Floral similarity and vegetative divergence in a new species of *Bletia* (Orchidaceae) from Mexico

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Abstract

*Bletia mixtecana*, a new species from Oaxaca, Mexico is described and illustrated. This species is florally similar to *B. parkinsonii* but differs in its aerial thickened stems (‘pseudobulbs’) and several floral attributes. A phylogenetic analysis of nuclear ribosomal ITS DNA sequences indicates that *B. mixtecana* and *B. parkinsonii* are not closely related, suggesting that floral similarity represents either parallelism or shared ancestral (symplesiomorphic) traits. The new species is a strict gypsothile known only from two populations and it qualifies as endangered based on the small number of known populations and individuals, high habitat specificity and the observed loss of plants at one of the two known locations.

Key words: *Bletia mixtecana*, endemism, ITS, gypsophily, Mixtec region, Oaxaca, phylogenetics

Introduction

The Neotropical genus *Bletia* Ruiz & Pavón (1794: 119) consists of about 30 species of terrestrial orchids and attains its greatest diversity in Mexico, where around 25 species have been recorded (Soto et al. 2007). *Bletia* belongs to subtribe Bletiinae Bentham (1881: 287, tribe Epidendreae, subfamily Epidendroideae), which, as currently delimited based on molecular phylogenetic studies, also includes the genera *Basiphyllaea* Schlechter (1921: 77), *Chysis* Lindley (1837: t. 1937) and *Hexalectris* Rafinesque (1825: 4, Sosa et al. 2005, van den Berg et al. 2005, Chase et al. 2015). Monophyly of *Bletia* is supported by molecular and morphological data (Sosa 2007), and the genus is characterized by usually underground, thickened stems (‘corms’) made up of several unequal internodes, plicate, deciduous leaves and relatively showy flowers with an usually three-lobed labellum provided with prominent longitudinal keels, arching column and eight pollinia (Sosa et al. 2005, Sosa 2007).

In the course of botanical exploration conducted recently in the Mixtec region, state of Oaxaca, Mexico, an undescribed species of *Bletia* was discovered which, while having a floral morphology typical for the genus, is vegetatively unusual in possessing aerial, thickened stems (‘pseudobulbs;’ Fig. 1). The peculiar vegetative structure of the new species poses interesting evolutionary questions, such as whether its aerial thickened stems represent a derived specialization or an ancestral trait in the genus. This sort of questions can only be meaningfully explored against a phylogenetic framework; hence, besides describing and illustrating the new species, in this work we assess its phylogenetic relationships analysing nucleotide sequences of the internal transcribed region of nuclear ribosomal DNA (nrITS, Baldwin et al. 1995). This marker has been used previously to assess relationships both within *Bletia* and among the genera of Bletiinae (Sosa 2007), as well as in many other orchid lineages (reviewed in Cameron 2007). We also provide a detailed comparison of the floral morphology of the new species with that of *Bletia parkinsonii* Hooker (1839: t. 3736), to which the new species is florally most similar (Figs. 2–3).
FIGURE 1. A. Tropical deciduous forest around La Cuchara gypsum mine, type locality of *Bletia mixtecana*. B. Plant of *B. mixtecana* growing on a gypsum wall. C. Massive plant on an unstable bank of a ravine, growing alongside *Selaginella* sp. D. Columnar cactus (*Neobuxbaumia* sp.) scrub on a gypsum outcrop at Cerro Jacaba, the second known locale for *B. mixtecana*. E. Plants of *B. mixtecana* and *Selaginella* sp. growing at the base of *Neobuxbaumia* sp. F. Pseudobulb of a plant of *B. mixtecana* at flowering time during the dry season, after shedding the leaves, showing the base of the inflorescence emerging from a lateral node. G. Inflorescence of the type plant (Chávez-Rendón et al. 5598R). Photographers: César Chávez-Rendón (A–E, G); Gerardo A. Salazar (F).
Materials and methods

Morphological observations:—We studied live, pickled and pressed material from the two known populations of the new species (six plants in total). Fresh flowers from one plant each of the new species and of B. parkinsonii were dissected and photographed in vivo under a stereomicroscope (Stemi SV 6, Carl Zeiss Mikroskopie, Jena, Germany) to compare their morphological features. Measurements of vegetative and floral parts of the new species were made on fresh and pickled material, complemented with herbarium specimens; flowers from herbarium material were softened in hot, soapy water prior to measuring.
**Taxa included in the phylogenetic analysis:**—We analysed sequences from 20 accessions of 18 species of *Bletia*, including two accessions each of the new species (hereafter referred to as *Bletia mixtecana* Salazar & Chávez-Rendón; see Taxonomy, later) and of *B. parkinsonii*. Additionally, we included one species each of *Basiphyllaea*, *Chysis* and *Hexalectris*, all belonging in Bletiinae sensu Chase et al. (2015), plus representatives of other subtribes of Epidendreae, namely *Arpophyllum giganteum* Hartweg ex Lindley (1840a: 384) and *Nidema boothii* (Lindley 1838: misc. 52–53) Schlechter (1922: 43), both Laeliinae; *Dilomilis montana* (Swartz 1788: 121) Summerhayes (1961: 253) and *Dracula chimaera* (Reichenbach 1872: 463) Luer (1978: 194), both Pleurothallidinae; *Helleriella guerrerensis* Dressler & Hágsater (1975: 36) and *Isochilus alatus* Schlechter (1912: 360), both Ponerinae. *Coelia triptera* (Smith 1793: t. 14) Don ex von Steudel (1840: 394), a member of Calypsoinae sensu Chase et al. (2015), was used as functional outgroup. Five of the sequences were generated newly for this study and the remaining 25 were downloaded from GenBank. A list of the species analysed, including voucher information for the new sequences and GenBank accessions, is given in Table 1. The aligned matrix was deposited in TreeBase (study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S19302).

### TABLE 1. Taxa analysed, voucher information or reference and GenBank accessions.

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<thead>
<tr>
<th>Taxon</th>
<th>Voucher or reference</th>
<th>ITS Accession</th>
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<tbody>
<tr>
<td><strong>Subtribe Bletiinae</strong></td>
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</tr>
<tr>
<td><em>Basiphyllaea hamiltoniana</em> Ackerman &amp; Whitten</td>
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<tr>
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<tr>
<td><em>Bletia catenulata</em> Ruiz &amp; Pav.</td>
<td>van den Berg et al. (2009)</td>
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<tr>
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<td>Mexico, Salazar et al. 7225 (MEUX)</td>
<td>KX241468</td>
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<tr>
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<td><em>Bletia reflexa</em> Lindl.</td>
<td>Sosa (2007)</td>
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<td><em>Bletia senophylla</em> Schltr.</td>
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<th>Taxon</th>
<th>Voucher or reference</th>
<th>ITS</th>
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<td>van den Berg et al. (2000)</td>
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<td><strong>Subtribe Ponerinae</strong></td>
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<tr>
<td><em>Helleriella guerrerensis</em> Dressler &amp; Hägsater</td>
<td>van den Berg et al. (2009)</td>
<td>AF260142</td>
</tr>
<tr>
<td><em>Isochilus alatus</em> Schltr.</td>
<td>van den Berg et al. (2000)</td>
<td>AY008482</td>
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</table>

**FIGURE 3.** Comparison of some floral attributes of *Bletia mixtecana* and *B. parkinsonii* (in all instances, placed on the left and right, respectively). A–B. Base of labellum; note in A the central channel formed by the thickened veins that turn into keels further up towards the apex. C–D. Midlobe of labellum. E. Column; note the prominent, yellow auricles on the ventral margins of *B. parkinsonii* (right). F. Ventral view of column apex. G. Dorsal view of column apex. H. Ventral view of column apex after dislodging the anther to show the pollinia. I–J. Pollinaria. Photographer: Gerardo A. Salazar.
Molecular data:—DNA extraction, amplification (PCR) and sequencing were carried out using standard methods and primers described in Sosa (2007). Bidirectional sequence reads were assembled and edited with Sequencher (GeneCodes Corp., Ann Arbor, Michigan, USA). Alignment was carried out using the L-INS-I method (Katoh et al. 2005) of the online version of the program MAFFT v. 7 (Katoh & Standley 2013), with minor manual adjustment in Mesquite v. 3.03 (Maddison & Maddison 2011). The individual gap positions were treated as missing data.

Phylogenetic analysis:—We analysed the ITS sequences using two different methods, namely maximum parsimony (MP) and maximum likelihood (ML), which allowed us to compare the results from MP with a method that uses explicit models of nucleotide substitution (ML). The MP analysis was conducted with the programme PAUP* v. 4.0a144 for 32-bit Microsoft Windows (Swofford 2015) and consisted of a heuristic search with 1,000 replicates with random order addition of taxa for the starting trees and TBR branch-swapping, saving in memory all the most-parsimonious trees (MPTs) found. Branch support was assessed by 1,000 bootstrap replicates (Felsenstein 2015), each with 20 heuristic searches with random order of taxa and TBR branch-swapping, saving up to 20 MPTs per search. The ML analysis was carried out using RaxML v. 8 (Stamatakis 2014), implemented at the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v. 3.3 (Miller et al. 2010). One thousand rapid bootstrap replicates (Stamatakis et al. 2008) were followed by a search for the tree that maximizes the likelihood function with the default value of 25 rate categories and using in both instances the GTR+GAMMA model for nucleotides.

Results

Phylogenetic analysis:—The aligned ITS matrix consisted of 713 characters, of which 311 were variable and 179 were potentially parsimony-informative. The MP analysis found six trees with a length of 652 steps, consistency index (CI, excluding uninformative characters) of 0.52 and retention index (RI) of 0.67. One of the six trees, on which the bootstrap percentages from the bootstrap analysis are indicated, is shown in Fig. 4A. The MP analysis recovered a strongly supported clade (bootstrap percentage, BP, 100) that includes all members of Bletiinae sensu Chase et al. (2015) except Chysis bractescens Lindley (1840b: misc. 61). The latter is sister to the remaining Bletiinae, although this relationship did not obtain bootstrap support greater than 50%. In the strict consensus (not shown), ‘core’ Bletiinae (except Chysis) consist of a polytomy formed by Bletia catenulata Ruiz & Pavón (1798: 229), the strongly supported pair Basiphyllaea hamiltoniana Ackerman & Whitten in Ackerman et al. (2001: 13)-Hexalectris revoluta Correll (1941: 19, BP 94) and a weakly supported group comprising all the other species of Bletia (BP 56). The latter in turn includes two main groups, marked as clades 1 and 2 in Fig. 4. Clade 1 is marginally supported (BP 51) and encompasses, on the one hand, a strongly supported subgroup with B. punctata Lexarza (1925: 15) sister to B. parkinsonii (BP 100), and on the other hand a subclade encompassing B. reflexa Lindley (1835: t. 1760) through B. stenophylla Schlechter (1919: 42, BP 79). Clade 2 is also weakly supported (BP 59) and, within it, the two accessions of B. mixtecana form a polytomy with a group that includes the remaining species (BP 69; Fig. 4A).

The ML tree, with the respective bootstrap percentages, is shown in Fig. 4B. Unlike MP, the ML analysis failed to group Chysis with the core Bletiinae. The latter are strongly supported (BP 100) but Bletia is paraphyletic, although the association of Basiphyllaea hamiltoniana-Hexalectris revoluta to Bletia catenulata is only weakly supported (BP 55). The same two major clades (1 and 2) of remaining Bletia recovered in the MP analysis were found by ML. In clade 1, B. punctata is the strongly supported sister of B. parkinsonii (BP 100), whereas in clade 2 the two accessions of B. mixtecana are collective sisters to the rest (BP 77). In contrast with the MP analysis, in which the two accessions of B. mixtecana did not receive support as sisters to one another (BP <50; relationship collapsed in the strict consensus), in the ML analysis the new species was recovered as monophyletic, although with low support (BP 58).

Discussion

The overall relationships among species of Bletia and other genera of Bletiinae have been explored by Sosa (2007) based on separate and combined MP analyses of DNA sequences of the ITS region and plastid trnL-trnF intergenic spacer, and morphological characters. In that study, the ITS matrix included more taxa than other datasets and had more phylogenetically informative characters. A detailed re-examination of Bletiinae relationships is beyond the focus of the present study. Our discussion is mainly focused on establishing the phylogenetic position of B. mixtecana, its relationship to B. parkinsonii as the species to which it is florally most similar (Figs. 3–4), and some implications of the inferred relationships for our understanding of the evolution of floral and vegetative morphology in the genus.
Other than the non-monophyly of *Bletia* implied by the marginally supported nesting of the *Basiphyllaea- Hexalectris* clade in that genus in our ML analysis, our results are similar to those of Sosa (2007) regarding interspecific relationships in *Bletia* (Fig. 4). Both studies recovered two main clades of *Bletia* and differ mainly in the position of *B. purpurata* Richard & Galeotti (1845: 23), for which we generated a new sequence from newly collected, fresh material. Whereas in the study of Sosa (2007) *B. purpurata* was sister to a clade formed by *B. punctata* and *B. parkinsonii*, here it belongs in the other major clade of the genus, particularly in the subgroup that also includes *B. urbana* Dressler (1968: 186), *B. campanulata* Lexarza (1825: 17) and *B. macristhmochila* Greenman (1878: 297). Sosa (2007) did not include *B. roezlii* Reichenbach (1877: 7) in her ITS analysis, but our sequence of this species groups with *B. gracilis* Loddiges (1833: t. 1977) as expected, since they share several morphological attributes (e.g. elliptic leaves tinged with purple on the underside, greenish-yellow flowers with lustrous red veining on the labellum, and prominent, somewhat reflexed anther cap).

**FIGURE 4.** Phylogenetic relationships in *Bletia* inferred by maximum parsimony (MP) and maximum likelihood (ML) analyses of ITS DNA sequences. A. One of the six shortest trees found by the MP analysis; an asterisk (*) indicates that the clade collapses in the strict consensus. B. ML tree. On both trees branch lengths are drawn proportional to the number of character changes; numbers associated to branches are bootstrap percentages.

Both our MP and ML analyses (Fig. 4) indicate that *B. mixtecana* is not particularly closely related to *B. parkinsonii*, in spite of their resemblance in floral morphology (Figs. 2–3). Such resemblance includes the flowers that open widely only in sunny weather, otherwise opening only partially, which gives them a tubular appearance. In other species of *Bletia* flower aperture is constant, i.e. it does not change depending on sunlight intensity. In both *B. mixtecana* and *B. parkinsonii* the labellum is distinctly longer than wide and shallowly three-lobed, lacking deep sinuses that sharply separate the midlobe from the lateral lobes (Fig. 3C–D, 5J). In other species of *Bletia* the labellum is about as wide as long or wider and deeply three-lobed. The columns of *B. parkinsonii* and *B. mixtecana* are similar, but in the former there are prominent, rounded ventral auricles near the middle that are absent in the latter. The main differences between these two species are indicated in Table 2.
Since *B. mixtecana* and *B. parkinsonii* do not share an immediate common ancestor, their floral similarity might represent either a parallelism resulting from adaptation to the same or similar pollinators, or a plesiomorphic set of traits in *Bletia* to the exclusion of *B. catenulata*. If the last scenario holds true, the more or less tubular flower with narrow labellum was modified subsequently in the last common ancestors of the remaining species in both major subclades of *Bletia* (clades 1 and 2 in Fig. 4).

*Bletia mixtecana* and *B. parkinsonii* diverged at or near the base of their respective clades, and beside their floral similarity they share a preference for seasonally dry tropical deciduous forests. Most other species of *Bletia* dwell in more mesic habitats such as pine-oak forests or, in the case of *B. purpurea* Lamark (1791: 515) de Candolle (1841: 97) and *B. stenophylla* Schlechter (1919: 42), savannas, steep hillsides, roadside banks and other locations with open vegetation in areas dominated by warm, moist or wet tropical forests. The ‘basal’ position of *B. mixtecana*, *B. parkinsonii* and *B. punctata* suggests that their seasonally dry tropical habitat may be plesiomorphic in the main clade of *Bletia*, with derived transitions toward mesic pine-oak forests and tropical moist forests and savannas.

Both *B. mixtecana* and *B. parkinsonii* flower during the dry season (mainly December to April), when the leaves are withered or absent altogether. *Bletia punctata*, a widespread Mexican species recovered both in this study and in that of Sosa (2007) as the sister of *B. parkinsonii*, also occurs in seasonally dry, warm areas but more commonly is found in moister pine-oak forests, blooming during the rainy season (June to September), when the leaves are present. The corms of *B. punctata* are more globose and less oblique than those of *B. parkinsonii* (Fig. 2E) and florally the former is very different from the latter, with the sepals and petals standing erect on the upper part of the flower and having a deeply three-lobed labellum. The divergent lateral lobes are separated from the obtrullate midlobe by deep sinuses. Line drawings of *B. parkinsonii* and *B. punctata* useful for comparison can be found in Espejo et al. (2002: 33 and 35, respectively).

*Bletia mixtecana* and *B. parkinsonii* occur sympotically at the type locality of the former. However, *B. mixtecana* grows on exposed chalk along the steep banks of a ravine (Fig. 1B−C), whereas *B. parkinsonii* occurs in flat, rocky areas with open grassland and shallow soil (G. A. Salazar, pers. obs.; cf. Fig. 2D). Both species flower at the same time, as demonstrated by flowering specimens of both species collected on the same day and a few metres apart (*B. mixtecana*: L. A. Hernández & A. Torres 869, MEXU!; *B. parkinsonii*: L. A. Hernández & A. Torres 870, MEXU!). The fact that *Bletia mixtecana* and *B. parkinsonii* coexist and flower simultaneously in at least one locality raises the question on how reproductive isolation is achieved, since these two species are similar in flower size and overall morphology (Fig. 2−3), and in the repetitive, partial opening and closure of the perianth apparently regulated by sunlight intensity. Other than early reports of pollination of *B. purpurea* and *B. catenulata* by various bees, and sparse observations on autogamy, nothing is known on natural pollination of *Bletia* (Dressler 1968; Sosa et al. 2005 and references therein; Salazar 2009). Nectar was absent in the fresh flowers of both *B. mixtecana* and *B. parkinsonii* examined by us, in agreement with previous observations on various other species of *Bletia*, and it is likely that pollination involves some sort of deceit (Salazar 2009). To our knowledge, no individuals with intermediate attributes suggesting hybridization

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>B. mixtecana</em></th>
<th><em>B. parkinsonii</em></th>
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<tbody>
<tr>
<td>Retorse mentum at base of perianth</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td>Adnation of base of labellum to column</td>
<td>marginal</td>
<td>intramarginal (the margins themselves are free)</td>
</tr>
<tr>
<td>Channel on proximal part of labellum</td>
<td>present, formed by the thickened veins that further up become keels</td>
<td>absent</td>
</tr>
<tr>
<td>Colour of labellum keels</td>
<td>deep magenta on their proximal two-thirds, white above</td>
<td>deep yellow on their proximal two-thirds, pale yellow above</td>
</tr>
<tr>
<td>Structure of labellum keels</td>
<td>entire on their proximal two-thirds, irregularly dentate and undulate above; surface smooth</td>
<td>entire on their proximal two-thirds, irregularly dentate-laciniate above; surface warty (more prominently so in front of the stigma)</td>
</tr>
<tr>
<td>Column</td>
<td>ventral surface flat, margins prominent near the middle but devoid of distinct auriciles</td>
<td>ventral surface channelled, each margin provided with a fleshy, yellow, somewhat involute round auricle near the middle</td>
</tr>
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between *B. mixtecana* and *B. parkinsonii* are known, although several instances of natural hybridization have been recorded elsewhere between co-occurring species of *Bletia* (e.g. Salazar 2009, Serguera & Sánchez 2011, Hágsater *et al.* 2005).

No other mainland member of *Bletia* has aerial thickened stems like those of *B. mixtecana*, which represent an autapomorphic, diagnostic attribute of the species. Aerial pseudobulbs are present in *B. patula* Graham (1836: 155), a species restricted to Florida (U.S.A.), Cuba, Haiti, the Dominican Republic and Puerto Rico, but in this case the pseudobulbs are globose to conical, not ovoid (Sosa & Díaz 2014). The pseudobulbs of *B. mixtecana* are reminiscent of those of some members of tribe Cymbidieae, such as certain species of *Acriopsis* Blume (1825: 376), *Cyrtopodium* Brown (1813: 216) and *Eulophia* Brown ex Lindley (1821: 573). At first, such resemblance suggested to us the possibility that it could represent the long-lost *Eulophia filicaulis* Lindley (1842: 184), a species supposedly collected in Mexico by Karwinskii in the first half of the nineteenth century (Lindley 1842), which was never collected again in this country. However, study of the type of *E. filicaulis* and other relevant material at K and M demonstrated that *E. filicaulis* is a genuine species of *Eulophia* and conspecific with *West African Eulophia ramifera* Summerhayes (1958: 50), most likely having been attributed to Mexico by error (Salazar & Cribb 2007).

*Bletia mixtecana* is the only recorded instance of strict edaphic endemism for *Bletia* in the mainland. Eastern Cuban *B. antillana* M.A. Díaz & Sosa in Sosa & Díaz (1997: 81) grows along the margin of streams in acid, humus-rich serpentine soil (Sosa & Díaz 1997), but there is no published information indicating edaphic specialization in other species of this genus. One of the two known populations of *B. mixtecana* occurs at Cerro Jucaba, which is the type (and only locality known so far) of *Galeoglossum cactorum* Salazar & Chávez-Rendón in Salazar *et al.* (2011: 262), another obligate gypsumophilous orchid. As noted above, *B. parkinsonii* co-occurs with *B. mixtecana* at La Cuchara. However, *B. parkinsonii* is one of the most widespread Mexican species of *Bletia* and is not restricted to gypsum, but it grows in a variety of soil conditions, both on volcanic and limestone or gypsum substrates (i.e. it is a ‘gypsovag’; see Palacio *et al.* 2007; Escudero *et al.* 2015). Gypsum soils have stressful physical and chemical properties for plants, including a hard surface crust that could limit plant establishment, mechanical instability, an excess of S, and Ca/Mg imbalances, among others (Parsons 1976; Palacio *et al.* 2007; Escudero *et al.* 2015). The aerial thickened stems of *B. mixtecana* may represent an adaptation to gypsum outcrops in ravines and steep slopes by allowing the plant to grow on the surface, instead of within and across the surface crust (Fig. 1A–E). This hypothesis, as well as the apparent reproductive isolation between co-occurring *B. mixtecana* and *B. parkinsonii* in spite of their striking floral similarity, are but two of the many aspects of these peculiar plants requiring further research.

**Taxonomy**

*Bletia mixtecana* Salazar & Chávez-Rendón, *sp. nov.*

Vegetatively it differs from all other species of *Bletia* in its ovoid pseudobulbs and its restriction to gypsum outcrops along ravines and steep slopes. Floraually it is similar to *B. parkinsonii*, but differs from the latter in the absence of a distinct retrorse mentum at the base of the floral tube; labellum marginally adnate to the column at base; proximal portion of the labellum provided with a central channel formed by prominent, thickened veins; labellum disc provided with 5–7 prominent, entire keels, these coloured deep magenta on their proximal two-thirds, irregularly dentate, undulate and cream-white above; column devoid of distinct ventral auricles near the middle.

**Type:**—MEXICO. Oaxaca: distrito Huajuapan, municipio Santo Domingo Tonalá, La Cuchara, 1538 m elev., collected 4 September 2002, pressed in cultivation 8 April 2006, Chávez-Rendón et al. **5598R** (holotype MEXU, isotypes IEB!, SERO!).

Terrestrial herb, 15–32 cm high including the inflorescence. Roots simple, terete, flexuous, dull white to pale brown, glabrous to pubescent-laniginose, 1.5–2.0 mm in diameter. Pseudobulbs epigean, ovoid, slightly compressed laterally, consisting of 6–7 internodes, when young completely concealed by sheaths, when old naked, green with reddish-brown nodes, smooth, longitudinally sulcate, 3.4–6 cm long, 1.0–2.3 cm in diameter. Leaves deciduous, shedding at maturity of the pseudobulb, absent at flowering time, when present 7–8, progressively larger toward the apex of the pseudobulb, the imbricating sheaths of the 4–5 distalmost leaves forming a pseudostem up to 3.5 cm long; leaf blades plicate, green, articulate to the sheaths that conceal the pseudobulb, linear to ensiform or the lowermost one sometimes lanceolate, long- acuminate at apex, truncate and channelled at base, 9-veined, 4–24 cm long, 0.8–1.4 cm wide; sheaths conspicuous, concealing completely the newly developed pseudobulb, tight, papery-fibrous, whitish,
eventually disintegrating (after about ca. 4 years), up to 4 cm long and 2 cm wide. Inflorescence racemose, 1–2 per pseudobulb, up to 23 cm long, arising from the nodes on the distal two-thirds of the mature pseudobulb, erect; peduncle slender, terete, rigid, ca. 11 cm long and 0.9 mm in diameter, with 4–5 internodes and an equal number of tight to loose, scarious bracts up to 7 mm long; raceme lax, ca. 10 cm long, rachis glabrous, 0.25–0.8 mm in diameter, slightly flexuous, producing 5–10 flowers in succession. Floral bracts thin, scarious, glabrous, covering less than one-fifth to one-fourth of the ovary, triangular-ovate or ovate, sometimes that of the lowermost flower tubular, apex acute or obtuse, apiculate, apiculate, 5–12-veined, (0.6–)2.4–4.4 mm long, 1–2 mm wide. Ovary glabrous, ascending, slightly arcuate, suberete, 6-sulcate on the distal two-thirds, gently tapering and obscurely twisting towards the base, pale green to purplish-red with three green ribs, 10.0–16.5 mm long, ca. 1 mm in diameter near the apex. Flowers resupinate, widely open in warm, sunny weather but the sepal and petals barely opening in cloudy weather; floral segments inconspicuously connate at base for up to 0.5 mm, bearing sparse, minute indoblasts apparently containing crystals. Sepals completely magenta or externally white with magenta suffusion on margin and internally magenta except for the white base, bearing at base 5 main veins that branch above; lateral sepal free except as noted above, variably spreading, obliquely oblong-ob lanceolate, slightly recurved near the acute apex, obscurely keeled dorsally, 15.5–20.0 mm long, 4.0–4.5 mm wide; dorsal sepal ob lanceolate, recurved, 15.0–19.3 mm long, 2.9–4.1 mm wide. Petals homogeneously magenta, free or fused basally to the label lum for 0.7–2.0 mm (degree of adnation variable even among flowers of the same inflorescence), bearing at the base 3 main veins that branch above, obliquely ob lanceolate or ob lanceolate-spathulate, minutely crenate-lacerate and slightly undulate on the distal one-fourth to one-third, acute to obtuse, 15–20 mm long, 2.9–5.0 mm wide. Labellum white on the proximal half with the lateral lobes and the margins of midlobe magenta, the centre of the midlobe white; base adnate to the column for 1.8–2.4 mm, otherwise free, obscurely three-lobed above the middle, in natural position with the lateral margins below the lateral lobes embracing the column, 16.8–19.5 mm total length, 9.4–9.5 mm wide across the lateral lobes when spread out; lateral lobes semiorbicular, somewhat divergent, rounded, erose and slightly undulate, protruding 1–2 mm at apex; midlobe obcordate to cuneate-obcordate, 5.0–6.5 mm long, 5.8–6.7 mm wide, deeply emarginate at apex, the resulting lobules obliquely ovate-flabellate, ruffled, margins erose and undulate, apex shortly apiculate (sometimes the apicule inconspicuous); base bearing 5 main veins that branch profusely above (the three central veins nearly reach the apex of the midlobe); disc provided with 5–7 prominent keels, these entire and deep magenta on their proximal two-thirds, irregularly dentate, undulate and cream-white above, sometimes a few more lateral veins slightly thickened but not distinctly keeled, base of the labellum channelled inside the short tube resulting from its partial adnation to the column, the channel formed by the basal portions of the keels that run at each side of the central one (the last one does not reach the base); no nectar is produced. Column elongate, slightly arching, white near the base and magenta above, ventrally flat with thin, slightly prominent margins, more noticeably so below the middle, 10.8–11.5 mm long including the short, inconspicuous column foot, 2 mm wide near the apex; anther incumbent, semi-globose, versatile, with slightly flat with thin, slightly prominent margins of midlobe magenta, the centre of the midlobe white; base adnate to the column for 1.8–2.4 mm, otherwise free, obscurely three-lobed above the middle, in natural position with the lateral margins below the lateral lobes embracing the column, 16.8–19.5 mm total length, 9.4–9.5 mm wide across the lateral lobes when spread out; lateral lobes semiorbicular, somewhat divergent, rounded, erose and slightly undulate, protruding 1–2 mm at apex; midlobe obcordate to cuneate-obcordate, 5.0–6.5 mm long, 5.8–6.7 mm wide, deeply emarginate at apex, the resulting lobules obliquely ovate-flabellate, ruffled, margins erose and undulate, apex shortly apiculate (sometimes the apicule inconspicuous); base bearing 5 main veins that branch profusely above (the three central veins nearly reach the apex of the midlobe); disc provided with 5–7 prominent keels, these entire and deep magenta on their proximal two-thirds, irregularly dentate, undulate and cream-white above, sometimes a few more lateral veins slightly thickened but not distinctly keeled, base of the labellum channelled inside the short tube resulting from its partial adnation to the column, the channel formed by the basal portions of the keels that run at each side of the central one (the last one does not reach the base); no nectar is produced. Column elongate, slightly arching, white near the base and magenta above, ventrally flat with thin, slightly prominent margins, more noticeably so below the middle, 10.8–11.5 mm long including the short, inconspicuous column foot, 2 mm wide near the apex; anther incumbent, semi-globose, versatile, with slightly prominent apical margin, 8-celled, ca. 1.5 mm long and wide; pollinia 8, yellow, laterally compressed, with granulose caudicles; rostellum laminar, semiorbicular, reflexed, provided on its adaxial surface (facing the stigma) with a lunate prominent apical margin, 8-celled, ca. 1.5 mm long and wide; pollinia 8, yellow, laterally compressed, with granulose caudicles; rostellum laminar, semiorbicular, reflexed, provided on its adaxial surface (facing the stigma) with a lunate viscarium. Stigma semiorbicular, concave, with margins slightly raised, covered by a shiny, transparent fluid in fresh condition. Capsules not seen.

**Distribution and ecology:**—*Bletia mixtecana* is known only from two populations distant from one another c. 76 air kilometres, both within the Mixtec region in the Sierra Madre del Sur, state of Oaxaca. The plants live on gypsum in banks along ravines and on steep slopes. At the type locality (La Cuchara; Fig. 1A–C) the dominant vegetation is tropical deciduous forest with *Acacia angustissima* (Miller 1768: *Mimosa* no. 19) Britton & Rose (1928: 100), *Agave aff. angustifolia* Haworth (1812: 72), *Brahea dulcis* (Kunth 1816: 300) Martius (1838: 244), *Bursera mirandae* Toledo (1984: 441), *Euphorbia aff. rossiana* Pax (1910: 162), *Fouquieria ochoterenae* Miranda (1942: 458), *Neobuxbaumia mezcalaensis* (Bravo 1932: 378) Backeberg (1941: 3), *Opuntia* Tournefort ex Miller (1754: *Opuntia*) sp., *Plumeria rubra* Linnaeus (1753a: 209), and the orchids *Bletia parkinsonii*, *Cypripedium calceolus* (Laxarza 1825: 42) Romero-González & Carnevali (1999: 331), *Dichromanthus cinnabarinus* (Laxarza 1825: 3) Garay (1982: 314) subsp. *galeottianus* (Schlechter 1918: 432) Soto Arenas & Salazar (in Hágsater & Soto Arenas 2003: xii) and *Ponthieva angustiptetala* Greenwood (1986: 9). At the other known locality (Cerro Jacaba; Fig. 1D–E) the steep gypsum slope is covered by a tall scrub of *Neobuxbaumia* Backeberg (1938: 20) sp. with sparse individuals of *Brahea dulcis*, *Hechta* Klotsch (1835: 401) sp. and *Agave Linnaeus* (1753a: 323) sp., with a sparse herbaceous stratum including *Selaginella Palisot de Beauvois* (1805: 101) sp., *Begonia Linnaeus* (1753b: 1056) sp., *Sedum Linnaeus* (1753a: 430) sp., *Pinguicula Linnaeus* (1753a: 17) sp., some grasses and xeromorphic ferns and two further orchid species, *Galeoglossum cactorum* and an unidentified species of *Malaxis* Solander ex Swartz (1788: 119).
**Conservation status:**—From the small number of known populations (two), the small area that they occupy (less than 1 km$^2$), the small number of individuals known (a few dozen) and its habitat specificity, this species qualifies as ‘Endangered’ according to the ‘MER-Plantas’ (the method for assessing the risk of extinction for plants established by the relevant Mexican legal norm; SEMARNAT 2010). Likewise, based on IUCN’s red list categories and criteria (IUCN 2012), B. mixtecana qualifies as ‘Endangered’ because of its area of occupancy estimated to be <500 km$^2$, less than five known locations and an observed decline in the number of mature individuals, at least at La Cuchara (criteria B2, a(iii)). Nevertheless, the two known populations of B. mixtecana occur on a rough, botanically little-explored area with seemingly extensive spans of potential habitat. It is likely that further exploration will lead to the discovery of additional populations, and its conservation status should be then revised accordingly.

**Etymology:**—The specific epithet refers to the Mixtec region of Oaxaca, an area to which the new species seems to be restricted.

**Additional specimens examined:**—MEXICO. Oaxaca: distrito Huajuapan, municipio Santo Domingo Tonalá, paraje Cerro de la Cuchara-Barranca, 1,383 m, 13 January 2009, Hernández & Torres 869 (MEXU!, SERO!); municipio Santo Domingo Tonalá, La Cuchara, 1,518 m, 10 February 2009, Torres & Hernández 910 (MEXU!); distrito Tlaxiaco, municipio San Bartolomé Yucuña, Cerro Jacaba, 1708 m elev., collected 15 August 2008, pressed in cultivation 17 March 2015, Chávez-Rendón et al. 7421C (MEXU!).

**Acknowledgements**
The authors thank the late Miguel Ángel Soto Arenas for discussions on this species; Jerónimo Reyes for plants and photographs of B. parkinsonii; Lidia I. Cabrera and Laura Márquez Valdelamar for assistance with DNA sequencing; the Curators of AMES, AMO, CHAPA, ENCB, K, MEXU, M, MO, NY, SERO, US and XAL for facilitating study of the collections in their charge; and Angel Vale and Cásio van den Berg for constructive criticisms to an earlier version of the manuscript. C.C.-R. and A.de Á.B. thank the authorities of Santo Domingo Tonalá and San Bartolomé Yucuña for permission for botanical collecting. This work was partially supported by an infrastructure grant from CONACYT (project 224743).

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