



Phylogenetic placement, taxonomic revision and a new species of *Nothosteale* (Orchidaceae), an enigmatic genus endemic to the cerrado of central Brazil

JOÃO A. N. BATISTA^{1*}, THIAGO E. C. MENEGUZZO^{2,3}, GERARDO A. SALAZAR⁴, ALINE J. RAMALHO¹ and LUCIANO DE BEM BIANCHETTI²

¹Universidade Federal de Minas Gerais, Departamento de Botânica, Avenue Antônio Carlos 6627, Pampulha, C.P. 486, Belo Horizonte, MG, 31270-910, Brazil

²Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Final Avenue. W5 Norte, C.P. 02372, Brasília, DF, 70770-901, Brazil

³Universidade de Brasília, Instituto de Ciências Biológicas, Departamento de Botânica, C.P. 4457, Brasília, DF, 70919-970, Brazil

⁴Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-367, 04510 Mexico, D.F., Mexico

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Nothosteale is a rare genus endemic to central Brazil. The taxonomy of the genus is controversial and almost every taxonomist has had a different point of view regarding its generic and subtribal classification. After the first collection 138 years ago, *N. acianthiformis* has been collected again and we report here a phylogenetic analysis of the genus based on nuclear ribosomal internal transcribed spacer and plastid *matK* and *trnL-trnF* sequence data. Our results show that *Nothosteale* belongs to subtribe Spiranthinae and is sister to *Eltroplectris* within the *Stenorrhynchos* clade. A reanalysis of gynostemium morphology shows that the presence of a hamulus was misinterpreted in *Nothosteale* and that the placement of the genus in Cranichidinae based on morphology of the pollinarium is unwarranted. Furthermore, the flattened, sessile, prostrate leaves of *N. acianthiformis*, which are described here for the first time, occur in some Spiranthinae but are atypical in Cranichidinae. Specimens from Brasília in the Brazilian central plateau are a distinct new species, which is described here as *N. brasiliaënsis*. Currently, the genus comprises two disjunct species restricted to the cerrado and rocky field vegetation of central Brazil. © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, 165, 348–363.

ADDITIONAL KEYWORDS: cranichidinae – conservation status – endemism – phylogenetic systematics – Spiranthinae.

INTRODUCTION

Nothosteale Garay is a monospecific genus endemic to central Brazil. The genus has attracted the attention of taxonomists because of its distinctive combination of floral characters and confusing phylogenetic position within tribe Cranichideae (Lindl.) Endl. The perianth morphology in general agrees with subtribe Spiranthinae Lindl., whereas the column and

pollinarium features have been considered similar to Cranichidinae Lindl. Since its description, nearly all taxonomists who have considered *Nothosteale* have expressed a different point of view regarding its position.

Nothosteale acianthiformis (Rchb.f. & Warm.) Garay was originally described as *Pelexia acianthiformis* Rchb.f. & Warm. (Reichenbach, 1881) based on plants collected by Warming in 1864 in Lagoa Santa, Minas Gerais, Brazil (Warming, 1884). Cogniaux (1893–1896) transferred it to *Stenorrhynchos* Rich. ex

*Corresponding author. E-mail: janb@icb.ufmg.br

Spreng. (as *Stenorrhynchus*) in *Flora Brasiliensis* and Hoehne (1945) transferred it to *Centrogenium* Schltr. in *Flora Brazilica*, now a synonym of *Eltroplectris* Raf. Garay (1982) recognized its distinctive characters and created the monospecific genus *Nothosteale*, including it in Spiranthinae, but based on pollinarium morphology, Dressler (1993) transferred the genus to Cranichidinae, and the phylogenetic position of *Nothosteale* has remained unclear. The flowers of *Nothosteale* are similar to some species of *Eltroplectris* of Spiranthinae, explaining its transfer to *Centrogenium* by Hoehne. However, *Nothosteale* differs from *Eltroplectris* and most Spiranthinae by its non-resupinate flowers and the overall morphological similarity could represent a case of floral convergence. Szlachetko (1995), Szlachetko & Rutkowski (2000), Pridgeon *et al.* (2003) and Salazar *et al.* (2003b, 2009) also supported its inclusion in Cranichidinae.

A major hindrance to clarifying the systematic position of *Nothosteale* has been the unavailability of suitable material. For example, in his revision of Spiranthinae, Schlechter (1920) did not make any reference to the species. Populations of *Nothosteale* are extremely rare and, for a long time, the genus was known only from the type material from the state of Minas Gerais. In the 1960s and 1990s, a few additional collections were made in Brasília, the Brazilian capital located in the central plateau (Pabst & Dungs, 1975; Batista & Bianchetti, 2003). Recently, two additional populations of *N. acianthiformis* were found in Minas Gerais. The availability of fresh material enabled DNA extraction and we present here a phylogenetic analysis of the genus based on nuclear and plastid DNA sequences. Furthermore, the examination of live material of *N. acianthiformis* from Minas Gerais, and its comparison with the material from Brasília, showed that the latter corresponds to a distinct species, which is described here as new. Finally, the observation in the field of one of the newly discovered populations enabled examination of the vegetative parts of *N. acianthiformis*, which are described here for the first time, as the species was known only from dried material including only inflorescence and flowers.

MATERIAL AND METHODS

TAXONOMIC SAMPLE FOR THE PHYLOGENETIC ANALYSES

Exemplars of 27 species/23 genera in subtribe Spiranthinae *sensu* Salazar (2003) and 43 species/12 genera of subtribe Cranichidinae *s.l.* (including Prescottiinae Dressler; after Chase, 2003; Chase *et al.*, 2003; Salazar, Chase & Cribb, 2003a; Salazar, 2009; Salazar *et al.*, 2003b, 2009) were analysed in this study. Representative species of all other subtribes of Cranichideae recognized by Chase *et al.* (2003), namely Achlydosinae M.A.Clem. & D.L.Jones (formerly Megastylidinae Schltr., in part), Chloraeinae Rchb.f., Galeottiellinae Salazar & M.W.Chase, Goodyerinae Klotzsch (including Pachyplectroninae Schltr.), Manniellinae Schltr. and Pterostylidinae Pfitzer, were used as outgroups. Nearly all the sequences analysed were produced by us for previous works and voucher information and GenBank accessions for these can be found in Salazar *et al.* (2003b, 2009); information for the newly sequenced accessions is provided in Table 1.

MOLECULAR MARKERS

We analysed nucleotide sequences of plastid and nuclear DNA. The plastid DNA regions include the pseudogene *matK* and part of the flanking *trnK* intron, the *trnL* intron, the *trnL-trnF* intergenic spacer and short exon portions of genes *trnL* and *trnF*. The nuclear region (nrITS) consists of the internal transcribed spacers (ITS1 and ITS2) and the intervening gene 5.8S of the nuclear ribosomal multi-gene family. All these regions have demonstrated their utility for inferring phylogenetic relationships at various taxonomic levels in Cranichideae and other lineages of Orchidaceae (reviewed in Salazar *et al.*, 2003b, 2009; Cameron, 2007; Álvarez-Molina & Cameron, 2009). Extraction, amplification and sequencing of DNA were carried out following standard protocols explained in Salazar *et al.* (2003b) and Figueroa *et al.* (2008). Bidirectional sequence reads

Table 1. Voucher information and GenBank accessions for the new sequences produced for this work

Taxon	Voucher	<i>trnL-F</i>	<i>matK</i>	ITS
<i>Eltroplectris triloba</i> (Lindl.) Pabst	Argentina, <i>Munich Bot. Gard.</i> 96/4474 (M)	–	FN868835	–
<i>Nothosteale acianthiformis</i> (Rchb.f. & Warm.) Garay	Brazil, <i>Viana 767</i> , BHCB	FN868836	FN868833	FN868838
<i>Pteroglossa roseoalba</i> (Rchb.f.) Salazar & M.W.Chase	El Salvador, <i>Salazar 6023</i> , MEXU	FN868837	FN868834	FN868839

ITS, internal transcribed spacer.

were obtained for all the DNA regions and the resulting chromatograms were edited and assembled with Sequencher version 4.8 (GeneCodes Corp.).

SEQUENCE ALIGNMENT AND CLADISTIC ANALYSIS

The edited sequences were aligned by visual inspection, trying to maximize sequence similarity (Simmons, 2004). No data were excluded from the analyses because of ambiguous alignment. Individual gap positions were treated as missing data. Previous molecular phylogenetic studies of Spiranthinae and Cranichidinae (Salazar *et al.*, 2003b, 2009; Figueroa *et al.*, 2008; Álvarez-Molina & Cameron, 2009) have shown that analysing the DNA sequence markers used here in combination increases resolution and clade support compared with separate analyses. Thus, we combined all our data sets and analysed them using cladistic parsimony. The analysis was conducted with the computer program PAUP* version 4.02b for Macintosh (Swofford, 2002) and consisted of a heuristic search with 1000 replicates of random taxon addition for the starting trees and tree rearrangements using the tree bisection–reconnection (TBR) option for branch swapping. The option MULTREES was activated to allow for storage of multiple trees in memory and all most-parsimonious trees (MPTs) were saved. All characters were treated as unordered and had equal weights. Internal support for clades was assessed by non-parametric bootstrapping (Felsenstein, 1985), performing 300 bootstrap replicates, each with 20 replicates with random taxon addition and TBR branch swapping, keeping up to 20 MPTs from each addition replicate.

TAXONOMIC ANALYSIS

Descriptions were based on live, pickled or herbarium material. Details of the flowers, particularly the gynostemium, were examined under a stereoscopic microscope and measured with a digital paquimeter (caliper). Data for flowering times, habitats and distribution were based on herbarium labels of collections or on field observations. Habit was examined from live material, perianth from live and dried material, and gynostemium details from live, dried or pickled material. Dried materials were rehydrated in 20% ammonium hydroxide. Materials and images were examined from the following herbaria: BHC, BR, C, CEN, HB, NY, P, UB and W. Descriptive terminology is based on Stearn (1992) and Simpson (2006).

RESULTS

CLADISTIC ANALYSIS

The combined data set consisted of 4674 characters, 2059 corresponding to *matK-trnK*, 1811 to the *trnL-*

trnF region and 804 to the nrITS region. Of the 4674 characters, 2009 (43%) were variable and 1306 (28%) were potentially parsimony-informative. The heuristic search found only two MPTs with a length of 5941 steps, consistency index (excluding uninformative characters) 0.41, and retention index 0.73. One tree is shown in Fig. 1A, and the strict consensus of the two trees, on which bootstrap support (BP) for clades is indicated, is depicted in Fig. 1B. The two cladograms found in the heuristic search differ from each other only in the position of the clade formed by *Prescottia* Lindl. and *Galeoglossum* A.Rich. & Galeotti, which in the tree depicted (Fig. 1A) is sister to Spiranthinae, whereas in the other tree *Prescottia/Galeoglossum* is recovered as sister to a 'core Cranichidinae' clade (Salazar *et al.*, 2009) comprising *Cranichis* Sw., *Pterichis* Lindl. and *Ponthieva* R.Br. (not shown). However, neither topology obtained BP > 50. The phylogenetic positions of Goodyerinae, Galeottiellinae and Manniellinae mirror the results of previous molecular studies of Cranichideae (Salazar *et al.*, 2003b, 2009; Álvarez-Molina & Cameron, 2009) and will not be dealt with further here; we will focus only on the relationships in Cranichidinae *s.l.* and Spiranthinae, the two groups in which most authors have included *Nothosteale*.

Cranichidinae *s.l.* are paraphyletic and consist of three strongly supported clades (a–c in Fig. 1B), the relationships of which to each other and to Spiranthinae (d–g) are not clearly supported. The three clades correspond to the *Stenoptera* C.Presl (a; BP 100), 'core' Cranichidinae (b; BP 100) and *Prescottia* (c; BP 93) clades of Salazar *et al.* (2009). *Nothosteale* is deeply embedded in a strongly supported Spiranthinae as the strongly supported sister of *Eltroplectris* (BP 100) within the *Stenorhynchos* Spreng. clade (e). Other relationships within Spiranthinae are similar to those found by previous DNA studies (Salazar *et al.*, 2003b; Górniak *et al.*, 2006; Salazar & Ballesteros-Barrera, 2010; Salazar & Dressler, in press).

DISCUSSION

PHYLOGENETIC POSITION OF *NOTHOSTELE*

Our phylogenetic analysis clearly supports the inclusion of *Nothosteale* in Spiranthinae, in agreement with Reichenbach (1881), Cogniaux (1893–1896), Hoehne (1945) and Garay (1982). In contrast, Dressler (1993) considered *Nothosteale* as a member of Cranichidinae *s.s.* (= core Cranichidinae *sensu* Salazar *et al.*, 2009) because of the shape of the pollinia as drawn by Garay (1982). In that drawing, the pollinarium was depicted as consisting of two (instead of four, as usual in Cranichidinae) clavate pollinia with distinct,

slender 'caudicles' joined at their tips to a globular, hooked viscidium. However, in the plate of this species published by Warming (1884), the pollinarium was represented as two longitudinally divided pollinia that gradually attenuate towards the apex; the viscidium was not attached at the apex of the pollinia, but in two views of the column it is globular and situated at the apex of the rostellum. Szlachetko (1995) likewise included *Nothostele* in Cranichidinae s.s., as did Szlachetko & Rutkowski (2000: 293), who described its viscidium as having 'a hook-like upcurved apex transformed into a hamulus'. On that basis, together with the non-resupinate flowers, Salazar *et al.* (2003b, 2009) argued for its inclusion in Cranichidinae, and a similar conclusion was reached by Álvarez-Molina & Cameron (2009). However, now it seems that Szlachetko & Rutkowski (2000) misinterpreted the structure of the viscidium of *Nothostele*. Close analysis of the corresponding description and illustration (Szlachetko & Rutkowski, 2000: fig. 357) shows that they interpreted as a hamulus a retrorse prominence present on the ventral (adaxial) side of the viscidium, i.e. on the opposite side of where the pollinia attach to the latter (Szlachetko & Rutkowski, 2000: fig. 357D). In contrast, the dorsal (abaxial) side of the viscidium (Szlachetko & Rutkowski, 2000: fig. 357E: 'apex of rostellum, view from above') lacks any indication of a true hamulus (the apical part of the rostellum bent towards the anther and to which pollinia adhere; Rasmussen, 1986). A confirmation of the nature of the viscidium of *Nothostele* will require a detailed developmental study, but so far we are unaware of evidence that it represents a hamulus. One of the main reasons for including this genus in Cranichidinae is therefore not upheld.

Non-resupination has been considered as a distinguishing feature of Cranichidinae s.l. (e.g. Lindley, 1840; Schlechter, 1926; Dressler, 1981; Salazar *et al.*, 2009). However, instances of particular species with non-resupinate flowers are known in various genera of the otherwise uniformly resupinate subtribe Spiranthinae, such as *Aracamunia liesneri* Carnevali & I. Ramírez, *Cyclopogon glabrescens* (T. Hashim.) Dodson and *Hapalorchis trilobatus* Schltr.

Another difference between *Nothostele* and Cranichidinae s.l. is the distinctive leaves, which are sessile, flattened and adpressed to the ground (Fig. 2), and are more reminiscent of some Spiranthinae such as *Discyphus scopulariae* (Rehb.f.) Schltr., *Cyclopogon saccatus* (A. Rich. & Galeotti) Schltr. and *Sarcoglottis biflora* (Vell.) Schltr. This type of leaf growth is also found across a broad range of tribes and genera in Orchideaceae, including *Liparis* Rich. (Malaxideae Lindl.), *Satyrium* Sw. (Diseae (Lindl. ex Benth.)) Dressler, *Satyriinae* Schltr.), *Holothrix* Rich. ex Lindl., *Bartholina* R.Br. and *Habenaria* Willd.

(Orchideae Dressler & Dodson, Habenariinae Benth.), but is rare in Cranichidinae s.l. The only member of this group with similar leaves is *Galeoglossum thysanochilum* (B.L. Rob. & Greenm.) Salazar (= *Pseudocranichis thysanochila* (B.L. Rob. & Greenm.) Garay) (Salazar, 2009), but in this case the three or four leaves that form the rosette are shortly but distinctly petiolate. In contrast, most Cranichidinae are characterized by the petiolate leaves being arranged in a basal, upright rosette. In semi-arid South Africa, geophytes with flattened leaves that lie prostrate on the soil surface occur in at least eight families (Amaryllidaceae, Asparagaceae, Colchicaceae, Geraniaceae, Iridaceae, Orchidaceae and Oxalidaceae). This growth form has been hypothesized to play several adaptive ecological roles, including avoidance of herbivory, reduction of water loss around the roots, reduction of water loss through transpiration, precipitation of dew on the leaves and maintenance of optimal leaf temperatures for growth (Esler, Rundel & Vorster, 1999).

On the one hand, *Nothostele* and its sister genus *Eltroplectris* share some floral morphological similarities such as a conduplicate labellum with glandular trichomes, an anther filament partially free from the column, clavate pollinia, a stiff rostellum/rostellar remnant and a deltoid, ventral stigma. However, most of these characters are also shared to a greater or lesser extent with other members of the *Stenorhynchos* clade. On the other hand, the differences between *Nothostele* and *Eltroplectris* are significant and include shorter and fewer roots, orbiculate, sessile, fleshy leaves adpressed to the ground, non-resupinate flowers, the lip not adherent to the column, and a short spur in *Nothostele*, vs. long-petiolate, elliptic or oblong-elliptic, membranaceous leaves, inflorescences with several to many flowers, resupinate flowers, a prominent, cylindrical to clavate spur formed by the joined bases of the lateral sepals and labellum with the apex of the column foot, a three-lobed labellum that is connate basally to the lateral sepals to form a spur and partially adherent to the column and a clinandrium devoid of membranaceous margins in *Eltroplectris* (Pridgeon *et al.*, 2003). Thus, despite some similarities, there are noticeable morphological differences that support maintaining *Nothostele* as a distinct genus from *Eltroplectris*.

Among the species of *Eltroplectris*, *Nothostele* is similar in overall morphology of the inflorescence and flowers to *Eltroplectris cogniauxiana* (Schltr.) Pabst and *E. longicornu* (Cogn.) Pabst (compare fig. 3 with fig. 7). Like *Nothostele*, these species are seasonal geophytes restricted to the cerrado of central Brazil. The leaves of these species have never been recorded and it would be interesting to compare them, when available, with those of *Nothostele*. The general similarity between *Nothostele* and these species of

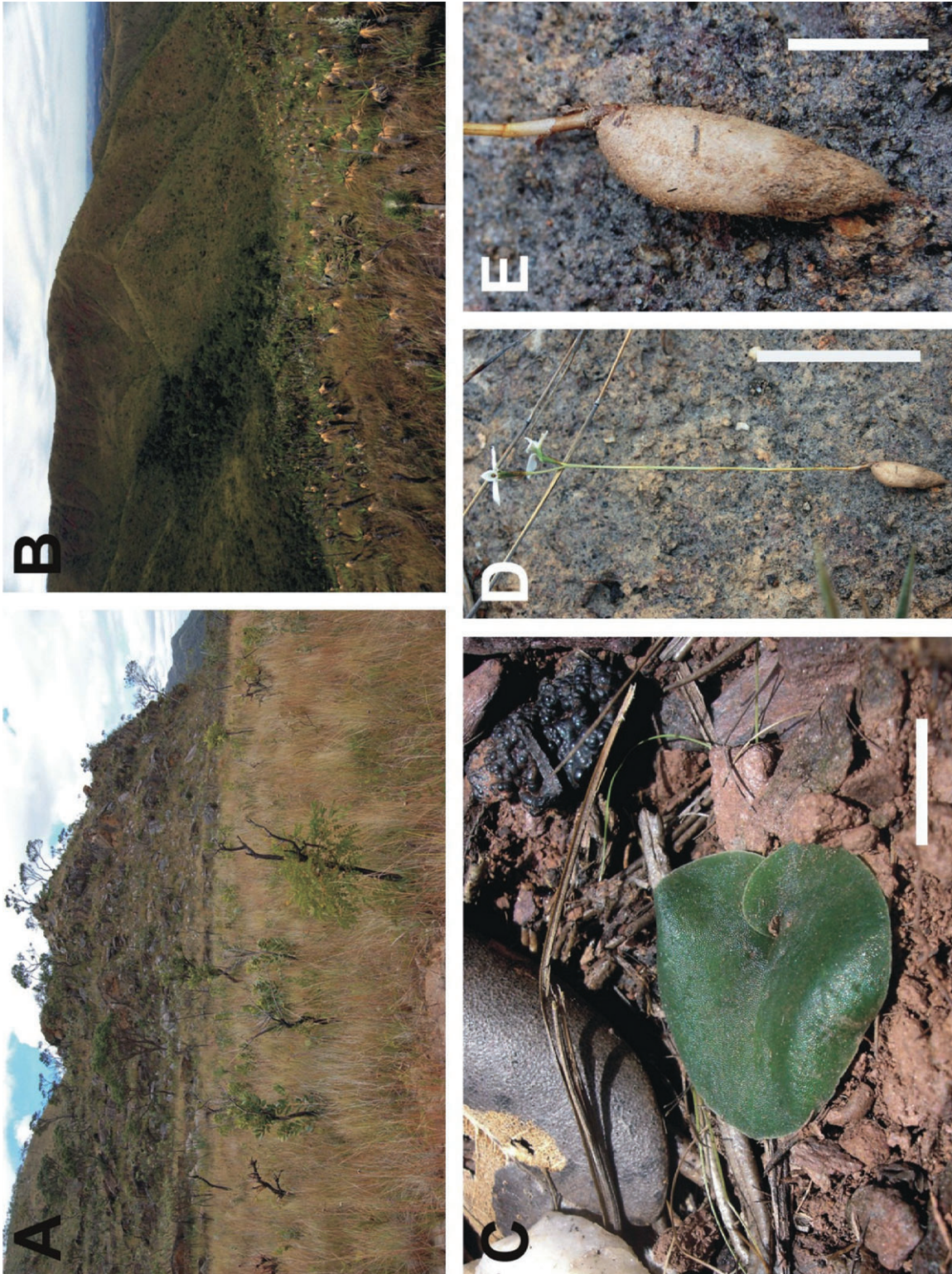


Figure 2. *Nothostele aciantiformis*. Habitat. A, dry grass-herb-subshrub field (campo sujo) in the foreground, associated with rocky fields (campos rupestres) in the background; Serra do Cipó during the dry season in August 2007; B, rock slopes with rocky fields with Velloziaceae at Serra da Calçada, in August 2008. Habit. C, leaf at the beginning of the rainy season, in late November. D, uprooted flowering plant at the peak of the dry season in August. E, root. Scale bars, 1 cm (C, E); 5 cm (D). C (Viana 767). D–E (Batista *et al.* 2097).

Eltroplectris could represent either the ancestral condition in the *Nothosteale/Eltroplectris* clade or convergence in response to similar ecological conditions and likely pollinators. However, material of these species of *Eltroplectris* has not been available for molecular study and it is unknown whether they represent an early branching or a derived lineage within the genus. Therefore, we prefer to maintain *Nothosteale* as a distinct genus until the relationships of its two known species to *Eltroplectris cogniauxiana* and *E. longicornu* are clarified by the inclusion of the latter in molecular phylogenetic analyses.

TAXONOMIC TREATMENT

NOTHOSTELE GARAY, BOT. MUS. LEAFL.
28: 340. 1982.

Type species: Nothosteale acianthiformis (Rchb.f. & Warm.) Garay, Bot. Mus. Leaf. 28: 340. 1982.

Description: HERB acaulescent, terrestrial; roots few, fasciculate, tuberous, fleshy, thick, fusiform to cylindrical, pilose. LEAVES one (or two), basal, fleshy, orbicular, sessile, adpressed to the ground, green, absent at anthesis. INFLORESCENCE terminal, erect, racemose, slender, lax, glabrous below, sparsely pubescent above, hairs short, peduncle provided with tubular sheaths, these being imbricate in the lower part and spaced above; floral bracts lanceolate, acuminate, slightly longer to approximately as long as the pedicel and ovary. FLOWERS few, ascending, non-resupinate, white to cream–whitish, pedicellate ovary fusiform, sparsely pubescent, brown to greenish. SEPALS spreading; dorsal sepal glabrous, lanceolate, narrowly acute to acuminate; lateral sepals shortly connate at base, basally adnate to ovary apex and base of the dorsal sepal, forming a short spur, sparsely pubescent at base, oblique, recurved. PETALS slender, obliquely spatulate to oblanceolate, adherent to the dorsal sepal. LABELLUM free, not adherent to the column, sessile, conduplicate–concave, ovate to obovate, base pubescent, apex glabrous, acute to cuspidate, gently recurved to strongly reflexed. COLUMN erect, slightly curved, the ventral side pubescent at base, convex, dorsal side concave, foot completely adnate obliquely to ovary apex; clinandrium concave, expanded laterally at each side of the column, expansion membranous, translucent, laterally embracing part of the pollinia. ANTHER dorsal, motile, persistent, membranous, apex thickened; filament free near the apex only. POLLINIA two, soft, clavate, cleft, yellow; viscidium single, terminal, positioned at the apex of the column, spheroid to ellipsoid, ventrally with a small concavity in which fits the apex of the rostellum

remnant. STIGMA ventral, entire, deltoid, slightly convex. ROSTELLUM triangular, fleshy, short and apiculate to elongate and acuminate. CAPSULE not seen.

Etymology: From the Greek *nothos* (false) and *stela* (column), in reference to the incomplete fusion between the stamen filament and the style.

Distribution: *Nothosteale* is a small genus of two species restricted to central Brazil in Minas Gerais, Goiás and the Distrito Federal (Fig. 4).

Habitat and ecology: *Nothosteale* occurs in the cerrado and rocky field vegetation of central Brazil. The cerrado is a species-rich savanna vegetation covering approximately 2 million km² of central Brazil (Ratter, Ribeiro & Bridgewater, 1997). Climate of the cerrado is marked by two well-defined seasons: a rainy season with an average precipitation of 800–2000 mm from October to April and a dry season during the winter from May to September, when the relative humidity falls to less than 20%. Average annual temperatures are 18–28 °C. *Nothosteale* spp. are deciduous terrestrials and grow in dry grass–herb–subshrub field (campo sujo) vegetation, often associated with rocky fields (campos rupestres), over shallow sandy or deep loamy, well-drained soils, at elevations of 700–1300 m. Plants flower at the peak of the dry season in August and September, when they are leafless. At this time of the year the plants have only a small underground, tuberous root that supports the inflorescences. As all collections of the species were made from flowering individuals, the leaves of the species were unknown until recently. Leaves develop during the rainy season, then wither and are eventually lost as the dry season approaches.

Bushfires are common in savanna-like habitats with a marked seasonally dry climate and affect the flowering of several species of Orchidaceae (Schelp, 1970). In the cerrado, flowering of several Spiranthinae such as *Veyretia sagittata* (Rchb.f. & Warm.) Szlach., *V. rupicola* (Garay) F.Barros, *Cyclopogon goyazensis* (Cogn.) Schltr. and *Sacoila lanceolata* (Aubl.) Garay, is significantly enhanced by fire. However, this is probably not the case for *Nothosteale*. The three observations of flowering specimens of *Nothosteale* by the authors were made in areas that were not burned. Because the flowering time of *Nothosteale* and the bushfire season are coincident, it is likely that burnings at this time may be disadvantageous to flowering specimens.

Both species in the genus are known only from a few collections and can be considered rare. However, as suitable habitats occur in many areas, plants can only be located when in flower and the cerrado is not frequently visited by botanists or collectors during the

KEY TO THE SPECIES OF *NOTHOSTELE*

- 1 Flowers two to six; dorsal sepal 10.5–12.0 mm long; labellum 11–14 × 6.0–7.5 mm, ovate, apex acute, gently recurved; column semi-terete; anther attached to the column for 1.5–2.2 mm from the apex of the ovary; viscidium spheroidal, not projected beyond the labellum; rostellum 0.5 mm long, apiculate; Minas Gerais.....
.....*N. acianthiformis*
- 1 Flowers (3) 7–10 (14); dorsal sepal 5.5–8.0 mm long; labellum 7–9 × 4.0–4.5 mm, obovate, apex cuspidate, strongly reflexed; column dorsiventrally flattened; anther attached to the column for c. 0.8 mm from the apex of the ovary; viscidium ellipsoidal, projected beyond the labellum; rostellum 1.2–1.5 mm long, caudate; Distrito Federal and Goiás.....
.....*N. brasiliaënsis*

dry season (when the plants are in bloom), it is likely that the species have been overlooked and may be more common than currently believed.

NOTHOSTELE ACIANTHIFORMIS (RCHB.F. & WARM.)

GARAY, BOT. MUS. LEAFL. 28: 340. 1982.

(Figs 2–3, 5D–F)

Basionym: *Pelexia acianthiformis* Rchb.f. & Warm.,
Otia Bot. Hamburg. 2: 83. 1881.

Type: BRAZIL. Minas Gerais: Lagoa Santa, paa grused campos, hvid bl. (in campos vegetation with much gravel, white flowers), 18.viii.1864 (fl), *J.E.B. Warming* (Holotype: not indicated; lectotype, designated here: W-R 23938; Isotypes: BR, C, P, W-R 23937).

Synonyms: *Stenorrhynchos acianthiformis* (Rchb.f. & Warm.) Cogn., Fl. Bras. (Martius) 3(4): 178. 1895. *Centrogenium acianthiforme* (Rchb.f. & Warm.) Hoehne, Fl. Bras. (Hoehne) 8(12; 2): 289. 1945.

Description: ROOTS one to four, 16–24 mm long, 7–9 mm in diameter. LEAVES one (or two), basal, fleshy, orbicular, sessile, c. 23 × 19 mm, adpressed to the ground, green. INFLORESCENCE few-flowered, 12–20 cm high, peduncle with five or six sheaths, sheaths 11–22 mm long; floral bracts 14 × 3.5–4.0 mm, brownish. FLOWERS two to six, medium-sized, white, pedicellate ovary 8–11 mm long, brown to greenish. SEPALS inside white, outside white-pinkish; dorsal sepal 10.5–12.0 × 2.5–3.2 mm, base concave, gently recurved above the base, lanceolate, acuminate, apiculate; lateral sepals forming a short spur approximately 3 mm long, 13–16 × 3.0–4.7 mm, obliquely oblanceolate, strongly recurved above the base, apex acute, base brownish. PETALS 10–12 × 2.3–3.0 mm, obliquely spatulate, free margin slightly recurved, white, base brownish to salmon. LABELLUM 11–14 × 6.0–7.5 mm, ovate, white, base cuneate, brownish to salmon, margins thickened, pubescent, apex acute, gently recurved. COLUMN semi-terete, mostly glabrous, pubescent at base, 7.6–9.8 mm long

from the column foot to the rostellum apex, free part 5.5–6.0 mm long, slightly curved, brownish. ANTHER ovate, 3.0–3.4 × 1.6–1.8 mm, centre reddish brown, sides dark brownish, attached to the column 1.5–2.2 mm from the apex of the ovary, filament 1.5–2.5 mm, whitish, free near the apex only, forming a keel along the dorsal side of the column. POLLINARIUM 3.8–4.0 mm long; pollinia deeply cleft, caudicles barely developed; viscidium whitish, not projected beyond the labellum, spheroidal. STIGMA whitish to brownish. ROSTELLUM approximately 0.5 mm long, brownish, apiculate, the apicule deflexed.

Etymology: In all probability, ‘*acianthiformis*’ was meant to signify ‘resembling *Acianthus*’ (a genus of Orchidaceae in tribe Diurideae). According to Jones (2001), *Acianthus* is derived from the Greek *akis* (point), and *anthos* (flower), referring to the slender apices of the sepals of some species.

Illustrations: Warming (1884), Hoehne (1945: table 158; reproduction of the drawings by Warming), Szlachetko & Rutkowski (2000: fig. 357; drawings of the gynostemium based on the collection by Warming located in C), Pridgeon *et al.* (2003: fig. 122.1; redrawn from the drawings by Warming and Szlachetko & Rutkowski).

Distribution, habitat and ecology: Endemic to the central region of the state of Minas Gerais, where it has been recorded from only three localities: Serra da Calçada, Serra do Cipó and Lagoa Santa. *Nothostele acianthiformis* is a deciduous terrestrial and grows in dry grass-herb-subshrub field (campo sujo) vegetation, usually associated with rocky fields (campos rupestres), over shallow, sandy, well-drained soils at elevations of 700–1300 m. The habitat of the collection from Lagoa Santa is described as ‘*campis tam graminosis fertilioribus prope silvas, quam glareosis sterilibus*’ (Warming, 1884). Flowering occurs at the peak of the dry season in July and August, when the plants are leafless. At this time, the plants have only a few, small, underground tuberous roots that support

the inflorescence. No information is available on the pollination of *N. acianthiformis*. A population of the species, observed in the Serra da Calçada in August 2009, with approximately a dozen flowering specimens, did not produce any fruit, indicating a low reproductive success and the absence of autogamy. The species is similar to *Eltroplectris cogniauxiana* in overall flower morphology (Fig. 7). Both these species occur in similar habitats and flower at the same time. As for *N. acianthiformis*, nothing is known about the pollination of *E. cogniauxiana*.

Conservation status: *Nothostele acianthiformis* is known so far from just three collections. The collection sites around Lagoa Santa where Warming collected in the 19th century are now completely urbanized and this population is almost certainly lost. The two known extant populations are small and located outside protected areas. Approximately a dozen specimens were observed in Serra da Calçada and only three in Serra do Cipó. The population in Serra da Calçada is close to the Serra do Rola Moça State Park and the population in Serra do Cipó is located in the vicinity of the Serra do Cipó National Park. The specific habitat and vegetation in both localities are also found in surrounding areas and it is likely that other populations of the species will be eventually discovered. Using the World Conservation Union Red List Categories and Criteria (IUCN, 2001) *N. acianthiformis* would be classified as Critically Endangered CR [criteria C2a(i)].

Additional material examined: BRAZIL. Minas Gerais: Brumadinho, Retiro das Pedras, Serra da Calçada, 7.viii.2002 (fl), P.L. Viana 767 (BHCB); Santana do Riacho, Serra do Cipó, 6.viii.2007 (fl), J.A.N. Batista et al. 2097 (BHCB).

Notes: *Nothostele acianthiformis* is similar to *Eltroplectris cogniauxiana* and *E. longicornu*. Dried material of these species can be easily confused with each other. All have overlapping distributions, occur in similar habitats, flower at the same time, are leafless at anthesis and have short inflorescences with few flowers with a white perianth. However, *E. cogniauxiana* and *E. longicornu* have resupinate flowers (vs. non-resupinate in *Nothostele*) and a longer spur (c. 9–11 mm long vs. 3 mm long in *N. acianthiformis*).

Nothostele acianthiformis was first collected by Warming in Lagoa Santa, Minas Gerais, in 1864 and described by Reichenbach f. and Warming in 1881. As far as we can determine, the species remained uncollected for the next 138 years until it was collected in 2002 by P. L. Viana in Serra da Calçada, Minas Gerais, located approximately 52 km south of Lagoa

Santa. Material for phylogenetic analysis and description of the leaves are from this population. A second population of the species was found in 2007 in Serra do Cipó, approximately 48 km east of Lagoa Santa. These are the only known extant populations of the species.

Some of the duplicates of the Warming collection of *Nothostele* are numbered as 128. This number is in a different handwriting and colour from the other writings in the collection labels. It seems that this is not Warming's original number but was added later, probably as a control when the material was distributed among the many taxonomic authorities who examined and identified his collections.

NOTHOSTELE BRASILIAËNSIS J.A.N.BAT.,

MENEGUZZO & BIANCH., SP. NOV. (Figs 5A–C, 6)

Type: BRAZIL. Distrito Federal: Brasília, Península Norte, ao lado do Clube do Congresso, campo sujo com transição para cerrado ralo, solo argilo-arenoso, plano, apenas 1 planta, flores toda branco-creme, base do labelo com dois pontos de pêlos de cor verde, 25.viii.1990 (fl), J.A.N. Batista 103 (Holotype: CEN).

Diagnosis: *Nothostelae acianthiformi* similis, sed inflorescentia generaliter 7–10 florum, floribus minoribus, labello obovato apice fortiter reflexo et cuspidato, columna brevior (5–6 mm) et dorsiventraliter complanata, anthera propter ovarii apicem connexa, rostello longiore (1.2 mm) caudatoque et distributione geographica ad bioma 'Cerrado' nucleum limitata differt.

Description: ROOTS not seen. LEAVES not seen. INFLORESCENCE (9)18–22(29) cm high, 1 mm in diameter, green, peduncle (8) 16–23 cm provided with three to six sheaths, sheaths 14–25 mm long; rachis (0.5) 4.0–7.4 cm; floral bracts brownish, 5.0–9.0 × 1.5–2.0 mm. FLOWERS (3) 7–10 (14), cream–whitish, pedicellate ovary green, 6.0–9.0 mm. SEPALS cream–whitish; dorsal sepal recurved, lanceolate, narrowly acute, 5.5–8.0 × 1.5–2.1 mm; lateral sepals recurved, obliquely lanceolate, falcate, shortly connate at base, forming a spur 2–3 mm long, acuminate, 8.0–11.0 × 2.0–2.5 mm. PETALS obliquely oblanceolate, falcate, cream–whitish, 5.0–8.0 × 1.0–1.2 mm. LABELLUM obovate, cream–whitish, base cuneate, margins thickened, green, apex cuspidate, strongly reflexed, 7–10 × 4.0–4.5 mm. COLUMN semi-fusiform, dorsiventrally flattened, 5.0–6.5 mm long from the column foot to the rostellum apex, free part 4–5 mm long; clinandrium extending from the base of the column to the rostellum. ANTHER lanceovate, 2.8–3.5 × 1.4–2.0 mm, attached to the column c. 0.8 mm from the apex of the ovary, filament short, c. 0.3 mm long, free near the

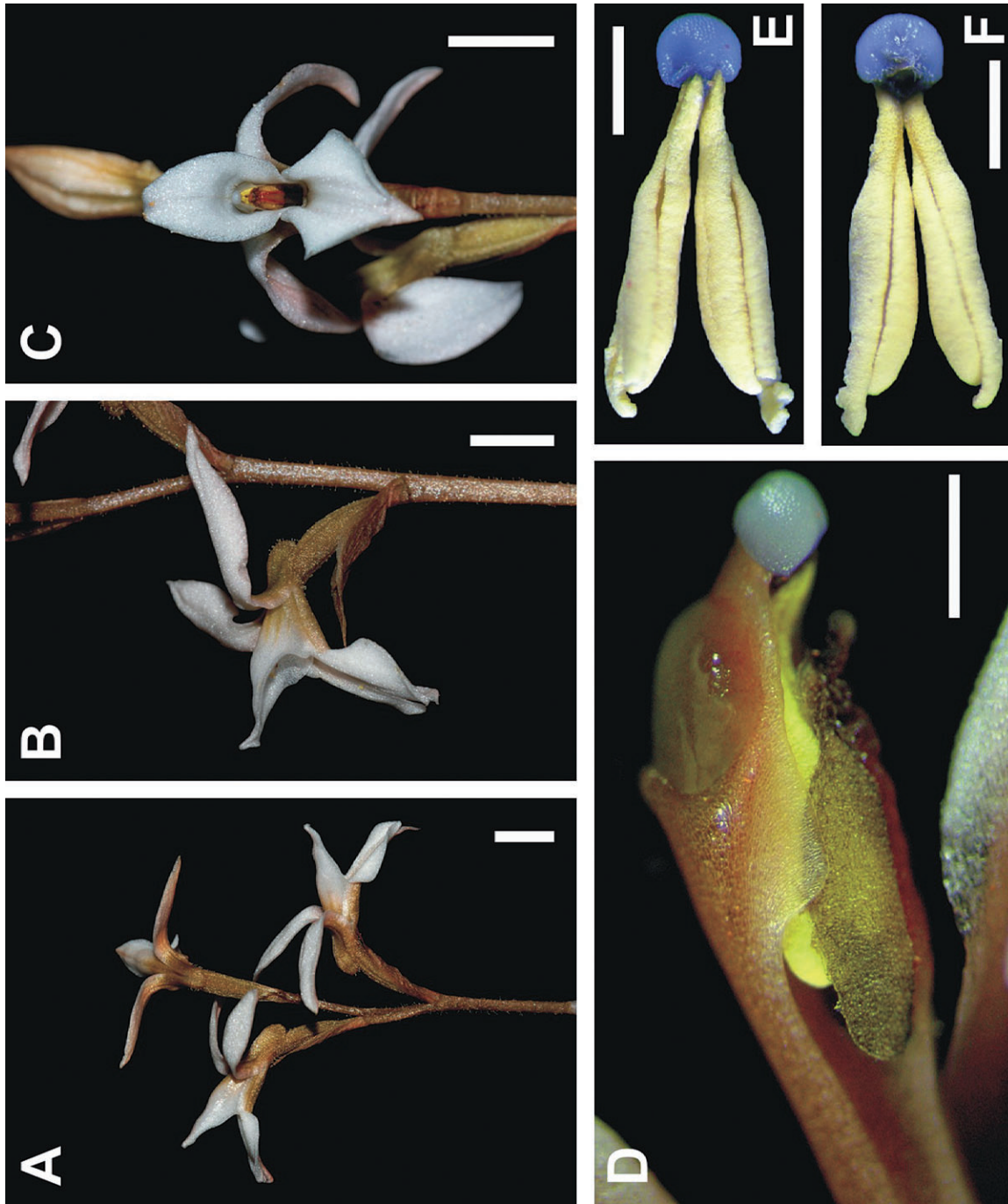


Figure 3. *Nothostele acianthiformis*. A, inflorescence. B, flower, lateral view. C, flower, front view. D, gynostemium including anther, part of filament, clinandrium, stigma and pollinarium, lateral view. E, pollinarium, dorsal view. F, pollinarium, ventral view. Scale bars, 5 mm (A–C); 1 mm (D–F). A–B, D–F (*Viana 767*), C (*Batista et al.* 2007).

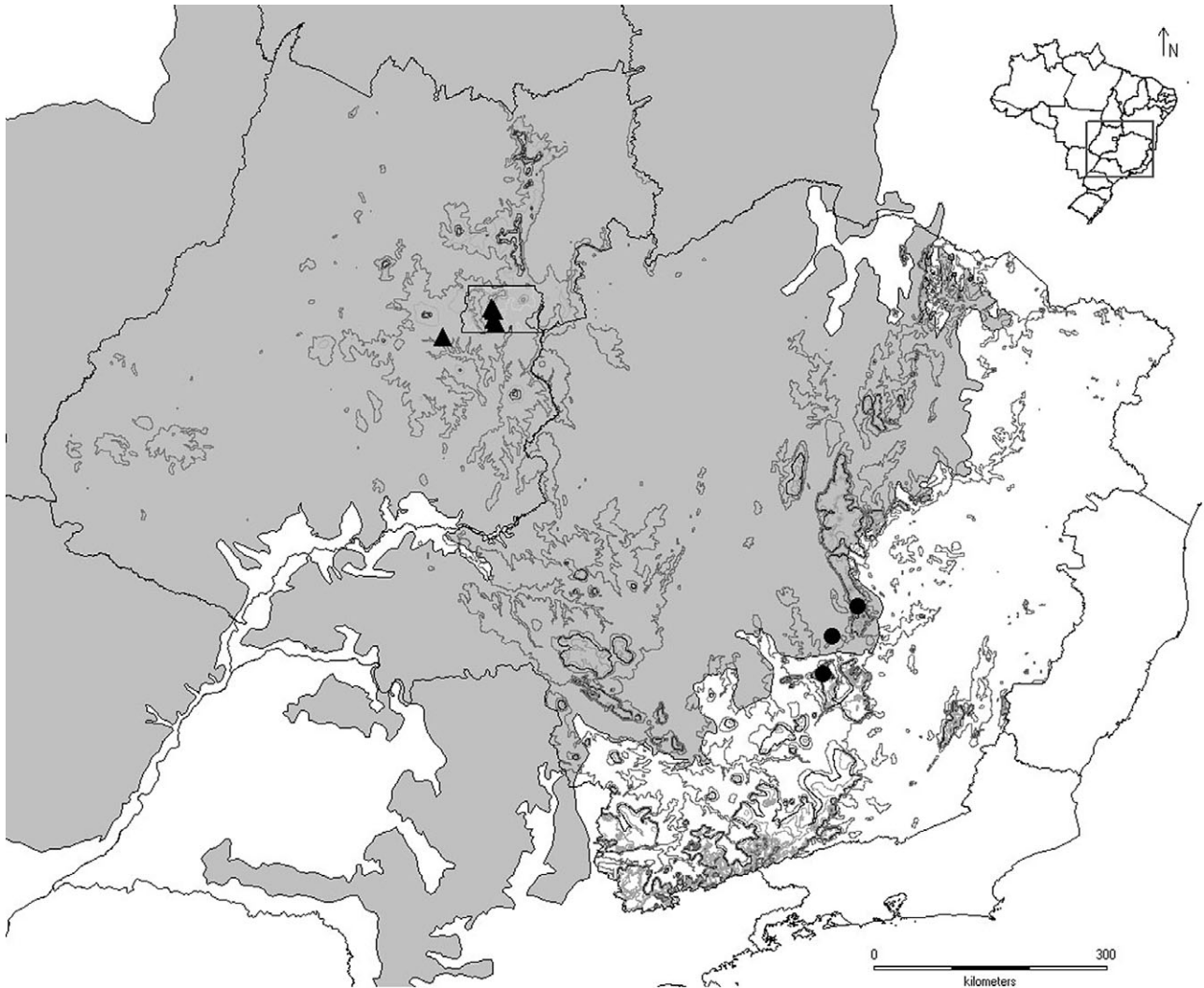


Figure 4. Distribution of *Nothosteles acianthiformis* (●) and *Nothostele brasiliaënsis* (▲). The cerrado area is shaded. Areas over 800 m are indicated by lines.

apex. POLLINARIUM 3.0–3.5 mm long; pollinia partially cleft, clavate; base elongated forming a prominent caudicle made of pollen grains; viscidium ellipsoidal, projected beyond the labellum. STIGMA ventral, entire, deltoid, slightly convex. ROSTELLUM terminal, elongate, 1.0–1.5 mm long, caudate.

Etymology: Named after the Brazilian capital, Brasília, from which most of the collections of the species came.

Illustrations: Garay (1982: fig. 38A), Pabst & Dungs (1975: fig. 491A), and Pabst & Dungs (1977: fig. 491A), all as *N. acianthiformis*. All figures based on the collection *Heringer 8761* (HB).

Distribution, habitat and ecology: The new species is restricted to the core region of the cerrado biome in

Goiás and the Distrito Federal. *Nothostele brasiliaënsis* is a deciduous terrestrial and grows in dry grass-herb-subshrub field (campo sujo) vegetation, over deep, well-drained, reddish, sandy clay oxisols at elevations of approximately 1000 m. Flowering occurs at the peak of the dry season in August when the plants are completely leafless. The leaves of this species have never been observed, but most likely develop during the rainy season and are probably similar to those of *N. acianthiformis*. During the rainy season the leaves are most likely protected from direct sunlight by the surrounding grassy vegetation.

Conservation status: *Nothostele brasiliaënsis* is a rare species known only from three collections from the Distrito Federal and one record from Alexânia in the state of Goiás. The record for Goiás was made by one of the authors, but unfortunately the single specimen

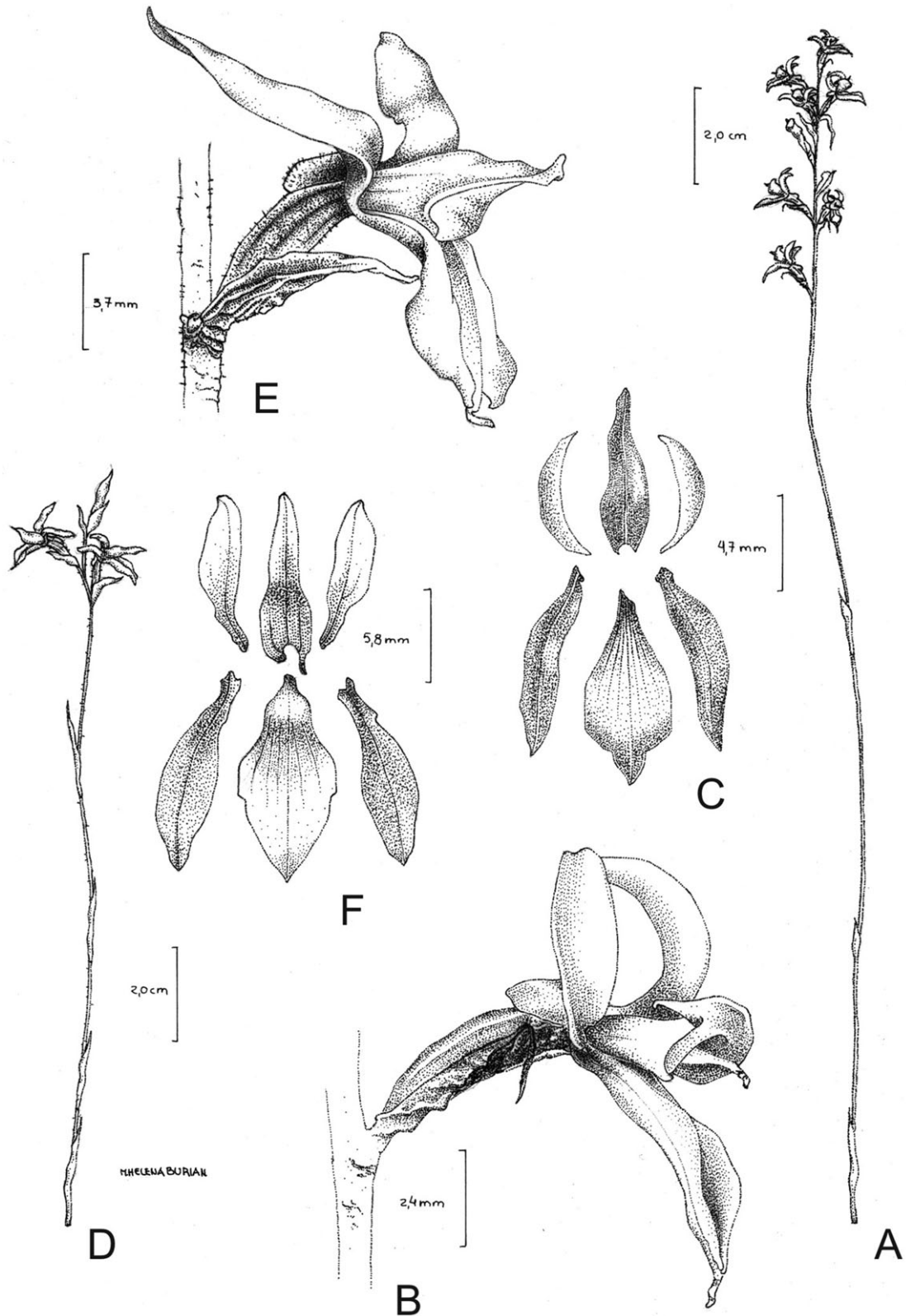


Figure 5. *Nothostele brasiliaënsis* sp. nov. A, habit. B, flower, lateral view. C, perianth. *Nothostele acianthiformis*. D, habit. E, flower, lateral view; F, perianth. A–C (Batista 103). D–E (Viana 767).

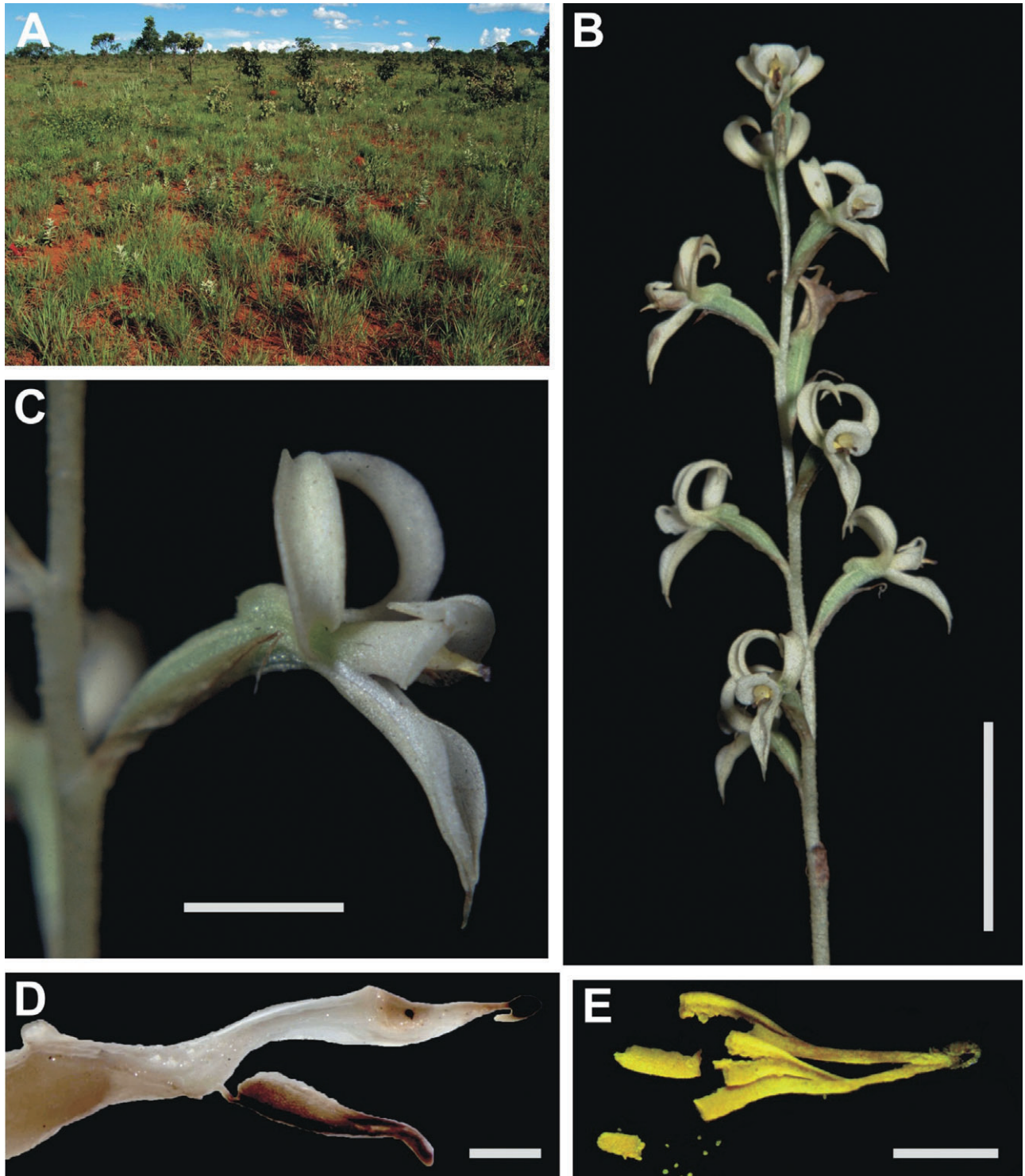


Figure 6. *Nothosteale brasiliaënsis* sp. nov. A, habitat. Dry grass-herb-subshrub field (campo sujo) over deep, well-drained, reddish, clay oxisols. B, inflorescence. C, flower, lateral view. D, gynostemium including anther, filament, clinandrium, stigma and viscidium attached to apex of rostellum, lateral view. E, pollinarium, ventral view. Scale bars, 2 cm (B); 5 mm (C); 1 mm (D–E). B–D (Batista 103). E (Heringer 8761).

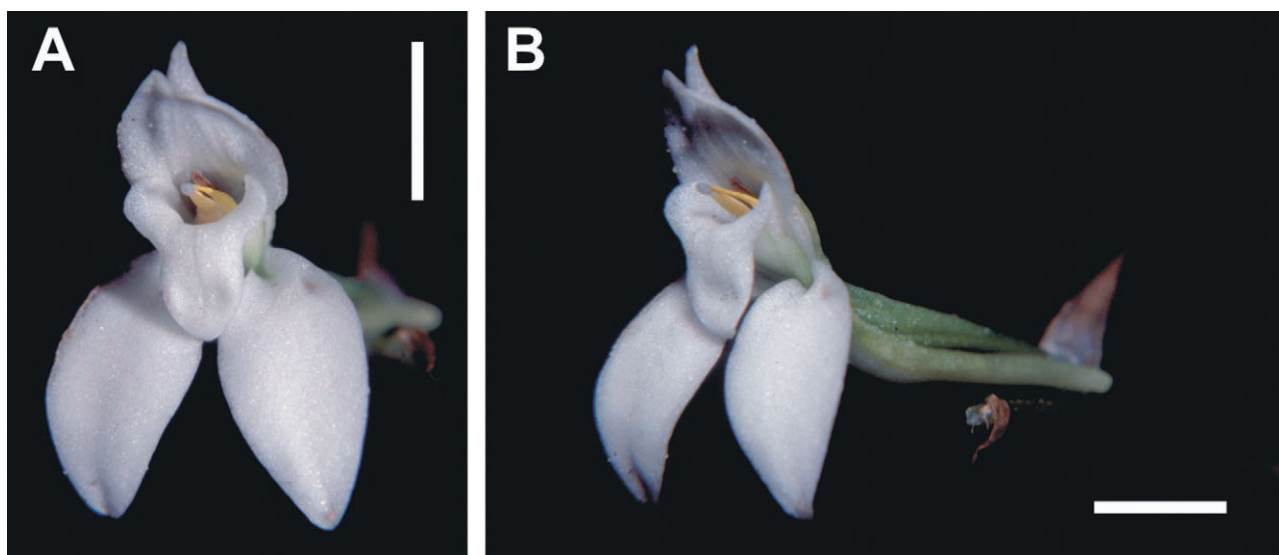


Figure 7. *Eltroplectris cogniauxiana*. A, flower, front view. B, flower, lateral view. Scale bars, 5 mm. A–B (Santuário Ecológico do Vaga Fogo, Pirenópolis, Goiás. Unvouchered).

observed and collected was lost. In the last collection of the species (1990) made by one of the authors, only a single specimen was observed. The Irwin & Soderstrom collection (see below) also comprises a single specimen. The sites where the species was collected in the Federal District are now completely urbanized. Possibly the only known extant site is at Alexânia, but this region is also under intense pressure from agriculture and urban expansion. Using the World Conservation Union Red List Categories and Criteria (IUCN, 2001) *N. brasiliaënsis* would be classified as Critically Endangered CR [criteria B2ab(iii); D].

Additional material examined: BRAZIL. Distrito Federal: Brasília, ao lado da usina de tratamento de água, entre as gramíneas de cerrado seco, 15.viii.1962 (fl), *E.P. Heringer 8761* (HB); Brasília, em frente ao Batalhão, 12.viii.1964 (fl), *E.P. Heringer 8761* (BHCB, UB); terrestrial, solitary, stem pale green, fls. white, the labellum green, 14.viii.1964 (fl), *H.S. Irwin & T.R. Soderstrom 5108* (NY).

Notes: As far as we have been able to determine, *N. brasiliaënsis* was first collected by E. P. Heringer in Brasília, Distrito Federal, in 1962. At that time, specimens in this collection were examined by G. F. J. Pabst and misidentified as *N. acianthiformis*. Material of this collection was also used by Garay for the characterization of the genus in his studies of Spiranthinae (Garay, 1982). Neither Pabst nor Garay, both of whom examined material or illustrations of *N. brasiliaënsis*, perceived the differences between the two species, probably because, in all probability, they never examined genuine material of *N. acianthifor-*

mis. The new species is similar to *N. acianthiformis* but the two species differ by the characters outlined in Table 2. In *Nothostele*, *N. brasiliaënsis* is restricted to the core area of the cerrado biome in Goiás and the Distrito Federal, whereas *N. acianthiformis* is restricted to the central part of Minas Gerais; these areas are approximately 700 km apart (Fig. 4).

The two collections of *N. brasiliaënsis* made by Heringer have the same collection number (*Heringer 8761*), but are from different localities and have different dates. Examination of other Heringer collections revealed similarly equivocal data. Nevertheless, considering the differences in the locality and data, we treat them here as distinct collections.

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Table 2. Diagnostic characters between *Nothostele acianthiformis* and *N. brasiliaënsis*

Characters	<i>N. acianthiformis</i>	<i>N. brasiliaënsis</i>
Flowers	2–6	(3) 7–10 (14)
Dorsal sepal length	10.5–12.0 mm	5.5–8.0 mm
Labellum	Ovate	Obovate
Dimensions	11–14 × 6.0–7.5 mm	7–9 × 4.0–4.5 mm
Apex	Acute, gently recurved	Cuspidate, strongly reflexed
Column	Semi-terete	Dorsiventrally flattened
Free part length	5.5–6.0 mm	4.0–5.0 mm
Anther	Attached to the column for 1.5–2.2 mm from the apex of the ovary	Attached to the column for c. 0.8 mm from the apex of the ovary
Viscidium	Spheroidal	Ellipsoidal
Position relative to labellum	Not projected beyond the labellum	Projected beyond the labellum
Rostellum length	0.5 mm	1.2–1.5 mm
Apex	Apiculate	Caudate
Substrate	Shallow, sandy soils	Loamy, deep oxisols
Habitat	Rocky fields (campos rupestres)	Grass–herb–subshrub field (campo sujo) vegetation
Distribution	Minas Gerais	Goiás and Distrito Federal

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