

Research article

A new species of *Habenaria* (Orchidaceae, Asparagales) and a checklist of Orchidaceae from limestone outcrops of BrazilJoão A.N. BATISTA^{1,*}, Vera L. FERREIRA², Camila I.G. ALVES³ & João R. STEHMANN⁴^{1,2,3,4}Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Botânica, Av. Antônio Carlos 6627, Pampulha, Belo Horizonte, 31270-910, Minas Gerais, Brazil.* Corresponding author: janb@icb.ufmg.br²Email: veralfbio@gmail.com³Email: camila.igalves@hotmail.com⁴Email: stehmann@gmail.com

Abstract. *Habenaria karstica* J.A.N.Bat. sp. nov., a new species from limestone outcrops (i.e., karst) in the State of Minas Gerais, Brazil, is described. The new species is ecologically very distinct since it is the only Neotropical species of the genus that grows on limestone outcrops under full sun, associated with mat-forming saxicolous plants. It is morphologically similar to species of the *Habenaria repens* complex, and separation is only possible through a combination of morphological characters. Bayesian and parsimony phylogenetic analyses based on nuclear (rDNA ITS, ETS) and plastid (*matK-trnK*, *rps16-trnK*) DNA markers revealed that *H. karstica* belongs to *H.* sect. *Spathaceae*. The new species, however, differs in floral morphology from the other species in this clade. A survey of the literature and public plants datasets revealed 122 species and 58 genera of Orchidaceae in karsts of Brazil. Although small compared to the family's diversity in the country, Orchidaceae is among the families with the highest number of species in some karst localities. Our results indicate that approximately 97% of Orchidaceae found in karsts of Brazil is not limited to limestone outcrops areas, and that species composition of each locality is primarily determined by the regional flora and the biome in which it is located.

Keywords. Brazil, Cerrado, karst, molecular phylogenetics, Orchidaceae.

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Introduction

Limestone outcrops (i.e., carbonate karsts) are sedimentary rock outcrops that primarily consist of calcium carbonate produced millions of years ago by marine organisms. After being lifted by tectonic movements and shaped by mechanical and chemical erosion, they form landscapes marked by deeply rugged terrain, often with steep columns and cliffs, deep crevices, and fissures, usually associated with underground drains and caves (Vermeulen & Whitten 1999; Clements *et al.* 2006; Espírito Santo *et al.* 2018). Carbonate karsts are distributed worldwide and are estimated to occur over 10–15% of the

planet's continental area (Ford & Williams 2007). Carbonate karsts are also widespread in Brazil and are estimated to cover between 5–7% of the country's total surface, where the most extensive formations occur in its central region covering the states of Bahia, Goiás, and Minas Gerais (Auler & Farrant 1996).

In many instances, karsts are important sources of species richness and endemism in plants and are seen as priority areas for biodiversity conservation, particularly in southeast Asia and southwest China (Xu 1995; Clements *et al.* 2006). In these areas, the Yunnan-Guizhou limestone plateau in China is recognised as a centre of plant diversity (Davis *et al.* 1994). In contrast, the karsts of Brazil contain approximately 1/3 of the Brazilian flora but are not significantly more species-rich or richer in endemic species than surrounding landscapes (Bystriakova *et al.* 2019).

The Lagoa Santa karst (LSK) is one of Brazil's most important limestone regions and is considered the country's cradle of palaeontology and archaeology due to the pioneering studies of Danish naturalist Peter Wilhelm Lund (Goodland 1975; Auler 2020a). The area is in the south-central region of the State of Minas Gerais, covering an area of approximately 350 km² in the municipalities of Confins, Funilândia, Lagoa Santa, Matozinhos, Pedro Leopoldo, Prudente de Morais, and Vespasiano. A classical and scenic karst landscape characterises the LSK with limestone cliffs, karst lakes, karst plains, and numerous solution dolines (Berbert-Born 2000; Auler 2020b). Despite its natural and historical importance, the flora of LSK has never been the subject of a systematic floristic study. The Danish botanist Eugen Warming collected and studied the vegetation of Lagoa Santa, including some limestone outcrops, between 1863 and 1866 (Warming 1892, 1908). Still, 155 years later, his studies constitute the main reference for the flora of this region (Moreira & Stehmann 2020). Since Warming, floristic studies of karsts of Minas Gerais have been mostly focused on other limestone formations of this state (Brandão & Magalhães 1991; Lombardi *et al.* 2005; Meguro *et al.* 2007; Santos *et al.* 2007; Melo 2008; Melo *et al.* 2013), with few additional case studies carried out in the LSK (Pedersoli & Martins 1972; Ferreira 2020; Ferreira & Stehmann 2020).

Orchidaceae is Brazil's second-largest family of flowering plants, with 251 genera and 2684 species (Flora e Funga do Brasil 2021). The phytogeographic domain with the richest diversity of species is the Atlantic Forest with 1393 species, but the family is also well represented in the Amazon (792 spp.) and Cerrado (642 spp.). Unlike Southeast Asia (Rusea & Yong 2005; Liang 2008; Rusea *et al.* 2009; Kumar *et al.* 2016), there are few studies of Orchidaceae in karsts of Brazil. In the only specific survey of Orchidaceae for a karst region in Brazil, Bianchetti *et al.* (2005) recorded 36 species and 25 genera for the Cafuringa Environmental Protection Area in the Federal District of Central Brazil. In turn, Melo (2008) and Melo *et al.* (2013) recorded 30 species in 23 genera in a survey of the vascular flora of 14 limestone outcrops in the states of Bahia, Minas Gerais, and Goiás, while Peixoto (2018) recorded 41 species and 26 genera of Orchidaceae in a survey of the vascular flora in karsts of the Atlantic Forest domain. Besides these, only a few additional studies recorded species of Orchidaceae in karsts of Brazil, such as Warming (1908) and Ferreira & Stehmann (2020), both for the LSK.

Habenaria Willd. is the largest genus of Brazilian Orchidaceae with 170 species (Govaerts *et al.* 2021). The genus is the largest among terrestrial orchids, with ca 910 species (Govaerts *et al.* 2021) distributed through tropical and subtropical regions worldwide (Cribb 2001). Species of *Habenaria* grow in grassland and forest habitats with a conspicuous seasonality, usually growing and flowering during rainy seasons and remaining dormant in the form of an underground tuberoid stem during the dry season (Cribb 2001). In Brazil, the genus is distributed in all regions, phytogeographic domains, and most vegetation types, except for markedly xerophilous vegetation (Flora e Funga do Brasil 2021). So far, four species have been recorded for karsts of Brazil, *H. cryptophila* Barb.Rodr., *H. distans* Griseb., *H. curvilabria* Barb.Rodr. (Bianchetti *et al.* 2005), and *H. glaucophylla* Barb.Rodr. (Warming 1908), but none of them is restricted to karsts, being widely distributed throughout the country or the Neotropical region.

During a survey of the saxicolous plants of two limestone massifs from the LSK, a very distinct species of *Habenaria* was found growing only within the saxicolous vegetation, a habitat still unreported for American species of *Habenaria*. The morphological and phylogenetic characterisation of this population of *Habenaria* revealed that it is distinct from all other Neotropical species of the genus, being described here as *H. karstica* J.A.N.Bat. sp. nov. To analyse the new species in the context of the diversity and endemism of Orchidaceae in karst vegetation, we also carried out a preliminary survey of this family in Brazilian karsts.

Material and methods

Taxon sampling for phylogenetic analyses

The datasets for the phylogenetic analyses were based on those used by Pedron *et al.* (2014) to infer the phylogenetic relationships of the *Habenaria* sect. *Pentadactylae* Kraenzl., including some species addressed in later works (Batista *et al.* 2014, 2016, 2017; Lau *et al.* 2021), excluding most duplicate terminals. For the outgroup, encompassing old world taxa, we used a selected sample based on the works of Batista *et al.* (2013), 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 101 terminals of 98 taxa, including 81 terminals of 78 Neotropical *Habenaria* taxa containing most subclades of Neotropical *Habenaria* (Batista *et al.* 2013). We used *Stenoglottis longifolia* Hook.f., from subtribe Orchidinae Dressler & Dodson, to root all trees, based on the results of Ngugi *et al.* (2020). Voucher information, geographic origins, and GenBank accession numbers can be found in Batista *et al.* (2013, 2014, 2016, 2017), Pedron *et al.* (2014), Jin *et al.* (2017), Ngugi *et al.* (2020), and Lau *et al.* (2021).

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 using the same primers and conditions as described by Monteiro *et al.* (2010), Batista *et al.* (2013), and Pedron *et al.* (2014). PCR products were purified by precipitation with 20% PEG (2.5 M NaCl and 20% polyethene glycol 8000) and sequenced with the primers used for amplification at Macrogen Inc. (Korea). All samples were sequenced bidirectionally. Sequence reads were assembled with the Staden software package (Staden 1996) and aligned with MAFFT ver. 7 (Kato & Standley 2013) as implemented in the EMBL-EBI bioinformatics web (Madeira *et al.* 2019). Acronyms of herbaria follow *Index Herbariorum* (Thiers continuously updated).

Phylogenetic analyses

DNA sequence alignments were analysed using parsimony and Bayesian inference. Searches were performed using a combined matrix, as no cases of strongly supported incongruences were detected in previous analyses with similar datasets (Pedron *et al.* 2014; Lau *et al.* 2021). In most cases, the concatenation was done with identical individuals that were sequenced for the different markers. We performed maximum parsimony (MP) phylogenetic analyses using PAUP* ver. 4 (Swofford 2002) with Fitch parsimony (equal weights, unordered characters; Fitch 1971) as the optimality criterion. Each search consisted of 1000 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 1000 replicates, simple addition, and TBR branch swapping, saving ≤ 10 trees per replicate. For bootstrap support levels, we considered bootstrap percentages (BS) of 50–70% as weak, 71–85% as moderate, and $> 85\%$ as strong (Kress *et al.* 2002).

We performed Bayesian analyses using MrBayes ver. 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 Akaike information criterion (AIC). The unlink command was used to estimate model parameters separately for each partition. Each analysis consisted of two independent runs, each with four chains, for 10 000 000 generations, sampling one tree every 1000 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 816 000 generations. After discarding the first 2000 trees (20%) as the burn-in, the remaining trees were used to assess topology and posterior probabilities (PP) in a majority-rule consensus. We considered 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

The habit, perianth, and gynostemium were all examined and illustrated from fresh material. For 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 field observations. Morphological terminology is based on Stearn (1992) and Simpson (2006).

Conservation assessment

We estimated the extent of occurrence and area of occupancy 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. 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).

Orchidaceae in Brazilian karsts

To evaluate the diversity of Orchidaceae associated with karsts in Brazil, we used the datasets of speciesLink (2021), Herbário Virtual Reflora (Flora e Funga do Brasil 2021), and the main floristic studies with records of Orchidaceae in karsts of Brazil (Warming 1908; Bianchetti *et al.* 2005; Melo 2008; Melo *et al.* 2013; Peixoto 2018; Ferreira & Stehmann 2020). For the dataset searches, we filtered Orchidaceae collections using the following keywords associated with karst: ‘calcário(a)’, ‘calcáreo(a)’, ‘limestone’, ‘carst’, ‘carste’, ‘karst’, and ‘cárstico(a)’. When available, we examined samples and images and revised their identification. To identify synonyms and standardise the application of names, we used the names accepted for Orchidaceae in the World Checklist of Selected Plant Families – WCSP (Govaerts *et al.* 2021).

Results

Phylogenetic analyses

Since no significant incongruences were detected between nuclear and plastid datasets in previous molecular phylogenetic analyses with similar datasets (Pedron *et al.* 2014; Lau *et al.* 2021), we performed searches using only the concatenated alignment. The concatenated ITS, ETS, *matK-trnK*, and *rps16-trnK* DNA matrix consisted of 4490 aligned characters, of which 810 (18%) were parsimony-informative. The parsimony analyses retained a total of 15 257 most parsimonious trees, with a tree length of 2929 steps, a consistency index (CI) of 0.64, 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. 1). For comparison, bootstrap percentages (BP) from the bootstrap analysis are shown in the Bayesian tree.

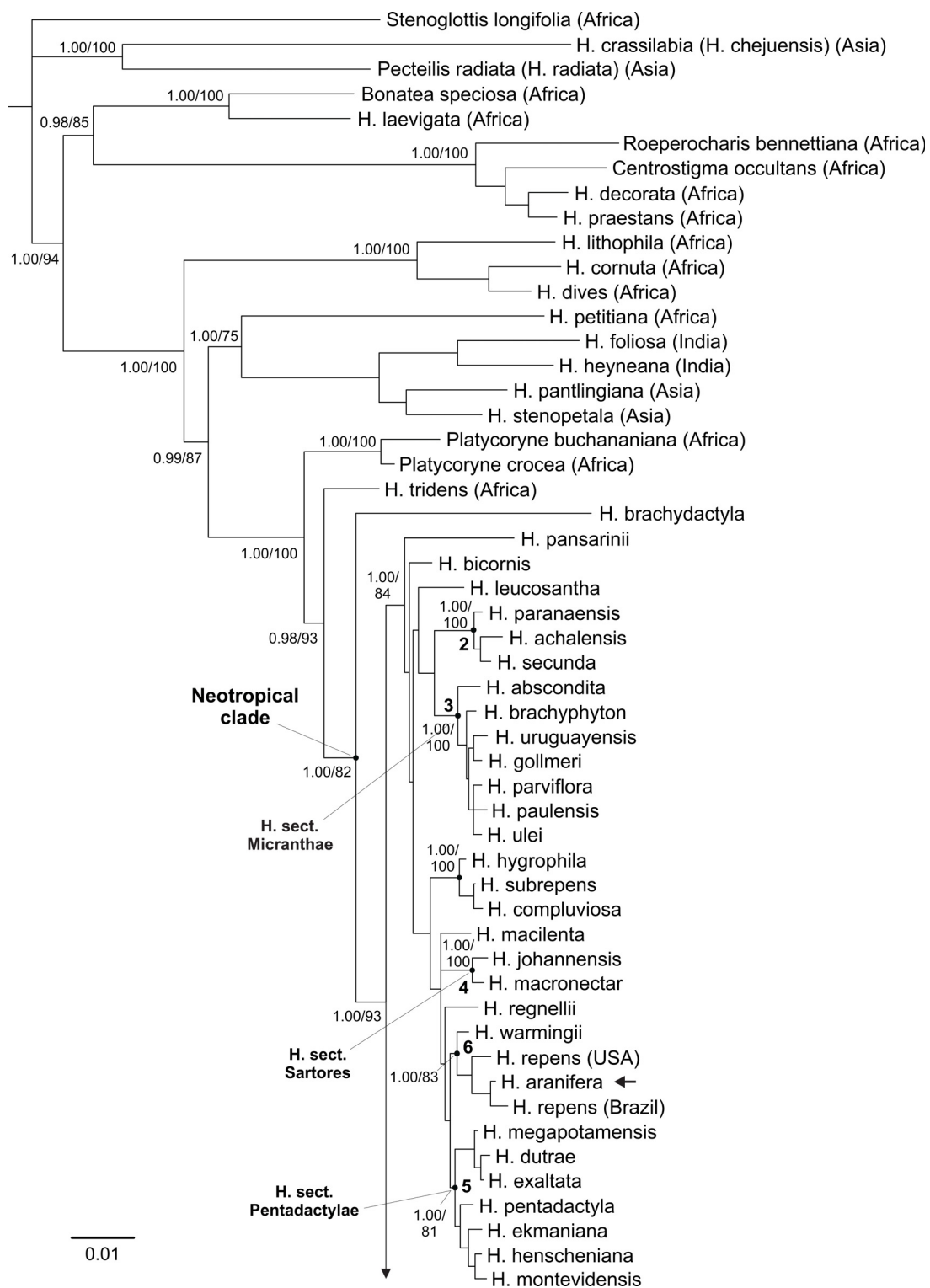


Fig. 1 (part 1). Bayesian 50% majority-rule consensus tree of the combined ITS, ETS, *matK-trnK* and *rps16-trnK* datasets. Numbers next to the nodes represent the posterior probabilities (PP) from the Bayesian analysis and bootstrap percentages (BP) from parsimony analyses. Only values of major clades are shown. Bootstrap percentages $\leq 50\%$ are indicated by a dash (-). Neotropical subclades are numbered according to Batista *et al.* (2013). *Habenaria karstica* J.A.N.Bat. sp. nov. is highlighted in bold. Species of the *Habenaria repens* complex morphologically similar to *H. karstica* are indicated by arrows.

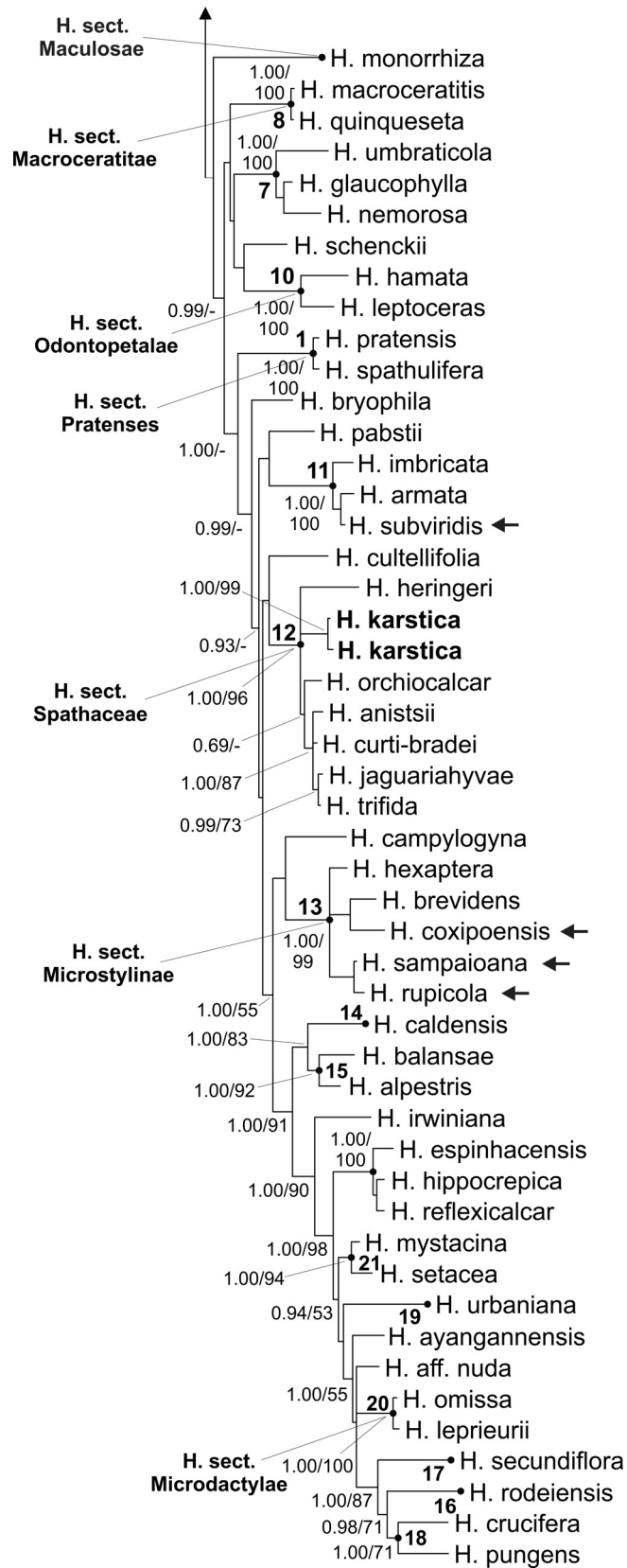


Fig. 1 (part 2). Bayesian 50% majority-rule consensus tree of the combined ITS, ETS, matK-trnK and rps16-trnK datasets.

The relationships recovered were similar to those of our earlier molecular phylogenetic studies (Batista *et al.* 2013, 2014, 2016, 2017; Pedron *et al.* 2014; Lau *et al.* 2021), with the New World species of *Habenaria* forming a well-supported monophyletic group (1.00 PP, 82% BP) and recovering several well-supported subclades within the New World clade that are numbered accordingly (subclades 1 to 21) (Fig. 1). Sequences for the ETS, complete *matK* gene, and *rps16-trnK* intergenic spacer were not available for species from subclade 9, therefore these were not included in the analyses. Subclades that could be associate with one of the sections of Kränzlin's (1901) sectional treatment are identified accordingly. The increase in the sampling of terminals and characters from the external group increased the support of the Neotropical clade compared to the results of Batista *et al.* (2017). The two sequenced samples of *H. karstica* sp. nov. were recovered with high support (1.00 PP, 96% BP) within subclade 12 (Fig. 1), which in Kränzlin's (1901) sectional classification corresponds to *H.* sect. *Spathaceae* Kraenzl.

Diversity of Orchidaceae in Brazilian karsts

Our survey retrieved 343 records of Orchidaceae from karsts throughout Brazil, comprising 122 species distributed in 58 genera (Table 1). The State of Minas Gerais is the federation unit with the most records (136 records), followed by São Paulo (64 records), Distrito Federal (52 records), Goiás (31 records), Mato Grosso (19 records), Mato Grosso do Sul (18 records), and Bahia (12 records). Other states (Espírito Santo, Paraná, Rondônia, Tocantins, and Pernambuco) accounted for only a total of 11 records. *Epidendrum* L., with nine species, is the genus with the largest number of species, followed by *Cyclopogon* C.Presl, *Cyrtopodium* R.Br., and *Maxillaria* Ruiz & Pav. (seven spp. each), *Encyclia* Hook., *Gomesa* R.Br., *Habenaria*, *Pabstiella* Brieger & Senghas, and *Trichocentrum* Poepp. & Endl. (five spp. each), and *Cattleya* Lindl. and *Stelis* Sw. with four species each. From the 58 genera, 38 (65% of the total) are represented by only a single species. From the total of 122 species recorded, 115 (94%) occur in forests associated with karsts, while only six species (*Cyrtopodium andersonii* (Lamb. ex Andrews) R.Br., *C. glutiniferum* Raddi, *C. holstii* L.C.Menezes, *C. virescens* Rchb.f. & Warm., *C. withneri* L.C.Menezes, and *Habenaria karstica* sp. nov.) grow directly on the rocky massif. One species, *Vanilla chamissonis* Klotzsch, occurs in forests and on the outcrops. Regarding the type of substrate, 81 (66%) species are epiphytes, 31 (25%) are terrestrial, five (4%) are rupicolous, and only two are scandent.

Among the Brazilian phytogeographic domains, the Cerrado has the greatest diversity, with 77 species (63% of the total species found in karsts) and 44 genera (76%) (Table 1). *Cyrtopodium* and *Epidendrum*, with six species each, are the genera with the highest number of species, followed by *Cyclopogon*, *Encyclia*, *Habenaria*, and *Trichocentrum*, with five species each. For the karsts of the Atlantic Forest domain, 53 species (43%) and 32 genera (55%) were recorded. The genera with the highest number of species are *Epidendrum* and *Maxillaria* (six species, each), and *Pabstiella* and *Stelis*, each with four species. For the Caatinga domain, seven genera and seven species were recorded. For the Amazon domain, a single species, *Cyrtopodium andersonii*, occurred in limestone outcrops in the Northern State of Mato Grosso.

Considering only karsts, the number of species unique to each of the two most diverse domains is high: 83% of the species registered for the Cerrado and 81% for the Atlantic Forest do not occur in karst from other phytogeographic domains (Fig. 2). The Caatinga has only one exclusive species (14%), but the total number of species and sampling in this domain is smaller than in the other two domains. A total of 13 species (11% of the total species of Brazilian karsts) are shared between the karsts of the Cerrado and Atlantic Forest domains. At the same time, only three (*Epidendrum secundum* Jacq., *Isochilus linearis* (Jacq.) R.Br., and *Oeceoclades maculata* (Lindl.) Lindl.) are common to the karsts of the Cerrado, Atlantic Forest, and Caatinga domains.

Regarding the general geographic distribution of the species, most have a wide distribution, occurring in various regions of Brazil or other countries (Table 1), in more than one phytogeographic domain and other vegetation types not associated with karsts. Only four species (3.3%) are potentially restricted to

Table 1 (continued on next five pages). Checklist of the Orchidaceae recorded for karsts in Brazil. Names used in some of the references are indicated in parentheses. Potentially endemic species of karsts are indicated by an asterisk. Abbreviations: Habit: Ep = epiphyte; T = terrestrial; Ru = rupicolous; Sc = scandent. Distribution: Biomes: AF = Atlantic Forest; Caa = Caatinga; Cer = Cerrado. Brazilian federation units: BA = Bahia; DF = Distrito Federal; ES = Espírito Santo; GO = Goiás; MG = Minas Gerais; MS = Mato Grosso do Sul; MT = Mato Grosso; PE = Pernambuco; PR = Paraná; RO = Rondônia; SP = São Paulo; TO = Tocantins. References: 1 = Warming (1908); 2 = Bianchetti *et al.* (2005); 3 = Melo (2008); 4 = Melo *et al.* (2013); 5 = Ferreira & Stehmann (2020); 6 = Peixoto (2018); 7 = speciesLink (2021); 8 = Flora e Funga do Brasil (2021).

Species	Habit	Habitat	Karsts distribution: biome (state)	General distribution	References
<i>Acianthera aphthosa</i> (Lindl.) Pridgeon & M.W.Chase	Ep	forest	Cer (MG)	W South America to SE & S Brazil	3, 4
<i>Acianthera pubescens</i> (Lindl.) Pridgeon & M.W.Chase	Ep	forest	AF (SP)	Mexico to Trop. America	6
<i>Acianthera saundersiana</i> (Rehb.f.) Pridgeon & M.W.Chase	Ep	forest	AF (SP)	Peru to Brazil and Argentina	7
<i>Anathallis microphyta</i> (Barb.Rodr.) C.O.Azevedo & Van den Berg	Ep	forest	AF (SP)	E & S Brazil to Argentina	6
<i>Aspasia variegata</i> Lindl.	Ep	forest	Cer (DF, MG)	Trinidad to S Trop. America	2, 7
<i>Barbosella miersii</i> (Lindl.) Schltr.	Ep	forest	AF (SP)	Brazil (RJ to PR)	7
<i>Brachystele guayanensis</i> (Lindl.) Schltr.	T	forest	Cer (GO)	Mexico to S Trop. America	7
<i>Brassavola flagellaris</i> Barb.Rodr. (as <i>B. tuberculata</i>)	Ep	forest	Caa, Cer (BA, DF, MG)	SE Brazil	5, 7
<i>Bulbophyllum insectiferum</i> Barb.Rodr.	Ep/Ru	forest	Cer (DF)	E Bolivia to Brazil	2
<i>Bulbophyllum malachadenia</i> (Lindl.) Cogn.	Ep	forest	AF (SP)	Brazil (RJ to PR)	6
<i>Bulbophyllum napellii</i> Lindl.	Ep	forest	AF (SP)	E & S Brazil to Argentina	6
<i>Campylocentrum neglectum</i> (Rehb.f. & Warm.) Cogn.	Ep	forest	Cer (DF, MG, MS)	Brazil to NE Argentina	2, 5, 7
<i>Catasetum fimbriatum</i> Rchb.f.	Ep	forest	Cer (MG)	S Trop. America	3, 4
<i>Cattleya bicolor</i> Lindl.	Ep	forest	Cer (MG)	WC & SE Brazil	3, 4, 7
<i>Cattleya cernua</i> (Lindl.) Van den Berg	Ep	forest	Cer (MG)	Brazil to NE Argentina	3, 4, 5
<i>Cattleya lundii</i> (Rehb.f. & Warm.) Van den Berg	Ep	forest	Cer (MG)	Bolivia to Argentina	5
<i>Cattleya walkeriana</i> Gardner	Ep	forest	Cer (DF, MG)	WC & SE Brazil	2, 3, 4, 7
<i>Cranichis scripta</i> Kraenzl. * (as <i>C. candida</i>)	T	forest	Cer (DF, GO, MG)	WC Brazil (to MG)	2, 3, 4, 7
<i>Cyclopogon bicolor</i> (Ker Gawl.) Schltr.	T	forest	Cer (MG)	S Trop. America to NE Argentina	1
<i>Cyclopogon congestus</i> (Vell.) Hoehne	T	forest	AF (SP)	SE & S Brazil to N Argentina	6

Table 1 (continued). Checklist of the Orchidaceae recorded for karsts in Brazil. Names used in some of the references are indicated in parentheses. Potentially endemic species of karsts are indicated by an asterisk.

Species	Habit	Habitat	Karsts distribution: biome (state)	General distribution	References
<i>Cyclopogon cf. elatus</i> (Sw.) Schltr.	T	forest	Cer (GO)	S Florida to Trop. America	7
<i>Cyclopogon cf. elegans</i> Hoehne	T	forest	Cer (DF)	E Bolivia to Brazil and N Argentina	2
<i>Cyclopogon graciliscapus</i> Schltr.	T	forest	Cer (DF)	SE & S Brazil	2
<i>Cyclopogon longibracteatus</i> (Barb.Rodr.) Schltr.	T	forest	Cer (MG)	SE & S Brazil to NE Argentina	3, 4
<i>Cyclopogon variegatus</i> Barb.Rodr.	T	forest	AF (SP)	Brazil to Paraguay	7
<i>Cyrtopodium andersonii</i> (Lamb. ex Andrews) R.Br.	Ru	massif	Ama (MT)	N South America and N Brazil	7
<i>Cyrtopodium eugenii</i> Rchb.f.	T	forest	Cer (DF, GO)	Brazil to Bolivia	7
<i>Cyrtopodium glutiniferum</i> Raddi	Ru	massif	AF, Cer (ES, MG)	SE Brazil (to BA)	3, 4, 5, 7
<i>Cyrtopodium holstii</i> L.C. Menezes	Ru	massif	Cer (MG)	E & SE Brazil	3
<i>Cyrtopodium saintlegerianum</i> Rchb.f.	Ep	forest	Caa, Cer (BA, GO)	Brazil to Paraguay	7
<i>Cyrtopodium virescens</i> Rchb.f. & Warm.	Ru	massif	Cer (GO,MS)	Peru to Brazil	7
<i>Cyrtopodium withneri</i> L.C.Menezes*	Ru	massif	Cer (DF, GO, MG)	WC Brazil (to MG)	2, 7
<i>Dichaea pendula</i> (Aubl.) Cogn.	Ep	forest	AF (SP)	Trop. America	6
<i>Eltroplectris brachycentron</i> Szlach.	T	forest	Cer (MS)	Bolivia to W Brazil	7
<i>Encyclia argentinensis</i> (Speg.) Hoehne	Ep	forest	Cer (MS)	W & C Brazil to N Argentina	7
<i>Encyclia linearifolioides</i> (Kraenzl.) Hoehne	Ep	forest	Cer (DF, GO, MT, MS)	Bolivia to C Brazil and Paraguay	2, 7
<i>Encyclia osmantha</i> (Barb.Rodr.) Schltr	Ep	forest	Cer (MG)	Venezuela to Brazil	5, 7
<i>Encyclia patens</i> Hook.	Ep	forest	Cer (MG)	E & S Brazil	7
<i>Encyclia seidelii</i> Pabst	Ep	forest	Cer (MG)	Brazil (MG)	7
<i>Epidendrum anceps</i> Jacq.	Ep	forest	Cer (DF, GO, MG)	Trop. America	2, 3, 4, 7
<i>Epidendrum avicula</i> Lindl.	Ep	forest	AF, Cer (DF, SP)	W South America to Brazil	2, 6
<i>Epidendrum cristatum</i> Ruiz & Pav.	Ep	forest	AF (SP)	Mexico to S Trop. America	7
<i>Epidendrum densiflorum</i> Hook. (as <i>E. paniculatum</i>)	Ep/Ru	forest	AF, Cer (DF, MG, MS, SP)	S Trop. America to Argentina	2, 3, 4, 5, 7, 6

Table 1 (continued). Checklist of the Orchidaceae recorded for karsts in Brazil. Names used in some of the references are indicated in parentheses. Potentially endemic species of karsts are indicated by an asterisk.

Species	Habitat	Habit	Habitat	Karsts distribution: biome (state)	General distribution	References
<i>Epidendrum geniculatum</i> Barb.Rodr.	Ep	forest	AF (SP)	SE & S Brazil	6	
<i>Epidendrum nocturnum</i> Jacq.	Ep	forest	Cer (DF)	S Florida, Mexico to Trop. America	7	
<i>Epidendrum pseudodifforme</i> Hoehne & Schltr. (as <i>E. difforme</i>)	Ep	forest	Cer (DF)	Brazil	2	
<i>Epidendrum secundum</i> Jacq. (as <i>E. ellipticum</i>)	Ep/T	forest	Caa, Cer (BA, MG, SP)	Trop. America	1, 6, 7	
<i>Epidendrum tridactylum</i> Lindl.	Ep	forest	AF (SP)	W South America to Brazil	7	
<i>Erycina pusilla</i> (L.) N.H.Williams & M.W.Chase	Ep	forest	Cer (MS)	S Mexico to S Trop. America	8	
<i>Galeandra beyrichii</i> Rehb.f. (<i>G. hysterantha</i>)	T	forest	Cer (GO, MG)	Trop. America	3, 4, 7	
<i>Gomesa fuscopetalata</i> (Hoehne) M.W.Chase & N.H.Williams	Ep	forest	Cer (DF)	E Bolivia to Brazil and Paraguay	2	
<i>Gomesa macropetalata</i> (Lindl.) M.W.Chase & N.H.Williams	Ep	forest	Cer (DF, GO)	Brazil to E Bolivia	2, 7	
<i>Gomesa recurva</i> R.Br.	Ep	forest	AF (SP)	E Brazil to Argentina	6	
<i>Gomesa uniflora</i> (Booth ex Lindl.) M.W.Chase & N.H.Williams	Ep	forest	AF (SP)	SE & S Brazil	6	
<i>Gomesa varicosa</i> (Lindl.) M.W.Chase & N.H.Williams	Ep	forest	Caa (BA)	Bolivia to Brazil and N Argentina	7	
<i>Govenia gardneri</i> Hook. (as <i>G. utriculata</i>)	T	forest	AF (PR, SP)	Panama to S Trop. America	6	
<i>Habenaria cryptophila</i> Barb.Rodr.	T	forest	Cer (DF, GO)	Bolivia to C Brazil	2, 7	
<i>Habenaria curvilabris</i> Barb.Rodr.	T	forest	Cer (DF)	Brazil	2	
<i>Habenaria distans</i> Griseb.	T	forest	Cer (DF)	SW Florida, Mexico to Trop. America	2	
<i>Habenaria glaucophylla</i> Barb. Rodr.	T	forest	Cer (MG)	WC & SE Brazil	1	
<i>Habenaria karstica</i> J.A.N.Bat. sp. nov. *	T	massif	Cer (MG)	Brazil (MG)	this work	
<i>Ionopsis utricularioides</i> (Sw.) Lindl.	Ep	forest	Cer (DF, GO, MG, MS)	S Florida, S Mexico to Trop. America	2, 3, 4, 7	
<i>Isochilus linearis</i> (Ruiz & Pav.) R.Br.	Ep	forest	AF, Caa, Cer (BA, MG, SP)	Mexico to Trop. America	3, 4, 6, 7	

Table 1 (continued). Checklist of the Orchidaceae recorded for karsts in Brazil. Names used in some of the references are indicated in parentheses. Potentially endemic species of karsts are indicated by an asterisk.

Species	Habit	Habitat	Karsts distribution: biome (state)	General distribution	References
<i>Laelia gloriosa</i> (Rchb.f.) L.O. Williams	Ep	forest	AF, Cer (DF, ES, MG)	N South America to Brazil	1, 2, 7
<i>Leptotes bicolor</i> Lindl.	Ep	forest	Cer (MG)	Brazil to Paraguay	3, 4
<i>Liparis nervosa</i> (Thunb.) Lindl.	T	forest	AF, Cer (DF, SP)	Tropics & Subtropics	2, 7
<i>Lockhartia goyazensis</i> Rchb.f.	Ep	forest	Cer (DF, MG)	E Bolivia to Brazil	2, 7
<i>Lockhartia lunifera</i> (Lindl.) Rchb.f.	Ep	forest	AF (SP)	Brazil	7
<i>Maxillaria leucomata</i> Barb.Rodr.	Ep	forest	AF (SP)	French Guiana to Brazil	6
<i>Maxillaria obtusa</i> (Lindl.) Molinari	Ep	forest	AF (SP)	S Trop. America	6
<i>Maxillaria ochroleuca</i> Lodd. ex Lindl.	Ep	forest	AF (SP)	S Trop. America	6
<i>Maxillaria paranaensis</i> Barb.Rodr.	Ep	forest	AF (SP)	SE & S Brazil and Argentina	6
<i>Maxillaria rufescens</i> Lindl.	Ep	forest	AF (SP)	Trop. America	6
<i>Maxillaria subrepens</i> (Rolfe) Schuit. & M.W.Chase (as <i>Trigonidium tenue</i>)	Ep	forest	Cer (MT)	S Trop. America	7
<i>Maxillaria valenzuelana</i> (A.Rich.) Nash	Ep	forest	AF (SP)	Trop. America	6
<i>Mesadenella cuspidata</i> (Lindl.) Garay	T	forest	Cer (DF, MG)	S Trop. America	2, 3, 4
<i>Microchilus arietinus</i> (Rchb.f. & Warm.) Ormerod	T	forest	AF (SP)	S Trop. America to Argentina	6
<i>Miltonia flavescens</i> Lindl.	Ep	forest	Cer (MG)	Brazil to Argentina	3, 4
<i>Myoxanthus lonchophyllus</i> (Barb. Rodr.) Luer	Ep	forest	Cer (MG)	SE Brazil	3, 4, 5
<i>Nolyia</i> cf. <i>hemiricha</i> Barb. Rodr.	Ep	forest	Cer (MG)	Brazil	3, 4
<i>Octomeria crassifolia</i> Lindl.	Ep	forest	AF (SP)	E Ecuador, Brazil to Argentina	6
<i>Octomeria gracilis</i> Lodd. ex Lindl.	Ep	forest	AF (SP)	SE & S Brazil	6
<i>Octomeria warmingii</i> Rchb.f.	Ep	forest	Cer (MS)	Brazil (MG)	7
<i>Oeceoclades maculata</i> (Lindl.) Lindl.	T	forest	AF, Caa, Cer (BA, DF, MG, MS, PE)	Africa & Trop. America	2, 3, 4, 7
<i>Orleanesia cuneipetala</i> Pabst	Ep	forest	Cer (MT)	N Brazil	7

Table 1 (continued). Checklist of the Orchidaceae recorded for karsts in Brazil. Names used in some of the references are indicated in parentheses. Potentially endemic species of karsts are indicated by an asterisk.

Species	Habitat	Habit	Habitat	Karsts distribution: biome (state)	General distribution	References
<i>Pabstiella hians</i> (Lindl.) Luer	Ep	forest	AF (SP)	SE & S Brazil	6	
<i>Pabstiella pristeoglossa</i> (Rchb.f. & Warm.) Luer (as <i>Pleurothallis</i> sp.1)	Ep	forest	Cer (MG)	SE & S Brazil	3, 4, 5, 7	
<i>Pabstiella trimeropetala</i> (Pabst) Luer	Ep	forest	AF (SP)	Brazil (RJ, SP)	6	
<i>Pabstiella tripterantha</i> (Rchb.f.) F.Barros	Ep	forest	AF (SP)	Costa Rica to Argentina	6	
<i>Pabstiella uniflora</i> (Lindl.) Luer	Ep	forest	AF (SP)	N South America to Brazil	6	
<i>Phymatidium falcifolium</i> Lindl.	Ep	forest	AF (SP)	SE & S Brazil to Uruguay	6	
<i>Plectrophora edwallii</i> Cogn.	Ep	forest	Cer (DF, GO, MS)	E Bolivia to Brazil (GO)	2, 7	
<i>Polystachya foliosa</i> (Hook.) Rchb.f. (as <i>P. estrellensis</i>)	Ep	forest	AF, Cer (MG, SP)	Mexico to Trop. America	3, 4, 6	
<i>Ponthieva phaenoleuca</i> (Barb.Rodr.) Cogn. (as <i>P. montana</i>)	T	forest	Cer (DF)	Brazil (MG)	2	
<i>Prescottia oligantha</i> (Sw.) Lindl. (as <i>P. microrhiza</i>)	T	forest	Cer (DF)	S Florida to Trop. America	2	
<i>Prescottia plantaginifolia</i> Lindl. ex Hook. (as <i>P. plantaginea</i>)	T	forest	AF (ES)	Brazil	7	
<i>Prosthechea bulbosa</i> (Vell.) W.E.Higgins	Ep	forest	AF (PR)	E & S Brazil to Paraguay	6	
<i>Pteroglossa macrantha</i> (Rchb.f.) Schltr.	T	forest	Cer (DF, GO)	S Trop. America	2, 7	
<i>Pteroglossa roseoalba</i> (Rchb.f.) Salazar & M.W.Chase	T	forest	Cer (MG)	S Mexico to S Trop. America	3, 4, 7	
<i>Rodriguezia brachystachys</i> Rchb.f. & Warm.*	Ep	forest	Cer (MG)	Brazil (MG)	5, 7	
<i>Rodriguezia decora</i> (Lem.) Rchb.f.	Ep	forest	AF (PR)	Brazil to Argentina	7	
<i>Sacoila lanceolata</i> (Aubl.) Garay	T	forest	Cer (GO, MS)	Trop. & Subtrop. America	7	
<i>Sarcoglottis curvisepala</i> Szlach. & Rutk. (as <i>S. fasciculata</i>)	T	forest	Cer (DF, MG)	Brazil (BA, DF, GO, MG)	2, 3, 4	
<i>Sarcoglottis homalogastra</i> (Rchb.f. & Warm.) Schltr.	T	forest	Cer (DF)	S Trop. America to Argentina	2	
<i>Sauroglossum elatum</i> Lindl.	T	forest	AF (SP)	Colombia to Brazil and N Argentina	6, 7	
<i>Scaphyglottis modesta</i> (Rchb.f.) Schltr.	Ep	forest	AF (SP)	Trop. America	6, 7	

Table 1 (continued). Checklist of the Orchidaceae recorded for karsts in Brazil. Names used in some of the references are indicated in parentheses. Potentially endemic species of karsts are indicated by an asterisk.

Species	Habit	Habitat	Karsts distribution: biome (state)	General distribution	References
<i>Specklinia grobyi</i> (Batem. ex Lindl.) F.Barros	Ep	forest	AF, Cer (GO, SP)	S Mexico to S Trop. America	6, 7
<i>Stanhopea lietzei</i> (Regel) Schltr.	Ep	forest	AF (SP)	E & S Brazil	6
<i>Stelis aprica</i> Lindl.	Ep	forest	AF (SP)	S Trop. America	6, 7
<i>Stelis deregularis</i> Barb.Rodr.	Ep	forest	AF (SP)	C & S Trop. America	6, 7
<i>Stelis papaquerensis</i> Rchb.f. (<i>S. fraterna</i>)	Ep	forest	AF (SP)	S Trop. America	6
<i>Stelis intermedia</i> Poepp. & Endl.	Ep	forest	AF (SP)	S Trop. America	6
<i>Trichocentrum albococcineum</i> Linden	Ep	forest	Cer (DF)	Brazil to Peru and E Bolivia	2
<i>Trichocentrum cepula</i> (Hoffmanns.) J.M.H.Shaw (as <i>Oncidium cebolleta</i>)	Ep	forest	Cer (BA, DF, GO, MG, MT)	Argentina, Bolivia, Brazil, Paraguay & Peru	2, 3, 4, 7
<i>Trichocentrum fuscum</i> Lindl.	Ep	forest	Cer (MG)	S Trop. America	7
<i>Trichocentrum jonesianum</i> (Rchb.f.) M.W.Chase & N.H.Williams	Ep	forest	Cer (DF, MG)	E Bolivia to Brazil and NE Argentina	2, 7
<i>Trichocentrum pumilum</i> (Lindl.) M.W.Chase & N.H.Williams	Ep	forest	Cer (DF, MG)	Brazil to NE Argentina	2, 3, 4, 7
<i>Trizeuxis falcata</i> Lindl.	Ep	forest	AF (SP)	Costa Rica to S Trop. America	6
<i>Vanilla chammissonis</i> Kraenzl.	Sc	forest/ massif	Cer (DF)	French Guiana, E Brazil, NE & E Bolivia	2
<i>Vanilla palmarum</i> (Salzm. ex Lindl.) Lindl.	Sc	forest	Caa, Cer (BA, MS)	S Trop. America	7
<i>Xylobium variegatum</i> (Ruiz & Pav.) Garay & Dunst.	Ep	forest	AF (SP)	S Trop. America	6
<i>Zootrophion atropurpureum</i> (Lindl.) Luer	Ep	forest	AF (SP)	Antilles & SE & S Brazil	6

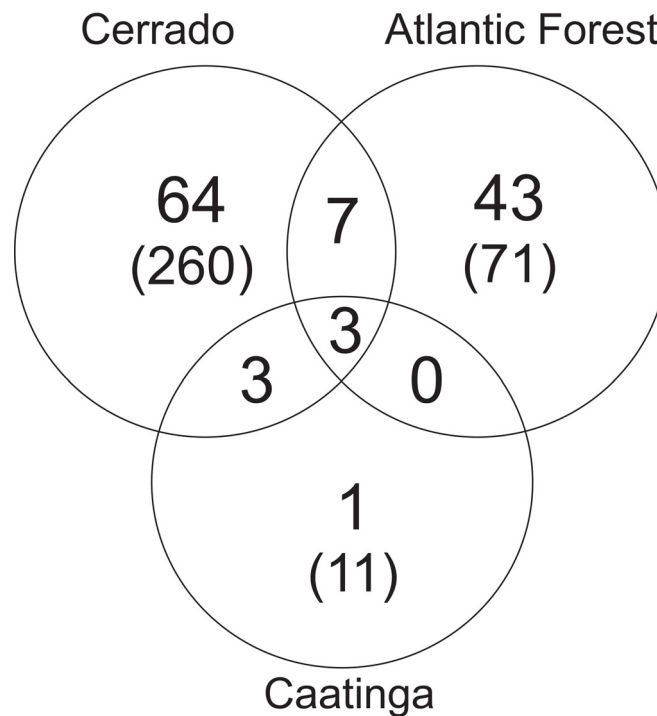


Fig. 2. Venn diagram of the distribution by biome of species of Orchidaceae recorded for karsts from Brazil. The number of samples for each biome is indicated in parentheses.

karsts: *Cranichis scripta* Kraenzl., *Cyrtopodium withneri*, *Habenaria karstica* sp. nov., and *Rodriguezia brachystachys* Rchb.f. & Warm. (Table 1). Of these, the last two are known only from the LSK.

Taxonomy

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

Habenaria karstica J.A.N.Bat. sp. nov.

urn:lsid:ipni.org:names:77299816-1

Figs 3–4

Diagnosis

Similar in general morphology to some species in the *Habenaria repens* complex, but differs from these and all other Neotropical species of the genus for growing on limestone outcrops, under full sun, associated with saxicolous plants, and by the combination of the following characters: leaves not pleated, pedicellate ovary 19–25 mm long, petal anterior segment about the same length as the posterior segment (ratio 0.9–1.2), lip lateral segment about the same length as the median segment (ratio 0.9–1.0), spur 16–19 mm long, and rostellum mid lobe apex acute and erect. Distinct from the phylogenetically

related species of the *Habenaria* sect. *Spathaceae* by the flowers completely green, pedicel 5–6 mm long, shorter than the ovary, pollinaria separate, and rostellum mid lobe completely held between the anther loculi.

Etymology

From the German 'karst' (limestone region), the specific epithet refers to the preference of this species for growing on limestone outcrops.

Type material

BRAZIL – Minas Gerais • Pedro Leopoldo, Lapa do Baú; 19°32'54.4" S, 43°59'34.1" W; 750 m a.s.l.; 7 Jan. 2020; fl; J.A.N. Batista, V.L. Ferreira & G.V.A. dos Santos 3649; holotype: BHCB[204961]; isotype: CEN; GenBank nrITS: ON197322 and ON197323; *matK* gene and *trnK* intron: ON168967 and ON168968; *rps16-trnK* intergenic spacer: ON168969 and ON168970.

Other material studied

BRAZIL – Minas Gerais • Pedro Leopoldo, Lapa do Baú; 19°32'55" S, 43°59'35" W; 715 m a.s.l.; 11 Dec. 2018; fl; V.L. Ferreira, J.R. Stehmann & G.V.A. Santos 126; BHCB.

Description

Geophytic herb, caulescent, sympodial. *Roots* 1.4–1.6 mm wide, up to 7 cm long; tuberoid ca 3.6 × 1.7 cm, ellipsoid. *Stem* 47–62 cm long including the inflorescence, 3.5–6.0 mm wide, erect. *Leaves* 4–6, spirally-alternate, lanceolate, membranaceous, the largest concentrated in the middle of the stem, 10–18 × 1.8–3 cm, reducing towards the apex of the stem, base sheathing, sheath closed, blade patent, apex acute. *Inflorescence* 10–13 cm long, spiral, 0.7–1.2 flowers/cm of the rachis; floral bracts 14–36 mm long, about the same size as the pedicellate ovary, decreasing in size towards the inflorescence apex, green, ovate to lanceolate, apex acuminate. *Flowers* 8–14, resupinate, green, glabrous; pedicellate ovary 19–25 mm long, mostly straight, more or less parallel to the rachis axis or ascending, apex curved; ovary 15–19 mm long, pedicel 5–6 mm long. *Sepals* green, margin smooth; dorsal sepal 8.0–9.0 × 6.0 mm, concave, ovate when flattened, apex mucronate; lateral sepals 10.0–11.0 × 4.0 mm, abaxially concave, obliquely lanceo-ovate, patent, the distal third reflexed, apex mucronate. *Corolla* light green. *Petals* bipartite; posterior segment 8.0–10.0 × 1.6–2.0 mm, narrowly triangular, falcate, apex acute, free from the dorsal sepal to connivent; anterior segment 8.0–9.0 mm long, 0.9–1.2 times as long as the posterior segment, filiform, inserted at the base of the posterior segment. *Labellum* tripartite; undivided basal part 1.3–2.2 × 2.5–3.0 mm; lateral segments 9–10 × 0.8–1.0 mm, 0.9–1.0 times as long as the median segment, reflexed; median segment 10–11 × 1.3–1.5 mm, linear, straight; spur 16.0–19.0 × 1.6–2.0 mm, 0.8–0.9 times as long as the pedicellate ovary, deflexed, parallel to the pedicellate ovary, apex covered by the bract, linear to slightly clavate, green. *Gynostemium* ca 3.3 mm high, erect; connective light green, apex emarginate; lateral appendages (auricles) 2.2–2.4 × 0.6 mm, fleshy, verrucose, erect, translucent. *Anther* 1.6–1.7 × 1.3 mm, bilocular, loculi parallel, translucent; canals parallel, projected forward, more or less perpendicular to the loculi; pollinaria 2, separate; caudicles 1.2 mm long, filiform, pale yellow; pollinia 4, 1.2 × 0.7 mm, elliptical, yellow; viscidia 0.4 × 0.3 mm, ellipsoidal, whitish, 1.0 mm apart from each other. *Stigmatophores* (stigma lobes) 2, ca 1.8 mm long, parallel, light green, receptive surface 0.7 mm long, convex, mostly turned frontwards, 0.5 mm wide each. *Rostellum* ca 2.7 mm long; mid lobe ca 1.2 mm high, triangular, erect, held between the anther loculi, fleshy, translucent, apex light green, acute; side-lobes 1.1 mm long, parallel throughout.

Ecology and phenology

Habenaria karstica sp. nov. grows in limestone outcrops, under full sun, in soil patches accumulated at the base of *Dyckia luxor* (L.B.Sm. & Read) Forzza, which forms large colonies on the rock, or in

small pockets of earth between rocks (Fig. 3). The plants begin to grow in late November and early December, at the beginning of the rainy season and bloom from mid-December to January. Several species of orchids also occur on the same limestone outcrop, but only *Cyrtopodium glutiniferum* grows under the same conditions. However, this species is much stouter and forms large clumps with exposed, fusiform pseudobulbs up to 90 cm in length (Batista & Bianchetti 2021). Other species such as *Cattleya cernua* (Lindl.) Van den Berg, *C. lundii* (Rchb.f. & Warm.) Van den Berg, *Campylocentrum neglectum* (Rchb.f. & Warm.) Cogn., *Myoxanthus lonchophyllus* (Barb.Rodr.) Luer, and *Trichocentrum pumilum* (Lindl.) M.W.Chase & N.H.Williams occur on the same outcrop, but all are epiphytes and grow on isolated trees or in the forests surrounding the rock massif.

Distribution and conservation assessment

Habenaria karstica sp. nov. is so far known from a single limestone outcrop called Lapa do Baú in the municipality of Pedro Leopoldo, in the State of Minas Gerais. This population is restricted to a small massif area, and in December 2020, we observed only about 15 individuals with growing inflorescences. The place is a private property inserted in a conservation unit of sustainable use (APA Carste de Lagoa Santa), and although the surrounding area has been completely converted to cultivated land and pasture, the massif is relatively preserved. However, there is an intense limestone mining activity in the region, and several outcrops have been destroyed (de Deus *et al.* 2013). According to the *IUCN Red List Categories and Criteria* and the guidelines (IUCN 2012, 2016), the species can tentatively be classified

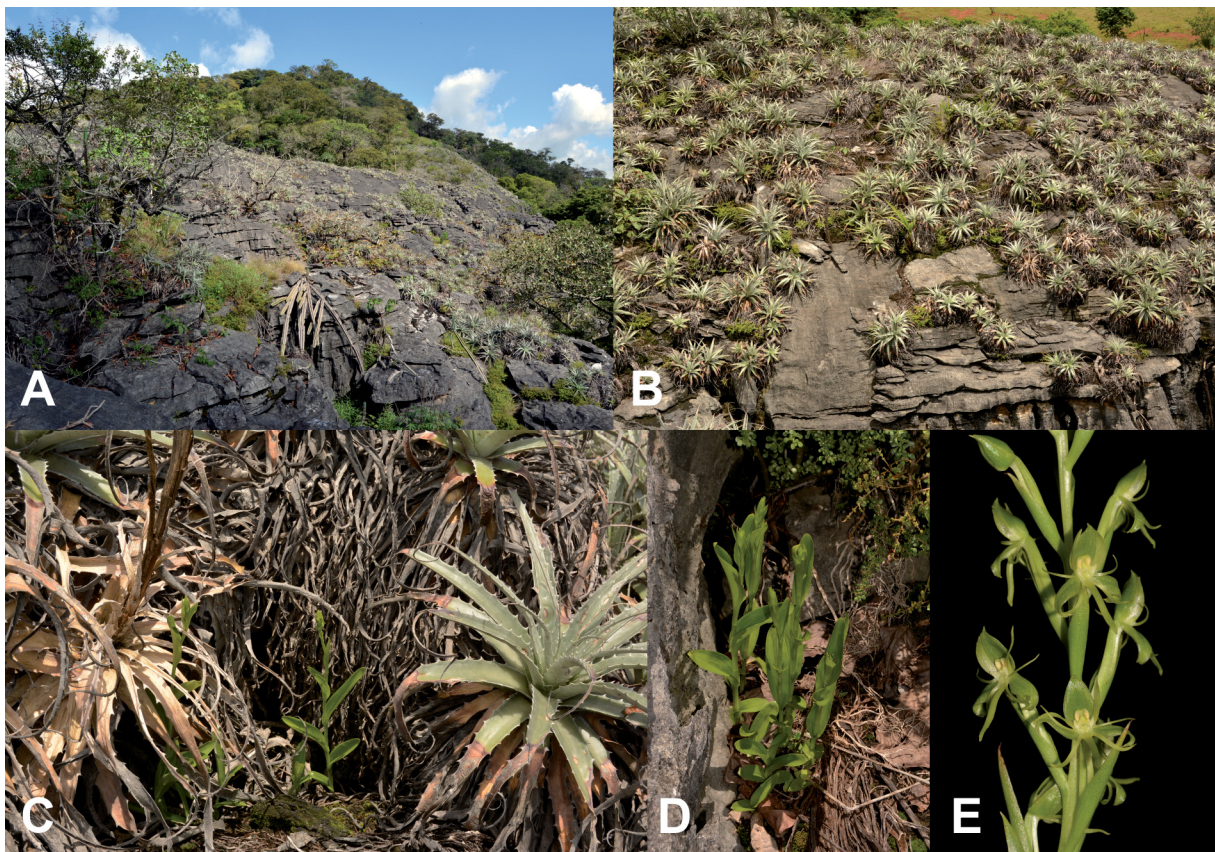


Fig. 3. Lapa do Baú, Pedro Leopoldo, during the rainy season. **A.** General view of the outcrop. **B.** View of the massif with predominance of *Dyckia luxor* (L.B.Sm. & Read) Forzza. **C–E.** *Habenaria karstica* J.A.N.Bat. sp. nov. **C.** Plants growing at the base and between *D. luxor*. **D.** Plants growing in small pockets of soil accumulated between rocky blocks. **E.** Flowers.

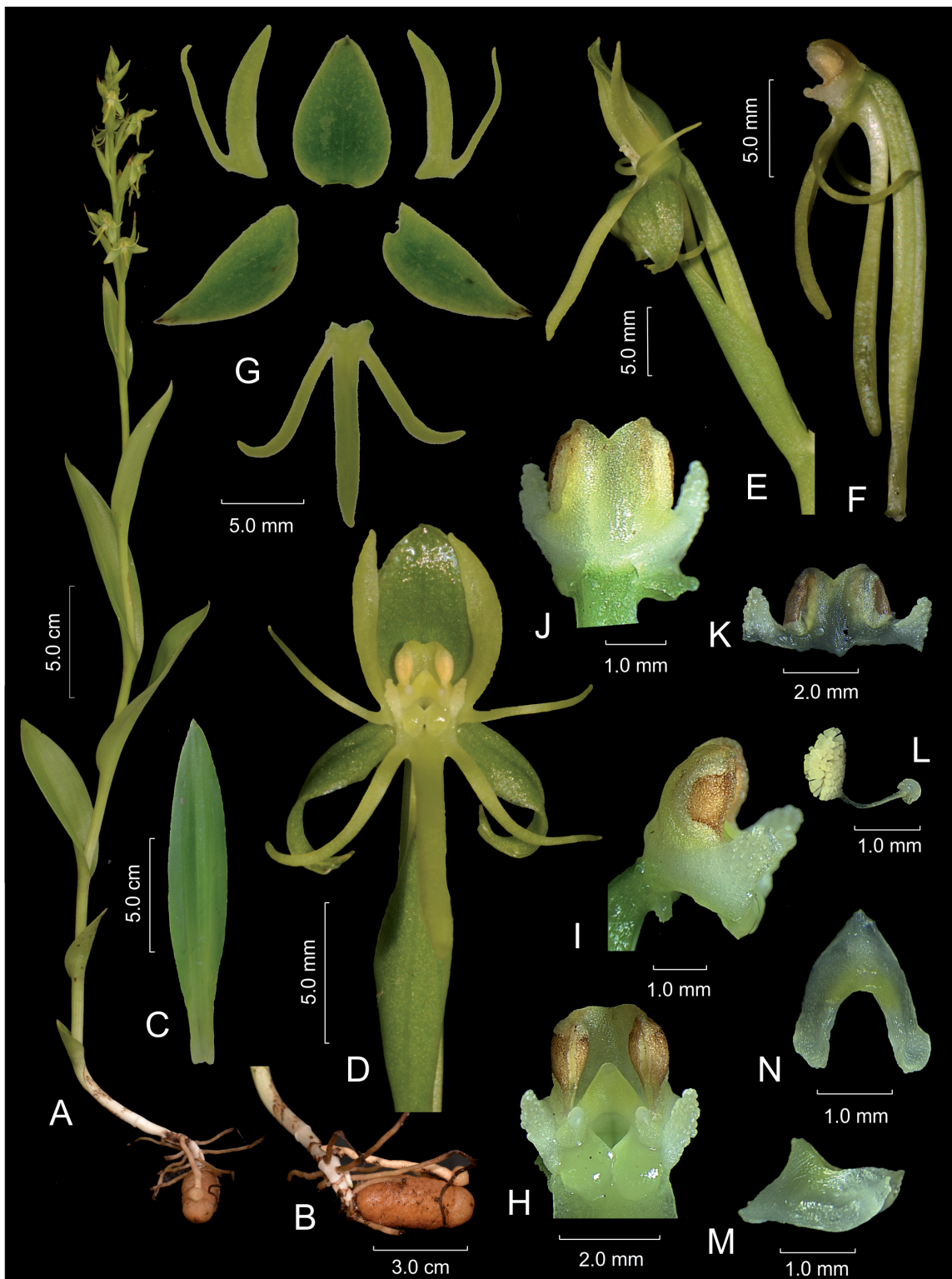


Fig. 4. *Habenaria karstica* J.A.N.Bat. sp. nov. A. Habit. B. Stem base, roots and tuberoid. C. Leaf. D. Flower, front view. E. Flower, side view. F. Pedicellate ovary, gynostemium, lip and spur, side view. G. Dissected perianth. H. Gynostemium, front view. I. Gynostemium, lateral view. J. Gynostemium, dorsal view. K. Dissected connective, anthers and auricles. L. Pollinaria. M. Rostellum, side view. N. Rostellum, upper view. All images from the type material, *J.A.N. Batista et al.* 3649 (BHCB).

Table 2. Comparison between *H. karstica* J.A.N.Bat. sp. nov. and morphologically similar species of the *H. repens* complex.

	<i>H. karstica</i>	<i>H. achuantha</i>	<i>H. amambayensis</i>	<i>H. aranifera</i>	<i>H. coxipoensis</i>	<i>H. novaesii</i>	<i>H. rupicola</i>	<i>H. sampaioana</i>	<i>H. subviridis</i>
Stem high, including the inflorescence (cm)	47–62	27–155	21–64	12–72	17–42	75–80	12–60	26–94	10–42
Leaf	flat	plicate	plicate	plicate	flat	plicate	flat	flat	plicate
Pedicellate ovary length (mm)	19–25	(11–)15–21	(9–)11–18	10–17	10–14	17–20	(10–)13–17	(10–)14–21	12–19(–25)
Dorsal sepal length (mm)	8–9	4.6–7.2	(5.1–)6.1–8.5	4.1–5.7	5.9–8.4	7.4–9.1	5.7–9.2	4.2–8.2	5.5–10
Petal anterior segment length (mm)	8–9	5.7–8.2	(7–)9.5–15	4.9–8.1	6.3–9.1	3.8–6	(4.5–)5.2–9.3	4.7–8.6	4.7–9.8
Petal anterior segment/posterior segment length ratio	0.9–1.2	1.4–1.5	1.3–1.8	1.2–1.9	1.0–1.6	0.6–0.9	0.9–1.3	1.1–1.4	(0.8)1.2–1.5
Lip median segment length (mm)	10–11	5.3–7.3	(5.5–)9.1–12	3.6–6.5	5–6.8	5.1–6.8	5.3–9.8	(4.3–)6.1–9.2	4.9–11
Lip lateral segment length (mm)	9–10	5.6–10	(8–)12–17	3.6–9	8.3–9.5	4.6–6.5	6–10	(5.5–)7.2–11	5.8–13
Lip lateral segment/median segment length ratio	0.9–1.0	1.1–1.4	1.4–1.6	1.4–1.6	1.1–1.6	0.9–1.1	1.1–1.3	1.1–1.5	1.3–1.5
Spur length (mm)	16–19	(7.5–)12–20	(9–)11–18	7.7–12	5.1–10	14–16	(4.5–)7–10	(9–)11–19	7.5–12
Rostellum mid lobe apex	acute	acute	rounded	rounded	obtuse	acute	rounded	rounded to obtuse	rounded to obtuse
Rostellum mid lobe apex direction	erect	curved forward	curved forward	curved forward	curved forward	curved forward	erect	erect	erect

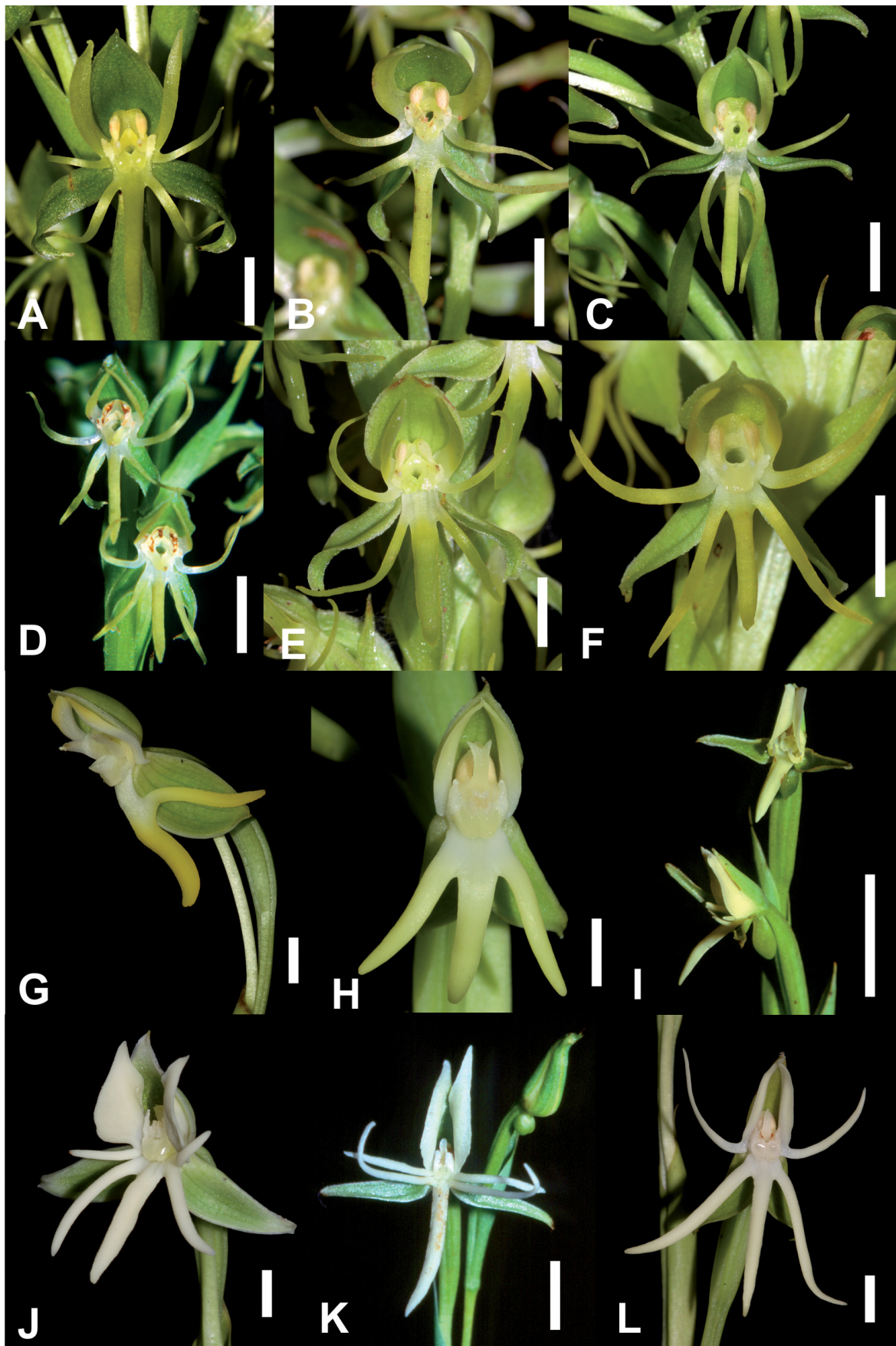
as Critically Endangered (CR), due to its small area of occupancy, estimated to be 4 km², and the fact that it is known from a single small population [B1ab(iii) + 2ab(iii); D].

Taxonomic notes

In the general morphology of flowers, *H. karstica* sp. nov. is similar to some species of the *Habenaria repens* complex (Lau *et al.* 2021), such as *H. coxipoensis* Hoehne, *H. rupicola* Barb.Rodr., *H. sampaiiana* Schltr., and *H. subviridis* Hoehne & Schltr. (Fig. 5). Based only on morphological characters, separating these species is difficult, and there is overlap with one or another species in all characters examined (Table 2). In addition to the very specific habitat, *H. karstica* can be morphologically differentiated from these species by a combination of characters, which include: leaves patent, distributed along the stem, leaf blade not plicate, pedicellate ovary 19–25 mm long, petal anterior segment about the same length as the posterior segment (ratio 0.9–1.2), lip lateral segment about the same length as the median segment (ratio 0.9–1.0), spur 16–19 mm long, and rostellum mid lobe apex acute and erect (Table 2).

Key to the species of *Habenaria* sect. *Spathaceae*

1. Flowers completely green; pedicellate ovary 19–25 mm long; pedicel 5–6 mm long, shorter than the ovary; rostellum mid-lobe completely held between the anther loculi *H. karstica* J.A.N.Bat.sp. nov.
 - Sepals green, petals and lip white or yellowish; pedicellate ovary (23–)30–115 mm long; pedicel 9–95 mm long, approximately the same size or longer than the ovary; rostellum mid-lobe mostly held beyond the anther loculi 2
2. Spur shorter than 6 mm 3
 - Spur 24–90 mm long 4
3. Spur scrotiform, 2.5–3.5 mm long; petal anterior segment and lip lateral segments at least half the length of the petal posterior segment and lip median segment, respectively *H. orchioalcar* Hoehne
 - Spur obovate, 4–6 mm long; petal anterior segment and lip lateral segments less than half the length of the petal posterior segment and lip median segment, respectively *H. heringeri* Pabst
4. Corolla yellowish; lateral sepals obliquely oblong; petal posterior segment oblanceolate to spatulate, largest width at the apical third; petal anterior segment 0–1.1 mm long; anther canals ca 5 mm long, parallel; pollinaria separate; stigma lobes with a prominent margin forming a keel *H. anisitsii* Kraenzl.
 - Corolla white; lateral sepals obliquely ovate to lanceolate; petal posterior segment not oblanceolate or spatulate, largest width at the middle; petal anterior segment 0–20 mm long; anther canals 1.5–3 mm long, convergent towards the apices; pollinaria joined by the viscidia; stigma lobes without a prominent margin, not forming a keel 5
5. Petals posterior segment rhombic to asymmetrically trullate *H. jaguariahyaе* Kraenzl.
 - Petals posterior segment lanceolate to narrowly elliptic 6
6. Anterior segment of the petal conspicuous, at least half or more of the length of the posterior segment *H. trifida* Kunth
 - Anterior segment of the petal absent, reduced to a tooth-like projection or less than half the length of the posterior segment *H. curti-bradei* Hoehne



Discussion

Phylogenetic relationships of *H. karstica* sp. nov.

Our results show that *H. karstica* sp. nov. belongs to the *H.* sect. *Spathaceae*. This result was unexpected since the new species differs from other species in the section in almost every respect, including morphology, habitat and distribution. In Kränzlin's (1901) classification, *H.* sect. *Spathaceae* corresponds to a morphologically highly polymorphic assemblage of species. On the other hand, the phylogenetic study of Batista *et al.* (2013) showed that the type species of the section, *H. spathacea*, a synonym of *H. trifida* Kunth (Batista *et al.* 2011b), forms a morphologically cohesive clade (subclade 12) which comprises six species (Fig. 5). These species occur in grasslands and are characterised by the few-flowered inflorescence (flowers 1–6), medium to large flowers (e.g., dorsal sepal 6–20 mm long), white or yellowish corolla and long pedicel (9–95 mm long), the same length or longer than the ovary. In terms of distribution, they are mostly restricted to the Cerrado of Central Brazil and are widely distributed throughout the domain. One species, *H. trifida*, has one of the broadest geographic ranges among the Neotropical species in the genus, occurring from Mexico to northern Argentina (Batista *et al.* 2011b).

The inclusion of *H. karstica* sp. nov. in *H.* sect. *Spathaceae* will make the morphological circumscription of the section particularly difficult, since the new species does not present any of the aforementioned characters shared by the other species of the group. The only apparent common morphological feature is the flat, non-plicate leaves, which differentiate *H. karstica* and the other species in *H.* sect. *Spathaceae* from *H. repens* and phylogenetically related species.

In general, both floral and vegetative morphology are conserved within the subclades identified in the phylogenetic analyses of the Neotropical species (Batista *et al.* 2013, 2014, 2016, 2017; Pedron *et al.* 2014; Lau *et al.* 2021), but there are exceptions. In *H.* sect. *Pentadactylae* Kraenzl., *H. montevidensis* Spreng. is distinct from other species in the section due to the reflexed dorsal sepal (vs the dorsal sepal forming a hood over the gynostemium) and the entire, orbicular, white petals, which form a hood over the gynostemium (vs bipartite, green, petals with posterior segment triangular-falcate to oblong-falcate, connivant with the dorsal sepal) (Pedron *et al.* 2014). The reflexed dorsal sepal is a widespread feature in the African species of the *H.* sect. *Replicatae* Kraenzl., but exclusive of *H. montevidensis* among Neotropical species. In a previous study, Pedron *et al.* (2012) showed that *H. montevidensis* is pollinated by butterflies, while moths pollinate other species characterised by green flowers and nocturnal odour, suggesting that the morphological differences of *H. montevidensis* are a result of a shift to diurnal pollinators. It could also be the case for *H. karstica* sp. nov., but currently, there is no information about pollination in the new species or other species in the section. Another possibility is that the morphological differences of *H. karstica* represent the plesiomorphic condition of the subclade. Still, the low internal resolution of subclade 12 does not allow inferences in this sense for the moment.

Fig. 5. A. *Habenaria karstica* J.A.N.Bat. sp. nov. (Batista *et al.* 3649 – BHCB). B–F. Species of the *Habenaria repens* complex morphologically similar to *H. karstica*. B. *Habenaria rupicola* Barb. Rodr. (Batista *et al.* 2782 – BHCB). C. *Habenaria sampaioana* Schltr. (Batista *et al.* 2426 – BHCB). D. *Habenaria coxipoensis* Hoehne (Batista 404 – CEN). E. *Habenaria subviridis* Hoehne & Schltr. (Batista 2605 – BHCB). F. *Habenaria aranifera* Lindl. (Batista 2472 – BHCB). G–L. Species phylogenetically related to *H. karstica* from *Habenaria* sect. *Spathaceae* Kraenzl. G. *Habenaria anistisii* Kraenzl. (Batista & Bianchetti 3087 – BHCB). H. *Habenaria curti-bradei* Hoehne (Batista *et al.* 2372 – BHCB). I. *Habenaria heringeri* Pabst (Batista 21 – CEN). J. *Habenaria jaguariahvyae* Kraenzl. (Batista *et al.* 1824 – BHCB). K. *Habenaria orchioalcar* Hoehne (Batista 173 – CEN). L. *Habenaria trifida* Kunth (Batista *et al.* 2724 – BHCB). Scale bars: A–F = 5 mm; G–L = 10 mm.

Diversity of Orchidaceae in Brazilian karsts

Compared to some floristic surveys of Orchidaceae in specific locations of Brazil, the diversity of Orchidaceae in Brazilian karsts is not particularly significant. For example, the Parque Natural da Serra do Caraça, with 11 233 ha in the State of Minas Gerais, shows 184 orchid species (Mota 2006), while the Parque Estadual das Fontes do Ipiranga, a small public park with 345 ha located within the city of São Paulo, has 125 species (Barros 1983). In contrast, our survey recorded a total of 122 species (Table 1), including all studies carried out so far in karsts of Brazil. Compared to the diversity of Orchidaceae in the country, this number represents only 4.5% of the total species known to Brazil. Likewise, the number of endemic species is small, and only four species (3.3% of total karst species) are potentially restricted to karsts of Brazil, representing 0.15% of Brazilian orchids.

Overall, this result seems to be a function of two main factors, the distribution of karsts in Brazil and the location of each sampled locality concerning the main phytogeographic domains of the country. Karsts of Brazil are mostly concentrated in areas within the Cerrado domain (Auler & Farrant 1996), a secondary centre of richness and diversity of Orchidaceae in Brazil, compared to the Atlantic Forest (Pabst & Dungs 1975; Flora e Funga do Brasil 2021). In line with this interpretation, the Cerrado is the domain with the largest number of sampled locations and the greatest diversity of species and genera. Still, the single locality sampled with the greatest number of species, the Alto Ribeira Tourist State Park (Peixoto 2018), is located within the Atlantic Forest domain.

As for the second factor, an analysis of each sampled location indicates that the species composition is associated with the phytogeographic domain where it is located or with the regional flora. Three cases exemplify this well: (a) the greatest diversity of Pleurothallidinae and Maxillariinae is found in karsts associated with the Atlantic Forest; (b) the occurrence of species and genera typical of the Atlantic Forest in Mata de Pains (Melo *et al.* 2013), located within the Cerrado domain, but in a transitional region with the Atlantic Forest; and (c) the distribution of species in the *Cyrtopodium andersonii* species complex (Batista & Bianchetti 2020), which is conditioned by the domain. *Cyrtopodium glutiniferum* occurs in karsts of the Atlantic Forest and bordering regions with the Cerrado (SE Brazil), *C. withneri* in karsts in the core region of the Cerrado (WC Brazil), and *C. andersonii* in the Amazon (N South America and N Brazil). As additional consequences, localities located in bordering regions between domains tend to have a mixed species composition. The floristic similarity between the domains (Fig. 2) and between the sampled localities is low (Melo 2008; Peixoto 2018; Ferreira & Stehmann 2020).

Despite the low diversity of Orchidaceae in Brazilian karsts compared to the family's diversity in the country, Orchidaceae is an important component of the local flora in some localities, both in the Cerrado and in the Atlantic Forest domains. In floristic surveys of three karsts in areas of rainforest in the States of São Paulo and Paraná (Peixoto 2018), within the Atlantic Forest domain, and of two calcareous massifs at Lagoa Santa karst (Ferreira & Stehmann 2020), Orchidaceae was the largest family in the number of species, while in Mata de Pains in the upper São Francisco river basin karst region it was the second-largest family (Melo *et al.* 2013). On the other hand, in a survey on calcareous outcrops in six locations along the west side of the Espinhaço Range in the State of Minas Gerais, in the Cerrado domain, Orchidaceae occupied the fortieth position out of a total of 70 families, with only two species out of a total of 296 species sampled (Meguro *et al.* 2007).

Another important component of the karstic flora in some areas of Brazil is the presence of locally exclusive species. In the Federal District, karsts are home to several species that do not occur in other localities or phytophysionomic types in the region, constituting an important component of the local diversity and contributing to the general diversity of the region (Bianchetti *et al.* 2005). However, this effect seems limited to areas within the Cerrado domain, where mesophytic forests associated with limestone outcrops present conditions not found in other local phytophysionomies.

Our results of the analysis of the diversity of Orchidaceae associated with karst are in line with previous studies carried out with other plant families in Brazil. According to Bystriakova *et al.* (2019), despite the great floristic diversity found in karst environments, this species richness is not significantly greater than in non-karst environments. Likewise, our results indicate that approximately 97% of the floristic diversity of Orchidaceae found in karsts of Brazil is not limited to karst areas. The species composition of each locality is primarily determined by the regional flora and the phytogeographic domain in which it is located.

Ecological and geographic notes on *H. karstica* sp. nov.

Habenaria karstica sp. nov. is unique among all Neotropical species of the genus for growing on limestone massifs, under full sun, associated with *Dyckia luxor*, which forms large clusters on the massif (Fig. 3). A few other Neotropical species of *Habenaria*, such as *H. cryptophila*, *H. curvilabria*, *H. distans*, *H. glaucophylla*, and *H. yookuaaensis* Mejía-Marín, Espejo, López-Ferr. & R. Jiménez also occur in karst (Warming 1908; Bianchetti *et al.* 2005; Mejía-Marín *et al.* 2017). However, they are rainforest species not exclusive to karsts, and most have a wide distribution. *Habenaria distans*, for example, occurs from Florida, Mexico, and Central America, the West Indies, and the Andes to southern Brazil (Batista *et al.* 2011a). In Southwest Asia, China and New Guinea, several species of *Habenaria* are reported for karsts (Rusea & Yong 2005; Kurzweil 2009; Rusea *et al.* 2009; Zeng *et al.* 2017; Kolanowska *et al.* 2021), a few of which seem to be restricted to these formations, such as *H. malipoensis* Q.Liu & W.L.Zhang (Wen-Liu *et al.* 2017). However, most grow under evergreen or deciduous forests and are not restricted to karsts (Kurzweil 2009), although a few such as *H. vidua* C.S.P.Parish & Rchb.f. and *H. svengsuksae* Schuiteman & P.Bonnet can also grow in crevasses in limestone on exposed rocks or open karst vegetation (Kurzweil 2009; Schuiteman & Bonnet 2014).

Habenaria karstica sp. nov. was collected for the first time only very recently, in 2018, during a floristic survey of the saxicolous vegetation of Lapa do Baú in the Lagoa Santa karst region (Ferreira 2020). An ongoing floristic survey and taxonomic study of the genus *Habenaria* in Brazil, including examining about 9500 samples in about 69 herbaria in Brazil, Europe, and the United States, did not reveal any additional specimens of the species. The Lapa do Baú was one of the sites visited by Eugen Warming in the XIX Century, who lived in Lagoa Santa between 1863 and 1866, and collected intensively in the region. His collections were the basis of *Florula Lagoensis*, an extensive list that recorded 2593 species for the region (Warming 1892, 1908). It was the first intensive and specific floristic inventory for a location in Brazil and the first floristic survey of the Brazilian Cerrado (Moreira & Stehmann 2020). For Orchidaceae, 40 genera and 120 species were recorded, of which 55 were described as new, 39 of which are still accepted. For *Habenaria*, Warming recorded 17 species, of which nine were proposed as new (Reichenbach f. 1881; Warming 1908).

Considering the intensive and continuous sampling carried out by Warming in the region, it is surprising that *H. karstica* sp. nov. was not among the species he reported, as it is a conspicuous plant, up to 62 cm tall, and when fully developed, it is difficult to go unnoticed (Figs 3–4). One possibility is that he visited the Lapa do Baú region at some point when the species was not fully developed or was dormant, as it occurs during the dry season. Another possibility is that the material of the species was lost, as he reported for some of his collections (Warming 1908). In addition to the absence of historical records, another noteworthy observation is the lack of additional records in other floristic inventories of karsts both in the region of LSK and in other areas in the State of Minas Gerais (Pedersoli & Martins 1972; Brandão & Magalhães 1991; Lombardi *et al.* 2005; Meguro *et al.* 2007; Santos *et al.* 2007; Melo 2008; Melo *et al.* 2013). Although the phytophysiognomies and ecological conditions seem similar between limestone outcrops, the study of Ferreira (2020) in the LSK pointed out a low similarity between two nearby massifs. This result indicates a high heterogeneity for limestone outcrops, as observed for other types of vegetation associated with rocky outcrops, such as the campos rupestres (Rapini *et al.* 2008) and

inselbergs (de Paula *et al.* 2020), and may explain the restricted distribution of the new species. Despite this heterogeneity, other limestone outcrops in the LSK have similar vegetation with a predominance of *Dyckia luxor*, so that *H. karstica* is likely to occur at least in some of these.

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