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ESTUDO TAXONÔMICO E ANÁLISE CLADÍSTICA DO COMPLEXO *BIFRENARIA* LINDL. (MAXILLARIEAE, ORCHIDACEAE)

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RESUMO

O complexo *Bifrenaria* constitui os gêneros sul-americanos *Adipe* (=*Stenocoryne*), *Bifrenaria* (sensu stricto), *Cydoniorchis* e *Rudolfiella,* e pode ser caracterizado pela presença de pseudobulbos tetrangulares e unifolados, folhas plicadas, e por flores com um esporão preminente, com um estipe bifurcado. Os objetivos deste estudo foram investigar o monofiletismo do complexo *Bifrenaria* assim como dos gêneros *Adipe* (=*Stenocoryne*), *Bifrenaria*, *Cydoniorchis* e *Rudolfiella,* com base em caracteres morfológicos e macromoleculares; determinar as relações filogenéticas do complexo *Bifrenaria*; e reavaliar carateres morfológicos tradicionalmente utilizados para delimitar taxa. Dezesseis espécies referentes ao complexo *Bifrenaria* e seis gêneros relacionados foram amostrados. As matrizes foram analisadas sob o critério de parcimônia. As análises dos conjuntos de caracteres em separado resultaram em árvores com topologias ligeiramente distintas e, por este motivos, nós combinamos os diferentes conjuntos de dados. Os resultados suportam o monofiletismo do complexo *Bifrenaria*, excluindo-se *Rudolfiella*, que constitui um gênero distinto e monofilético. Nós consideramos *Bifrenaria* como um gênero monofilético englobando os gêneros *Adipe* (=*Stenocoryne*), *Cydoniorchis* e *Bifrenaria (*sensu stricto). Dentro de *Bifrenaria,* seus dois clados basais são altamente suportados: o clado referente às espécies amazônicas e o clado referentes às espécies do sul-sudeste brasileiro. O gênero *Adipe* é parafilético. Apesar do gênero *Cydoniorchis* ser monofilético e apresentar várias sinapomorfias morfológicas, seu reconhecimento demanda muitas mudanças nomenclaturais e, por isso é proposto o reconhecimento amplo do gênero *Bifrenaria.* Os resultados também sugerem a origem amazônica do gênero *Bifrenaria.* A segunda parte deste estudo apresenta o estudo taxonômico do gênero *Bifrenaria.* Os principais objetivos foram apresentar uma sinopse taxonômica para o gênero *Bifrenaria*, determinando quais taxa devem ser reconhecidos, as distribuições e quais caracteres tradicionalmenete utilizados para reconhecer espécies são taxonomicamente informativos. São apresentadas chaves de identificação, descrições, mapas de distribuição e ilustrações.

ABSTRACT

The *Bifrenaria* complex constitutes the South American genera *Adipe* (=*Stenocoryne*), *Bifrenaria* (sensu stricto), *Cydoniorchis* and *Rudolfiella,* which are characterized by four-angled, unifoliate pseudobulbs, plicate leaves, and by flowers bearing a conspicuous spur and a forked stipe. The aims of the present study were to investigate the monophyly of the *Bifrenaria* complex as well as the genera *Adipe* (=*Stenocoryne*), *Bifrenaria*, *Cydoniorchis* and *Rudolfiella,* based on morphology and DNA sequence data; to determine phylogenetic relationships within the *Bifrenaria* complex; and to reevaluate morphological characters traditionally used to delimit taxa. Sixteen species from the *Bifrenaria* complex and six related genera were sampled. Matrices were analysed using maximum parsimony. Separate analyses of data partitions resulted in slightly different topologies, therefore we combined all the data sets. The results support the monophyly of the *Bifrenaria* complex, excluding *Rudolfiella*, which constitutes a monophyletic and distinct genus. Therefore we consider *Bifrenaria* a monophyletic genus comprising *Adipe* (=*Stenocoryne*), *Cydoniorchis* and *Bifrenaria (*sensu stricto). Within *Bifrenaria,* two clades are strongly supported: the Amazonian species clade and the southern Brazilian clade. The genus *Adipe* is a paraphyletic grade. Although the genus *Cydoniorchis* is monophyletic and presents many morphological synapomorphies, the acceptance of this genus would demand many nomenclatural changes. The results also suggest an Amazonian origin for *Bifrenaria.* In the second part of the study a taxonomic study for the genus *Bifrenaria* is presented. The main goals of this study were to present a taxonomic synopsis for the genus *Bifrenaria*, determining which taxa should be recognized, their distributions and which characters traditionally used to discriminate species are taxonomically informative. Identification keys, descriptions, distribution maps, and illustrations of the species are also provided.

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APRESENTAÇÃO

A família Orchidaceae constitui a maior família das monocotiledôneas, com cerca de 700 gêneros e 19.500 espécies, em sua maioria epífitas ocorrentes nos trópicos (Dressler 1993). As orquídeas apresentam micorrizas e raízes dotadas de velame, um tecido epidérmico, geralmente multisseriado, com função de proteção mecânica, redução de perda de água, e absorção de água e nutrientes. O caule é geralmente rizomatoso e, em orquídeas simpodiais, pode desenvolver pseudobulbos, estruturas espessadas que armazenam basicamente água (Pridgeon et al. 1999). As flores são geralmente zigomorfas, bissexuadas e ressupinadas, ou seja, apresentam o labelo em posição inferior em relação à coluna (Fig. 1). O perianto é diferenciado em três sépalas petalóides, unidas ou livres entre si, e em três pétalas, sendo duas geralmente semelhantes entre si e às sépalas (Fig. 1). Muitas espécies de orquídeas, incluindo as do gênero *Bifrenaria*, apresentam um esporão formado pelo prolongamento das sépalas laterais (Fig. 1). A pétala mediana, referida como labelo, é geralmente bastante diferenciada e estruturalmente complexa, podendo apresentar protuberâncias com as mais diversas formas, usualmente denominadas de calo (Fig. 1). A parte central da flor é caracterizada pela redução do número de partes florais e pela fusão dos órgãos femininos e masculinos em uma estrutura denominada coluna (Fig. 1). Em muitas orquídeas a base da coluna apresenta uma extensão ventral, denominada pé da coluna, onde o labelo se fixa (Fig. 1). O androceu é, em geral, composto por um estame fértil, sendo os outros dois estéreis ou ausentes. Na maioria das espécies da subtribo Epidendroideae, a antera, ereta quando ainda em botão, curva-se gradativamente para baixo ao longo do seu desenvolvimento, situando-se como um capuz, no ápice da coluna (Fig. 1). Este tipo de antera é denominada incumbente ou operculada. Os grãos de pólen são geralmente agrupados em polínias (Fig. 1), massas compactas de grãos de pólen, cuja consistência varia de fina e delicada a endurecida. Na maioria dos casos, o número e forma das polínias reflete a forma e as divisões da própria antera. As polínias são transferidas para outras flores com auxílio de estruturas acessórias, que se originam no estigma. O conjunto de todas as estruturas envolvidas na transferência de pólen é denominado polinário. Além das polínias, o polínario pode apresentar um viscídio (Fig. 1), uma região

de adesão secretada pelo rostelo (região estéril do lobo mediano do estigma que separa a antera da região fértil do estigma, ver Fig. 1), e estipes, região do rostelo alongada e contínua ao viscídio, porém sem função de aderência (Fig. 1). Polínias extremamente compactas e endurecidas freqüentemente apresentam caudículos, que são extensões produzidas pela antera, que permitem a fixação das polínias às estruturas acessórias do polinário. Os carpelos são três, conados, apresentando estigma trilobado, sendo muitas vezes parte do lobo mediano, ou rostelo, estéril e muito maior do que os lobos laterais (Fig. 1). O ovário é ínfero, unilocular, de placentação geralmente parietal com numerosos óvulos. Em vários grupos de espécies, como em *Bifrenaria*, é difícil a distinção entre o pedicelo e o ovário, e geralmente os óvulos completam seu desenvovimento somente após a ocorrência da polinização . O fruto é, em geral, do tipo cápsula com (1-) 3 a 6 aberturas longitudinais apresentando sementes numerosas, muito pequenas (10μm a 5 mm de comprimento), sem fitomelanina. A semente contém um embrião muito reduzido e, em geral, nenhum endosperma (Dressler 1993, Judd et al. 1999, Pridgeon et al. 1999).

Devido ao grande número de espécies e à ampla distribuição geográfica da família, muitos aspectos da morfologia e evolução das orquídeas ainda precisam ser compreendidos (Dressler 1993, Pridgeon et al. 1999). A escassez de conhecimentos desta natureza demanda, conseqüentemente, a elaboração de revisões taxonômicas e estudos filogenéticos em gêneros e níveis hierárquicos superiores.

O gênero *Bifrenaria* (tribo Maxillariae, subtribo Lycastinae) constituí-se de cerca de 20 espécies de distribuição restrita à América do Sul. A grande maioria das espécies ocorre apenas nas regiões sul e, principalmente, sudeste do Brasil, sendo as três espécies restantes restritas à região amazônica. São plantas epifíticas, raro rupícolas ou terrestres, de crescimento simpodial, apresentando pseudobulbos monófilos, de internó único, e formato tetrangular, cônico a comprimido. As folhas são plicadas e as inflorescências apresentam de uma a muitas flores. As flores são ressupinadas com grande variação de tamanho, forma, coloração e odor. As sépalas laterais são unidas ao pé da coluna, constituindo um esporão. A coluna é alongada, prolongando-se no pé. A antera é operculada, com 4 polínias superpostas. O polinário apresenta dois estipes, raramente apenas um, em geral pouco desenvolvidos e com viscídio proeminente.

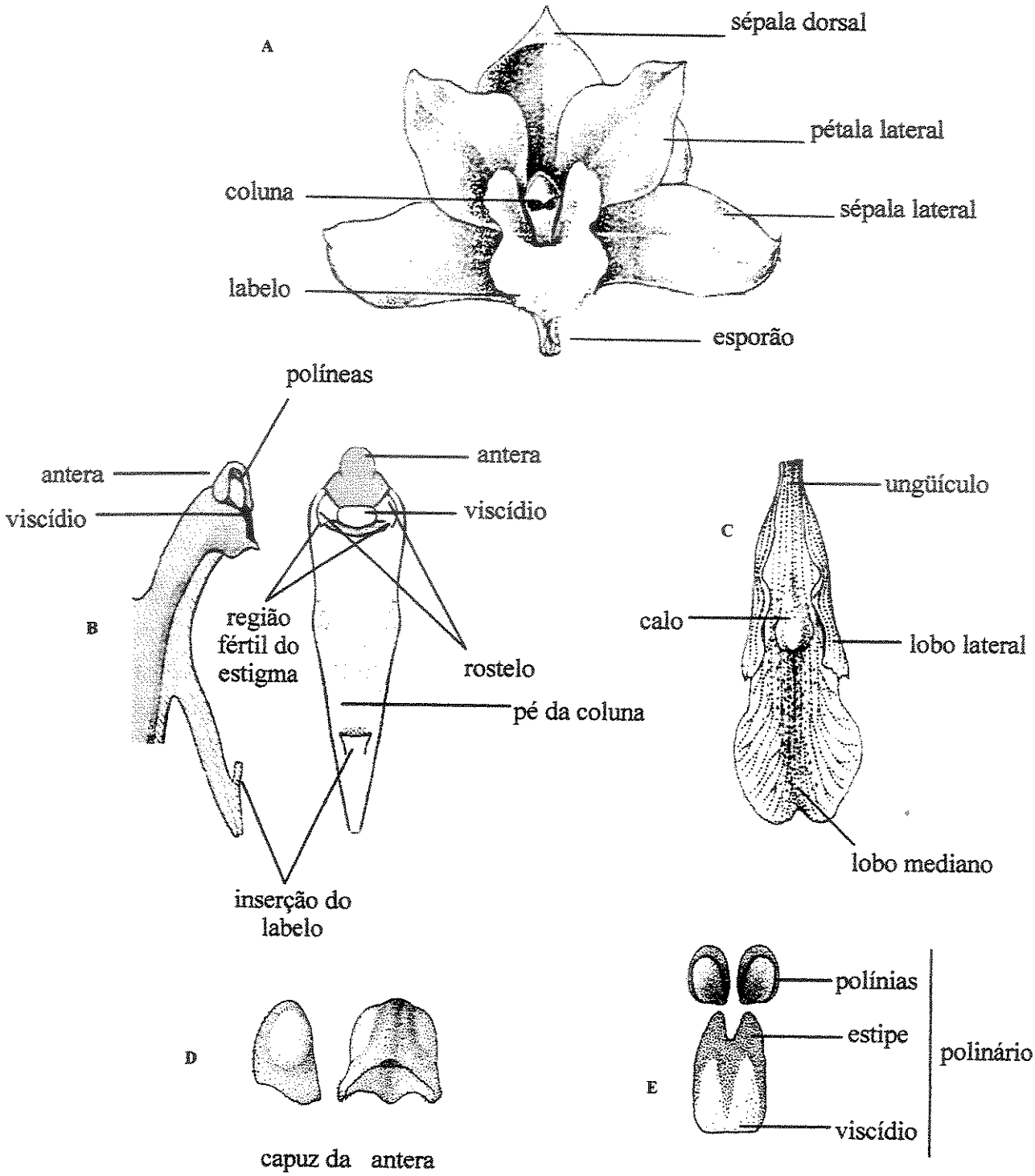

Fig. 1. Estruturas reprodutivas de *Bifrenaria* [(A) modificado a partir de Hoehne (1953); (B) modificado a partir de Reichbach (1896); (C-E) desenhos originais de E. Kickhöfel].

O complexo *Bifrenaria* apresenta um histórico taxonômico de clara instabilidade e incerteza quanto a sua delimitação genérica. Além do próprio gênero *Bifrenaria*, três outros gêneros, *Adipe* Raf. (=*Stenocoryne* Lindl.), *Cydoniorchis* K.Senghas e *Rudolfiella* Hoehne foram propostos para incluir espécies anteriormente consideradas em *Bifrenaria*. Várias classificações foram propostas, mas nenhuma considerou a aplicação de análises filogenéticas para compreensão da história evolutiva deste grupo e para delimitação de grupos monofiléticos. Além disso, as classificações existentes baseiam-se fortemente em caracteres reprodutivos, que por sua vez estão intrinsicamente ligados à polinização, mas não necessariamente refletem as relações de parentesco entre espécies e grupos de espécies (Benzing 1986).

SISTEMÁTICA FILOGENÉTICA

A sistemática filogenética está baseada em métodos para elaboração de hipóteses sobre as relações de parentesco entre espécies e grupos de espécies, estando apoiada na teoria geral da evolução (Hennig 1966). A abordagem filogenética da sistemática implica no reconhecimento de grupos monofiléticos e permite, desta forma, a compreensão de uma variedade de fenômenos evolutivos em um contexto histórico, tais como processos de adaptação e especiação, diversificação e especialização ecológica, coevolução e biogeografia (Brooks and Mclennan 1991).

Os avanços teóricos e metodológicos da sistemática filogenética, a disponibilidade de programas de computador que podem lidar com grande número de dados e o acesso a novas fontes de informação, como dados moleculares, vêm contribuindo, cada vez mais, para a compreensão das relações de parentesco entre organismos, permitindo o desenvolvimento de hipóteses filogenéticas mais robustas (Donoghue and Sanderson 1992).

Caracteres moleculares são vantajosos pois, geralmente, podem ser determinados objetivamente, são abundantes e podem ser extraídos muitas vezes a partir de pequenas porções de material biológico. Não obstante, a detecção de relações de homologia em dados moleculares é problemática e as dificuldades podem ser ainda maiores do que as encontradas com dados morfológicos, sobretudo devido a possibilidade de ocorrer transferência horizontal e conversão gênica (Meyer 1997). Assim, apesar das vantagens da utilização de dados moleculares, elas não justificam a exclusão de caracteres morfológicos

no estudo de relações de parentesco entre espécies (Donoghue and Sanderson 1992). Além disso, pouco vale uma árvore filogenética construída com caracteres moleculares se não há caracteres morfológicos claros e bem definidos que caracterizem os clados.

Espaçadores internos de transcrição do DNA ribossomal nuclear

A região de espaçamento interno (ITS) do DNA nuclear ribossomal (nrDNA) tem sido amplamente utilizada para compreensão das relações de parentesco de plantas, geralmente ao nível genérico ou infragenérico (*e.g.* Hsiao et al. 1994; Sang et al. 1995; Ryan et al. 2000). Esta região inclui a unidade 5.8S, altamente conservada, e dois espaçadores denominados ITS-1 e ITS-2 (Fig. 2a), que são parte integrante da unidade de transcrição do nrDNA mas não são incorporados aos ribossomos maduros (Baldwin et al. 1995). Estudos preliminares indicaram a conservação intraespecífica do comprimento das sequências de ITS-1 e ITS-2 e alta variabilidade de seus nucleotídeos, sugerindo a aplicação do seqüenciamento destes fragmentos em estudos comparativos em baixos níveis taxonômicos (Baldwin et al. 1995). Além disso a família de genes de nrDNA, em que os espaçadores ITS-1 e ITS-2 estão incluídos, apresenta diversas cópias por todo genoma nuclear vegetal, permitindo a fácil detecção, amplificação, clonagem e seqüenciamento dos fragmentos desejados (Hills and Dixon 1991; Hamby and Zimmer 1992; Baldwin et al. 1995). Este grupo de genes sofre rápida evolução combinada (*sensu* Arnheim et al. 1983) através de crossing-over desigual e conversão gênica, promovendo uniformidade das unidades repetidas. Além disso, o pequeno tamanho destes espaçadores e suas posições adjacentes a porções extremamente conservadas do genoma permitem a fácil amplificação destas seqüências através da elaboração de *primers* universais, conforme proposto por White et al. (1990).

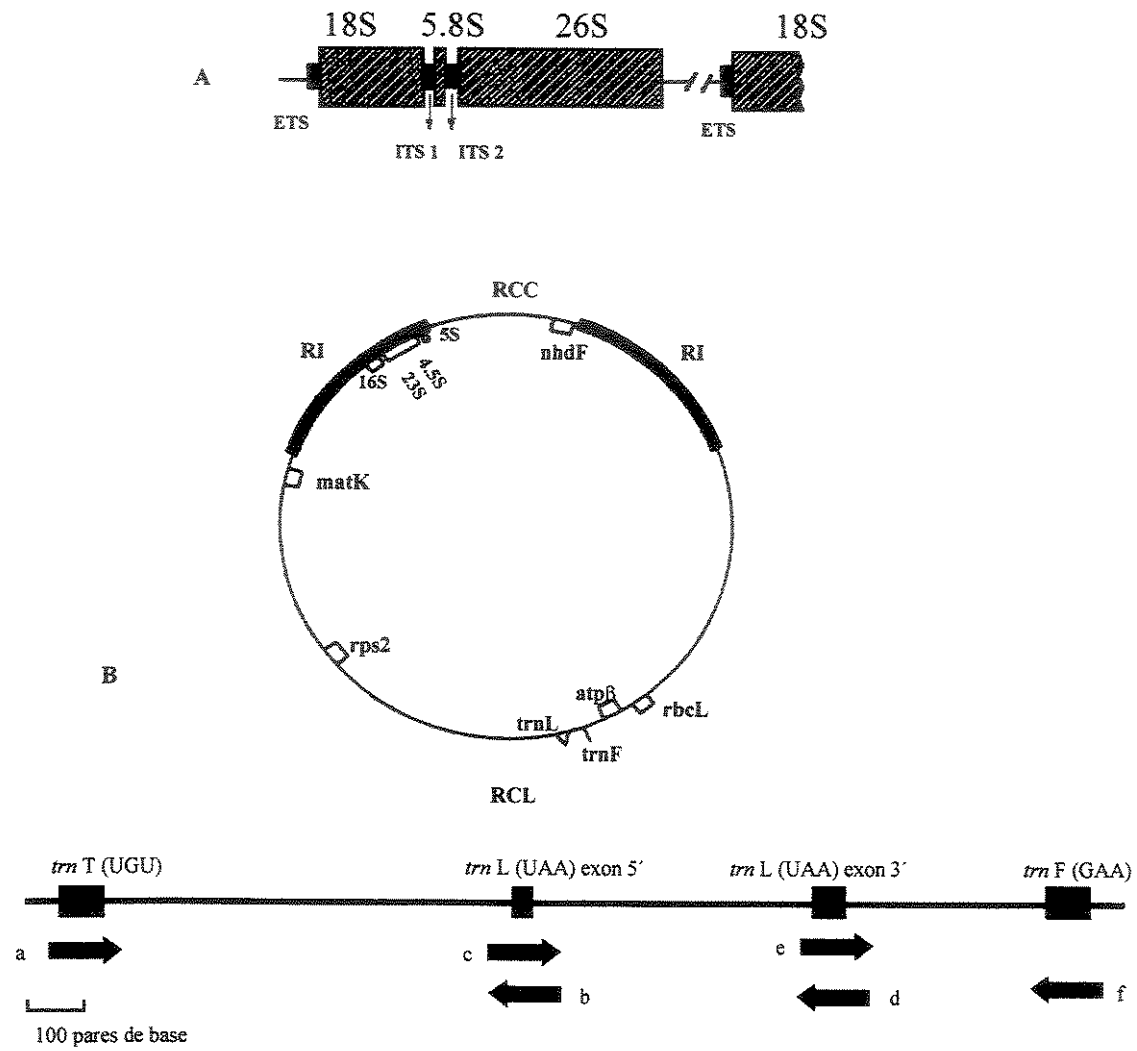

Fig. 2. (A) Unidade típica de repetição do DNA ribossomal nuclear vegetal, em escala. Espaçador de transcrição externo (ETS); espaçadores de transcrição internos ITS-1 e ITS-2; a região hachurada corresponde às unidades de codificação [modificado a partir de Donoghue & Sanderson (1992)]. (B) Genoma de cloroplasto com a localização de várias regiões utilizadas na sistemática molecular de plantas. No detalhe regiões não-codificadoras do gene *trn* com as posições e direções dos *primers* disponíveis para amplificação. A extremidade das setas indica direção 3´dos *primers*. RI (repetição inversa); RCC (região curta de cópia única); RCL (região longa de cópia única) [modificado a partir de Taberlet et al. (1991) e Soltis e Soltis (1998)].

Apesar das limitações impostas pela interferência da estrutura secundária de nrDNA na amplificação de seqüências e do fenômeno da evolução combinada, dados obtidos a partir de nrDNA têm se mostrado informativos para estudos filogenéticos. permitindo muitas vezes a elaboração de hipóteses de relações de parentesco entre espécies. (Baldwin et al. 1995).

Regiões não-codificadoras dos genes *trn* (*tranfer* RNA) do genoma de cloroplasto

Diferentes regiões do DNA de cloroplasto têm sido intensamente utilizadas para investigação de relações filogenéticas em plantas (Palmer et al. 1988), embora a relativa baixa taxa de mutação em muitas regiões seja uma séria limitação no estudo de relação de parentesco ao nível interespecífico. Entretanto, as regiões não-codificadoras do cloroplasto apresentam maior freqüência de mutações (Palmer et al. 1988) e, por isso, têm sido utilizadas em estudos envolvendo táxons também em baixos níveis hierárquicos de classificação.

Um DNA genômico de cloroplasto típico constitui uma molécula circular, caracterizada por dois segmentos repetidos e inversos entre si, que separam duas regiões únicas, uma mais longa e outra mais curta (Fig. 2b). As vantagens de se estudar o genoma de cloroplastos incluem seu tamanho reduzido (geralmente entre 120 e 200 kb) e o fato de que a maioria dos genes de cloroplasto são cópias únicas, ao contrário do ITS e outros genes nucleares que pertencem a famílias multigênicas, conforme explicado no item anterior. Genomas de cloroplasto também são úteis para compreender processos evlutivos ao nível populacional. Entretanto, devido a sua baixa taxa de mutação, sua aplicabilidade em estudos populacionais é, ainda hoje, bem mais restrita em relação aos genomas mitocondriais. Além disso, genomas de cloroplasto são mais suscetíveis aos efeitos de eventos de introgressão, ou transferência de genoma de outras espécies através de hibridização, devido ao seu reduzido tamanho em relação ao genoma nuclear A transferência de DNA entre espécies, quando não detectada, pode causar estimativas errôneas de relações filogenéticas, embora possam ser muito informativas para compreensão de processos evolutivos (Soltis and Soltis 1998).

O fragmento *trn*L-*trn*F constitui uma região não-codificadora do genoma de cloroplasto, que inclui o íntron *trn*L (UAA), com aproximadamente 350-600 pares de

bases, e o espaçador intergênico localizado entre o éxon *trn*L (UAA) 3´e o gene *trn*F (GAA), apresentando entre 120-350 pares de base, conforme ilustrado na Fig. 2b (Taberlet et al. 1991; Soltis and Soltis 1998). Vários estudos já demonstraram a utilidade desta região em estudos filogenéticos considerando gêneros e espécies (Gielly and Taberlet 1996; Baker et al. 1999; Whitten et al. 2000).

OBJETIVOS

O presente estudo está dividido em duas partes. O primeiro capítulo apresenta o estudo filogenético de *Bifrenaria* s.l., desenvolvido a partir de análises cladísticas que utilizaram caracteres morfológicos e moleculares. A partir da investigação das relações de parentesco entre espécies e grupos de espécies do complexo *Bifrenaria,* foi possível definir o gênero *Bifrenaria* e propor delimitações intergenéricas. Assim sendo, o segundo capítulo constitui o estudo taxonômico do gênero *Bifrenaria*, onde são apresentadas descrições sobre anatomia e morfologia das espécies estudadas, uma chave de identificação para as espécies de *Bifrenaria*, descrições taxonômica, mapas de distribuição, fotos e ilustrações das espécies estudadas.

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CAPÍTULO 1

PHYLOGENY OF THE *BIFRENARIA* LINDL. (ORCHIDACEAE) COMPLEX BASED ON MORPHOLOGY AND SEQUENCE DATA FROM NUCLEAR RDNA INTERNAL TRANSCRIBED SPACERS (ITS) AND CHLOROPLAST TRNL-TRN-F REGION

ABSTRACT

The aims of this study were to investigate the monophyly of the genera *Adipe* (=*Stenocoryne*), *Bifrenaria*, *Cydoniorchis* and *Rudolfiella*, based on morphology and DNA sequence data; to determine phylogenetic relationships within the *Bifrenaria* complex; and to reevaluate morphological characters traditionally used to delimit taxa. Sixteen species from the *Bifrenaria* complex and six related genera were sampled. Matrices were analysed using maximum parsimony. Separate analyses of data partitions resulted in slightly different topologies, therefore we combined all the data sets. The results support the monophyly of the *Bifrenaria* complex as well as of the genus *Rudolfiella*. Within *Bifrenaria*, two clades are strongly supported: the Amazonian species clade and the southern Brazilian clade. The genus *Adipe* is a paraphyletic grade. Although the genus *Cydoniorchis* is monophyletic and presents many morphological synapomorphies, the acceptance of this genus would demand many nomenclatural changes; we propose recognizing a broad *Bifrenaria*. The results also suggest an Amazonian origin for *Bifrenaria*.

Bifrenaria Lindl. is a South American genus of Orchidaceae comprising approximately 20 species (Hoehne 1944, 1953; Castro 1991a, 1991b, 1991c, 1996). The majority of the species occurs in southern Brazil as epiphytes in the Atlantic Rain Forest and, less frequently, as rupicolous plants in the Brazilian "campos rupestres" vegetation. Three species are distributed exclusively in the Amazonian region. Species of *Bifrenaria* are characterized by four-angled, unifoliate pseudobulbs, by plicate leaves and by flowers bearing a conspicuous spur and a forked stipe.

The genus *Bifrenaria* has traditionally been classified in the *Bifrenaria* alliance (subfamily Epidendroidae, tribe Maxillariae, subtribe Lycastinae), earlier denominated subtribe Bifrenariinae (Dressler 1981), together with *Horvatia* Garay, *Rudolfiella* Hoehne, *Teuscheria* Garay, and *Xylobium* Lindl. (Dressler 1993). Recently two species formerly included in the genus *Bifrenaria* have been transferred to two new monotypic genera of the *Bifrenaria* alliance, *Guanchesia maguirei* (C. Schweinf.) G.A. Romero and Carnevali and *Hylaeorchis petiolaris* (Schltr.) Carnevali and G.A. Romero (Carnevali and Romero 2000).

Whitten et al. (2000) suggested the fusion of the subtribes Lycastinae, Bifrenariinae, and Maxillariinae into a broader Maxillarinae s.l., based on sequence data from ITS nrDNA, *mat-K*, and *trnL-F*, that suggested the recognition of *Xylobium* as a separate clade, apart from Maxillariinae s.str., Lycastinae and Bifrenariinae subtribes.

Generic delimitation within *Bifrenaria* has long been controversial. Floral morphology seems to be the main source of information used in previous classifications to construct infrageneric classifications for the *Bifrenaria* complex (Hoehne 1953, Pabst & Dungs 1977), as demonstrated in Tab. 1. The *Bifrenaria* complex currently comprises three genera: *Adipe* Raf. (=*Stenocoryne* Lindl.), defined by delicate, long-inflorescence plants with small flowers; *Bifrenaria* Lindl. (sensu stricto), comprising robust plants with large flowers and inflorescences shorter than pseudobulbs; and *Cydoniorchis* K. Senghas, based on flowers with an entire stipe and inflorescences with erect perianth segments (Wolff 1990, Senghas 1994). The last taxonomic treatments considering the *Bifrenaria* complex (Pabst and Dungs 1977, Castro 1991a, 1991b, 1991c, 1996) suggested the union of the genera *Adipe* (=*Stenocoryne*) and *Cydoniorchis* under the genus *Bifrenaria*, retaining *Rudolfiella* as a separate genus, even though Cogniaux (1902) considered some *Rudolfiella* species as part of the *Bifrenaria* complex. Recent studies including molecular data suggested that the genus *Bifrenaria* is monophyletic and closely related to *Rudolfiella*, but only species of *Bifrenaria* s.str. were considered (Ryan et al. 2000, Whitten et al. 2000).

Infrageneric classification within the *Bifrenaria* complex as well as the relationships among the genera of the *Bifrenaria* alliance have not yet been examined in a phylogenetic context. Thus, the position of the *Bifrenaria* complex within the *Bifrenaria* alliance is still uncertain, especially regarding the genus *Rudolfiella*. Data based on DNA sequences have provided useful information for the understanding of the evolutionary history of many different groups of plants, including the Orchidaceae (e.g. Cameron et al. 1999, Ryan et al. 2000, Van den Berg et al. 2000, Whitten et al. 2000). The nuclear ribosomal DNA internal transcribed spacer (ITS) and the *trnL-F* region of cpDNA have proved to be useful sources of information to understand phylogenetic relationships at low taxonomic levels (e.g. Taberlet et al. 1991, Soltis and Soltis 1998 and references therein). The ITS region corresponds to two non-coding transcribed spacers, ITS 1 and ITS 2, flanking the 5.8S

Tab. 1. Comparison of classifications developed for the *Bifrenaria* complex. Nomenclatural types are indicated below each name.

Authors	Classifications proposed	Taxonomical changes proposed	Characters used
Cogniaux (1902)	**Genus *Bifrenaria* Lindl.** (1832) **Sect. *Eu-Bifrenaria*** (nom. inval.) [3 species] *B. atropurpurea* (Lodd.) Lindl. **Sect. *Stenocoryne*** [17 species] *S. longicornis* Lindl.	Included *Adipe* (*Stenocoryne*), *Bifrenaria s.str.*, and *Rudolfiella* in *Bifrenaria*	length and form of the spur
Hoehne (1944, 1953)	**Genus *Bifrenaria* Lindl.** (1832) [11 species] *B. atropurpurea* (Lodd.) Lindl. **Genus *Stenocoryne* Lindl.** (1838) [6 species] *S. longicornis* Lindl. **Genus *Rudolfiella* Hoehne** (1944) [5 species] *R. aurantiaca* (Lindl.) Hoehne	Considered 3 genera: *Stenocoryne*, *Bifrenaria*, and *Rudolfiella*	form of pseudobulbs, length of the inflorescence flower size, length of the spur, length o the labellum claw, form of labellum
Wolff (1990)	**Genus *Adipe* Raf.** (1837) [9 species] *A. racemosa* Raf.	Revalidated the older name *Adipe* Raf. and tranfered species previously considered in *Stenocoryne* to *Adipe* but with no criteria for synomnization of taxa – ignored other genera	none
Senghas (1995)	**Genus *Cydoniorchis* K. Senghas** (1994) [2 species] *C. tetragona* (Lindl.) K. Senghas	Transfered 2 species from *Bifrenaria* to *Cydoniorchis*, ignored other genera	form of stipe, number of flowers, position of the floral segments.
Castro (1991a, 1991b, 1991c, 1996)	**Genus *Bifrenaria* Lindl.**, 1832 **Sect. *Cydoniorchis*** (2 species) *B. tetragona* (Lindl.) Schltr. **Sect. *"Atropurpurea" Bifrenaria*** (1 species) *B. atropurpurea* (Lodd.) Lindl. **Sect. *Mellicolor*** (2 species) *B. mellicolor* Reichb. f. **Sect. *Harrisoniae*** (2 species) *B. harrisoniae* (Hook.) Reichb. f. **Sect. *Stenocoryne*** (10 species, 2 new) *S. longicornis* Lindl.	Included *Adipe* (*Stenocoryne*), *Bifrenaria s. str.* and *Cydoniorchis* as *Bifrenaria*. Considered *Rudolfiella* as a separate genus.	form of the spur, color of sepals and petals, size of callus, form of stipe

nuclear ribosomal DNA gene. The *trnL-trnF* region is composed of the intron *trnL*, 350-600 bp long, and the *trnL-trnF* spacer, 120-350 bp long, located between the *trnL* and *trnF* (GAA) genes (Soltis and Soltis 1998).

The aims of this study were: (1) to test the monophyly of the genera *Adipe* (= *Stenocoryne*), *Bifrenaria* (sensu stricto), *Cydoniorchis* and *Rudolfiella;* (2) to determine phylogenetic relationships among species and groups of species of the *Bifrenaria* complex, thus evaluating generic limits within this group; and (3) to reevaluate morphological characters traditionally used to delimit taxa.

MATERIAL AND METHODS

Voucher materials of all 34 species sampled in the molecular analysis are listed in Tab. 2. The species *Bifrenaria melanopoda* Klotzsch., *B. racemosa* (Hook.) Lindl., *B. silvana* V.P. Castro and *B. steyermarkii* (Foldats) Garay and Dunsterv. were excluded from all the analyses due to lack of appropriate material. Sixteen out of 21 known species from the *Bifrenaria* complex (Castro 1996) were sampled.

Outgroups were chosen based on previous molecular phylogenetic studies within the tribe Maxillarieae (Ryan et al. 2000, Whitten et al. 2000). The genera included in the present analyses were *Anguloa* Ruiz and Pavón, *Cryptocentrum* Benth., *Lycaste* Lindl., *Maxillaria* Ruiz and Pavón, *Neomoorea* Rolfe, *Rudolfiella* Hoehne, *Trigonidium* Lindl. and *Xylobium* Lindl. In order to have a better understanding of the generic relationships within the Bifrenariinae, we also sampled six additional species, *Hylaeorchis petiolaris* and representative species of the genera *Rudolfiella, Scuticaria* Lindl. and *Teuscheria* Garay of the *Bifrenaria* alliance.

DNA isolation: DNA was extracted from fresh, herbarium and silica gel-dried material (Chase and Hills 1991) according to Doyle and Doyle (1987) and scaled down to 5µl extraction volumes. DNA was precipitated overnight at -20°C with 0.65 volumes of isopropanol, centrifuged, washed twice with 70% ethanol, and dried. The pellet was resuspended in 75µl of Tris-EDTA buffer (TE) and stored at -20°C.

PCR amplification and sequencing: Amplification was performed using 50 µl reactions with 35 cycles, 2.5 mmol/L $MgCl_2$, and a hot start, using Sigma (Sigma Inc., St. Louis, MO.) buffers and *Taq* polymerase. In all ITS samples, betaine was added (1.0 mol/L

Tab. 2. Sources of plant material for the taxa included in this study.

Taxon	Voucher/Source information
Anguloa hohenlohii C. Morren	Whitten 94083 (FLAS)
Bifrenaria atropurpurea (Lodd.) Lindl.	Koehler 99/04 (FLAS,UEC)
Bifrenaria aureo-fulva (Hook.) Lindl. (= *Adipe, Stenocoryne*)	Simões et al. s.n. (FLAS, UEC)
Bifrenaria calcarata Barb. Rodr.	Koehler 00/21 (FLAS, UEC)
Bifrenaria charlesworthii Rolfe (= *Adipe, Stenocoryne*)	Koehler 00/26(FLAS, UEC)
Bifrenaria clavigera Reichb.f. (= *Adipe, Stenocoryne*)	Koehler 99C (FLAS, UEC)
Bifrenaria harrisoniae (Hook.) Reichb.f.	Simões et al. s.n. (FLAS, UEC)
Bifrenaria inodora Lindl. (= *Stenocoryne*)	Whitten 93197 (FLAS)
Bifrenaria leucorrhoda Reichb.f. (= *Adipe, Stenocoryne*)	Koehler 56 (FLAS, UEC)
Bifrenaria) longicornis Lindl. (= *Adipe, Stenocoryne*)	Koehler 101C (FLAS, UEC)
Bifrenaria mellicolor Reichb.f.	Koehler 00/30 (FLAS, UEC)
Bifrenaria stefanae V.P. Castro (= *Adipe*)	Koehler 62C (FLAS, UEC)
Bifrenaria tetragona (Lindl.) Schltr. (= *Cydonirchis*)	Whitten 93156 (FLAS)
Bifrenaria tyrianthina (Lodd.) Reichb.f.	Faria s.n (FLAS, UEC)
Bifrenaria venezuelana C. Schweinf.	Romero 5 (FLAS, UEC)
Bifrenaria vitellina (Lindl.) Lindl. (= *Adipe, Stenocoryne*)	Koehler 65C (FLAS, UEC)
Bifrenaria wittigii (Reichb.f.) Hoehne (= *Cydonirchis*)	Koehler 00/27 (FLAS, UEC)
Cryptocentrum calcaratum (Schltr.) Schltr.	Whitten s.n. (FLAS)
Hylaeorchis petiolares Carnevali and G.A. Romero	Baptista sn (FLAS, UEC)
Lycaste cruenta Lindl.	Whitten 97021 (FLAS)
Maxillaria umbratilis L.O. Williams	Whitten SEL 1995-0397
Maxillaria violaceopunctata Reichb.f.	Whitten SEL 1981-2139
Neomoorea wallisii (Reichb.f.) Schltr.	Whitten 90010 (FLAS)
Rudolfiella aurantiaca (Lindl.) Hoehne	Williams 246 (FLAS)
Rudolfiella saxicola (Schltr.) C. Schwienf.	Whitten 97020 (FLAS)
Rudolfiella (Lacaena) aff. grandis (Kraenzl.)	SEL 1996-0341A (FLAS)
Scuticaria hadwenii (Lindl.) Hook.	Whitten 97019 (FLAS)
Scuticaria salesiana Dressler	SEL 2000-0295A (FLAS)
Teuscheria wageneri (Rchb. f.) Garay	SEL 2000-0446A (FLAS)
Trigonidium egertoniamun Bateman ex Lindl.	Whitten 93099 (FLAS)
Xylobium leontoglossum (Reichb.f.) Rolfe	Whitten 91384 (FLAS)
Xylobium pallidiflorum (Hook.) G. Nicholson	Whitten 90241 (FLAS)
Xylobium zarumense Dodson	Whitten 90176 (FLAS)

final concentration) to the PCR mix to relax secondary structure. A touchdown thermal cycling program was used for ITS amplifications. The initial annealing temperature was 76°C, decreasing 1°C per cycle for 15 cycles, followed by 20 cycles at 59°C. The annealing temperature for amplification of *trnL-F* was 57°C. Amplification and sequencing primers used for ITS and *trnL-F* were designed by Sun et al. (1994) and Taberlet et al. (1991), respectively. Amplified products were purified with QIAquick PCR cleaning column and filtration kit (Qiagen Inc.) and directly sequenced on an PE Biosystems, Inc. 373 or 377 automated sequencer using standard dye-terminator manufacturer's protocols, except that cycle sequencing reactions were scaled down to 5µl. Both strands were sequenced to assure accuracy in base calling. The ABI software packages "Sequence Navigator™" and "Autoassembler™" were used to edit and assemble complementary and overlapping sequences. Each individual base position was examined for agreement of the two strands. DNA sequences were aligned manually, and gaps were coded as missing values. The ends of the matrix were trimmed to exclude sequence artifacts.

Morphological data. Herbarium material from BHCB, BHMH, CESJ, HB, HRCB, IAN, INPA, MBML, MO , MG, NY, PACA, R, RB, SP, SPF, UEC and VIC, as well as cultivated specimens with voucher material at UEC and AMES, were used to investigate morphological characters. Polarization of morphological characters was performed following the outgroup comparison method (Nixon and Carpenter 1993) according to the root defined by the molecular tree. When states varied among the outgroup taxa we followed the criteria of Maddison et al. (1984), given that outgroup relationships are resolved. We chose *Hylaeorchis petiolaris*, *Scuticaria hadwenii* (Lindl.) Hook., *Rudolfiella aurantiaca* (Lindl.) Hoehne, and *Teuscheria dodsoni* Dressler as outgroups in the morphological matrix because the all molecular analyses indicated they are sister to the ingroup. In addition, *Rudolfiella, Scuticaria* and *Teuscheria* present low intrageneric variance of most characters selected for the morphological analysis. The morphological matrix is presented in Tab. 3. In order to minimize inapplicable states for taxa lacking particular structures, absence was included as a state of a multistate character (Maddison,1993). Quantitative characters were considered only when no overlapping

Tab. 3. Morphological data matrix. (?) = missing data; (-) = inapplicable data. All characters are unordered.

Taxon	000000000111111111222 123456789012345678901 2
Teuscheria dodsoni	00000000000000-0000100
Hylaeorchis petiolares	00010001-00100-0000000
Rudolfiella aurantiaca	00001001-0000-10111000
Scuticaria hadwenii	00?001?0-0001200110100
Bifrenaria atropurpurea	001101?100000100120000
B. aureo-fulva	00000001000002001100 11
B. calcarata	00110111001100000200 01
B. charlesworthii	00001001110000001100 00
B. clavigera	00000001110000001100 00
B. harrisoniae	001-1101000002001100 00
B. inodora	00111101000001001100 00
B. leucorrhoda	00000001000002001100 00
B. longicornis	11000001100005001300 10
B. mellicolor	00110111001100000200 00
B. stefanae	00000001000002001100 00
B. tetragona	00110101000114110211 01
B. tyrianthina	00101101001001001300 00
B. venezuelana	11010001000002001100 00
B. vitellina	00001001000002001100 00
B. wittigii	00110101000014110211 01

Tab. 4. Morphological characters and character polarization

1. **Pseudobulb distribution.** aggregate (0); distant (1)
2. **Form of pseudobulbs (in transverse section).** elliptic to round (0); four-angled (1)
3. **Ratio sterile bract length/floral bract length.** 1.44-3.01 (0); 0.80-1.12 (1)
4. **Ratio pseudobulb length/inflorescence length.** 0.19-0.74 (0); 1.29-5.25 (1)
5. **Sepal position.** divergent (0); parallel to each other (1)
6. **Flower size.** 14.5-22.9mm (0); 34.0-65.2mm (1) [measurements were taken from the base of the spur to the apex of the central sepal]
7. **Sepal nectaries.** absent (0); present (1)
8. **Prolonged spur.** absent (0); present (1) [we considered the prolongation of the spur the extension of the sepals from the column foot downwards]
9. **Fusion of sepals on spur region.** sepals free (0); sepals connate at base (1)
10. **Form of labellum.** 3-lobed (0); entire (1)
11. **Position of labellum to inflorescence axis.** oblique (0); parallel (1)
12. **Margin of median labellum lobe.** crenate (0); entire (1)
13. **Position of median labellum lobe.** curved downwards (0); straight (1)
14. **Form of labellum callus.** fleshy on apex, entire (0); fleshy on apex, 2-lobed (1); fleshy on apex, 3-lobed (2); entirely fleshy (4); callus absent (5) [a callus, as defined here, extends from the base of the labellum to its median region]
15. **Form of column foot.** straight (0); arched (1)
16. **Column wings.** absent (0); present (1)
17. **Stipe number.** 1 (0); 2(1)
18. **Viscidium form.** arched (0); truncate (1); cuneate (2); round (3)
19. **Polinia form.** round (0); ovate (1)
20. **Flower position on inflorescence.** straight to downwards (0); upwards (1)
21. **Apex of lateral petals.** round (0); acuminate (1)
22. **Form of median labellum apex.** round (0); acute (1)

morphometric states were obtained. Morphological characters and character state coding are described in Tab. . 4.

Phylogenetic analyses. All cladistic analyses were performed using PAUP version 4.0b4 - (Swofford, 2000), using the criteria of Fitch parsimony (unordered characters, equal weights to all changes; Fitch 1971), and ACCTRAN optimization with zero-length branches collapsed. We performed individual heuristic searches on 34 taxa for ITS, *trnL-F* and combined molecular matrices, as well as for molecular and morphological data matrices combined. The search strategy for the molecular and combined data sets used 1000 replications of random taxon entry additions, option MULTREES (saving multiple trees) in effect, and tree-bisection- reconnection swapping (TBR), holding 10 trees/replicate, to minimize finding huge numbers of suboptimal trees, and saving all shortest trees. To assess the internal support of the clades we performed 1000 bootstrap replicates (Felsenstein 1985) with 10 replicates of random taxon entry additions, holding one tree for each replicate. We used the following descriptions for categories of bootstrap support: unsupported, <50%; weak, 50-74%; moderate, 75-84%; strong 85-100%. The morphological matrix comprising 22 characters and 19 taxa was analysed using a branch-and-bound search. No bootstrap replicates were performed for the morphological data set due to the low internal support of branches. In order to reduce the effect of homoplastic characters on the tree topologies, successive weighting (Farris 1969) was applied to the morphological and combined data sets. The morphological character state changes were traced with MacClade version 4.0 (Maddison and Maddison 2000).

RESULTS

Tree statistics for each analysis (Tab. 5) consist of the number of characters included 7in the matrix, the number of variable characters, the number of phylogenetically informative characters, the number of Fitch trees, the tree length, consistency indices, retention indeces and the number of highly supported clades in bootstrap consensus trees.

Tab. 5. Values and statistics from PAUP analyses of separate and combined data matrices. (SW= successive weighting values)

Matrix	**ITSnrDNA**	***trn*L-F**	**molecular data combined**	**morphology**	**all data combined**
No. included characters in matrix	743	1117	1860	22	1882
No. variable characters	250	187	437	22	459
No. phylogenetically informative characters	107	67	174	22	195
No. Fitch trees	135	2249	1694	253 71 (SW)	36 2 (SW)
No. steps	406	245	659	46 22,6 (SW)	708 451.9(SW)
CI	0.764	0.829	0.778	0.59 0.76 (SW)	0.76 0.94 (SW)
RI	0.785	0.841	0.795	0.71 0.86 (SW)	0.78 0.94 (SW)
No. clades in bootstrap consensus with >85% support	10	8	11	-	11

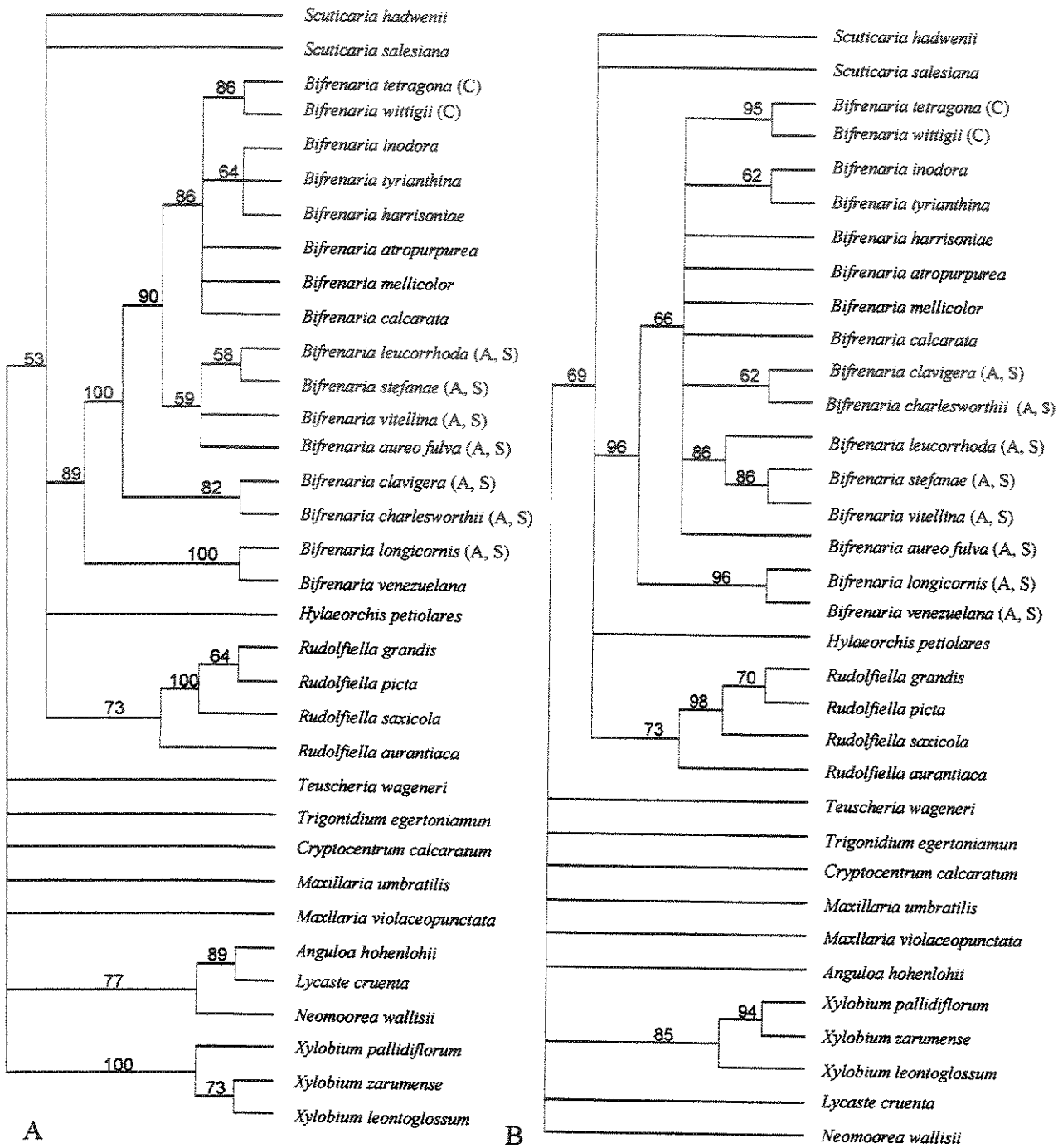

Fig. 1. (A) Bootstrap consensus tree from unweighted ITSnrDNA analysis. (B) Bootstrap consensus tree from unweighted *trnL-F* analysis. Only bootstrap values above 50% are reported. Species considered in the genus *Adipe* Raf. (A), *Cydoniorchis* K. Senghas (C) and *Stenocoryne* Lindl. (S) are indicated in the trees.

ITS nrDNA. The aligned ITSnrDNA matrix consists of 743 positions. A total of 250 characters (33.6%) were variable and 107 (14.4%) were potenially parsimony informative. The analysis produced 135 Fitch trees of 406 steps (CI= 0.76 RI =0.78). Considering data sets separately, the ITS data produced the most resolved and best supported trees (Fig. 1a). The *Bifrenaria- Hylaeorchis-Rudolfiella - Scuticaria* clade presents weak levels of support and there is no resolution for the intergeneric relationships within this clade. The genus *Rudolfiella* has weak support and there is no support for the genus *Scuticaria*, but only two species were sampled. The *Bifrenaria* complex is strongly supported as monophyletic, as it is also the clade of the Amazonian species of *Bifrenaria* (*B. longicornis* and *B. venezuelana)*. The Amazonian clade appears as the sister group of the also well supported clade comprising the southern Bifrenarias. Within the southern Bifrenarias, only the *Bifrenaria* (sensu stricto) clade is strongly supported. Terminal nodes, with the exception of the clade consisting of *B. tetragona* and *B. wittigii* have weak to no support. There is no support for the genus *Adipe*, which appears as a paraphyletic group.

trnL-F. The aligned *trnL-F* matrix consists of 1117 positions, of which 187 (16.7%) were variable and 67 (6%) were potenially phylogenetically informative. One 30 bp region was considered unalignable and was excluded. A total of 2249 Fitch trees of 245 steps (CI =0.83, RI =0.84) were obtained. Although the *trnL-F* bootstrap consensus tree is less resolved than the ITS one, they are highly congruent (Fig. 1b). The *Bifrenaria-Hylaeorchis-Rudolfiella- Scuticaria* clade presents higher, although still weak, level of support compared to the ITS data. There is also weak support for the genus *Rudolfiella* and the genus *Scuticaria* remains unsupported. The *Bifrenaria* complex appears as strongly monophyletic, as well as its internal clades (*B. longicornis*, *B. venezuelana*) and (*B. tetragona*, *B. wittigii*). Contrary to the ITS data, the plastid data also provides strong support for the clade (*B. stefanae*, *B. vittelina*, *B. leucorrhoda*). There is no support for the *Bifrenaria* (sensu stricto) clade nor for the genus *Adipe*.

Combined molecular data. The combined molecular matrix consisted of 1860 included positions, of which 437 (23.5%) were variable and 174 (9.4%) were potentially phylogenetically informative. Molecular data were combined because the trees estimated from different data partitions presented differences restricted to a few terminal clades without strong bootstrap support that may be due to sampling error or local incongruence effects, and not because of conflicting phylogenetic signals between the data partitions (Huelsenbeck et al 1996; Baker et al. 2001). High levels of support are observed for the *Bifrenaria, Hylaeorchis, Rudolfiella, Scuticaria* clade, for the genus *Rudolfiella* as well as for *Bifrenaria* s.s (Fig. 2a). Within *Bifrenaria* there is also strong support for the terminal clades (*B. inodora, B. harrisoniae, B. tyrianthina*) and (*B. clavigera, B. charlesworthii*). The genus *Adipe* remains unsupported as well as the genus *Scuticaria.*

Morphology. The analysis of morphological data resulted in 253 trees (CI 0.59; RI 0.71) with 46 steps (Fig. 2b). Morphology does not support the *Bifrenaria* complex clade (Fig. 2b). The Amazonian clade collapses together with the other species of *Adipe*, which, according to morphological data, are also not monophyletic. The only clades that remain in the strict consensus tree are the (*B. tetragona, B. wittigii*) clade, which appears sister to the (*B. calcarata, B. mellicolar)* clade and the (*B. clavigera, B. charlesworthii*). Successive weighting analysis resulted in 71 trees (tree length 22.6; CI= 0.76; RI= 0.88). The clades supported in the strict consensus tree of sucessive weighted data are the same ones obtained from equally weighted analysis.

Combined morphological and molecular analysis. Although the morphological tree is poorly resolved, there were no strongly supported incongruent patterns in the trees obtained for the different data sets. Differences are probably due to sampling error and not because of hard polytomies and thus we combined the data sets into a single analysis (Huelsenbeck et al. 1996, Baker et al. 2001). In the combined data analysis 1882 characters were included, of which 459 (24.4%) were variable and 195 (10.4%) were potentially parsimony informative. The number of trees produced was 36, with a length of 708 steps (CI= 0.76; RI= 0.78). There is strong support for the genera *Bifrenaria* and *Rudolfiella* (Fig. 3). The Amazonian species clade (Fig. 3, clade A) has strong support and, according to all data

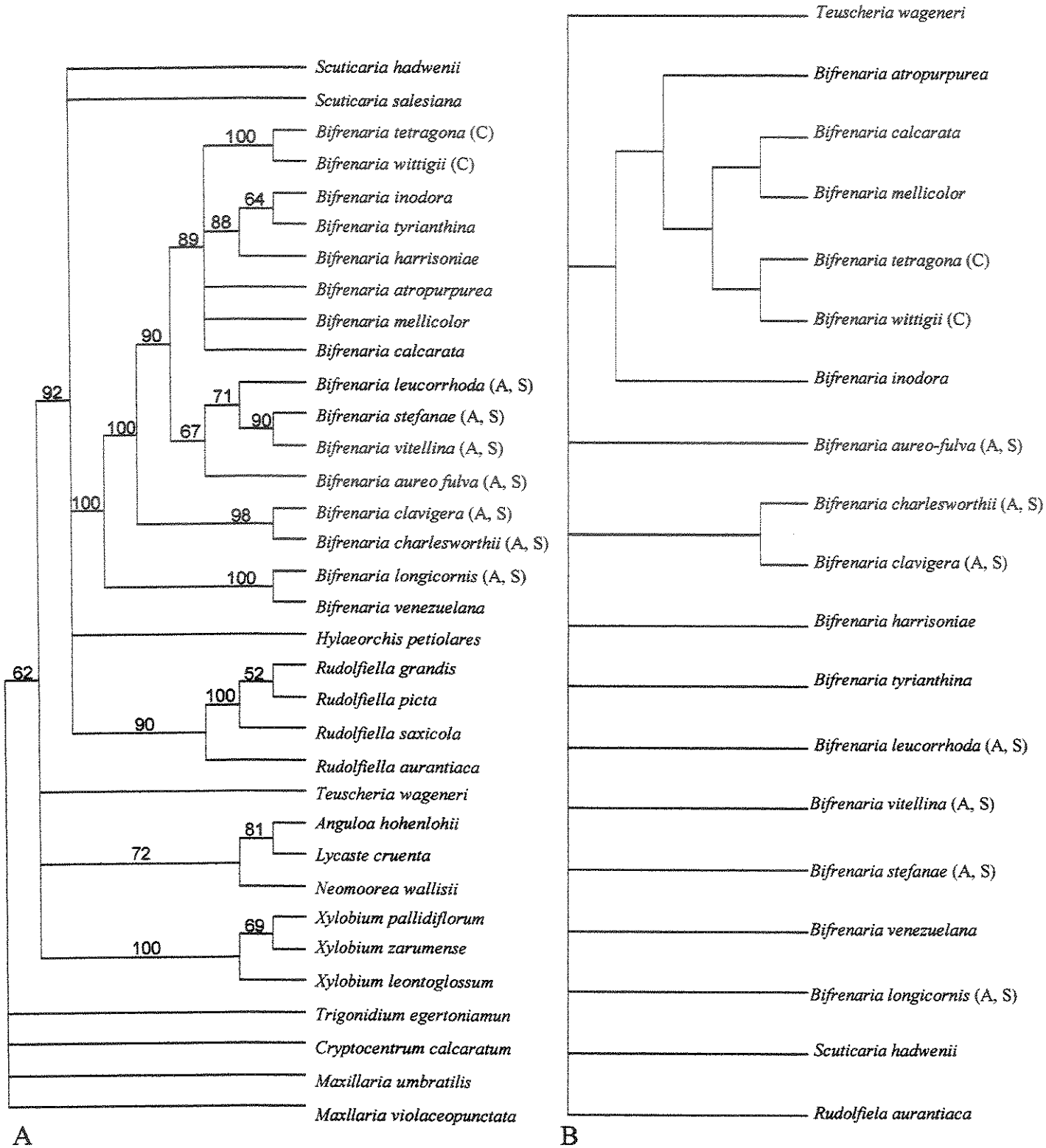

Fig. 2. (A) Bootstrap consensus tree from unweighted combined molecular analysis. (B) Strict consensus tree from unweighted morphological analysis. Only bootstrap values above 50% are reported. Species considered in the genus *Adipe* Raf. (A), *Cydoniorchis* K. Senghas (C) and *Stenocoryne* Lindl. (S) are indicated in the trees.

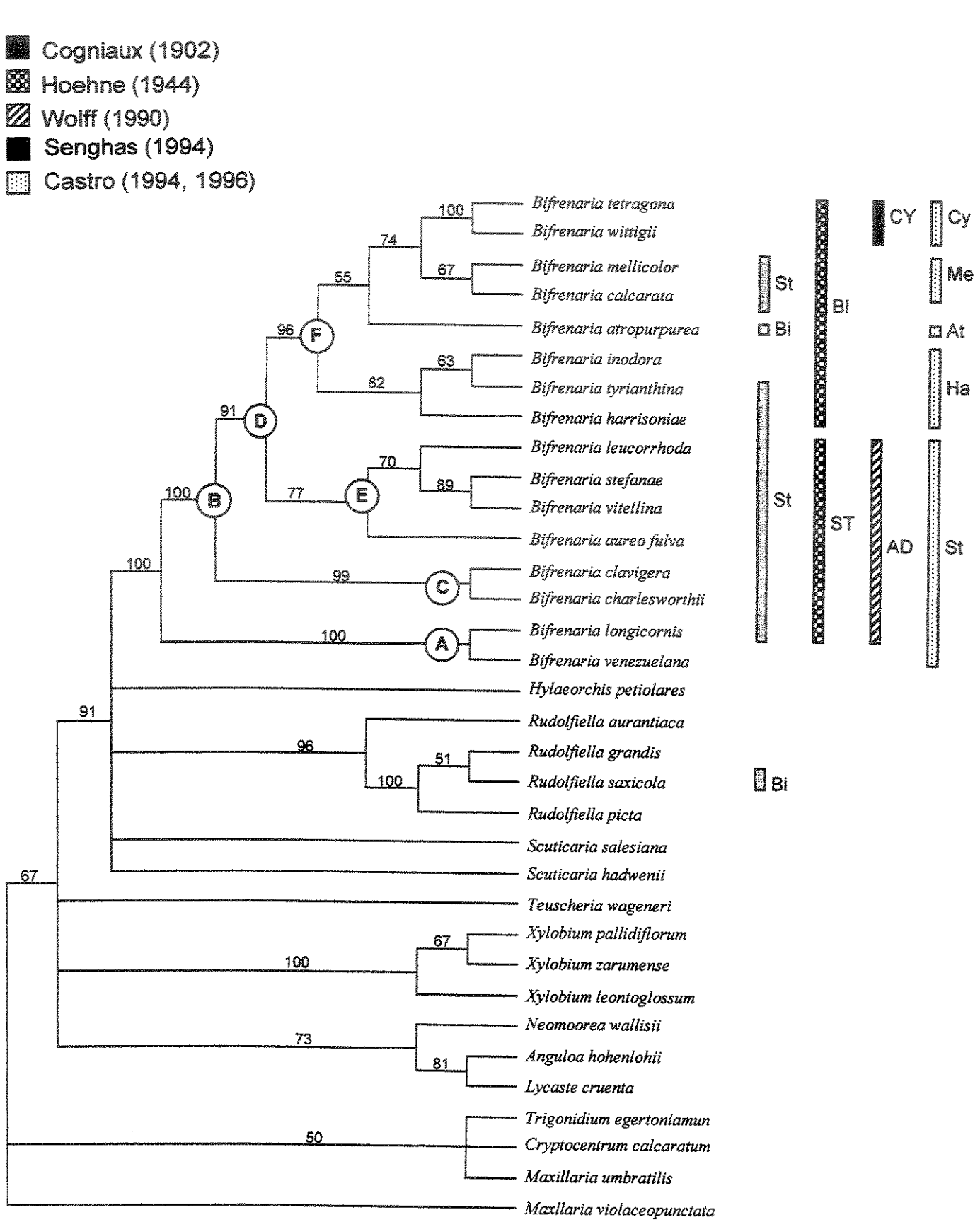

Fig. 3. Bootstrap consensus tree from unweighted combined analysis. Only bootstrap values above 50% are reported. Previous classifications are indicated by the right hand bars. (Bi) section "Eu-Bifrenaria", (St) section *Stenocoryne*, (BI) genus *Bifrenaria*, (ST) genus *Stenocoryne*, (CY) genus *Cydoniorchis*, (AD) genus *Adipe*, (Cy) section *Cydoniorchis*, (Me) section "Mellicolor", (At) section "Atropurpurea", (Ha) section "Harrisoniae", (St) section *Stenocoryne*

sets, it is the sister group of the Southern species clade (Fig. 3, clade B), which also has strong bootstrap support (Fig. 3). Within this clade, the *Bifrenaria* s.str. (Fig. 3, clade F) has strong support as well as the (*B. tetragona*, *B. wittigii*) clade (Fig. 3, clade C). Other terminal clades show strong (*B. charlesworthii*, *B. clavigera*) to moderate (*B. calcarata*, *B. mellicolor*) (*B. aureo-fulva* (*B. leucorrhoda* (*B. stefanae*, *B. vitellina*))) bootstrap support (Fig. 3, clades C and E, respectively). Successive weighting resulted in 2 trees with 451.9 steps, of which the strict consensus tree presented very similar topology to the equally weighted bootstrap consensus tree. The only differences between the tree topologies are the positions of the sister groups of the *Bifrenaria* complex.

DISCUSSION

Even though the monophyly of *Bifrenaria* s.l. is highly supported by both separate molecular data sets (Figs. 1, 2) as well as by the combined data set (Fig. 3), this clade is morphologically characterized only by the presence of four-angled pseudobulbs and of a prolonged spur (Fig. 4). Outgroup species generally present ovoid to oblong, compressed, smooth to sulcate pseudobulbs, and, although a prolonged spur is very common in many Maxillarieae (Dressler 1993), the spur is extremely reduced or absent on closely related groups of *Bifrenaria* (*Hylaeorchis, Rudolfiella* and *Teuscheria*). Most characters commonly used to characterize the genus, such as the forked stipe and unifoliate pseudobulbs, are also present in the genera *Rudolfiella* and *Scuticaria*.

Basal relationships within the genus *Bifrenaria* were also strongly resolved with molecular and combined data. Both ITS and *trnL-F* data supported two basal clades, named here as clade A and clade B (Fig. 3). Clade A represents the Amazonian species of *Bifrenaria*, *B. longicornis* and *B. venezuelana* (Fig. 3). Even though clade A collapsed in the morphological analysis, propably due to striking differences in reproductive traits, such as inflorescence lenght, flower morphology, viscidium format, they can be defined by the presence of long-creeping rhizomes (char.1, Fig. 4). Clade B comprises the other species of *Bifrenaria*, all restricted to Southern Brazil (Figs. 3, 4), and is characterized by extremely short rhizomes (char.1), a character also present in species of the outgroup.

Within clade B, there is the highly supported clade C, consisting of *B. charlesworthii* and *B. clavigera* (Fig. 3). This clade is morphologically defined by a basally connate spur (char.9) and by an entire, red-spotted labellum (char.10, Fig. 4). Furthermore, the species of clade C usually present very short stipes. As sister to clade C is clade D, containing two sister groups: *B. aureo-fulva, B. leucorrhoda, B. stefanae* and *B. vitellina* (clade E) and the *Bifrenaria* (sensu stricto) clade, which we called clade F. Clade D has no morphological synapomorphies, and the only feature connecting the species is the southern Brazilian geographical distribution. Clade E received weak to moderate support in the analyses performed, and species of this clade can be recognized only by plesiomorphic character states: the long inflorescence with small flowers up to 23mm (char.6, Fig. 4) and lateral sepals parallel to each other (char.5, with a reversion to divergent lateral sepals in *B. vitellina* – see Fig. 4). The monophyly of *Bifrenaria* s.str. (clade F), is not surprising, since this group presents many conspicuous morphological traits: large-sized plants with flower size greater than 34 mm (char.6); the length of the sterile bracts is similar to the floral bracts (char.3); and the inflorescence is shorter than the pseudobulbs (char.4, with a reversion in *B. harrisoniae* and *B. tyrianthina*) (Fig. 4). Phylogenetic relationships within clade F are not well resolved and probably comparison of faster evolving DNA regions is needed to resolve relationships in these terminal clades. The only highly resolved group in clade F is the (*B. tetragona-B. wittigii*) clade (= *Cydoniorchis*). It can be distinguished from all the other *Bifrenaria* species by an inflorescence with flowers in the upward position (char.20); a straight labellum (char.13); a entirely fleshy callus (char.14); an arched column foot (char.15); a column with wings (char.16); and by a pollinarium with oval pollinia (char.19) (Fig. 4).

Bifrenaria species are mostly epiphytes inhabiting forest habitats. Three species (*B. harrisoniae, B. inodora, B. tyrianthina*), however, are commonly found in open places in rocky, or sandy soil of montane fields ("campos rupestres") in the states of Bahia and Minas Gerais, but only *B. tyrianthina* is restrictively rupiculous. This condition appears to have originated once, since these species form a monophyletic group. The phylogenetic pattern observed in *Bifrenaria* s.l. in the molecular as well as in the combined analyses suggests an Amazonian origin for the genus, since the most closely related genera are mainly distributed in northern South America, followed by an initial diversification in

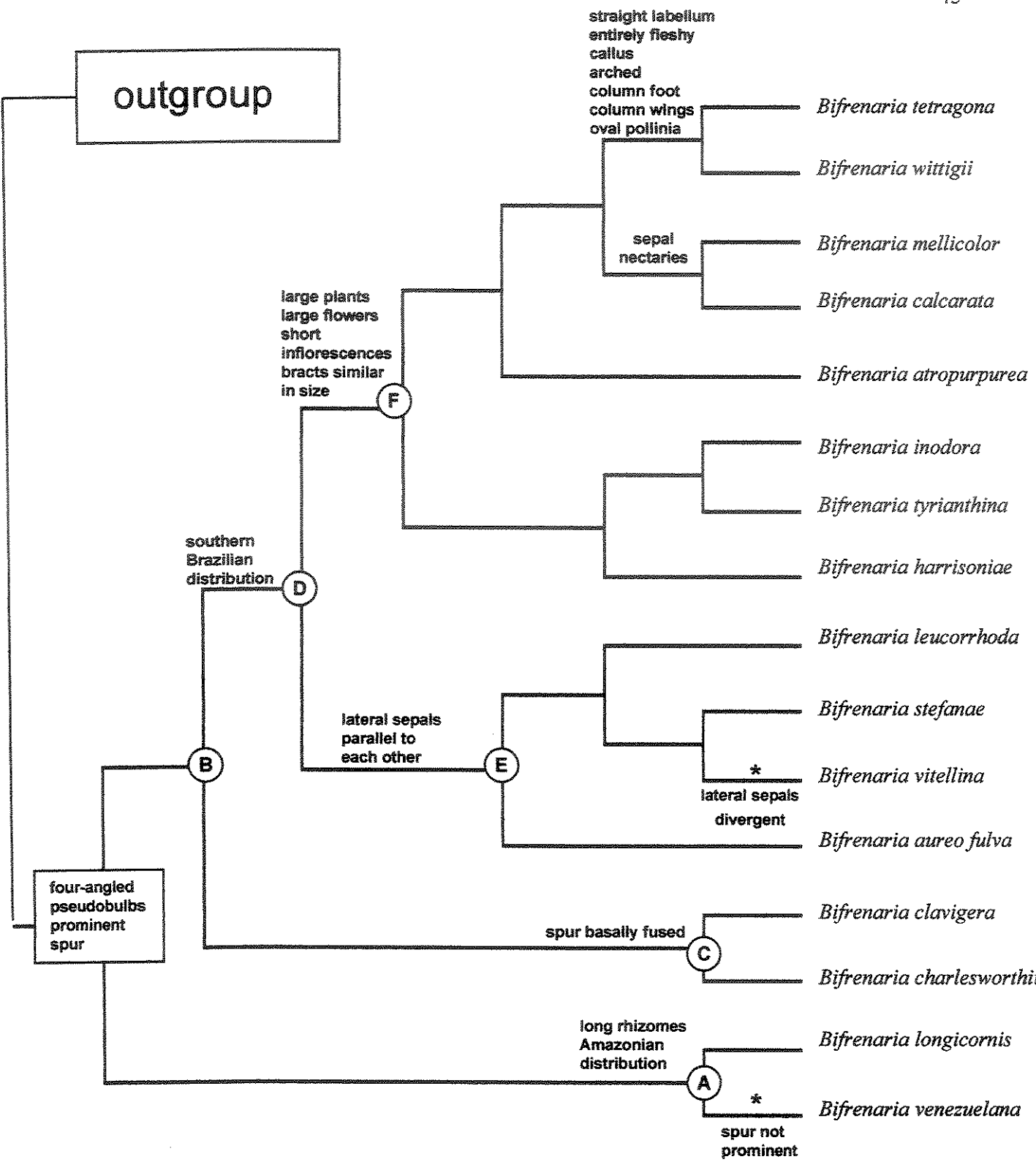

Fig. 4. Bootstrap consensus tree from unweighted combined analysis, showing only the *Bifrenaria* complex clade. Main clades are indicated by letters according to discussion in text. The most important synapomorphies are indicated in the tree (* = reversion of character).

southern Brazil. The biogeographic pattern for the southern *Bifrenaria* species as a whole is less clear, since the species are distributed throughout the Brazilian Atlantic Forest, predominantly in the States of Espirito Santo, Minas Gerais, Rio de Janeiro and São Paulo. Species included in these analyses were assigned to genera or sections according to Cogniaux (1902), Hoehne (1944), Wolff (1990), Senghas (1994) and Castro (1991a, 1991b, 1991c, 1996) in Tab. 1. These previous classifications were used to lable the same species in one of the most parsimonious trees obtained from combined data sets (Fig. 3).

Cogniaux (1902) considered in the genus *Bifrenaria* to consist of all *Adipe* (*Stenocoryne*) and *Bifrenaria* s.str. species and also two species of the later described genus *Rudolfiella*. Our results indicate that the genera *Bifrenaria* and *Rudolfiella* are closely related but phylogenetically distinct (Fig. 3). The circumscription of *Stenocoryne* by Hoehne as well as its later nomination to *Adipe* by Wolff (1990) comprises a paraphyletic group of species which have been defined by the following plesiomorphic states: inflorescences longer than pseudobulbs (char. 4) and small, delicate flowers (char. 6). Other characters commonly used by Hoehne (1944, 1953) to separate *Bifrenaria* from *Stenocoryne*, such as the number of flowers in the inflorescence, the length of the labellum claw and the form of the labellum, were found to show continuous variation or to be erroneously defined and, therefore, not applicable to the character delimitation procedure. Four of the five sections of Castro (1991a, 1991b, 1991c, 1996) are monophyletic and correspond to clades of *Bifrenaria* s.str. (clade F). Castro, however, also used the small size of the flowers (1991a), a plesiomorphic character state (char. 6), to define the section "*Stenocoryne*", making it paraphyletic. The genus *Cydoniorchis* of Senghas (1994), comprising *B. tetragona* and *B. wittigii*, is definitely monophyletic and can be recognized by many morphological characters, as previously cited. However, despite the various morphological synapomorphies supporting *Cydoniorchis*, the acceptance of this genus is problematic. It would require the creation of at least five new genera within the *Bifrenaria* complex to avoid paraphyly of *Bifrenaria s.str.* Such taxonomic changes does not seem reasonable, especially because the low levels of divergence detected among species of clade F could lead to erroneous placements and, thus, to nomenclatural instability. Also, the new genera would not be as clearly morphologically distinct as *Cydoniorchis*.

We conclude that all the species previously considered under the genera *Adipe* (=*Stenocoryne*), *Bifrenaria* s.str. and *Cydoniorchis* comprise a clade that represents the genus *Bifrenaria*. The genus *Rudolfiella* represents a monophyletic and morphologically distinct group, characterized by closely related to the genus *Bifrenaria*. For a complete understanding of the phylogenetic relationships and character evolution patterns within the *Bifrenaria* alliance, it is necessary to gather more data of the genera *Hylaeorchis, Rudolfiella* and *Scuticaria.*

The importance of using multiple sources of phylogenetic information has become increasingly important as numerous studies combining several data sets have consistently demonstrated the limited ability of single data partitions to accurately reconstruct phylogenetic hypothesis (Baker at al. 2001, Soltis et al. 1998). This study illustrates the utility of ITS nrDNA, *trnL-F,* and morphology for this purpose, as many other studies in the Orchidaceae have demonstrated before (e.g. van den Berg et al. 2000, Whitten et al. 2000, Williams et al. 2001). It is obvious that additional information about molecular, morphological, biochemical and ecological data is needed to produce a more comprehensive view of phylogenetic relationships and character evolution patterns within the tribe Maxillariae in particular and in Orchidaceae as a whole.

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Apêndice 1. Protocolo utilizado no presente estudo para extração e precipitação de DNA (modificados a partir de Doyle e Doyle 1987)

Material necessário: solução CTAB, mercaptoetanol, solução clorofórmio/álcool isoamílico 24:1, acetato de sódio 3M (pH 4.8), isopropanol 100%, etanol 70%, tampão TE, centrífuga, tubos eppendorf 1.5ml, pipetas automáticas (1000µl, 20-200µl e 1-10µl), vórtice, água miliq. autoclavada, material vegetal (folhas, flores, raízes), pistilo e recipiente para macerar, banho ou placa aquecedora, regulado em 65 °C.

Extração:

1. Coloque 50-100mg de tecido no recipiente próprio para macerar (tecido em excesso reduz a quantidade de DNA extraído). Adicione 1.2 ml de solução de CTAB e 8µl de mercaptoetanol. Macere tudo até a completa homogeinização da solução
2. Transfira 1ml da solução para os tubos eppendorf (não esqueça de numerar e anotar suas amostras em um protocolo apropriado).
3. Incubar amostras a 65 °C por 20 minutos. No caso de amostras difíceis, deixar aquecendo por várias horas ou até o dia seguinte. Misturar as amostras ocasionalmente com auxílio de vórtice.
4. Adicione 500µl de clorofórmio/álcool isoamílico (se a ponteira encostar no tubo, troque-a) e misture em vórtice rapidamente até que seja obtida uma da solução leitosa.
5. Centrifugar por 5 minutos a 10.000 r.p.m. para separar fases (o clorofórmio ficará abaixo e a fase aquosa, contendo o DNA, acima).
6. Pipetar 750µl da solução aquosa e transferi-la para um novo tubo. Evitar aspirar a camada contendo clorofórmio. Caso ocorra a mistura das camadas, centrifugar novamente. Descarte o clorofórmio em um recipiente apropriado.

Precipitação:

1. Adicionar acetato de sódio 3M à fase aquosa, de acordo com a fórmula: vol. de acetato de sódio em µl = vol. da fase aquosa em µl X 0.04 (750µl X 0.04 = 30µl).
2. Adicionar isopropanol 100% de acordo com a fórmula: isopropanol em µl = vol. fase aquosa X 0.65 (= 780 X 0.65 = ca. 510µl).

3. Inverter os tubos manualmente. Ocasionalmente você poderá ver o DNA precipitando, mas não se preocupe se não conseguir ver. Coloque tubos no freezer (-20C) por 3-4 horas ou preferencialmente até o dia seguinte.

4. Centrifugar amostras na velocidade máxima (13 mil r.p.m.) por 20 min. Após a centrifugação um pellet deverá estar visível. Caso contrário, deixe precipitar por mais um dia e repita o procedimento.

5. Descarte cuidadosamente o sobrenadante e enxugue a boca do tubo em papel absorvente.

6. Adicione 1ml de etanol 70%. Inverta o tubo até que o pellet se destaque (alguns pellets simplesmente não saem do lugar!). Descarte o sobrenadante e repita o procedimento. Pressione tubos em papel absorvente e coloque-os para secar. Espere até que o etanol tenha evaporado completamente.

7. Adicione 75µl de tampão TE. Incube amostras a 65 °C por 15 min para assegurar ressuspensão do DNA. Armazene amostras a curto prazo a -4 °C ou, a longo prazo, a -20 °C

8. Correr um gel de agarose a 1% com a solução de DNA extrádo para checar qualidade do DNA. Corar gel em brometo de etídeo e visualizar sob luz U.V. Se necessário, fotografar.

Apêndice 2. Protocolos utilizados no presente estudo para amplificação das regiões ITS1 e ITS2 (nrDNA) e *trn*L-F

Material necessário: tubos 0.2 ml (numerados), pipetas automáticas (20-200µl, 1-10µl e 0.1-1µl), ponteiras descartáveis NOVAS, *primers* para a amplificação das regiões desejadas, água miliq. autoclavada, DNA a ser amplificado, *Taq* polimerase, Solução Premix I, nucleotídeos (DNTP MIX)

Solução Premix I (volumes referentes a uma amostra):

Tampão Sigma (10X)	5µl
$MgCl^2$ Sigma (2.5 mmol/L)	7µl
DNTP Mix[1]	1µl
Betaína, solução saturada	12µl
(somente necessária na amplificação de ITSnrDNA)	
Total	25µl

1. Utilize luvas descartáveis
2. Organize os tubos numerados na máquina de PCR
3. Prepare a solução (Premix I em um tubo de 1,5 ml com os reagentes (sempre calcule 1 amostra a mais para cada 10, para evitar erros de pipetagem):

Premix I	25 µl
água miliq.	23 µl
primer 3´- 5´	1 µl
primer 5´- 3´	1 µl
Total	49 µl

[1] 40 µl de solução estoque de cada nucleotídeo (dATP. DCTP, dGTP, dTTP) totalizando 160 µl mais 240 µl de água miliq. autoclavada.

4. Com auxílio de um vórtice, agite o tubo rapidamente para assegurar a completa mistura dos reagentes.
5. Pipete 49µl da solução MIX em cada tubo numerado na máquina de PCR (se você está trabalhando com muitas amostras deixe a máquina de PCR a 4 °C)
6. Adicione 1µl de DNA. Feche os tubos.
7. Ligar a máquina e esperar a temperatura do bloco chegar a 80 °C. Não feche a tampa da máquina neste momento. Quando atingida esta temperatura, pause o programa.
8. Rapidamente adicione 0.2µl de *Taq* polimerase em cada tubo numerado na máquina de PCR e feche os tubos o mais rápido possível para evitar evaporação. Feche a tampa da máquina de PCR e dê continuidade ao programa. Retorne a *Taq* polimerase ao freezer imediatamente.
9. O programa leva de 2:30 a 3hs para terminar. Após o término, coloque os produtos de PCR imediatamente no freezer.
10. Correr gel de agarose a 1% com produtos de PCR para checar sua qualidade. Corar gel em brometo de etídeo e visualizar sob luz U.V. Se necessário, fotografar.

Programa para amplificação de ITS:

1. 94 °C 3 min
2. 94 °C 1 min
3. 76 °C 1 min, diminuindo 1 °C por ciclo
4. 72 °C 1 min
5. Repetir passos 2-4 mais 15 vezes (estratégia "touchdown")
6. 94 °C 1 min
7. 59 °C 1 min
8. 72 °C 1 min
9. Repetir passos 6-8 mais 21 vezes
10. 72 °C 4 min (extensão final para garantir sequências completas)
11. 4 °C indefinitivamente (esfria bloco para parar ação da *Taq* polimerase)

*Programa para amplificação de trn*L-F:

1. 94 °C 2 min
2. 94 °C 1 min
3. 57 °C 45 s
4. 72 °C 1 min 20 s
5. Repetir passos 2-4 mais 32 vezes
6. 72 °C 5 min (extensão final para garantir sequências completas)
7. 4 °C indefinitivamente (esfria bloco para parar ação da *Taq* polimerase)

Apêndice 3. Protocolo utilizado no presente estudo para purificação dos produtos de PCR

Material necessário: produtos de PCR, tubos 1.5ml, Kit Quiaquick, centrífuga (Nota: O tampão PE do Kit Quiaquick vem em solução concentrada. É necessário adicionar etanol 95% para diluir a solução)

1. Numere os tubos coloridos do Kit Quiaquick de acordo com a numeração das suas amostras e adicione a coluna para filtração ao tubo .
2. Adicione à coluna de filtração 250µl de tampão PB e 50µl do produto de PCR. Misture bem com a pipeta.
3. Centrifuge em máxima rotação (13 000 rpm) por 1 min.
4. Descarte líquido no fundo do tubo. Adicione 750µl de tampão PE.
5. Centrifuge em máxima rotação (13 000 rpm) por 1 min.
6. Descarte líquido no fundo do tubo. Centrifuge novamente em máxima rotação (13 000 rpm) por 1.5 min.
7. Descarte tubo e transfira a coluna filtradora para tubo de 1,5ml (enumerado apropriadamente). Adicione 50µl de solução EB.
8. Centrifuge a 3 000 rpm por 1min - ATENÇÃO: os tubos devem ser arrumados na centrífuga com a tampa aberta voltada para baixo. Não tente centrifugar com velocidade superior a 3000 rpm pois a alta velocidade destruirá as tampas livres.
9. Descarte a coluna filtradora. O produto de PCR limpo está no fundo do tubo.
10. Corra um gel para verificar a qualidade e presença dos produtos de PCR.

Apêndice 4. Protocolo utilizado no presente estudo para reação de seqüenciamento

A reação de seqüenciamento é essencialmente uma segunda reação de PCR, com algumas diferenças importantes: o DNA utilizado é o produto de PCR purificado, a solução para reação contém etiquetas fluorescentes marcadoras, os oligosnucleotídeos sintéticos são utilizados separadamente e são utilizados apenas 25 ciclos.

Material necessário: tubos 1,5 ml, tubos 0,2 ml, BIG DYE cycle sequencing premix, *primers* (apropriadamente diluídos), tampão de sequenciamento (5X, 400 mM Tris, 10 mM MgCl2,pH 9), água miliq. autoclavada, etanol 70%, etanol 95%, acetato de sódio (pH 4.8, 3M).

(Nota: Os oligosnucleotídeos sintéticos devem ser diluídos para um concentração de 1 picomole/µl, que equivale a 1/10 da concentração do *primer* utilizado no PCR).

1. Para cada amostra numerar dois tubos de 0,2ml (um para cada *primer*).
2. Prepare um mix em um tubo de 1,5ml de acordo com o protocolo abaixo (multiplique o volume final dos produtos por 10% para evitar erros de pipetagem). Os volumes estão ajustados para um volume total igual a 20µl:

Solução Mix: (ATENÇÃO: como você estará adicionando os dois *primers* separadamente, deverão ser feitas duas soluções mix, uma para cada oligonucleotídeo).

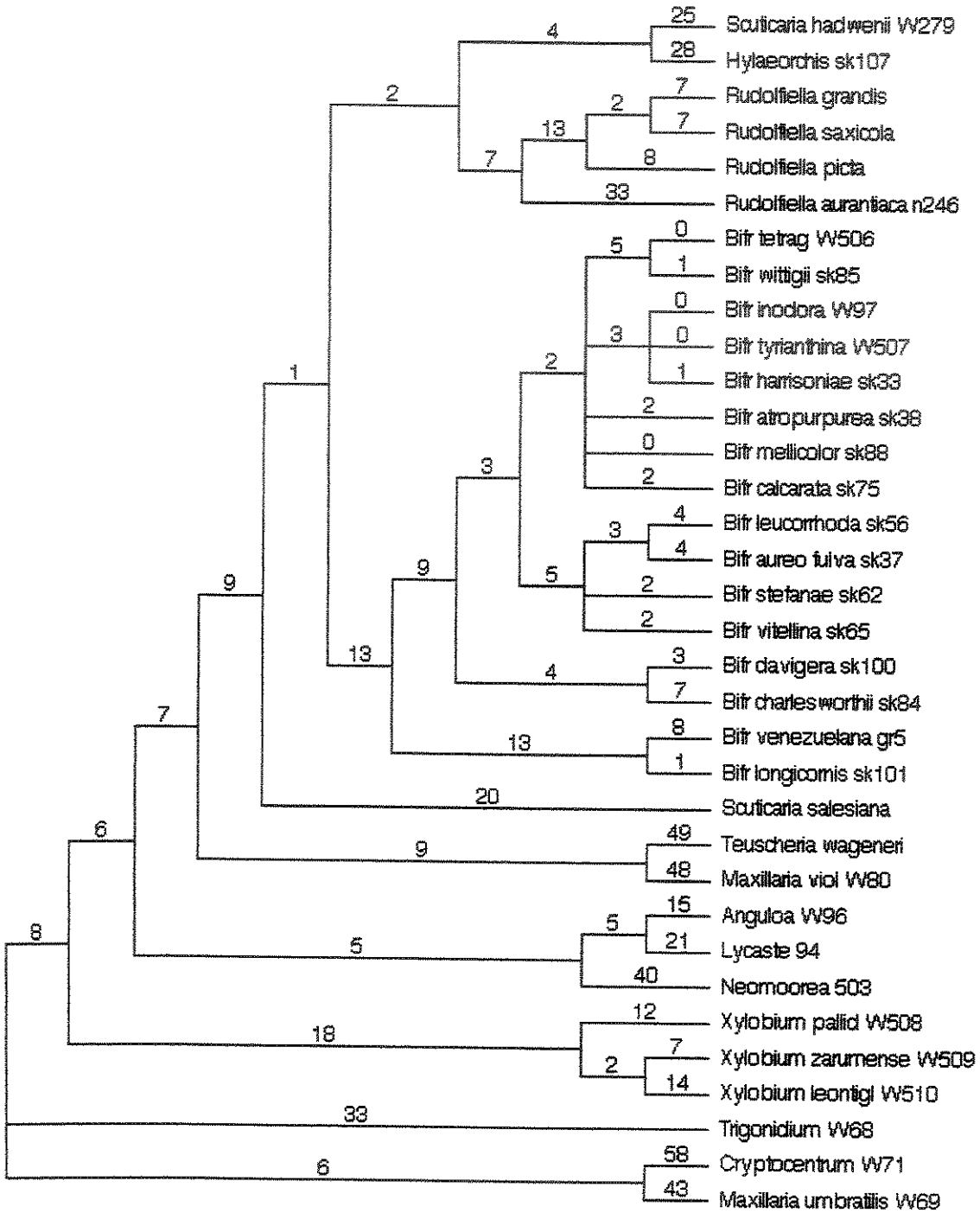
Reagente	Quantidade
Terminator Mix (Big Dye)	1.5 µl X (# amostras + 10%)
Tampão 5X	3.5 µl
primer (somente um!)	1.0 µl
água miliq.	13.0 µl
Total	19.0µl

1. Programe a máquina de PCR para 4 °C.
2. Ao atingir a temperatura de 4 °C, adicione 19µl da solução MIX em cada tubo. A solução MIX 1 (contendo o oligonucleotídeo 3'-5') deverá ser adicionada na primeira metade dos tubos e a solução MIX 2 (contendo o oligonucleotídeo 5'-3') na segunda metade.
3. Adicione 1.0 µl de DNA em cada tubo.
4. Feche tubos e assegure-se de que a solução está concentrada no fundo do tubo, sem gotas espalhadas pelas paredes.
5. A reação dura aproximadamente 2hs. Após o final, os produtos poderão ser armazenados em freezer indefinidamente (as amostras deverão sempre ser armazenadas no escuro, pois os marcadores fluorescentes são sensíveis à luz).

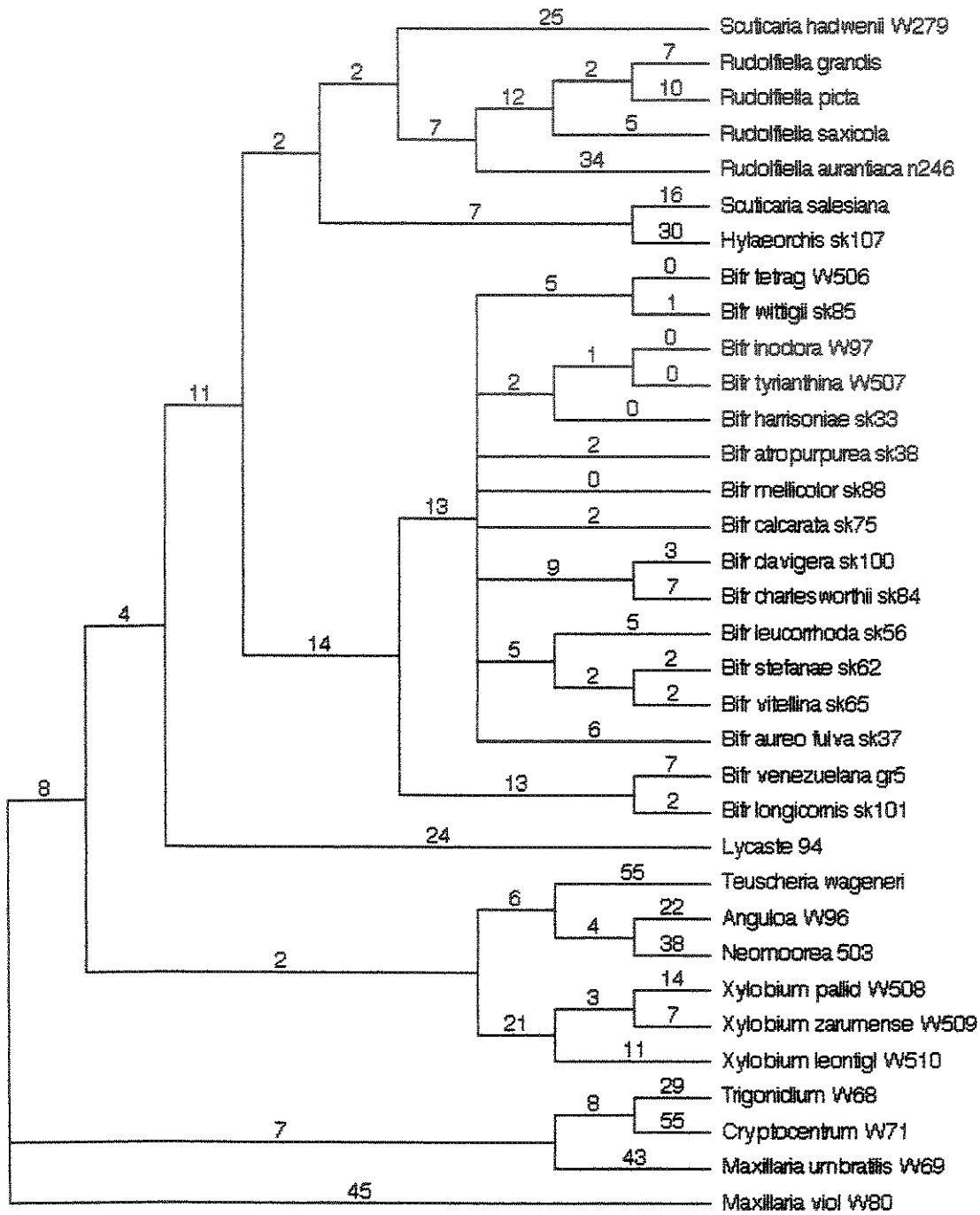
Reação de seqüenciamento:

1. Após o término do programa, os produtos deverão ser transferidos para tubos de 1,5ml (NUMERE OS TUBOS FACILMENTE, ex.: 1, 2, etc. E NÃO WG4456-09! - pois estes serão os tubos que serão levados para o seqüenciador)
2. Em um tubo de 1,5ml, prepare uma solução MIX de 1000 µl de 95% ethanol, mais 40µl de acetato de sódio 3M (esta quantidade é suficiente para 19 tubos).
3. Pipete 52 µl da solução MIX e em seguida adicione 20µl do produto da reação de seqüenciamento. Agite tubo rapidamente em vórtice.
4. Centrifugue por 20 min em velocidade máxima (13,000 rpm). O pellet NUNCA é visível neste estágio.
5. Descarte o sobrenadante.
6. Adicione 250 µl de etanol 70% em cada tubo, descarte o sobrenadante novamente e seque os tubos em papel toalha. Repita o procedimento.
7. Deixe os tubos secarem COMPLETAMENTE até que nenhuma gota de etanol esteja visível, pois o etanol interfere no gel de sequenciamento. IMPORTANTE: deixe os tubos secando em um lugar escuro, pois os marcadores degradam na presença dea luz.
8. Armazene os produtos no escuro e no freezer até serem encaminhados para o seqüenciador.

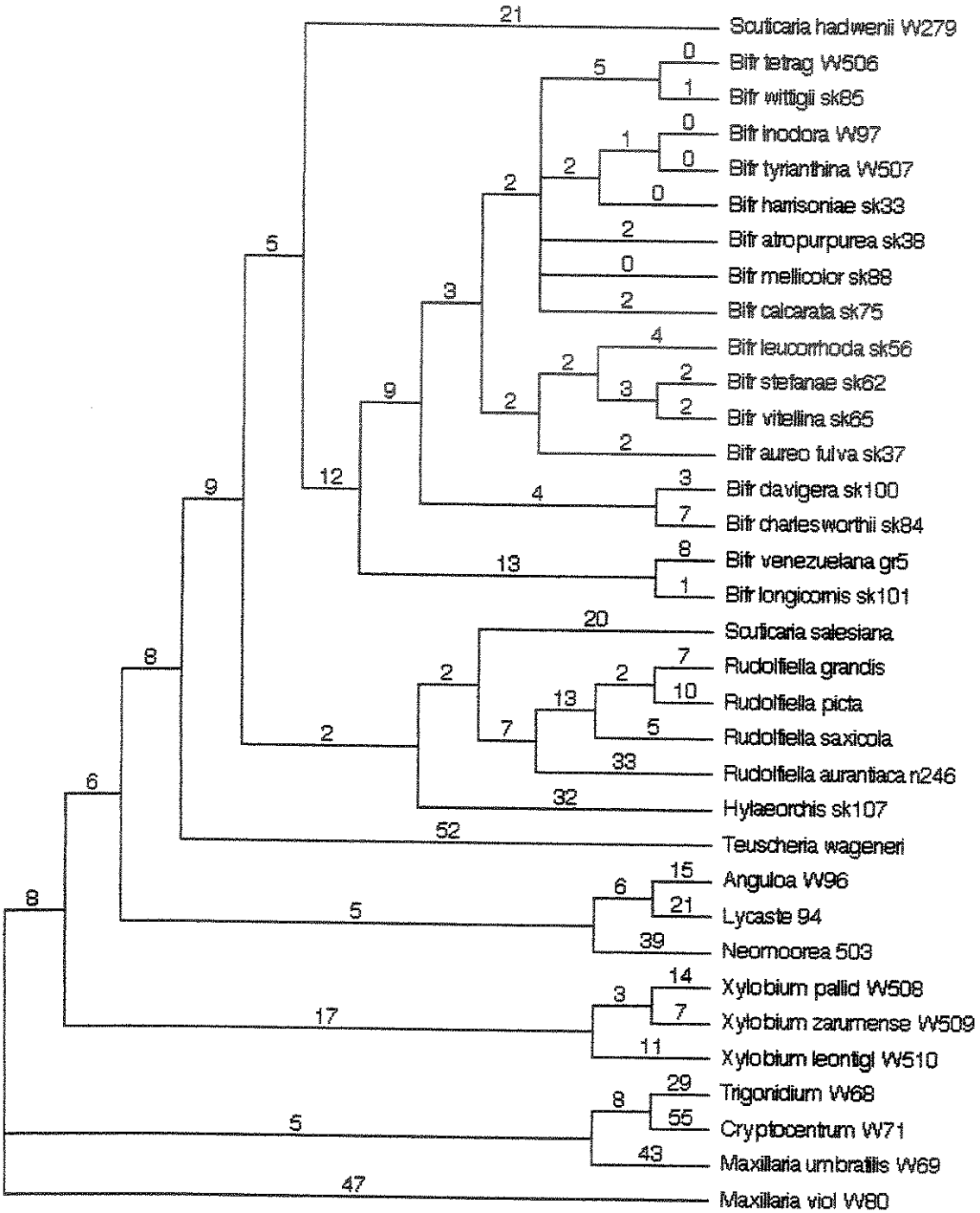
Apêndice 5. Uma das 743 árvores mais parcimoniosas obtidas na análise de sequências das regiões ITS 1 e ITS 2 do DNA ribossômico nuclear. O número de passos está indicado acima de cada ramo.



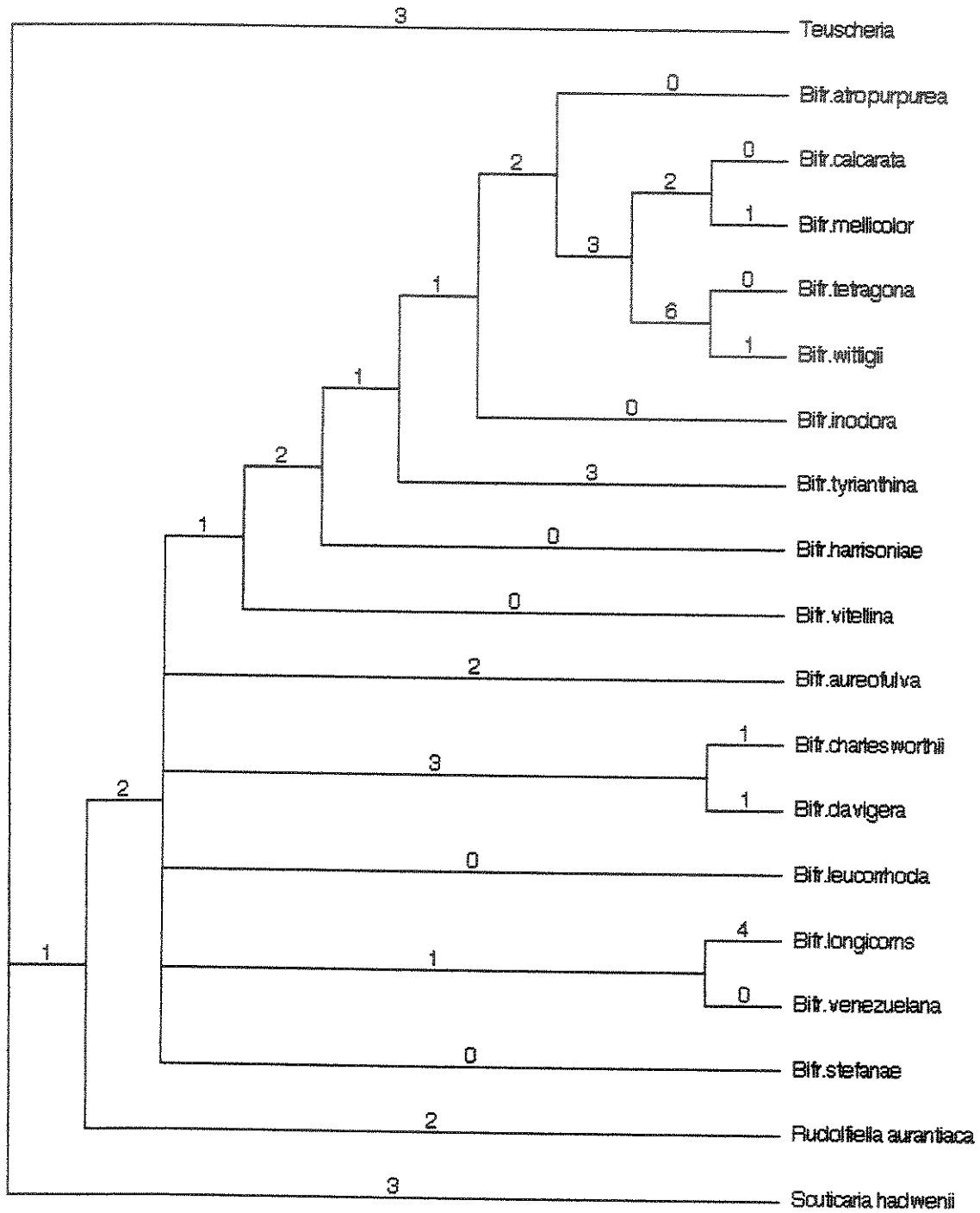
Apêndice 6. Uma das 1117 árvores mais parcimoniosas obtidas na análise de sequências da região *trnL-F* do DNA de cloroplastos. O número de passos está indicado acima de cada ramo.



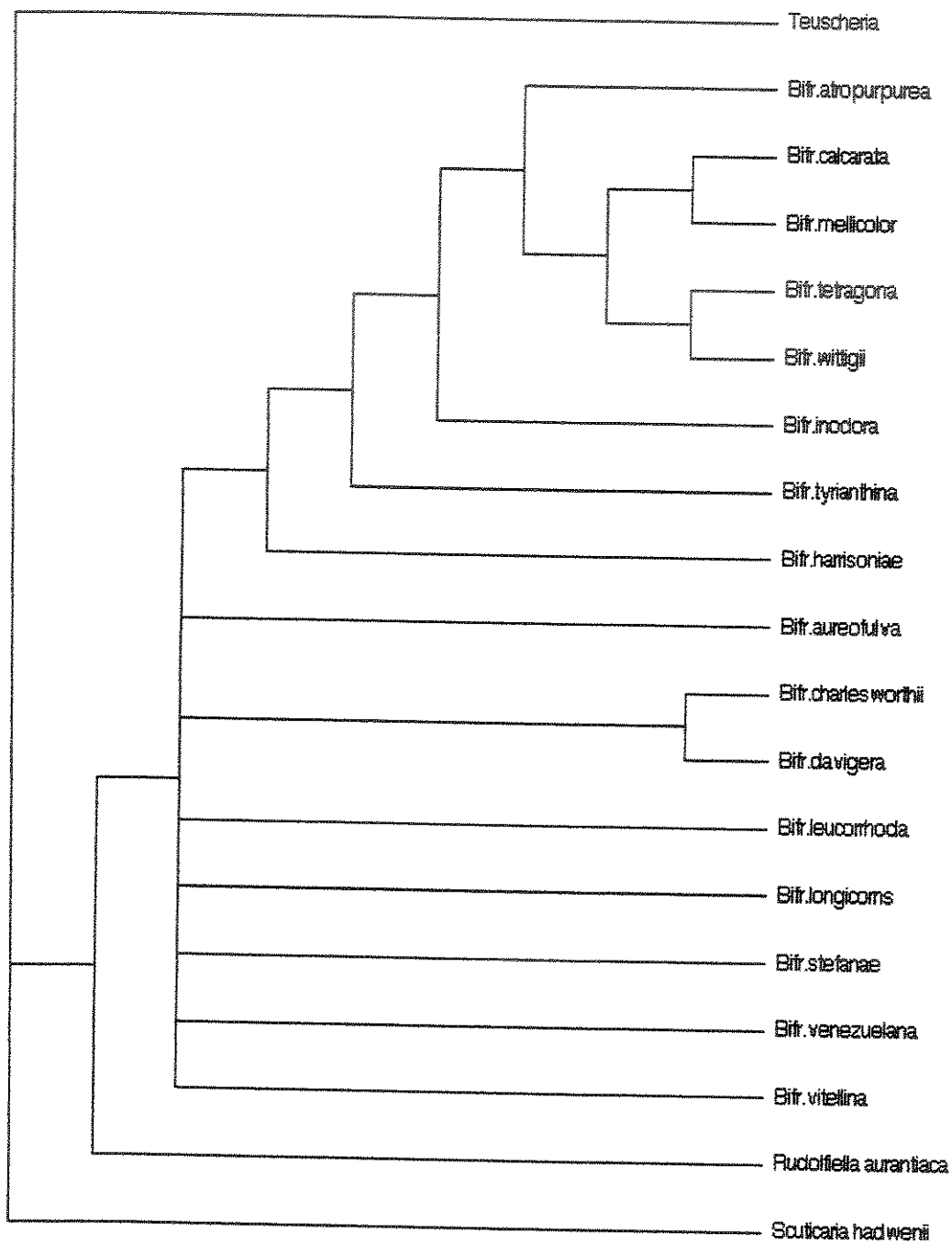
Apêndice 7. Uma das 1860 árvores mais parcimoniosas obtidas na análise de sequências das regiões ITS 1 e ITS 2 do DNA ribossômico nuclear e da região *trnL-F* do DNA de cloroplastos. O número de passos está indicado acima de cada ramo.



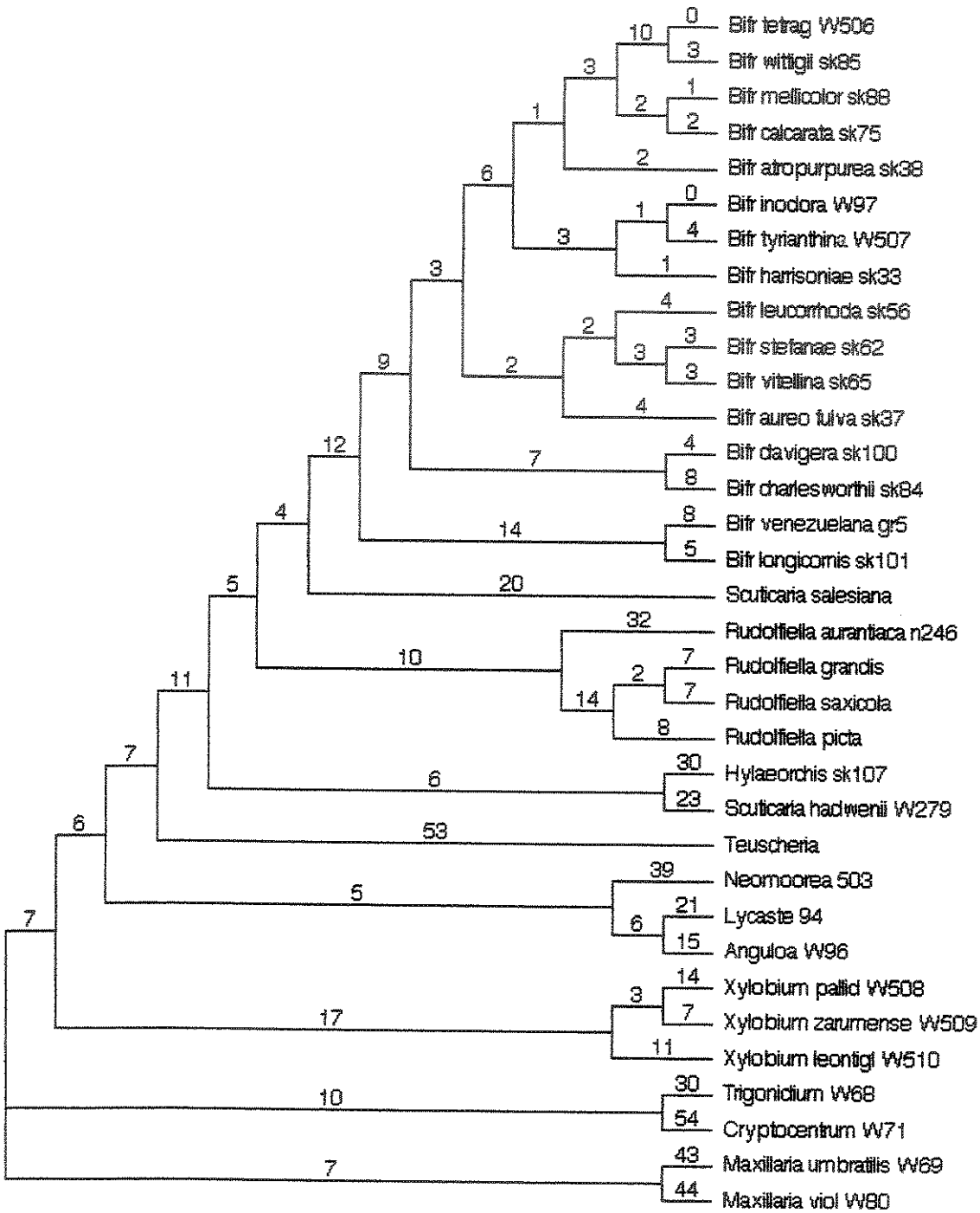
Apêndice 8. Uma das 253 árvores mais parcimoniosas obtidas na análise de caracteres morfológicos. O número de passos está indicado acima de cada ramo.



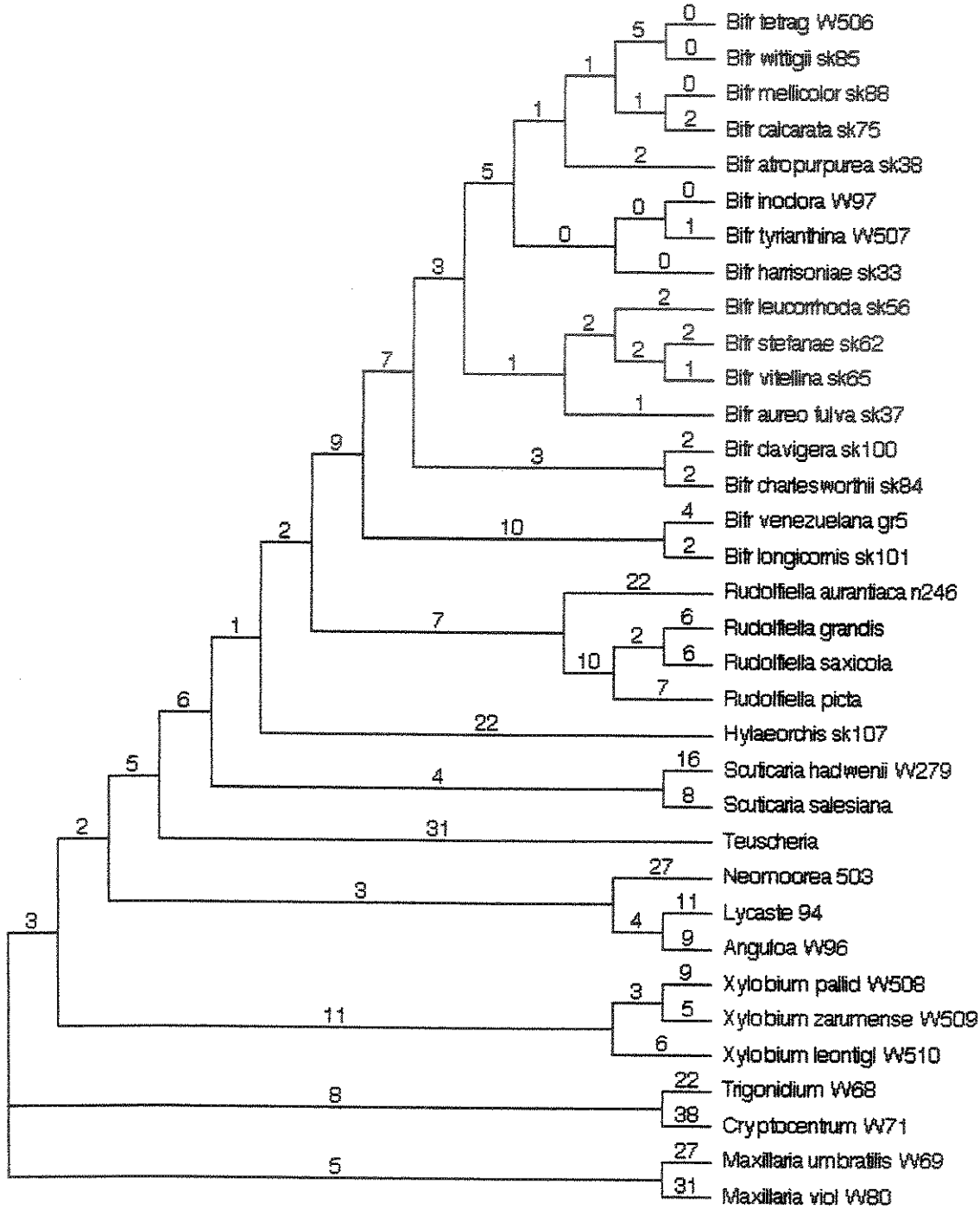
Apêndice 9. Uma das 71 árvores mais parcimoniosas obtidas na análise, com pesagem sucessiva, de caracteres morfológicos. O número de passos está indicado acima de cada ramo.



Apêndice 10. Uma das 708 árvores mais parcimoniosas obtidas na análise combinada de sequências de DNA (ITS 1 e 2; *trnL-F)* e de caracteres morfológicos. O número de passos está indicado acima de cada ramo.



Apêndice 11. Uma das duas árvores mais parcimoniosas obtidas na análise combinada, com pesagem sucessiva, de sequências de DNA (ITS 1 e 2; *trnL-F)* e de caracteres morfológicos. O número de passos está indicado acima de cada ramo.



CAPÍTULO 2

A TAXONOMIC STUDY OF *BIFRENARIA* LINDL. (ORCHIDACEAE)

ABSTRACT

A taxonomic study is presented for the South American orchid genus *Bifrenaria* Lindl. (Epidendroideae, Maxillarieae), which is characterized by four-angled, unifoliate pseudobulbs, plicate leaves, and by flowers bearing a conspicuous spur and a forked stipe. We consider *Bifrenaria* a monophyletic group comprising the genera *Adipe* (=*Stenocoryne*), *Cydoniorchis* and *Bifrenaria* sensu stricto. The main goals of this study were to present a taxonomic synopsis for the genus *Bifrenaria*, determining which taxa should be recognized, their distributions and which characters traditionally used to discriminate species are taxonomically informative. Identification keys, descriptions, distribution maps, and illustrations of the species are also provided.

Bifrenaria Lindl. is a South American genus of the Orchidaceae (subfamily Epidendroideae, tribe Maxillarieae) easily recognized by four-angled, unifoliate pseudobulbs, plicate leaves, and, generally, by flowers bearing a conspicuous spur and a forked stipe. The majority of the species occurs in southern Brazil as epiphytes in the Atlantic Forest and, less frequently, as rupicolous plants in the Brazilian "campos rupestres" vegetation. Two species occur exclusively in the Amazonian Region.

TAXONOMIC HISTORY

Bifrenaria was first described by Lindley in 1832, based on *B. atropurpurea*, a robust plant with large flowers and with the inflorescence shorter than the pseudobulbs. In 1843, Lindley also described the genus *Stenocoryne*, based on a delicate, long-inflorescence specimen with small flowers, type of the species *S. longicornis*. Curiously, Rafinesque in 1836 had described the genus *Adipe* based on *Adipe racemosa*, a species very similar to *S. longicornis* in regarding vegetative and inflorescence characters, although undoubtedly different from it regarding the floral morphology. Apparently, the genus *Adipe* was ignored by Lindley when describing *Stenocoryne* as well as by all subsequent authors (Kraenzlin 1896, Cogniaux 1902, Hoehne 1944, Pabst and Dungs 1977, Castro 1991a, 1991b, 1991c). It was only in 1990, that Wolff proposed new combinations for the genus *Adipe*, including the transference of *S. longicornis*. He did not present, however, the criteria he used to include or exclude species from the genus *Adipe*. Schlechter (1914) described a new genus

belonging to the *Bifrenaria* complex, which he named *Lindleyella* (nom. illeg., later validated under the name *Rudolfiella* by Hoehne, 1944), based on *Bifrenaria aurantiaca* Lindl. This new genus was defined by the abruptly divided lateral lobes of the labellum; the presence of a prominent claw at the base of the labellum and of a conspicuous callus.

It was only in 1944 that Hoehne provided the first taxonomic revision of the complex *Bifrenaria*. In this revision Hoehne separated *Bifrenaria, Stenocoryne* and *Rudolfiella* based on plant size, pseudobulb shape, inflorescence length, flower size, form of the labellum, length of the labellum claw, presence and shape of the spur and pollinarium structure. Although this classification was based on several characters, Hoehne (1953) himself admitted to be uncertain about the position of some species. Classification within the *Bifrenaria* complex was further complicated by the description of *Cydoniorchis* by Senghas in 1994. He followed previous authors when giving much emphasis on floral characters and transferred *Bifrenaria tetragona* (Lindl.) Schltr. and *B. wittigii* (Rchb. f.) Hoehne to *Cydoniorchis* due to the presence of an entire stipe on the pollinarium, inflorescences with numerous flowers and the erect position of the perianth segments. The latest taxonomic treatment available for this complex (Castro 1991a, 1991b, 1991c; 1996) recognizes only the genus *Bifrenaria*, excluding *Rudolfiella*, with 19 species and five sections.

A phylogenetic analysis based on DNA sequence data and morphology (Koehler et al., unpubl. data) concluded that *Bifrenaria* constitutes a monophyletic group comprising the genera *Adipe*, *Cydoniorchis* and *Bifrenaria* sensu stricto. They also concluded that *Bifrenaria* sensu stricto and *Cydoniorchis* are monophyletic, but not *Adipe*. Any attempt to maintain *Cydoniorchis* as a separate genus would demand the creation of seven new genera, with no strong support. Consequently, widening the circumscription of *Bifrenaria* is the best way to maintain nomenclatural stability. Based on such evidences, we propose the reduction of the genera *Adipe*, *Stenocoryne* and *Cydoniorchis* to synonymy with *Bifrenaria*.

The main goals of this study are to present a taxonomic synopsis for the genus *Bifrenaria*, determining which taxa should be recognized, what are their distributions and which characters traditionally used to discriminate species are taxonomically informative. Identification keys, descriptions, distribution maps, and illustrations of the species are also provided.

MATERIAL AND METHODS

Herbaria material from BHCB, BHMH, CESJ, HB, HRCB, IAN, INPA, MBML, MO, MG, NY, PACA, R, RB, SP, SPF, UEC and VIC, as well as cultivated specimens were analysed for morphological studies. Leaf surfaces and flowers structures (labellum, column, anther cap) were analysed with a low vaccum electron microscope. Plant material was either fixed in ethanol 70% or obtained from herbaria material. Delicate structures were dehydrated through a graded ethanol series and posteriorly critical-point dried with CO_2 and coated in vacuo with gold-palladium. Plant segments were attached to aluminim stubs with double-sided sticky carbon tabs and examined by means of back-scattered electron imaging using a Jeol 5800 LV scanning electron microscope at an accelerating voltage of 10 kv. Anatomical studies were performed with fresh material and formalin-acetic acid-alcohol (FAA) fixed material, fixed for at least 24h, and then stored in ethanol 70%. Freehand transversal sections of mature leaves and pseudobulbs were made in a standardized region equidistant from the base and apex of the lamina. Sections were then stained in Safranine-astral blue (Maácz ¨Vagás 1961), observed and photographed under bright field with a XX microscope.

MORPHOLOGY

The genus *Bifrenaria* comprises plants with sympodial growth, 10-60 cm high. They are typically epiphytes, but some species are facultatively terrestrials (*Bifrenaria atropurpurea* Lindl.) or rupicolous (*B. harrisoniae* Rchb. f., *B. inodora* Lindl.). *B. tyrianthina* (Lodd.) Rchb. f. is the only species exclusively rupicolous. The roots are cylindrical, fleshy and present a well-developed velamen.

The stem system is composed of rhizome and pseudobulbs. Pseudobulbs of *Bifrenaria* are composed of a single, thick internode commonly bearing papery sheaths at the base and a single leaf at the apex. The leaves are convolute in development, plicate and leathery. The basal portion of the leaf forms a slender cylindrical pseudo-petiole.

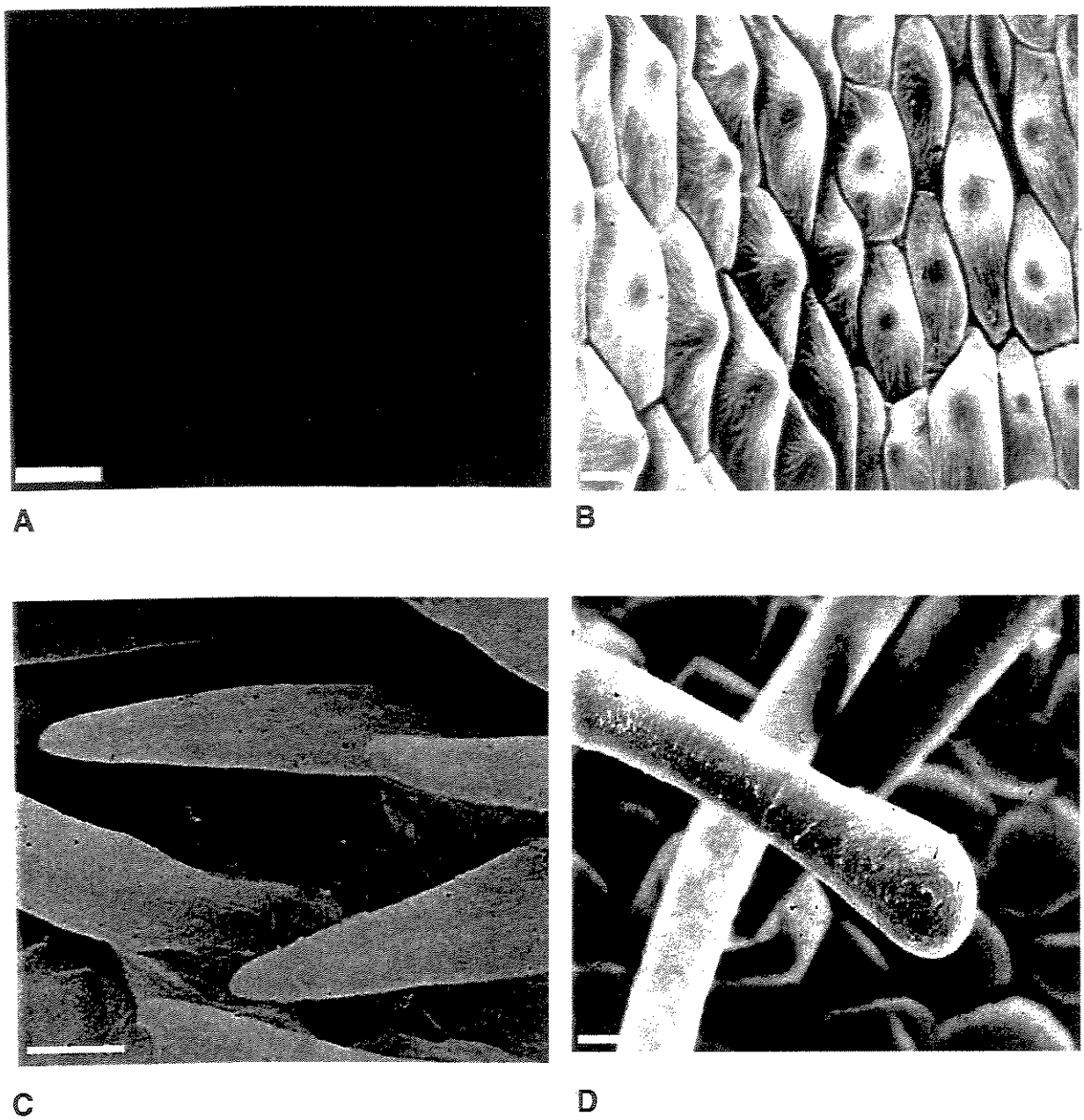


Fig. 1. (A-B) Trichomes from the spur. (A) *Bifrenaria tetragona* (scale= 10m), (B) *B. longicornis* Lindl. (scale = 10m). (C-D) Trichomes from the labellum. (C) *B. harrisoniae* (scale= 20m), (D) *B. aureo-fulva* (scale= 20m).

Inflorescences are racemose, arising laterally from the base of pseudobulbs and are subtended by bracts. Species formerly included in the genus *Bifrenaria* sensu stricto have traditionally been characterized by few-flowered inflorescences, whereas species placed in the genus *Adipe* (*Stenocoryne*), here considered as synonymous to *Bifrenaria*, by inflorescences with several flowers. The analysis of a large sample of specimens showed that many species of *Bifrenaria* sensu stricto, such as *B. inodora* and *B. tetragona*, may present up to 10 flowers in a single inflorescence, while *B. charlesworthii* and *B. stefanae*, earlier placed in the genus *Adipe* (=*Stenocoryne*) may bear only two-flowered inflorescences.

The flowers are zygomorphic, resupinate, with each pedicel subtended by a floral bract. Most species present strong to delicate, pleasant fragrances. The three sepals are usually slightly larger than the lateral petals, and the two lateral sepals are united to the column foot to form the spur. The spur may present trichomes and/or papillae (Figs. 1a-b). The labellum is differentiated considerably from the other perianth segments. It varies from entire to distinctively 3-lobed, usually bearing a claw. Stripes and dots may be present, as well as trichomes (Figs.1c-d). Its basal portion is modified into a callus, which generally consists of vertically oriented ridges that end in a fleshy lump, often divided into two or three lobes. In *B. tetragona* and *B. wittigii*, the callus consists of a fleshy plate that occupies all the basal portion of the labellum. The callus also may present trichomes, papillae, and stomata, or it may have a completely glabrous surface (Fig. 2). Histochemicals assays for polysaccharide were made on fresh labellum tissue (callus and midlobe regions) and on spur tissues of the species *Bifrenaria charlesworthii* Rolfe and *B. harrisoniae* with ruthenium red 1% (Baas and Gregory 1985). The same regions were also stained with Sudan Black B 1% to test for the presence of fatty acids (Jensen 1962). In all tissue regions stained with Sudan Black B the cytoplasm of trichomes revealed the lipidic contain of these cells (fig 3c). Tests with ruthenium red suggested the absence of nectar in the cytoplasm of trichomes, since the cytoplasm of these cells did not stain or stained weakly (Fig 3d).

The column is semi-cylindrical, elongated, slightly bend downward, usually with no lateral expansions, bearing trichomes or not (Figs. 3a-b). Its base bears a ventral extension, the column foot, to which the labellum is attached. The foot may also bear trichomes.

The anther is incumbent and terminal, and it bears four laterally flattened, superposed pollinia. The pollinarium also presents two, rarely one, well-developed stipe and an extended viscidium. There is no distinct external division between the ovary and the pedicel.

The fruits are either pendent or erect (Figs. 4a-b), green, with stomata (Fig. 4c), and always with thick outer cell walls. They may take up to eight months to mature. According to Dressler (1993) *Bifrenaria* present seeds of the *Maxillaria* type, described as dust seeds, 250-500 μm long, yellowish to brownish with terminal testa cells isodiametric (or nearly so) and medial sector cells strongly elongate; marginal ridges are present and round in section, cell-border ridges are fine and may be concealed by marginal ridges; the periclinal walls have prominent reticulate or longitudinal thickenings – fine warts or micropapillae may be present (Chase and Pippen 1988; Dressler 19981; 1993). Seeds examined by us vary from brownish to yellow (Fig 2), ranging from 180-350μ in length by 90-100μ in width. They are oblong to elongate, with one end not funneled (Figs. 5a-b). The testa cells are ovate to rectangular, elongated or only at the median region, with no expansions. Fine warts have been observed in the testa cell walls of *B. harrisoniae* and *B. tyrianthina* (Figs. 5d).

ANATOMY

Roots

The velamen of *B. atropurpurea, B. clavigera, B. harrisoniae, B. inodora* and *B. tyrianthina* was analysed by Pridgeon (1983, 1987), Porembski and Barthlott (1988) and Bezerra et al. (2001). The velamen of these species is composed of 2 to 12 layers of hexagonal epidermal cells, bearing helical thickenings, which defines the *Cymbidium* velamen type (Porembski and Barthlott 1988). The external layer consists of smaller and lignified cells, with external periclinal walls thicker, bearing unicellular hairs (Sandford and Adanlawo 1973; Arditti 1992, Bezerra et al. 2001). Tilosomes, or lignified excrescences from the walls of cells of the innermost velamen cell layer adjacent to thin-walled passage cells of the exodermis of roots (Pridgeon et al. 1999), have not been reported for the genus, although they are found in the genera *Lycaste, Neomoorea* and *Xylobium* (Pridgeon et al. 1983). The cortex bears poliedrical cells. Raphide-containing idioblasts, tracheiodal

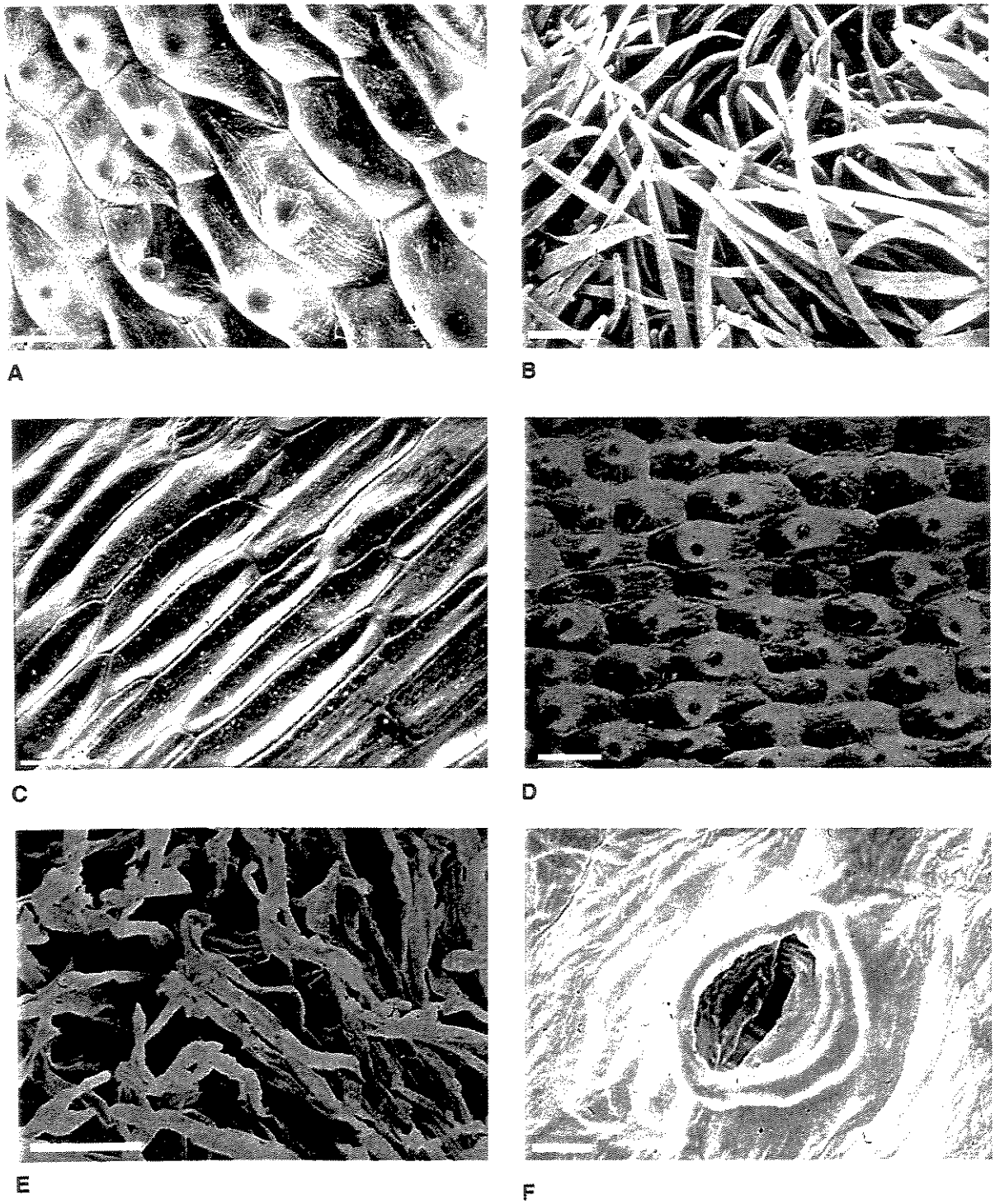


Fig. 2. (A-E) Trichomes and papillae from the callus, anterior, protuberant region. (A) *Bifrenaria longicornis* (scale = 20m), (B) *B. harrisoniae* (scale = 200m), (C) callus glabrous *B. aureo-fulva* (scale = 10m), (D) trichomes or papillae from the callus, posterior, flat region (D) *B. longicornis* (scale = 20m), (E) *B. clavigera* (scale = 50m). Stomata from callus, (E) *B. calcarata* (scale = 5m).

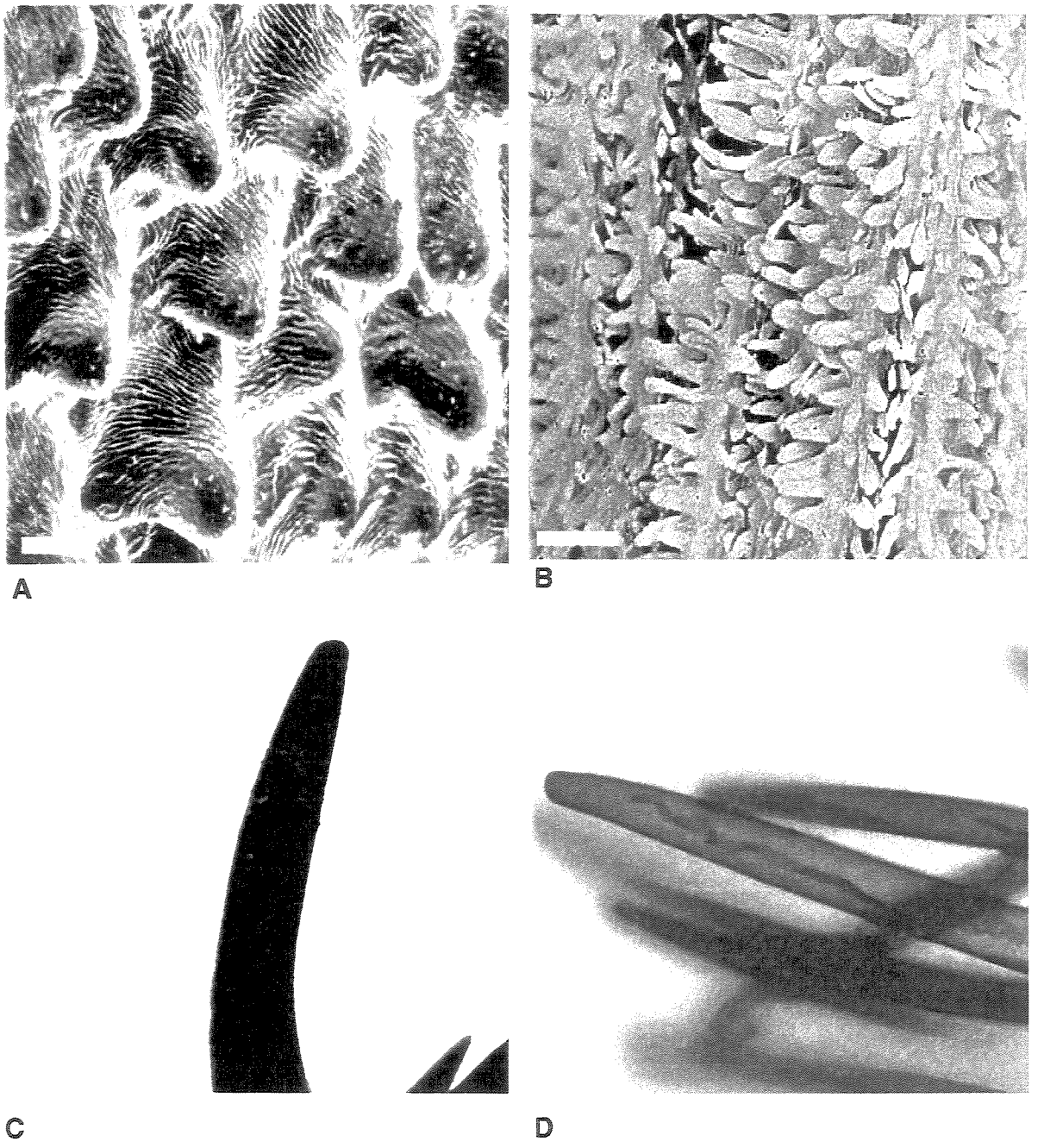
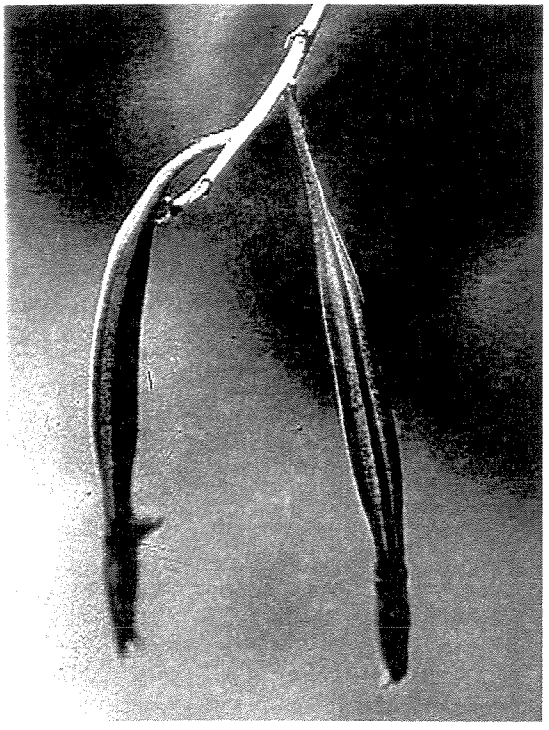
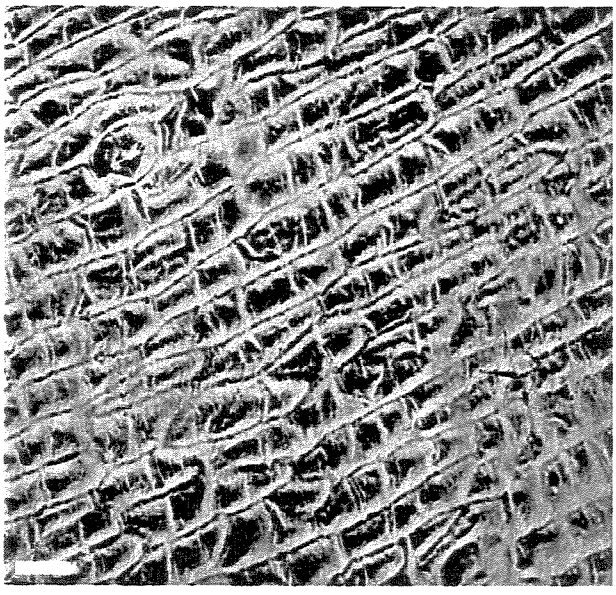


Fig. 3. (A-B) Trichomes from column. (A) *Bifrenaria aureo-fulva* (scale = 10m), (B) *B. calcarata* (scale = 100m). (C-D) Trichomes from the labellum of *B. harrisoniae* stained with Sudan Black B 1% (C) and with ruthenium red 1% (D), indicating the presence of fatty acids (C) and the absence of polissacarids (D) in the cytoplasm of the trichome.



A

B



C

Fig. 4. (A) Pendent fruit of *Bifrenaria aureo-fulva* (B) Erect fruit of *B. calcarata*, after dehiscence (C) Stomata on ovary wall of *B. clavigera* (scale = 20m).

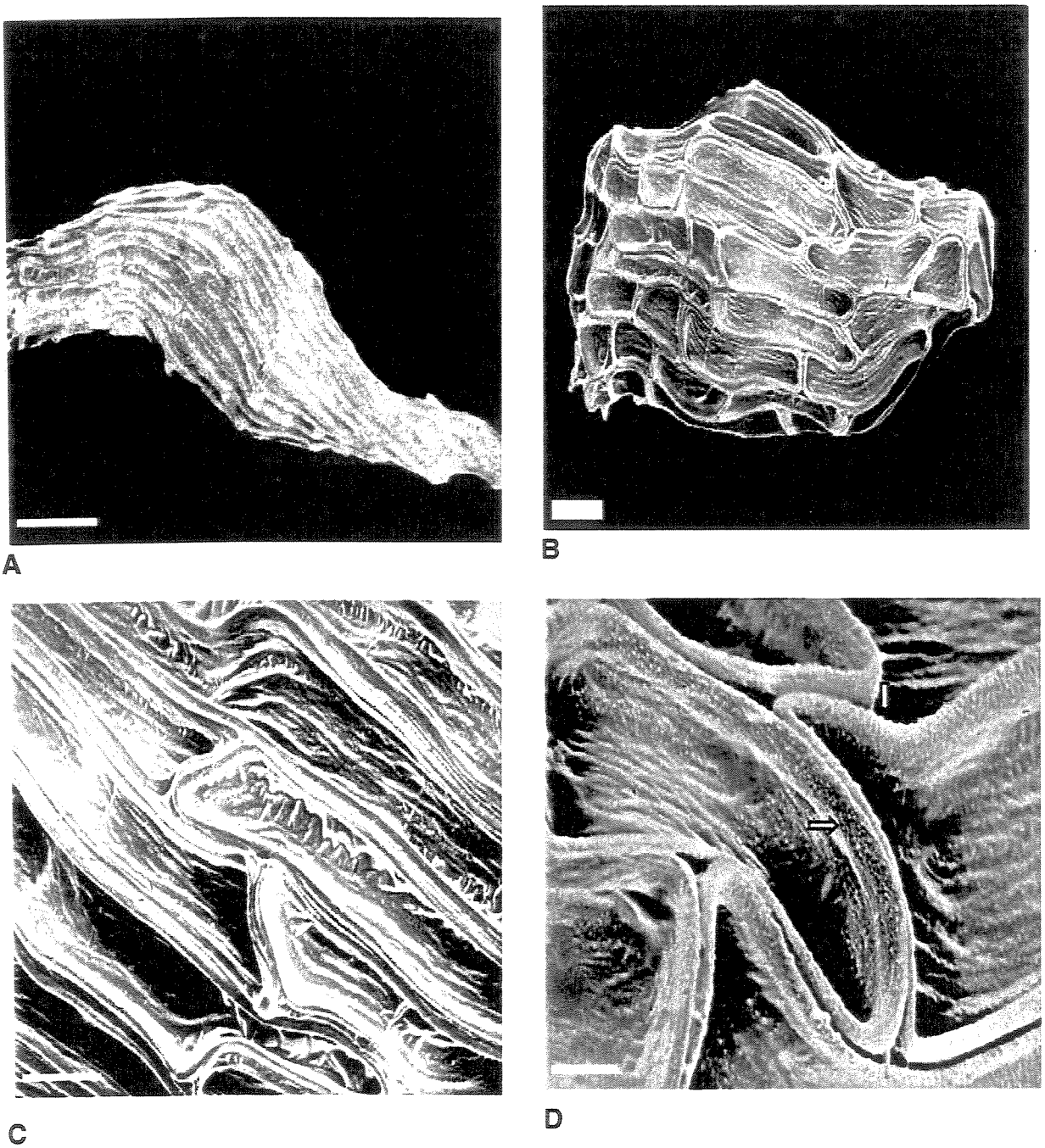


Fig. 5. (A-B) Seeds. (A) *B. harrisoniae*. (scale = 50m), (B) *B. aureo-fulva*. (C-D) Testa walls. (C) *B. aureo-fulva*, smooth walls (scale = 20m). (D) *B. tyrianthina*, ornamented walls are indicated (scale = 5m).

idioblasts and sclerenchimatic cells are present throughout the cortex. The endoderm is composed of a single layer of poliedrical cells with "O" thickenings (Porembski and Barthlott 1988, Bezerra et al. 2001). Thin-walled passage cells are present. The pericycle is composed by cells with lignified and thickened cell walls with thin-walled cells close to the protoxylem. The vascular cylinder with vascular sclerenchyma bordering the phloem. The pith is composed by parechymatic cells bearing lignified and thickened walls, with central cells not lignified (Bezerra et al. 2001)

We did not examined additional taxa due to the lack of variation observed in previous anatomical studies. According to Porembski and Barthlott (1988) and Pridgeon (1987) the genera *Rudolfiella, Xylobium* and *Lycaste*, closely related to *Bifrenaria*, present similar number of velamen layers as well as tracheoidal idioblasts. The genera *Anguloa, Lycaste* and *Xylobium* present outer tangential walls and radial walls thickned, while *Lycaste denningiana* Rchb. f. and all the species of *Bifrenaria* analysed present outer tangential walls thickened. Although tilosomes are frequent in all species of *Xylobium, Lycaste, Neomoorea* studied, as well as in all species of the closely related subtribe Maxillarinae, they are absent in all species of *Bifrenaria* examined. Studies considering the sister genera of *Bifrenaria* (*Rudolfiella, Scuticaria, Hyleorchis*) are necessary to assess the diagnostic value of this character.

Pseudobulbs

We have studied the pseudobulbs of seven species: *Bifrenaria aureo-fulva, B. calcarata, B. charlesworthii, B. harrisoniae, B. stefanae* and *B. wittigii.* The cuticule is thick, hairs and stomata are absent. Epidermal cells are isodiametric with irregular thickened walls (Fig. 6a), a feature also registered for for species of *Maxillaria* and *Xylobium* (Solereder and Meyer 1930). Pits were observed in the cellulose part of outer walls of *Bifrenaria atropurpurea*, as well as in species of *Lycaste* and *Xylobium* (Solereder and Meyer 1930). A hypodermis 2-3 layered, bearing sclerified cells was recorded for *Bifrenaria harrisoniae*, *Lycaste aromatica* Lindl. and *Xylobium squalens* (Lindl.) Lindl. The ground tissue comprises three to four layers composed of small-sized, elliptic living cells with thin walls intermingled with water storage cells, which are large and dead (Fig. 6a-b). The vascular system is composed of scattered collateral vascular bundles (Fig. 6b-c).

The phloem sclerenchyma is well developed and composed of thick-walled fibres, whereas the xylem sclerenchyma is less developed (Fig. 6c). Similar patterns have been recorded for *Lycaste aromatica* and *Xylobium squalens*. Starch grains were observed in parenchima cells of *B. aureo-fulva* (Fig. 6d).

According to Pridgeon (1999) the axis (stems, rhizomes and pseudobulbs) is the least useful organ in systematic studies. The anatomy of pseudobulbs can be rather homogenous or it may vary even within a species (Arditti 1992). All the information available for pseudobulb anatomy of *Bifrenaria* and related genera was synthesized by Solereder and Meyer (1930). None additional studies have been developed since then. Characters with taxonomical value were not recorded for the species examined in this study. It seems to be no anatomical features that characterizes the genus *Bifrenaria* nor the genera of Bifrenariinae and Lycastinae.

Leaves

We have studied the leaf anatomy of ten species: *Bifrenaria atropurpurea, B. aureo-fulva, B. calcarata, B. charlesworthii, B. clavigera, B. harrisoniae, B. leucorrhoda, B. longicornis, B. stefanae* and *B. tetragona.* The cuticule is thick, like in many other orchid species (Solereder and Meyer 1930), especially on the adaxial side, and rugulose (Figs. 7a-b) or smooth. The hairs (Fig. 7a) are multicellular, solitary, sunken in crypts, and usually present on both surfaces. This type of trichome has been recorded for many species of Orchidaceae, including the genera *Lycaste*, *Maxillaria* and *Xylobium* (Solereder and Meyer 1930). Epidermis cells are isodiametric and present thin to irregular thickened walls (Figs. 7a-b). Stomata are abaxial only, always with cuticular ridges (Figs. 7b-d). An hypodermis is absent, as it is also in the genera *Lycaste* and *Xylobium.* An abaxial hypodermislike cell layer has been recorded for *Scuticaria steelii* Lindl. Mesophyll fibre bundles are small-sized, comprising thickened wall fibres regularly occurring in a uniseriate row throughout the abaxial mesophyll. They are also characteristic for *Lycaste, Maxillaria, Scuticaria* and *Xylobium.* The mesophyll is undifferentiated and composed of round, chlorenchymatous, thin-walled cells. Raphide-containing idioblasts are present throughout the mesophyll. Vascular bundles are moderately sized to large, collateral, in one linear series. Lignified idioblasts may be present around and between vascular bundles (Figs. 7e-f), but the

presence and absence of such structure seems to vary within species.

Leaf anatomy showed very little intrageneric variation. The anatomical surveys considering other genera of the Bifrenariinae, Lycastinae and Maxillarinae are still restricted to few species (Solereder and Meyer 1930), but based on the data available so far, anatomical leaf characters seem to be of some taxonomical value at generic and subtribal levels.

Based on our preliminary survey, vegetative anatomy does not appear to be of great taxonomic utility within the *Bifrenaria* complex. All characters described display either continuous variation across taxa or showed to be phenotypically plastic, as the lignified idioblasts. Future anatomical studies considering additional genera, such as *Hylaeorchis, Guanchesia, Scuticaria, Rudolfiella* and *Xylobium* may be of some phylogenetic utility as well as detailed anatomical studies of secretory structures of *Bifrenaria* and related genera.

TAXONOMY

BIFRENARIA Lindl. Gen. Sp. Orchid. Pl.152. 1832. TYPE SPECIES: *B. atropurpurea* Lindl.

Adipe Raf. Fl. Tellur. 2. 101. 1836. TYPE SPECIES: *A. racemosa* Raf.

Stenocoryne Lindl. Edward's Bot. Reg. 29. misc. 53. 1843. TYPE SPECIES: *S. longicornis* Lindl.

Cydoniorchis K. Senghas. J. Orchideenfreund 1. 11. 1994. TYPE SPECIES: *C. tetragona* (Schltr.) K. Senghas.

Epiphytes, rarely terrestrial or rupicolous plants to 60cm high. *Roots* cylindrical, white, glabrous. *Rhizome* rigid, very short to elongate, horizontal. *Pseudobulbs* erect, four-angled, pyramidal to laterally compressed, commonly bearing a black ring at the apex, apically unifoliate, and becoming irregularly wrinkled with age. *Leaves* membranaceous to coriaceous, plicate, lanceolate to oblong, acuminate, and attenuate at the base into a pseudo-petiole. *Inflorescence* racemose, lateral, erect or pendent, 1-10 flowered. *Bracts* subtending the inflorescence, brownish, membranaceous to coriaceous, concave, oblong, acuminate. The scape bracts are numerous, truncate, concentrated at the inflorescence base. Floral bracts are fixed at the base, similar in size and form to the scape bracts or smaller. *Flowers* resupinate, zygomorphic, fragrant or not, and of various colors. *Sepals* 3, concave,

ovate, rarely lanceolate or oblong, truncate, rarely attenuate, mucronate. Lateral sepals are parallel to each other or divergent, and prolonged into a spur, constituted by overlapping or fusioned sepal segments. *Lateral petals* obovate, rarely rhombic or lanceolate, slightly asymmetric and always smaller than sepals. *Labellum* entire to 3-lobed, lanceolate to obovate, attached to the column foot by a straight, rarely recurved, claw, the lateral lobes are involute, the midlobe is recurved, rarely straight, glabrous to pubescent, with entire, undulate or crenate margins. The callus extends from the labellum base up to its median region, along the midvein, as a low, thick rib; the anterior region is protuberant, entire to 3-lobed, glabrous to pubescent. The column is clavate, fleshy, arcuate, with an attenuate, rarely apiculate, apex, glabrous to pubescent; the basal portion is prolonged into a foot, erect to arched, glabrous to pubescent. *Anther* helmet-shaped, 4-celled, terminal, incumbent, externally papillose. Pollinarium with 4 asymmetric, superimposed, laterally flattened, ovate to round pollinia; stipes strap-like, 2, rarely 1; viscidium prominent, basely acute, cuneate or truncate. *Stigma* concave; ovary erect to curved. Ovary sulcate. *Capsules* erect or pendent, green, with flower segments persistent until dehiscence.

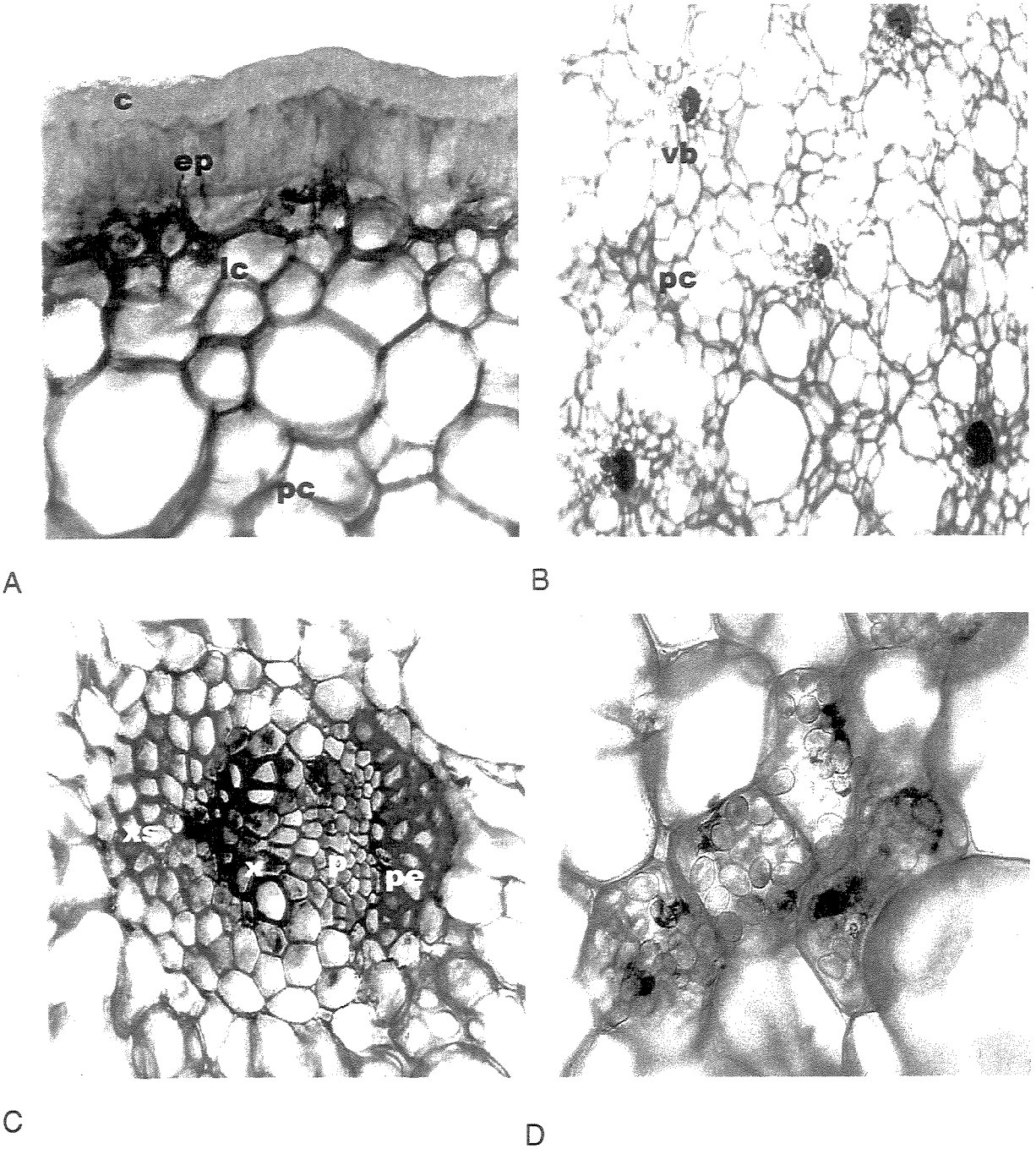


Fig. 6. (A-D) Transections of pseudobulb. (A) *Bifrenaria harrisoniae*; cuticule (c), epidermis (ep), parenchymatic cells with lignified walls (lc) parenchymatic cells (pc) (scale = 20X), (B) *B. harrisoniae,* ground tissue, parenchymatic cells (pc), vascular bundles (vb) (scale = 4X), (C) *B. aureo-fulva.*, central vascular bundle: phloem sclerenchyma (p), xylem (x), xylem sclerenchyma (xs) (scale 10X), (D) *B. aureo-fulva*, parenchymatic cells with starch grains (scale=20X).

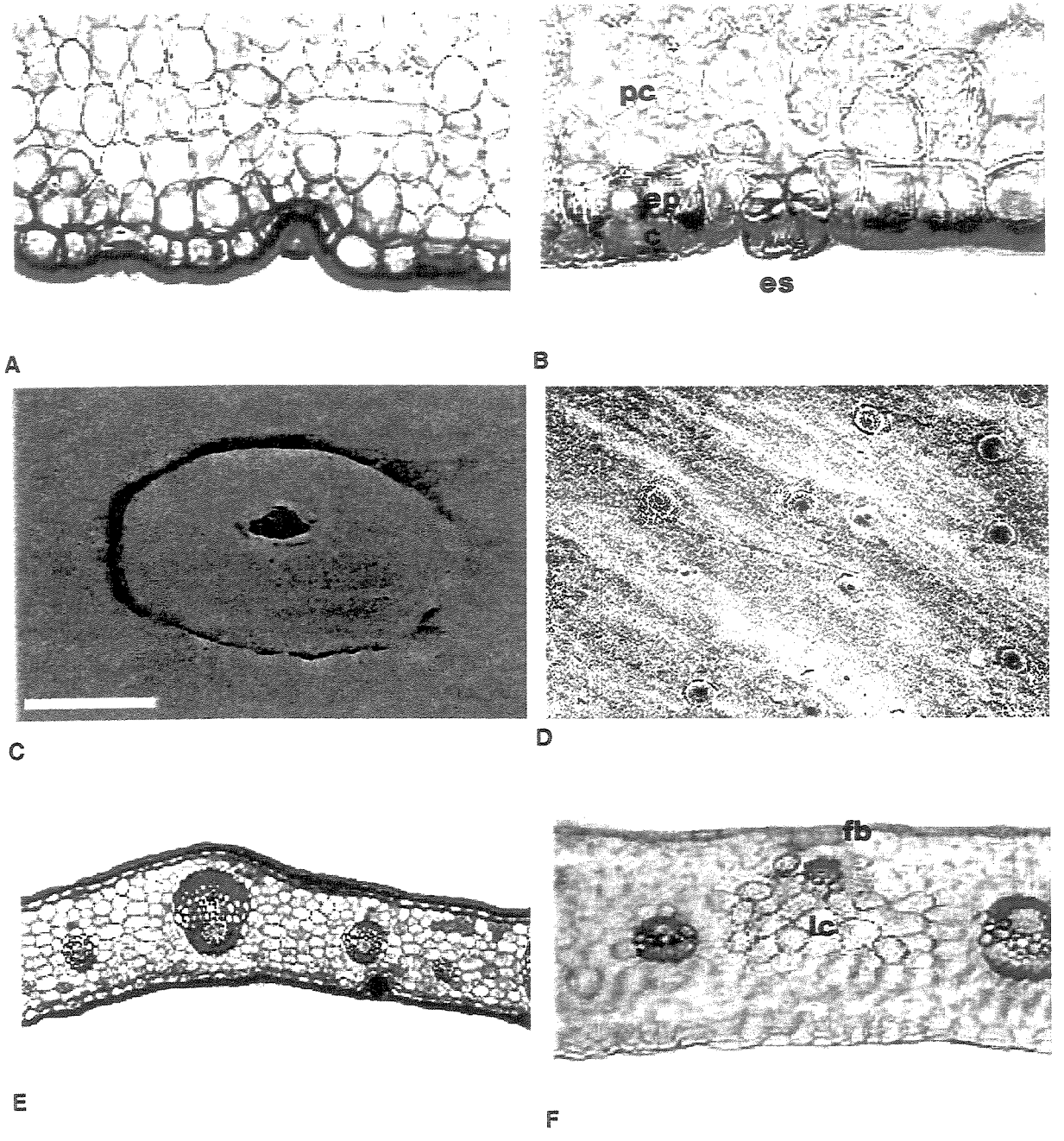


Fig. 7. (A-F) Transections of leaf. (A) *Bifrenaria aureo-fulva*, abaxial epidermis showing a trichome (scale 10X), (B) *B. harrisoniae*, abaxial surface showing stomata (es) with cuticular ridges, cuticule (c), epidermis (e), parenchymatic cells (pc) (scale 40X), (C) *B. leucorrhoda*, abaxial surface showing stomata (scale = 10m), (D) *B. stefanae*, abaxial surface showing stomata (scale=10m), (E) *B. harrisoniae*, mesophyll showing collateral vascular bundles in uniseriate row (scale =10X), (F) *B. aureo-fulva*, mesophyll showing fibre bundles (fb), lignified cells between vascular bundles (lc) (scale = 10X).

KEY TO THE SPECIES OF *BIFRENARIA*

1. Pseudobulbs distant, long rhizomes, plants from northern South America
   2. Inflorescence longer than pseudobulbs, spur prominent, flowers white yellowish with brownish red spots ..... *B. longicornis*
   2. Inflorescence shorter than pseudobulbs, spur inconspicous, flowers vinaceous to pale brown ..... *B. venezuelana*
1. Pseudobulbs aggregate, short rhizomes, plants from southern and southeastern Brazil
   3. Pollinarium with an entire stipe
      4. Callus protuberant on the anterior region only
         5. Labellum midlobe subacute, lateral lobes of labellum truncate, the midlobe subacute ..... *B. calcarata*
         5. Labellum midlobe rounded to truncate, lateral lobes of labellum rounded, the midlobe rounded, folded lenghtwise ..... *B. mellicolor*
      4. Callus completely fleshy
         6. Labellum midlobe glabrous, subacute ..... *B. tetragona*
         6. Labellum midlobe velutinous, rounded ..... *B. wittigii*
   3. Pollinarium with forked stipe
      7. Scape bracts similar in size with floral bracts, flowers with 4 cm diam. or more
         8. Viscidium cuneate ..... *B. atropurpurea*
         8. Viscidium truncate or rounded, never cuneate
            9. Viscidium round ..... *B. tyrianthina*
            9. Viscidium truncate
               10. Flowers greenish to yellow (when old), callus 2-lobed ..... *B. inodora*
               10. Flowers white, yellowish, pink to vinaceous, callus 3-lobed ..... *B. harrisoniae*
      7. Scape bracts generally greater than floral bracts, flowers with 2 cm diam. or less, seldom to 3cm diam.
         11. Sepals and petals lanceolate ..... *B. aureo-fulva*
         11. Sepals and petals ovate, obovate to oblanceolate
            12. Petals parallel to column

13. Lateral sepals divergent to each other, labellum pubescent and orbicular .... *B. charlesworthii*

13. Lateral sepals parallel to each other, labellum scantly pubescent and ovate to elongate ..... *B. racemosa*

12. Petals oblique to column

14. Lateral sepals and petals with spots

15. Labellum flabellate, spur with sepal segments fusioned ..... *B. clavigera*

15. Labellum orbicular, spur with sepal segments superposed ..... *B. silvana*

14. Lateral sepals and petals without spots

16. Flowers white to pale pink ..... *B. leucorrhoda*

16. Flowers pale brown , yellow or orange

17. Lateral sepals parallel to each other, flowers 1cm wide, pale brown to yellow-greenish, without a vinaceous spot ..... *B. stefanae*

17. Lateral sepals divergent to each other, flowers 2.5-3cm wide, yellowish to orange, labellum with a conspicous vinaceous spot ..... *B. vittelina*

1. BIFRENARIA ATROPURPUREA (Lodd.) Lindl., Gen. Sp. Orchid. Pl. 152. 1832. *Maxillaria atropurpurea* Lodd., Bot. Cab. 19: t. 1877. 1832. TYPE: BRAZIL. Rio de Janeiro: s. loc., 1932, *Warre s.n.*. (HOLOTYPE: K-n.v., photo at AMES).

Bifrenaria caparoensis Brade, Orquidea 6: 16. 1943; Arq. Serv. Florest., 2 (1): 6. 1943. *Bifrenaria atropurpurea* (Lodd.) Lindl. var. *caparoensis* (Brade) Hoehne, Arq. Bot. Estado São Paulo n.s. 2 (5): 116. 1950. TYPE: BRAZIL. Minas Gerais: Serra do Caparaó, 2000 m elevation, Oct 1941, *Brade 17141* (HOLOTYPE: RB).

(Figs. 8a, 10a, 11a)

Epiphytes or terrestrials to 40 cm tall. *Pseudobulbs* aggregate, conical, dark green to brownish, 5-8 x 2-2.5 cm. *Leaves* lanceolate to ovate, membranaceous to sub-coriaceous, 15-30 x 6.5-10 cm, pseudo-petiole 0.5-2 cm long. *Inflorescence* 3-6 flowered, erect, up to 10 cm long, concealed by scape bracts, scape and floral bracts 2-3 x 1-1.5 cm. *Flowers* with a sweet and strong fragance, dark red to vinaceous on the exterior, greenish yellow on the interior, the labellum yellowish pink, with red stripes, 3.5 cm long, 2.5 cm wide. *Sepals* ovate, truncate, dorsal sepal 2.5-3.3 x 1.8 cm, lateral sepals parallel to each other, 2-3 x 1.5 cm, spur with sepal segments superposed, 0.5 cm long. *Petals* oblanceolate, truncate, oblique to column, 1.8-2.3 x 1-1.4 cm. *Labellum* 3-lobed, obovate, puberulous, 1.7-1.8 x 1.2-1.3 cm when expanded, lateral lobes erect, involute, obovate, 0.8-1 x 0.3 cm, the midlobe obtuse, rounded, emarginate, recurved, with undulate margins, the claw 0.4 cm long, the callus 1.2 cm long, puberulous, on the anterior region 2-lobed to 3-lobed, protuberant and glabrous. *Column* glabrous, 0.9 – 1 cm, the foot straight, puberulous, 1-1.3 cm long. *Anther* with a prominent apex, 0.3-0.4 x 0.2-0.4 cm, the pollinia rounded, the stipe forked, 0.1 cm long, the viscidium cuneate, 0.1 cm long. *Ovary-pedicel* 3.5-5 cm long.

Habitat. — Native to wet montane forests at 1000-1200 m elevation. Flowering occurs from October to December.

Distribution. — Brazil, Minas Gerais and Rio de Janeiro states. According to Pabst and Dungs (1977) and Castro (1996) this species is also found in the state of Espírito Santo, however we could not find any specimen from this region neither by the examination of herbaria material, nor on field trips to this state.

Brade (1943a, 1943b) described a new species, *Bifrenaria caparoensis* Brade, based on the yellow color of the flowers, and on the format and size of the labellum. He considered this new species very similar to *B. tyrianthina* Rchb. f. and *B. aurea* Barb. Rodr. An examination of the type specimen revealed that *B. caparoensis* is, indeed, very similar to *B. atropurpurea* considering floral morphology and vegetative traits. The only character that separates both species is the color of the flower, red to vinaceous in *B. atropurpurea* and yellow in *B. caparoensis*. For this reason, we followed Castro (1996), reducing *B. caparoensis* to synonymy with *B. atropurpurea*.

Bifrenaria atropurpurea can be distinguished from other species of the genus by its red-vinaceous or yellow flowers, the small labellum and the cuneate viscidium.

Additional specimens examined: BRAZIL. **Minas Gerais:** Serra do Caparaó, Sep 1941, *Brade 17111* (RB). **Rio de Janeiro:** Serra dos Órgãos, Dec 1958, *Abendroth p-126* (HB); Friburgo, Alto Macaé, 1300 m elevation, Dec 1962, *Dungs s.n.* (HB20017); same locality, Nov 1975, *Waras s.n.* (HB63382); same locality, Macaé de Cima, Oct 1999, *Koehler 99/02* (UEC); Teresópolis, Parq. Nac. Serra dos Órgãos, Dec 1952, *Vidal II-5777* (R).

2. BIFRENARIA AUREO-FULVA (Hook.) Lindl., Edward's Bot. Reg. 29: Misc. 52. 1843. *Maxillaria aureo-fulva* Hook. Bot. Mag. 65: t. 3629. 1838. *Stenocoryne aureo-fulva* (Hook.) Kraenzl. in Rchb. f., Xenia Orchid. 3: 142. 1896. *Adipe aureo-fulva* (Hook.) M.Wolff, Orchidee 41(2): 36. 1990. TYPE: BRAZIL. Rio de Janeiro, s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: Bot. Mag.: t. 3629. 1838).

Epidendrum secundum Vell. Fl. Flumin. 9: t. 9. 1827, nom. illeg., non Jacq. (1760). *Stenocoryne secunda* (Vell.) Hoehne, Arq. Bot. Estado São Paulo 2(1): 14. 1944. *Bifrenaria secunda* (Vell.) Pabst, Orquidea 29: 165. 1967. TYPE: BRAZIL. Rio de Janeiro: s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: Fl. Flum. 9: t. 9. 1827).

(Figs. 8b, 10b, 11a)

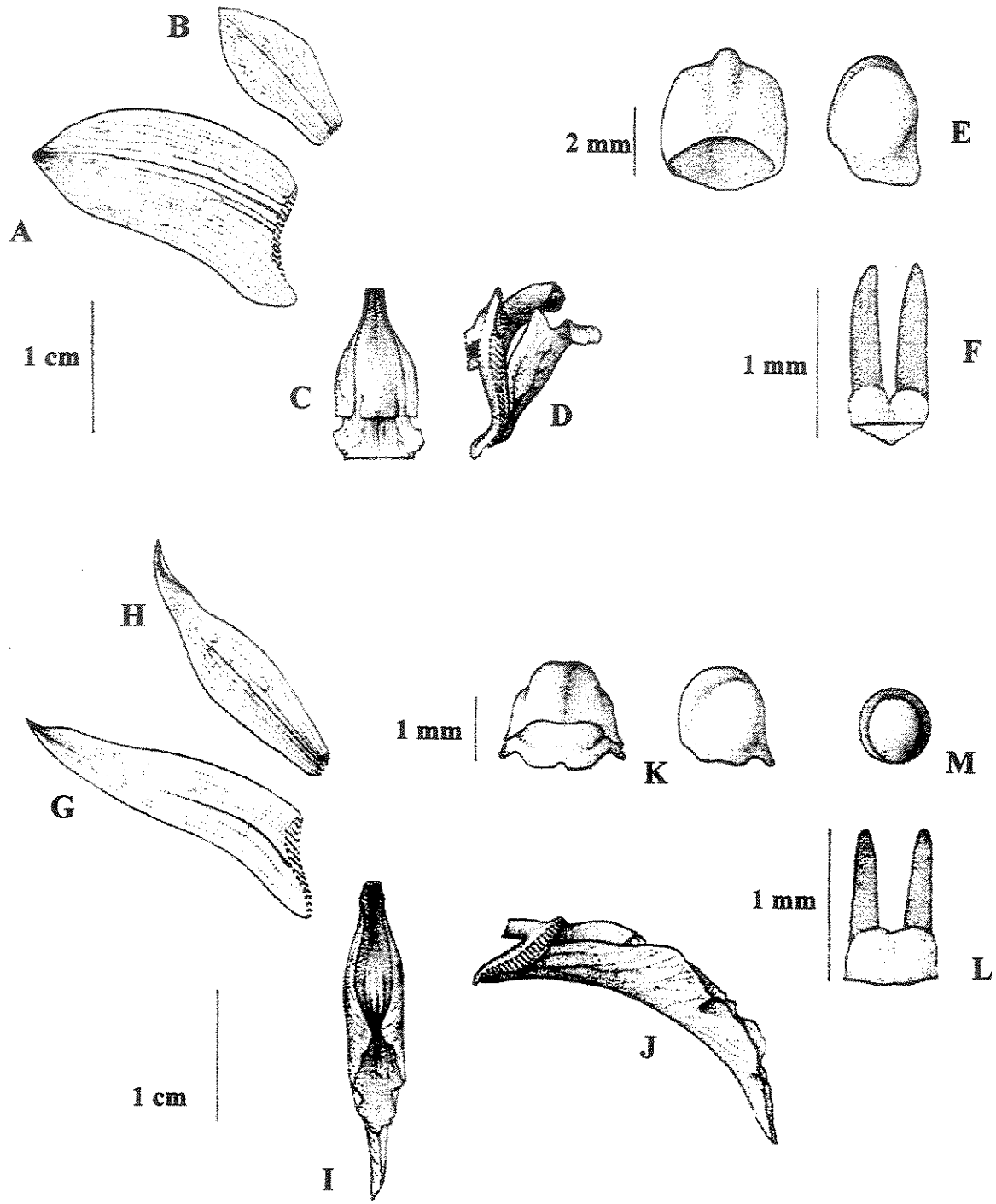


Fig. 8. (A-F) *Bifrenaria atropurpurea*, (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia. (G-M) *B. aureo-fulva*, (G) lateral sepal, (H) lateral petal, (I) labellum, (J) lateral view of flower showing the position of the labellum, (K) anther cap, (L) pollinarium without pollinia, (M) pollinia.

Epiphytes, rarely rupicolous plants, 10-25 cm tall. *Pseudobulbs* aggregate, conical to laterally compressed, dark green to brownish, 2.5-5 x 0.8-3 cm. *Leaves* lanceolate, membranaceous, 7-25 x 2.2-4 cm, pseudo-petiole 0.5-6.5 cm long. *Inflorescence* 2-7 flowered, erect to pendent, 12.5-21 cm long; scape bracts 0.6-1.5 x 0.1-0.5 cm, floral bracts 0.4-0.6 x 0.1-0.2 cm. *Flowers* odorless, orange to dark yellow, labellum with pink to red stripes, 1.5-2.5 cm long, 0.5-1.5 cm wide. *Sepals* lanceolate, acuminate, apically recurved, dorsal sepal 1.5-2.4 x 0.3-0.5 cm, lateral sepals parallel to each other, 1.2-2 x 0.4-0.5 cm, spur with sepal segments superposed, 0.4-1.5 cm long. *Petals* lanceolate, acuminate, oblique to column, 1.3-2.4 x 0.3-0.5 cm. *Labellum* slightly 3-lobed, lanceolate, glabrous, 1.8-2.5 x 0.7-2 cm when expanded, lateral lobes involute, acute, 0.8-1.7 x 0.2-0.5 cm, the midlobe lanceolate, acute, recurved, with undulate margins, the claw 0.1-0.5 cm long, the callus 0.5-1 cm long, glabrous, rarely pubescent, on the anterior region 3-dentate, slightly protuberant and glabrous. *Column* glabrous, 0.5-0.7 cm, the foot straight, glabrous, 0.5-0.7 cm long. *Anther* 0.15-0.2 x 0.1-0.15 cm, the pollinia rounded, the stipe forked, 0.03-0.05 cm long, the viscidium truncate, 0.03 x 0.05-0.06 cm. *Ovary-pedicel* 2.2-3 cm long.

Habitat. — Native to wet montane forests, riparian forests, and in the Brazilian "campos rupestres" vegetation, at 300-1700 m elevation. Flowering occurs from November to May.

Distribution. — Brazil, states of Bahia, Minas Gerais, Paraná, Rio de Janeiro and São Paulo.

Hooker in 1838 described *Maxillaria aureo-fulva*, which turned out to be the same species described by Velloso as *Epidendrum secundum* in 1827. Based on that Hoehne (1944) and Pabst (1967) published the new combinations *Stenocoryne secunda* (Vell.) Hoehne and *Bifrenaria secunda* (Vell.) Pabst. However, the name *Epidendrum secundum* Vell. is illegitimate, since Jacquin described a different species under the same name in 1760. Therefore the legitimate basionym for this species is *Maxillaria aureo-fulva* Hook.

Bifrenaria aureo-fulva can be easily distinguished from other species of the genus by its dark yellow to orange flowers, by its lanceolate and acuminate perianth segments and by its lateral sepals parallel to each other. Blumenschein and Packer (1961) have reported a chromosome number of 2n= 38 for this species.

Additional specimens examined: BRAZIL. (RB49328); *Sampaio s.n.* (R36210). **Bahia:** Abaíra, Campo Ouro Fino, 13°15′N 41°54′W, Mar 1992, *Laessoe et al. H53331* (UEC). **Minas Gerais:** Baipendi, São Tomé das Letras, Nov 1950, *Brade and Apparicio 19* (RB); Serra da Cachoeira do Campo, s. loc., *Schwacke 10471* (RB); Carrancas, Apr 2000, *Simões et al. s.n.* (UEC117751); Caxambu, Sep 1949 (RB); P.F.E Ibitipoca, Mar 1994, *Forzza 88* (CESJ); Itabirito, Serra dos Inconfidentes, Jan 1994, *Teixeira s.n.* (BHCB23374); Santa Bárbara, Serra do Caraça, Dez 1978, *Leitão Filho et al. 9780* (UEC); Santana do Riacho, Serra do Cipó, May 1994, *Campos and Souza s.n.* (SPF106820); Serra do Gongo-Socco, Mar 1921, *Hoehne s.n.* (SP5454). **Paraná:** Jan 1909, *Dúsen 7678* (NY). **Rio de Janeiro:** Friburgo, Colégio Anchieta, Feb-Mar 1936, *Amarante s.n.* (RB150853); same locality, Morro da Cruz, Feb-Mar 1936, *Amarante 8* (RB). **São Paulo:** 23°59′16′′S 46°44′01′′W, P.E. Serra do Mar, Dez 1996, *Garcia et al. 975* (UEC); Paranapiacaba, Estação Biológica, Mar 1918, *Hoehne 1598* (NY, SP); Santo Amaro, Estr. Rio Bonito a 9Km de Santo Amaro, s. loc., *Gehrt s.n* (SP27485); São Paulo, Jardim Botânico, Mar 1939, *Handro s.n.* (SP1568).

3. BIFRENARIA CALCARATA Barb. Rodr., Gen. Sp. Orchid. 2: 213. 1881. *Bifrenaria barbosae* V.P. Castro, Proceedings of the 15th World Orchid Conference, Rio de Janeiro. 377 1996, nom. illeg. TYPE: BRAZIL. Espírito Santo, s. loc. (HOLOTYPE: destroyed, LECTOTYPE: Iconographie des Orchidées du Brésil 5: t.227 at Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil).

(Figs. 9a, 10c, 11b)

Epiphytes, rarely rupicous plants, 25-45 cm tall. *Pseudobulbs* aggregate, conical, dark green to brownish, 4-7.3 x 1-3 cm. *Leaves* lanceolate, membranaceous to sub-coriaceous, 15-33 x 4-6.3 cm, pseudo-petiole 1-12 cm long. *Inflorescence* 1-3 flowered, erect, 1.5-2 x 0.5-0.8 cm long; scape and floral bracts 1.5-2 x 0.5-0.8 cm. *Flowers* fragrant, vinaceous to brownish on the exterior, yellow to pale green with vinaceous spots on the interior, labellum pink to vinaceous, white to pale yellow with vinaceous spots at the base, 4-6 cm long, 2.5 cm wide. *Sepals* ovate, truncate, dorsal sepal 2.5-3 x 1.5-1.7 cm, lateral sepals

parallel to each other, 2.7-3.4 x 1.3-1.7 cm, spur with sepal segments superposed, hooked, 2-3 cm long. *Petals* oblanceolate, rounded at the apex, truncate, oblique to column, 2-2.7 x 1-1.3 cm. *Labellum* 3-lobed, obovate, pubescent, parallel to column, 2.7-4.7 x 2.2-4.3 cm when expanded, lateral lobes involute, truncate, 1.5-2 x 0.7-0.8 cm, the midlobe subacute, with entire margins, the claw 0.6-0.7 cm long, the callus 1.8-2 cm long, glabrous, on the anterior region entire to slightly lobate, glabrous, protuberant. *Column* glabrous to puberulous, 1-1.3 cm, the foot straight, anteriorly recurved, glabrous, 1-1.3 cm long. *Anther* elongate, 0.4 x 0.3 cm, the pollinia rounded, the stipe entire, 0.2 cm long, the viscidium cuneate, 0.1 cm long. *Ovary-pedicel* 4.5-5 cm long.

Habitat. — Native to wet montane forests, at 500-1500 m elevation. Flowering occurs from February to June.

Distribution. — Brazil, states of Bahia, Minas Gerais and Rio de Janeiro.

Velloso described *Epidendrum calcaratum* in 1827, which turned out to be the same species described by Lindley in 1831 as *Maxillaria tetragona* (= *Bifrenaria tetragona* (Lindl.) Schltr.). Ignoring the fact that the specific epithet of *E. calcaratum* Vell was already occupied by *B. calcarata* Barb. Rodr. (1882), which was based on a different type, Castro (1996) reduced *B. tetragona* (Lindl.) Schltr. to synonymy of his new combination, *Bifrenaria calcarata* (Vell.) V. P. Castro, nom. ileg., and published, in the same publication, another illegitimate name, *B. barbosae* V. P. Castro, as an alternative name for *B. calcarata* Barb. Rodr.

Bifrenaria calcarata is very similar to *B. mellicolor*, and may be distinguished from it especially by its hooked spur and by the format of the labellum.

Additional specimens examined: BRAZIL. **Bahia:** Itororó, Serra da Ouricana, May 2000, *Silva s.n.* (UEC117740). **Minas Gerais:** Serra do Cipó (?), Apr 1963, *Magalhães s.n.* (VIC3711).

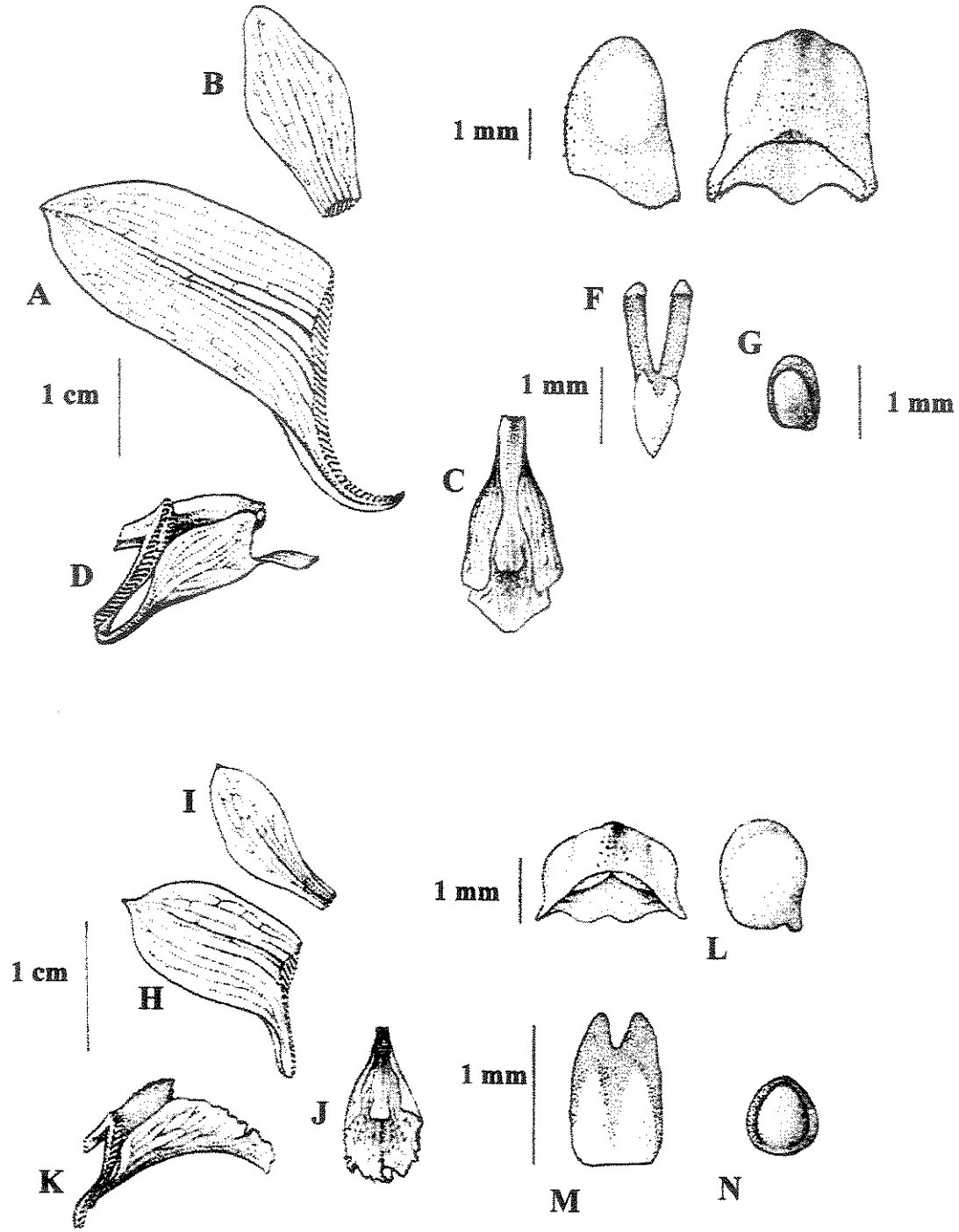


Fig. 9. (A-G) *Bifrenaria calcarata*, (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia, (G) pollinia. (H-N) *B. charlesworthii*, (H) lateral sepal, (I) lateral petal, (J) labellum, (K) lateral view of flower showing the position of the labellum, (L) anther cap, (M) pollinarium without pollinia, (N) pollinia.

4. BIFRENARIA CHARLESWORTHII Rolfe, Kew Bull. 184. 1894. *Stenocoryne charlesworthii* (Rolfe) Hoehne, Arq. Bot. Estado São Paulo 2(1): 14. 1944. *Adipe charlesworthii* (Rolfe) M.Wolff, Orchidee 41(2): 36. 1990. TYPE: BRAZIL. Minas Gerais. s. loc. (HOLOTYPE: K-n.v., photo at AMES).

Bifrenaria villosula Brade, Arch. Jard. Bot. Rio de Janeiro 9: 11-12. 1949. *Stenocoryne villosula* (Brade) Brade, Arch. Jard. Bot. Rio de Janeiro 10: 149. 1950. *Adipe villosula* (Brade) M.Wolff, Orchidee 41(2): 36. 1990. TYPE: BRAZIL. Espírito Santo: Castelo, Braço Sul, 20 Oct 1948, *Brade 19138* (HOLOTYPE: RB).

(Figs. 9b, 10d, 11c)

Epiphytes, 10-25 cm tall. *Pseudobulbs* aggregate, laterally compressed, dark green to brownish, 3.5-5 x 1-2 cm. *Leaves* lanceolate, membranaceous, 16-22 x 3.6-3.7 cm, pseudo-petiole 2.3-4.5 cm long. *Inflorescence* 1-9 flowered, erect to pendent, 15-20 cm long; scape and floral bracts 0.3-1 x 0.1-0.3 cm. *Flowers* greenish yellow to brownish on the exterior, labellum white to yellowish with red dots, 1-2.5 cm long, 2 cm wide. *Sepals* ovate, acuminate, truncate, dorsal sepal 1.3-1.5 x 0.6-0.8 cm, lateral sepals divergent to each other, 1.1-1.5 x 0.6-0.9 cm, spur with sepal segments fusioned, 0.4-0.6 cm long. *Petals* oblanceolate, parallel to column, 1-1.4 x 0.5-0.6 cm. *Labellum* entire, orbicular, emarginate, recurved, laterally involute, with crenulate margins, pubescent, 0.8-1.5 x 1.2-1.3 cm when expanded, the claw 0.2-0.3 cm long, the callus 0.4-0.6 cm long, pubescent, on the anterior region entire to slightly lobate, glabrous, protuberant, yellow. *Column* ventrally pubescent, 0.5-0.8 cm, the foot straight, puberulous to pubescent, 0.4-0.7 cm long. *Anther* 0.1-0.15 x 0.1-0.2 cm, the pollinia rounded, the stipe forked, 0.005-0.01 cm long, the viscidium truncate, 0.02-0.05 cm long. *Ovary-pedicel* 1.1-2.3 cm long.

Habitat. — Native to wet montane forests, at 300-1000 m elevation. Flowering occurs from October to April.

Distribution. — Brazil, states of Espírito Santo and Rio de Janeiro.

Brade (1949) described a new species, *Bifrenaria villosula* Brade, considering it to be an intermediate species between *B. charlesworthii* Rolfe and *B. racemosa* (Lindl.) Lindl. An examination of the type specimen revealed that *B. villosula* is, indeed, very similar to *B. charlesworthii* considering floral morphology. There is no good character that separates both species and, for this reason, we have followed Castro and Campacci (2000), reducing *B. villosula* to synonymy under *B. charlesworthii.*

Bifrenaria charlesworthii is also very similar to *Bifrenaria racemosa*, but apparently *B. charlesworthii* can be distinguished from it by the lateral sepals divergent to each other, and by the labellum with a yellow callus, while *B. racemosa* has lateral sepals parallel to each other and a labellum with a white callus.

Additional specimens examined: BRAZIL. **Espírito Santo:** s. loc. Apr 2000, *Castro s.n.* (UEC117752); Santa Leopoldina, rio Nove, Oct 1986, *Boudet Fernandes 2037* (MBML). **Rio de Janeiro:** Rio de Janeiro, s.d., *Delfina s.n.* (UEC117757); Petrópolis, Orquidário Binot (cult.), Feb 2000, *Koehler 80c* (UEC).

5. BIFRENARIA CLAVIGERA Rchb. f., Hamburger Garten- Blumenzeitung 21: 269. 1865. *Stenocoryne clavigera* (Rchb. f.) Kraenzl. in Rchb. f., Xenia Orchid. 3: 142. 1896. *Adipe clavigera* (Rchb. f.) M.Wolff, Orchidee 41(2): 36. 1990. TYPE: BRAZIL. s. loc. (HOLOTYPE: W-n.v., photo at AMES and UEC).

Stenocoryne wendlandiana Kraenzl. in Rchb. f., Xenia Orchid. 3: 154, t. 289. 1896. *Bifrenaria wendlandiana* (Kraenzl.) Cogn. in Mart., Fl. Bras. 3(5): 489. 1902. TYPE: BRAZIL. s. loc. (HOLOTYPE-destroyed, LECTOTYPE, selected here: in Xenia Orchid. 3: 154, t. 289. 1896).

(Figs. 10e, 11b, 12a)

Epiphytes, 5-12 cm tall. *Pseudobulbs* aggregate, laterally compressed, dark green to brownish, 1-3 x 1.5-2 cm. *Leaves* lanceolate, coriaceous to membranaceous, 5.5-11 x 1.2-3 cm, pseudo-petiole 0.5-1 cm long. *Inflorescence* 1-11 flowered, erect to pendent, 5-8 cm long; scape bracts 0.5-0.8 x 0.1-0.3 cm, floral bracts 0.2-0.4 x 0.1-0.2 cm. *Flowers* green to

red on the exterior, white pink on the interior, labellum with red dots, 1-1.5 cm long, 1.5 cm wide. *Sepals* ovate, truncate, dorsal sepal 0.9-1.2 x 0.5-0.6 cm, lateral sepals parallel to each other, 0.8-1.1 x 0.4-0.6 cm, spur with sepal segments fusioned, 0.3-0.5 cm long. *Petals* oblanceolate, attenuate, oblique to column, 1-1.3 x 0.7-1 cm. *Labellum* entire, flabellate, recurved, pubescent, laterally involute, with crenate margins, 1-1.3 x 0.7-1 cm, when expanded the claw 0.2 cm long, the callus 0.5-0.8 cm long, pubescent, on the anterior region entire to slightly lobate, glabrous, protuberant. *Column* puberulous, 0.5-0.6 cm, the foot straight, glabrous to puberulous, 0.3-0.5 cm long. *Anther* 0.1 x 0.2 cm, the pollinia rounded, the stipe forked, 0.05-0.07 cm long, the viscidium truncate, 0.05 cm long. *Ovary-pedicel* 1.1-1.5 cm long.

Habitat. — Native to wet montane forests, at 1000-1500 m elevation. Flowering occurs on June (in Friburgo, Rio de Janeiro) and from September to Dezember.

Distribution. — Brazil, states of Espírito Santo and Rio de Janeiro.

Kraenzlin (1896) described a new species, *Stenocoryne wendlandiana* Kraenzl., considering it to be a smaller version of *B. longicornis* Lindl., which Cogniaux transfered to *Bifrenaria* in 1902. An examination of the original illustration revealed that *B. wendlandiana* is very similar to *B. clavigera* Rchb. f. and, for this reason, we have followed Castro and Campacci (2000), reducing *B. wendlandiana* to synonymy under *B. clavigera.*

Bifrenaria clavigera can be distinguished from congeneric species by its flabellate labellum, unique in this genus.

Additional specimens examined: BRAZIL. **Espírito Santo:** Santa Teresa, Jun 1987, *Fernandes 2142* (MBML); same locality, Reserva Biológica Santa Lúcia, Aug 1996, *Sartori 163 et al.*(UEC). **Rio de Janeiro:** Nova Friburgo, Macaé de Cima, Oct 1999, *Koehler s.n.* (UEC117739); Teresópolis, Sep1929, *Brade 9374* (R).

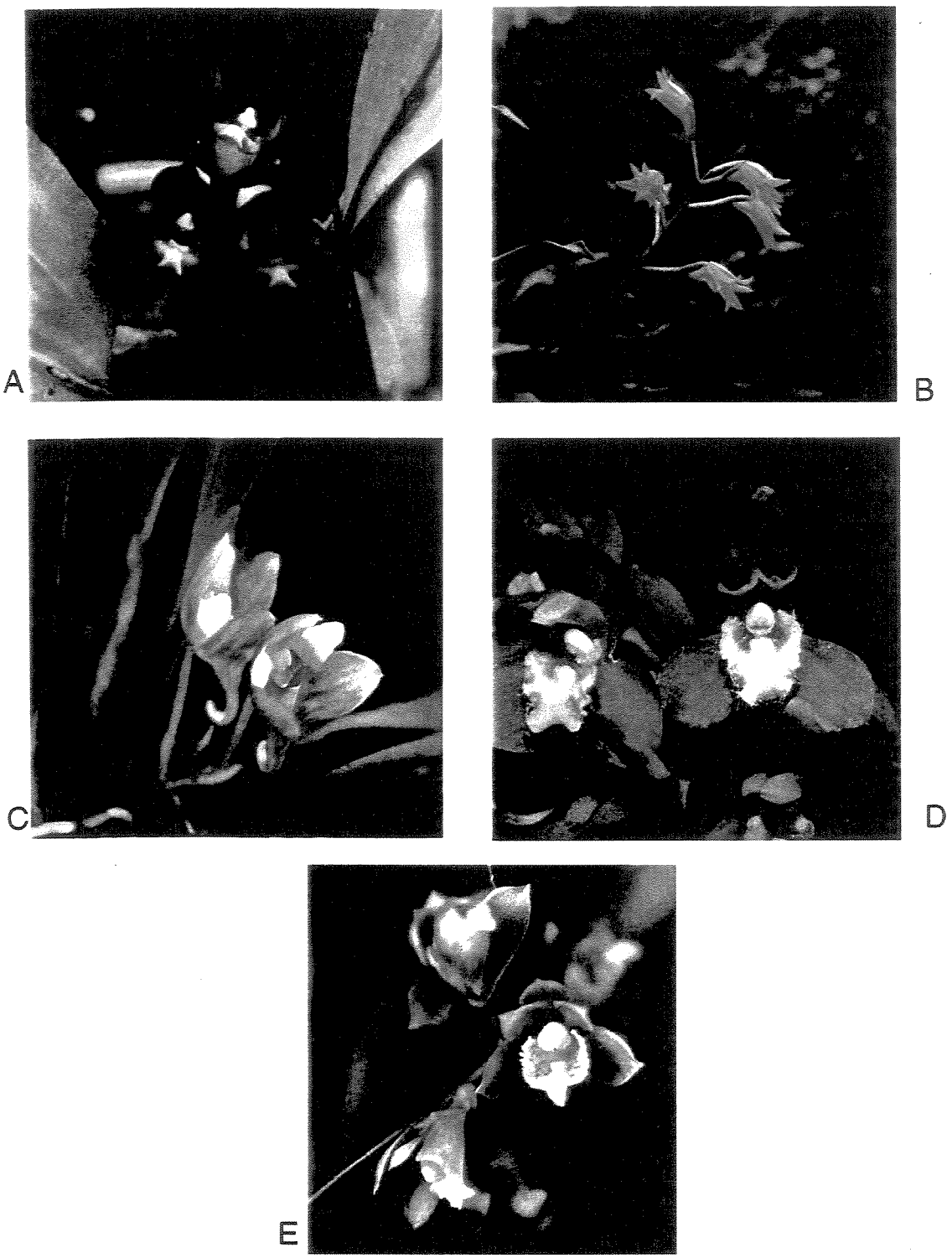
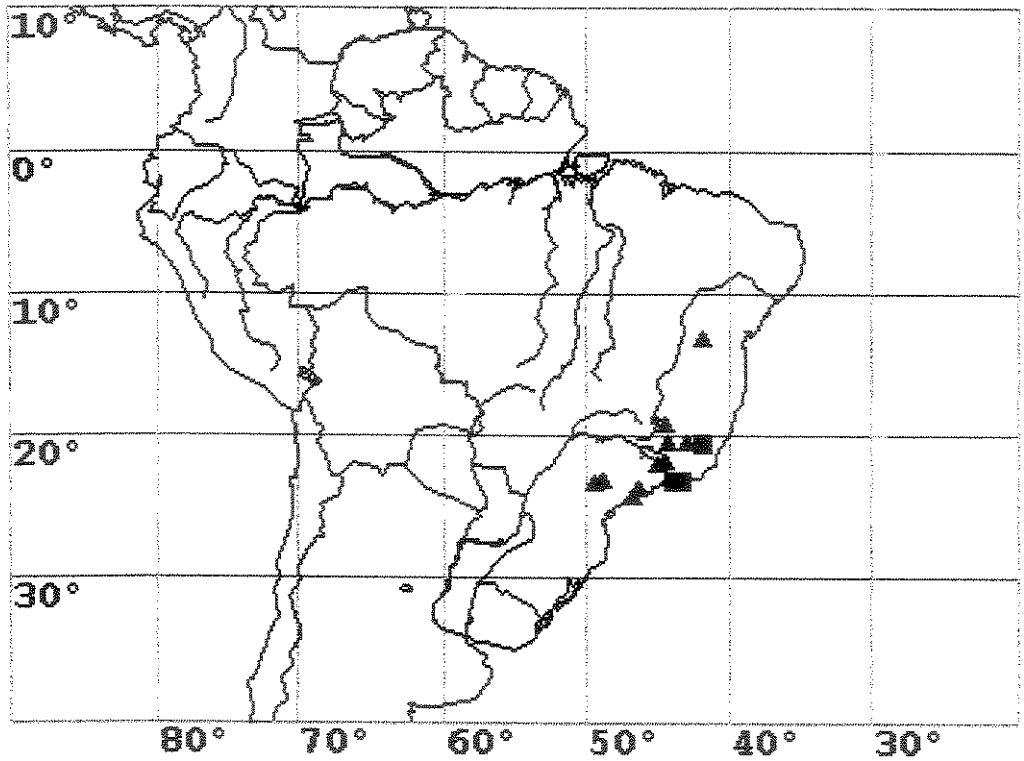
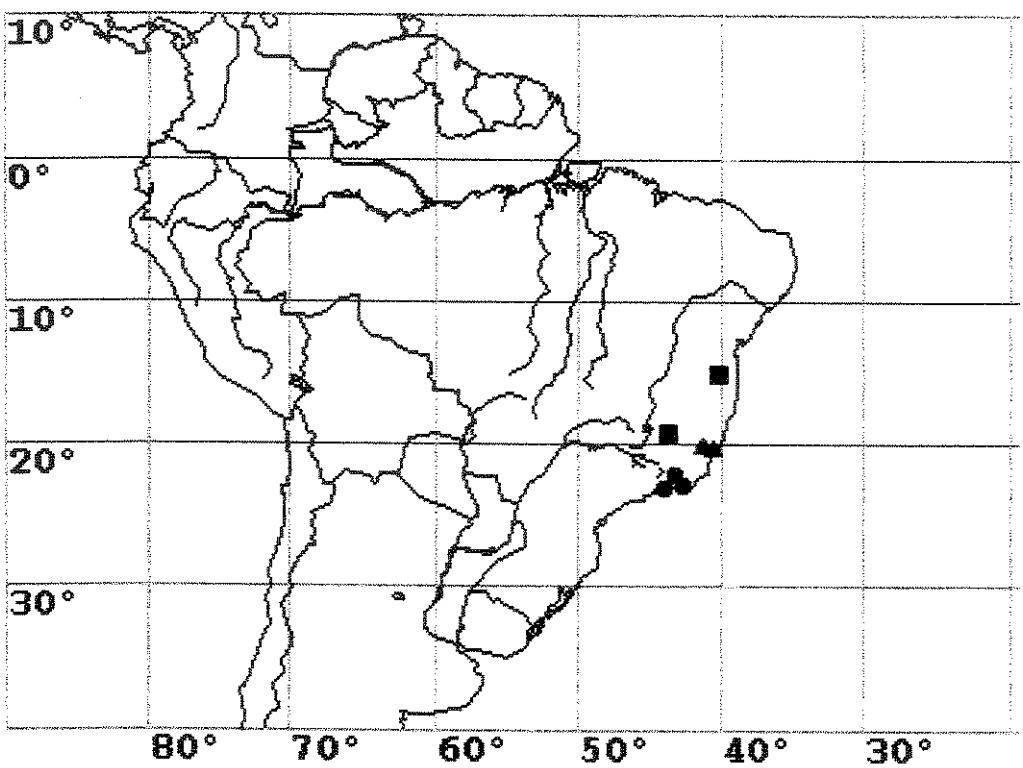


Fig. 10. (A) *Bifrenaria atropurpurea* (foto V.P. Castro) (B) *B. aureo-fulva* (C) *B. calcarata* (D) *B. charlesworthii* (foto E. Pansarin) (E) *B. clavigera.*



A



B

Fig. 11. (A) *Bifrenaria atropurpurea* (■); *B. aureo-fulva* (▲). (B) *B. calcarata* (■); *B. charlesworthii* (▲); *B. clavigera* (●).

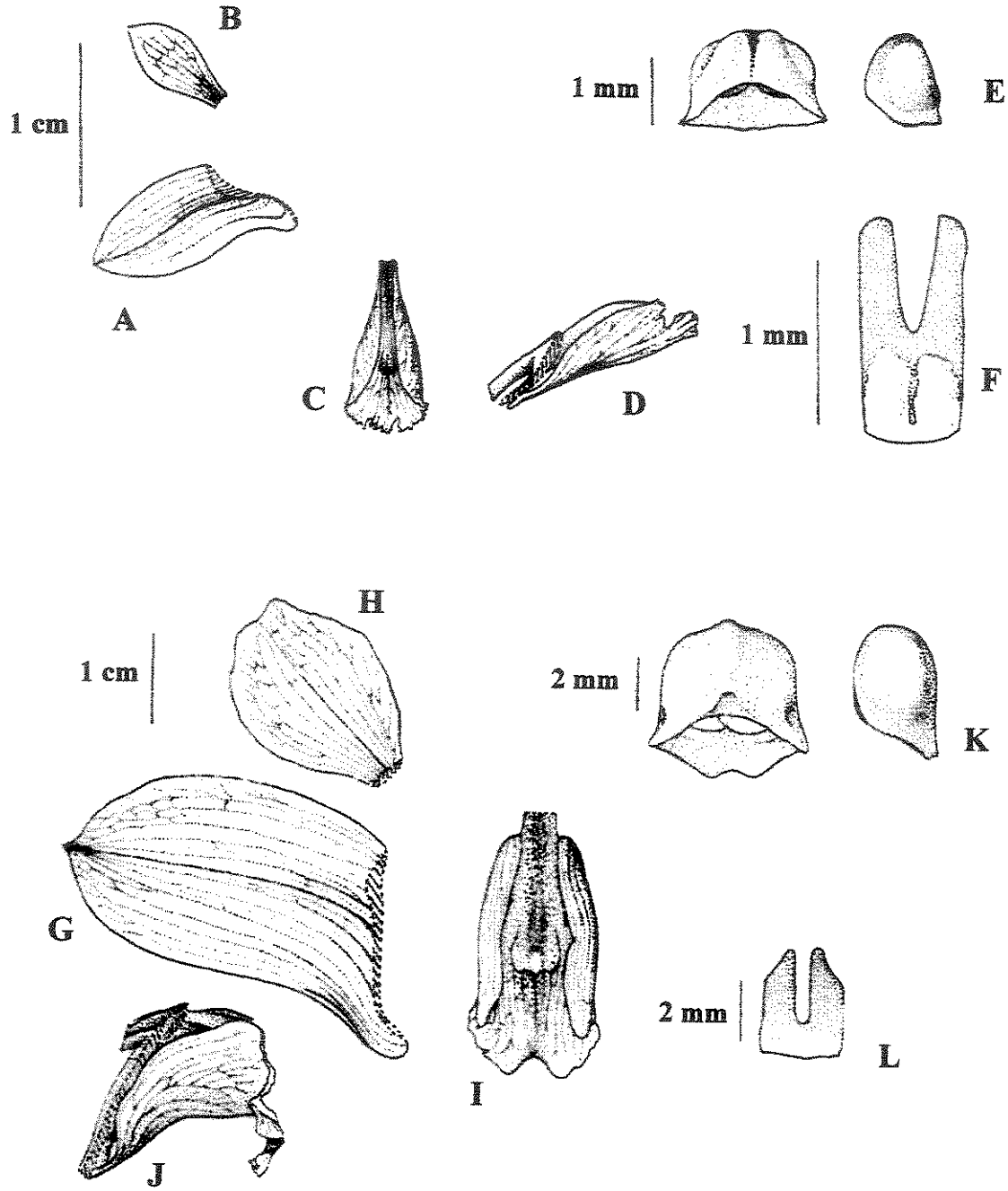


Fig. 12. (A-F) *Bifrenaria clavigera*, (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia. (G-L) *B. harrisoniae*, (G) lateral sepal, (H) lateral petal, (I) labellum, (J) lateral view of flower showing the position of the labellum, (K) anther cap, (L) pollinarium without pollinia.

6. BIFRENARIA HARRISONIAE (Hook.) Rchb. f., Bonplandia, 3 277. 1855. *Dendrobium harrisoniae* Hook., Exot. Fl.: t. 120. 1825. *Maxillaria harrisoniae* (Hook.) Lindl., Bot. Reg. 7: t. 897. 1826. *Colax grandiflorus* (Lindl.) Raf., Fl. Tellur. 2: 85. 1836. TYPE: BRAZIL. Rio de Janeiro. s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: Exot. Fl.: t. 120. 1825).

Colax harrisoniae Lindl. ex Spreng. Syst. Veg. 3: 727. 1826. TYPE: unknown.

Bifrenaria aurea Barb. Rodr., Gen. Spec. Orchid. Nov. 2: 212. 1881. TYPE: BRAZIL Rio de Janeiro, Serra dos Órgãos, s. d. (HOLOTYPE: RB-destroyed, LECTOTYPE, selected here: Iconographie des Orchidées du Brésil 5: t.226 at Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil).

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *alba* Kranzl. Gartenflora 38: 651. 1889. TYPE: BRAZIL, s. loc. (HOLOTYPE: unknown).

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *alba-plena* Pabst. Bradea 2(40): 274. 1978. TYPE: BRAZIL, Espírirto Santo, s. loc., *Kautsky 383* (HOLOTYPE: HB).

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *angustior* Cogn., in Mart. Fl. Bras. 3(5): 483. 1902. *Maxillaria harrisoniae* Hook. var. *angustior* Lindl. Paxton´s fl. gard. 30: 92. 1852. TYPE: s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: Paxton´s fl. gard. 30: 92. 1852).

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *buchaniana* Rchb. f. Gard. Chron. n.s. 11. 430. 1879. TYPE: unknown.

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *citrina* Stein. Orchid.-Buch. 85 1892. TYPE: unknown.

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *eburnea* Stein. Orchid.-Buch. 85 1892. TYPE: unknown.

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *flavo-purpurea* Hoehne, Arq. Bot. Estado São Paulo, 2(5): 116. 1950. TYPE: BRAZIL. s. loc. (HOLOTYPE: missing).

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *glabra* W. Zimm. Biblioth. Bot. 109: 18, fig. 12; pl. 4. 1934. TYPE: BRAZIL. Minas Gerais, s.d. (HOLOTYPE: unknown).

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *insularis* Hoehne, Arq. Bot. Estado São Paulo, 2 (5): 116. 1950. TYPE: BRAZIL. São Paulo: Ilha dos Alcatrazes, 25 Ago 1922, *Luederwaldt s.n.* (HOLOTYPE: SP7928).

Bifrenaria harrisoniae (Hook.) Rchb. f. var. *minor* Hoehne, Arq. Bot. Estado São Paulo 2(5): 117. 1950. TYPE: BRAZIL. São Paulo: Alto da Serra, 5 Oct 1921, *Gehrt s.n.* (HOLOTYPE: SP2510).

Bifrenaria tyrianthina (Lodd.) Rchb. f. var. *albescens* Hoehne, Arq. Bot. Estado São Paulo, 2 (5): 117. 1950. TYPE: BRAZIL. s. loc., Dec 1945 (HOLOTYPE: missing, LECTOTYPE, selected here: Fl. Bras. (Hoehne) 12 (7): t. 18. 1953).

(Figs. 12a, 13, 14a)

Epiphytes or rupiculous plants, 10-40 cm tall. *Pseudobulbs* aggregate, conic, dark yellow to greenish, 3-6.5 x 1-3 cm. *Leaves* lanceolate to ovate, coriaceous, 8-38.5 x 3-7cm, pseudo-petiole 0.5-10 cm long. *Inflorescence* 1-3 flowered, erect, 2-20 cm long; scape and floral bracts 1-3 x 0.5-3 cm. *Flowers* with a sweet and strong fragrance, white, yellowish, pink to vinaceous, labellum pink to vinaceous, rarely white yellowish with red stripes, 4.5-6 cm long, 4-7 cm wide. *Sepals* ovate, truncate, dorsal sepal 2.3-4.5 x 0.9-2.7 cm, lateral sepals divergent to parallel to each other, 1.8-4.5 x 1.1-2.9 cm, spur with sepal segments superposed, 0.3-2.5 cm long. *Petals* obovate, oblique to column, 1.3-3.9 x 1-2 cm. *Labellum* 3-lobed, obovate, 2.2-4.6 x 0.7-4 cm when expanded, lateral lobes oblong, involute, with undulate margins, midlobe obovate, recurved, puberulous to pubescent, the claw 0.2-0.6 cm long, the callus 1.3-2.6 cm long, puberulous to pubescent, on the anterior region 3-lobed, puberulous to pubescent. *Column* puberulous, 1-2.3 cm, the foot straight, puberulous to pubescent, 1.5-3.3 cm long. *Anther* 0.3-0.6 x 0.4-0.6 cm, the pollinia rounded, the stipe forked, 0.1-0.2 cm long, the viscidium truncate, 0.2-0.4 x 0.1-0.2 cm long. *Ovary-pedicel* 2.5-6 cm long.

Habitat. — Native to wet montane forests, riparian forests, and to Brazilian "campos rupestres", at 750-1800 m elevation. Flowering occurs from July to December.

Distribution. — Brazil, states of Espírito Santo, Minas Gerais, Paraná, Rio Grande do Sul, Rio de Janeiro, Santa Catarina and São Paulo.

Fig. 13. (A-E) *Bifrenaria harrisoniae* (fotos A e D de V.P. Castro) (F) *Bombus atratus* with pollinarium of *B. harrisoniae* (foto R. B. Singer).

This species has been commonly mistaken for the related species *Bifrenaria tyrianthina* in several taxonomic treatments. *B. harrisoniae* may be distinguished from *B. tyrianthina* mainly by the truncate viscidium, a generally short spur, the puberulous column, and the 3-lobed callus. Also, *B. harrisoniae* presents a much wider geographic distribution, growing as epiphytes on wet forests, as well as on open areas.

This is a rather variable species considering the lenght of the scape and the size and color of the flowers. The analysis of types and herbarium material from many different localities showed that the varieties described for *B. harrisoniae* correspond to a continuous range of morphological variation. Therefore we here do not consider the varieties *B. harrisoniae* (Hook.) Rchb. f. var. *minor* Hoehne, *B. harrisoniae* (Hook.) Rchb. f. var. *flavo-purpurea* Hoehne, *B. harrisoniae* (Hook.) Rchb. f. var. *insularis* Hoehne and *B. tyrianthina* (Lodd.) Rchb. f. var. *albescens* as good taxa. Although *B. tyrianthina* var. *albescens* was described as a variety of another species, the analysis of the type material leaves no doubts of its identity with *B. harrisoniae*, especially as regards the pollinarium and the format of the labellum.

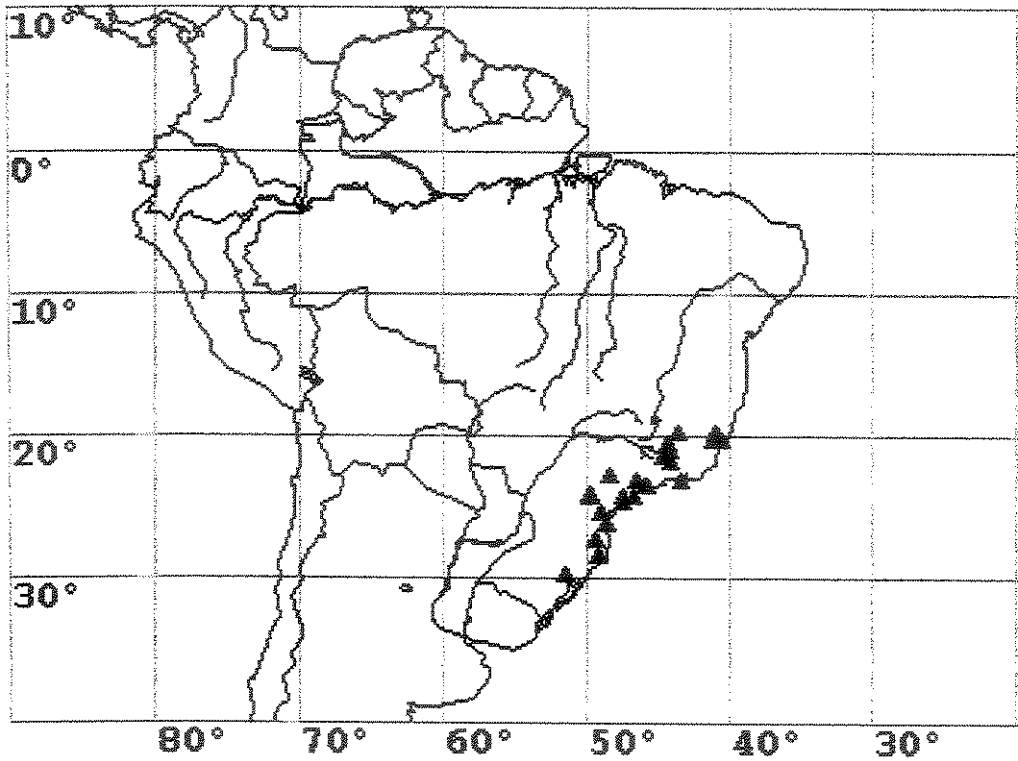
We follow Castro and Campacci (2000) on the reduction of *B. aurea* to synonymy under *B. harrisoniae*, based on the format of the labellum and on the color of the flower.

Blumenschein and Packer (1961) have reported a chromosome number of 2n= 38 for this species. Two different species of bees, *Bombus atratus* and *Eufrisia violaceae* (Hymenoptera, Apidae), have been collected bearing pollinaria of *Bifrenaria harrisoniae* in Paranapiacaba, São Paulo state (I. Gajardo, pers. comm.).

Additional specimens examined: BRAZIL. (MO2157696) **Espírito Santo:** Castelo, Reserva Florestal de Forno Grande, Nov 1991, *Valpassos 27* (MBML); Pedra Azul, Oct 1973, *Kautsky s.n.* (HB52617); same locality, Oct 1973, *Kautsky s.n.* (HB52619); same locality, prox. Cachoeiro do Itapemirim, Oct 1964, *Kautsky 63* (HB); Santa Teresa - Itarana, alto da Pedra Alegre, Feb 1988, *Boudet Fernandes 2298 et al.* (MBML). **Minas Gerais:** 44°10´S, 21°05´W, Serra de São José, Oct 1989, *Alves 1131f* (RB); Bom Jardim de Minas, Sep1961, *Saléh 77* (HB); Caeté, Serra da Piedade, Nov 1915, *Hoehne 6427* (R); same locality, Dez 1921, *Gehrt s.n.* (SP8143); same locality, Aug 1922, *Gehrt s.n.* (SP8144); same locality, 1ª Estação, Oct 1987, *Reis et al. 37* (BHCB); Carrancas, Oct

1999, *Simões et al. s.n.* (UEC117753); Ibitipoca, Pico do Peão, 1750 m elevation, Oct 1970, *Braga 1938, Sucre and Krieger* (RB), Itabirito, Pico do Itabirito, Sep 1994, *Teixeira s.n.* (BHCB26073); Santa Bárbara, Serra do Caraça, Sep 1990, *Tameirão Neto s.n.* (BHCB28333). **Paraná:** Arapoti, Rio das Cinzas, Oct 1968, *Hatschbach 20052* (HB); Guaratuba, Serra de Araçatuba, Morro do Pinhais, Nov 1998, *Santos, Candido and Hassegawa 605* (SP); Jaguariaiva, Fda. Cajuru, Oct 1968, *Hatschbach 20070* (MO); Ponta Grossa, Vila Velha, Sep 1976, *Hatschbach 38864* (UEC); Piraquara, Serra do Emboque, Dec 1970, *Hatschbach 25751* (HB); same locality, Santa Maria, Oct 1969, *Hatschbach 22428* (HB); São José dos Pinhais, Nov 1952, *Hatschbach s.n.* (HB3000). **Rio Grande do Sul:** Zimmersberg, prox. Montenegro, Oct 1935, *Rambo s.n.* (PACA2067). **Rio de Janeiro:** Cabo Frio, Oct 1951, *Brasilino s.n.* (RB84158); Friburgo, Sep 1954 (HB); same locality, Macaé de Cima, Oct 1999, *Koehler s.n.* (UEC117758); Rio de janeiro, Pedra da Gávea, Oct 1941, *Carris s.n* (RB49329). SANTA CATARINA. São Francisco do Sul, Garuvá, Monte Crista, Oct 1960, *Reitz and Klein 10011* (HB); Limeira, Tigipó, Oct 1970, *Klein et al. 9181* (HB); Orleãs, Sítio Rio Novo, Jul 1962, *Itarga and Bicalho s.n.* (SP168436); Vidal Ramos, Sabiá, Oct 1956, *Reitz 5908* (SP). **São Paulo:** Atibaia, Pedra Grande, Oct 2000, *Koehler 00/35.* (UEC); Biritiba Mirim, Estação Ecológica da Boracéia, 23°38′-23°49′S, 45°52′-49°53′W, X.1983, *Custódio Filho 1731* (SP); same locality, Nov 1983, *Custódio Filho 1851* (SP); Bocaina, reserva florestal, Aug 1968, *Sucre 3497 and Braga 1075* (RB); Iguapé, Morro das Pedras, *Brade 8307* (HB8438, 8440); Ilha Comprida, Dec 1999, *Belinello 34.* (UEC); Miracatú, Sítio Irapuã Km 345,5, BR116, Nov 1984, *Martuscelli 87* (SP); Salesópolis, Estação Ecológica da Boracéia, Casa Grande, Nov 1981, *Custódio Filho 712* (SP); São Paulo, Butantan, Oct 1918, *Hoehne s.n.* (2510); same locality, Reserva Biológica Parque Estadual das Fontes do Ipiranga, Nov 1981, *Barros 656* (SPF).

A



B

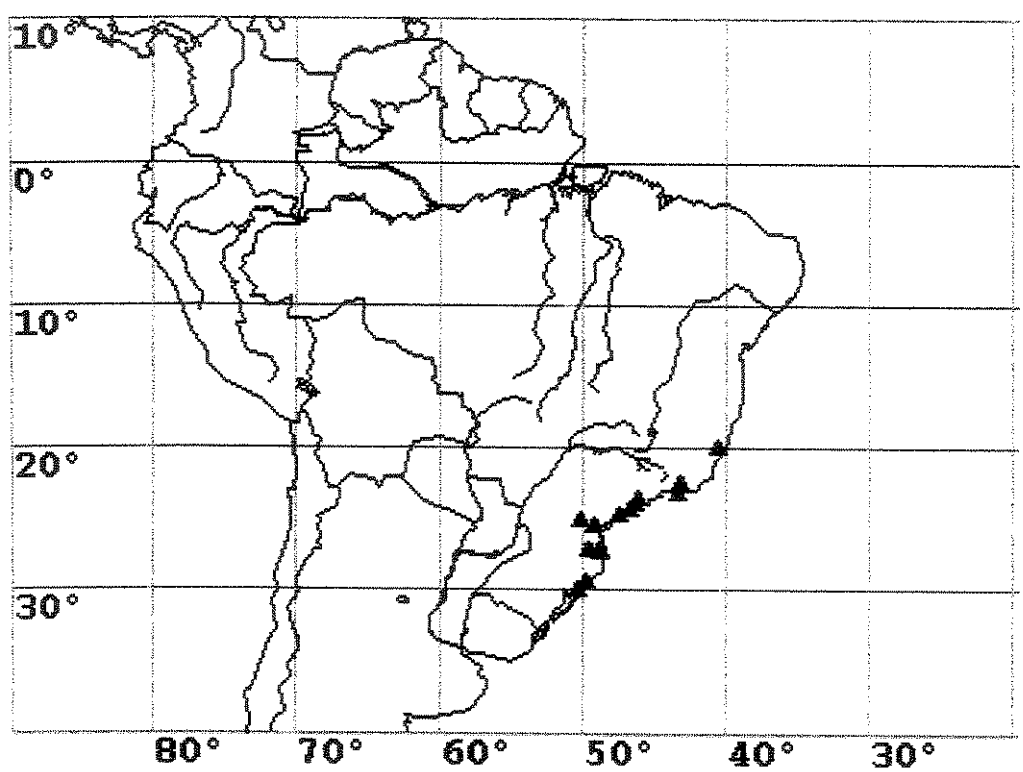


Fig. 14. (A) *Bifrenaria harrisoniae* (▲). (B) *B. inodora* (▲).

7. BIFRENARIA INODORA Lindl., Edward´s Bot. Reg. 29: Misc. 48. 1843. *Bifrenaria fragans* Barb. Rodr., Gen. Spec. Orchid. 2: 214. 1881, nom. illeg. *Stenocoryne inodora* (Lindl..) Kraenzl., in Rchb. f., Xenia Orchid. 3: 142. 1896. TYPE: BRAZIL. Rio de Janeiro, Apr 1843 (HOLOTYPE: missing, LECTOTYPE, selected here: Edward´s Bot. Regist. 30: misc. 48. 1843, original illustration at K).

(Figs. 14b, 15a, 16a)

Epiphytes or rupiculous plants 15-60 cm tall. *Pseudobulbs* aggregate, conical, dark green to yellowish, 0.5-7.5 cm. *Leaves* lanceolate-obovate, coriaceous, 18-52.2 x 5.3-8.5 cm, pseudo-petiole 0.5-7.5 cm long. *Inflorescence* 2-10 flowered, erect, 1.9-15 cm long; scape and floral bracts 1-2.5 x 0.5-1.5 cm. *Flowers* with a sweet and strong odor, light green to yellowish, the labellum pink to red, with red stripes, 4-7 cm long, 4.5-6.5 cm wide. *Sepals* ovate, truncate, dorsal sepal 2.9-4.2 x 1.1-2.1 cm, lateral sepals divergent to each other, 2.7-4 x 1-2.5 cm, spur with sepal segments superposed, 0.5-2.5 cm long. *Petals* obovate to rhombic, oblique to column, 2.2-3.5 x 1.11.7 cm. *Labellum* 3-lobed, obovate, puberulous, 2.9-4.8 x 1.8-3.3 cm when expanded, lateral lobes involute, oblong, 1-2.6 x 0.6-1.7 cm, the midlobe obovate, emarginate, recurved, with undulate margins, the claw 0.2-0.7 cm long, the callus 1-2 cm long, glabrous to puberulous, on the anterior region 2-lobed, protuberant, glabrous to puberulous. *Column* puberulous to pubescent, 0.8-1.5 cm, the foot straight, puberulous, rarely glabrous, 2-2.3 cm long. *Anther* with a prominent apex, 0.4-0.5 x 0.3-0.4 cm, the pollinia rounded, the stipe forked, 0.25 cm long, the viscidium truncate, 0.1-0.15 x 0.1-0.2 cm long. *Ovary-pedicel* 3.2-5.5 cm long.

Habitat. — Native to wet montane forests, 200-1000 m elevation, flowering occurs from August to January.

Distribution. — Brazil, states of Espiríto Santo. Paraná, Rio Grande do Sul, Rio de Janeiro, Paraná, Santa Catarina and São Paulo.

Bifrenaria inodora is close related to *B. harrisoniae* and *B. tyrianthina* and can be distinguished from them by its greenish to yellow flowers with lateral sepals divergent to each other, the pinkish to vinaceous labellum with a the prominent midlobe, the truncate viscidium, and by the 2-lobed callus.

Additional specimens examined: BRAZIL. Dec 1954 (HB2508). **Espírito Santo:** Nov 1994, *Ruschy s.n.* (RB46099); Santa Teresa, Reserva Biológica do Museu Nacional, Jul 1976, *Emmerich s.n.* (MBML388). **Paraná:** Estr. Curitiba-Joinville Km70, Serra do Mar, Dec 1958, *Leinig 65* (HB); Jacarehy, Jan 1910, *Dúsen s.n.* (NY); Vila Velha, Oct 1914, *Dúsen s.n.* (NY). **Rio Grande do Sul:** Brasília, Osório-Porto da Cachoeira, Dec 1934, *Dutra 1163* (HB); pr. Torres, *Seidel 979* (HB). **Rio de Janeiro:** Nova Friburgo, Macaé de Cima, *Miller s.n.* (UEC117748); Petrópolis, Oct 1933, *Spannagel 376* (SP); same locality, Oct 1937, *Magalhães s.n.* (RB49330). **Santa Catarina:** *Rohr s.n.* (HB860); Brasília, prox. Nova Trento, Nov 1952, *Welton SCJ36* (HB); Ibirama, Feb 1953, *Gevieski 5* (HB); Brusque, Nov 1951, *Reitz 4194* (HB); N.S.Bom Socorro, Nova Trento, Nov 1957, *Reitz 4195* (HB); Ribeirão, Dec 1970, *Bresolin 35* (HB). **São Paulo:** *Brade 6880-b* (HB); Cubatão, Nov 1923, (SP); Iguape, *Brade s.n.* (HB8443, HB8444); Morro Caiuvá, *Brade 6877* (HB); Morro das Pedras, Oct 1919, *Brade 8308* (HB); Paranapiacaba, Dec 1929, *Hoehne s.n.* (SP25610); São Paulo, nativa do Jardim Botânico, Dec 1937, *Handro s.n.* (SP).

8. BIFRENARIA LEUCORRHODA Rchb. f. , Hamburger Garten- Blumenzeitung 15: 54. 1859. *Stenocoryne leucorrhoda* (Rchb. f.) Kraenzl. in Rchb. f., Xenia Orchid. 3: 142. 1896. *Adipe leucorrhoda* (Rchb. f.) M.Wolff, Orchidee 41(2): 36. 1990. TYPE: s. loc. (HOLOTYPE: W-n.v., photo at UEC)

Bifrenaria vitellina Lindl. var. *leucorrhodia* Rchb. f. Bonplandia 3 (15). 217. TYPE: missing.

Bifrenaria leucorrhoda Rchb. f. var. *macaheensis* Brade, Arq. Serv. Florest. 2(1): 7 1943. *Stenocoryne leucorrhoda* (Rchb. f.) Kranzl. var. *macaheensis* (Brade) Hoehne, Fl. Bras. (Hoehne) 12 (7): 16. 1953. *Adipe leucorrhoda* (Rchb. f.) M.Wolff var. *macaheensis* (Brade) M. Wolff, Orchidee 41 (2): 36 1990. TYPE: BRAZIL. Rio de Janeiro: Frade de Macaé, 19 Jun 1937, *Brade 15872* (HOLOTYPE: RB).

(Figs. 15b, 16b, 20a)

Epiphytes, 20-40 cm tall. *Pseudobulbs* aggregate, conic, dark green, 3.5-7 x 1.4-2.6 cm. *Leaves* lanceolate, membranaceous to sub-coriaceous, 14.7-30 x 3-5 cm, pseudo-petiole 3-10.5 cm long. *Inflorescence* 3-5 flowered, erect, 12-38 cm long; scape bracts 1-1.5 x 0.5-0.6 cm, floral bracts 0.3-0.5 x 0.1-0.2 cm. *Flowers* with a subtle fragrance, white on the exterior, pink to white on the interior, labellum with pink to red stripes, 2.2-2.3 cm long, 1.2-1.5 cm wide. *Sepals* ovate, truncate, dorsal sepal 1.2-2 x 0.4-1 cm, lateral sepals parallel to each other, 1.1-1.7 x 0.5-0.9 cm, spur with sepal segments superposed, 0.4-0.6 cm long. *Petals* ovate, entire to 3-lobed, oblique to column, 1.2-1.7 x 0.5-1 cm. *Labellum* 3-lobed, obovate, puberulous, with crenate margins, 1.2-2 x 1-2.1 cm when expanded, lateral lobes oblong, involute, midlobe strongly emarginate, recurved, the claw 0.1-0.3 cm long, the callus 0.4-0.8 cm long, puberulous, on the anterior region 3-lobed, glabrous to puberulous. *Column* puberulous to pubescent, 0.5-0.7 cm, the foot straight, glabrous to puberulous, 0.4-1 cm long. *Anther* 0.15-0.2 x 0.15-0.2 cm, the pollinia rounded, the stipe forked, 0.1-0.15 cm long, the viscidium truncate, 0.05 x 0.1 cm long. *Ovary-pedicel* 1.5-2.8 cm long.

Habitat. — Native to wet montane forests, mainly litoranean, at 500-1500 m elevation. Flowering occurs from December to May.

Distribution. — Brazil, states of Espírito Santo, Rio de Janeiro, and São Paulo.

Brade (1943) described *Bifrenaria leucorrhoda* Rchb. f. var. *macaheensis* based on the format of petals and of the lateral lobes. We believe *B. leucorrhoda* is insufficiently represented in herbarium collections and, therefore, the assessement of its morphological variation is problematic. Also the analysis of the herbarium material available corroborates that there is a great variation considering the size and format of floral segments. We therefore consider the variety *B. leucorrhoda* var. *macaheensis* not a good taxon.

Bifrenaria leucorrhoda can be distinguished from congeneric species by its white to pinkish perianth and by its lateral sepals parallel to each other.

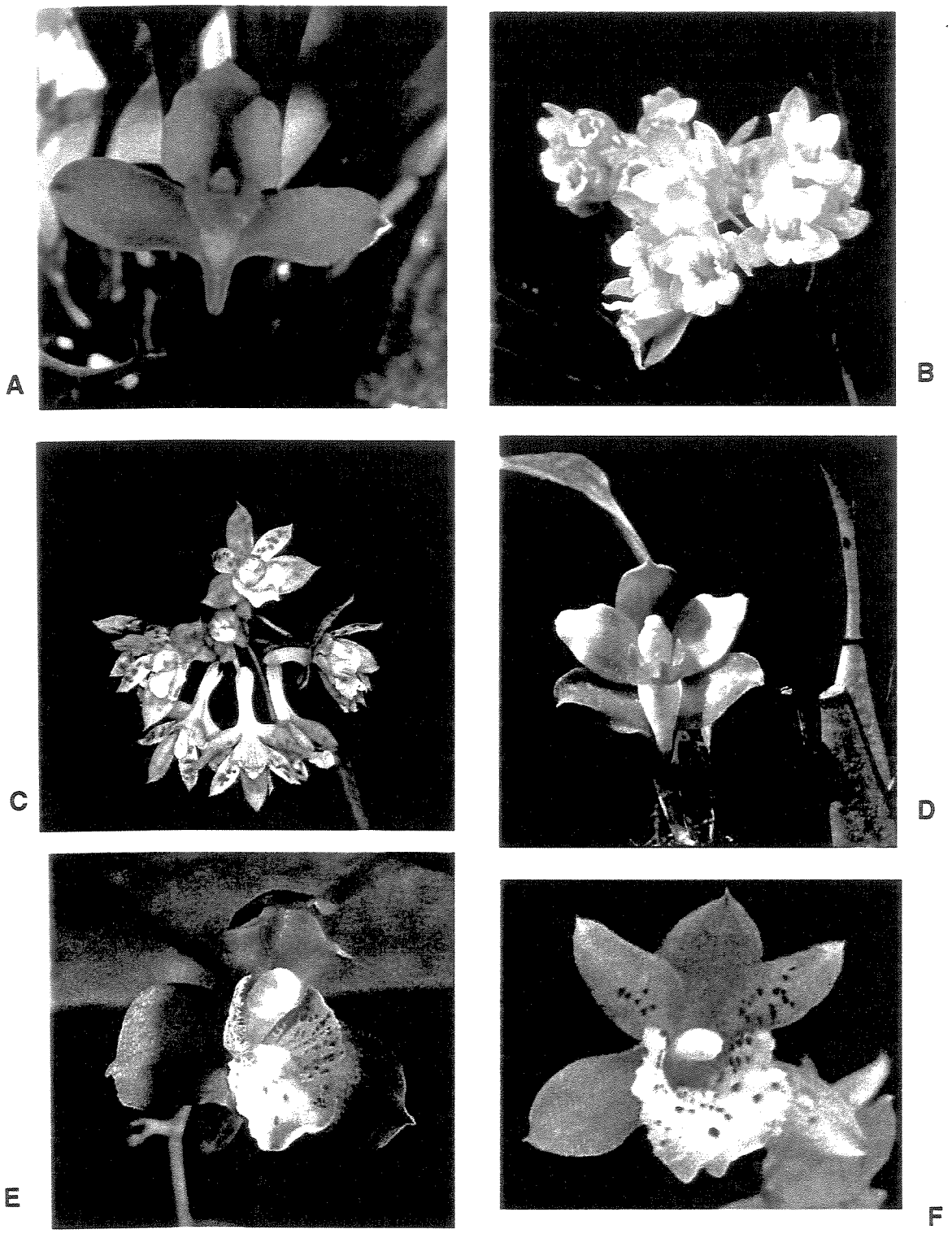


Fig. 15. (A) *B. inodora* (B) *Bifrenaria leucorrhoda* (C) *B. longicornis* (D) *B. mellicolor* (E) *B. racemosa* (F) *B. silvana* (fotos B-F de V. P. Castro).

Additional specimens examined: BRAZIL. **Espírito Santo:** Castelo, Forno Grande, May1949, *Brade 19834* (RB). **Rio de Janeiro:** Itatiaia, Dec 1941, *Lanius s.n.* (SP46177); Petrópolis, Jan 1930, *Geoffroy s.n.* (SP25611); same locality, Feb 2000, ex hort., Orquidário Binot, *Koehler 56c* (UEC); Rio de Janeiro, Corcovado, 1934, *Carris s.n.* (RB28969); Teresópolis, Serra dos órgãos, Vale do Jacob, Mar-Apr 1952, *Vidal 52-1735* (R). **São Paulo:** Picinguaba, Jan 2000, *Singer et al. s.n.* (UEC117746).

9. BIFRENARIA LONGICORNIS Lindl., Edward's Bot. Reg. 24: t. 93. 1838. *Stenocoryne longicornis* (Lindl.) Lindl., Bot. Reg. 29: Misc. 53. n. 68. 1843. *Adipe longicornis* (Lindl.) M.Wolff., Orchidee 41(2). 36. TYPE: s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: Edward's Bot. Reg. 24: t. 93. 1838.)

Bifrenaria sabulosa Barb. Rodr., Gen. Sp. Orchid.. 1: 111. 1877. *Rudolfiella sabulosa* (Barb. Rodr.) Hoehne, Arq. Bot. Estado São Paulo. 2(1). 13-14. 1944. TYPE: s. loc. (HOLOTYPE: RB-destroyed, LECTOTYPE, selected here: Iconographie des Orchidées du Brésil 5: t.228 at Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil).

(Figs. 15c, 17a, 20a)

Epiphytes, 10-25 cm tall. *Pseudobulbs* distant, compressed, dark green to brownish with dark spots, 1,5-10 cm long. *Leaves* lanceolate, membranaceous to sub-coriaceous, 10-20 x 3-6.6 cm, pseudo-petiole 0.5-2 cm long. *Inflorescence* 4-15 flowered, erect to pendent, 4-16 cm long; scape bracts 0.7-2 x 0.3 cm, floral bracts 0.5-1 x 0.1-0.2 cm. *Flowers* white yellowish with brownish red dots, the labellum with pink to red stripes, 2 cm long, 1 cm wide. *Sepals* ovate to oblong, truncate, acuminate, dorsal sepal 1.5 x 0.3-0.5 cm, lateral sepals parallel to each other, 1.5 x 0.3-0.5 cm, spur with sepal segments fusioned, 1 cm long. *Petals* oblanceolate, truncate, oblique to column, 0.8-1 x 0.3-0.5 cm. *Labellum* 3-lobed, rhombic, glabrous, 1.2-2 x 0.5-1.5 cm when expanded, lateral lobes obtuse, involute, midlobe obtuse, recurved, the claw 1-1.2 cm long, the callus 0.3-0.5 cm long, glabrous to pubescent, on the anterior region 3-dentate, elongate, glabrous, not protuberant. *Column* glabrous to puberulous, 0.4-0.6 cm, yellowish, the foot straight, glabrous to puberulous, 1-1.5 cm long. *Anther* 0.1 cm long, the pollinia rounded, the stipe forked, 0.05 cm long, the

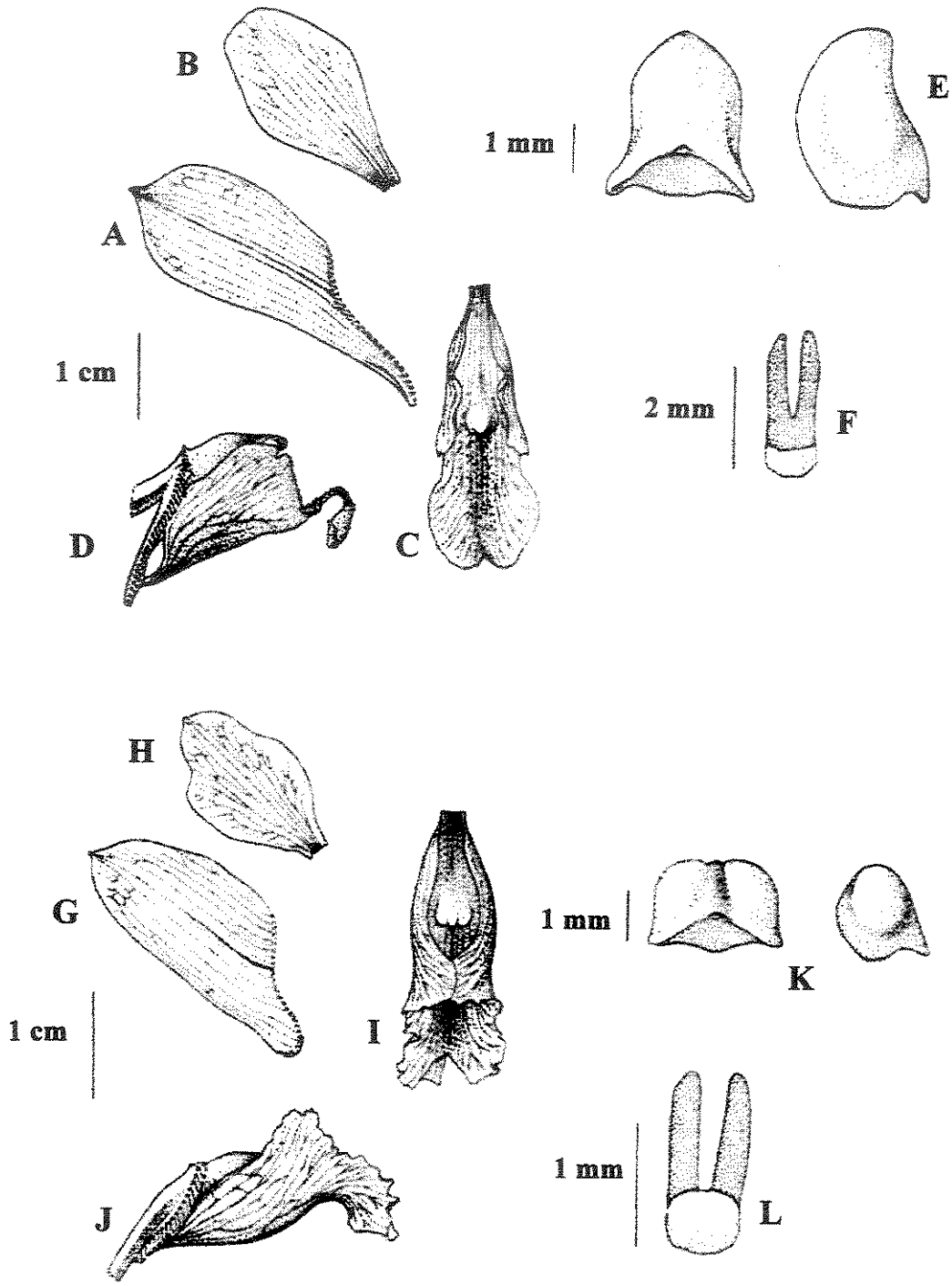


Fig. 16. (A-F) *Bifrenaria inodora* Lindl., (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia. (G-L) *B leucorrhoda* Rchb. f., (G) lateral sepal, (H) lateral petal, (I) labellum, (J) lateral view of flower showing the position of the labellum, (K) anther cap, (L) pollinarium without pollinia.

viscidium broadly rounded, 0.05 cm long. *Ovary-pedicel* 1-1.5 cm long.

Habitat. — Native to wet lowland and riparian Amazonian forests, at 80-450 m elevation. Flowering occurs along the whole year.

Distribution. — Brazil, states of Amazonas, Acre and Pará; Colombia, Guiana, Peru, Suriname and Venezuela. We agree with Castro and Campacci (2000) when regarding *Bifrenaria sabulosa* Barb. Rodr. to synomyn of *Bifrenaria longicornis.*

Bifrenaria longicornis is a very common species on the Amazonian region. It is easily recognizable by its long spur and by the yellow flowers with red dots.

Additional specimens examined: COLOMBIA. Amazonas, rio Caquetá, cerca al chorro Córdoba, Mar1990, *Galeano et al. 2086* (NY). BRASIL. Apr 1954, *Pires 4645* (IAN). **Acre:** Projeto Radam, Sub-base Cruzeiro do Sul, Ponto 10 SB182D, Feb1976, *Marinho 84* (IAN). **Amazonas:** Campo Petrolífero do rio Urucu, Coari, May 1993, *Cruz 186* (INPA); same locality, road to LUC-16, IX.1992, *Cruz 187* (INPA); Manaus, Mauá Road, Mar 1971, *Prance at al. 11518* (NY, R); Manaus-Itacoatiara Road Km64, Feb 1989, *Byron and Coelho 69-128* (UEC); Manaus-São Gabriel, Morro dos Seis Lagos, ca. 80km N of São Gabriel; Jul 1979, *Poole 2051* (NY); Nova Olinda, Mari Mari river, Laranjal, Jul 1983, *Todzia 2293 et al.* (INPA, NY); Bacia do rio Madeira, Posto da FUNAI, Vila dos Indios Munducurus, rio Marimarí, Jul 1983, *Cid 3998* (INPA); Parque Nac. Jaú, Campina do Patauá, Quadrante K8, Sep 1998 (INPA); South bank of Rio Negro, Baia de Bueussu, 15Km, Mar 1969, *Prance et al. 10433* (NY); Urubu river, between cachoeira de Iracema e Manaus-Caracaraí road, Jun 1968, *Prance 5025* (NY). **Pará**. Serra do Cachimbo, Mar 1958, *Pires 6695* (IAN). GUIANA. Bartica, Essequibo river, Lat. 6°25´, Dec 1922, *De la Cruz 1964* (NY); same locality, Dec 1922, *De la Cruz 1905* (NY); Mazaruni river, 60°10´W; Sep-Oct 1922, *De la Cruz 2182* (MO, NY); Pomeroon, Pasanalley Island, Aug 1921, *De la Cruz 1084* (NY). PERU. Depto Loreto, Prov. Requena, 73°45´W, 4°55´S, Feb 1987, *Gentry 56251* (MO). VENEZUELA. Amazonas, rio Atabapo, Jun 1922, *Wurdack and Adderley 42994* (NY); Alto Orinoco, Jul 1951, *Croizat 117* (NY); Bolivar, 4°20´N, 61°48´W, Dec 1978, *Steyermarkii et al.*

117743 (MO). Depto Rio Negro, Rio Pasimoni, 1°53´N, 66°35´-66°32´W, Jul 1984, *Davidse 27742* (MO, NY); same locality 1°10´N, 66°25´W, Apr 1984, *Liesner 17144* (MO, NY); 4Km east of San Carlos de Rio Negro, IVIC study area, 1°56´N, 67°4´W, Nov 1977, *Liesner 3319* (MO); Santa Cruz, Amazonas, rio Atacaví, Sep 1960, *Foldats 3649* (NY).

10. BIFRENARIA MELLICOLOR Rchb. f., Gard. Chron.1: 622. 1878. TYPE: BRAZIL, s. loc. (HOLOTYPE: W-n.v., photo at UEC).

(Figs. 15d, 17b, 20b)

Epiphytes, 15-30 cm tall. *Pseudobulbs* aggregate, conic, dark yellow to green, 1,5-10 cm long. *Leaves* lanceolate, membranaceous to sub-coriaceous, 13,5-26 x 3,3-6 cm, pseudo-petiole 1-6.5 cm long. *Inflorescence* 1-3 flowered, erect, 3-7 cm long; scape and floral bracts 2 x 0.6-0.8 cm. *Flowers* light yellow to greenish yellow, with the labellum white-yellowish to pink with red stripes, 2.5-3.5 cm long, 2.5 cm wide. *Sepals* ovate, truncate, dorsal sepal 1.2-3.2 x 0.6-2 cm, lateral sepals parallel to each other, 2.2-3 x 1-1.5 cm, spur with sepal segments superposed, 1-2 cm long. *Petals* obovate to rhombic, oblique to column, 1.9-2.6 x 0.9-1.2 cm. *Labellum* 3-lobed, obovate to ovate, parallel to column, 2-3 x 1-2.6 cm when expanded, lateral lobes oblong, rounded, involute, 1-1.6 x 0.5-0.7 cm, midlobe folded lengthwise, emarginate and recurved, pubescent, rounded, the claw 0.5-0.8 cm long, the callus 0.7-2 cm long, scantly pubescent, on the anterior region entire to slightly lobed, glabrous, protuberant. *Column* scantly puberulous, glabrous ventrally, white to yellow, 0.7-1 cm, the foot straight, puberulous, 2-3 cm long. *Anther* 0.4 x 0.4 cm long, the pollinia rounded, the stipe entire, 0.1-0.2 cm long, the viscidium cuneate, 0.1 cm long. *Ovary-pedicel* 3.5-5 cm long.

Habitat. — Native to wet montane forests, at 500-1000 m elevation. Flowering occurs from June to October.

Distribution. — Brazil, states of Espírito Santo and Rio de Janeiro.

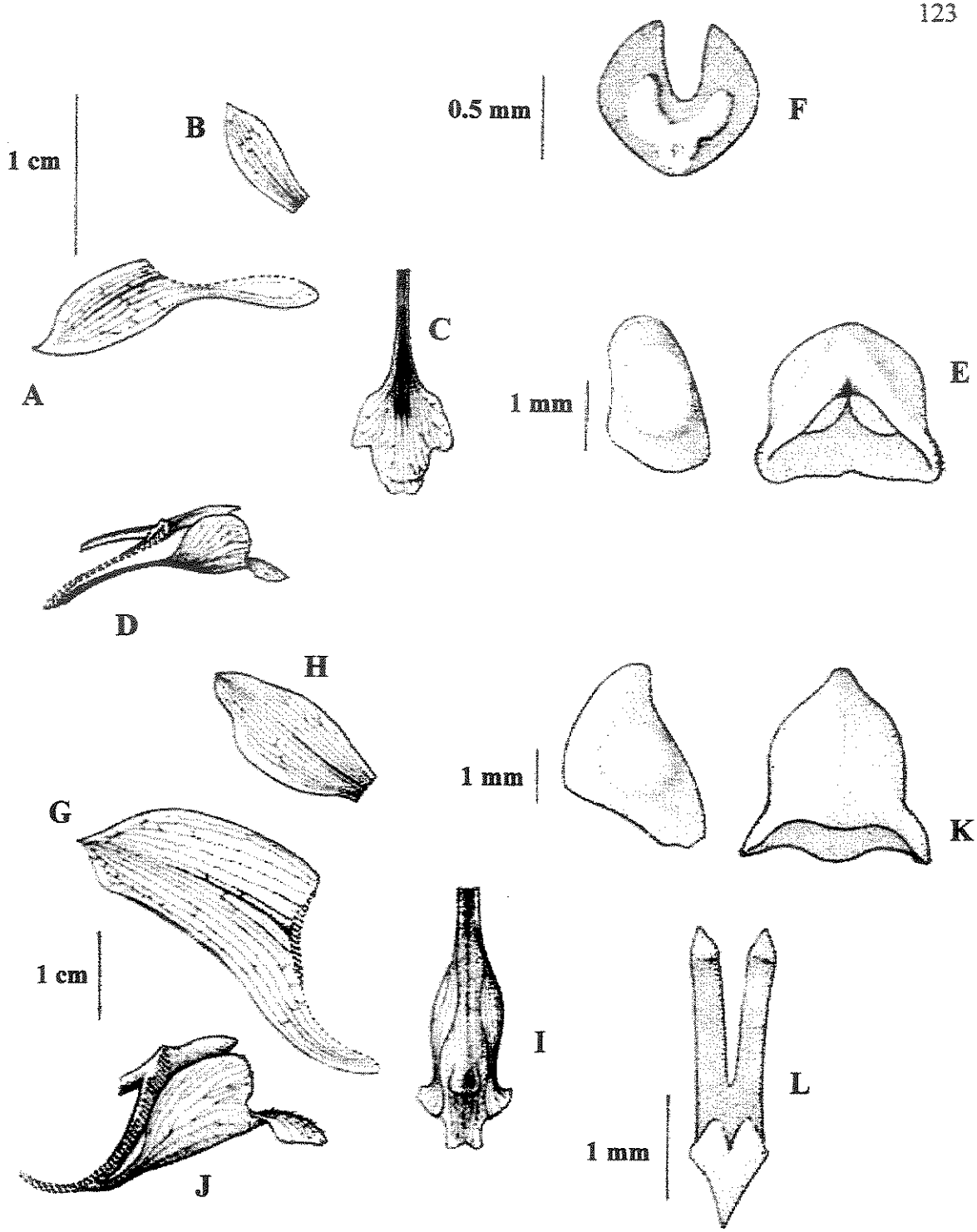


Fig. 17. (A-F) *Bifrenaria longicornis*, (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia. (G-K) *B mellicolor*, (G) lateral sepal, (H) lateral petal, (I) labellum, (J) lateral view of flower showing the position of the labellum, (K) anther cap, (L) pollinarium without pollinia.

Bifrenaria melllicolor is very similar to *B. calcarata*, and can be distinguisged from it by the shorter spur, and the straight, rounded to truncate midlobe of the labellum.

Additional specimens examined: BRASIL. **Espírito Santo:** May 1988, *Kollmann 6* (MBML); Domingos Martins, Oct 1970, *Kautsky 289* (HB); same locality, June 2000, *Schunk s.n.* (UEC117746); Santa Leopoldina, Jul 1961, *Seidel 3* (HB); same locality, Morro da estação repetidora de TV, May 1985, *Boone 436* (MBML); same locality Reserva Santa Lúcia, Feb 1996, *Lombardi and Temponi 1100* (BHCB); Santa Teresa, Pátio do Museu de Biologia, Aug 1985, *Boone 705* (MBML), same locality, Reserva Biológica Nova Lombardia, 800-1000 m elevation, May 1985, *Martinelli 10941, Zuloaga, Varques, Caruso* (RB)

11. BIFRENARIA RACEMOSA (Hook.) Lindl., Edward's Bot. Reg. 29: Misc. 52. 1843. *Maxillaria racemosa* Hook., Bot. Mag. 54: t. 2789. 1827. *Adipe racemosa* (Hook.) Raf., Fl. Tellur. 2: 101. 1836. *Stenocoryne racemosa* (Hook.) Kraenzl., in Rchb. f., Xenia Orchid. 3: 142 1896. TYPE: BRAZIL. Rio de Janeiro: Jun 1827 (HOLOTYPE: missing, LECTOTYPE, selected here: in Bot. Mag. 54: t. 2789. 1827).

Adipe fulva Raf., Fl. Tellur. 2: 101. 1836, pro syn.

(Figs. 15e, 18, 20b)

Epiphytes, 10-25 cm tall. *Pseudobulbs* aggregate, compressed, dark green to brownish, 3.2-5 x 2-2.3 cm. *Leaves* lanceolate, membranaceous, 14-22 x 2.5- 4.8 cm, pseudo-petiole 2.3-7 cm long. *Inflorescence* 2-8 flowered, pendent, 15-40 cm long; scape bracts 0.6-1.2 x 0.2-0.4 cm, floral bracts 0.3-1 x 0.1-0.3 cm. *Flowers* yellow-greenish to pale brown, the labellum white-yellowish with red dots, 1-2.5 cm long, 2 cm wide. *Sepals* ovate, truncate, dorsal sepal 1.1-1.7 x 0.5-0.8 cm, lateral sepals parallel to each other, 0.9-1.5 x 0.6-0.8 cm, spur with sepal segments fusioned, 0.4-0.8 cm long. *Petals* oblanceolate, parallel to column, 1.1 x 0.5 cm. *Labellum* entire, obovate to orbicular, involute, margins crenate, laterally involute, midlobe emarginate and prominent, scantly pubescent, 0.9-1.5 x 0.5-1.3 cm when expanded, the claw 0.3-0.4 cm long, the callus 0.4-0.7 cm long, scantly

pubescent, on the anterior region entire, glabrous, protuberant, white. *Column* pubescent, 0.5 cm, the foot straight, glabrous to puberulous, 0.4-0.7 cm long. *Anther* 0.1 x 0.15 cm long, with a prominent apex, the pollinia rounded, the stipe forked, 0.03 cm long, the viscidium truncate, 0.07 cm long. *Ovary-pedicel* 1.8-2.2 cm long.

Habitat. — Native to wet montane forests, at 300-1000 m elevation. Flowering occurs from April to May.

Distribution. — Brazil, states of Espírito Santo, Rio de Janeiro and São Paulo.This species is very similar to *Bifrenaria charlesworthii*.

We considered *B. racemosa* to be different of *B. charlesworthii* based on its lateral sepals parallel to each other and the slightly pubescent, ovate to elongate labellum. Due to the fact these two species are sympatric and very dfficult to distinguish in herbarium material, a careful study of morphological variation considering different populations is necessary to clarify the species boundaries, if there are any at all.

Additional specimens examined: BRASIL. **Rio de Janerio:** ex hort. Jardim Botânico do Rio de Janeiro, Mar 1951 (RB); Itatiaia, Monte Serrat, 1914, *Porto 22* (RB); Petrópolis, Apr 1930, *Spannagel 272* (SP); Morro Queimado, May 1972, *Sucre and Soderstrom 9149* (RB); Rio de Janeiro, Alto da Boa Vista, Corcovado, May 1874 (R); same locality, Serra da Tijuca, Apr 1931, *Brade s.n.* (R24925); same locality, Jan 1932, *Brade and Santos Lima s.n.* (R35694); same locality, May 1948, *Koch s.n.* (RB622339); same locality, trilha Pico da Tijuca, Nov 1946, *Walter, Aparicio, Altamiro and Edmundo s.n.* (RB55790); Matas do Bal Ricardo, May 1943, *Occhioni 68* (RB). **São Paulo:** Guarujá, Ilha de Santo Amaro, May 1934, *F. Zoega* s.n. (SP31829).

Fig. 18. *Bifrenaria racemosa* (from Bot. Magaz. 54: t. 2789. 1827).

12. BIFRENARIA SILVANA V. P. Castro. Bol. Coord. Assoc. Orquid. 3(4): 42-44. 1994. TYPE: BRAZIL. Bahia: Itororó, Serra da Ouricana, 500 m elevation, 25 Nov 1987, *Silva s.n.* (HOLOTYPE: SP246656)
(Figs. 15f, 19, 20b)

Epiphytes, to 17 cm tall. *Pseudobulbs* aggregate, compressed, dark green, 1.5-2 x 1.2-1.7 cm. *Leaves* lanceolate, coriaceous, 8 x 2-3 cm, pseudo-petiole 3 cm long. *Inflorescence* 2-6 flowered, pendent, 3 cm long; scape and floral bracts 0.4 x 0.1 cm *Flowers* pale brown to white, the labellum white with red dots. *Sepals* ovate, truncate, with red dots at the base, dorsal sepal 1.2 x 0.6 cm, lateral sepals divergent to each other, 1.5 x 0.9 cm, spur with sepal segments superposed, 0.5 cm long. *Petals* oblanceolate, oblique to column, 1.1 x 0.7 cm. *Labellum* entire to slightly 3-lobed, orbicular, puberulous, margins crenate, laterally involute, 1.3 x 1.6 cm when expanded, midlobe prominent, emarginate, recurved, the claw 0.1 cm long, the callus 0.6 cm long, glabrous, on the anterior region 3-lobed, glabrous, protuberant. *Column* 0.6 cm, the foot straight, 0.5 cm long. *Anther* with the pollinia rounded, the stipe forked, the viscidium truncate. *Ovary-pedicel* 2 cm long.
Habitat. — Native to wet montane forests in the state of Bahia, at 700 m elevation.
Flowering occurs from November to December.
Distribution. — Brazil, states of Bahia.

This species is apparently restricted to the state of Bahia. There is no herbarium material available other than the type. It is similar to *Bifrenaria charlesworthii* and *B. racemosa*, but can be distinguished from them by its white perianth, with red spotted labellum and sepals, by lateral sepals divergent to each other and petals oblique to column.

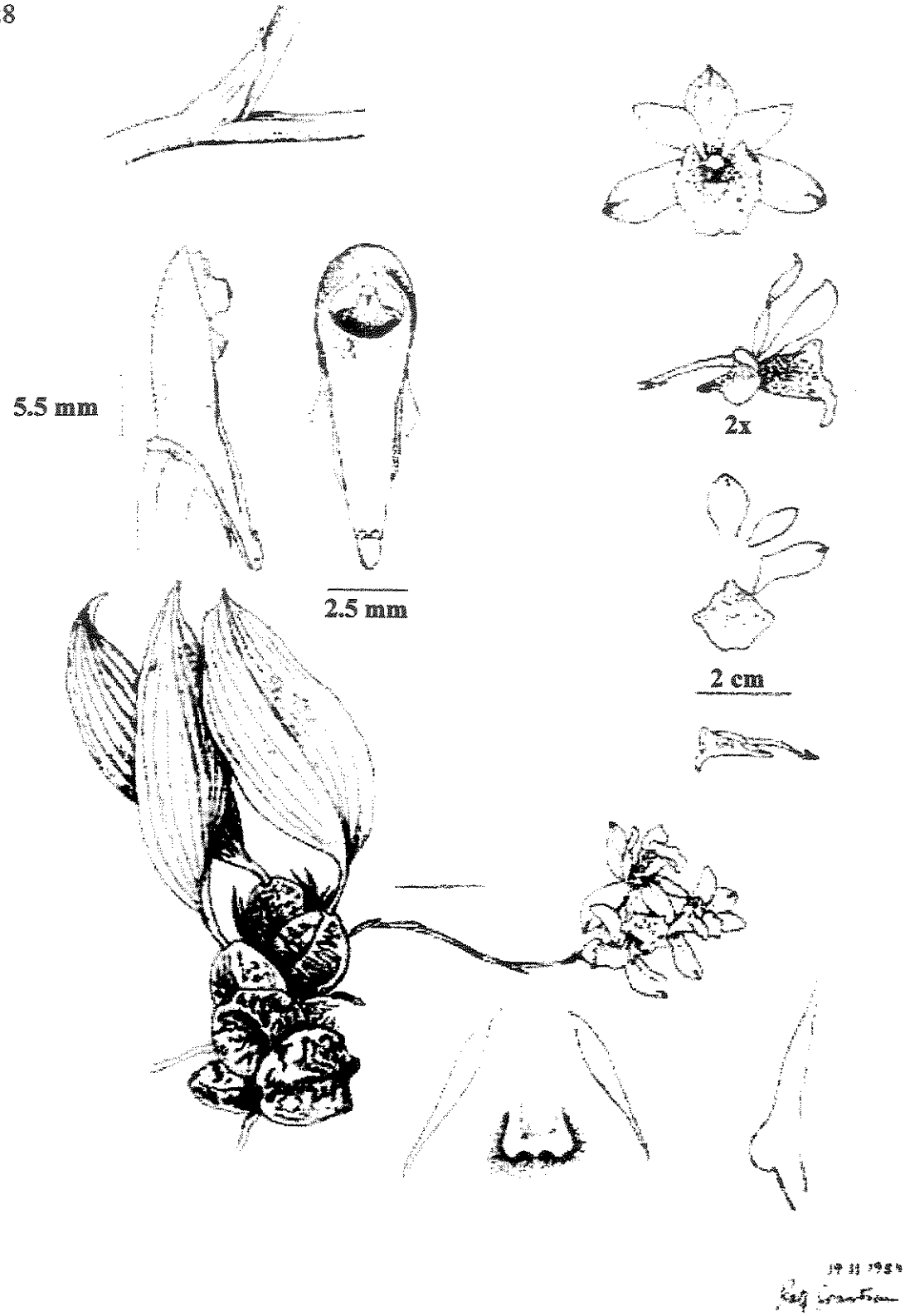
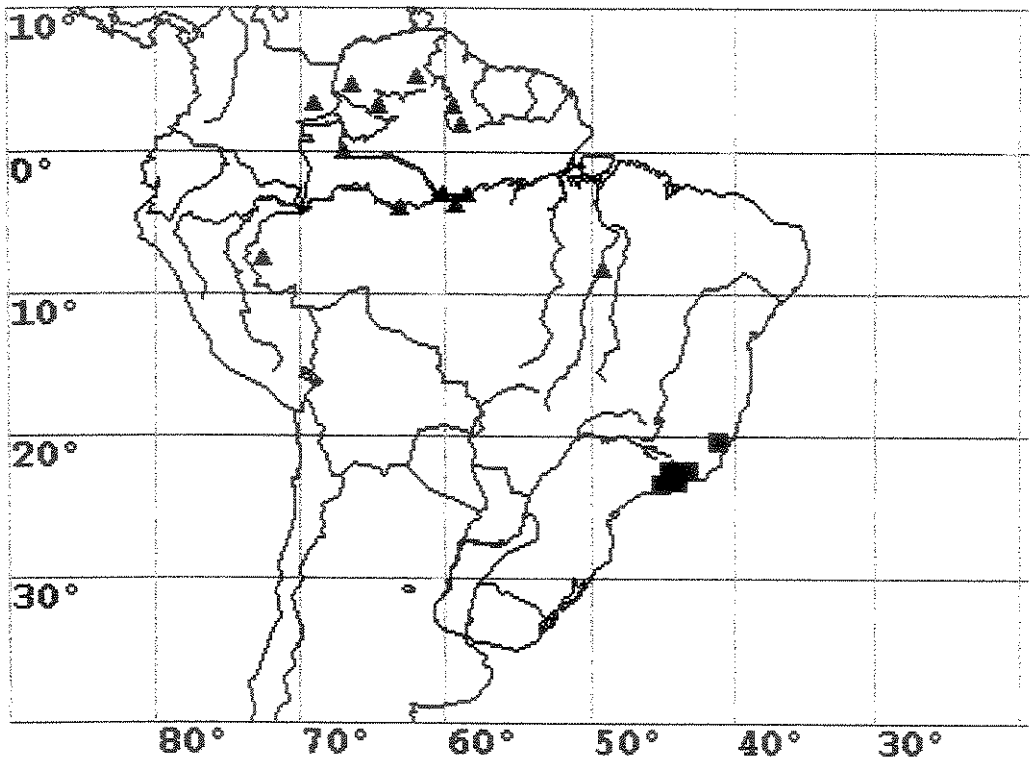


Fig. 19. *Bifrenaria silvana* (from Boletim Coord. Assoc. Orquid. 3 (4):43. 1991).

A



B

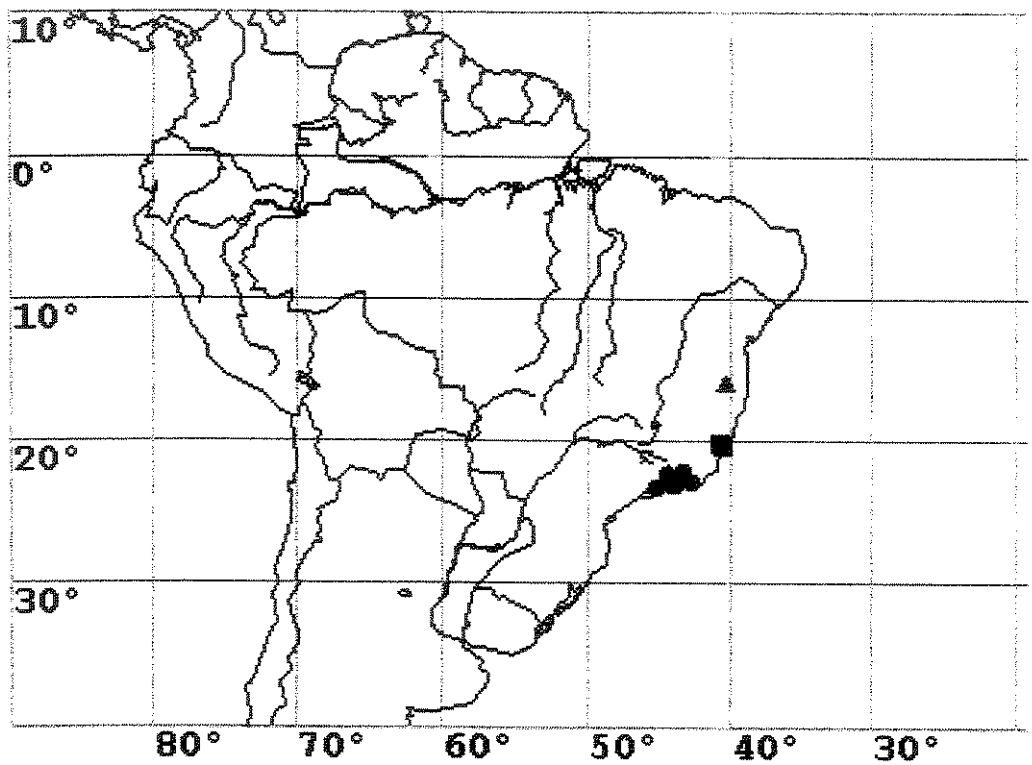


Fig. 20. (A) *Bifrenaria leucorrhoda* (■); *B. longicornis* (▲). (B) *B. mellicolor* f. (■); *Bifrenaria racemosa* (●) *Bifrenaria silvana* (▲).

13. BIFRENARIA STEFANAE V.P. Castro, Bol. Coord. Assoc. Orquid. 3 (4): 42-43. 1991. *Adipe stefanae* (V.P.Castro) K. Senghas, Schlechter Orchideen. ed. 3, 1/B (29): 1837. 1994. TYPE: BRAZIL. São Paulo: Salesópolis, Serra do Mar, 500-600 m elevation, 16 Apr 1984, *Castro s.n.* (HOLOTYPE: SP246655, ISOTYPE: AMES74688).
(Figs. 21a, 25a, 26a)

Epiphytes, rarely rupiculous plants, 10-35 cm tall. *Pseudobulbs* aggregate, conic, dark green to brownish, 2.2-6 x 0.9-2.9 cm. *Leaves* lanceolate, membranaceous to sub-coriaceous, 8-31 x 1.5-6.2 cm, pseudo-petiole 2-13 cm long. *Inflorescence* 1-8 flowered, pendent, 8-20 cm long; scape bracts 0.7-1.2 x 0.1 cm, floral bracts 0.2-0.5 x 0.1-0.2. *Flowers* pale brown to yellow greenish, the labellum pale yellow with red stripes, 1.5 cm long, 1 cm wide. *Sepals* ovate, truncate, apically recurved, dorsal sepal 1-1.4 x 0.3-0.6 cm, lateral sepals parallel to each other, 1-2.2 x 0.4-0.8 cm, spur with sepal segments superposed, 0.4-0.8 cm long. *Petals* oblanceolate to obovate, oblique to column, 1.1-1.5 x 0.4-0.6 cm. *Labellum* 3-lobed, obovate, scantly pubescent, margins undulate, 1.2-1.9 x 1.2-2.5 cm, lateral lobes obovate, 0.7-1.4 x 0.3-0.4 cm, when expanded, midlobe emarginate, recurved, the claw 0.1 cm long, the callus 0.6-1.2 cm long, scantly pubescent, on the anterior region 3-lobed, glabrous, protuberant, yellow. *Column* puberulous, 0.4-1.6 cm, the foot straight, puberulous, 0.4-1.2 cm long. *Anther* 0.2 x 0.1-0.2 cm long, the pollinia rounded, the stipe forked, 0.1 cm long, the viscidium truncate, 0.1 cm long. *Ovary-pedicel* 1.3-2.3 cm long.

Habitat. — Native to wet montane forests, at 900-1350 m elevation. Flowering occurs from November to April.

Distribution. — Brazil, states of Minas Gearis, Paraná, Rio de Janeiro and São Paulo.

This recently described species is, curiously, very common on the wet Atlantic forests of Southern Brazil. For a long time it was mistaken by *Bifrenaria vitellina* Lindl., due to the color of the flowers and vegetative traits. Castro, in 1994, described *Bifrenaria stefanae* based on its lateral sepals parallel to each other, smaller size of the flowers and on the shorter labellum midlobe.

Additional specimens examined: BRASIL. VI.1918 (NY2649) **Minas Gerais:** Caeté, Serra da Piedade, 19′49′′S 43′40′′W, May 1987, *Paula s.n.* (BHCB9022); Carrancas, Apr 2000, *Simões s.n.* (UEC); Ibitipoca, Apr 1993, *Barros s.n.* (CESJ26469); Santa Bárbara, Serra do Caraça, Apr 1933, *Barreto 5334* (BHMH); same locality, 1350 m, Dec 1977, *Martinelli and Távora 2734* (RB); Santa Luzia, Serra do Cipó, Oct 1924, *Sampaio 6906* (R). **Paraná:** Curitiba, Apr 1943, *Guimarães s.n.* (RB48258). **Rio de Janeiro:** Cunha, Parati, ex hort. Instituo de Botânica de São Paulo, Mar 2000, *Catharino s.n.* (SP); Itatiaia, Jan 1938, *Zoéga s.n.* (SP39160); same locality, rio Campo Belo, Lote 37, Mar 1942, *Brade 17288* (RB);

same locality, Maromba, Jan 1928, *Porto 1677* (RB); same locality, rio Campo Belo at 1000 m, Jan 1950, *Brade 20231* (RB); Nova Friburgo, Macaé de Cima, Mar 2000, *Koehler 54c* (UEC); Petrópolis, Serra dos Órgãos, Feb 1991, *Castro s.n.* (SP246836); Serra dos Órgãos, ex hort., Jan 1942, *Brade s.n.* (RB); Serra dos Penitentes, Feb?, Moura 1888 (RB). **São Paulo:** Bananal, P. N. Serra da Bocaina, margens rio Bonito, 1000 m, Apr 1980, *Martnelli 6715 and Simões Lopes*; Rebouças, Jan 1944, *Krakowzier s.n.* (SP50364); Rio Bonito, Mar 1945, *Vianna s.n.* (R196510).

14. BIFRENARIA TETRAGONA (Lindl.) Schltr., Gard. Chron. 43: 422. 1908. *Maxillaria tetragona* Lindl., Edward′s Bot. Reg. 17: t. 1428. 1832. *Lycaste tetragona* (Lindl.) Lindl., Edward′s Bot. Reg. 29: Misc. 49. 1843. *Cydoniorchis tetragona* (Lindl.) K. Senghas, J. Orchideenfreund 1: 11. 1994. TYPE: BRAZIL. Rio de Janeiro: Jun 1830 (HOLOTYPE: missing, LECTOTYPE, selected here: Edward′s Bot. Reg. 17: t. 1428. 1832).

Bifrenaria tetragona (Lindl.) Schltr. var. *umbrophila* Hoehne, Arq. Bot. Estado São Paulo 2 (5): 116. 1950. TYPE: BRAZIL. São Paulo: Serra de Paranapiacaba, prox. Est. Biológica Alto da Serra, 25 Jan 1922, *Gehrt s.n.* (HOLOTYPE: SP7519).

Bifrenaria tetragona (Lindl.) Schltr. var. *rupicola* Hoehne, Arq. Bot. Estado São Paulo 2 (5): 116. 1950. TYPE: s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: in Fl. Bras. (Hoehne) 12 (7): 16, t. 11. 1953).

Epidendrum calcaratum Vell., Fl. Flumin. 8: t. 8. 1827. *Bifrenaria calcarata* (Vell.) V.P.Castro Proceedings of the 15th World Orchid Conference, Rio de Janeiro. 377. 1996, nom. illeg., non *B. calcarata* Barb. Rodr (1882). TYPE: s. loc. (HOLOTYPE: destroyed, LECTOTYPE, selected here: Fl. Flumin. 8: t. 8. 1827).

(Figs. 21b, 25b, 26b)

Epiphytes or rupiculous plants, 30-50 cm tall. *Pseudobulbs* aggregate, conic, dark green to yellow, 2.5-10 x 1-2.5 cm. *Leaves* lanceolate to obovate, coriaceous, 19-42.5 x 4.2-11.5 cm, pseudo-petiole 4.3-13 cm long. *Inflorescence* 4-7 flowered, pendent, 5-5.5 cm long; scape and floral bracts 1.5-2 x 0.5-1 cm. *Flowers* turned upward, fragrant, brownish yellow to greenish yellow, the labellum purplish with vinaceous stripes, 3.5-4.2 cm long, 3.4-4.5 cm wide. *Sepals* ovate, truncate, acute, dorsal sepal 2.5-3.6 x 1.5-2 cm, lateral sepals parallel to each other, 3-3.9 x 2.5-3.8 cm, spur with sepal segments superposed, 0.3-0.6cm long. *Petals* ovate, truncate, acute, oblique to column, 2.2-3 x 1-1.6 cm. *Labellum* 3-lobed, convex, margins entire, 2-2.9 x 2.4-3.8 cm when expanded, lateral lobes involute, truncate to laterally acuminate, midlobe prominent, subacute, glabrous, 0.5-1.7 x 0.7-1.5 cm, the claw 0.3-0.5 cm, the callus 1.1-1.5 cm long, fleshy, puberulous, on the anterior region emraginated. *Column* glabrous, winged, yellow-greenish, 1.2-1.5 cm long, the foot arched, glabrous, 1.8-3 cm long. *Anther* 0.5-0.6 x 0.4 cm, the pollinia oval, the stipe entire, 0.1-0.2 cm long, the viscidium cuneate, 0.2-0.3 cm long. *Ovary-pedicel* 2-4 cm long.

Habitat. — Native to wet montane forests, elevation 500-1000 m elevation. Flowering occurs from December to February.

Distribution. — Brazil, states of Rio de Janeiro Santa Catarina and São Paulo. at 700 m elevation. Flowering occurs from November to December.

Lindley described *Maxillaria tetragona* in 1831, which turned out to be the same species described by Velloso as *Epidendrum calcaratum* in 1827. Based on the latter name Castro (1996) published the new combination *Bifrenaria calcarata* (Vell.) V.P. Castro, which is not legitimate since Barbosa Rodrigues described *Bifrenaria calcarata*, a different species, in 1886 based on a different type. Therefore the legitimate basionym for this species is *Maxillaria tetragona* Lindl.

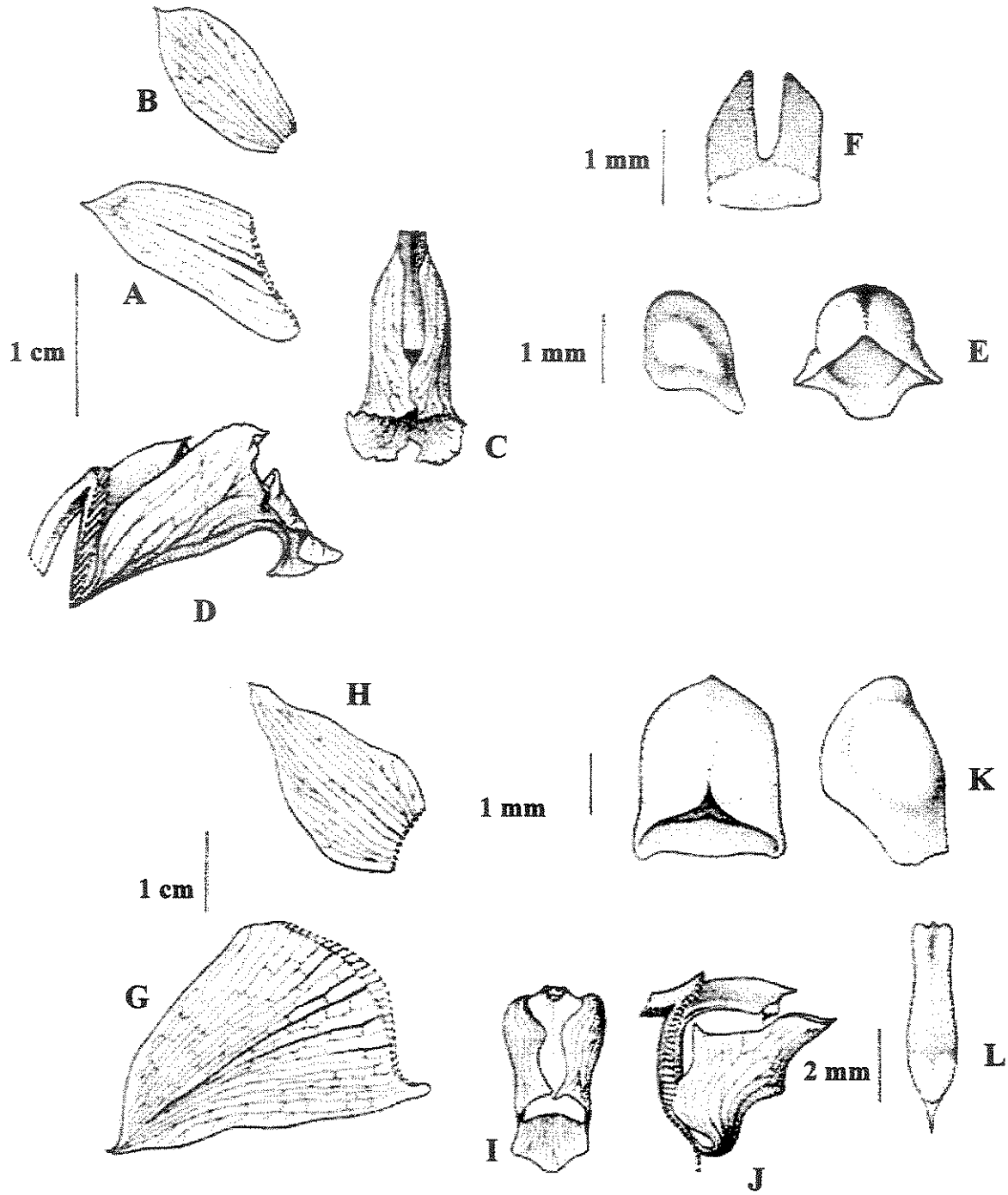


Fig. 21. (A-F) *Bifrenaria stefanae* (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia. (G-L) *B tetragona* (G) lateral sepal, (H) lateral petal, (I) labellum, (J) lateral view of flower showing the position of the labellum, (K) anther cap, (L) pollinarium without pollinia.

Bifrenaria tetragona is closely related to *B. wittigii*, but can be distinguished from it by the sub-acute labellum midlobe, and by the lateral lobes truncate to laterally acuminate.

Blumenschein and Packer (1961) have reported chromosome numbers of 2n= 38 and 4n=76 for this species.

Additional specimens examined: BRASIL. **Rio de Janeiro:** Dec 1918, *Magalhães s.n.* (SP2661); Petrópolis, Feb 2000, *Koehler 00/20b.* (UEC); Rio de Janeiro, Serra da Tijuca, Jan 1932, *Brade 11321* (R). **Paraná:** Bacaíva do Sul, Rio Capivari, Jan 1969, *Hatschbach 20715* and *Koczicki* (HB). **Santa Catarina:** Brasília, Nova Trento, Jan 1952, *Rohr 2175* (HB); Brusque, próx. Vida Ramos, Dec 1952, *Walter SCJ41* (HB); Correa, Corupá, Jaguará Sul, Jan 1958, *Reitz and Klein 6202* (HB); Itajaí, Morro do Baú, I.1953, *Reitz 5178* (HB). **São Paulo:** Alto da Serra, Estação Biológica, Jan 1922. *Gehrt 7519* (NY); Iguape, Boa Vista de Peroupava, Dec 1920, *Brade 8078* (HB); São Paulo, nativa do Jardim Botânico, Jan 1952, *Handro 216* (SP); same locality, Dec 1937, *Handro s.n.* (SP1566); Dec 1969, *Handro 2108* (SPF).

15. BIFRENARIA TYRIANTHINA (Lodd.) Rchb. f., Xenia Orchid. 1: 61. 1854. *Lycaste tyrianthina* Lodd. Hort. Brit. Suppl. 3: 582. 1850. TYPE: unknown. (NEOTYPE, selected here: BRAZIL. Bahia. Rio de Contas, Pico das Almas, Vertente Leste, Campo do Queiroz, 13°32′S, 47°57′W, Nov 1988, *Harley 26104 and Hind,* UEC; ISONEOTYPES, selected here: SP, SPF).

Bifrenaria tyrianthina (Lodd.) Rchb. f. var. *magnicalcarata* Hoehne, Arq. Bot. Estado São Paulo, 2(5): 117. 1950. *Bifrenaria magnicalcarata* (Hoehne) Pabst, Bradea 2(14): 86. 1976. TYPE: BRAZIL. Minas Gerais: pr. Datas, 14 Nov 1974, *Seidel 1122* (HOLOTYPE: HB)

(Figs. 22a, 25c, 26b)

Rupiculous plants 10-30 cm tall. *Pseudobulbs* aggregate, conical, yellowish, 4-8 x 1.5-4 cm. *Leaves* lanceolate to ovate, coriaceous, 9.5-30.5 x 4.5-6.5 cm, pseudo-petiole up to 3.2 cm long. *Inflorescence* 1-3 flowered, erect, 5-25.5 cm long; scape and floral bracts 1.1-2.7 x 0.7-2 cm. *Flowers* with a sweet and strong odor, pink to vinaceous, the labellum white to pink-vinaceous, with red stripes, 5.5-7.5 cm long, 6-7 cm wide. *Sepals* ovate, truncate, dorsal sepal 3-4 x 1-3 cm, lateral sepals parallel to each other, 3.5-4.5 x 1.8-2.2 cm, spur with sepal segments superposed, 1.5-3 cm long. *Petals* obovate, attenuate, oblique to column, 3-4.5 x 1.7-2.8 cm. *Labellum* 3-lobed, obovate, puberulous to pubescent, margins undulate to crenate, 3-4.5 x 2-3 cm when expanded, lateral lobes involute, oblong, 1-2.8 x 0.5-1.8 cm, the midlobe obcordate, recurved, the claw 0.2-0.6 cm long, the callus 1.5-2.5 cm long, puberulous to pubescent, on the anterior region 2-lobed, protuberant, glabrous. *Column* pubescent, 1.4-1.7 cm, the foot straight, pubescent, 1.8-3.2 cm long. *Anther* 0.5 x 0.5 cm, the pollinia rounded, the stipe forked, 0.2-0.3 cm long, the viscidium rounded, 0.15-0.4 x 0.1-0.2 cm long. *Ovary-pedicel* 3.5-6.5 cm long.

Habitat. — Native to Brazilian "campos rupestres" vegetation, 1000-1300 m elevation, flowering occurs from October to December.

Distribution. — Brazil, states of Bahia, Minas Gerais and São Paulo.

Pabst (1976) elected the variety *Bifrenaria* tyrianthina (Lodd.) Rchb. f. var. *magnicalcarata* Hoehne to species level based on the longer spur and on the form of the labellum. The careful analysis of herbarium material indicated that there is no difference between *B. magnicalcarata* and *B. tyrianthina*. For this reason, we have followed Castro (1996), reducing *B. magnicalcarata* to synonymy under *B. tyrianthina.*. *Bifrenaria magnicalcarata* is indeed very different from *B. harrisoniae*, especially considering the length of the spur and the form of the labellum. Since species delimitation between *B. harrisoniae* and *B. tyrianthina* was confused at the time Pabst transfered *B. tyrianthina* var. *magnicalcarata* to *B. magnicalcarata*, he could have mistaken *B. harrisoniae* for B. *tyrianthina*.

B. tyrianthina is restricted to the states of Bahia and northern Minas Gerais, where it grows on open areas, always as a rupiculous plant. It can be distinguished from them by the rounded viscidium, the pubescent column, the long spur, the 2-lobed callus, and by the parellel position of the labellum to the column.

Blumenschein and Packer (1961) have reported chromosome numbers of 2n= 38 and 4n=76 for this species.

Additional specimens examined: BRAZIL. **Bahia:** Ibiraquara, Cascavel, trilha para o Rumo (Machobengo), entre Riachão e Morro do Chapéu, Nov 1989, *Ferreira et al. 216* (RB); Morro do Chapéu, Nov 1980, *Furlan et al. s.n.* (SPF17952). **Minas Gerais:** Camarinhas, *Alves s.n.* (R5887); same locality, 1963, *Rfeiffer 173* (R); Datas, Estr. Diamantina-Curvelo, a 21Km de Diamantina, Nov 1981, *Menezes et al. 2648* (SP, SPF); same locality, Nov 1981, Estr. Diamantina-Extração, a 12Km de Diamantina, Oct 1981, *Giulietti et al. 2229* (SP, SPF); Itabirito, Dec 1970, *Krieger 9774* (CESJ); Jaboticatubas, Km 132 da Estr. Lagoa Santa a Conceição do Mato Dentro, Santana do Riacho, Serra do Cipó, Nov 1972, *Sazima s.n.* (UEC13413); same locality, Dec 1971, *Sazima s.n.* (UEC13386); same locality, Dec 1971, *Sazima s.n.* (UEC13341); Ouro Preto, cachoeira das Andorinhas, 1200 m, Nov 1978, *Martinelli 4704* (RB); Santana do Riacho, Serra do Cipó, Oct 1997, *Sano et al. 545* (SPF); same locality, Oct 1995, *Forzza 137* (SPF); same locality, Nov 1990, *Queiroz 101* (BHCB); same locality, Nov 1991, *Pereira 900 and Lucca* (BHCB); Serras de Belo Horizonte, Dec 1943. *Gehrt s.n.* (SP50368); Serra do Cipó, Km 138 de Conceição, Dec 1949, *Duarte 2126* (RB); Serra de Grão Mogol, 1000 m, Nov 1938, *Markgraf 3500, Mello Barreto and Brade* (RB).

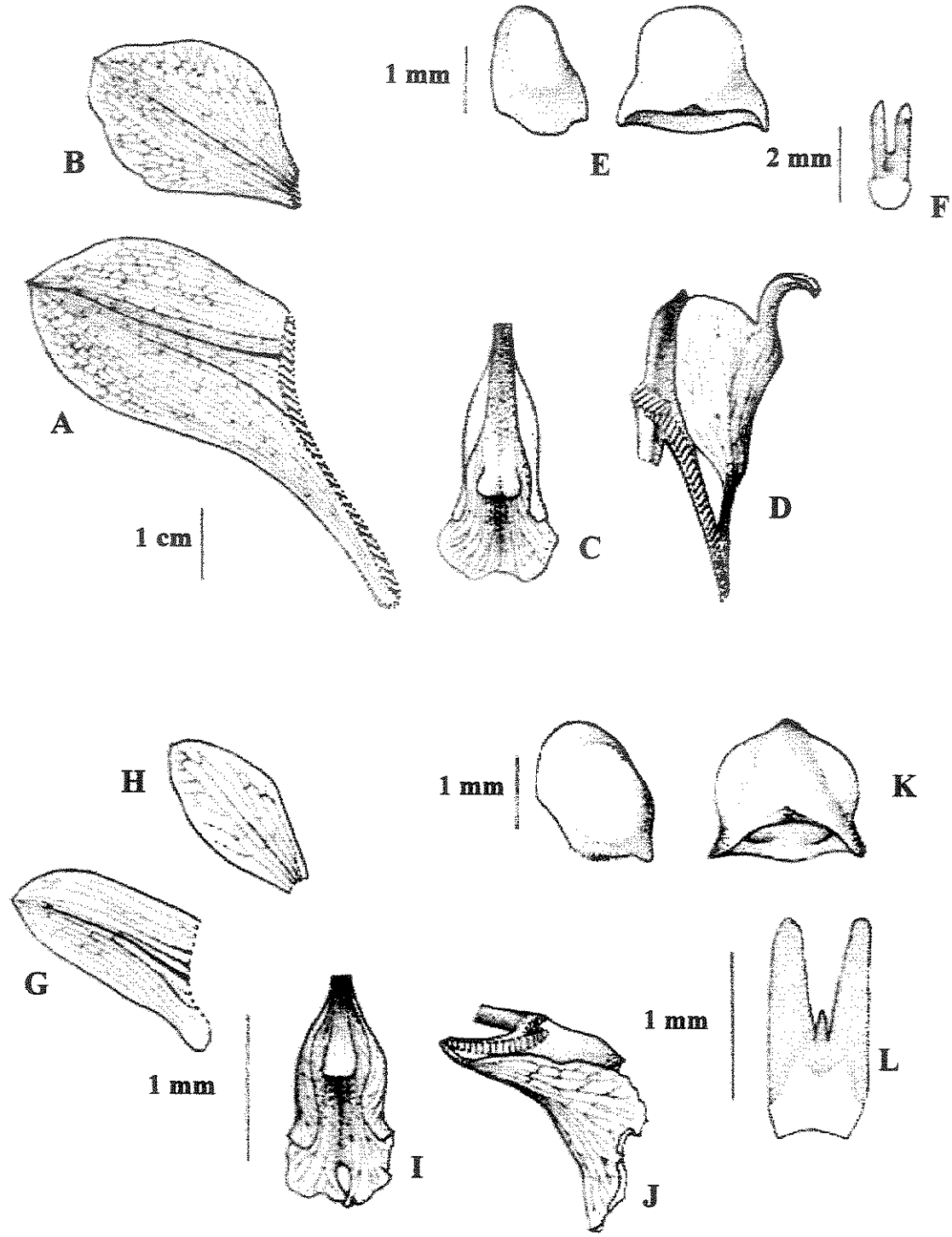


Fig. 22. (A-F) *Bifrenaria tyrianthina* (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia. (G-L) *B vitellina* (Lindl.) Lindl. (G) lateral sepal, (H) lateral petal, (I) labellum, (J) lateral view of flower showing the position of the labellum, (K) anther cap, (L) pollinarium without pollinia.

16. BIFRENARIA VENEZUELANA C. Schweinf., Amer. Orch. Soc. Bull. 34: 38. 1965. TYPE: VENEZUELA. State of Bolivar: Chimatá-tepuí (Tororno-teouí), 1000-1400 m elevation, May 15 1953, *Steyermark 75375* (HOLOTYPE: VEN-n.v).

(Figs. 23, 25d, 27)

Epiphytes, 10-15 cm tall. *Pseudobulbs* distant, compressed, green, 2.5-7.5cm long. *Leaves* lanceolate to ovate, coriaceous, 3.4-9 x 1.3-2 cm, pseudo-petiole inconspicous. *Inflorescence* 1-2 flowered, erect, 1.5 cm long; scape and floral bracts 0.4 cm long. *Flowers* vinaceous to pale brown, labellum with red stripes, ca. 1 cm long. *Sepals* ovate, truncate, dorsal sepal 1-1.3 x 0.4-0.6 cm, lateral sepals parallel to each other, 1.2-1.5 x 0.5-0.9 cm, spur with sepal segments superposed, inconspicous. *Petals* oblanceolate to oblong, oblique to column, 1 x 0.2-0.4 cm. *Labellum* slightly 3-lobed, obovate, laterally involute, 1-1.4 x 0.8-1.2 cm when expanded, the midlobe rounded, recurved, the claw 0.2 cm long, callus 0.6 cm long, puberulous, on the anterior region 3-lobed, protuberant, glabrous. *Column* glabrous, 0.5 cm, the foot straight, puberulous, 0.8-1.5 cm long. *Anther* 0.2 x 0.2 cm, the pollinia rounded, the stipe forked, 0.7 cm long, the viscidium truncate. *Ovary-pedicel* 1 cm long.

Habitat. — Native to the Venezuelan amazonian forest, 100-800 m elevation. Flowering occurs from October to December.

Distribution. — Venezuela.

Bifrenaria venezuelana C. Schweinf. is a rare species from Venezuela, known by its distant pseudobulbs and vinaceous, small flowers. Flowering is apparently rare due to the large amount of sterile herbarium material observed.

Additional specimens examined: VENEZUELA. Amazonas, 5-3Km NE of San Carlos de Rio Negro, 1°51´N, 67°03´W, Jan 1980, *Liesner 8563* (MO); Depto Rio Negro, Cerro de la Neblina Camp. IV, 0°51´N, 65°57´W, Mar 1984, *Liesner 16705* (MO); same locality, slope of Cerro Aracamuni, 0°24´N, 65°38´W, Oct 1987, *Liesner and Carnevali 22280* (MO); same locality, *Liesner and Carnevali 22372* (MO).

Fig. 23. *Bifrenaria venezuelana* (from Amer. Orch. Soc. Bull. 34: 38. 1965).

17. BIFRENARIA VITELLINA (Lindl.) Lindl., Edwards's Bot. Reg. 29: Misc. 52. 1843. *Maxillaria vitellina* Lindl., Edwards's Bot. Reg. 24: Misc. 62. 1838. *Stenocoryne vitellina* (Lindl.) Kraenzl. in Rchb. f., Xenia Orchid. 2: 142. 1896. *Adipe vitellina* (Lindl.) M.Wolff, Orchidee 41(2): 37. 1990. TYPE: BRAZIL. s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: Edwards's Bot. Reg. 24: Misc. 62. 1838).

(Figs. 22b, 25e, 27)

Epiphytes, 10-30 cm tall. *Pseudobulbs* aggregate, conic, dark green to brownish, 3-5 x 1.5-3 cm. *Leaves* lanceolate, membranaceous to sub-coriaceous, 11.5-26.5 x 2.3-5.6 cm, pseudo-petiole 2-8 cm long. *Inflorescence* 1-6 flowered, erect to pendent, 4-16 cm long; scape bracts 0.5-1.2 x 0.5 cm, floral bracts 0.3-1.1 x 0.1-0.5 cm. *Flowers* yellowish to orange, the labellum with red stripes and a conspicous vinaceous spot, 1.5 cm long, 2.5-3 cm wide. *Sepals* ovate to oblong, truncate, dorsal sepal 1.2-1.6 x 0.4-0.6 cm, lateral sepals divergent to each other, 1-1.8 x 0.4-0.6 cm, spur with sepal segments superposed, 0.3-0.7 cm long. *Petals* obovate, oblique to column, 1-1.5 x 0.4-0.7 cm. *Labellum* 3-lobed, obovate, pubescent, margins crenate, 1.3-1.7 x 0.7-1.6 cm, when expanded, recurved, lateral lobes involute, rounded, midlobe emarginate, 0.7-1.2 x 0.3-0.4 cm, the claw 0.1-0.4 cm long, the callus scantly pubescent, 0.4-0.8 cm long, on the anterior region 3-lobed, glabrous to pubescent, protuberant, yellow. *Column* puberulous, 0.4-0.7 cm, the foot straight, glabrous to puberulous, 0.5-0.7 cm long. *Anther* 0.15-0.2 x 0.15-0.2 cm long, the pollinia rounded, the stipe forked, 0.1 cm long, the viscidium truncate, 0.1 cm long. *Ovary-pedicel* 1.7-3.5 cm long.

Habitat. — Native to wet montane forests. Flowering occurs from August to May.

Distribution. — Brazil, states of Espírito Santo, Minas Gearis, Rio de Janeiro and São Paulo.

Bifrenaria vitellina is very similar to *B. stefanae*, although a rarer species. It can be disinguished by its lateral sepals divergent to each other, bigger flowers, bearing a prominent labellum midlobe with a conspicous vinaceous spot.

Additional specimens examined: BRASIL. **Espírito Santo:** Jan 1981. *Campacci s.n.* (SP295745); Castelo, Braço do Sul, Aug 1948, *Brade 19435* (RB). **Minas Gerais:** Ibitipoca, Dec 1986, *Souza s.n.* (BHCB9082). **Rio de Janeiro:** Petrópolis, Feb 2000, Orquidario Binot, *Koehler 65c.* (UEC). **São Paulo:** Cunha, Dec 1952, *Pereira s.n.* (SP68448); Serra do Mar, Jan 1952, *Pires s.n.* (SP56324); Bocaina, Apr 1951, *Bsrthah 12* (R); Bananal, May 1936, *Brade 8541* (RB).

18. BIFRENARIA WITTIGII (Rchb. f.) Hoehne., Fl. Bras. (Hoehne) 12(7): 30. 1953. *Cydoniorchis wittigii* (Rchb. f.) K. Senghas, J. Orchideenfreund 1: 11. 1994. *Lycaste wittigii* Rchb. f. Gard. Chron. 2: 654. 1878. TYPE: BRAZIL. s. loc. (HOLOTYPE, missing, NEOTYPE: BRAZIL, Rio de Janeiro, Orquidário Binot, 1200 m, *Castro s.n.* UEC117742).

(Figs. 24, 25f, 27)

Epiphytes 20-30 cm tall. *Pseudobulbs* aggregate, conic, dark green to brownish, 5-8 x 2-3 cm. *Leaves* lanceolate to obovate, coriaceous, 22-27 x 4.5-8 cm, pseudo-petiole 1-2 cm long. *Inflorescence* 2-5 flowered, erect, 4-5 cm; scape and floral bracts 1.5-2 x 0.5-1 cm. *Flowers* turned upward, fragrant, brown to greenish, the labellum vinaceous to yellowish with vinaceous stripes, 3.5-4 cm long. *Sepals* ovate, truncate, acute, dorsal sepal 3-3.4 x 1.5-2 cm, lateral sepals parallel to each other, 3.5-3.7 x 2-2.5 cm, spur with sepal segments superposed, 0.5-0.8 cm long. *Petals* ovate, acute, oblique to column, 2.5-2.7 x 1.1-1.7 cm. *Labellum* 3-lobed, convex, margins entire, 2.7-3 x 2 cm when expanded, midlobe rounded, velutinous, lateral lobes involute, acute, 1.2-1.5 cm, the claw 0.2-0.4 cm, the callus 1.2-1.5 cm long, fleshy, puberulous, on the anterior region 2-lobed. *Column* puberulous, winged, 1-1.8 cm long, the foot arched, glabrous, 1.6-2 cm long. *Anther* 0.5 x 0.4 cm, with a prominent apex, the pollinia oval, the entire linear, 0.3-0.4 cm long, the viscidium cuneate, 0.1 cm long. *Ovary-pedicel* 2.6-3.5 cm long.

Habitat. — Native to wet montane forests, elevation 800-1200 m elevation. Flowering occurs from December to January.

Distribution. — Brazil, states of Espírito Santo and Rio de Janeiro.

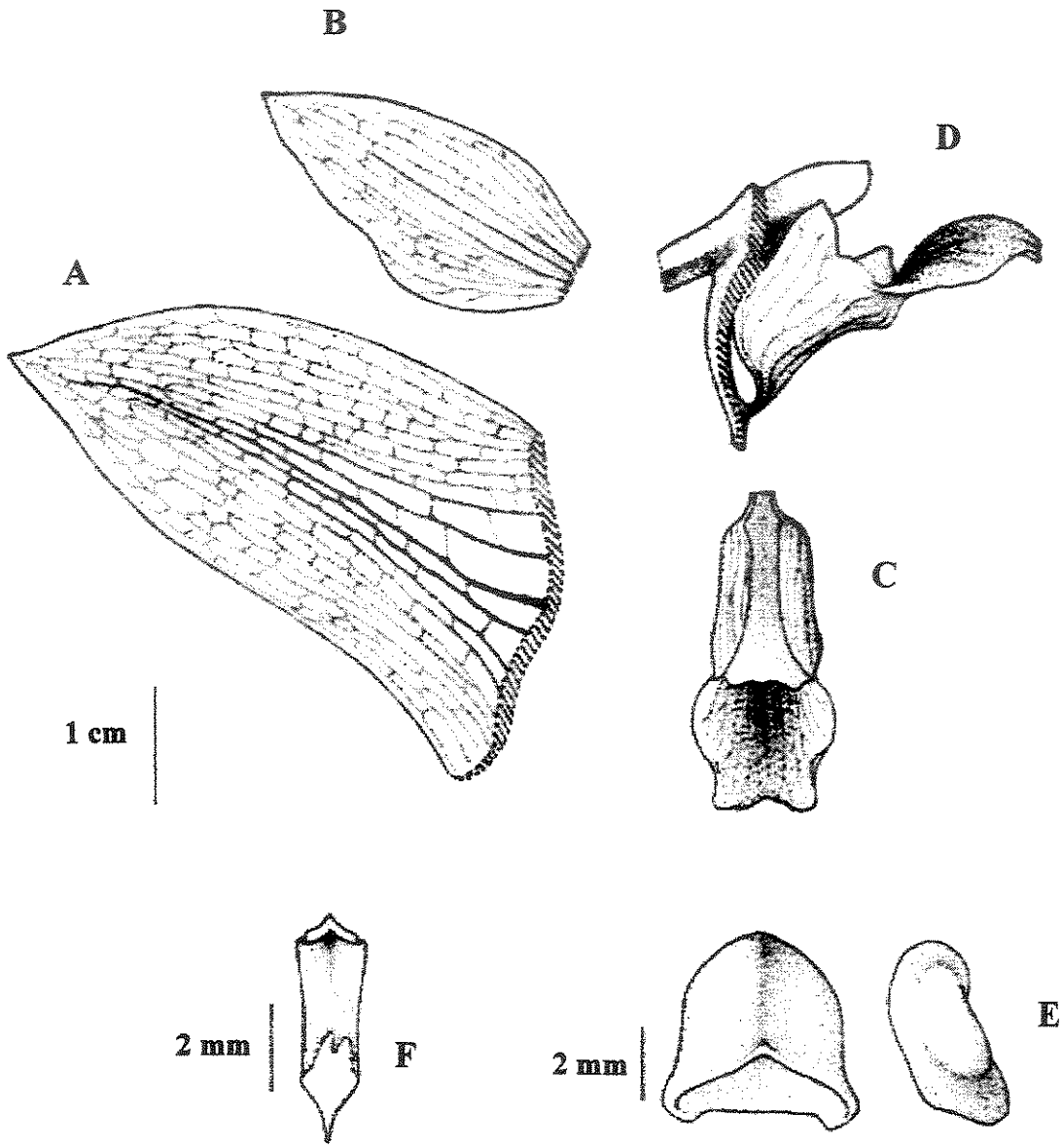
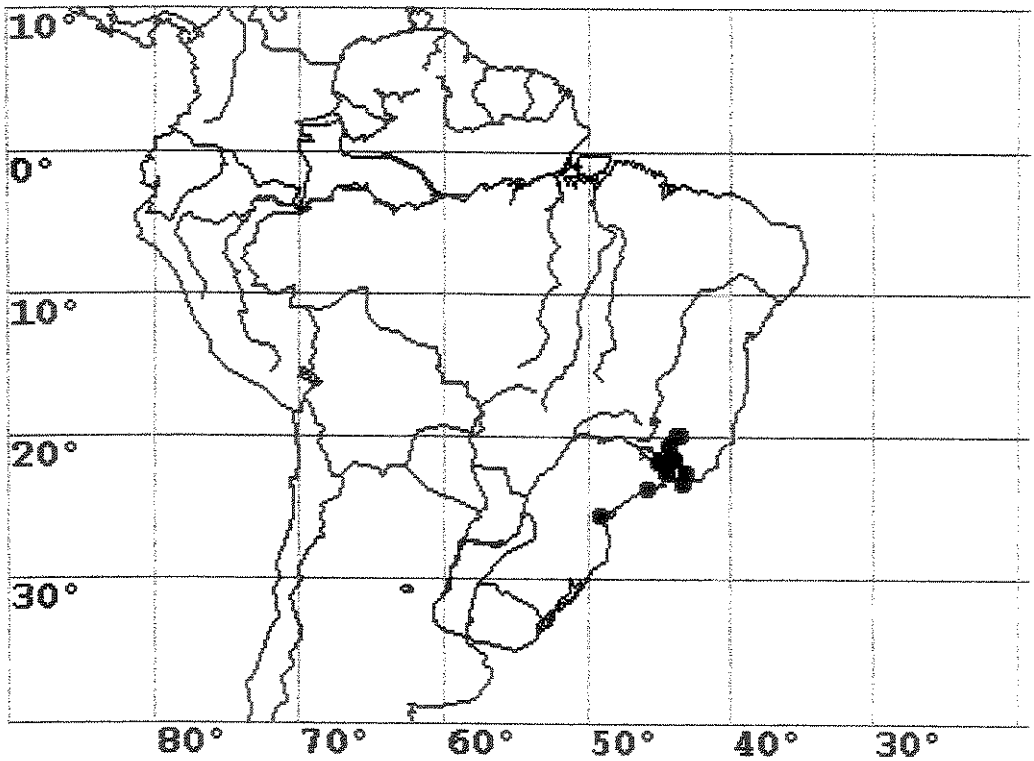


Fig. 24. (A-F) *Bifrenaria wittigii* (A) lateral sepal, (B) lateral petal, (C) labellum, (D) lateral view of flower showing the position of the labellum, (E) anther cap, (F) pollinarium without pollinia.

Fig. 25. (A) *B. stefanae* (foto E. Pansarin), (B) *Bifrenaria tetragona,* (C) *B. tyrianthina* (foto V. P. Castro), D) *B. venezuelana* (foto J. Baptista), (E) *B. vitellina,* (F) *B. wittigii* (foto V. P. Castro).

A



B

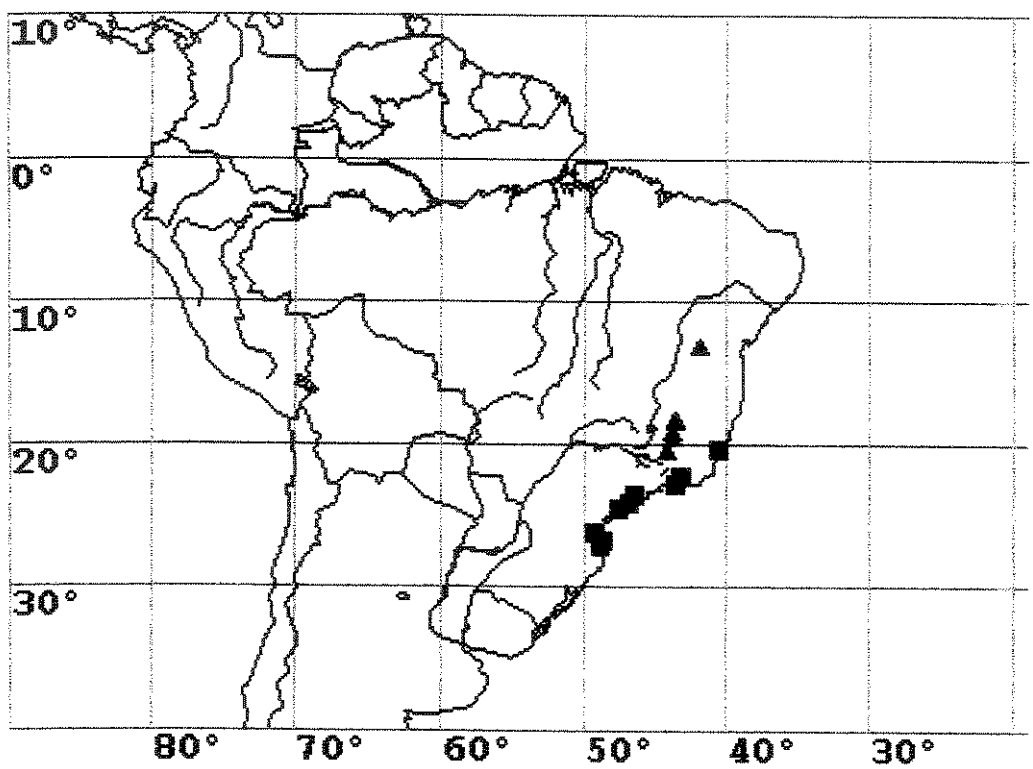


Fig. 26. (A) *Bifrenaria stefanae* (●). (B) *B. tetragona* (■); *B. tyrianthina* (▲).

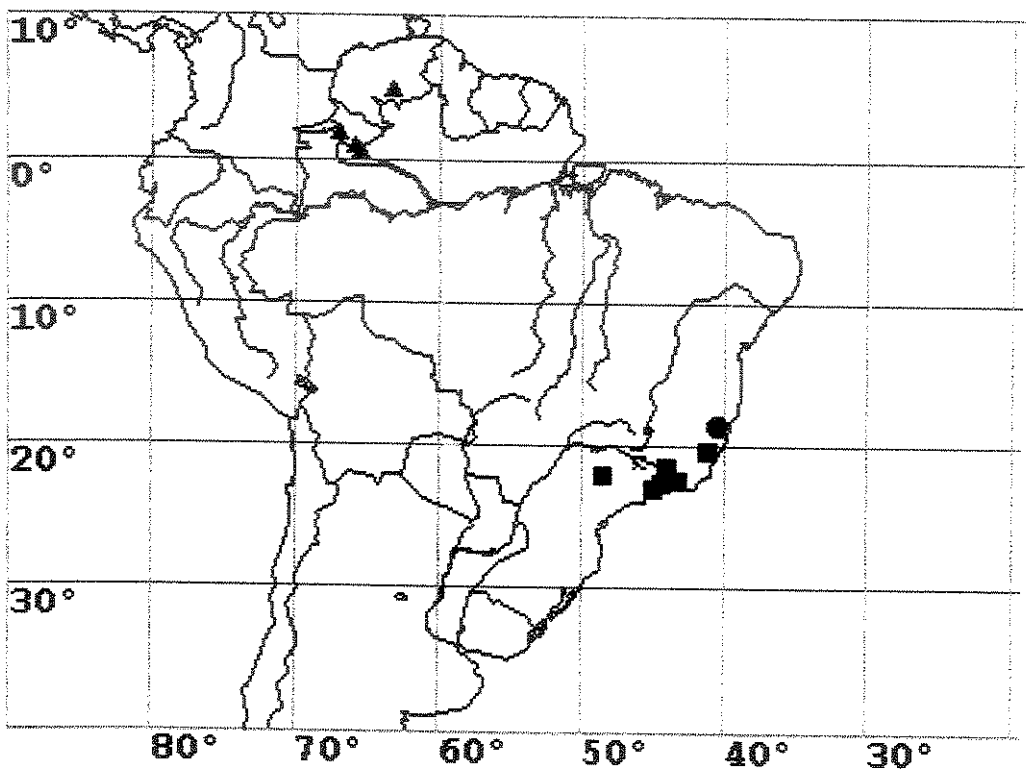


Fig. 27. *Bifrenaria venezuelana* (▲); *B. vitellina* (■); *B. wittigii* (●).

Bifrenaria wittigii is closely related to *B. tetragona*, but can be distinguished from it by a rounded and velutinous labellum midlobe and acute lateral lobes. This is a rare species only known from the state of Espírito Santo.

Additional specimens examined: BRASIL. **Espírito Santo:** Dec 1961 (HB19742); Dec 1960, *Machado s.n.* (HB19481); Domingos Martins, Jan 1970, *Kaustky 216* (HB).

INVALID AND DUBIOUS SPECIES

1. BIFRENARIA CHLOROLEUCA Barb. Rodr. Gen. Spec. Orchid 2: 212. 1881, *nomen nudum.*

2. BIFRENARIA MELANOPODA Klotzsch, in Otto and Dietr., Allg. Gartenzeitung. 23: 105. 1855. *Stenocoryne melanopoda* (Klotzsch) Hoehne, Arq. Bot. Estado São Paulo. 2(1): 14. 1944. *Adipe melanopoda* (Klotzsch) M. Wolff, Orchidee 41(2): 36. 1990. TYPE: unknown.

Bifrenaria melanopoda seems to be very close related to *B. charlesworthii* and *B. racemosa*. It has been characterized by the short inflorescence and by the two vinaceous spots at the base of the labellum. Since we were unable to locate the type material and the herbaria material available is scarce and without localities we did not include accept this species in our taxonomic treatment.
Blumenschein and Packer (1961) have reported chromosome numbers of 2n= 38 for this species (*Bifrenaria clavigera* Rchb. f.)

3. BIFRENARIA LEUCOPETALA Barb. Rodr., Gen. Spec. Orchid. 2: 212. 1881, *nomen nudum.*

4. BIFRENARIA PARVULA (Hook.) Rchb. f. , Ann. Bot. Syst. 6: 547. 1854. *Maxillaria parvula* Hook. Exot. Fl. 3: t. 217. 1826. *Colax parvula* (Hook.) Spreng., Syst. Veg. ed. 16, Cur. Post. 307. 1826. TYPE: s. loc. (HOLOTYPE: missing, LECTOTYPE, selected here: Exot. Fl. 3: t. 217. 1826).

The absence of herbaria material does not allow a complete analysis of this species. The original illustration (lectotype) does not resemble a *Bifrenaria* at all: the pollinaria lacks a stipe and a viscidium, the callus looks rather different from the type found in the genus *Bifrenaria*. There is no indication how the leaves look like.

5. BIFRENARIA STEYERMARKII (Foldats) Garay and Dunsterv., Venez. Orchid. Ill. 6: 56. 1976. *Xylobium steyermarkii* Foldats, Noved. Cient. Contrib. Ocas. Hist. Bat. La Salle Bot. 35, ser. Bot. 1: f. 1. 1970. TYPE: Venezuela, Edo. Bolivar, 125KM al sur de El Dorado, 1155m, *Steyermark and Dunsterville 62185-A* (HOLOTYPE, VEN-n.v.).

Garay and Dunsterville (1976) transfered *Xylobium steyermarkii* Foldats to the genus *Bifrenaria* based on its resemblance to *Bifrenaria aureo-fulva* (Hook.) Lindl. Besides the fact that *X. steyermarkii* occurs in Venezuela and, probably, in Guiana, and that *B. aureo-fulva* is restricted to southern Brazil, *X. steyermarkii* presents up to two leaves in a pseudobulb, a character not observed in any other *Bifrenaria* species. Also, the pollinarium is rather different, bearing apparently no stipe, the callus is not protuberant and the column foot is much shorter than the column, a feature not common for other *Bifrenaria* species. Maybe *X. steyermarkii* is really an intermediate species between the genera *Bifrenaria* and *Xylobium*, as stated by Foldats (1970), that be put into a different genus, but the analysis of this complex species is hampered by the absence of herbaria material other than the type.

6. BIFRENARIA VERBOONENII G. Romero and V. P. Castro, Harvard Papers in Botany 5(1): 187-188. TYPE: BRAZIL. Minas Gerais: Serra do Cipó, 1200-1400 m elevation, Sep 1995, ex Hort., Orquidário Binot s.n. (HOLOTYPE: SP, missing, ISOTYPE: AMES-n.v.).

Romero and Castro (2000) described *Bifrenaria verboonenii* based on the longer inflorescence, the straight spur, parallel to the ovary, the shape of the labellum, petals and sepals, and on the yellowish green flowers. The analysis of herbarium material of *Bifrenaria tyrianthina* (Lodd.) Rchb f. showed that the inflorescence in this species may reach 25.5 cm, which is longer than the 23 cm inflorescence of *B. verboonenii*. Also the measurements of sepals and petals of *B. tyrianthina* include those presented for *B. verboonenii*. The position of the spur is a rather difficult character to evaluate, since it is not preserved in herbaria material. The only character that really distinguishes both species is the color of the yellowish green flower, pink to vinaceous in *B. tyrianthina*. Unfortunatelly the holotype deposited in SP is missing and we could not to examine the isotype deposited at AMES. For this reason, we decided to exclude this species from our taxonomical treatment.

7. BIFRENARIA VINOSA Barb. Rodr., Gen. Spec. Orchid. 2: 212. 1882, *nomen nudum.*

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CONCLUSÃO GERAL

O presente estudo demonstrou que o complexo *Bifrenaria*, incluindo os gêneros *Adipe* Raf., *Bifrenaria* Lindl. (sensu stricto) e *Cydoniorchis* K. Senghas, constitui um grupo monofilético, suportado por dados moleculares (regiões ITS 1 e 2 do DNA nuclear e *trn*L-F, do DNA de cloroplasto) e morfológicos. Os caracteres morfológicos que definem o gênero são a presença de pseudobulbos tetrangulares e de um esporão proeminente. A maioria dos outros caracteres tradicionalmente utilizados para definir o gênero, tais como a presença de dois estipes no polinário e de pseudobulbos unifoliados, também estão presentes no gênero *Rudolfiella.* e portanto não representam sinapomorfias de *Bifrenaria.*

O grupo irmão do gênero *Bifrenaria* ainda ainda não está definido, pois a relação deste com *Hylaeorchis petiolares* e os gêneros *Rudolfiella* e *Scuticaria* ainda permanece incerta. A espécie *Hylaeorchis petiolares* foi considerada em *Bifrenaria* até quando Carnevali e Romero (2000) transferiram esta espécie para um gênero a parte. Apesar desta espécie apresentar uma flor cuja estrutura geral se aproxima muito das flores de *Bifrenaria*, o gênero monotípico *Hylaeorchis* é morfologicamente bastante distinto de *Bifrenaria* por apresentar pseudobulbos cônicos, não angulosos, polinário com viscídio luniforme, como no gênero *Maxillaria* e folhas marcadamente crassas.

Já a posição do gênero *Rudolfiella* em relação às bifrenarias sempre foi incerta e algumas espécies já foram inclusive descritas como pertencentes à *Bifrenaria.* De acordo com estudos filogenéticos desenvolvidos por Whitten et al. (2000), e complementados aqui, o monofiletismo de *Rudolfiella* aparece bem suportado em todas as análises em que foi considerado. O gênero *Rudolfiella* pode ser caracterizado por plantas com um labelo distintamente trilobado, apresentando lobos laterais amplos e erguidos, um calo constituído por saliências pequenas e numerosas e um ungüículo longo e recurvado.

O gênero *Scuticaria*, também filogeneticamente muito próximo de *Bifrenaria*, era considerado como pertencente à subtribo Maxillarinae (sensu Dressler 1993). Análises filogenéticas baseadas em caracteres moleculares indicaram que *Scuticaria* é filogeneticamente mais próximo dos gêneros de Bifrenariinae do que de Maxillariinae

(Whitten 2000 et al.). Apesar de *Scuticaria* apresentar caracteres vegetativos bastante distintos dos outros gêneros de Bifrenariinae e Lycastinae, tais como pseudobulbos pouco evidentes e roliços e folhas cilíndricas, o gênero apresenta o padrão básico de flor dos gêneros próximos. Será necessária a amostragem de mais espécies pertencentes a estes gêneros próximos a *Bifrenaria*, utilizando-se marcadores moleculares distintos, para que a relação de parentesco entre os gêneros de Bifrenariinae seja melhor compreendida.

A análise de dados combinados (morfológicos e moleculares) também indicou que *Bifrenaria* está dividido em dois clados basais bem suportados: um clado representado pelas espécies amazônicas, *Bifrenaria longicornis* e *B. venezuelana* e outro, representado por todas as demais espécies, restrito à região sul-sudeste do Brasil (mais a região sul da Bahia). É interessante notar que apesar das espécies *B. longicornis* e *B. venezuelana* apresentarem caracteres reprodutivos bastante distintos, tais como o comprimento da inflorescência, cor e consistência das flores, comprimento do esporão e o formato do polinário, estas são as únicas espécies de *Bifrenaria* que apresentam um rizoma distintamente longo, caracterizando pseudobulbos espaçados. Provavelmente este clado não foi identificado na análise cladística considerando apenas caracteres morfológicos devido à grande quantidade de estados de caracteres florais distintos destas duas espécies.

As relações de parentesco entre espécies do clado do sul-sudeste brasileiro não estão tão bem definidas, mas foi possível caracterizar grupos de espécies principais. As espécies *Bifrenaria charlesworthii* e *B. clavigera* constituem um clado com 99% de suporte (de acordo com análises de *bootstrap*, Felsenstein 1985), sendo caracterizado morfologicamente por apresentar um esporão basalmente fundido e estipes muito curtos. A espécie *B. silvana*, embora não tenha sido considerada na análise devido a ausência de material apropriado para extração de DNA, também apresenta as duas sinapomorfias morfológicas que definem este clado e, por este motivo, acredita-se que *B. silvana* seja filogeneticamente próxima de *B. charlesworthii* e *B. clavigera*.

As análise também indicaram que as espécies *Bifrenaria aureo-fulva, B. vitellina, B. stefanae* e *B. leucorrhoda* representam um grupo monofilético. Este grupo de espécies apresenta como sinapomorfia sépalas laterais paralelas entre si e não divergentes como nas outras *Bifrenarias*. Aparentemente este caráter sofreu uma reversão em *B. vitellina*, que apresenta sépalas divergentes.

Um outro clado bem suportado está representado pelas espécies *Bifrenaria inodora*, *B. tyrianthina* e *B. harrisoniae*. Este clado não apresenta nenhuma sinapomorfia morfológica, mas suas espécies podem ser descritas como plantas robustas que apresentam flores grandes, de fragância muito doce e intensa. Este clado é o único a apresentar espécies inteiramente (*B. tyrianthina*) ou parcialmente (*B. inodora*, *B. tyrianthina* e *B. harrisoniae*) rupícolas.

Embora as espécies *Bifrenaria calcarata* e *B. mellicolor* apresentem caracteres morfológicos muito semelhantes (presença de nectários nas sépalas e de labelos eretos e glabros), este clado não foi identificado na análise cladística considerando apenas caracteres moleculares, mas sim nas análise morfológica e combinada, ilustrando a importância de considerar caracteres de diferentes fontes em estudos filogenéticos.

Como grupo irmão dete clado, foi identificado o clado que engloba as espécies *Bifrenaria tetragona* e *B. wittigii*, descritas por Senghas (1994) como pertencentes ao gênero *Cydoniorchis*. Este gênero é definitivamente monofilético, sendo caracterizado por inúmeras sinapomorfias morfológicas, tais como a posição das flores na inflorescência voltadas para cima, o calo carnoso, coluna com expansões laterais, pé da coluna arqueado e políneas ovais. Entretanto, a aceitação do genêro *Cydoniorchis* requer a criação de sete novos gêneros, que englobem os outros clados de *Bifrenaria*. Tal mudança taxonômica não foi considerada satisfatória, pois muitos clados basais apresentam baixos suportes e sua elevação a categoria de gêneros seria prematura. Além disso, não há caracteres morfológicos que claramente definam todos estes clados, o que dificulta a identificação e aceitação de novos nomes.

De acordo com os resultados obtidos o gênero *Adipe* (=*Stenocoryne* Lindl.), conforme circunscrito, constitui um grupo parafilético, ou seja, definido por caracteres que também estão presentes nos grupos externos ao gênero *Bifrenaria* e, portanto, não podem ser utilizados para definir clados. Dentre os caracteres comumente utilizados para definir este gênero, pode-se citar: plantas delicadas, flores pequenas e inflorescências maiores que pseudobulbos.

Considerando os resultados obtidos neste estudo através de análises filogenética considerando espécies do complexo *Bifrenaria* e gêneros afins, os gêneros *Adipe*, *Cydoniorchis* e *Stenocoryne* são sinonimizados aqui ao gênero *Bifrenaria* Lindl.

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