

Labellar Micromorphology of Bifrenariinae Dressler (Orchidaceae)

K. L. DAVIES^{1,*} and M. STPICZYŃSKA²

¹School of Earth, Ocean and Planetary Sciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3YE, UK and ²Department of Botany, Agricultural University, Akademicka 15, 20-950 Lublin, Poland

Received: 5 July 2006 Returned for revision: 24 July 2006 Accepted: 14 August 2006 Published electronically: 28 September 2006

• **Background and Aims** The two closely related subtribes Bifrenariinae Dressler and Maxillariinae Benth. are easily distinguished on morphological grounds. Recently, however, molecular techniques have supported the inclusion of Bifrenariinae within a more broadly defined Maxillariinae. The present paper describes the diverse labellar micromorphology found amongst representatives of Bifrenariinae (*Bifrenaria* Lindl., *Rudolfiella* Hoehne, *Teuscheria* Garay and *Xylobium* Lindl.) and compares it with that found in *Maxillaria* Pabst & Dungs and *Mormolyca* Fenzl (Maxillariinae).

• **Methods** The labella of 35 specimens representing 22 species of Bifrenariinae were examined by means of light microscopy and scanning electron microscopy and their micromorphology compared with that of *Maxillaria sensu stricto* and *Mormolyca* spp. The labellar epidermis of representatives of *Bifrenaria*, *Xylobium* and *Mormolyca* was tested for protein, starch and lipids in order to ascertain whether this tissue is involved in the rewarding of pollinators.

• **Key Results and Conclusions** The labella of *Bifrenaria* spp. and *Mormolyca* spp. are densely pubescent but those of *Xylobium*, *Teuscheria* and *Rudolfiella* are generally papillose. However, whereas the trichomes of *Bifrenaria* and *Mormolyca* are unicellular, those found in the other three genera are multicellular. Hitherto, no unicellular trichomes have been described for *Maxillaria*, although the labella of a number of species secrete a viscid substance or bear moniliform, pseudopollen-producing hairs. Moniliform hairs and secretory material also occur in certain species of *Xylobium* and *Teuscheria* and these genera, together with *Maxillaria*, are thought to be pollinated by stingless bees (Meliponini). Differences in the labellar micromorphology of *Bifrenaria* and *Mormolyca* are perhaps related to Euglossine- and/ or bumble bee-mediated pollination and pseudocopulation, respectively. Although *Xylobium* and *Teuscheria* share a number of labellar features with *Maxillaria sensu stricto*, this does not necessarily reflect taxonomic relationships but may be indicative of convergence in response to similar pollinator pressures.

Key words: *Bifrenaria*, Bifrenariinae, *Maxillaria*, Maxillariinae, Meliponini, papillae, pollination, pseudopollen, *Rudolfiella*, *Teuscheria*, trichomes, *Xylobium*.

INTRODUCTION

In his treatment of the tribe Maxillarieae Pfitzer, Dressler (1990) assigned five genera, namely, *Bifrenaria* Lindl., *Horvatiia* Garay, *Rudolfiella* Hoehne, *Teuscheria* Garay and *Xylobium* Lindl. to the newly erected subtribe Bifrenariinae Dressler since they share the following set of characters. They may be epiphytic or lithophytic with pseudobulbs consisting of a single node, sometimes covered with hard cataphylls. The leaves are articulate, terminal or distichous and convolute, plicate or subconduplicate and the inflorescence, which is lateral, bears one to several, spirally arranged, small or large flowers. These are generally resupinate with a column of variable length and usually have a pronounced foot. The anther is terminal, operculate with reduced partitions and the four pollinia are superposed with a prominent viscidium. The pollinia may be sessile or possess one, or more usually two, stipes. The stigma is entire (Dressler, 1990).

The distinct column-foot and mentum, the four rounded or ovoid pollinia and the broad, open stigma found in the Bifrenariinae are also shared by members of Maxillariinae Benth. and Lycastinae Schltr. However, members of Maxillariinae *sensu stricto*, are distinguished by their conduplicate leaves and usually crescent-shaped viscidium, whereas members of Lycastinae have plicate leaves and a

strap-like viscidium (Whitten *et al.*, 2000). Similarly, *Xylobium* is distinguished from *Maxillaria* Ruiz & Pav. by its plicate leaves and several-flowered, racemose inflorescences, the four pollinia arising from a transverse, scale-like viscidium (Bechtel *et al.*, 1981). As a result, genera formerly assigned to Bifrenariinae and Lycastinae have recently been incorporated into Maxillariinae (Dressler, 1993; Ryan *et al.*, 2000; Whitten *et al.*, 2000; Koehler *et al.*, 2002; Chase *et al.*, 2003; Chase, 2005) thereby creating a Neotropical, species-rich assemblage that displays diverse vegetative morphology and growth patterns (Dressler, 1993; Atwood and Mora de Retana, 1999) whilst retaining a relatively conservative floral morphology (Dressler, 1993; Atwood and Mora de Retana, 1999; Ryan *et al.*, 2000; Whitten *et al.*, 2000; Koehler *et al.*, 2002).

Both molecular and non-molecular evidence strongly agree that Maxillariinae is monophyletic (Dressler, 1993; Holtzmeier *et al.*, 1998; Whitten *et al.*, 2000; Dathe and Dietrich, 2006) but generic boundaries are poorly defined, particularly so with regard to the very morphologically diverse genus *Maxillaria*. This genus in its broader sense is polyphyletic (Singer and Koehler, 2004), whereas in its current, narrower circumscription, it is considered paraphyletic (Dathe and Dietrich, 2006). Parsimony analyses of combined nuclear ribosomal and plastid DNA sequence data strongly support the four clades Maxillariinae, Bifrenariinae, Lycastinae and *Xylobium* (Whitten *et al.*,

* For correspondence. E-mail kevinldavies@btinternet.com

2000) but, since there is little support for the position of *Xylobium* relative to Maxillariinae *sensu stricto* and Lycastinae, this would necessitate either the erection of a separate subtribe or entail that *Xylobium* be left *incertae sedis* (Whitten *et al.*, 2000). Consequently, Whitten, Chase and co-workers (Whitten *et al.*, 2000; Chase *et al.*, 2003; Chase, 2005) favour the lumping of these four clades into a single, broadly defined Maxillariinae thereby reflecting their close relationships whilst avoiding the creation of a monogeneric subtribe.

Lindley (1832) based his genus *Bifrenaria* on the robust species *B. atropurpureum* Lindl. which has large flowers borne upon an inflorescence that does not exceed the pseudobulbs in height. Some years later, he erected the genus *Stenocoryne* Lindl. which he based upon *S. longicornis* (Lindl.) Lindl. (Lindley, 1843). This genus differed from *Bifrenaria* in that the small flowers were borne upon a relatively tall inflorescence. However, it would appear that Lindley, and all subsequent authors, had overlooked the fact that Rafinesque (1836) had already erected the genus *Adipe* Raf. based upon *A. racemosa* Raf., a species whose vegetative and inflorescence characters closely resemble those of *S. longicornis*, although florally the plants are very different (Koehler and Amaral, 2004, and references therein). Eventually, Wolff (1990) transferred *S. longicornis* to *Adipe*.

Meanwhile, Schlechter (1914) had described a new genus belonging to the *Bifrenaria* complex and named it *Lindleyella* (*nom. illeg.*). This was validated in 1944 by Hoehne under the name *Rudolfiella*. This genus shares a forked stipe and unifoliate pseudobulbs with *Bifrenaria* (Koehler *et al.*, 2002) but can be distinguished from that genus by its compressed pseudobulbs, the strongly divided lobes to the labellum, the prominent claw at the back of the labellum and the conspicuous callus (Koehler and Amaral, 2004). *Rudolfiella* is monophyletic, and, like *Teuscheria*, is closely related to, but distinct from, *Bifrenaria* although its position within Bifrenariinae has hitherto not been satisfactorily resolved (Koehler *et al.*, 2002).

Garay (1958) had erected the genus *Teuscheria* to accommodate a newly discovered orchid species, *T. cornucopiae* Garay, from the Ecuadorian Andes. Since then, a number of *Teuscheria* spp. have been discovered (Garay, 1970; Dressler, 1972; Dodson, 1978; Jenny and Braem, 1987) and these share the following features. They are epiphytic, caespitose or rhizomatous with unifoliate, conical, pyriform or ovate pseudobulbs with cataphylls. The leaves are petiolate, narrowly elliptic to oblong and plicate. The inflorescence is lateral and 1-flowered. Flowers are resupinate or not with a short to pronounced spur. The lateral sepals and tri-lobed labellum are fused to the column-foot. Column short; pollinia 4, unequal.

During his revision of the *Bifrenaria* complex, Hoehne (1944) considered a number of characters such as plant size, pseudobulb shape, length of inflorescence, flower size, shape of labellum and length of claw, presence and shape of spur, and pollinarium structure, and used these to separate *Bifrenaria*, *Stenocoryne* and *Rudolfiella*. Castro (1991a–c, 1996), in his treatment of the complex, recognized only the genus *Bifrenaria* excluding *Rudolfiella* and Senghas (1994)

transferred *B. tetragona* (Lindl.) Schltr. and *B. wittigii* (Rchb.f.) Hoehne, on the grounds that these species possess several-flowered inflorescences, erect perianth segments and an entire pollinarium stipe, to *Cydoniorchis* Senghas. Likewise, Carnevali and Romero (2000) erected two new monotypic genera, *Guanchezia* G.A. Romero & Carnevali and *Hylaorchis* Carnevali & G.A. Romero in which they placed *B. maguirei* C. Schweinf. and *B. petiolaris* (Schltr.) G.A. Romero & Carnevali, respectively.

More recently, morphological studies and phylogenetic analyses based on DNA sequence data (Koehler *et al.*, 2002; Chase *et al.*, 2003; Koehler and Amaral, 2004) concluded that *Bifrenaria sensu lato* constitutes a monophyletic group comprising *Adipe*, *Cydoniorchis* and *Bifrenaria sensu stricto*, but not *Rudolfiella*. These same studies showed that *Cydoniorchis* is monophyletic but that *Adipe* and *Bifrenaria sensu stricto* are not. Even so, since retaining *Cydoniorchis* as a separate genus would demand the erection of seven new genera for which there is little bootstrap support, Koehler and co-workers (Koehler *et al.*, 2002; Koehler and Amaral, 2004) consider that the widening of the circumscription of *Bifrenaria* and the reduction of *Adipe*, *Stenocoryne* and *Cydoniorchis* to synonymy under *Bifrenaria* is the best way to maintain nomenclatural stability. Thus, according to the latest treatment (Koehler and Amaral, 2004), the South American genus *Bifrenaria*, as it is currently circumscribed, contains about 20 species. Morphological and molecular data indicate that there are two distinct clades. The first comprises species that occur mainly in the Atlantic Forest of south-eastern Brazil or less frequently as rupicolous plants in the Brazilian ‘campos rupestres’, whereas the other is represented by two species that grow exclusively in the Amazonian region. The genus is distinguished from other members of the Maxillariinae *sensu lato* by the four-angled pseudobulbs, the plicate leaves, the conspicuous floral spur and the forked pollinarium stipe.

By contrast, the genus *Xylobium* has been largely neglected since the revision of Schlechter (1913) in which he recognized 24 species most of which had previously been described as species of *Maxillaria*. Several of these are to be found in cultivation (Teuscher, 1974; Senghas, 1995; Röth, 2004) but are often wrongly labelled.

Since general morphology and molecular approaches currently support the incorporation of genera formerly assigned to Bifrenariinae into a broadly defined Maxillariinae (Whitten *et al.*, 2000; Chase *et al.*, 2003; Chase, 2005), it is reasonable to suppose that comparison of the labellar micromorphology of *Bifrenaria* and allied genera with that of *Xylobium* and *Maxillaria sensu stricto* could also yield useful information, especially since the labellar micromorphology of *Maxillaria sensu stricto* has already been extensively studied (Davies and Winters, 1998; Davies *et al.*, 2000, 2003a, b; Davies and Turner, 2004a; Matusiewicz *et al.*, 2004).

Mormolyca Fenzl is distinguished from *Maxillaria sensu stricto* on morphological grounds by the inflorescence, which is as long as the leaves, the absence of a foot and the lunate viscidium (Garay and Wirth, 1959; Bechtel *et al.*, 1981) but Holtzmeier *et al.* (1998) and Dathe and

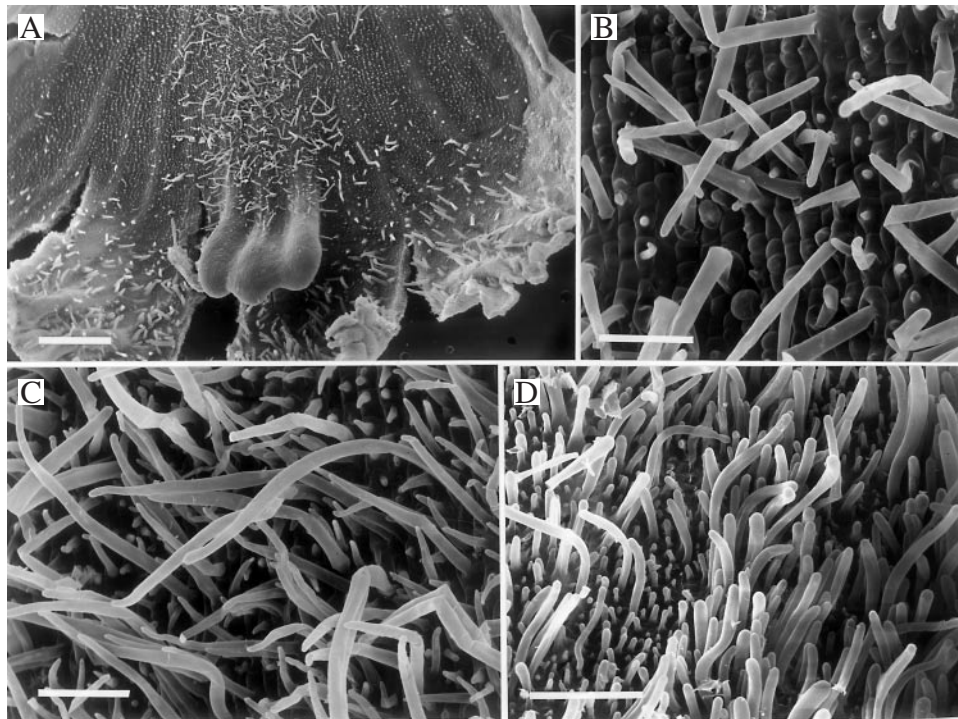


FIG. 1. (A–C) Labellum of *Bifrenaria wendlandianum* (K57074) showing densely pubescent median region (A), stages in the development of short, unicellular trichomes from conical papillae (B) and fully formed, elongate trichomes (C). Scale bars: A = 1 mm; B and C = 100 μm . (D) Labellar surface of *Bifrenaria harrisoniae* (KLD200501) showing similar, unicellular trichomes. Scale bar = 500 μm .

Dietrich (2006) have shown by means of phylogenetic and maximum parsimony and Bayesian analysis that this genus, as represented by *M. ringens* (Lindl.) Schltr., is deeply embedded within the cladistic structure of *Maxillaria* and that, if *M. ringens* is to be accepted as a member of a distinct genus, then *Maxillaria* should be considered paraphyletic.

Consequently, the aim of this present paper is to describe the labellar micromorphology of *Bifrenaria*, *Xylobium* and representative species of *Rudolfiella* and *Teuscheria* and to compare it with that of *Maxillaria sensu stricto* and *Mormolyca*, with the intention of gaining greater insight into the evolution and pollination biology of these genera.

MATERIALS AND METHODS

Thirty-one spirit-preserved specimens, representing 20 taxa, were obtained from the herbarium of the Royal Botanic Gardens, Kew, UK and supplemented with living and preserved material from the first author's collection and from Swansea Botanical Complex, UK (Table 1). Their accession numbers are prefixed 'K', 'KLD' and 'S', respectively. The names by which these specimens were originally collected have been retained but recent changes in nomenclature have been noted. Preserved material was stored in 'Copenhagen mix' (70 cm³ industrial methylated spirit : 2 cm³ glycerol : 28 cm³ water) and the authorities for plant names follow Brummit and Powell (1992). Following preliminary examination by means of light microscopy,

pieces of labellum were excised and prepared for scanning electron microscopy (SEM) as previously described (Stpiczyńska *et al.*, 2003; Davies and Turner, 2004b) and examined by means of a JSM 5200 LV-SEM or TESLA BS-300 at an accelerating voltage of 20–25 kV.

On the basis of SEM results, labellum samples derived from representative specimens of the genera *Bifrenaria* (*B. harrisoniae* KLD200501), *Xylobium* (*X. squalens* K13837, K14424, *X. leontoglossum* KLD200601, *X. cf. corrugatum* S20030489) and *Mormolyca* (*M. ringens* S19980091) were subjected to histochemical analysis for protein, starch and lipids (Davies *et al.*, 2000, 2002, 2003a–c; Davies and Turner 2004a, b) in order to determine whether particular labellar structures are involved in the production of pollinator rewards. Wherever possible, these tests were carried out on living tissue (KLD200501, S19980091, KLD200601). However, in the absence of living material, preserved tissue (K13837, K14424) was used reluctantly and only when SEM dictated that this was necessary. Although not ideal, there is evidence that these compounds are preserved and can be detected in plant material even after prolonged storage in 'Copenhagen mix' or formalin solution (Davies and Turner, 2004b).

RESULTS

The labellum of *Bifrenaria* is papillose and densely pubescent, the papillae and trichomes intergrading (Fig. 1A–D). The papillae are conical with pointed tips (Fig. 2A–D) and, in certain species such as *B. tetragona*,

TABLE 1. *Specimens studied and their provenance*

Taxon	Accession no.	Collector	Provenance	Date of Collection	Taxonomic notes
<i>Bifrenaria aurea</i> Barb. Rodr.	K54349	Donated by Sander, F.	Brazil	Cult. 1998	syn. <i>B. harrisoniae</i> (Hook.) Rchb.f. - Koehler & Amaral, 2004
<i>B. fuerstenbergiana</i> Schltr.	K14273	Purchased from Blossfield, R.	Brazil	Cult. 1944	syn. <i>B. inodora</i> Lindl. - Pabst & Dungs 1977
<i>B. harrisoniae</i> (Hook.) Rchb.f.	KLD200501	Davies, KL.		Cult. 2005	
<i>B. tetragona</i> Lindl.	K50008	Wyld Court Nursery	Brazil	Cult. 1986	syn. <i>Cydoniorchis tetragona</i> (Lindl.) Senghas - Senghas, 1994
<i>B. tetragona</i> Lindl.	KLD200502	Davies, KL.		Cult. 2005	syn. <i>Cydoniorchis tetragona</i> (Lindl.) Senghas - Senghas, 1994
<i>B. tyrianthina</i> (Lodd. ex Loudon) Rchb.f.	KLD200602	Davies, KL.		Cult. 2006	
<i>B. wendlandiana</i> (Kraenzl.) Cogn.	K57074	Warren, R.	Brazil	1993	syn. <i>B. clavigera</i> Rchb.f. - Koehler & Amaral, 2004
<i>Stenocoryne secunda</i> (Vell.) Hoehne	K37726	Gailer, J. 100		Cult. 1975	syn. <i>B. aureofulva</i> (Hook.) Lindl. - Koehler & Amaral, 2004
<i>Rudolfiella aurantiaca</i> (Lindl.) Hoehne	K61278	Sothers, CA; Pereira, E.DA C. 601	Manaus-Itacoatiara, Brazil	1995	
<i>Rudolfiella aurantiaca</i> (Lindl.) Hoehne	K57061	da Silva, JBF.	Amazonas, Brazil		
<i>Teuscheria wagneri</i> (Rchb.f.) Garay	K41855	Dunsterville, GCK. 474	Venezuela	1958	
<i>Xylobium bractescens</i> (Lindl.) Kraenzl.	K14421	From N.B.G. Glasnevin		Cult. 1961	
<i>X. colleyi</i> (Bateman ex Lindl.) Rolfe	K37071	Mason, LM. 1079	Guyana	Cult. 1976	
<i>X. colleyi</i> (Bateman ex Lindl.) Rolfe	K13833	Donated by Mason, LM.	Guyana	Cult. 1957	
<i>X. corrugatum</i> (Lindl.) Rolfe	K49592	Hodgson, I. 264	Ecuador	Cult. 1986	
<i>X. cf. corrugatum</i> (Lindl.) Rolfe	S20030489	Gregg, A.		Cult. 2004	
<i>X. elongatum</i> (Lindl.) Hemsl.	K14422	Donated by Mason, LM.	Panama	Cult. 1959	
<i>X. foveatum</i> (Lindl.) G. Nicholson	K14423	Lankester, CH.	Costa Rica	Cult. 1934	
<i>X. latilabium</i> C. Schweinf.	K45854	Jenny, R. vo 178/82	Peru	Cult. 1982	syn. <i>X. ornatum</i> (Klotzsch) Rolfe
<i>X. latilabium</i> C. Schweinf.	K6811	Donated by Mason, LM.	Peru	Cult. 1965	syn. <i>X. ornatum</i> (Klotzsch) Rolfe
<i>X. leontoglossum</i> (Rchb.f.) Benth. ex Rolfe	KLD200601	Davies, KL.		Cult. 2006	
<i>X. pallidiflorum</i> (Hook.) G. Nicholson	K47185	Hodgson, IG. 280	Ecuador	Cult. 1983	
<i>X. pallidiflorum</i> (Hook.) G. Nicholson	K13835	Donated by Lawrance, AE. 280	Venezuela	Cult. 1931	
<i>X. palmifolium</i> (Sw.) Fawc.	K37706	Mason, LM. 1040	West Indies	Cult. 1976	syn. <i>Maxillaria palmifolia</i> (Sw.) Lindl.
<i>X. powellii</i> Schltr.	K8480	Donated by Mahoney, LM. Mason	Panama	Cult. 1959	
<i>X. scabrilingue</i> (Lindl.) Rolfe ex Gentil	K31551	Mason, LM. 2146	Peru	Cult. 1968	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981
<i>X. squalens</i> (Lindl.) Lindl.	K10492	Donated by Sander, F.	Venezuela	Cult. 1956	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981
<i>X. squalens</i> (Lindl.) Lindl.	K12658	Bought from Binot, J.		Cult. 1937	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981
<i>X. squalens</i> (Lindl.) Lindl.	K13837	Purchased at sale by Protheroe & Morris		Cult. 1937	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981
<i>X. squalens</i> (Lindl.) Lindl.	K13838	Donated by Lawrance, AE.	Venezuela	Cult. 1931	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981

TABLE 1. *Continued*

Taxon	Accession no.	Collector	Provenance	Date of Collection	Taxonomic notes
<i>X. squalens</i> (Lindl.) Lindl.	K14424	Mason, LM.	Guyana	1960	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981
<i>X. squalens</i> (Lindl.) Lindl.	K14425	Donated by Garnett, CS.		Cult. 1935	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981
<i>X. squalens</i> (Lindl.) Lindl.	K32139	Dunsterville, G.	Venezuela	Cult. 1968	syn. <i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst. - Bechtel <i>et al.</i> , 1981
<i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst.	K46104	Dunsterville, G.	Venezuela	Cult. 1982	
<i>X. variegatum</i> (Ruiz & Pav.) Garay & Dunst.	K43855	Storr, R. 077	Brazil	Cult. 1980	
<i>Mormolyca ringens</i> (Lindl.) Schltr.	S19980091	Gregg, A.		Cult. 1998	
<i>M. schweinfurthiana</i> Garay & Wirth	K27543				

bear longitudinal, cuticular striations (Fig. 2E). At first, the epidermal cells have convex outer tangential walls. A small protrusion appears at its centre (Fig. 2C) and this forms a papilla which eventually develops into a trichome (Fig. 2D). The hairs are simple, unicellular and relatively long with rounded tips (40–620 µm; mean 322.4 µm in *B. harrisoniae*) (Figs 1B–D and 2F) but in *B. tetragona*, 2–3-celled trichomes were occasionally observed. The trichomes of *B. aurea* are shorter (36.3–131.6 µm; mean 69.5 µm) and much more closely resemble papillae (Fig. 2A, D) than those of the other *Bifrenaria* species studied. By contrast, the labellum of *B. secunda* [syn. *B. aureofulva* (Hook.) Lindl.], which was formerly assigned to *Stenocoryne*, differs from the other *Bifrenaria* spp. studied in that it is largely glabrous except for a few, sparsely arranged, relatively short, straight hairs (Fig. 3A, B).

The labellum of *Xylobium*, however, shows greater micromorphological diversity. It is generally papillose (Figs 3C, D, 4A–F, 5A–F, 6A–D, 7A–F, 8A–F and 9A–D), although it may be glabrous or minutely papillose as in *X. colleyi* (Fig. 9E, F). In certain species such as *X. squalens*, even when papillae are present, hairs are usually absent.

In *X. squalens* (considered by some authors, together with *X. scabrilingue*, conspecific with *X. variegatum*), the outlines of the labellar epidermal cells, especially those at the tips of the verrucae, like those of *X. leontoglossum*, are often indistinct (Fig. 8D). The epidermal, convex, outer tangential wall may bear traces of a film-like deposit (K12658), interpreted here as secreted material (Fig. 8E and F), and this may explain poor cell definition. Sparse, multicellular, 2–8-celled, uniseriate hairs with swollen, possibly glandular tips were observed on the proximal, adaxial surface of one specimen (K13837). Plants collected as *X. squalens*, however, may differ from those assigned to *X. scabrilingue* and *X. variegatum* in that the labellum of the two latter species often bear obpyriform to spherical papillae (Figs 7E, F and 9B). Again, in *X. variegatum*, unlike *X. scabrilingue*, these cells may be rather poorly

defined towards the tips of the verrucae (Figs 7E, F and 9C, D) but no obvious secreted material was observed in *X. variegatum* nor in *X. scabrilingue*.

Obpyriform or spherical papillae also occur upon the labellum of *X. corrugatum* (Fig. 5A, B) and *X. palmifolium* (Fig. 4A, B). Moreover, there is some evidence that the papillae of *X. corrugatum* (K49592) produce a viscid secretion that accumulates at their bases (Fig. 5B). However, the rounded papillae of *X. powellii*, *X. pallidiflorum* and *X. bractescens*, which are arranged longitudinally along the labellar carinae, are laterally compressed and are paddle-like or resemble ‘lollipops’ (Figs 4C–F, 5C–F and 6A, B).

Unlike the other *Xylobium* spp. studied, hairs are present upon the labella of *X. elongatum*, *X. foveatum* and *X. latilabium* [syn. *X. ornatum* (Klotzsch) Rolfe] (Figs 3D, 6C and 7A–C). The trichomes of *X. elongatum* and *X. foveatum* are similar in that they are 1–2-celled but, whereas the hairs of the former species have rounded terminal cells (Fig. 6C), those of *X. foveatum* have clavate terminal cells (Fig. 3D). These hairs are interspersed between obpyriform to spherical papillae or conical to obpyriform papillae in *X. elongatum* and *X. foveatum*, respectively (Figs 3D and 6C, D) and the conical papillae have rounded tips. The trichomes of *X. latilabium*, however, are very different. They occur mainly on the mid-lobe of the labellum (Fig. 7A), arise from obpyriform to spherical papillae and are 4–8-celled, uniseriate and moniliform with rounded to clavate terminal cells (Fig. 7B, C).

Moniliform, 2–10-celled, uniseriate trichomes consisting of oval to rounded cells also occur in *Teuscheria wageneri* (Fig. 10A, C, D) but much of the epidermal surface is obscured by a film of secreted material (Fig. 10B, D). The epidermis, where visible, is papillose and consists of conical papillae with wide bases (Fig. 10B).

Similarly, the labellum of *Rudolphiella aurantiaca* is papillose with conical to obpyriform papillae (Figs 10E, F) bearing well-defined, cuticular striations (Fig. 10F).

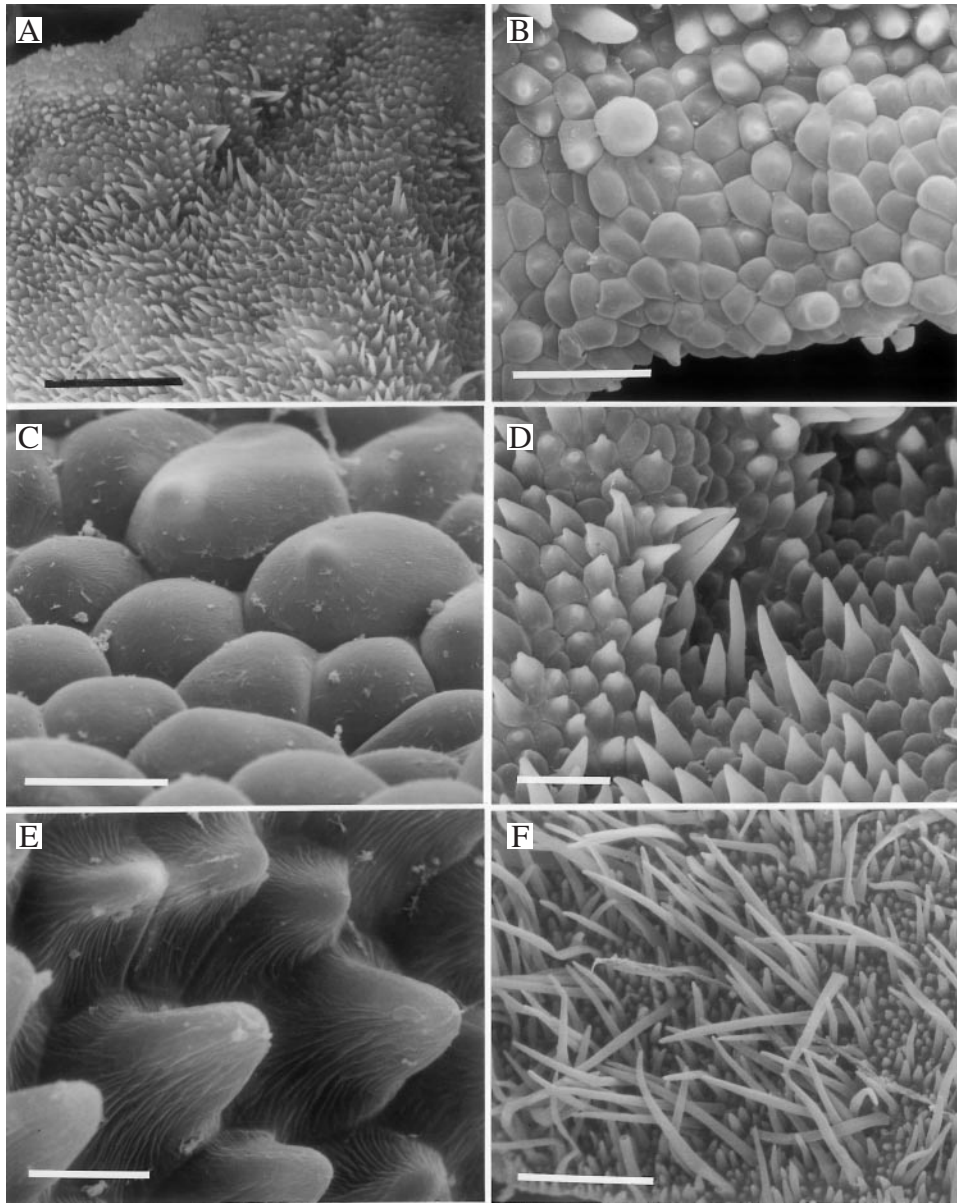


FIG. 2. (A–D) Labellum of *Bifrenaria aurea* (K54349) showing short, conical papilla-like trichomes (A), conical to obpyriform papillae (B) and stages in trichome formation (C, D). The latter process commences with the development of a small protrusion upon the papilla (C). Further development of this protrusion results in the formation of a conical papilla and eventually a trichome (D). Scale bars: A = 500 μm ; B = 100 μm ; C = 25 μm ; D = 100 μm . (E, F) Labellar surface of *Bifrenaria tetragona* (KLD200502) showing conical papillae with longitudinal, cuticular striations (E) and stages in the development of unicellular trichomes (F). Scale bars: E = 10 μm ; F = 200 μm .

Uniseriate, 3–5-celled trichomes with pointed or rounded tips and cylindrical component cells are present at the point of attachment of the labellum.

The labella of both *Mormolyca ringens* (Figs 11A–D) and *M. schweinfurthiana* (Fig. 11E) are densely pubescent with conical papillae having pointed ends and narrow points of insertion intergrading to form simple, unicellular trichomes. These hairs measure 32–64 μm (mean 48 μm) and 25–92 μm (mean 53.2 μm) for *M. ringens* and *M. schweinfurthiana*, respectively.

Since food materials also have a constitutive role, their presence alone does not necessarily indicate that they

function as pollinator rewards. Only when they are present at elevated concentrations within secretions or structures such as hairs and papillae, and there is strong evidence that these are foraged and ingested by presumed pollinators or their larvae, can the role of these substances as pollinator rewards be established with any degree of certainty. In the absence of relevant field data, this was not possible, although histochemistry revealed that, in some cases, high concentrations of food materials were indeed present.

Histochemical analysis of *B. harrisoniae* (KLD 200501) revealed that the labellar trichomes did not contain starch or

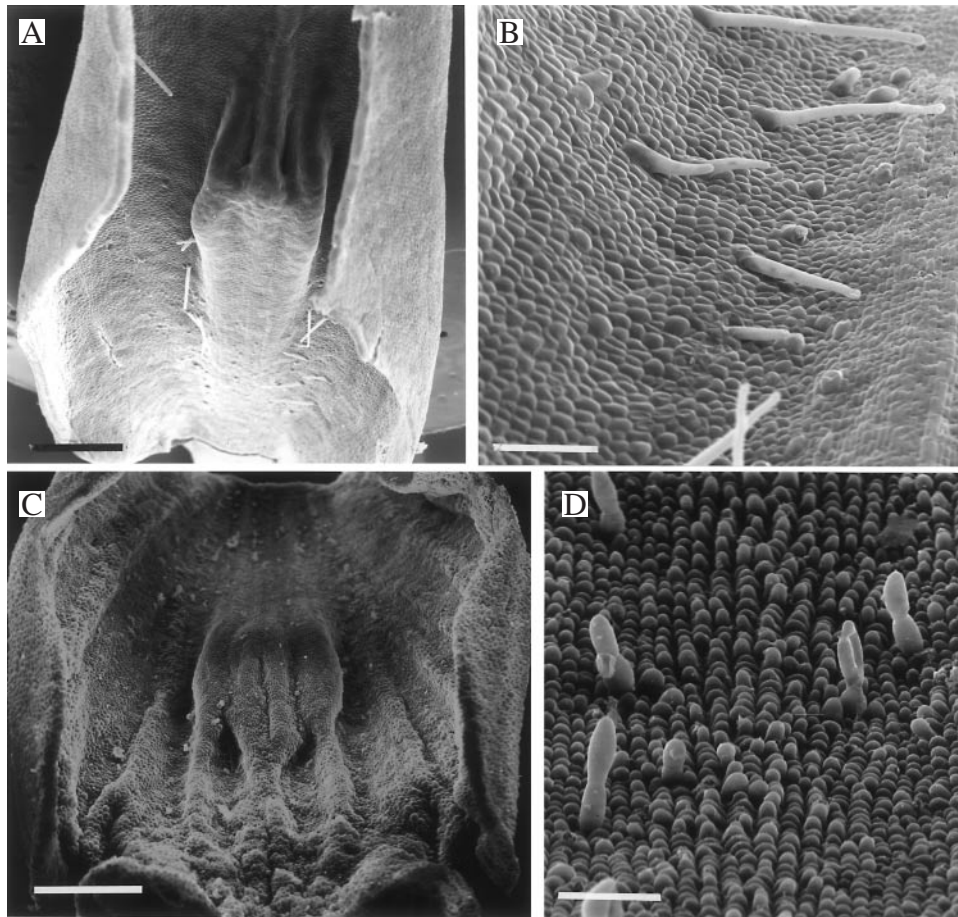


FIG. 3. (A, B) Labellum of *Stenocoryne secunda* (syn. *Bifrenaria aureofulva*) (K37726) showing sparse (A), unicellular trichomes (B). Scale bars: A = 1 mm; B = 250 μ m. (C, D) Papillose labellum of *Xylobium foveatum* (K14423) (C) showing obpyriform papillae and 2-celled trichomes with clavate terminal cells (D). Scale bars: C = 1 mm; D = 100 μ m.

lipid nor were there higher concentrations of protein present here than in any other part of the labellum. In *X. squalens* (K13837 and K14424) and *X. leontoglossum* (KLD200601), treatment with an ethanolic solution of Sudan III revealed the presence of lipid along some of the epidermal cell walls and especially at those points where three (or more) adjacent cells meet. The labella of flowers of unknown provenance (S20030489), identical to those collected as *X. corrugatum* (K49592) but preserved in 5% formalin solution, stained even more intensely with Sudan III. In each case, the location of lipid-rich material corresponded to that of the presumed secretion observed using SEM (Figs 5B and 8E, F). The walls of some of the epidermal hairs of *X. squalens* (K13837) also stained with Sudan III. Histochemistry of *Mormolyca ringens* was frustrated by the presence of pigment in the epidermal hairs. At first, it appeared that the epidermis had stained more intensely for protein than underlying tissues but careful comparison of the extent of staining in non-pigmented trichomes with that of underlying parenchyma in hand-cut sections showed that the hairs did not contain greater quantities of protein, nor indeed starch and lipids, than other labellar tissues.

DISCUSSION

The labellar micromorphology of *Maxillaria sensu stricto* has been extensively studied (Davies and Winters, 1998; Davies *et al.*, 2000, 2003a, b; Davies and Turner, 2004a; Matusiewicz *et al.*, 2004). It may be glabrous and often rugose as in members of the *M. cucullata* Lindl. alliance but is generally papillose. The papillae are usually conical with wide bases and rounded or pointed tips but are frequently obpyriform or almost spherical, and some of the latter are modified and secrete wax or a resinous, viscid material (Davies *et al.*, 2003a, b; Davies and Turner, 2004a; Matusiewicz *et al.*, 2004). This contains aromatic amino acids, lipoidal compounds and triterpenoids (Davies *et al.*, 2003a, b; Davies and Turner, 2004a; Flach *et al.*, 2004; Singer *et al.*, 2006) and it is thought that it may function as a pollinator reward. Trichomes, where present, tend to be simple, multicellular and uniseriate with pointed or rounded tips (Davies and Winters, 1998; Davies *et al.*, 2003a; Davies and Turner, 2004a) and, although multiseriate hairs have been observed in this genus (Davies and Turner, 2004a), simple, unicellular hairs have not. Moniliform trichomes occur in certain *Maxillaria* spp., in particular those of the *M. grandiflora* (Humb., Bonpl. &

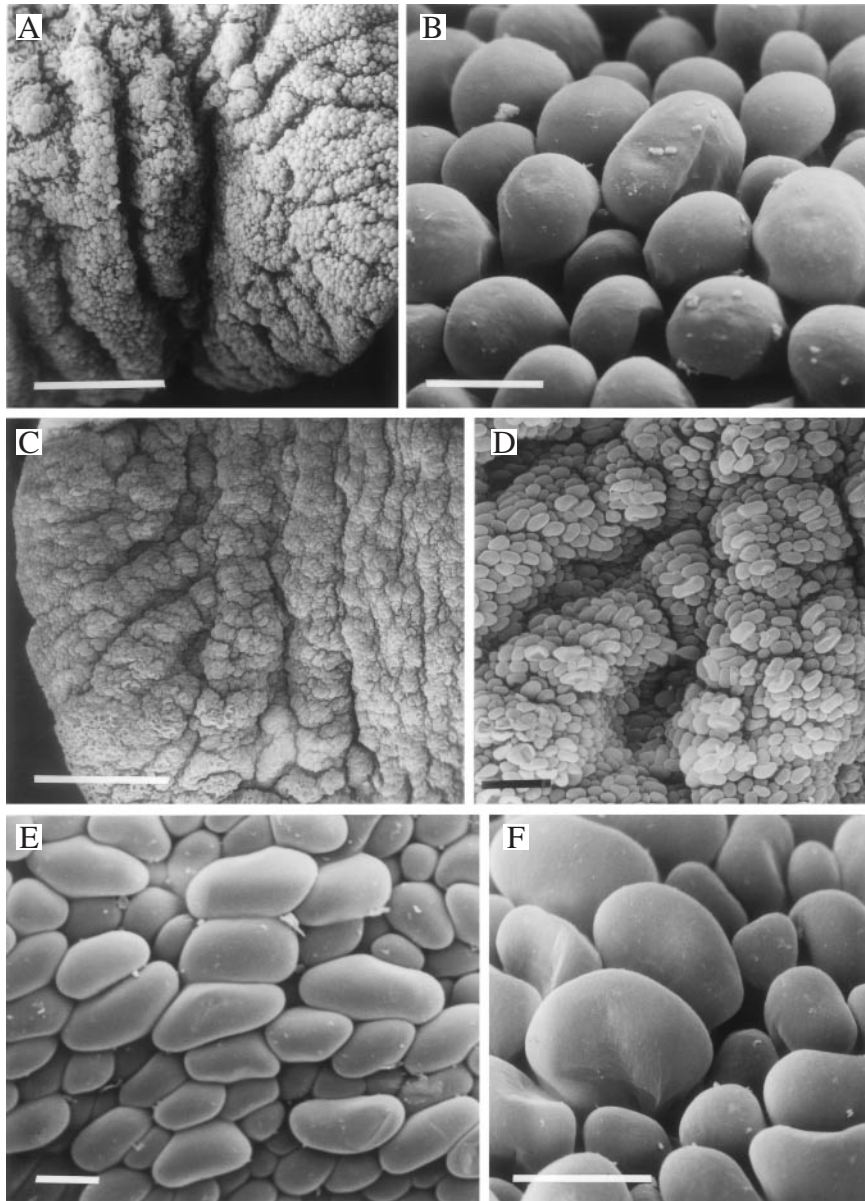


FIG. 4. (A, B) Papillose labellum of *Xylobium palmifolium* (K37706) (A) with obpyriform papillae (B). Scale bars: A = 500 μ m; B = 25 μ m. (C–F) Labellum of *Xylobium pallidiflorum* (K47185) (C) showing laterally compressed papillae (D, E) that have a lollipop- or paddle-like profile (F). Scale bars: C = 500 μ m; D = 100 μ m; E and F = 25 μ m.

Kunth) Lindl. complex and *M. discolor* (Lodd. ex Lindl.) Rchb.f. alliance (Davies and Winters, 1998; Davies *et al.*, 2000, 2003a; Davies and Turner, 2004a; Matusiewicz *et al.*, 2004) and their cells contain reserves of protein and, often, starch. They may fragment to form individual or short chains of cells (pseudopollen) and are gathered by stingless bees (Meliponini) that pollinate the flowers (Singer and Koehler, 2004), although, to date, there is no direct evidence that they are ingested by the pollinator. A small number of maxillaries secrete nectar and, although largely pollinated by stingless bees (Davies *et al.*, 2005, and references therein), some are probably pollinated by hummingbirds (Stpiczyńska *et al.*, 2003). In a study involving 100 species of *Maxillaria*, Davies *et al.* (2005)

showed that some 13 % of species produce wax or viscid material, 16–23 % produce pseudopollen and 8 % produce nectar. The majority of species, some 56 %, however, do not produce any rewards and attract potential pollinators, mainly *Trigona* spp., solely by deceit (Singer and Cocucci, 1999; Singer and Koehler, 2004; Davies *et al.*, 2005).

The labellum of *Bifrenaria* spp. resembles that of *Maxillaria sensu stricto* in that it bears conical papillae but the densely pubescent labellum of *Bifrenaria* at once distinguishes it from that genus. Moreover, the simple, unicellular type of trichome found in *Bifrenaria* has not been recorded for *Maxillaria sensu stricto* but these hairs are present, albeit sparsely, even in *B. aureofulva*, a species formerly placed in *Stenocoryne*. Generally, the distinction

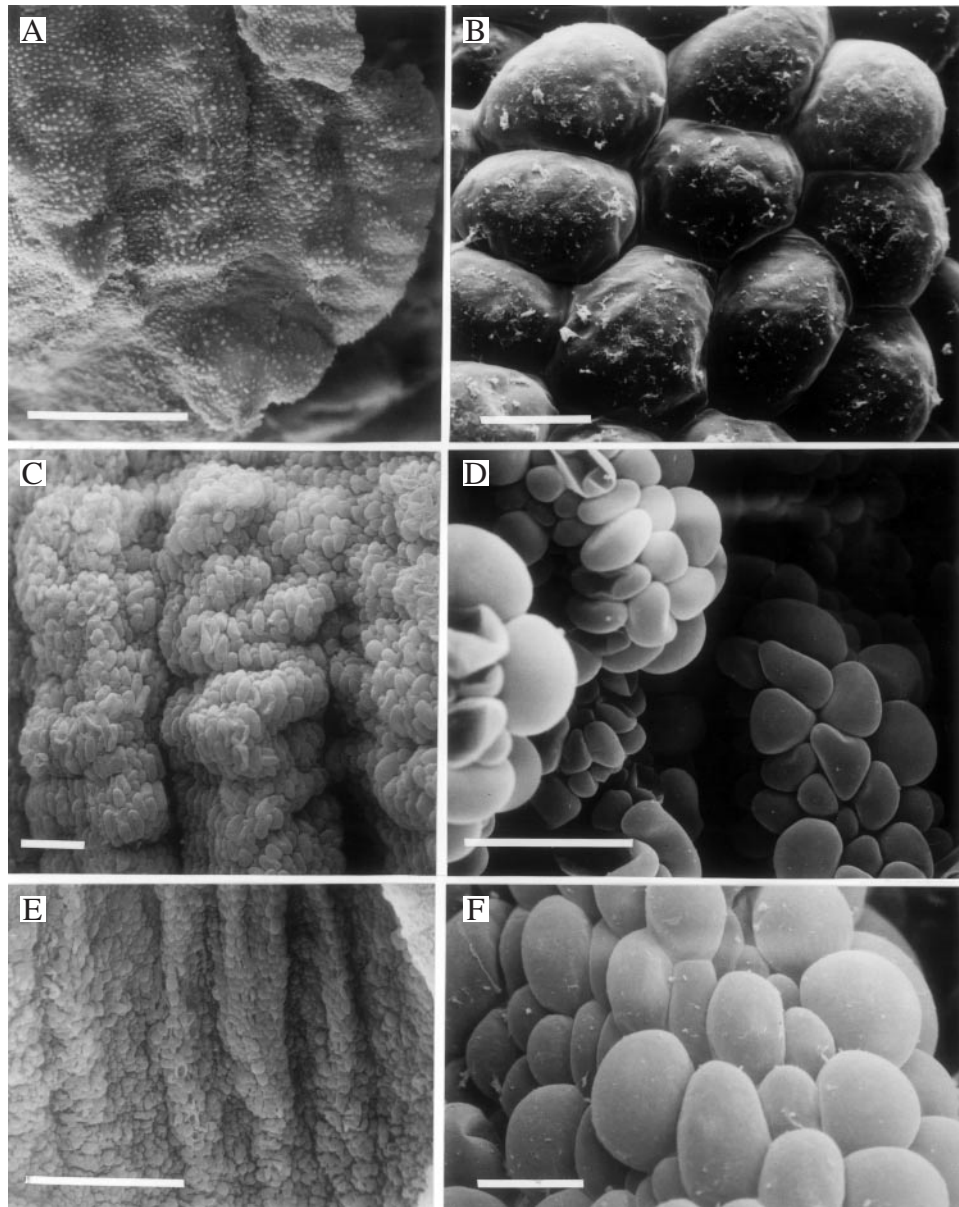


FIG. 5. (A, B) Labellum of *Xylobium corrugatum* (K49592) (A) showing papillae with traces of secreted material (B). Scale bars: A = 500 μ m; B = 10 μ m. (C, D) Labellar surface of *Xylobium bractescens* (K14421) showing the arrangement (C) of laterally compressed, lollipop- or paddle-like papillae (D) along the carinae. Scale bars: C and D = 100 μ m. (E, F) Labellum of *Xylobium powellii* (K8480) (E) showing laterally compressed papillae (F). Scale bars: E = 500 μ m; F = 25 μ m.

between papilla and trichome is simply a matter of degree, although usually, trichomes are more than twice as tall as they are wide with pointed or rounded tips and a narrow point of insertion. This distinction is much more clearly demarcated in *Maxillaria* than in *Bifrenaria* where there is a greater degree of intergrading. Differences in labellar micromorphology, the absence of secreted material upon the labellar surface of *Bifrenaria* and the absence of elevated concentrations of food substances in the labellar hairs of this genus make it unlikely that *Bifrenaria* and *Maxillaria* share the same pollinator. In fact, it would appear from the paucity of published data that the pollination of *Bifrenaria* has seldom been observed and

much of the evidence available was arrived at indirectly. For example, Dressler (1990) reports *Bifrenaria* pollinaria on males of *Eufriesia violacea* (Euglossini) and Singer and Koehler (2004) cite the observation of I. Gajardo who reported seeing pollinaria of *B. harrisoniae* on *Eufriesia violacea* and *Bombus brasiliensis* (Bombini) in Paranapiacaba, São Paulo State. Smaller bees may be responsible for pollinating small-flowered species such as *B. mellicolor* Rchb.f. and it has even been suggested that *B. aureofulva* may be pollinated by hummingbirds which may explain the paucity of labellar hairs.

Koehler and Amaral (2004), in their review of *Bifrenaria*, concur with Castro and Campacci (2000) in

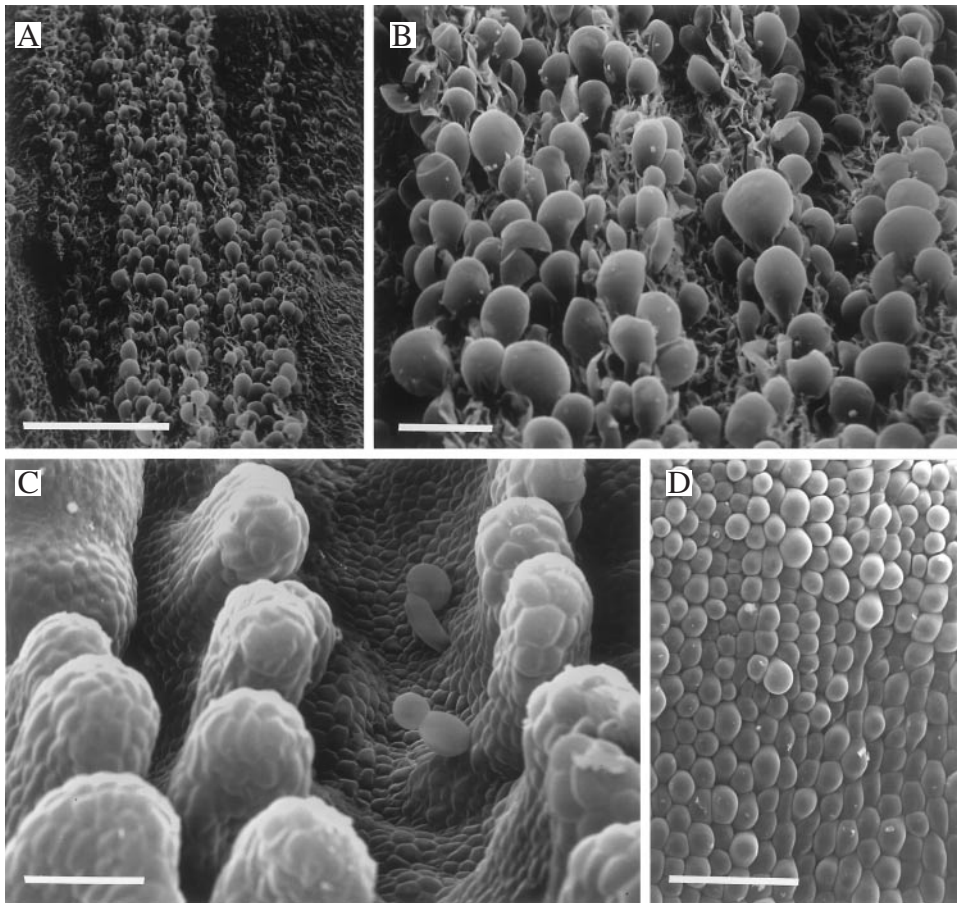


FIG. 6. (A, B) Labellar surface of *Xylobium powellii* (K8480) showing arrangement (A) of laterally compressed, lollipop- or paddle-like papillae (B) along carinae. Scale bars: A = 500 μ m; B = 100 μ m. (C, D) Labellar surface of *Xylobium elongatum* (K14422) showing verrucae, simple, 2-celled trichomes (C) and obpyriform papillae (D). Scale bars: C and D = 200 μ m.

reducing *B. aurea* to synonymy under *B. harrisoniae* and, although the present specimen of *B. aurea* generally conformed to the description of *B. harrisoniae* (Koehler and Amaral, 2004) in the dimensions, shape and proportions of all its floral parts, there were nonetheless, significant differences. For example, the callus was glabrous, the petal apices were not as rounded and the labellar hairs were much shorter and more papilla-like than those of *B. harrisoniae*. Unfortunately, it was not possible to examine the viscidium as the pollinarium was no longer present.

The labellar micromorphology of *Xylobium* more closely approaches that of *Maxillaria* than does *Bifrenaria*, in that the epidermis may be glabrous or papillose. A glabrous to minutely papillose labellum, however, was observed only for *X. colleyi*. Unlike other Brazilian species of *Xylobium*, which tend to have 2–3-leaved pseudobulbs and small flowers, *X. colleyi* has unifoliate pseudobulbs with relatively large flowers (Singer and Koehler, 2004). However, a number of *Xylobium* species such as *X. corrugatum*, *X. latilabium*, *X. pallidiflorum* and *X. subintegrum* C. Schweinf., from other parts of S. America, also have unifoliate pseudobulbs and flowers similar in size to those of *X. colleyi* (Dunsterville and

Garay, 1965; Bennett and Christenson, 1993, 1995) but their labella, unlike that of *X. colleyi*, are distinctly papillose. In *Xylobium*, the papillose labellum bears conical, obpyriform or almost spherical papillae. The conical papillae have wide bases with pointed or rounded tips. Obpyriform to spherical papillae occur in *X. palmifolium*, a species retained in *Maxillaria* by some authorities. Indeed, in terms of labellar micromorphology alone, there is little to distinguish it from that genus, although its several-flowered, racemose inflorescence and plicate leaves justify its inclusion in *Xylobium*. In some species of *Xylobium*, trichomes are present. As in *Maxillaria*, there is greater demarcation between the papillae and trichomes here than in *Bifrenaria*, and the trichomes of *Xylobium*, like those of *Maxillaria*, are multicellular rather than unicellular as in *Bifrenaria*. However, the laterally compressed, paddle- or lollipop-like papillae found in certain *Xylobium* spp. do not appear to occur in *Maxillaria*. Nevertheless, moniliform hairs, similar to those found in pseudopollen-forming species of *Maxillaria*, occur in *X. latilabium*, and lipoidal, secreted material, like that found in members of the *Maxillaria acuminata* Lindl., *M. discolor* and *M. rufescens* Lindl. alliances as well as in *M. lepidota* Lindl., *M. reichenheimiana*

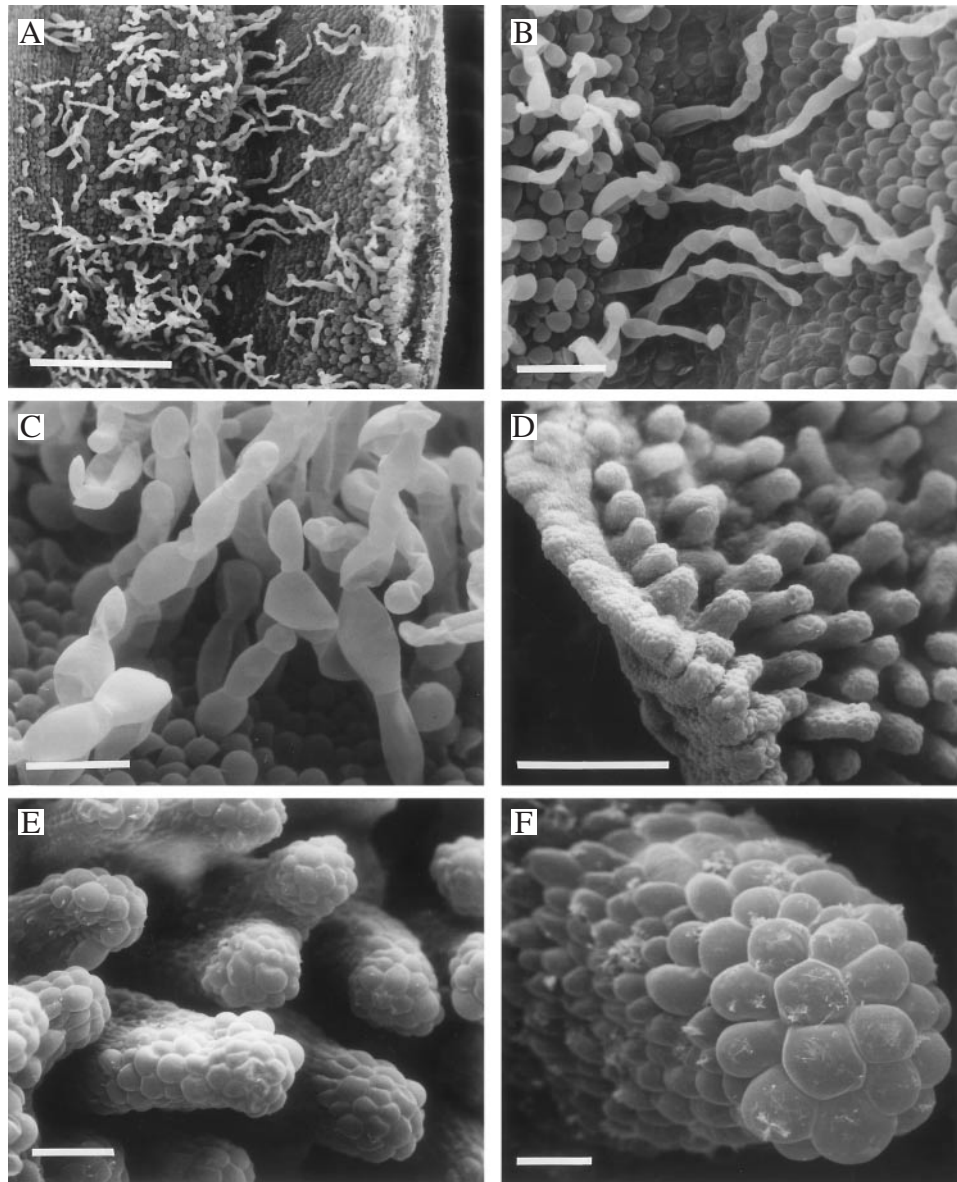


FIG. 7. (A–C) Labellum of *Xylobium latilabium* (K6811) showing distribution (A) and detail (B, C) of uniseriate, moniliform trichomes and obpyriform papillae. Scale bars: A = 500 μ m; B = 100 μ m; C = 50 μ m. (D–F) Labellum of *Xylobium scabrilingue* (K31551) showing verrucae (D, E) with well-defined cells at their tips (F). Scale bars: D = 500 μ m; E = 100 μ m; F = 25 μ m.

Endres & Rchb.f. and *M. pseudoreichenheimiana* Dodson (Davies *et al.*, 2003a, b; Davies and Turner, 2004a; Flach *et al.*, 2004; Matusiewicz *et al.*, 2004; Singer *et al.*, 2006), was detected in fresh *X. leontoglossum* and preserved specimens of *X. corrugatum* and *X. squalens*, in some cases, even after 69 years in spirit! Dunsterville and Garay (1965) described the lip of *X. colleyi* as having a ‘wet and ‘sticky’’ lustre’. Although this may be due to viscid, secreted material, none was observed for this species in the present study, although cell outlines were often indistinct, possibly due to the presence of an overlying film. Generally, most *Xylobium* spp., like the majority of *Maxillaria* spp., are nectarless and are visited and presumably pollinated by Meliponini such as species of *Trigona* (Dressler, 1990) including *Trigona amalthea*,

T. silvestriana, *Scaptotrigona postica*, *Partamona orizabaensis* and *P. musarum* (Roubik, 2000) although, to date, no foraging activity has been recorded. Unfortunately, Roubik does not name the *Xylobium* spp. studied but does give locality data. However, van der Pijl and Dodson (1969) report that *X. squalens* is pollinated by *Trigona postica* (syn. *S. postica*). Pintaudi *et al.* (1990) have also observed the pollination of *X. squalens* by *S. postica*, and the nectarless flowers of *X. latilabium* are visited and pollinated by *T. amalthea* in Peru (van der Pijl and Dodson, 1969). Furthermore, it has been reported that smaller bees, also identified as *T. amalthea*, pollinate *X. variegatum* in Peru and Costa Rica. Interestingly, these bees did not visit flowers of *X. latilabium* even though they had to fly past them to reach *X. variegatum* (van der Pijl and Dodson,

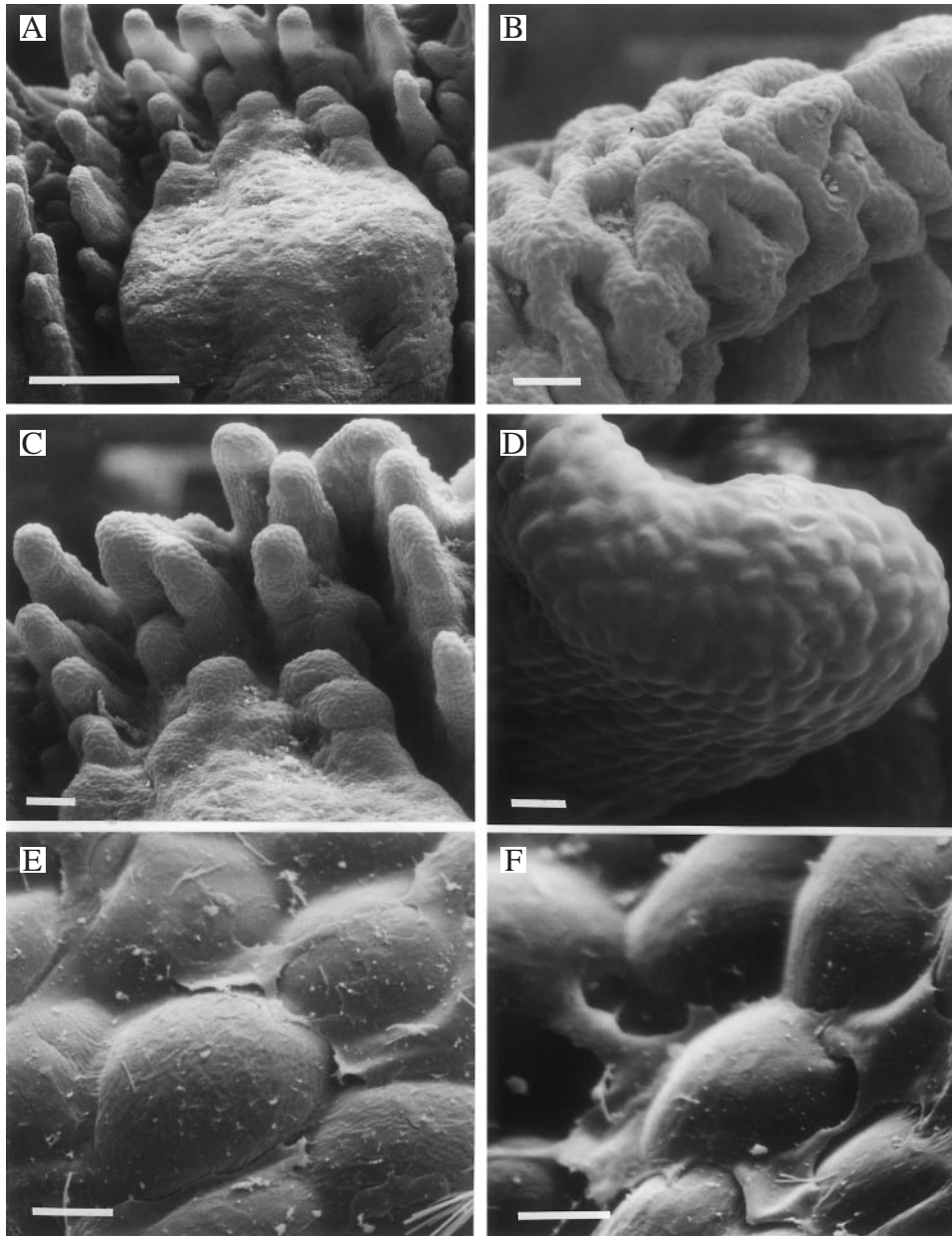


FIG. 8. (A–F) Labellar surface of *Xylobium squalens* (K12658) showing callus (A) and verrucae (A–C) with poorly defined cells at their tips (D). This may be due to the presence of a film of secreted material, traces of which can be seen upon and between the labellar papillae (E, F). Scale bars: A = 500 μm ; B and C = 100 μm ; D = 25 μm ; E and F = 5 μm .

1969). Thus, if *X. squalens* and *X. variegatum* truly are conspecific, then it would appear that the taxon is visited by at least two species of Meliponini. However, only when the transfer of pollinia has been unequivocally demonstrated in the field can it be claimed with certainty that pollination has taken place.

The labella of *Teuscheria wagneri* also bear conical papillae but profiles of the epidermal cells are obscured by a thick, viscid layer through which papilla tips protrude. Moniliform hairs are also present in this species and Dunsterville and Garay (1961) and Bennett and Christenson (1995) have described the labellar callus of *T. venezuelana* Garay (syn. *T. wagneri*) and *T. dodsonii*

Dressler as ‘covered with golden farinose material’ and ‘covered with bright yellow farinaceous trichomes’, respectively. Although, in the absence of field observations and histochemical data, it cannot be unequivocally claimed that the moniliform hairs of *T. wagneri* function as pseudopollen, there is every indication from their morphology that this is the case. Indeed, Vogel (1979) and Kjellsson and Rasmussen (1987) have argued that hairs which fragment into rounded component cells, even when devoid of food reserves, still have the potential to function as pseudopollen and attract pollinators by deceit. Although relatively uncommon, the co-occurrence of both secreted material and food-hairs within a single species has already

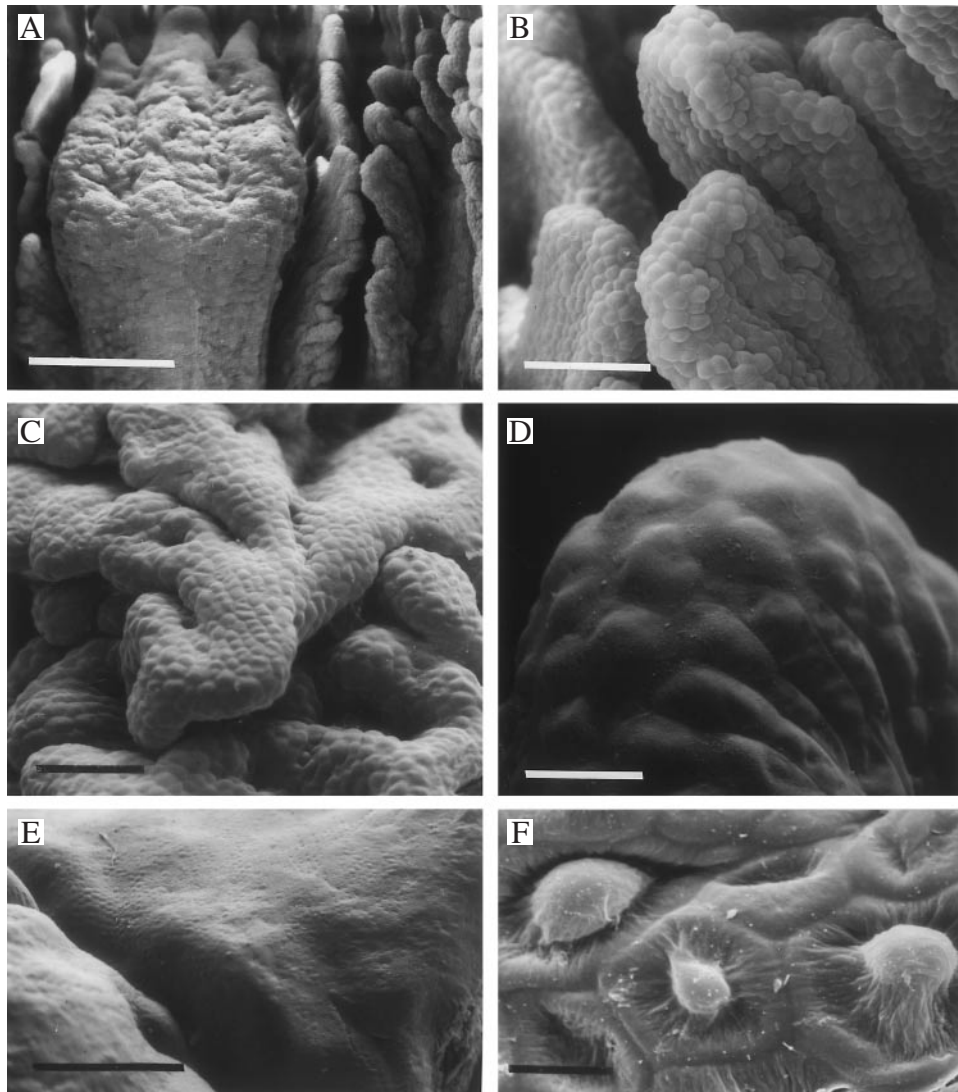


FIG. 9. (A–D) Labellar surface of *Xylobium variegatum* (K46104) showing callus (A) and verrucae (A–C) whose tips appear to be coated with a film of secreted material, thus obscuring cell outlines (D). Scale bars: A = 500 μm ; B and C = 100 μm ; D = 25 μm . (E, F) Glabrous or minutely papillose labellum of *Xylobium colleyi* (K13833) (E) showing detail of papillae (F). Scale bars: E = 500 μm ; F = 10 μm .

been recorded for members of the *Maxillaria discolor* alliance (Davies *et al.*, 2003a; Singer *et al.*, 2006) and *M. lepidota* (Matusiewicz *et al.*, 2004). Unfortunately, the pollinator of *Teuscheria wagneri* has yet to be identified. Even so, the presence of moniliform hairs and secreted material indicate that the pollinator, as in entomophilous species of *Maxillaria*, is possibly a member of the Meliponini.

The labellum of *Rudolfiella aurantiaca* mainly bears conical to obpyriform papillae with well-defined cuticular striations, together with simple trichomes at its point of attachment. Unlike *Bifrenaria* spp., where unicellular trichomes predominate, those of *Rudolfiella* are multicellular with cylindrical cells, and there is clear demarcation between papillae and trichomes. This supports the view of Koehler and co-workers (Koehler *et al.*, 2002; Koehler and Amaral, 2004) that *Bifrenaria* and *Rudolfiella* are phylogenetically distinct. Braga (1977) has proposed that

the hymenopteran pollinator of *R. aurantiaca* feeds upon labellar hairs. However, the present study showed that, in this species, hairs occur only at the point of attachment of the labellum, and these are neither moniliform nor is there any indication whatsoever that they fragment or are easily detached. Furthermore, they appear not to contain food reserves. Singer and co-workers, however, have reported putative labellar elaiophores in this species as in certain oil-producing members of the Oncidiinae (van der Cingel, 2001; Singer *et al.*, 2006). If this is confirmed, then the pollinator is likely to be one of the specialized oil-gathering bees (van der Cingel, 2001; Singer *et al.*, 2006) rather than a member of Meliponini as the observations of Braga (1977) would imply. Until further field work is undertaken and the pollinator identified, the matter cannot be resolved.

The densely pubescent labella of *Mormolyca ringens* and *M. schweinfurthiana* bear conical papillae with pointed tips and narrow points of insertion, and these papillae

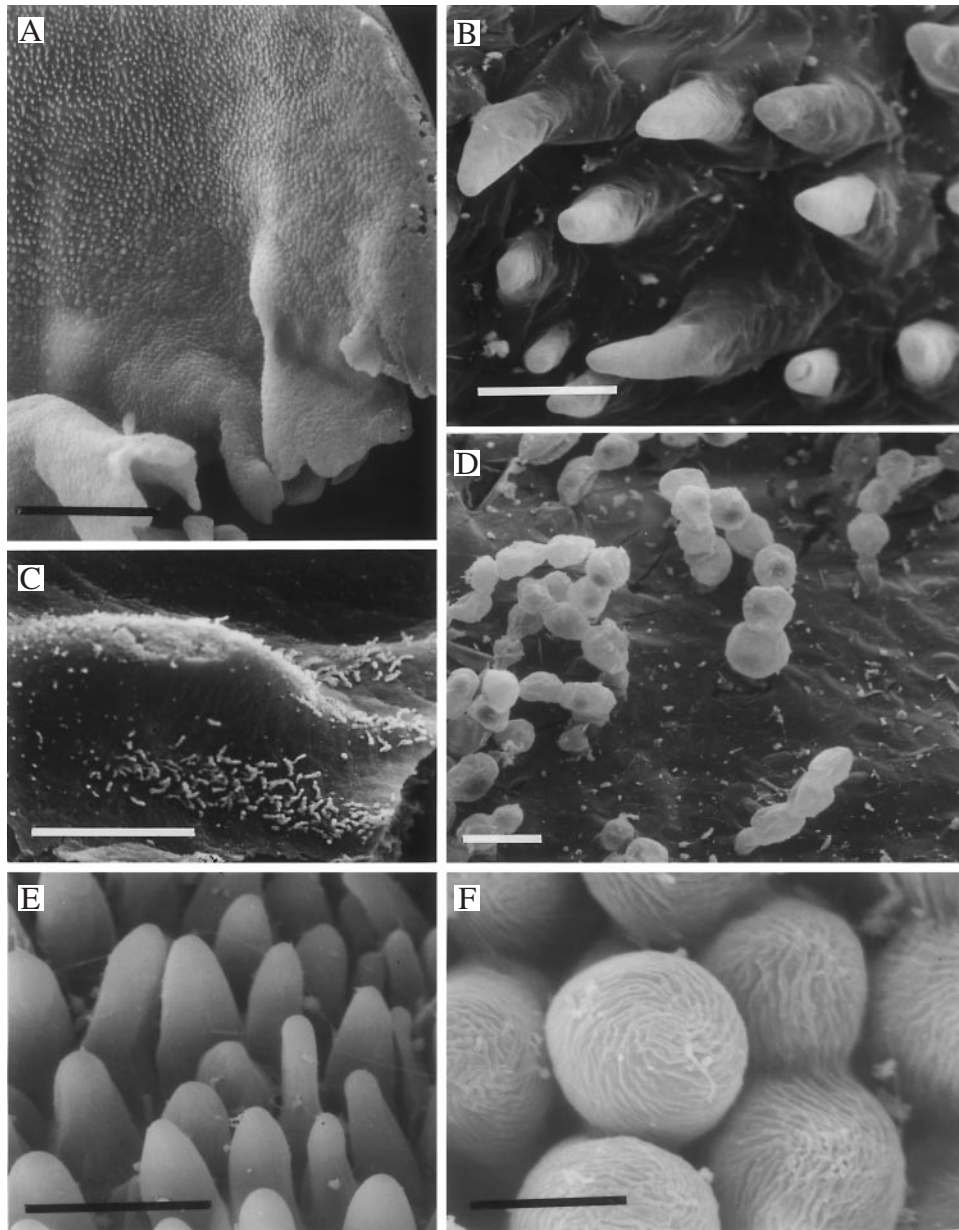


FIG. 10. (A–D) Papillose labellum of *Teuscheria wagneri* (K41855) (A) showing conical papillae protruding through a thick layer of secreted material (B). Uniseriate, multicellular, moniliform trichomes also occur upon the labellum (C, D) and these too, penetrate the secreted material (D). Scale bars: A = 500 μm , B = 25 μm ; C = 500 μm ; D = 25 μm . (E, F) Labellar surface of *Rudolphiella aurantiaca* (K57061) showing conical papillae (E) with cuticular striations (F). Scale bars: E = 25 μm ; F = 10 μm .

intergrade to form unicellular trichomes that lack food reserves. Phylogenetic and molecular studies (Holtzmeier *et al.*, 1998; Chase *et al.*, 2003; Singer *et al.*, 2004; Dathe and Dietrich, 2006) all indicate that *Mormolyca* is nested within *Maxillaria*. However, despite the fact that such labellar trichomes have never been reported from *Maxillaria sensu stricto*, this does not necessarily conflict with molecular data. Instead, the presence of this type of hair may simply reflect the occurrence of sexual mimicry in the genus. That pseudocopulation occurs in *M. ringens* is now well established (Singer *et al.*, 2004; Flach *et al.*, 2006). In this species, sexually excited drones of *Nannotrigona*

testaceicornis and *Scaptotrigona* sp. (Meliponini) pollinate the flower when attempting to copulate with the labellum. The labellar indumentum is said to resemble hairy areas found on the insect, and the hairs are concentrated at the basal, lateral margins of the labellum and on a purple, triangular area just below the column (Singer *et al.*, 2004). The densely trichromatic and insect-like shape of the flower of *M. ringens* parallels that of the Old World genus *Ophrys* L. (Kullenberg, 1961). Furthermore, the labellar papillae and trichomes of *M. ringens* closely resemble those of *Ophrys* spp. (Servettaz *et al.*, 1994; Ascensão *et al.*, 2005). The chemical composition of the fragrance which

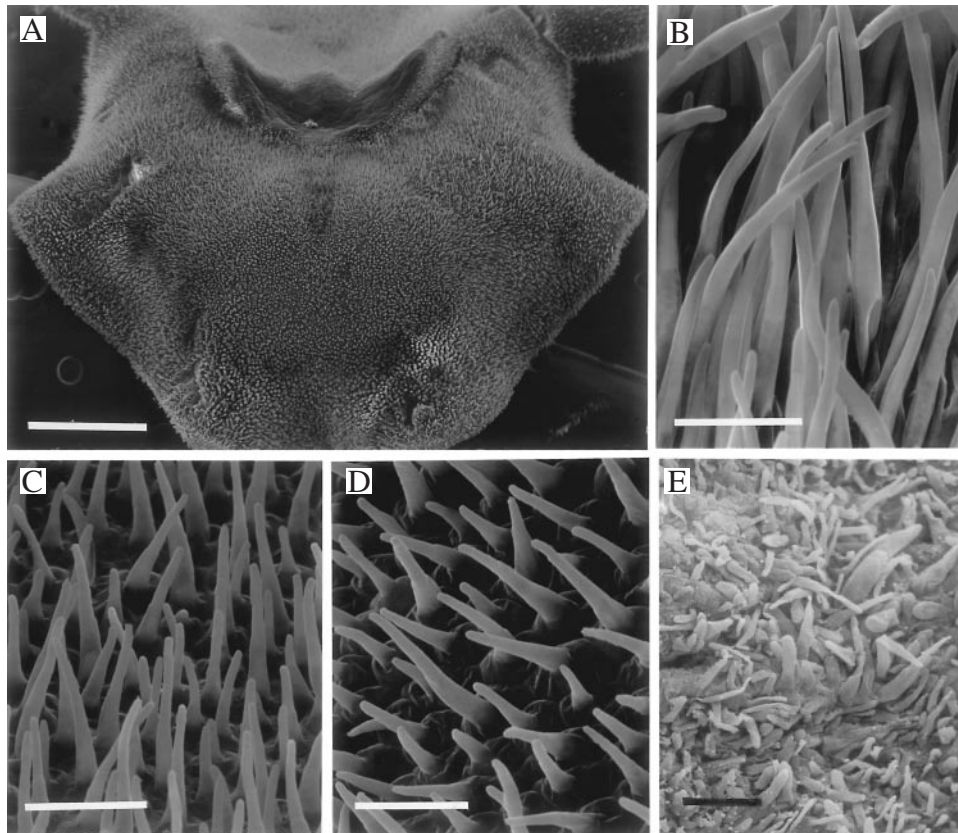


FIG. 11. (A–D) Densely pubescent labellum of *Mormolyca ringens* (S19980091) (A) showing unicellular trichomes with pointed tips and narrow points of insertion from proximal (B), median (C) and lateral (D) parts of lip. Scale bars: A = 1 mm; B–D = 50 μ m. (E) Labellar surface of *Mormolyca schweinfurthiana* (K27543) showing similar unicellular trichomes to those found in *M. ringens*. Scale bar = 50 μ m.

attracts the insect pollinator to *M. ringens* has been shown to resemble that of pheromones produced by virgin queens of *Scaptotrigona* sp. (Flach *et al.*, 2006). Both types of bee hover for a few seconds in front of the flower before alighting. The pollinator is then guided along the flower by visual and tactile cues provided by the labellar hairs (Singer *et al.*, 2004). Attempts at pseudocopulation are indicated by spasmodic abdominal movements and extrusion of the genitalia, and in *Nannotrigona* drones (but not *Scaptotrigona*) this is accompanied by an audible buzzing caused by the vibrating of wings (Singer *et al.*, 2004). *Nannotrigona* visits are much more common than those of *Scaptotrigona* but both types of bee are able to dislodge and deposit pollinaria (Singer *et al.*, 2004). Although the pollination of *M. schweinfurthiana* has not yet been described, the micromorphology of its labellum and the similar length of the labellar hairs would suggest a pollination mechanism close to that of *M. ringens*.

The genera *Bifrenaria* and *Mormolyca* are atypical in having unicellular hairs, whereas *Xylobium*, *Teuscheria* and *Rudolfiella*, in possessing multicellular hairs, more closely resemble *Maxillaria sensu stricto*. Moreover, *Xylobium* and *Teuscheria* share a number of other labellar features such as moniliform hairs and secretory papillae with *Maxillaria sensu stricto* and this, at first sight, would appear to support the case for the inclusion of these genera in Maxillariinae *sensu lato*. However, Stern *et al.* (2004)

have shown that anatomical characters alone are of limited value in determining relationships within the Maxillarieae and, more recently, this has been reiterated by Dathe and Dietrich (2006) who claim that ‘the value of morphological characters in phylogenetic reconstruction of Maxillariinae is limited by the high degree of homoplasy’. Benzing (1986) has also warned that the use of pollination-related traits alone to infer relationships among species and groups of species may lead to erroneous conclusions because of convergence. Indeed, the unusual, unicellular hairs of *Bifrenaria* and *Mormolyca* can certainly be related to pollination biology since *Bifrenaria*, unlike the other genera studied here, is thought not to be pollinated by Meliponini, whereas *Mormolyca* alone displays pseudocopulation. Conversely, the occurrence of moniliform hairs or secreted substances in certain species of *Maxillaria* and *Xylobium* can perhaps be explained in terms of a shared pollinator (Meliponini). Although the occurrence of both these labellar features in *Teuscheria* suggests that this genus too is pollinated by Meliponini, this may not necessarily be the case, and taxa that share such features may not necessarily be closely related. For example, almost identical moniliform hairs to those found in *Maxillaria*, *Xylobium* and *Teuscheria* also occur amongst representatives of *Polystachya* Hook. sect. *Polystachya*, an unrelated genus largely pollinated by halictid bees (Davies *et al.*, 2002, and references therein). It is thus probable that

several labellar features, but in particular moniliform hairs, have arisen on a number of occasions in Maxillariinae *sensu lato* as a result of convergence in response to similar pollinator pressures. Likewise, it has been demonstrated that convergence is responsible for a number of other shared morphological characters, both vegetative and reproductive, in Maxillariinae (Dathe and Dietrich, 2006) thus, simultaneously highlighting the need for caution in determining taxonomic relationships based solely on morphological data and stressing the importance of a multidisciplinary approach to orchid phylogeny.

ACKNOWLEDGEMENTS

K.L.D. is grateful to the Stanley Smith (UK) Trust for their generous grant. The authors also acknowledge the help of the staff of the Royal Botanic Gardens, Kew, UK, M.P. Turner, School of Biosciences, Cardiff University, UK and Alan Gregg, Swansea Botanical Complex, Swansea, UK.

LITERATURE CITED

- Ascensão L, Francisco A, Cotrim H, Pais MS. 2005. Comparative structure of the labellum in *Ophrys fusca* and *O. lutea* (Orchidaceae). *American Journal of Botany* 92: 1059–1067.
- Atwood JT, Mora de Retana DE. 1999. Orchidaceae: tribe Maxillarieae: subtribes Maxillariinae and Oncidiinae. *Fieldiana* 40: 1–182.
- Bechtel H, Cribb P, Launert E. 1981. *The manual of cultivated orchid species*. Poole: Blandford Press.
- Bennett Jr DE, Christenson EA. 1993. *Icones Orchidacearum Peruvianum*. Christenson EA, ed. Sarasota, FL, pp. 198, 199, 200.
- Bennett Jr DE, Christenson EA. 1995. *Icones Orchidacearum Peruvianum*. Christenson EA, ed. Sarasota, FL, pp. 389, 399.
- Benzing DH. 1986. The genesis of orchid diversity: emphasis on floral biology leads to misconceptions. *Lindleyana* 1: 73–89.
- Braga P. 1977. Aspectos biológicos das Orchidaceae da Amazônia Central. *Acta Amazonica, Manaus* 7: 1–89.
- Brummitt RK, Powell CE. 1992. *Authors of plant names*. London: Royal Botanic Gardens, Kew.
- Carnevali G, Romero GA. 2000. *Orchids of Venezuela, a field guide*. Caracas: Armitano.
- Castro VP. 1991a. Estudo taxonômico de Bifrenarias seção *Stenocoryne* no Brasil. 1a. parte. *Boletim Coordenadoria Associações Orquidófilas do Brasil* 3: 6–5.
- Castro VP. 1991b. Estudo taxonômico de Bifrenarias seção *Stenocoryne* no Brasil. 2a. parte. *Boletim Coordenadoria Associações Orquidófilas do Brasil* 3: 44–55.
- Castro VP. 1991c. Estudo taxonômico de Bifrenarias seção *Stenocoryne* no Brasil. 3a. parte. *Boletim Coordenadoria Associações Orquidófilas do Brasil* 3: 39–45.
- Castro VP. 1996. Contribution to the study of the genus *Bifrenaria* Lindl section *Harrisoniae* Pabst. In: *Proceedings of the 15th World Orchid Conference*, Rio de Janeiro. Turriers, France: Naturalia Publications, 376–383.
- Castro VP, Campacci M. 2000. *Icones Orchidacearum Brasiliensis*, Vol. 1. Castro VP, ed. São Paulo.
- Chase MW. 2005. Classification of Orchidaceae in the age of DNA data. *Curtis's Botanical Magazine* 22: 2–7.
- Chase MW, Barret RL, Cameron KN, Freudenstein JV. 2003. DNA data and Orchidaceae systematics: a new phylogenetic classification. In: Dixon KM, ed. *Orchid conservation*. Kota Kinabalu, Sabah, Malaysia: Natural History Publications, 69–89.
- van der Cingel NA. 2001. *An atlas of orchid pollination: America, Africa, Asia and Australia*. Rotterdam: A.A. Balkema.
- Dathe S, Dietrich H. 2006. Comparative molecular and morphological studies in selected Maxillariinae orchids. *Willdenowia* 36: 89–102.
- Davies KL, Turner MP. 2004a. Morphology of floral papillae in *Maxillaria* Ruiz & Pav. (Orchidaceae). *Annals of Botany* 93: 75–86.
- Davies KL, Turner MP. 2004b. Pseudopollen in *Eria* Lindl. section *Mycaranthes* Rehb.f. (Orchidaceae). *Annals of Botany* 94: 707–715.
- Davies KL, Winters C. 1998. Ultrastructure of the labellar epidermis in selected *Maxillaria* species (Orchidaceae). *Botanical Journal of the Linnean Society* 126: 349–361.
- Davies KL, Winters C, Turner MP. 2000. Pseudopollen: its structure and development in *Maxillaria* (Orchidaceae). *Annals of Botany* 85: 887–895.
- Davies KL, Roberts DL, Turner MP. 2002. Pseudopollen and food-hair diversity in *Polystachya* Hook. (Orchidaceae). *Annals of Botany* 90: 477–484.
- Davies KL, Turner MP, Gregg A. 2003a. Atypical pseudopollen-forming hairs in *Maxillaria* Ruiz & Pav. (Orchidaceae). *Botanical Journal of the Linnean Society* 143: 151–158.
- Davies KL, Turner MP, Gregg A. 2003b. Lipoidal labellar secretions in *Maxillaria* Ruiz & Pav. (Orchidaceae). *Annals of Botany* 91: 439–446.
- Davies KL, Turner MP, Gregg A. 2003c. Pseudopollen in *Dendrobium unicum* Seidenf. (Orchidaceae): reward or deception? *Annals of Botany* 94: 129–132.
- Davies KL, Stpiczynska M, Gregg A. 2005. Nectar-secreting floral stomata in *Maxillaria anceps* Ames & C. Schweinf. (Orchidaceae). *Annals of Botany* 96: 217–227.
- Dodson CH. 1978. Two orchids from Rio Palenque, Ecuador. *Selbyana* 2: 289–290.
- Dressler RL. 1972. Una *Teuscheria* nueva del Ecuador. *Orquideologia* 7: 3–6.
- Dressler RL. 1990. *The orchids—natural history and classification*. London: Harvard University Press.
- Dressler RL. 1993. *Phylogeny and classification of the orchid family*. Cambridge: Cambridge University Press.
- Dunsterville GCK, Garay LA. 1959–1966. *Venezuelan Orchids illustrated*, Vols 1–4. London: Andre Deutsch.
- Flach A, Dondon RC, Singer RB, Koehler S, Amaral MCE, Marsaioli AJ. 2004. The chemistry of pollination in selected Brazilian Maxillariinae orchids: floral rewards and fragrance. *Journal of Chemical Ecology* 30: 1045–1056.
- Flach A, Marsaioli AJ, Singer RB, Amaral MCE, Menezes C, Kerr WE, et al. 2006. Pollination by sexual mimicry in *Mormolyca ringens*: a floral chemistry that remarkably matches the pheromones of virgin queens of *Scaptotrigona* sp. *Journal of Chemical Ecology* 32: 59–70.
- Garay LA. 1958. A new orchid genus from the Ecuadorian Andes. *American Orchid Society Bulletin* 27: 820–823.
- Garay LA. 1970. Orquídeas Colombianas nuevas o críticas Decena IV. *Orquideologia* 5: 15–22.
- Garay LA, Wirth M. 1959. On the genera *Mormolyca* Fenzl and *Cyrtoglottis* Schltr. *Canadian Journal of Botany* 37: 479–490.
- Hoehne FC. 1944. Revisão taxonômica do gênero *Bifrenaria* Lindl. *Arquivos de Botânica do Estado de São Paulo* 2: 17–20.
- Holtzmeier MA, Stern WL, Judd WS. 1998. Comparative anatomy and systematics of Senghas's cushion species of *Maxillaria* (Orchidaceae). *Botanical Journal of the Linnean Society* 127: 43–82.
- Jenny R, Braem GJ. 1987. *Teuscheria horichiana*, a new orchid from Costa Rica. *Orchid Digest* 51: 187–189.
- Kjellsson G, Rasmussen FN. 1987. Does the pollination of *Dendrobium unicum* Seidenf. involve pseudopollen? *Die Orchidee* 38: 183–187.
- Koehler S, Amaral MCE. 2004. A taxonomic study of the South American genus *Bifrenaria* Lindl. (Orchidaceae). *Brittonia* 56: 314–345.
- Koehler S, Williams NH, Whitten WM, Amaral MCE. 2002. Phylogeny of the *Bifrenaria* (Orchidaceae) complex based on morphology and sequence data from nuclear rDNA internal transcribed spaces (ITS) and chloroplast *trnL-trnF* region. *International Journal of Plant Sciences* 163: 1055–1066.
- Kullenberg B. 1961. Studies in *Ophrys* pollination. *Zoologiska Bidrag Uppsala* 34: 1–340.
- Lindley J. 1832. *Bifrenaria atropurpurea*. In: *The genera and species of orchidaceous plants*: 152. London: Ridgways.
- Lindley J. 1843. *Stenocoryne longicornis*. *Edwards's Botanical Register* 29 (Misc.): 53. London: James Ridgway.

- Matusiewicz J, Stpiczynska M, Davies KL. 2004.** Pseudopollen in the flowers of *Maxillaria lepidota* Lindl. (Orchidaceae). *Proceedings of the 53rd Meeting of the Polish Botanical Society, Toruń*, p. 14.
- Pabst GFJ, Dungs F. 1977.** *Orchidaceae Brasilienses*, Band II. Hildesheim: Brücke-Verlag Kurt Schmiersow.
- van der Pijl L, Dodson CH. 1969.** *Orchid flowers: their pollination and evolution*. Coral Gables, FL: University of Miami Press.
- Pintaudi CT, Stort MNS, Marin-Morales MA. 1990.** Artificial and natural pollination of *Xylobium squalens* Lindl. (Orchidaceae). *Naturalia* **15**: 67–80.
- Rafinesque CS. 1836.** *Adipe racemosa*. Fl. Tell. 2: 101.
- Röth J. 2004.** *Xylobium foveatum* (Lindl.) Nicholson. *Die Orchidee* **55**: 592–593.
- Roubik DW. 2000.** Deceptive orchids with Meliponini as pollinators. *Plant Systematics and Evolution* **222**: 271–279.
- Ryan A, Whitten WM, Johnson MAT, Chase MW. 2000.** A phylogenetic assessment of *Lycaste* and *Anguloa* (Orchidaceae: Maxillarieae). *Lindleyana* **15**: 33–45.
- von Schlechter R. 1913.** Die Gattung *Xylobium* Lindl. *Orchis* **7**: 21–24.
- Schlechter FRR. 1914.** *Die Orchideen*. Berlin: Verlagsbuchhandlung Paul Parey.
- Senghas K. 1994.** *Cydoniorchis*. *Journal für den Orchideenfreund* **1**: 11–15.
- Senghas K. 1995.** *Xylobium pallidiflorum* (Hook.) Nichols. 1887. *Die Orchidee* **46**: 789–790.
- Servettaz O, Bini Maleci L, Grünanger P. 1994.** Labellum micromorphology in the *Ophrys bertolonii* agg. and some related taxa (Orchidaceae). *Plant Systematics and Evolution* **189**: 123–131.
- Singer RB, Cocucci AA. 1999.** Pollination mechanisms in four sympatric southern Brazilian Epidendroideae orchids. *Lindleyana* **14**: 47–56.
- Singer RB, Koehler S. 2004.** Pollinarium morphology and floral rewards in Brazilian Maxillariinae (Orchidaceae). *Annals of Botany* **93**: 39–51.
- Singer RB, Flach A, Koehler S, Marsaioli AJ, Do Carmo E, Amaral M. 2004.** Sexual mimicry in *Mormolyca ringens* (Lindl.) Schltr. (Orchidaceae: Maxillariinae). *Annals of Botany* **93**: 755–762.
- Singer RB, Marsaioli AJ, Flach A, Reis MG. 2006.** The ecology and chemistry of pollination in Brazilian orchids: recent advances. In: Teixeira da Silva J, ed. *Floriculture, ornamental and plant biotechnology*, Vol. IV. Isleworth, Middlesex: Global Science Books, 569–582.
- Stern WL, Judd WS, Carlswald BS. 2004.** Systematic and comparative anatomy of Maxillarieae (Orchidaceae), sans Oncidiinae. *Botanical Journal of the Linnean Society* **144**: 251–274.
- Stpiczynska M, Davies KL, Gregg A. 2003.** Nectary structure and nectar secretion in *Maxillaria coccinea* (Jacq.) L.O. Williams ex Hodge (Orchidaceae). *Annals of Botany* **93**: 87–95.
- Teuscher H. 1974.** The genus *Xylobium*. *American Orchid Society Bulletin* **43**: 207–212.
- Vogel S. 1979.** Evolutionary shifts from reward to deception in pollen flowers. In: Richards AJ, ed. *The pollination of flowers by insects*. London: Academic Press, 89–96.
- Whitten WM, Williams NH, Chase MW. 2000.** Subtribal and generic relationships of Maxillarieae (Orchidaceae) with emphasis on Stanhopeinae: combined molecular evidence. *American Journal of Botany* **87**: 1842–1856.
- Wolff M. 1990.** *Adipe* Raf., Ein ‘vergessener Name’. *Die Orchidee* **41**: 35–37.