black at the apex, semiterete, 2 mm long x 1 mm wide; column foot 2 mm long; anther cap white.

Etymology: Named for the distinctive black-tipped petals that appear triangular when viewed from the front of the flower.

Distribution: *Dracula trigonopetala* has been found recently in small numbers along the Maldonado/El Chical road in extreme northern Ecuador. Because of the close proximity of these sites to the Colombian border, it would be no surprise to find this species inside the Nariño department of Colombia.

Diagnostic Characters: This species is distinguished from all others of the genus by the following combination of characters: 1) hypochile nearly as broad as the epichile; 2) a pair of prominent lateral alae along the margins of the hypochile; 3) a pair of prominent medial alae tapering into forward-projecting points that nearly touch over the midline cleft of the hypochile; 4) rounded deeply connate sepals often with involute margins; 5) shell-shaped semiorbicular epichile with smooth, unbroken, unbranched, radiating lamellae that dissipate just before reaching the margin of the epichile; 6) petals closely approximate to the column, nearly black, forming three acute points at the apices when viewed from the front of the flower.

History and taxonomic notes: The species described here as *Dracula trigonopetala* has been found occasionally in cultivated collections, and until recently a wild origin for the plant had been undocumented. To the best of the authors' knowledge, the plants currently in cultivation were distributed in small numbers by Ecuagenera Ltda of Gualaceo, Ecuador, labeled as *D. radiosa. Dracula radiosa* shows a reasonable similarity to these plants, but they are distinguished by multiple anatomic and genetic features (discussed below) noted by Meyer prior to the discovery of these plants in the wild. Given the frequency with which natural and greenhouse hybrids within the genus *Dracula* have been described as species without adequate knowledge of their natural provenance, Meyer was unwilling to describe this potential new taxon until a wild origin could be proven, despite the compelling genetic and visual observations.

In 2009, Baquero located *Dracula trigonopetala* in the wild along the road between Maldonado and El Chical in the Ecuadorian province of Carchi, mere kilometers from the border with Colombia. Plants collected from this region have since bloomed in cultivation in Ecuador and match the plants cultivated in the U.S. (Figure 3). The holotype for *D. trigonopetala* is from this recent wild collection.

Dracula trigonopetala shows a general similarly to *D. radiosa* in its overall aspect and coloration. However, notable anatomic features distinguish the two. The flowers of *D. radiosa* are on the order of three times the size of those of *D. trigonopetala*, not including the tails, which may be five times longer in *D. radiosa*. Comparison of the lips of the two flowers (Fig. 4) shows that *D. radiosa* lacks the midline and lateral projections on the hypochile of *D. trigonopetala*; further, the hypochile is nearly as large as the epichile of *D. trigonopetala*. The sepals of *D. radiosa* are covered densely in papillae of varied height, while the sepals of *D. trigonopetala* are more sparsely covered in short papillae (Figure 5 A and B).

Dracula fuliginosa Luer (Luer) (recently corrected from the moniker *D. radiella* Luer, Thoerle, 2011) grows in the vicinity of *D. trigonopetala*, and it is likely the two could be sympatric. The flowers of both are of similar size, save the longer tails of *D. fuliginosa*. Again, *D. fuliginosa* lacks the elaborate hypochile of *D. trigonopetala* (Figure 4) and has rounded, not sharply angular, petal apices. Some of the lamellae within the hypochile of *D. fuliginosa* are non-continuous between the hypochile and the margin of the epichile. The sepals of *D. fuliginosa* are densely covered in short bumps rather than the distinct papillae of *D. trigonopetala* (Figure 5 B and C).

D. syndactyla Luer has been a difficult species to study because, despite years of searching, Meyer had not been able to find a convincing living specimen. The plants are reported to persist in the wild near Ricuarte in the department of Nariño; Meyer has attempted to go there to study them but has been thwarted by ongoing unrest in the area. Thus, delineation from *D. syndactyla* is dependent on drawings and written descriptions (Luer, 1993) and paintings (Luer & Dalström, 1996). It would appear that *D. syndactyla* is a close relative based on vegetative and floral size and morphology, but distinguishable by its less connate sepals, less elaborate hypochile, epichile lamellae projecting unattenuated to the epichile margin, causing a denticulate edge to the epichile, and hirsute sepals. *Dracula syndactyla* has not been reported from the Maldonado area, but a photo of a plant thought to be *D. syndactyla* has been taken at the Reserva Los Cedros in

Imbabura, much further to the south (Endara *et al.*, 2009). Thus, it is possible that *D*. *trigonopetala* and *D. syndactyla* could be sympatric across some aspect of their ranges.

Dracula x radio-syndactyla Luer is a plant that has been collected several times near Ricuarte, department of Nariño, Colombia and is reported to be a hybrid between the sympatric D. radiosa and D. syndactyla. (Interestingly, D. x radio-syndactyla does not show DNA sequence features suggestive of a hybrid origin in the nuclear marker ITS1, nor does it match the plastid marker sequences of D. radiosa, ruling out D. radiosa as a potential egg parent, Meyer and Cameron, in preparation). The sepals of D. x radiosyndactyla are covered in papillae that are long enough to give a hirsute appearance at the sepaline margins. Additionally, the lip lacks the prominent hypochile projections of D. trigonopetala and has incomplete lamellae in the epichile.

Dracula trigonopetala cannot be mistaken for D. gastrophora Luer & Hirtz, which to date has only been collected once somewhat further south in Pichincha. Dracula morleyi Luer & Dalström is similarly distinct and appears to be restricted to the Reserva Los Cedros in the Imbabura province. Dracula carcinopsis Luer & R.Escobar has only been collected once, quite far away in the Colombian department Valle del Cauca, and is also quite obviously distinct.

Phylogenetic Relationships: The original cultivated specimen of *Dracula trigonopetala* was analyzed along with more than 200 other dracula specimens (encompassing all but five of the currently recognized taxa in the genus) as part of an ongoing molecular phylogenetic project conducted by Meyer and Cameron (Cameron & Meyer, 2007, and additional manuscripts in preparation). DNA sequences from markers commonly used in orchid molecular taxonomy studies (*matK*, *trnL-F*, *trnH-psbA*, *rpoC*, *rbcL*, *ycf1*, and ITS1) were obtained for the sampled taxa. The most sequence variation across the sample set can be found in the biparentally inherited nuclear marker ITS1 and the maternally inherited plastid markers *trnH-psbA* and *ycf1*.

Analysis of *trnH-psbA* and *ycf1* provides strong evidence that *Dracula trigonopetala* is a unique species distinct from all other dracula species, including morphologically related taxa in the "radiosa group". Figure 6 (top panel) shows a sequence segment of *trnH-psbA* from *D. trigonopetala*, as well as two distinct clones of *D. radiosa*, *D. fuliginosa*, *D. x radio-syndactyla*, and *D. morleyi*. *Dracula trigonopetala* has a 26 base pair (bp) insertion that is unique to it among all sampled dracula species. In *ycf1* (Figure 6, lower panel), the two clones of *D. radiosa* have a unique 24 bp insertion not shared with *D. trigonopetala*, *D. fuliginosa*, *D. x radio-syndactyla*, or *D. fuliginosa*, *D. x radio-syndactyla*, or *D. trigonopetala* has 4 single nucleotide substitutions that are not found in *D. radiosa*, *D. fuliginosa*, *D. x radio-syndactyla*, or *D. morleyi*.

The biparentally inherited ITS1 is the only marker sampled in this project that has the potential to provide evidence of hybrid origins. Careful examination of the chromatograms produced in the sequencing process will sometimes reveal multiple single base polymorphisms in a species sample, suggesting that the allelic copies of ITS1 were inherited from interspecies hybridization. Occasionally one ITS allele will be missing one or more nucleotides in comparison to the other allele; this, too, produces a distinct pattern in the sequence chromatogram and suggests a hybrid origin. The ITS1 sequence

for *Dracula trigonopetala* does not show any of the features that would suggest it arose through a recent hybridization event (data not shown). It is important to note that this approach, when considered in isolation from plastid sequence data, morphology, and population data, is not an infallible test for hybrid origin. For example, it relies only on sequence data from a single small nuclear DNA marker, and hybrids between two closely related taxa that show no ITS1 sequence differences (numerous such taxa exist in the sample set) will not yield any of the chromatogram features that point to hybrid origin. However, combining the lack of hybrid features in the ITS1 sequence of *D. trigonopetala* with its unique *trnH-psbA* and *ycf1* sequences and its existence in the wild argues strongly against a hybrid origin for the taxon, and strongly for its being a distinct species.

Dracula trigonopetala in cultivation: Dracula trigonopetala has not proven to be an easy plant to cultivate in the U.S., even in conditions where most draculas thrive. The plants that predate the recent wild collections remain significantly smaller in leaf dimensions than wild plants and grow very slowly. To date, Meyer is only aware of two collections in the U.S. with *D. trigonopetala* (his and his original source; ergo all plants in the U.S. are probably divisions of a single clone). Plants collected at the time of the wild discovery are being cultivated in Ecuador with greater success, suggesting the timid growth observed in U.S. cultivation can be overcome. No efforts have been made to self-pollinate the plants in U.S. cultivation as they are too small to bear capsules, nor are they sufficiently large to donate material for herbarium submission. Flowers in spirits have been prepared by Meyer as part of the voucher material for his ongoing molecular phylogeny studies.

Conservation status: *Dracula trigonopetala* had escaped discovery in a region of Ecuador that has been scoured mercilessly for draculas over the past 30 years. This region continues to be actively collected by local growers. Good habitat immediately to the north inside Colombia cannot be explored adequately while unrest continues. The inability to assess this region renders hypothetical the existence of *D. trigonopetala* north of the Ecuadorian/Colombian border, and we feel it is important to assign conservation status based on what is currently known about the distribution of the species.

Dracula trigonopetala should therefore be considered Vulnerable (VU) according IUCN Red List criterion B1, as the extent of occurrence is unlikely to exceed 20,000 km², the confirmed range of the species near Maldonado and El Chical is suspected to be subject to threats to the quality and area of suitable habitat (criterion b iii) and the number of subpopulations is expected to be declining due to continued orchid collecting in the area (criterion b iv).

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Figure Legends

Figure 1. Flower of *Dracula trigonopetala*. From a plant originally procured commercially in the U.S. as *D. radiosa*, believed to have been imported originally from Ecuador by Ecuagenera LTDA.

Figure 2. Illustration of Dracula trigonopetala Gary Mey. & Baquero

Figure 3. Flowers of *Dracula trigonopetala*. A. Photo of a bloom from a wild-collected plant cultivated in Ecuador. B. Ecuadorian *D. trigonopetala* clone producing a less open bloom. C. Close-up of the lip and petals from the *D. trigonopetala* clone originally grown in the U.S. as *D. radiosa*. Note the prominent lateral and midline projections on the hypochile and the triangular shape of the petals. D. Lateral view of *D. trigonopetala*.

Figure 4. Comparison of lips from *Dracula radiosa* (left), *D. trigonopetala* (center), and *D. fuliginosa* (right). Arrows indicate the lateral (white) and midline (black) alae on the hypochile of *D. trigonopetala*, lacking in both *D. radiosa* and *D. fuliginosa*. Also note the incomplete lamellae in the epichiles of both *D. radiosa* and *D. fuliginosa*. (Note that photos were prepared from alcohol-pickled specimens in the specimen collection of Meyer, so coloration is not representative of live flowers.)

Figure 5. Close-up photographs of sepaline indumentum from A. *Dracula radiosa*, B. *D. trigonopetala*, and C. *D. fuliginosa*. (Note that sepals were obtained from alcohol-pickled specimens in the specimen collection of Meyer, so coloration is not representative of live flowers.)

Figure 6. DNA sequence information supports *Dracula trigonopetala* as a distinct species. Top panel: Excerpt from sequences of the plastid *trnH-psbA* spacer. Note the 26 bp insertion unique to *D. trigonopetala*. Lower panel: Excerpt from sequences of *ycf1*. Note the 24 bp insertion unique to both *D. radiosa* samples and the $T \rightarrow G$ base substitution (yellow) in *D. trigonopetala*. Three other unique single base substitutions are present in the *ycf1* sequence of *D. trigonopetala* (data not shown). Methodolgy is as described in Cameron & Meyer, 2007. Complete sequences have been submitted to Genbank (Table 1) and are additionally available on request from Meyer. Figure prepared with Geneious sequence analysis software v. 5.5.2.

Table 1. GenBank accession numbers for *ycf1* and *trnH-psbA* sequences











Figure 5



Figure 6

trnH-psbA	190	200	210	220	230	240	250	260
Consensus Sequence	GGTGAAGT	ATAGTATAAGA	TAAA			TCAGAAAAG	CTAAGTCTT!	ATTA
D. trigonopetala D. radiosa 1 D. radiosa 2 D. fuliginosa D. radio-syndactyla D. morelyi	GGTGAAGT GGTGAAGT GGTGAAGT GGTGAAGT GGTGAAGT	ATAGTATAAGA ATAGTATAAGA ATAGTATAAGA ATAGTATAAGA ATAGTATAAGA ATAGTATAAGA	ТААА <mark>СТАСС</mark> ТААА — — — — ТААА — — — — — ТААА — — — — — ТААА — — — — — — ТААА — — — — —	TGAAG TA TAG I	AT AAGA T AA <i>I</i>	TCAGAAAAG - TCAGAAAAG - TCAGAAAAG - TCAGAAAAG - TCAGAAAAG - TCAGAAAAG	CTAAGTCTT CTAAGTCTT CTAAGTCTT CTAAGTCTT CTAAGTCTT CTAAGTCTT	ATTA ATTA ATTA ATTA ATTA ATTA
ycf1	100	410	420	430	440	450	4	50 470
Consensus Sequence	TATTGGC	ATCAAGATT	AAAAAA	ACTAAGAAT	GAAA TT CAG	AA		T T
D. trigonopetala D. radiosa 1 D. radiosa 2 D. fuliginosa D. radio-syndactyla D. morelvi	TA T <mark>G</mark> GGC TA TTGGC TA TTGGC TA TTGGC TA TTGGC TA TTGGC	A T C A A G A T T (A T C A A G A T T (A T C A A G A T T (A T C A A G A T T (A T C A A G A T T (A T C A A G A T T (СААААААА Т СААААААА Т СААААААА Т СААААААА Т СААААААА Т САААААА Т	ACTAAGAAT ACTAAGAAT ACTAAGAAT ACTAAGAAT ACTAAGAAT ACTAAGAAT	GAAATTCAG GAAATTCAG GAAATTCAG GAAATTCAG GAAATTCAG GAAATTCAG	AA AA <mark>AAA TACT</mark> AA AA AA AA	AAGAA TGAI AAGAA TGAI	AATTCAGAA AATTCAGAA TT TT TT TT

Table 1

Taxon	Voucher	Collector	trnH-psbA accession	ycf1 accession
Dracula fuliginosa	DR032	Meyer	JQ701725	JQ701731
Dracula morleyi	DR017	Meyer	JQ701726	JQ701732
Dracula radiosa	DR094	Meyer	JQ701728	JQ701734
Dracula radiosa	DR202	Meyer	JQ701729	JQ701735
Dracula trigonopetala	DR203	Meyer	JQ701730	JQ701736
Dracula x radio-syndactyla	DR043	Meyer	JQ701727	JQ701733