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Research article

Checklist and molecular phylogenetics reveal three taxonomic novelties in *Habenaria* (Orchidaceae, Orchidoideae) from Chapada dos Veadeiros, Goiás, Brazil

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Abstract. A comprehensive checklist of *Habenaria* from Chapada dos Veadeiros, State of Goiás, was performed alongside morphologic and molecular phylogenetic studies, revealing three new taxa endemic to this region. A total of 61 taxa (59 species and two varieties) of *Habenaria* are recorded for Chapada dos Veadeiros, representing a two-fold increase compared to previous lists and comprising one of the greatest diversities of the genus in Brazil. Of this total, four taxa are locally endemic. *Habenaria cultellifolia*, until recently known only from the type collection, was rediscovered in the region after 127 years without records and represents this species' only known extant population. Three proposed new taxa of *Habenaria* (*H. minuticalcar* J.A.N. Bat. & Bianch. sp. nov., *H. proiteana* J.A.N. Bat., A.A. Vale & Bianch. sp. nov., and *H. lavrensis* var. *xanthodactyla* J.A.N. Bat.& Bianch. var. nov.) are corroborated by molecular phylogenetic analyses based on nuclear and plastid markers. They are described, illustrated, tentatively assessed as threatened, and compared to phylogenetically and morphologically related species. Since some areas of this mountain range have not yet been floristically sampled, additional taxonomic novelties and new records are still expected in the future.

Keywords. Biodiversity, Cerrado, grasslands, Habenariinae, taxonomy.

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Introduction

Habenaria Willd. (Orchidaceae, Habenariinae) is a genus of terrestrial orchids distributed throughout tropical and subtropical regions of the world (Cribb 2001). Currently, around 910 species of *Habenaria* are accepted (Govaerts *et al.* 2021), making the genus one of the largest in the family. Brazil, with 164 to 177 taxa (Flora e Funga do Brasil 2022; Govaerts *et al.* 2021) has the largest number of *Habenaria* species in the New World and possibly also worldwide and this genus is the largest of the family Orchidaceae in the country. Despite the recent progress made in the taxonomic knowledge of this genus in Brazil, there are still large gaps in almost all other aspects, such as the distribution, the regional and the local diversity, the ecology, the pollination and the reproductive biology of the species. Higher regions, with altitudes above 1,000 m a.s.l., are the ones with the greatest diversity (Brade 1951; Toscano de Brito 1995; Batista & Bianchetti 2003; Carvalho *et al.* 2013; Vieira & Barros 2017), but surveys are few and a systematic analysis of the distribution of the genus diversity in Brazil is lacking.

A particular area with great potential for terrestrial orchids is the Chapada dos Veadeiros region, State of Goiás, due to the large extension of savannic grasslands and the mild climate. The floristic knowledge of this region is still incomplete, with the first floristic inventory being the study of Munhoz & Proença (1998) for the municipality of Alto Paraíso de Goiás. This study listed 1,310 species and 120 families of vascular plants, including 47 species and 18 genera of Orchidaceae. In a more recent checklist for Chapada dos Veadeiros, Mendonça *et al.* (2007) listed 2,661 species distributed in 150 families. Orchidaceae, with 84 species, appears as the eighth family with the highest number of species. Both in the checklist by Munhoz & Proença (1998) and by Mendonça *et al.* (2007), *Habenaria* corresponds to the Orchidaceae genus with the largest number of species in this region, with 17 (35% of the family total) and 28 (33%) species, respectively. Besides checklists, some studies described new species of Orchidaceae for Chapada dos Veadeiros, including *Encyclia chapadensis* L.C.Menezes (Menezes 1992), *Cleistes pusilla* Pansarin (Pansarin 2004), *Sacoila cerradicola* Meneguzzo (Meneguzzo 2013), *Habenaria irwiniana* J.A.N.Bat. & Bianch., and *H. minuta* J.A.N.Bat. & Bianch. (Batista *et al.* 2017). Additionally, taxonomic treatments for *Bulbophyllum* Thouars (Santos & Silva 2019) and *Epidendrum* L. (Santos & Silva 2020) are also available for the Chapada dos Veadeiros National Park (PNCV).

Since 1991, two of the authors of the present study (JANB and LBB) have been exploring the diversity of Orchidaceae from the Chapada dos Veadeiros region within the scope of a larger Orchidaceae study from the Cerrado biome. Among the specimens found and collected throughout these years, several specimens of *Habenaria* did not correspond to any of the currently recognised Neotropical species and could not be assigned to any species rank. Subsequent morphological and molecular studies of these specimens revealed that some correspond to new species. In this study, we present the results of the *Habenaria* checklist from Chapada dos Veadeiros alongside morphological and molecular studies revealing three new taxa of *Habenaria* endemic to this region.

Material and methods

Study area

The Chapada dos Veadeiros covers an area of 21 475.60 km^2 comprising eight municipalities in the north of the State of Goiás: São João d'Aliança, Alto Paraíso de Goiás, Campos Belos, Cavalcante, Colinas do Sul, Monte Alegre de Goiás, Nova Roma, and Teresina de Goiás (Lima & Franco 2014). The region's climate is Aw type (according to the Köppen classification system), and the relief is mild to strongly undulate with altitudes ranging from 500–1 650 m a.s.l., including the highest point of the Brazilian Central Plateau. The region has a notable variety of phytophysiognomies such as 'cerrado' sensu stricto (semideciduous xeromorphic medium tall forest), 'cerrado rupestre', 'campo rupestre' (rocky fields), 'campo úmido' (wet grasslands), 'campo limpo' (savannic grasslands), 'campo sujo' (grass-herb-sub-

shrub field), 'veredas' (damp grasslands with buriti palm trees *Mauritia flexuosa* L.f.), 'mata seca' (dry forests), and gallery forests (Munhoz & Proença 1998).

Checklist of *Habenaria* from Chapada dos Veadeiros

The checklist was carried out through 14 botanical expeditions from 1991 to 2018, complemented with the examination of specimens from the AMES, BHCB, CEN, ESA, HB, HBG, HEPH, HRBC, HTO, HUEFS, HUFU, IAN, IBGE, K, M, MBM, NY, P, RB, SP, SPF, UB, UEC and UFG herbaria (acronyms according to Thiers 2022). For herbarium specimens with a mixture of species in the same sheet, each species was counted as a separate record. Species identification was made by comparisons with protologues and type specimens. The main consulted taxonomic studies were those of Lindley (1830–1840), Reichenbach (1847, 1881), Barbosa Rodrigues (1877, 1882), Cogniaux (1893–1896, 1906), Kränzlin (1892, 1911), Renz (1992), and Batista *et al.* (2017). Type specimens and images of them were examined from the AMES, BHCB, BM, BR, C, CEN, G, HB, HBG, K, M, MBM, MPU, NY, P, R, RB, RENZ, S, SP, U, UPS, US and W herbaria. The taxonomic status and circumscription of the species follow a synopsis of the Neotropical species of *Habenaria* (Batista *et al.* 2011a, b).

Taxon sampling for the phylogenetic analyses

To investigate the phylogenetic relationships of the putative new taxa we performed an initial analysis using the same dataset as Batista *et al.* (2013), which comprises ca 51% of the Neotropical species of *Habenaria* and 1,389 bp of the complete nuclear internal transcribed spacer (ITS) region and part of the plastid *matK* gene. Based on this analysis, we selected some terminals for a second analysis using the dataset of Pedron *et al.* (2014), including some species addressed in later studies (Batista *et al.* 2014, 2016, 2017; Cruz-Lustre *et al.* 2022; Lau *et al.* 2021) and 37 new sequences from 11 species (12 terminals) that were generated for this study. For the outgroup, encompassing old world taxa, we used the same dataset as Batista *et al.* (2022a), which consists of a selected sampling based on the studies of Jin *et al.* (2017) and Ngugi *et al.* (2020), comprising representatives of the main clades of Asian and African *Habenaria* and related genera. The final data set consisted of the combined ITS, ETS, *matK-trnK* and *rps16-trnK* DNA sequences of 105 terminals of 103 taxa, including 85 terminals of 83 Neotropical *Habenaria* taxa containing most subclades of Neotropical *Habenaria* (Batista *et al.* 2013). We used *Stenoglottis longifolia* Hook.f., from subtribe Orchidinae, to root all trees based on the results of Ngugi *et al.* (2020). Voucher information, geographic origins, and GenBank accession numbers are provided in Supplementary file 1.

DNA extraction and molecular markers

The rDNA ITS, *matK-trnK*, *rps16-trnK* (Batista *et al.* 2013; Pedron *et al.* 2014) and rDNA ETS (Monteiro *et al.* 2010; Lau *et al.* 2021) markers were used. DNA was extracted from fresh or silica gel-dried samples, according to Doyle & Doyle (1987), and amplified the samples using the same primers and conditions described by Batista *et al.* (2013), Pedron *et al.* (2014) and Monteiro *et al.* (2010). PCR products were purified by precipitation with 20% PEG (2.5 M NaCl and 20% polyethene glycol 8000) and sequenced with the same primers used for amplification at Macrogen Inc. (Korea). All samples were bidirectionally sequenced, assembled with the Staden software package (Staden 1996), and aligned with MAFFT v.7 (Katoh & Standley 2013) as implemented in the EMBL-EBI bioinformatics web (Madeira *et al.* 2019).

Phylogenetic analyses

DNA sequence alignments were analysed using parsimony and Bayesian inference. To assess incongruences between the datasets we performed searches using both individual and combined matrices. In most cases, the concatenation was done with the same individuals sequenced for the different markers. We performed maximum parsimony (MP) phylogenetic analyses using PAUP* version 4 (Swofford 2002) with Fitch parsimony (equal weights, unordered characters; Fitch 1971) as the optimality criterion. Each search

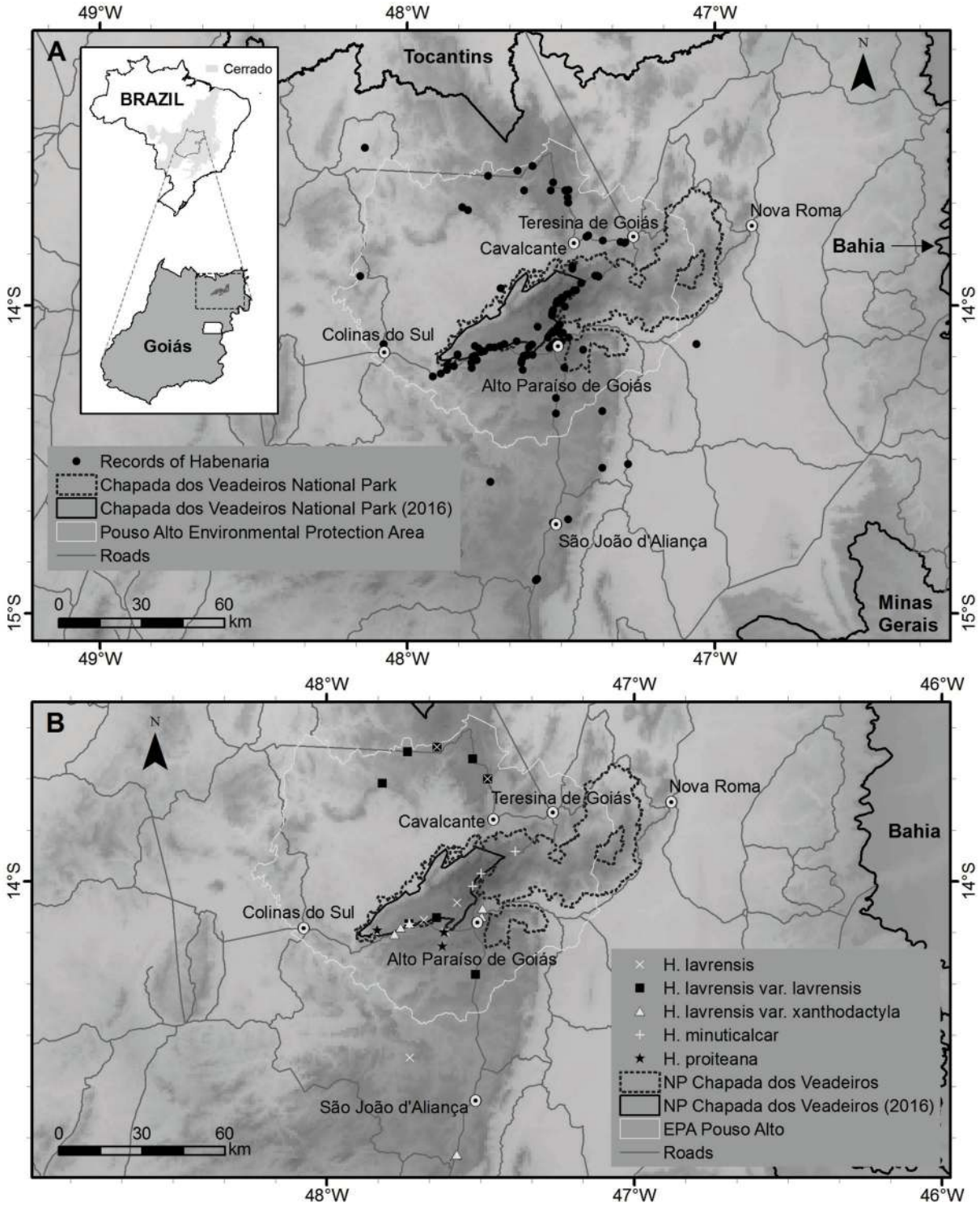


Fig. 1. Distribution map of *Habenaria* records from Chapada dos Veadeiros. **A.** Map with all records. **B.** Map with records of new taxa plus *H. lavrensis* Hoehne var. *lavrensis*. The area incorporated into the park in 2016 is indicated by a dashed line. *Habenaria lavrensis* denotes specimens with unknown intraspecific identification.

consisted of 2,000 replicates of random taxon additions, with branch swapping using the tree-bisection and reconnection (TBR) algorithm, saving ≤ 10 trees per replicate to avoid extensive swapping on suboptimal islands. We evaluated internal support by character bootstrapping (Felsenstein 1985) using 2,000 replicates, simple addition, and TBR branch swapping, saving ≤ 15 trees per replicate. For bootstrap support levels, we considered bootstrap percentages (BP) of 50–70% as weak, 71–85% as moderate, and > 85% as strong (Kress *et al.* 2002).

We performed Bayesian analyses using MrBayes 3.2.7a (Ronquist *et al.* 2012) as implemented in the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal 2.0 (Miller *et al.* 2010), treating each DNA region as a separate partition. We selected an evolutionary model for each DNA region in MrModeltest 2 (Nylander 2004) using the Hierarchical Likelihood Ratio Tests (hLRTs). The unlink command was used to estimate model parameters separately for each partition. Each analysis consisted of two independent runs with four chains for 5,000,000 generations, sampling one tree every 1,000 generations and a temperature parameter of 0.2. Convergence between the runs was evaluated using the average standard deviation of split frequencies (< 0.01) and the Potential Scale Reduction Factor – PRSF (= 1.0) and was achieved after 705,000 generations. After discarding the first 1,500 trees (30%) as the burn-in, the remaining trees were used to assess topology and posterior probabilities (PP) in a majority-rule consensus. Because PP in Bayesian analysis are not equivalent to BP but are generally much higher (Erixon *et al.* 2003), we used criteria similar to a standard statistical test, considering groups with PP > 0.95 as strongly supported, groups with PP ranging from 0.90–0.95 as moderately supported, and groups with PP < 0.90 as weakly supported.

Morphological analyses

Characters regarding habit, perianth, and gynostemium were examined from herbarium specimens, dissected flowers, and alcohol-fixed flowers. For the analyses of the gynostemium, we used a stereoscopic microscope and measured it using a digital calliper. Data concerning flowering times, habitats, and geographic distributions were based on herbarium labels and field observations. Morphological terminology is based on Stearn (1992) and Simpson (2006).

Conservation assessment

The distribution map was elaborated with ArcMap 10.5 (ESRI 2016). Occurrence records were obtained from herbaria and all records included had their identification revised according to the species protologues and the taxonomic circumscriptions of Batista *et al.* (2011 a, b). For specimens without coordinates, the latitude and longitude were inferred using Google Earth. We estimated the extent of occurrence (EOO) and area of occupancy (AOO) using a beta version of GeoCAT (Bachman *et al.* 2011) as implemented in the Royal Botanic Gardens, Kew Web site, with the default setting of 2 km cell width for AOO. Conservation status was inferred using the World Conservation Union Red List Categories and Criteria (IUCN 2012) and the guidelines for using the IUCN red list categories and criteria (IUCN 2016). To estimate the number of populations of each species, we considered each known record, if geographically separated, as a population.

Results

Survey of *Habenaria* in Chapada dos Veadeiros

A total of 61 taxa (59 species and two varieties) of *Habenaria* were recorded for Chapada dos Veadeiros (Table 1) in the municipalities of Alto Paraíso de Goiás, Cavalcante, Colinas do Sul, São João d'Aliança, and Teresina de Goiás (Fig. 1). The species with the highest number of records and the most common were: *H. obtusa* Lindl. (50 records), *H. magniscutata* Catling (32), *H. subviridis* Hoehne & Schltr. (29), *H. pabstii* J.A.N. Bat. & Bianch. (27), *H. hamata* Barb. Rodr. (25), *H. trifida* Kunth (25), *H. schwackei* Barb. Rodr. (24), *H. irwiniana* J.A.N. Bat. & Bianch. (20), *H. psammophila* J.A.N. Bat., Bianch. & B.M.

Table 1 (continued on the next page). *Habenaria* Willd. taxa recorded for Chapada dos Veadeiros, Goiás. Endemic taxa are indicated by an asterisk. Vouchers correspond to the first record of the taxon for the region. PNCV = Parque Nacional da Chapada dos Veadeiros (Chapada dos Veadeiros National Park).

Taxa	N º records	N º records PNCV	Voucher (Herbaria)
H. anisitsii Kraenzl.	7	0	*Batista et al. 719* (CEN, SP)
H. armata Rchb.f.	5	0	*Anderson 6412* (UB)
H. ayangannensis Renz	2	1	*Irwin et al. 32069* (UB)
H. balansae Cogn.	3	3	*Batista & Pansarin 1144* (CEN, UEC)
H. brevidens Lindl.	1	0	*Irwin et al. 24106* (NY)
H. caldensis Kraenzl.	15	7	*Irwin et al. 12406* (HB)
H. candolleana Cogn.	4	0	*Batista et al. 710* (BHCB, CEN, SP)
H. ciliatisepala J.A.N.Bat. & Bianch.	5	2	*Gates & Estabrook 43* (HB, RB, UB)
H. cryptophila Barb.Rodr.	1	0	*Batista et al. 1488* (CEN)
H. cultellifolia Barb.Rodr.	5	0	*Batista et al. 1487* (CEN)
H. curvilabria Barb.Rodr.	4	0	*Anderson 6542* (HB, SP, UB)
H. depressifolia Hoehne	1	0	*Batista 837* (CEN, HUFU, SP)
H. edwallii Cogn.	14	4	*Irwin et al. 12385* (UB UB0018542)
H. egleriana J.A.N.Bat. & Bianch.	13	6	*Irwin et al. 12385* (UB UB0018542_1)
H. gourlieana Gill. ex Lindl.	2	0	*Hatschbach et al. 54669* (MBM)
H. guilleminii Rchb.f.	5	2	*Irwin et al. 12384a* (HB, K)
H. hamata Barb. Rodr.	25	7	*Irwin et al. 12725* (HB, K, UB)
H. heptadactyla Rchb.f.	3	1	*Batista et al. 718* (CEN)
H. heringeri Pabst	2	0	*Batista 839* (CEN, SP)
H. hexaptera Lindl.	2	1	*Glaziou 22153a* (K, P)
H. imbricata Lindl.	8	2	*Batista et al. 705* (CEN)
H. irwiniana J.A.N.Bat. & Bianch.	19	10	*Irwin et al. 12383* (NY, UB)
H. jaguariahyvae Kraenzl.	2	0	*Irwin et al. 12309* (NY, UB)
H. johannensis Barb.Rodr.	1	0	*Batista 836* (CEN)
H. juruenensis Hoehne	5	0	*Batista & Pansarin 1166* (CEN)
H. lavrensis Hoehne var. *lavrensis*	15	4	*Glaziou 2215* (P)
H. lavrensis var. *xanthodactyla* J.A.N.Bat. & Bianch. var. nov. *	10	4	*Gates & Estabrook 12* (HB, RB, SP, UB)
H. leprieurii Rchb.f.	15	5	*Hatschbach et al. 36335* (MBM)
H. leucosantha Barb.Rodr.	3	1	*De Haas et al. 356* (HB, M)
H. ludibundiciliata J.A.N.Bat. & Bianch.	3	1	*Batista et al. 1539* (BHCB)
H. macilenta (Lindl.) Rchb.f.	10	2	*Glaziou 22153* (K, P)
H. magniscutata Catling	31	11	*Irwin et al. 12525* (HB, IAN, UB)
H. minuta J.A.N.Bat. & Bianch. *	3	1	*Gates & Estabrook 216* (RB)
H. minuticalcar J.A.N.Bat. & Bianch. sp. nov. *	5	4	*Batista 843* (CEN)
H. cf. *montis-wilhelminae* Renz	2	0	*Pereira-Silva et al. 4600* (CEN)
H. nasuta Rchb.f. & Warm.	1	0	*Anderson 7610* (UB)
H. nuda Lindl. var. *nuda*	2	2	*Batista et al. 1217* (CEN)

Table 1 (continued). *Habenaria* taxa recorded for Chapada dos Veadeiros, Goiás. Endemic taxa are indicated by an asterisk. Vouchers correspond to the first record of the taxon for the region. PNCV = Parque Nacional da Chapada dos Veadeiros (Chapada dos Veadeiros National Park).

H. nuda var. *pygmaea* Hoehne	2	2	*Walter 2142* (CEN)
H. aff. *nuda* Lindl. (1)	1	0	*Pastore 1870* (HUEFS)
H. aff. *nuda* Lindl. (2)	5	3	*Irwin et al. 12854* (UB)
H. aff. *nuda* Lindl. (3)	3	0	*Batista et al. 1490* (BHCB)
H. obtusa Lindl.	50	10	*Irwin et al. 24045* (HB, RB, UB)
H. omissa J.A.N.Bat. & Bianch.	2	0	*Batista et al. 728* (CEN)
H. orchiocalcar Hoehne	14	5	*Irwin et al. 24996* (HB, K, UB)
H. pabstii J.A.N.Bat. & Bianch.	28	13	*Irwin et al. 12382* (IAN, NY, UB)
H. petalodes Lindl.	2	0	*Anderson 6579* (UB)
H. pratensis (Salzm. ex Lindl.) Rchb.f.	1	1	*Martinelli 16578* (RB)
H. proiteana J.A.N.Bat., A.A.Vale & Bianch. sp. nov.*	3	1	*Hatschbach et al. 58383* (MBM)
H. psammophila J.A.N.Bat., Bianch. & B.M.Carvalho	21	7	*Irwin et al. 12360* (UB)
H. cf. *pseudoculicina* J.A.N.Bat. & B.M.Carvalho	13	8	*Gates & Estabrook 156* (HB)
H. aff. *rodeiensis* Barb.Rodr.	2	0	*Hatschbach et al. 58372* (HBG, MBM)
H. schwackei Barb.Rodr.	24	12	*Wanderley et al. 1695* (SP)
H. secundiflora Barb.Rodr.	4	1	*Pereira-Silva et al. 5887* (CEN)
H. spanophytica J.A.N.Bat. & Bianch.	1	0	*Pastore & Suganuma 579* (CEN)
H. spathulifera Cogn.	11	1	*Irwin et al. 24119* (HB, K, P)
H. sprucei Cogn.	2	0	*Batista & Bianchetti 3086* (BHCB)
H. subfiliformis Cogn.	7	4	*Irwin et al. 24201* (UB)
H. subrepens J.A.N.Bat., B.L.Lau & I.G.C.Machado-Costa	5	2	*Hatschbach 36847* (MBM)
H. subviridis Hoehne & Schltr.	29	13	*Irwin et al. 12384* (HB, UB)
H. tamanduensis Schltr.	20	4	*Glaziou 22156* (K, P)
H. trifida Kunth	24	7	*Irwin et al. 12957* (K, UB)

Carvalho (20), and *H. tamanduensis* Schltr. (20) (Table 1). 27 taxa in this study are known from three or fewer records and can be considered rare or uncommon in the region. Among these stands out, *H. minuta*, endemic to Chapada dos Veadeiros and known so far from only three collections. Another important result was the rediscovery of *H. cultellifolia* (Fig. 5G–I). This species was described in 1877 from material collected in Uberaba, located 600 km to the south, in the State of Minas Gerais, and never collected again, and until its collection in Chapada dos Veadeiros was known only from the type collection.

Regarding distribution, four taxa (7% of the total) are known only from Chapada dos Veadeiros: *H. lavrensis* var. nov. *xanthodactyla* J.A.N. Bat. & Bianch., *H. minuta*, *H. minuticalcar* J.A.N. Bat. & Bianch. sp. nov., and *H. proiteana* J.A.N.Bat., A.A.Vale & Bianch. sp. nov. (Table 1). On a larger scale, four taxa are restricted to the Brazilian Central Plateau: *H. egleriana* J.A.N.Bat. & Bianch., *H. irwiniana*, *H. pabstii* J.A.N.Bat. & Bianch., and *H. spanophytica* J.A.N.Bat. & Bianch. (Flora e Funga

do Brasil 2022), while six taxa are shared with other montane regions of the States of Minas Gerais and Bahia: *H. ciliatisepala* J.A.N.Bat. & Bianch., *H. lavrensis* Hoehne, *H. magniscutata* Catling, *H. nuda* Lindl. var. *nuda*, *H. psammophila*, and *H. subrepens* J.A.N.Bat., B.L.Lau & I.G.C.Machado-Costa (Flora e Funga do Brasil 2022; Batista *et al.* 2022b). Regarding distribution of records and taxa within the PNCV, of the total records, 175 (33%) were made in the PNCV, and of the total taxa recorded for the region, 22 (36%) have no record for the PNCV, while another 17 (28%) taxa have only 1–2 records for this park (Table 1).

From a historical perspective, the first records for the region were made by A.F.M. Glaziou, who collected four species in 'Chapadão dos Viadeiros' between 1894 and 1895. The following records were only made in the 60s, when H.S. Irwin and colleagues intensively collected in the region between 1966 and 1971, resulting in 19 new records of *Habenaria* for Chapada dos Veadeiros (Table 1). In the 70s, the main collectors were W.R. Anderson, G. Hatschbach, and Gates & Estabrook, whose collections resulted in 13 new records for the region. From the 1990s onwards, the collections in the region were made mainly by researchers and postgraduate students from regional institutions such as CENARGEN, Universidade de Brasília and Jardim Botânico de Brasilia, contributing with five new records. And from 1991 onwards, field collections within this study began and lasted until 2018, contributing to 17 new records of *Habenaria* for this region. According to our data, of a total of 63 collectors, the ones with the highest number of collections are J.A.N. Batista (165 collections), H.S. Irwin (46), A.A. Vale (32), J.F.B. Pastore (29), A.E.H. Salles (28), and G. Hatschbach (25). The number of collections of the genus in the region is high (542 herbarium collections; 9 collections/taxa), which reflects both the specific sampling effort for Orchidaceae carried out by the authors and the high frequency of the genus in the region.

Phylogenetic analyses

The concatenated ITS, ETS, *matK-trnK,* and *rps16-trnK* DNA matrix consisted of 4,372 aligned characters, of which 841 (19%) were parsimony-informative. Table 2 presents the general features of the datasets and parsimony statistics along with a summary of the models implemented for each partition. Missing data accounted for 8.4% of the combined matrix, most of which were from the complete *matK-trnK*, *rps16-trnK* and ETS sequences that were missing from Old World taxa. Missing data for the Neotropical sequences accounted for just 2.4% of the combined matrix. Comparable to previous molecular phylogenetic analyses with similar datasets (Pedron *et al.* 2014; Lau *et al.* 2021), no significant incongruences were detected between nuclear and plastid datasets, therefore only the results of the combined matrix are presented and discussed. The parsimony analyses retained a total of 17,551 most parsimonious trees, with a tree length of 3,013 steps, a consistency index (CI) of 0.63, and a retention index (RI) of 0.78. The strict consensus tree from the parsimony analysis was, for the most part, congruent with the Bayesian majority-rule consensus tree. Still, as the latter was more fully resolved and had stronger overall support, it was chosen for presentation and discussion (Fig. 2). For comparison, bootstrap percentages from the bootstrap analysis are shown in the Bayesian tree.

The relationships recovered were similar to those of earlier molecular phylogenetic studies (Batista *et al.* 2013, 2014, 2016, 2017, 2022a; Pedron *et al.* 2014; Lau *et al.* 2021), with the New World *Habenaria* species forming a well-supported monophyletic group (1.00 PP, 83% BP) and recovering several well-supported subclades within the New World clade that are numbered accordingly (subclades 1 to 21) (Fig. 2). The two new species addressed in this study (see Taxonomic treatment) were recovered within the Cerrado clades (1.00 PP, 96% BP), which comprises clades and species distributed mainly in the Cerrado Phytogeographic Domain of Central Brazil (Batista *et al.* 2013). *Habenaria minuticalcar* sp. nov. (Figs. 33A–E, 4) was recovered with high support (1.00 PP, 83% BP) as sister to *H. pseudoculicina* J.A.N.Bat. & B.M.Carvalho, while *H. proiteana* sp. nov. (Figs. 5A–F, 6) was recovered with high support (1.00 PP, 100% BP) as sister to *H. psammophila*. *Habenaria lavrensis* var. *xanthodactyla* var. nov. was not sampled in this study, but *Habenaria lavrensis* var. *lavrensis* was also recovered in the Cerrado clades.

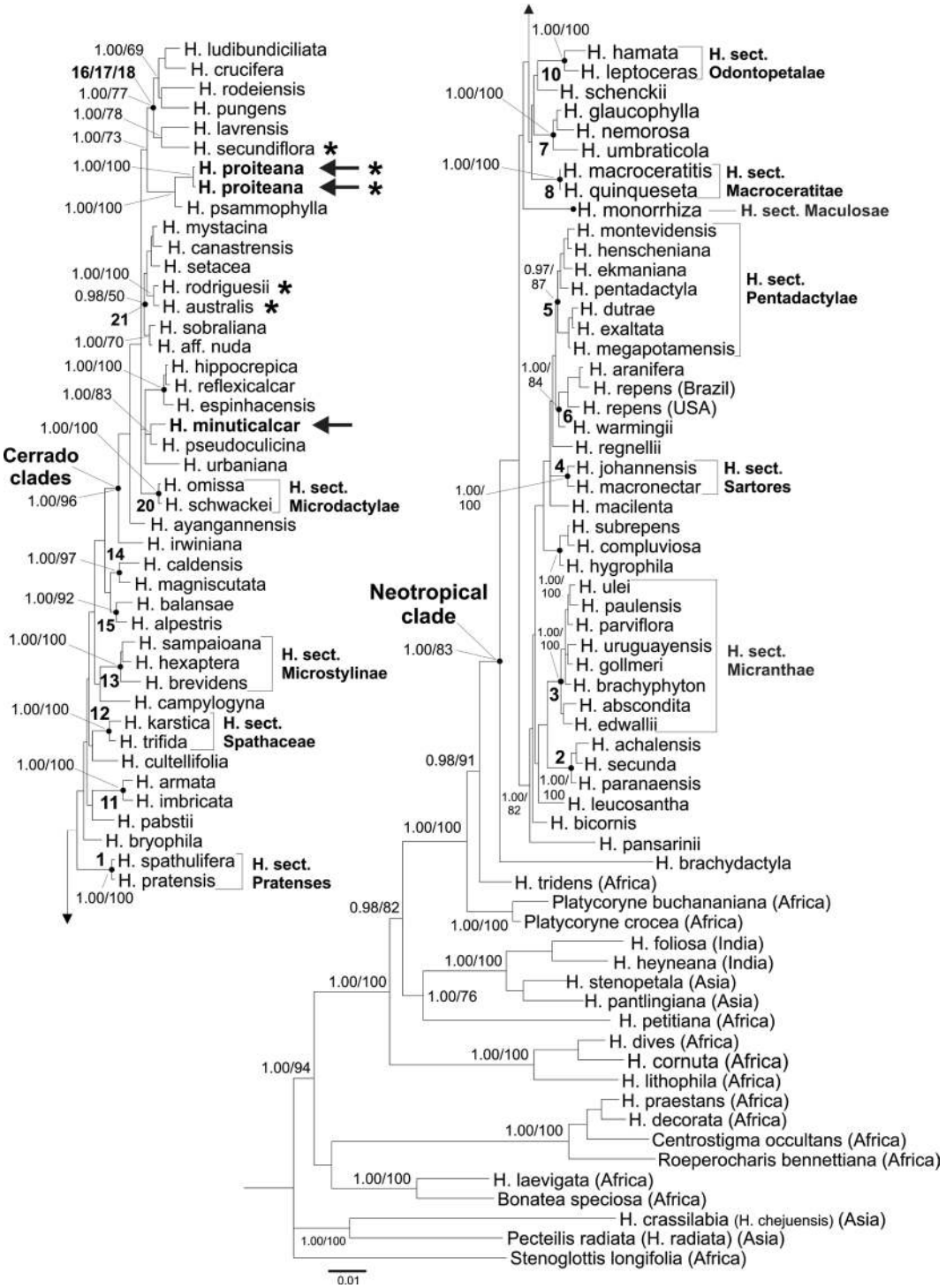


Fig. 2. Bayesian 50% majority-rule consensus tree of the combined ITS, ETS, *matK-trnK* and *rps16–trnK* datasets. Numbers next to the nodes represent the posterior probabilities (PP) from the Bayesian analyses and bootstrap percentages (BP) from the parsimony analyses. Only the values of the main clades are shown. Neotropical subclades are numbered according to Batista *et al.* (2013). The new species described here are highlighted in bold and indicated by an arrow. Species with the petal anterior segment inserted above the base of the posterior segment are highlighted by an asterisk. Subclades in which it was possible to associate with one of the sections of Kränzlin's sectional treatment are identified accordingly.

Taxonomy

Class Equisetopsida C.Agardh
Order Asparagales Link
Family Orchidaceae Juss.
Subfamily Orchidoideae Lindl.
Tribe Orchideae Verm.
Subtribe Habenariinae Benth.
Genus *Habenaria* Willd.

Habenaria minuticalcar J.A.N.Bat. & Bianch. sp. nov.
urn:lsid:ipni.org:names:77325126-1
Figs 3A–E, 4

Diagnosis

Similar to *H. guilleminii* Rchb. f. in the short spur, but distinguished by the leaves linear, narrow, 2–3 mm wide (vs lanceolate or lance-ovate, 5–23 mm wide), pedicellate ovary 7–12 mm long (vs5–7 mm long), lateral sepals linear, 0.9–1.2 mm wide (vsobliquely ovate or obliquely lance-ovate, 2–3 mm wide), petal anterior segment filiform, 1.5–2.2 mm long. (vsreduced to a tooth-like projection, 0.2–0.7 mm long), and lip lateral segments shorter than the median segment (vslonger).

Etymology

From the Latin 'minutus' (very small) and 'calcar' (spur), referring to the very small spur, an unusual feature in the genus and one of the smallest among New World *Habenaria*.

Material examined

Holotype

BRAZIL – **Goiás** • Alto Paraíso de Goiás, 21 km N. de Alto Paraíso, campo a margem esquerda da GO-118, em direção a Teresina de Goiás; Parque Nacional da Chapada dos Veadeiros; 1 Jan. 1999; fl.; *J.A.N. Batista 843*; GenBank nrITS: HM777691; nrETS: OP589155; *matK* gene and *trnK* intron: OP589167; *rps16-trnK* intergenic spacer: OP589998; holotype: CEN[32585].

Paratypes

BRAZIL – **Goiás** • Teresina de Goiás, Chapada dos Veadeiros, GO-118, em direção a Alto Paraíso, ca de 37 km ao N e antes de Alto Paraíso; 9 Jan. 2001; fl.; *J.A.N. Batista & E.R. Pansarin 1149*; CEN[CEN00037136]•Alto Paraíso de Goiás, Chapada dos Veadeiros; 30 Dec. 2010; fl.; *J.A.N. Batista & L.B. Bianchetti 3099*; BHCB[151375]•Alto Paraíso de Goiás, Chapada dos Veadeiros; 30 Dec. 2010; fl.; *J.A.N. Batista & L.B. Bianchetti 3104*; BHCB[151377]• Alto Paraíso de Goiás, estrada para a Vila de São Jorge, ca 26,5 km da GO-118; 23 Jan. 2005; fl.; *J. Paula-Souza et al. 4528*; ESA[090123].

Description

Geophytic herb, caulescent, sympodial. Rootsand tuberoid not examined. *Stem* 13–28 cm long including the inflorescence, 0.8–1.2 mm wide, slender, erect, straight to somewhat sinouse. *Leaves* 5–8, spirally-alternate, linear, narrow, the largest concentrated in the middle of the stem, 1.5–8 × 0.2–0.3 cm, reducing towards the apex of the stem, base sheathing, sheath closed. *Inflorescence* 2–6 cm long, spiral, few flowered, lax; floral bracts 5–17 × 3–4 mm, elliptical, caudate, apex acuminate, about the same size as the pedicellate ovary, decreasing in size towards the inflorescence apex, green. *Flowers* 2–7, resupinate, green; pedicellate ovary 7–12 mm long, mostly straight, more or less parallel to the rachis axis or ascending, apex curved; ovary 6–10 mm long, pedicel 1–2 mm long. *Sepals* green, margin smooth, apex

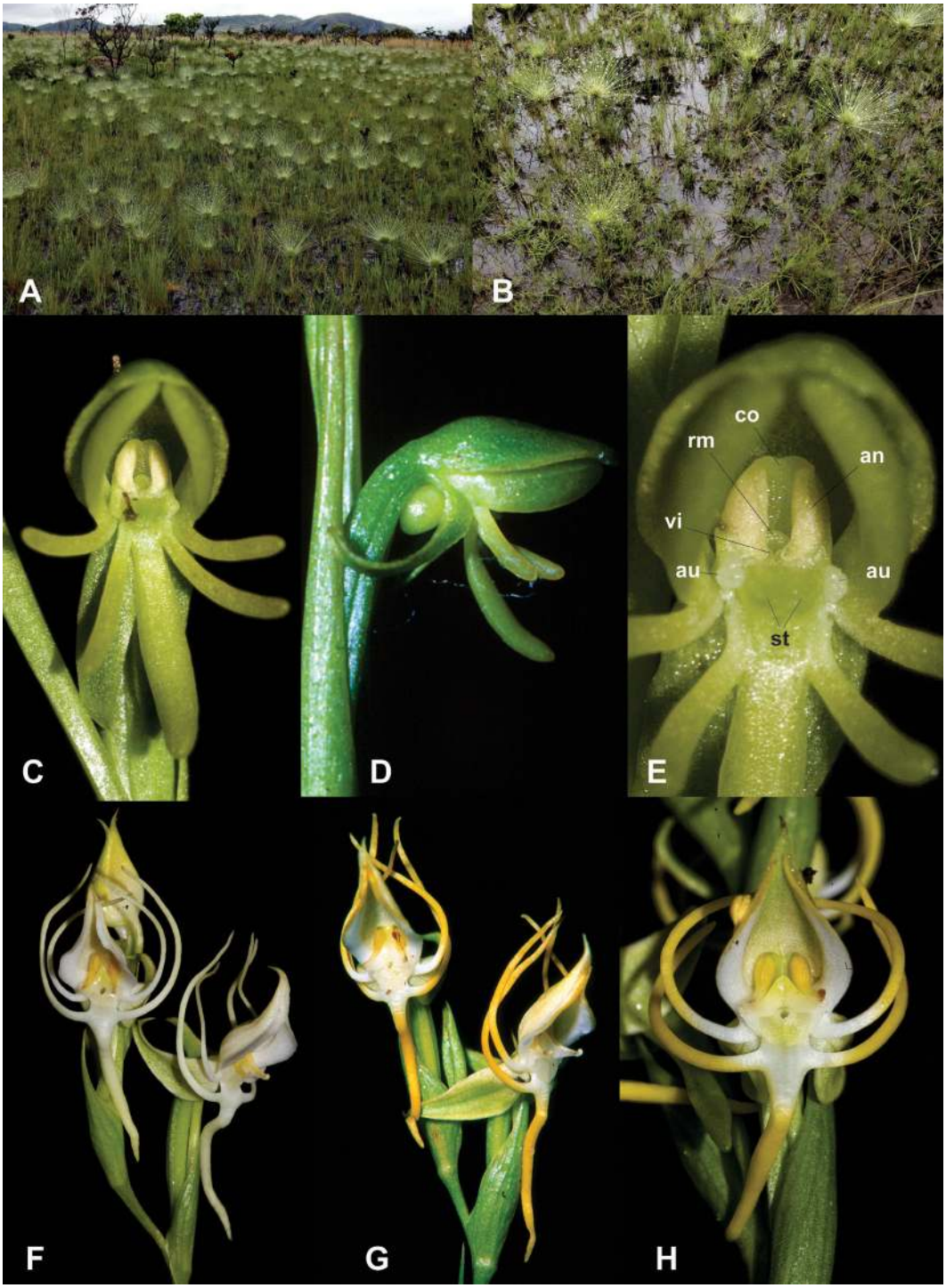


Fig. 3. *Habenaria minuticalcar* J.A.N. Bat. & Bianch. sp. nov. **A–B.** Habitat: seasonally humid grassland with *Paepalanthus* sp. during the rainy season in December 2010, in Chapada dos Veadeiros. **C.** Flower, front view. **D.** Flower, side view. **E.** Gynostemium, front view. *Habenaria lavrensis* var. *lavrensis*. **F.** Inflorescence. *Habenaria lavrensis* var. *xanthodactyla* var. nov. **G.** Inflorescence. **H.** Flower, front view. C, E from *Batista & Bianchetti 3099* (BHCB); D from *Batista 843* (CEN); F from *Batista 2940* (BHCB); G from *Batista & Proite 1494* (CEN); H from *Batista & Bianchetti 3095* (BHCB). Abbreviations: an = anther; au = auricles; co = connective; rm = rostellum mid lobe; st = stigmatophores; vi = viscidia. All photographs by J.A.N. Batista.

discreetly mucronate or obtuse; dorsal sepal 3–5 × 3–3.5 mm, concave, ovate or elliptical when flattened; lateral sepals 3–4.5 × 0.9–1.2 mm, obliquely linear to obliquely linear-lanceolate, deflexed or reflexed, apex obtuse. Corolla base whitish, towards the apex light green or greenish yellow. *Petals* bipartite; posterior segment 2.5–4.2 × 1–1.2 mm, oblong, slightly falcate, apex obtuse, connivent with the dorsal sepal; anterior segment 1.5–2.2 mm long, shorter than the posterior lobe, linear-filiform, inserted at the base of the posterior segment. *Lip* tripartite; undivided basal part 0.5 mm long; lateral segments 1.9–3 mm long, shorter than the median segment, linear-filiform, bent forward; median segment 3–4.5 × 0.7–0.9 mm, linear, bent forward; spur 1–1.6 × 1–1.3 mm, shorter than the pedicellate ovary, free from the bract, ovate, green. *Gynostemium* 1.5 mm high, reclinate; connective green, apex emarginate; lateral appendages (auricles) 0.9–1.2 × 0.4 mm, fleshy, verrucose, apex obtuse, whitish. *Anther* 0.7–1 mm high, bilocular, loculi parallel, translucent; canals very short, slightly convergent; pollinaria 2, 0.7–0.8 mm long each, joined by the viscidia; caudicles 0.2 mm long, filiform; pollinia 0.5–0.6 mm long, yellow; viscidia 0.15 × 0.15 mm, enclosed between the anthers. *Stigmatophores* (stigma lobes) 2, ca 1.6 mm long, closely parallel, green, receptive surface 0.9 mm long, convex, turned frontwards, 0.4 mm wide each, apex obtuse. *Rostellum* ca 1 mm long, green; mid lobe ca 0.6 mm long, 0.4–0.5 mm high, triangular, erect, held between the anther loculi, fleshy, apex obtuse; side-lobes 0.3–0.4 mm long, convergent towards the apex.

Distribution, Habitat and Phenology

Habenaria minuticalcar sp. nov. is restricted to Chapada dos Veadeiros, State of Goiás, central Brazil. The new species grows in seasonally humid grassland (Fig. 3A), associated with 'murundus' or 'campos rupestres' (rocky fields), with dark, shallow, rocky, sandy-clay soils, at 1,100 to 1,600 m a.s.l. The soil can be humid during some periods in the rainy season (Fig. 3B) but usually dries out completely during the dry season. The plants are difficult to find in their habitat as they are small and slender, grow among grasses and can only be found when they are in flower. Flowering occurs during the peak of the rainy season, from December to January. Like other *Habenaria* and terrestrial grassland orchids, the flowering of the new species is enhanced by seasonal brush fires. All our collections of the species were made in areas burned from the previous dry season.

Conservation Assessment

Habenaria minuticalcar sp. nov. is currently known from only four populations, all from Chapada dos Veadeiros, and shows an EOO estimated at 194 km^2 and a small AOO of 16 km^2. Based on the IUCN Red List Categories and Criteria and its guidelines (IUCN 2012, 2016), the species can be tentatively classified as Endangered EN B1ab(iii)+2ab(iii); C2a(i).

Taxonomic notes

Habenaria minuticalcar sp. nov. resembles *H. guilleminii* in the size and morphology of the spur. However, it is distinguished by the linear, narrow, 2–3 mm wide leaves (Fig. 4D–F) (vs lanceolate or elliptical, 5–23 mm wide in *H. guilleminii*), narrow, 0.9–1.2 mm wide lateral sepals (vs 2–3 mm wide), petal anterior segment 1.5–2.2 mm long, reaching half the size of the petal posterior segment (vs reduced to a tooth-like projection) and lip lateral segments at most half the length of the median segment (Fig. 4G–I) (vs lateral segments longer than the median segment). *Habenaria minuticalcar* sp. nov. is also similar to *H. pansarinii* var. *minuscula* J.A.N.Bat. & Bianch. in general morphology and size of the spur. However, it differs from this taxon by the floral bract measuring 5–17 mm long, approximately the same length as the pedicellate ovary (Fig. 4J–K) (vs 2.2–3.5 mm long, shorter than the pedicellate ovary), the slightly larger flowers (e.g., dorsal sepal 3–5 × 3–3.5 mm vs 2.4–3 × 1.8–2.4 mm in *H. pansarinii* var. *minuscula*), the petal posterior segment 2.5–4.2 mm long, oblong with an obtuse apex (vs 2.1–2.7 mm long, lanceolate, apex acute), and the geographical distribution restricted to Chapada dos Veadeiros (vs restricted to the southern and central regions of the Espinhaço Range in the State of Minas Gerais).

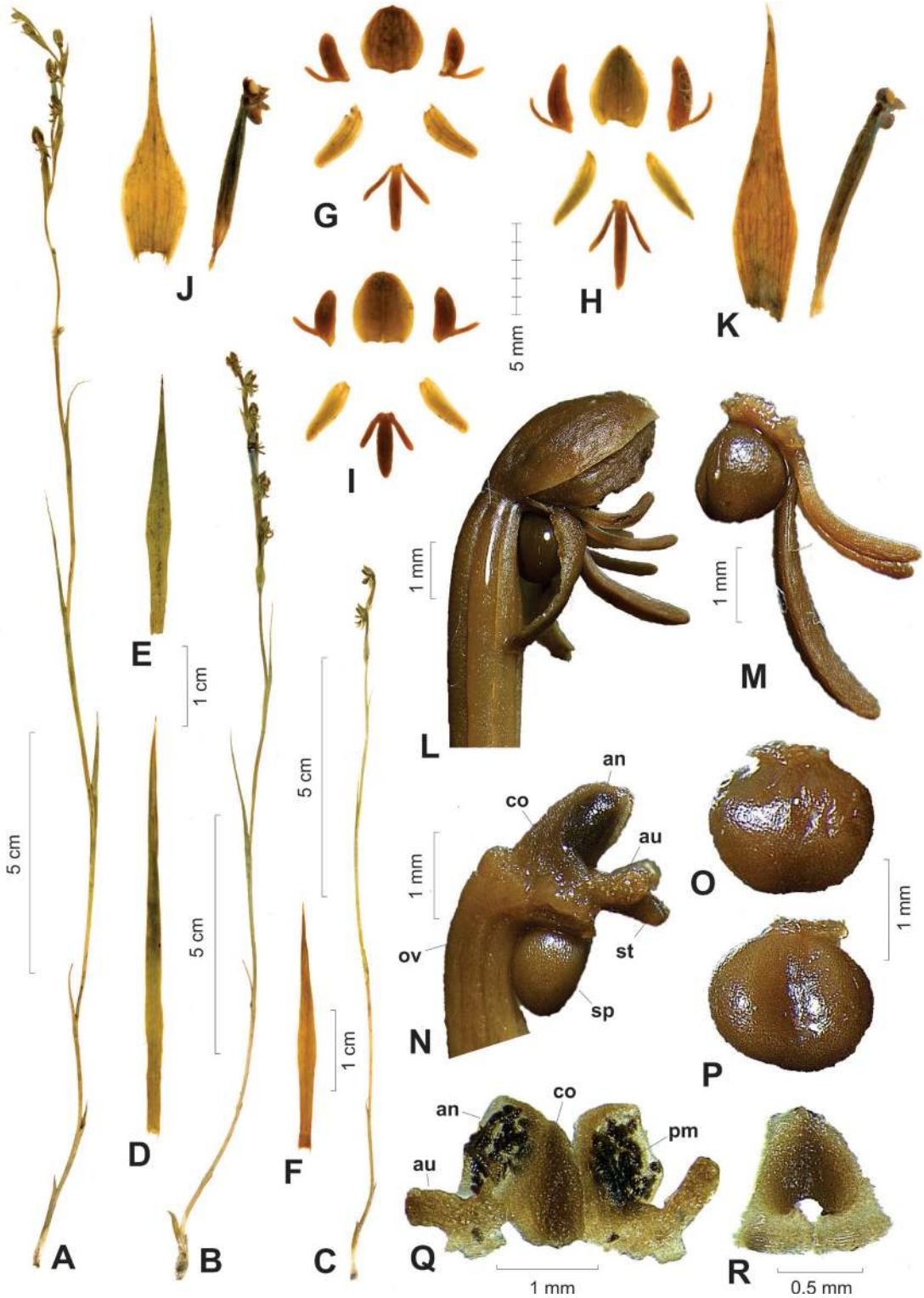


Fig. 4. *Habenaria minuticalcar* J.A.N. Bat. & Bianch. sp. nov. **A–C.** Habit. **D–F.** Leaves. **G–I.** Dissected perianth. **J–K.** Bract, pedicellate ovary, gynostemium and spur, lateral view. **L.** Flower, side view. **M.** Lip and spur, side view. **N.** Distal part of the ovary, gynostemium and spur, side view. **O–P.** Spur. **Q.** Flattened connective, anther and lateral appendages (auricles). **R.** Rostellum, upper view. A–D and F–R from *Batista 843* (CEN); E from *Batista & Bianchetti 3099* (BHCB). Abbreviations: an = anther; au = auricles; co = connective; ov = ovary; sp = spur; st = stigmatophores; pm = pollen massulae.

Key to the Brazilian species of Habenaria with a reduced spur

1. Pedicel shorter than the ovary; dorsal sepal ≤ 5 mm long 2
– Pedicel the same length or longer than the ovary; dorsal sepal ≥ 8 mm long 4

2. Leaves lanceolate or elliptical, 5–23 mm wide; lip lateral segments longer than the median segment; viscidia separated ..... ***H. guilleminii*** Rchb.f.
– Leaves linear, narrow, 1–4 mm wide; lip lateral segments shorter than the median segment; viscidia united 3

3. Floral bracts 5–17 × 3–4 mm, elliptical, about the same size as the pedicellate ovary, decreasing in size towards the inflorescence apex; pedicellate ovary 7–12 mm long; dorsal sepal 3–5 × 3–3.5 mm; petal posterior segment 2.5–4.2 mm long, oblong, apex obtuse; restricted to the Chapada dos Veadeiros, State of Goiás, Brazil ..... ***H. minuticalcar*** sp. nov.
– Floral bracts 2.2–3.5 × 1.2–1.8 mm, ovate, shorter than the pedicellate ovary; pedicellate ovary 6.7–8.1 mm long; dorsal sepal 2.4–3 × 1.8–2.4 mm; petal posterior segment 2.1–2.7 mm long, lanceolate, apex acute; restricted to the southern and central regions of the Espinhaço Range in the State of Minas Gerais, Brazil ..... ***H. pansarinii*** var. ***minuscula*** J.A.N.Bat. & Bianch

4. Dorsal sepal 11–20 mm long; petal anterior segment 8–11(–16) mm long, about the same size as the posterior segment; spur ovoid, scrotiform, ventrally compressed, 3–3.5 mm long..... ..... ***H. orchiocalcar*** Hoehne
– Dorsal sepal 8–12 mm long; petal anterior segment 1–4 mm long, less than half the length of the posterior segment; spur elliptical, 5–6 mm long ..... ***H. heringeri*** Pabst

Habenaria lavrensis Hoehne var. ***xanthodactyla*** J.A.N.Bat. & Bianch. var. nov.
urn:lsid:ipni.org:names:77326615-1
Figs 3G–H

Diagnosis

Similar to *H. lavrensis* var. *lavrensis* in general morphology, habitat and phenology, but distinguished by the yellow segments of the petals and lip (vs white) and by the geographical distribution restricted to the Chapada dos Veadeiros region (vs broadly distributed in the States of Goiás, Minas Gerais, and Distrito Federal).

Etymology

From the Greek ‘xanthos’ (yellow) and ‘dactylos’ (finger), referring to the yellow segments of the petals and lip.

Material examined

Type

BRAZIL – **Goiás** • São João da Aliança. GO-118, cerca de 22 km S de São João da Aliança; 9 Feb. 2004; fl.; *J.A.N. Batista & K. Proite 1494*; holotype: BHCB[BHCB199457]; isotypes: CEN, RB, SP.

Paratypes

BRAZIL – **Goiás** • Alto Paraíso de Goiás, Chapada dos Veadeiros; 27 Jan. 1993; fl.; *J.A.N. Batista & L.B. Bianchetti 386*; CEN[CEN00018264]•Alto Paraíso de Goiás, Chapada dos Veadeiros, na estrada de terra em direção a cachoeira da Água Fria; 8 Jan. 2003; fl.; *J.A.N. Batista et al. 1365*; CEN[CEN00046058]•Alto Paraíso, Chapada dos Veadeiros, em direção ao Vale da Lua, cerca de 1,7 km do entroncamento com a GO-239; 30 Dec. 2010; fl.; *J.A.N. Batista & L.B. Bianchetti 3095*;

BHCB[151368]•Alto Paraíso de Goiás, Chapada dos Veadeiros; 30 Dec. 2010; fl.; *J.A.N. Batista & L.B. Bianchetti 3100*; BHCB[151373]•Chapada dos Veadeiros, 5 km E of Alto Paraíso; 1500 m; 24 Jan. 1979; fl.; *Gates & Estabrook 12*; HB[70548], NY[NY01031853], RB[RB00257176], SP[195098], UB[UB0018813]•Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, trilha para as corredeiras; 23 Jan. 2009; fl.; P.L. *Ribeiro et al. 396*; HUEFS[HUEFS0162888]•Alto Paraíso de Goiás, estrada para a Vila de São Jorge; 23 Jan. 2005; *J. Paula-Souza 4555*; ESA[090150]•Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, trilha para as Cachoeiras; 24 Jan. 2005; *J. Paula-Souza 4581*; ESA[090176]•São João da Aliança, 21 km ao Sul de São João da Aliança; 23 Feb. 1997; fl.; *J.A.N. Batista et al. 729*; CEN[CEN00026177]•ca 20,5 km S de São João da Aliança; 5 Feb. 2010; *J.A.N. Batista et al. 2965*; BHCB[137850].

Distribution, Habitat and Phenology

Habenaria lavrensis var. *xanthodactyla* var. nov. is so far only known from Chapada dos Veadeiros, Goiás, in the municipalities of Alto Paraíso de Goiás and São João da Aliança. In the northern part of Chapada dos Veadeiros, in the Distrito Federal and the state of Minas Gerais, where we have collected or examined specimens of this species, only *H. lavrensis* var. *lavrensis* is known. *Habenaria lavrensis* var. *xanthodactyla* var. nov. grows in dry, hillside or *campo rupestre*, in shallow, dry, gravelly soil. Flowering occurs during the peak of the rainy season, from January to February. In common with other grassland species of the genus, bushfires favoured flowering during the dry period. All populations we observed in flowering grew in areas that burned in the previous dry period. The habitat and the flowering period are about the same between the two varieties.

Conservation Assessment

Habenaria lavrensis var. *xanthodactyla* var. nov. is currently known from only seven populations from six localities and shows an EOO estimated at 1,647 km^2 and an AOO of 32 km^2. Based on the IUCN Red List Categories and Criteria and its guidelines (IUCN 2012, 2016), the species can be tentatively classified as Vulnerable VU B1ab(iii)+2ab(iii).

Taxonomic notes

Habenaria lavrensis var. *xanthodactyla* var. nov. is identical in vegetative and flower morphology to *H. lavrensis* var. *lavrensis* but distinct by the yellow lateral segments of petals and lip (Fig. 3G–H). In contrast, the entire flower is completely white or, at most segments, slightly creamy-white in *H. lavrensis* var. *lavrensis* (Fig. 3F). Both *H. lavrensis* var. *lavrensis* and *H. lavrensis* var. *xanthodactyla* var. nov. grow at the Chapada dos Veadeiros as homogeneous populations, spatially separated, and we have never found mixed populations or intermediate specimens. In defining the taxonomic rank of this taxon, we followed Stace (1989), who defined a variety as "a population of one or several biotypes, forming more or less distinct local races of a species."

Habenaria proiteana J.A.N. Bat., A.A.Vale & Bianch. sp. nov.
urn:lsid:ipni.org:names:77325125-1
Figs 5A–F, 6

Diagnosis

Similar to *H. rodriguesii* Cogn. in general morphology, but distinguished by the dorsal sepal 6–9 mm long (vs 10–14 mm long), petal anterior segment 11–16 mm long (vs 20–33 mm long), spur 13–17 mm long, genuflexus (vs 16–21 mm long, straight or only slightly curved), and anther canals curved upwards (vs straight, perpendicular to the anther loculi). Also similar to *H. sobraliana* J.A.N.Bat., Vale & Menini in general morphology, but distinct by the petal anterior segment 11–16 mm long, inserted a few millimetres above the posterior segment's base (vs 5–8 mm long, inserted at the base of the posterior segment).

Etymology

We named this species after Karina Proite for her support, companionship, help in the field and contribution to numerous collections, and for finding the population used to characterise, describe, and typify the newly proposed species.

Material examined

Type

BRAZIL – **Goiás** • Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, próximo as corredeiras do Rio Preto; 3 Jan. 2008; fl.; *J.A.N. Batista, K. Proite & P. Proite 2376*; GenBank nrITS: OP584473 and OP584474; nrETS: OP589157 and OP589158; *matK* gene and *trnK* intron: OP589169 and OP589170; *rps16-trnK* intergenic spacer: OP590000; holotype: BHCB[120057]; isotypes: CEN, RB, SP.

Paratypes

BRAZIL – **Goiás** • Alto Paraíso de Goiás, rodovia para Colinas, Rio das Cobras; 2 Dec. 1992; fl.; *G. Hatschbach et al. 58383*; MBM[158574]•Alto Paraíso de Goiás, Chapada dos Veadeiros, GO-239, entre Alto Paraíso e São Jorge, cerca de 3,1 km após a entrada para a fazenda São Bento; 30 Dec. 2010; fl.; *J.A.N. Batista & L.B. Bianchetii 3101*; BHCB[151374]•Alto Paraíso de Goiás, Parque Nacional Chapada dos Veadeiros, Corredeiras; 19 Dec. 2012; *A.A. Vale & J.A. Vale 180*; BHCB[174606].

Description

Geophytic herb, caulescent, sympodial. *Roots* 1–1.6 mm wide, few, sparsely hairy. *Tuberoid* not examined. *Stem* 31–64 cm long including the inflorescence, 1.7–2.5 mm wide, erect, straight. *Leaves* 4–7, linear, narrow, the largest in the middle of the stem, 2.5–12 × 0.2–0.7 cm, reducing towards the apex of the stem, base sheathing, sheath closed, blade for the most part appressed to the stem, apex acuminate. *Inflorescence* 9–23 cm long, spiral, few flowered, lax; floral bracts 15–32 × 3.7–8 mm, ovate, lance-ovate, broadly elliptical or orbicular, apex acuminate, shorter than the pedicellate ovary, decreasing in size towards the inflorescence apex, green. *Flowers* 3–8, resupinate, green, glabrous; pedicellate ovary 19–21(–30) mm long, strongly arched; ovary 12–16 mm long, pedicel 5–15 mm long. Sepals green, apex apiculate; dorsal sepal 6–9 × 4.5–6.5 mm, concave, broadly elliptical when flattened; lateral sepals 8.5–12 × 3–4 mm, obliquely lance-ovate, deflexed. *Petals* bipartite; posterior segment 6–9 × 1.5–2.1 mm, falcate, apex acute-acuminate, connivent with the dorsal sepal; anterior segment 11–16 mm long, 1.6–2.2 times longer than the posterior segment, linear-filiform, inserted above the base of the posterior segment, directed backwards, bent upwards. *Lip* tripartite; undivided basal part 1–1.6 × 1.7–2.6 mm; lateral segments 13–18 mm long, 1.4–1.9 times longer than the median segment, linear-filiform, reflexed or bent upward; median segment 8–12 × 1–1.5 mm, linear, reflexed or slightly deflexed, more or less parallel to the distal part of the ovary; spur 13–17 mm long, shorter than the pedicellate ovary, clavate, base 1–1.3 mm wide, apex 1.6–2 mm wide, free or with the apex embraced by the bracts, more or less genuflexus, green. *Gynostemium* 4–6 mm high; connective 0.5 mm wide, green, apex retuse; lateral appendages (auricles) 0.5–1 × 1–1.6 mm, fleshy, verrucose, apex truncate, green. *Anther* 1.5–2 mm high, bilocular, loculi parallel, translucent; canals 1.8–2.3 mm long, parallels, curved upwards; pollinaria 2, 2.2–3.8 mm long each, separate; caudicles 3–4 mm long, filiform; pollinia 1.4–2 × 1–1.5 mm, yellow; viscidia ca 0.24 × 0.27 mm. *Stigmatophores* (stigma lobes) 2, 2–3 mm long, closely parallel, green, receptive surface 1.3–1.7 mm long, slightly convex, turned frontwards, inner margins around the spur entrance thickened, apex 1.6–2.3 mm wide, truncate or rounded. *Rostellum* 4–6 mm long, green; mid lobe 1–1.4 mm long, 1.8–2.3 mm high, triangular, fleshy, apex narrowly acute, held between the anther loculi; side-lobes 2.5–3.7 mm long, base ca 1 mm wide, apex ca 0.5 mm wide, curved, apex facing upwards, parallels. Fruit not seen.

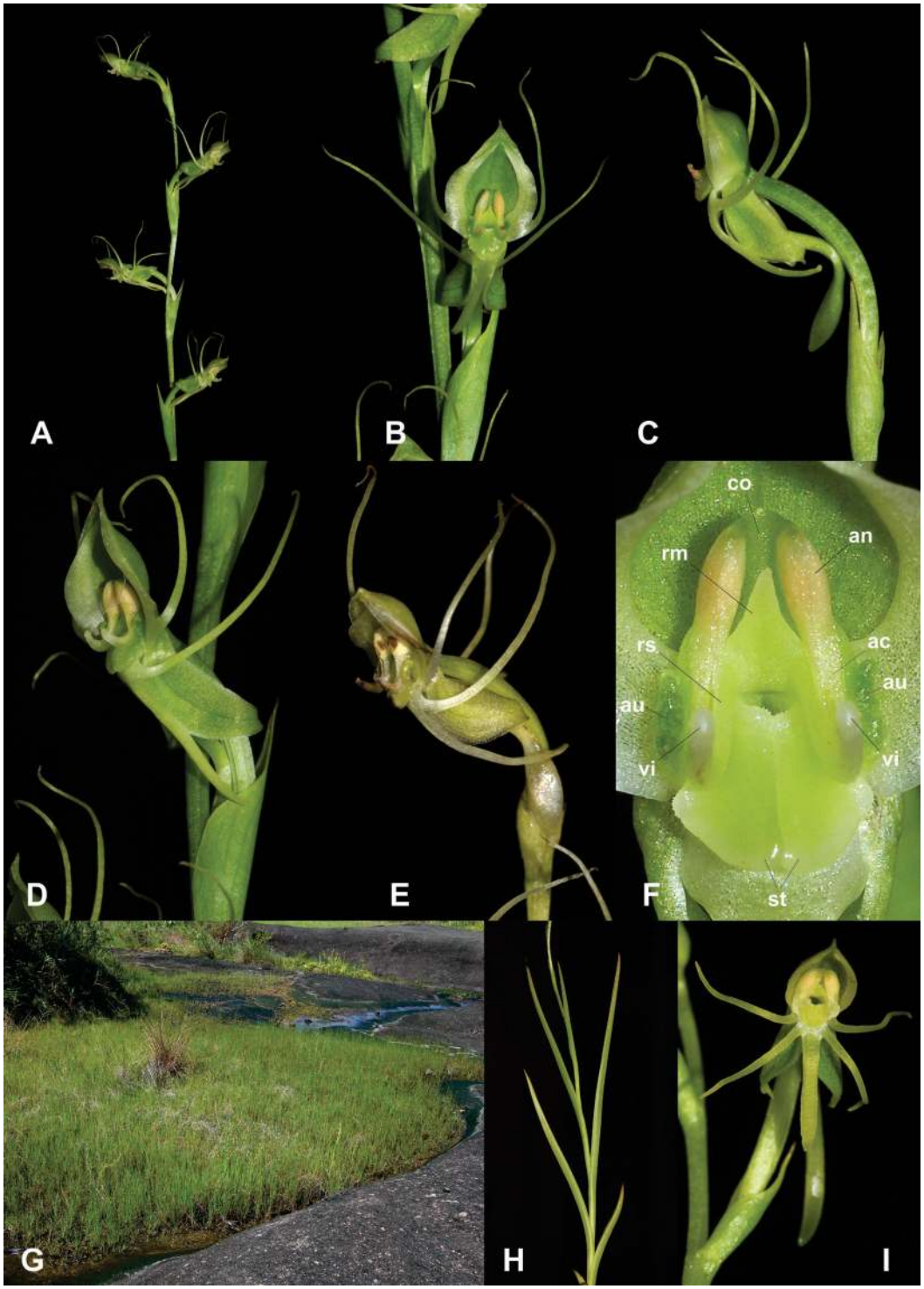


Fig. 5. *Habenaria proiteana* J.A.N. Bat., A.A.Vale & Bianch. sp. nov. **A.** Inflorescence. **B.** Flower, front view. **C.** Flower, side view. **D–E.** Flower, ¾ lateral view. **F.** Gynostemium, front view. *Habenaria cultellifolia*. **G.** Habitat (Chapadas dos Veadeiros, Goiás). **H.** Vegetative part. **I.** Flower, front view. A–D, F from Batista *et al.* 2376 (BHCB); E from *Batista & Bianchetii 3101* (BHCB); G–I from *Batista et al. 2959* (BHCB). Abbreviations: ac = anther canals; an = anther; au = auricles; co = connective; rm = rostellum mid lobe; rs = rostellum side-lobes; st = stigmatophores; vi = viscidium. All photographs by J.A.N. Batista.

Distribution, Habitat and Phenology

It is restricted to Chapada dos Veadeiros in the State of Goiás, occurring in *campos rupestres*, amid rocky outcrops in sandy, dry soil or seasonally humid campo limpo, in dark, sandy-clay soils. It flowers during the peak of the rainy season, in December and January.

Conservation Assessment

Habenaria proiteana sp. nov. is currently known from only three populations, all from Chapada dos Veadeiros, and shows a small EOO estimated at 37 km^2 and a small AOO of 12 km^2. Based on the IUCN Red List Categories and Criteria and its guidelines (IUCN 2012, 2016), the species can be tentatively classified as Critically Endangered CR B1ab(iii)+2ab(iii); C2a(i).

Taxonomic notes

Habenaria proiteana sp. nov., *H. australis* J.A.N.Bat., Vale & Menini, and *H. rodriguesii* present the anterior segment of the petal inserted a few millimetres above the posterior segment's base, not at the base as in the great majority of Neotropical *Habenaria*. However, *H. proiteana* sp. nov. differs from these species in the following characters: the petal anterior segment is proportionally longer (2.2–2.8 times longer than the posterior segment vs 1.2–1.8 times longer in *H. australis*), and the spur genuflexus (Figs. 5C, 6J–L) (vs straight or only slightly curved in *H. australis* and *H. rodriguesii*). In addition to these three species, only two other Neotropical species also have this distinctive character: *H. secundiflora* and *H. meeana* Toscano. Nonetheless, *H. secundiflora* has smaller flowers (dorsal sepal 4–6 × 3–4.5 mm), the lateral segments of the lip deflexed, and pollinaria joined by the viscidia, while *H. meeana* also has smaller flowers (dorsal sepal 5–6 × 3–4 mm), the anterior segment of the petal inserted only about 2 mm above the base of the posterior segment, and lateral sepals and lip segments strongly reflexed, embracing the ovary.

Habenaria proiteana sp. nov. is also similar to *H. sobraliana* in general morphology, but distinct by the petal anterior segment 11–16 mm long, inserted a few millimetres above the posterior segment's base (vs 5–8 mm long, inserted at the base of the posterior segment in *H. sobraliana*) and distribution restricted to the Chapada dos Veadeiros (vs restricted to the State of Rio Grande do Sul).

Key to the Brazilian species of Habenaria with the petal anterior segment inserted above the base of the posterior segment

1. Pedicellate ovary 19–28 mm long; dorsal sepal 10–14 mm long; petal anterior segment 20–33 mm long; spur 16–21 mm long ..... ***H. rodriguesii*** Cogn.
– Pedicellate ovary 12–21(–30) mm long; dorsal sepal 5–10 mm long; petal anterior segment 8–16 mm long, occasionally absent; spur 8.5–17 mm long 2

2. Lip lateral segments erect (turned upwards) 3
– Lip lateral segments deflexed (turned downwards) 4

3. Pedicellate ovary 19–21(–30) mm long, strongly arched; dorsal sepal broadly elliptical when flattened; petal anterior segment 11–16 mm long; spur 13–17 mm long, more or less genuflexus; restricted to the Chapada dos Veadeiros, State of Goiás, Brazil ..... ***H. proiteana*** sp. nov.
–. Pedicellate ovary 12–18 mm long, slightly arched; dorsal sepal ovate or elliptical when flattened; petal anterior segment (0)8–12 mm; spur 8.7–10.3 mm long, more or less straight; restricted to the Serra Gaúcha in the State of Rio Grande do Sul, Brazil ..... ***H. australis*** J.A.N. Bat., A.A. Vale & Menini

4. Pedicellate ovary erect, more or less parallel with the rachis; dorsal sepal ovate or elliptical not reclinate, covering the gynostemium; lateral sepal 2–2.5 mm wide; lip lateral segments deflexed, longer

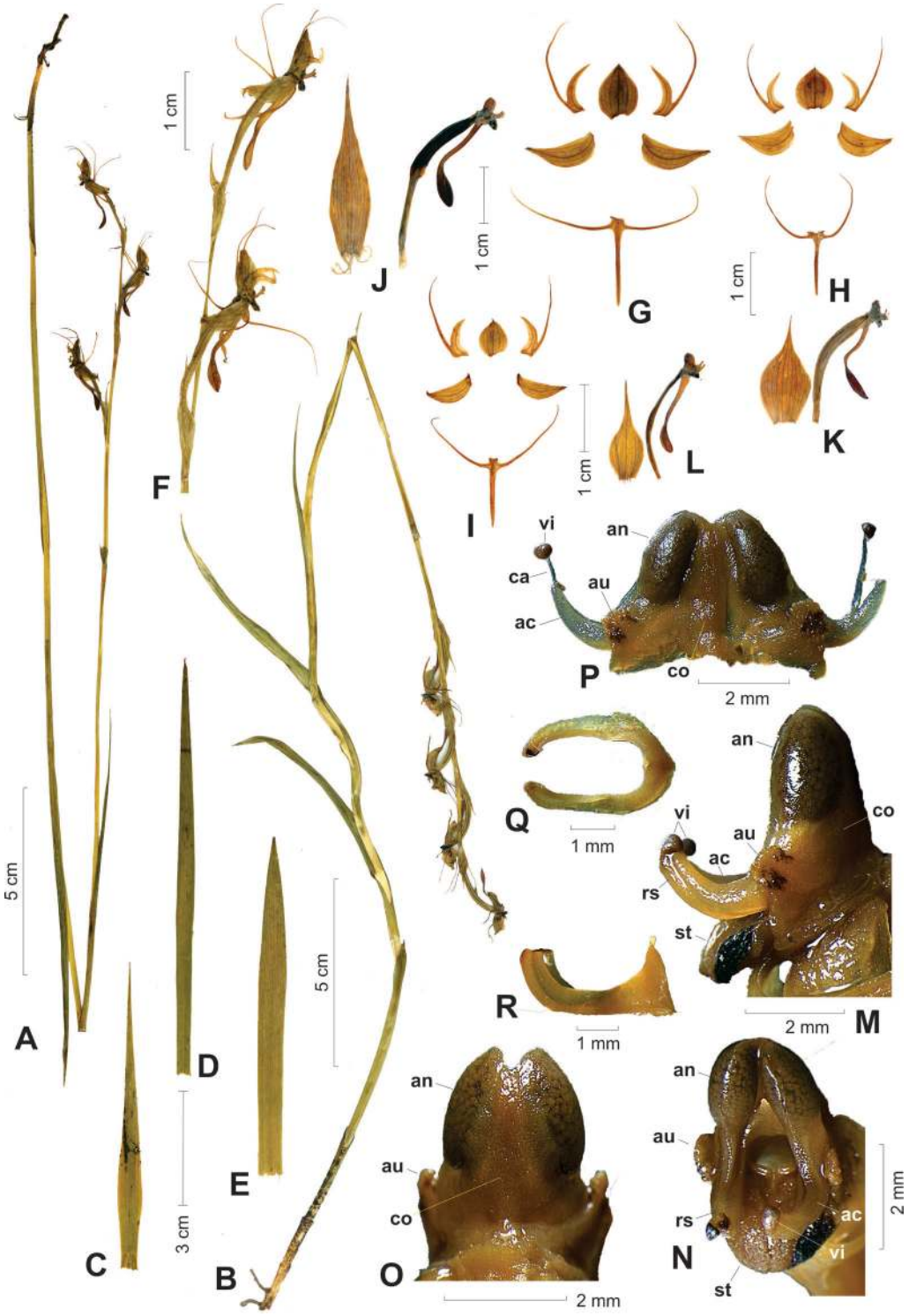


Fig. 6. *Habenaria proiteana* J.A.N. Bat., A.A.Vale & Bianch. sp. nov. **A–B.** Habit. **C–E.** Leaves. **F.** Flowers. **G–I.** Dissected perianth. **J–L.** Bract, pedicellate ovary, gynostemium and spur, lateral view. **M.** Gynostemium, side view. **N.** Gynostemium, front view. **O.** Gynostemium, back view. **P.** flattened connective, anther and lateral appendages (auricles). **Q.** Rostellum, upper view. **R.** Rostellum, lateral view. A–H, J–K and M–R from Batista *et al.* 2376 (BHCB); I and L from *Hatschbach et al. 58383* (MBM). Abbreviations: ac = anther canal; an = anther; au = auricles; ca = caudicle; co = connective; rs = rostellum side-lobes; st = stigmatophores; vi = viscidium.

than the median segment; anther canals and rostellum side-lobes held between the anther loculi; viscidia united; widely distributed from northern South America to southern Brazil..... ***H. secundiflora*** Barb.Rodr.

– Pedicellate ovary perpendicular to the rachis; dorsal sepal obovate, partially reclinate, not covering the gynostemium; lateral sepals 3–4 mm wide; lip lateral segments reflexed, about the same size as the median segment; anther canals and rostellum side-lobes projected beyond the anther loculi; viscidia separated; restricted to the Chapada Diamantina in the State of Bahia, Brazil..... ***H. meeana*** Toscano

Discussion

Survey and conservation of *Habenaria* in Chapada dos Veadeiros

The 61 taxa of *Habenaria* recorded for Chapada dos Veadeiros correspond to 74% of the 80 species of the genus recorded for the State of Goiás, according to Flora e Funga do Brasil (2022), while the region's area corresponds to only 6.3% of the state's total, reinforcing its importance for the preservation of the diversity of the genus in the state. The Chapada dos Veadeiros is one of the regions with the greatest diversity of the genus in Brazil, surpassed only by the Distrito Federal with 74 species (Batista & Bianchetti 2003), and followed by Serra do Cipó with 53 species (J.A.N. Batista, pers. comm.), and Serra da Canastra with 28 species (Carvalho *et al.* 2013) (Table 3).

Similar to other regions with surveys for *Habenaria* (Brade 1951; Batista & Bianchetti 2003; Batista *et al.* 2004, 2008; Carvalho *et al.* 2013), the number of endemic species is small, reinforcing that cases of restricted endemism are rare for the genus in Brazil (Table 3). This result contrasts with other groups that present high diversity in mountainous complexes of Southeastern and Central Western Brazil, such as Eriocaulaceae and the genus *Mimosa* (Simon & Proença 2000), which present a high number of endemic species. For the subfamily Paepalanthoideae (Eriocaulaceae), for example, more than 50% of the 42 species that occur in Chapada dos Veadeiros are endemic to the area (Trovó & Echternacht 2022).

Our results suggest that a significant portion of the diversity of the genus in the region does not occur or is underrepresented within the PNCV (Table 1). However, this data must be considered with reservations since many records reported in this study do not show coordinates or precise geographical locations, making it impossible to know exactly where they were collected. Collections in the region are centred along access roads, mainly on the GO-118 highway between Alto Paraíso de Goiás and Teresina de Goiás and GO-239 between Alto Paraíso and the village of São Jorge (Fig. 1). Large extensions of the region have not yet been sufficiently botanically collected or have no collections at all, including the central and north-western parts of the park as well as the extensive area added to the park in 2016 (Fig. 1), which includes some localities with the highest altitudes in the region and for which there are currently no herbarium collections available. More comprehensive and systematic explorations are expected to reveal new records and eventual taxonomic novelties within the *Habenaria* from this region, considering the exceptional richness of the genus in the Chapada dos Veadeiros. In this work, for example, we describe three new taxa and record the only known extant population of *H. cultellifolia*, a species known only from type material collected at least 127 years ago. These discoveries indicate that although some parts of the region have been extensively explored, new novelties are still to be expected, particularly among the terrestrial, small and ephemerous species.

Efforts to resolve the taxonomy of Brazilian species of *Habenaria* have intensified in the last years with the study of species complexes and the description of several new species (Batista *et al.* 2017, 2022a, b; Cruz-Lustre *et al.* 2020, 2022; Lau *et al.* 2021). Nevertheless, for the Chapada dos Veadeiros at least three morphotypes of the *Habenaria nuda* complex and some taxa associated with *H. montis-*

Table 2. Characterization of the markers used in the phylogenetic analyses. Ter = number of terminals; Char = number of aligned characters in base pairs; MD = percentage of missing data; VU = number of variable uninformative characters; VI = number of variable informative characters; CI = consistency index; RI = retention index. hLRTs = hierarchical likelihood ratio tests.

Marker	Ter	Char	Total MD	Neotropical MD	VU	VI (%)	CI	RI	Model (hLRTs)
ITS	105	760	0.2%	0%	111	291 (38.3%)	0.60	0.77	GTR+I+G
ETS	82	554	24.5%	9%	157	157 (28.4%)	0.72	0.79	HKY+G
Nuclear	105	1314	10.4%	3.8%	268	448 (34%)	0.64	0.77	–
trnK	100	419	9%	2.8%	49	52 (12.4%)	0.70	0.85	F81+G
matK	105	1594	3.8%	1.6%	156	207 (13%)	0.66	0.83	GTR+I+G
rps16-trnK	86	1045	12.6%	1.9%	114	134 (12.8%)	0.70	0.84	GTR+I+G
Plastid	105	3058	7.5%	1.8%	319	393 (12.8%)	0.66	0.82	–
Total	105	4372	8.4%	2.4%	587	841 (19.2%)	0.63	0.78	–

wilhelminae Renz, *H. rodeiensis* Barb. Rodr., and *H.pseudoculicina* (Table 1) are still unresolved and additional studies are needed to uncover the identity and taxonomy of these taxa.

Phylogenetic analyses

In terms of overall results of the phylogenetic analyses, the main differences between our results and some of the earlier molecular phylogenetic studies (Batista *et al.* 2013, 2014, 2016, 2018; Cruz-Lustre *et al.* 2022; Lau *et al.* 2021) were the position of *H. monorrhiza* (Sw.) Rchb.f. and subclade 1 (*H.* sect. *Pratenses* Kraenzl.), which were recovered in a polytomy at the base of the Neotropical clade, while in our results, they were internally recovered in the Neotropical clade (Fig. 2). Nevertheless, in both cases, the results had low support, showing that the phylogenetic relationships of these taxa within the Neotropical clade are not yet resolved. In another difference, species from subclades 16, 17 and 18 were intermingled into a single subclade, while in the topology of Batista *et al.* (2013) these subclades were recovered separately with high support. Although our dataset included a greater number of markers and characters (five markers and 4,372 characters vs two markers and 1,389 characters in the study of Batista *et al.* 2013), our sampling of taxa from these subclades was smaller (6 taxa vs 15 taxa), which may have reduced the resolution of the relationships between them.

As for the new species described here, both were recovered with high support as sister to species with different floral morphology, but with similar vegetative morphology, habitat and ecology. Both *H. minuticalcar* sp. nov./ *H. pseudoculicina* and *H. proiteana* sp. nov./ *H. psammophila*, occur in 'campos rupestres', in seasonally humid, sandy soils in isolated montane areas of central Brazil. In the pair *H. minuticalcar* sp. nov./ *H. pseudoculicina* both plants are slender with linear and reduced leaves, while in *H. pseudoculicina* the anterior segment of the petals (5.0–6.5 mm long) is about 1.7 times longer than the posterior segment (2.8–3.8 mm long), the lateral segments of the lip (5.0–7.5 mm long) are 1.3 to 1.4 times longer than the median segment (3.5–5.0 mm long) and the spur

Table 3. *Habenaria* Willd. data for localities or regions in Brazil with checklists of the genus with records above 10 species. Abbreviations: APA = Área de Proteção Ambiental; P.E. = Parque Estadual; P.N. = Parque Nacional; R.P.P.N. = Reserva Particular do Patrimônio Natural. Abbreviations for Brazilian states: BA = Bahia; GO = Goiás; MG = Minas Gerais; RJ = Rio de Janeiro.

Locality or region (State)	N º spp. (infra-especific taxa)	N º endemic taxa (% total)	Area (km^2)	Reference
Distrito Federal	74 (3)	8 (10.4%)	5,783	Batista & Bianchetti 2003
Chapada dos Veadeiros (GO)	59 (2)	4 (6.6%)	21,475	This work
Serra do Cipó/APA Morro da Pedreira (MG)	52 (3)	2 (3.8%)	1,317	Batista, pers. comm.
R.P.P.N. Santuário do Caraça (MG)	33 (1)	1 (2.9%)	112	Mota 2006
P.N. da Serra da Canastra (MG)	28 (0)	2 (7.1%)	2,000	Carvalho *et al.* 2013
Lagoa Santa (MG)	17 (0)	0	108	Warming 1892
P.N. do Itatiaia (RJ)	17 (0)	0	119	Brade 1951
P.E. da Serra do Ouro Branco (MG)	17 (1)	0	27	Vieira & Barros 2017
P.E. do Itacolomi (MG)	15 (0)	1 (6.7%)	75	Batista *et al.* 2004
Pico das Almas (BA)	12 (0)	0	170	Toscano-de-Brito 1995

(8.0–11.7 mm long) is about the same length as the pedicellate ovary (12–15 mm long). On the other hand, the morphology of the column is similar between the two species. In particular, the rostellum is very similar, is completely held between the anther loculi, and the pollinaria are joined by the viscidia in both species. However, these features are not unique and are shared with other non-directly related species groups, such as the species in subclade 20 (*H.* sect. *Microdactylae* Kraenzl.) and subclade 15, among others.

For the pair *H. proiteana* sp. nov./ *H. psammophila* the differences are greater than the similarities. Vegetatively, *H. psammophila* has sub-patent, papery, somewhat rigid leaves with the limb mostly detached from the stem. In floral morphology, the flowers of *H. psammophila* are smaller, the spur (3.0–4.5 mm long) is shorter than the pedicellate ovary (7–11 mm long), and the morphology of the column is quite different between the two species. The only obvious similarities between *H. proiteana* sp. nov./ *H. psammophila* are the anterior segment of the petals longer than the posterior segment and the lateral segments of the lip longer than the median segment. However, these features are also found in all species of the *H. nuda* complex and in several other Neotropical groups and species of the genus. An additional and perhaps more striking, but less obvious similarity, is the spur of *H. psammophila*. Although much smaller, it is also clavate and occasionally also more or less genuflexus, which are distinctive features of *H. proiteana* sp. nov.. Our results show that *H. proiteana* sp. nov. is not directly related to the species of the *H. nuda* complex (subclade 21), with which it shares a similar overall morphology in both vegetative and floral terms. This result suggests that both this general morphology as also the anterior petal segment inserted above the posterior segment's base, a characteristic shared with *H. rodriguesii*, and *H. australis*, are probably a result of convergence. On the other hand, *H. rodriguesii* and *H. australis* form a highly supported species pair and this morphological feature probably constitutes a synapomorphy for this pair within subclade 21.

Most subclades of Neotropical *Habenaria*, such as subclades 1, 2, 3 (Cruz-Lustre *et al.* 2022), 4, 8, 15, 20 (Batista *et al.* 2018) and the subclade formed by *H. espinhacensis* J.A.N.Bat. & A.A.Vale and

related species (Batista *et al.* 2016) have a high morphological uniformity. On the other hand, some subclades are otherwise homogeneous, but present one morphologically distinct species, as is the case of *H. montevidensis* Spreng. in subclade 5 (*H.* sect. *Pentadactylae* Kraenzl.) (Pedron *et al.* 2014) and *H. karstica* J.A.N.Bat. in subclade 12 (*H.* sect. *Spathaceae* Kraenzl.) (Batista *et al.* 2022a). In addition to these differences within the subclades, some species pairs from the Cerrado Phytogeographic Domain recovered as sisters with high support, such as *H. caldensis* Kraenzl. / *H. magniscutata* and now *H. minuticalcar* sp. nov. / *H. pseudoculicina* and *H. proiteana* sp. nov. / *H. psammophila* (Fig. 2) differ greatly in floral morphology. Since habitat, ecology and vegetative morphology are similar between these species pairs, and the pairs are often sympatrical, these results suggest speciation driven by different pollinators (Harder & Johnson 2009). In line with this interpretation, Pedron *et al.* (2012) showed that *H. montevidensis* is pollinated by butterflies, while other species of *H.* sect. *Pentadactylae*, are pollinated by moths or present floral morphology associated with moth pollination, suggesting that the morphological differences of *H. montevidensis* are a result of a shift to diurnal pollinators.

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Supplementary file 1 (continued on the next 7 pages)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	**Voucher**	**Origin**	**ITS**	***matK-trnK***	***rps16-trnK***	**ETS**
Neotropical						
Habenaria abscondita J.A.N.Bat. & G.Cruz-Lustre	*Cruz-Lustre 206* (BHCB)	Brazil, BA	MW567131	MW675883	MW675857	MW675870
Habenaria achalensis Kraenzl.	*Batista 2506* (BHCB)	Brazil, RS	HM777526	KJ021403	KJ021457	MT623244
Habenaria alpe stris Cogn.	*Batista 2539* (BHCB)	Brazil, MG	HM777688	KJ021361	KJ021416	MT623218
Habenaria aranifera Lindl.	*Batista 2472* (BHCB)	Brazil, RS	HM777626	KJ021394	KJ021448	MT623173
Habenaria armata Rchb.f.	*Batista 1297* (CEN)	Brazil, DF	HM777677	KJ021367	KJ021422	–
Habenaria australis J.A.N. Bat., A.A. Vale & Menini	*Batista 2496* (BHCB)	Brazil, RS	HM777724	OP589163*	OP589994*	OP589151*
Habenaria ayangannensis Renz	*Batista 1919* (BHCB)	Brazil, MG	HM777706	KJ021360	KJ021415	–
Habenaria balansae Cogn.	*Batista 2336* (BHCB)	Brazil, MG	HM777683	KJ021362	KJ021417	MT623217
Habenaria bicornis Lindl.	*Felix 10803* (EAN)	Brazil, PB	KF998087	KX784170	KX784174	MT623247
Habenaria brachydactyla J.A.N.Bat. & Bianch.	*Batista & Proite 3109* (BHCB)	Brazil, DF	KX784176	KX784171	KX784175	MT623252
Habenaria brachyphyton Schltr.	*Batista 2515* (BHCB)	Brazil, RS	HM777557	MW675872	MW675846	MW675859
Habenaria brevidens Lindl.	*Batista 2616* (BHCB)	Brazil, MG	HM777535	MW675873	MW675847	MW675860
Habenaria bryophila J.A.N.Bat., B.L.Lau & I.G.C.Machado-Costa	*Batista et al. 1962* (BHCB)	Brazil, MG	HM777629	MT623320	MT623273	MT623199
Habenaria caldensis Kraenzl.	*Batista 250* (CEN)	Brazil, GO	HM777645	KJ021363	KJ021418	MT623219

Supplementary file 1 (continued)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	Voucher	Origin	ITS	*matK-trnK*	*rps16-trnK*	ETS
Habenaria campylogyna J.A.N.Bat. & Bianch.	*Batista 1901* (BHCB)	Brazil, MG	HM777581	KJ021364	KJ021419	MT623220
Habenaria canastrensis J.A.N. Bat. & B.M.Carvalho	*Batista 1806* (BHCB)	Brazil, MG	HM777726	OP589164*	OP589995*	OP589152*
Habenaria compluviosa J.A.N.Bat., B.L.Lau & I.G.C.Machado-Costa	*Lau 23* (BHCB)	Brazil, MG	MT582228	MT623313	MT623267	MT623194
Habenaria crucifera Rchb.f. & Warm.	*Batista 1826* (BHCB)	Brazil, MG	HM777574	KJ021356	KJ021411	MT623212
Habenaria cultellifolia Barb.Rodr.	*Batista 1487* (CEN)	Brazil, GO	HM777675	MW675874	MW675848	MW675861
Habenaria dutrae Schltr.	*Pedron 3* (ICN)	Brazil, RS	KJ021339	KJ021387	KJ021441	–
Habenaria edwallii Cogn.	*Batista 1717* (BHCB)	Brazil, MG	HM777564	KJ021402	KJ021456	MT623243
Habenaria ekmaniana Kraenzl.	*Radins s.n.* (BHCB)	Argentina	KJ021340	KJ021383	KJ021438	MT623233
Habenaria espinhacensis J.A.N.Bat. & A.A.Vale	*Batista 2814* (BHCB)	Brazil, MG	HM777701	MT623325	MT623277	MT623211
Habenaria exaltata Barb.Rodr.	*Batista 2771* (BHCB)	Brazil, MG	HM777621	KJ021388	KJ021442	MT623234
Habenaria glaucophylla Barb. Rodr.	*Batista 761* (CEN)	Brazil, DF	HM777631	KJ021373	KJ021428	MT623231
Habenaria gollmeri Schltr.	*Castro 2000* (COL)	Colombia	MW567127	MW675875	MW675849	MW675862
Habenaria hamata Hoehne	*Batista 1519* (CEN)	Brazil, DF	HM777586	KJ021371	KJ021426	MT623228
Habenaria henscheniana Barb. Rodr.	*Batista 2802* (BHCB)	Brazil, MG	HM777623	KJ021376	KJ021431	–

Supplementary file 1 (continued)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	Voucher	Origin	ITS	*matK-trnK*	*rps16-trnK*	ETS
Habenaria hexaptera Lindl.	*Batista 2399* (BHCB)	Brazil, MG	HM777538	KJ021366	KJ021421	MT623221
Habenaria hippocrepica J.A.N.Bat. & Bianch.	*Batista 1948* (BHCB)	Brazil, MG	HM777698	MT623326	MT623278	MT623209
Habenaria hygrophila J.A.N.Bat., B.L.Lau & I.G.C.Machado-Costa	*Batista 2538* (BHCB)	Brazil, MG	MT582221	MT623321	MT623274	MT623200
Habenaria imbricata Lindl.	*Batista 1123* (CEN)	Brazil, DF	HM777648	KJ021368	KJ021423	MT623226
Habenaria irwiniana J.A.N.Bat. & Bianch.	*Batista 249* (CEN)	Brazil, GO	HM777642	KX784167	KX784172	MT623251
Habenaria johannensis Barb.Rodr.	*Mota 2777* (BHCB)	Brazil, MG	HM777609	KJ021398	KJ021452	MT623240
Habenaria karstica J.A.N.Bat.	*Batista et al. 3649* (BHCB)	Brazil, MG	ON197322	ON168967	ON168969	–
Habenaria lavrensis Hoehne	*Batista 673* (CEN) & *Batista 1497* (CEN)	Brazil, DF	HM777711	OP589165*	OP589996*	OP589153*
Habenaria leptoceras Hook.	*Batista 2658* (BHCB)	Brazil, RJ	HM777597	KJ021370	KJ021425	MT623227
Habenaria leucosantha Barb.Rodr.	*Batista 1604* (BHCB)	Brazil, DF	HM777568	KJ021406	KJ021460	MT623248
Habenaria ludibundiciliata J.A.N.Bat. & Bianch.	*Batista 1372* (CEN)	Brazil, MA	HM777638	MT623328	MT623280	MT623215
Habenaria macilenta (Lindl.) Rchb.f.	*Batista 2393* (BHCB)	Brazil, MG	HM777606	KJ021400	KJ021454	MT623241
Habenaria macroceratitis Willd.	*Whitten 2827* (FLAS)	USA, FL	MT582231	MT623329	MT623281	MT623230
Habenaria macronectar (Vell.) Hoehne	*Batista 2519* (BHCB)	Brazil, PR	HM777614	KJ021399	KJ021453	MT623239

Supplementary file 1 (continued)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	Voucher	Origin	ITS	*matK-trnK*	*rps16-trnK*	ETS
Habenaria magniscutata Catling	*Batista 1227* (CEN)	Brazil, GO	HM777641	OP589166*	OP589997*	OP589154*
Habenaria megapotamensis Hoehne	*Pedron 10* (ICN)	Brazil, RS	KJ021345	KJ021384	KJ021439	MT623235
Habenaria minuticalcar J.A.N.Bat. & Bianch. sp. nov.	*Batista 843* (CEN)	Brazil, GO	HM777691	OP589167*	OP589998*	OP589155*
Habenaria monorrhiza (Sw.)Rchb.f.	*Whitten 2721* (FLAS)	Ecuador	MT582232	MT623330	MT623282	MT623249
Habenaria montevidensis Spreng.	*Batista 2479* (BHCB)	Brazil, RS	HM777619	KJ021378	KJ021433	–
Habenaria mystacina Lindl.	*Batista 1812* (BHCB)	Brazil, MG	HM777728	KJ021353	KJ021408	MT623205
Habenaria nemorosa Barb.Rodr.	*Batista 2567* (BHCB)	Brazil, MG	HM777634	MW675876	MW675850	MW675863
Habenaria aff. *nuda* Lindl.	*Batista 2869* (BHCB)	Brazil, MG	HM777718	KJ021354	KJ021409	MT623206
Habenaria omissa J.A.N.Bat. & Bianch.	*Batista 1576* (BHCB)	Brazil, DF	HM777655	KJ021357	KJ021412	MT623207
Habenaria pabstii J.A.N.Bat. & Bianch.	*Batista 2360* (BHCB)	Brazil, DF	HM777666	OP589168*	OP589999*	OP589156*
Habenaria pansarinii J.A.N.Bat. & Bianch.	*Batista et al. 1926* (BHCB)	Brazil, MG	HM777520	KX784168	KX784173	MT623250
Habenaria paranaensis Barb.Rodr.	*Batista 2436* (BHCB)	Brazil, MG	HM777528	KJ021405	KJ021459	MT623246
Habenaria parviflora Lindl.	*Batista 1813* (BHCB)	Brazil, MG	HM777560	KJ021401	KJ021455	MT623242
Habenaria paulensis Porsch	*Batista 2481* (BHCB)	Brazil, RS	HM777556	MW675878	MW675852	MW675865

Supplementary file 1 (continued)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	Voucher	Origin	ITS	*matK-trnK*	*rps16-trnK*	ETS
Habenaria pentadactyla Lindl.	*Pedron 11* (ICN)	Brazil, RS	KJ021348	KJ021380	KJ021435	MT623236
Habenaria pratensis (Salzm. ex Lindl.) Rchb.f.	*Batista 2686* (BHCB)	Brazil, BA	HM777546	KJ021374	KJ021429	MT623225
Habenaria proiteana J.A.N.Bat., A.A.Vale & Bianch. sp. nov.	*Vale 180* (BHCB)	Brazil, GO	OP584473*	OP589169*	OP590000*	OP589157*
Habenaria proiteana J.A.N.Bat., A.A.Vale & Bianch. sp. nov.	*Batista* 2376 (BHCB)	Brazil, GO	OP584474*	OP589170*	–	OP589158*
Habenaria psammophila J.A.N. Bat., Bianch. & B.M. Carvalho	*Batista* 1794 (BHCB)	Brazil, MG	HM777550	OP589171*	OP590001*	OP589159*
Habenaria pseudoculicina J.A.N.Bat. & B.M.Carvalho	*Batista 1808* (BHCB)	Brazil, MG	HM777707	OP589172*	OP590002*	OP589160*
Habenaria pungens Cogn.	*Batista 2095* (BHCB)	Brazil, GO	HM777570	KJ021355	KJ021410	MT623213
Habenaria quinqueseta (Michx.) Sw.	*Whitten 3324* (FLAS)	USA, FL	MT582230	MT623331	MT623283	MT623229
Habenaria reflexicalcar J.A.N.Bat. & B.M.Carvalho	*Batista 1744* (BHCB)	Brazil, MG	HM777700	MT623332	MT623284	MT623210
Habenaria regnellii Cogn.	*Batista 2801* (BHCB)	Brazil, MG	HM777603	KJ021391	KJ021445	–
Habenaria repens Nutt.	*Braun 01* (FLAS)	USA, FL	MT582195	MT623296	–	MT623180
Habenaria repens Nutt.	*Batista 2522* (BHCB)	Brazil, PR	HM777627	KJ021395	KJ021449	MT623178
Habenaria rodeiensis Barb.Rodr.	*Mota 2824* (BHCB)	Brazil, MG	HM777577	KJ021359	KJ021414	MT623216
Habenaria rodriguesii Cogn.	*Vale 122* (BHCB)	Brazil, MG	OP584475*	OP589173*	OP590004*	OP589162*

Supplementary file 1 (continued)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	Voucher	Origin	ITS	*matK-trnK*	*rps16-trnK*	ETS
Habenaria schenckii Cogn.	*Batista 2882* (BHCB)	Brazil, BA	HM777580	KJ021372	KJ021427	MT623232
Habenaria schwackei Barb.Rodr.	*Batista 2524* (BHCB)	Brazil, PR	HM777657	MT623327	MT623279	MT623208
Habenaria secunda Lindl.	*Batista 2640* (BHCB)	Brazil, RJ	HM777525	KJ021404	KJ021458	MT623245
Habenaria secundiflora Barb.Rodr.	*Batista 2392* (BHCB)	Brazil, MG	HM777637	KJ021358	KJ021413	MT623214
Habenaria setacea Lindl.	*Mota 3019* (BHCB)	Brazil, MG	HM777731	KJ021352	KJ021407	MT623204
Habenaria sobraliana J.A.N.Bat., Vale & Menini	*Batista 2499* (BHCB)	Brazil, RS	HM777704	HM777990	OP590003*	OP589161*
Habenaria spathulifera Cogn.	*Bringel 411* (CEN)	Brazil, GO	MT582233	MT623333	MT623285	MT623224
Habenaria subrepens J.A.N.Bat., B.L.Lau & I.G.C.Machado-Costa	*Mota 2285* (BHCB)	Brazil, MG	MT582217	MT623307	MT623264	MT623191
Habenaria trifida Kunth	*Batista 1783* (BHCB)	Brazil, DF	HM777672	KJ021369	KJ021424	MT623223
Habenaria ulei Cogn.	*Cruz-Lustre 281* (BHCB)	Brazil, SC	MW567130	MW675880	MW675854	MW675867
Habenaria umbraticola Barb.Rodr.	*Mota 3569* (BHCB)	Brazil, MG	HM777605	MW675881	MW675855	MW675868
Habenaria urbaniana Cogn.	*Batista 911* (CEN)	Brazil, MG	HM777658	MT623334	MT623286	–
Habenaria uruguayensis Garay	*Batista 2477* (BHCB)	Brazil, RS	KC257475	MW675882	MW675856	MW675869
Habenaria warmingii Rchb.f. & Warm.	*Batista 2409* (BHCB)	Brazil, MG	HM777616	KJ021396	KJ021450	MT623238

Supplementary file 1 (continued)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	Voucher	Origin	ITS	*matK-trnK*	*rps16-trnK*	ETS
Paleotropical						
Bonatea speciosa (L.f.) Willd.	GenBank	Africa	MT500586	MT533490	–	–
Centrostigma occultans (Welw. ex Rchb.f.) Schltr.	GenBank	Africa	MT500664	MT533569	–	–
Habenaria crassilabia Kraenzl. (*H. chejuensis*)	GenBank	Korea	KT338765	NC_046821	NC_046821	MN221405
Habenaria tridens Lindl.	GenBank	Africa	DQ522080	DQ522101	–	–
Habenaria cornuta Lindl.	GenBank	Africa	MT500657	MT533559	–	–
Habenaria decorata Hochst. ex A.Rich.	GenBank	Africa	MT500609	MT533514	–	–
Habenaria dives Rchb.f.	GenBank	Africa	MT500592	MT533497	–	–
Habenaria foliosa A.Rich.	GenBank	India	JN114529	JN004457	–	–
Habenaria heyneana Lindl.	GenBank	India	JN114547	JN004469	–	–
Habenaria laevigata Lindl.	GenBank	Africa	MT500591	MT533496	–	–
Habenaria lithophila Schltr.	GenBank	Africa	MT500589	MT533493	–	–
Habenaria petitiana (A.Rich.) T.Durand & Schinz	GenBank	Africa	MT500613	MT533518	–	–
Habenaria praestans Rendle	GenBank	Africa	MT500605	MT533510	–	–
Habenaria stenopetala Lindl.	GenBank	China	MF944324	MF945467	–	–
Habenaria stenopetala Lindl.	GenBank	China	MF944309	MF945513	–	–
Pecteilis radiata (Thunb.) Raf. (*Habenaria radiata*)	GenBank	Korea	KT338720	NC_035834	NC_035834	MN221407
Platycoryne buchananiana (Kraenzl.) Rolfe	GenBank	Africa	MT500587	MT533491	–	–

Supplementary file 1 (continued)**.** GenBank accessions and voucher data of the terminals used in the phylogenetic analyses. Acronyms for the Brazilian states: BA=Bahia; GO=Goiás; MG=Minas Gerais; MT=Mato Grosso; PB=Paraíba; PR=Paraná; RJ=Rio de Janeiro; RS=Rio Grande do Sul; SC=Santa Catarina. DF=Distrito Federal. USA: United States of America; FL = Florida. New sequences generated for this work are indicated by an asterisk.

Taxon	**Voucher**	**Origin**	**ITS**	***matK-trnK***	***rps16-trnK***	**ETS**
Platycoryne crocea Rolfe	GenBank	Africa	MT500614	MT533519	–	–
Roeperocharis bennettiana Rchb.f.	GenBank	Africa	MT500659	MT533562	–	–
Stenoglottis longifolia Hook.f.	Batista 3279 (BHCB)	Africa	MT582234	MT623335	MT623287	MT623253