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Comparative floral micromorphology and anatomy of species of *Bulbophyllum* section *Napelli* (Orchidaceae), a Neotropical section widely distributed in forest habitats

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Bulbophyllum section *Napelli*, as recently circumscribed based on molecular analyses, comprises 12 species. These occur as epiphytes in the Brazilian Atlantic Rain Forest and in gallery forests in the cerrado vegetation, and thus differ from other Neotropical sections of *Bulbophyllum*, which are more variable in habit and habitat. To identify diagnostic characteristics that are representative of this section and to confirm whether there are characteristics that are related to their habit and habitat, their floral micromorphology and anatomy were studied using conventional techniques. *Bulbophyllum* section *Napelli* is characterized by sepals with sunken glandular trichomes and stomata on the abaxial surface, reduced petals with one vascular bundle (sometimes lacking), an entire labellum with a secretory sulcus on the adaxial surface and a keel with stomata or glandular trichomes on the abaxial surface, two pollinia, and crystalliferous idioblasts in all floral parts. Our data enable us to distinguish between species and show congruence with the present circumscription of *B.* section *Napelli*. The labellar epidermal surface of three closely-related species was unusual in its possession of unicellular trichomes and these appear to function as osmophores. A relationship between striate surfaces, iridescence and pollinator attraction related to the epiphytic habit and forest habitat is proposed. *Botanical Journal of the Linnean Society*, 2015, ••, ••–••.

ADDITIONAL KEYWORDS: *Bulbophyllum atropurpureum* – *Bulbophyllum campos-portoi* – *Bulbophyllum granulosum* – *Bulbophyllum malachadenia* – *Bulbophyllum regnellii* – cell wall striation – nectary – petal reduction osmophore – secretory epidermis.

INTRODUCTION

The genus *Bulbophyllum* Thouars is represented by 62 species in the Neotropics that were recently allocated to six monophyletic sections, based mostly on molecular data. The sections are *Bulbophyllum* section *Bulbophyllaria* (Rchb.f.) Griseb., *Bulbophyllum* section *Furvescens* E.C.Smidt, Borba & Van den Berg, *Bulbophyllum* section *Napelli* Rchb.f., *Bulbophyllum* section *Micranthae* Barb.Rodr., *Bulbophyllum* section *Didactyle* (Lindl.) Cogn., and *Bulbophyllum* section *Xiphizusa* (Rchb.f.) Cogn. (Smidt *et al*., 2011). Most species occur both as epiphytes and lithophytes in open and wooded areas of the cerrado, Atlantic Rain Forest,

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Andean vegetation, and Central American Forest (Smidt *et al*., 2007, 2011, 2014).

Bulbophyllum section *Napelli* currently comprises 12 species that mostly occur in the Brazilian Atlantic Rain Forest and also in gallery forests in cerrado vegetation. However, one species has a disjunct distribution in Venezuelan forests: *Bulbophyllum dunstervillei* Garay & Dunst. (Smidt *et al*., 2011). This section differs from the other Neotropical sections of *Bulbophyllum* in its almost exclusively epiphytic habit and its forest habitat (Smidt *et al*., 2011, 2014). The flowers of *B*. section *Napelli* are characterized by the presence of a pedicel, the distichous arrangement of the flowers on the inflorescence axis, lateral sepals with attenuate to caudate apices, usually reduced petals, an entire labellum that is usually fleshy and glabrous, a column with teeth, and a foot that exceeds the column in length (Smidt *et al*., 2011, 2014).

There are only a few studies on the floral anatomy of Neotropical orchids. Recently, Nunes *et al*. (2014), when studying floral micromorphology and anatomy of *B*. section *Didactyle* (Lindl.) Cogn., found this approach useful for distinguishing between species. Furthermore, congruence was demonstrated with the proposed circumscription of Smidt *et al*. (2011). Apart from this study, the only report on the floral anatomy of Neotropical *Bulbophyllum* is that of Teixeira, Borba & Semir (2004), who investigated the labellar anatomy of six Brazilian and one Asian species of *Bulbophyllum*. The study included two species belonging to *B*. section *Napelli* that have osmophores on their adaxial labellum surface, although, despite the presence of nectar, no nectariferous tissue was found.

The floral morphology of Neotropical *Bulbophyllum* is quite diverse, and some studies have suggested environmental pressures and pollinator specificity as possible drivers for such variation (Borba & Semir, 1998; Verola, 2002; Teixeira *et al*., 2004). The reproductive biology of two species belonging to *B*. section *Napelli* was studied by Verola (2002), who found that *Bulbophyllum glutinosum* Cogn. is pollinated by female flies of a species of Tachinidae, whereas *Bulbophyllum regnellii* Rchb.f. was not pollinated during the course of the study. The reported pollination mechanism was similar to that of *Bulbophyllum macranthum* Lindl., as described by Ridley (1890); the fly lands on the labellum, which is displaced by the pollinator weight and, as the fly moves towards the base of the labellum, there is a shift in the centre of gravity causing the labellum to return to its usual position, thus causing the fly to come into contact with and to remove the pollinarium.

Therefore, the present study aimed investigate: (1) the micromorphological and anatomical floral features that are common in representatives of *B*. section *Napelli*; (2) the micromorphological and anatomical floral features that can be used to distinguish between the species of this section; and (3) any characteristics that may be related to the habit and habitat of representatives of this section.

MATERIAL AND METHODS

Mature flowers of six species representative of lineages within *B*. section *Napelli* were collected in the field or obtained from the botanical gardens (Table 1). The samples were fixed in FAA 50 (3.7% v/v formaldehyde, 50% ethanol, 5% acetic acid; Johansen, 1940) for 48 h at room temperature or 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer (pH 7.2) (modified from Karnovsky, 1965) for 24 h at 8 °C.

Their micromorphological and anatomical characteristics were assessed using scanning electron (SEM) and light (LM) microscopy. For each species, when-

Table 1. List of studied species and collection/collector data

Species	Collection data
Bulbophyllum atropurpureum Barb.Rodr.	SP 12014 (*V.L. Gil*, *M. Sakane & P. Brólio s.n.*: Santo André, São Paulo State, Brazil).
Bulbophyllum campos-portoi Brade	SP 6027 (*P. Brólio s.n.*: Nova Friburgo, Rio de Janeiro State, Brazil).
	SP 6028 (*P. Brólio s.n.*: Nova Friburgo, Rio de Janeiro State, Brazil).
Bulbophyllum granulosum Barb.Rodr.	UPCB 67312 (*W.S. Mancinelli 1012*: Joinville, Santa Catarina State, Brazil).
Bulbophyllum malachadenia Cogn.	SP 2691 (*P. Brólio s.n.*: Bauru, São Paulo State, Brazil).
	SP 10225 (*M.B. da Silva s.n.*: Ilha do Cardoso, São Paulo State, Brazil).
	UPCB 72468 (*W.S. Mancinelli 1396*: Adrianópolis, Paraná State, Brazil).
Bulbophyllum napelli Lindl.	UPCB 72452 (*W.S. Mancinelli 1367*: Campo Alegre, Santa Catarina State, Brazil).
	E.L.P. Nunes 10: São Miguel Arcanjo, São Paulo State, Brazil.
Bulbophyllum regnellii Rchb.f.	*E.C. Smidt 791*: Caldas, Minas Gerais State, Brazil.

SP, living collection from the State Orchidarium of Instituto de Botânica de São Paulo; UPCB, Herbarium of Universidade Federal do Paraná.

ever possible, at least four flowers from different specimens sourced from different localities were analyzed (Table 1).

The samples for SEM analysis were transferred from 70% ethanol to absolute formaldehyde-dimethyl acetal for dehydration for 16–24 h (Gerstberger & Leins, 1978), subjected to critical-point drying (CPD 030; Bal-Tec AG) and sputter-coated with gold (SCD 050; Bal-Tec AG). SEM observations were made using a Zeiss DSM 950 scanning electron microscope (Carl Zeiss), and images were obtained using Digital Image Processing Software version 2.2 (DIPS-Leipzig, Germany).

For LM analysis, the samples were dehydrated in an ethanol series, embedded in Leica historesin (Leica Microsystems GmbH) or Technovit 7100 (Heraeus Kulzer GmbH) in accordance with the manufacturer's instructions, and sectioned at 5–8 μm on a rotary microtome (RM 2065; Leica Microsystems GmbH) using disposable blades. The sections were stained with 0.05% Toluidine blue O in 0.1 M sodium phosphate buffer (pH 6.8) (O'Brien, Feder & McCully, 1965) for general analysis. Histochemical tests were performed on resin-embedded sections: iodine/potassium iodide solution for starch (Johansen, 1940); ruthenium red solution for pectic acids/mucilage (Johansen, 1940); Coomassie Brilliant Blue R250 for proteins (Southworth, 1973); Calcofluor white M2R for cellulose (Hughes & McCully, 1975); and the periodic acid–Schiff reaction for total insoluble polysaccharides (Feder & O'Brien, 1968).

Flowers fixed in Karnovsky's fixative were washed in 0.1 M phosphate buffer (pH 7.2), hand-sectioned and tested with: 10% (w/v) aqueous solution of ferric chloride for phenolic compounds (Johansen, 1940); Sudan III (O'Brien & McCully, 1981) and neutral red under ultraviolet excitation (Kirk, 1970) for total lipids; acidified phloroglucinol for lignin (Sass, 1951); Ruthenium red solution for pectic acids/mucilage (Johansen, 1940); the periodic acid–Schiff reaction for total insoluble polysaccharides (Feder & O'Brien, 1968); Fehling's reactive (Sass, 1951) for reducing sugars; and Acridine orange for acid polysaccharides (Armstrong, 1956). The appropriate controls were run simultaneously whenever applicable. Entire fresh flowers were immersed in a 1 : 10 000 neutral red aqueous solution (Kearns & Inouye, 1993) for 1 h, in the field, to locate osmophore/nectary.

Photomicrography was achieved using a Leica DMLB microscope coupled with a digital camera using LAS, version 3.3.0 (Leica).

RESULTS

Flowers of the studied species of *B.* section *Napelli* were characterized by the possession of free sepals (Fig. 1A–D, lateral sepals in red and dorsal in pink; some of the sepals were partly or totally removed to improve visualization) with an attenuate to caudate apex; reduced petals (Fig. 1A–J; in purple), except for *B. napelli* Lindl. and *B. regnellii* Rchb.f. (Fig. 1E, F); entire, linguiform glabrous labellum (Fig. 1A–L, in green); a gynostemium with inconspicuous stelidia and short or long teeth (Fig. 1A–L, in orange); long column-foot (Fig. 1G–L, in orange); and short anthers (Fig. 1A–J, L, in yellow). The labellum usually has a fleshy keel on its abaxial surface (Fig. 1G–L).

The ornamented wall of the epidermal cells of the adaxial surface of the dorsal sepal is striate (Fig. 2A), irregular (Fig. 2B) or granular (Fig. 2C). In frontal view, the epidermal cells are transversely elongate (Fig. 2D), longitudinally elongate (Fig. 2A) or irregular (Fig. 2C). In transverse section, the outer periclinal wall is mostly convex (Fig. 2E) but flat in *B. napelli* and *Bulbophyllum atropurpureum* Barb. Rodr. (Fig. 2F). The abaxial surface is longitudinally striate (Fig. 2G) or irregular (Fig. 2H). The cell shape is not clearly visible because it is obscured by the pattern of the cuticle. In transverse section, the outer periclinal wall is flat or slightly convex (Fig. 2E, F). Sunken multicellular glandular trichomes are present abaxially in all of the studied species (Fig. 2G–J, arrows).

The margin of the dorsal sepal is smooth (Fig. 2J), except for that of *B. atropurpureum*, which is papillose (Fig. 2K); in transverse section, the margin is formed both by epidermal and mesophyll cells (Fig. 2L). The mesophyll is homogeneous with regular parenchyma and two types of idioblasts: the first contains with raphides, the other with cellulose helical wall thickenings (Fig. 2E, F, L–O; Table 2). The number of collateral vascular bundles may be one (Fig. 2O), three (Fig. 3A), four (Fig. 3B) or five (Fig. 3C).

Adaxially, the lateral sepals either have striate ornamentation (Fig. 3D) or lack obvious ornamentation (Fig. 3E). In frontal view, the epidermal cells are similar to those of the dorsal sepal. In transverse section, the outer periclinal wall is mostly flat (Fig. 3F). Abaxially, the surface ornamentation is similar to that of the dorsal sepal. The outer periclinal wall in transverse section is mostly convex (Fig. 3F, G). Sunken multicellular glandular trichomes are also present on the abaxial surface of the lateral sepal. The margin, mesophyll and vascular bundles are identical to those of the dorsal sepal, although the number of bundles varies between two (Fig. 3G), three (Fig. 3H), and seven (Fig. 4A).

The petals have a striate wall pattern and irregularly shaped cells occur on both the adaxial and abaxial surfaces (Fig. 4B, C). The outer periclinal walls are usually convex in transverse section (Fig. 4D, E). The margin is usually papillose (Fig. 4F)

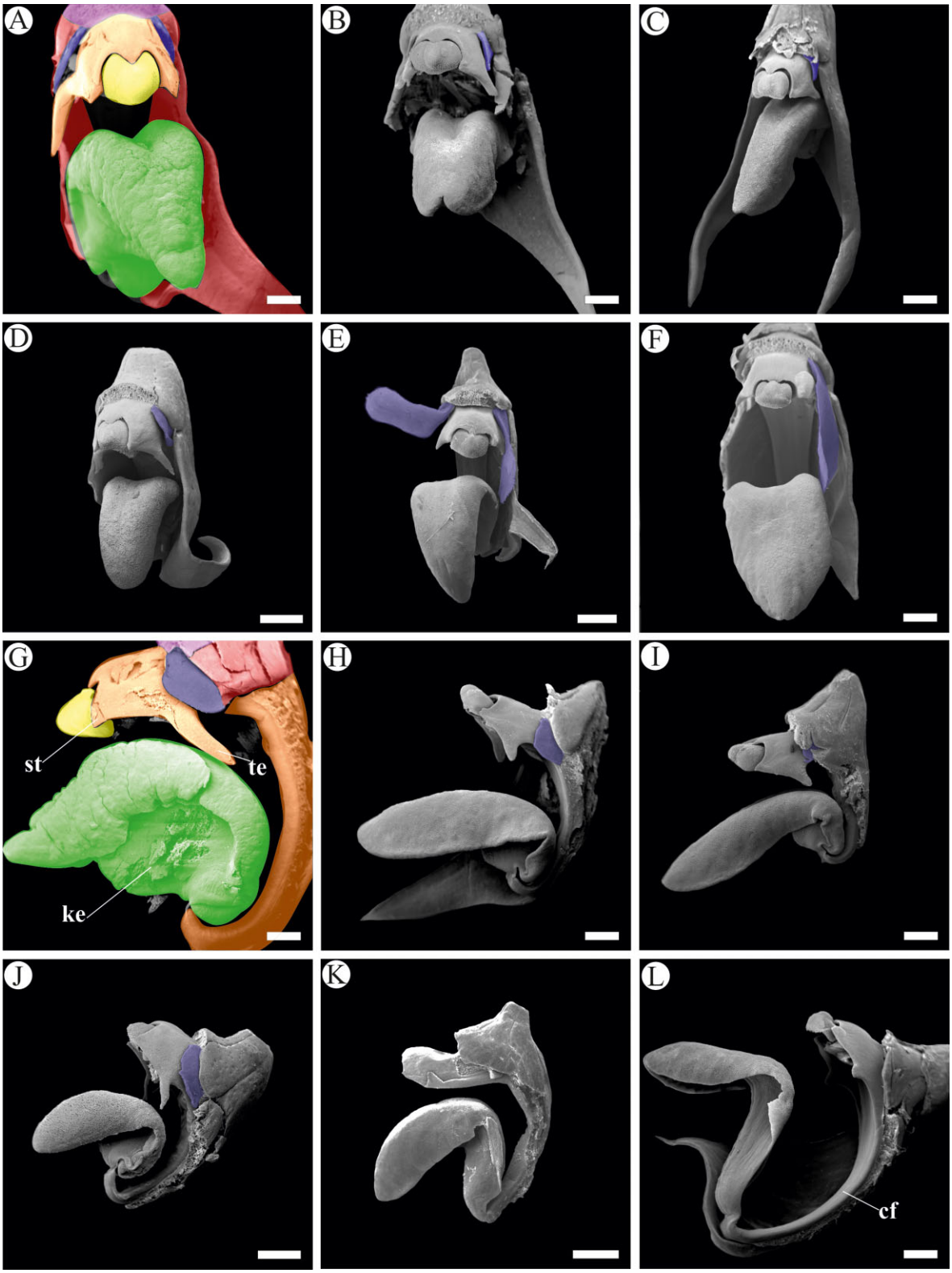

Figure 1. General floral morphology of the studied species of *Bulbophyllum* section *Napelli* in frontal (A–F) and lateral view (G–L). A, G, *Bulbophyllum malachadenia*. B, H, *Bulbophyllum campos-portoi*. C, I, *Bulbophyllum atropurpureum*. D, J, *Bulbophyllum granulosum*. E, K, *Bulbophyllum regnellii*. F, L, *Bulbophyllum napelli*. cf, column-foot; ke, keel; st, stelidia; te, teeth. Scale bars = 1 mm.

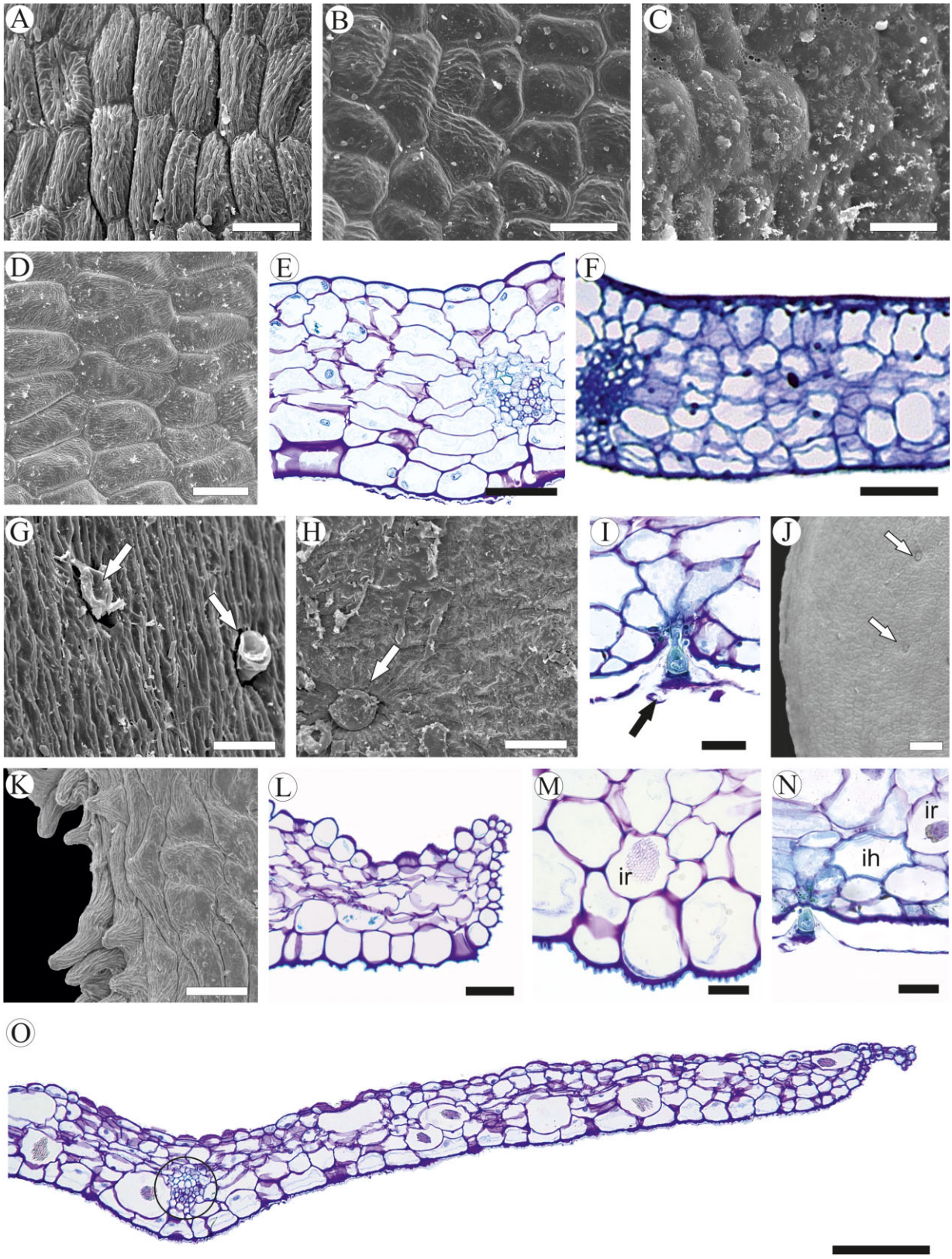

Figure 2. See caption on next page.

Figure 2. Micromorphology and anatomy of the dorsal sepal of *Bulbophyllum* section *Napelli*. A–D, G, H, J, K, scanning electron microscope photomicrographs. E, F, I, L–N, transverse sections. A, adaxial epidermis with striate ornamentation and longitudinally elongate cells in *Bulbophyllum malachadenia*. B, adaxial epidermis with irregular ornamentation and irregular-shaped cells, *Bulbophyllum atropurpureum*. C, adaxial epidermis with granulose ornamentation and irregular-shaped cells, *Bulbophyllum campo-portoi*. D, adaxial epidermis with striate ornamentation and transversely elongate cells, *Bulbophyllum regnellii*. E, transverse section (TS) of a sepal showing epidermal cells with convex adaxial outer periclinal walls and with the abaxial ones slightly convex, *B. campos-portoi*. F, TS of a sepal showing epidermal cells with flat adaxial and abaxial outer periclinal walls, *B. atropurpureum*. G, abaxial epidermis with longitudinally striate ornamentation and sunken glandular trichomes (arrows), *B. malachadenia*. H, abaxial epidermis with irregular ornamentation and sunken glandular trichomes (arrows) *B. atropurpureum*. I, TS of a sepal, featuring a sunken glandular trichome with the head disrupted. J, detail of the smooth margin of the dorsal sepal of *Bulbophyllum granulosum*. K, detail of the papillose margin of the dorsal sepal of *B. atropurpureum*. L, TS of the margin of the dorsal sepal composed of both mesophyll and epidermal cells. M, TS of a sepal showing idioblasts with raphides within the mesophyll and abaxial epidermal cells with ornamented outer periclinal walls, *B. regnellii*. N, TS of a sepal showing idioblasts with raphides and idioblasts with cellulose helical wall thickenings within the mesophyll, *B. malachadenia*. O, TS of a sepal showing a homogeneous mesophyll and featuring a single collateral vascular bundle, *B. granulosum*. ir, idioblast with raphides; ih, idioblast with helical wall thickenings. The circles indicate the vascular bundles. Scale bars = 40 μm in (A–D), (G–I), and (K); 200 μm in (E), (F), and (J); 50 μm in (L); 20 μm in (M); 100 μm in (N).

Table 2. List of the histochemical tests performed on species of *Bulbophyllum* section *Napelli* along with common results and reaction sites

Reagent	Reaction	Location
Acidified phloroglucinol	Lignin stains red	Secondary walls of xylem elements
Acridine orange under ultraviolet (UV) light	Double-stranded DNA fluoresces green; single-stranded DNA, RNA and polysaccharides fluoresce orange to red	Cytoplasm of the secretory epithelium of the labellum (sulcus)
Calcofluor white M2R	Cellulose cell walls fluoresce bright blue	Idioblasts with helical wall thickenings; epidermis striate wall
Coomassie brilliant blue	Proteins stains blue	Cytoplasm of the secretory epithelium of the labellum (sulcus)
Fehling's reactive	When reducing sugars are present, an orange to red precipitate is formed	Cytoplasm of the secretory epithelium of the labellum (sulcus) – inconclusive
Ferric chloride	Phenolic compounds stain dark grey or blue–black	Sepal glandular trichomes – faint
Iodine/potassium iodide solution	Starch stains blue-black	Labellar mesophyll starch; cytoplasm of the secretory epithelium of the labellum (sulcus)
Neutral red *in vivo*	Lipids/volatile compounds stain red	Sepal glandular trichomes; adaxial surface of the labellum
Neutral red under UV light	Lipids fluoresce greenish blue	Callus papillae; adaxial surface of the labellum; cuticle
Periodic acid–Schiff	Polysaccharides stain purplish red	Cell walls; labellar mesophyll starch; cytoplasm of the secretory epithelium of the labellum (sulcus)
Ruthenium red	Pectic acids stain pink to red.	Sepal glandular trichomes – faint
Sudan III	Lipids stain orange to red	Labellum – small drops in the mesophyll cells; cuticle

and formed of epidermal and mesophyll cells in transverse section (Fig. 4D, G). In transverse section, the mesophyll is homogeneous, comprising regular parenchyma and idioblasts containing raphides (Fig. 4D, E, G). Furthermore, idioblasts with cellulose helical wall thickenings may be present or absent. A single collateral vascular bundle is present (Fig. 4D, E), except for *B. granulosum* Barb. Rodr. and *B. atropurpureum* (Fig. 4G), which have reduced and nonvascularized petals.

Figure 3. Anatomy and micromorphology of the sepals of *Bulbophyllum* section *Napelli*. A–C, F–H, transverse sections. A, E, scanning electron microscope photomicrographs. A, transverse section (TS) of a sepal showing a homogeneous mesophyll and featuring three collateral vascular bundles, *Bulbophyllum campos-portoi*. B, TS of a sepal showing a homogeneous mesophyll and four collateral vascular bundles, *Bulbophyllum napelli*. C, TS of a sepal showing a homogeneous mesophyll and featuring five collateral vascular bundles, *Bulbophyllum malachadenia*. D, adaxial epidermis with striate ornamentation and irregular-shaped cells, *Bulbophyllum granulosum*. E, adaxial epidermis without conspicuous ornamentation and irregular-shaped cells, *B. campos-portoi*. F, TS of a lateral sepal showing epidermal cells with flat adaxial outer periclinal walls and with the abaxial ones slightly convex, *B. malachadenia*. G, TS of a lateral sepal showing epidermal cells with convex abaxial outer periclinal walls and featuring two collateral vascular bundles, *B. granulosum*. H, TS of a lateral sepal featuring three collateral vascular bundles, *B. campos-portoi.* ir, idioblast with raphides; ih, idioblast with helical wall thickenings. The circles indicate the vascular bundles. Scale bars = 500 μm in (A–C) and (H); 40 μm in (D) and (E); 200 μm in (F–G).

Distally and in the mid third, the labellar surface is adaxially striate (Fig. 4H). The epidermal cells are isodiametric but vary in shape and arrangement according to species. In *B. granulosum* (Fig. 4H, I), *B. atropurpureum* (Fig. 4J), and *Bulbophyllum campos-portoi* Brade (Fig. 5A), the epidermis has an extra layer of trichomes in which cells become connected by 'bridges', resulting in the formation of an extensive system of intercellular spaces. In *B. atropurpureum* (Fig. 4J) and *B. campos-portoi* (Fig. 5A), the linking of such trichomes produces a reticulate pattern. In *Bulbophyllum malachadenia* Cogn., *B. napelli*, and *B. regnellii*, the epidermal cells are papillose (Fig. 5B) and, in transverse section, display a pattern intermediary between that of *B. campos-portoi/B. atropurpureum* and *B. granulosum* (Fig. 5C).

Distally and in the mid third of the labellum, the abaxial surface is also striate (Fig. 5D), except for *B. napelli*, in which it is inconspicuous (Fig. 5E). In frontal view, the epidermal cells are either longitudinally elongate (Fig. 5F) or irregularly shaped (Fig. 5D, E). The outer periclinal walls are usually convex, although the epidermis may consist of papillose and nonpapillose cells in combination (Fig. 5D–I). Here, stomata were observed in some species (Fig. 5D, E, arrows). The mesophyll is homogeneous, with regular parenchyma and idioblasts containing raphides (Fig. 5G–I). The vascular bundles of the mid third are collateral and vary between five (Fig. 5G), seven (Fig. 5H), and nine (Fig. 5I) in number.

The adaxial surface of the proximal third of the labellum possesses similar epidermal cells and surfaces to those of the other floral parts. However, this region shows a well-developed, secretory sulcus (Figs 1A–F, 5J, K). A small quantity of secretion was observed on the sulcus in at least three species in the field (*B. campos-portoi*, *B. atropurpureum*, and *B. malachadenia*). The sulcus is mostly shallow (Fig. 5J), except for that in *B. malachadenia* (Figs 5K, 6A). In frontal view, the epidermal cells of the sulcus were either isodiametric (Fig. 6A) or irregularly shaped (Fig. 6B); the outer periclinal wall of the epidermal cells being mostly convex (Fig. 6A) but papillose in *B. campos-portoi* (Fig. 6C).

The abaxial surface of the proximal third of the labellum bears a conspicuous keel (Fig. 1G–K), except for *B. napelli*, in which it is inconspicuous (Fig. 1L). In frontal view, the epidermal cells are longitudinally elongate (Fig. 6D), isodiametric or irregularly shaped (Fig. 6E); stomata are present in all species (Fig. 6D, E); multicellular glandular trichomes are present in *B. campos-portoi* and *B. atropurpureum* (Fig. 6E, F). The mesophyll and vascular bundles are similar to those described for the other parts of the labellum, although the bundles vary in number between five (Fig. 5J, K) and seven (Fig. 6G).

The gynostemium in frontal view is covered by longitudinally elongate cells, mostly with striate ornamentation (Fig. 6H), except for *B. malachadenia*, in which they are smooth (Fig. 6I). The mesophyll is homogeneous with regular parenchyma and idioblasts containing raphides or cellulose helical wall thickenings (Fig. 6J). Cells of the column-foot have striate ornamentation and are longitudinally elongate (Fig. 6K, L). These cells have convex outer periclinal walls (Fig. 6K) but, in *B. atropurpureum* (Fig. 6L), they are papillose. The mesophyll is similar to that of the gynostemium (Fig. 6J).

The anther comprises isodiametric, papillose epidermal cells with striate ornamentation (Fig. 7A). The pollinarium is composed of only two pollinia (Fig. 7B, C). In *B. atropurpureum*, two extra, vestigial pollinia may occur (Fig. 7D). In addition to pollinia, the pollinarium comprises a viscidium formed by the degradation of part of the rostellum (Figs 6J, 7E, F).

The stigma is wide (Fig. 7F), except in *B. malachadenia*, where it is longitudinally elongate (Fig. 7E); the stigmatic cavity contains elongate cells and a mucilaginous matrix (Figs 6J, 7E, F, K). The ovary is a more complex six-lobed structure, the three outermost lobes of which are derived from the sepals (Fig. 7G). In transverse section, the outer epidermis of the ovary is seen to consist of columnar cells, each having a convex outer periclinal wall (Fig. 7G, H); the

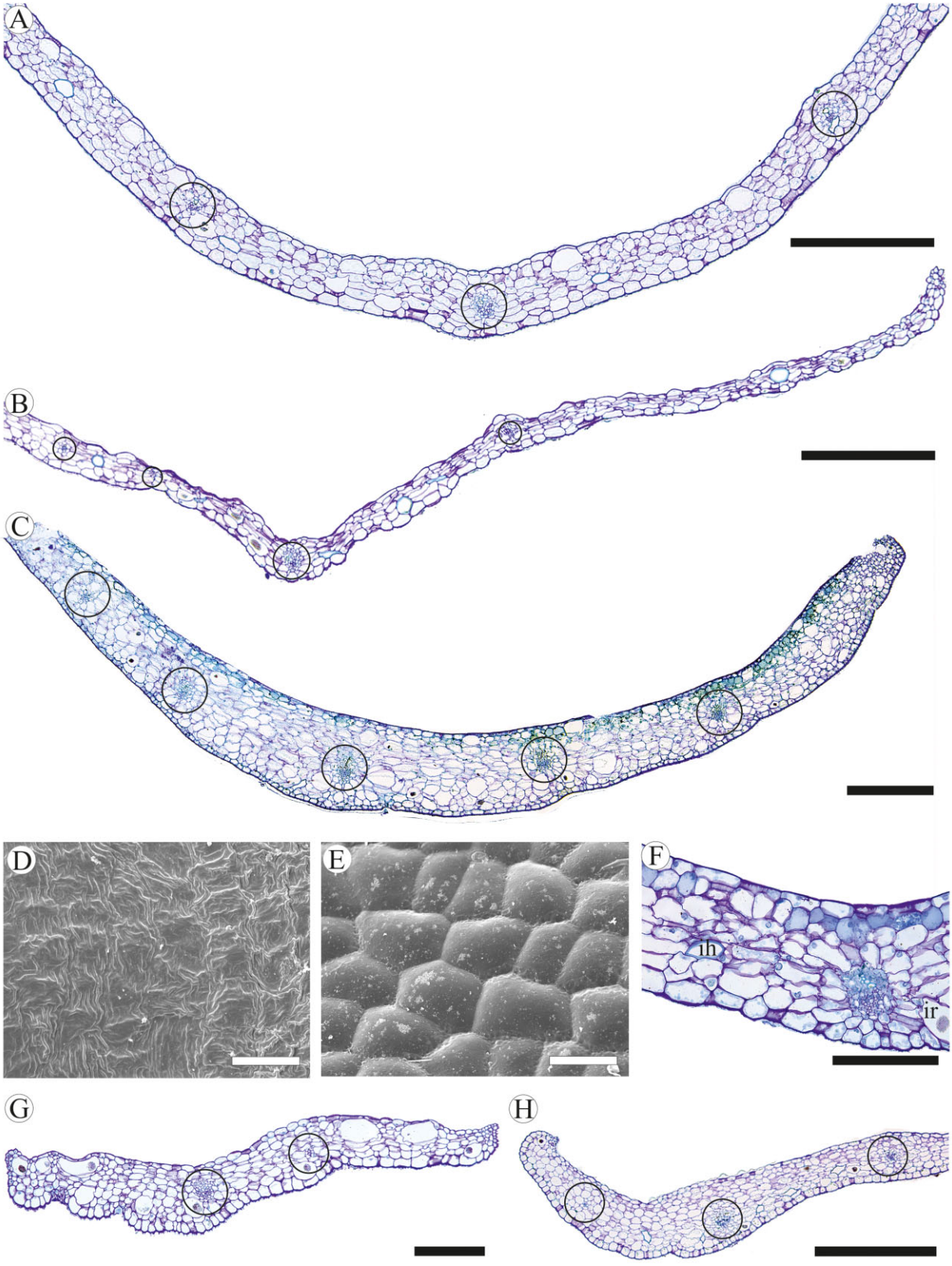

Figure 3. See caption on previous page.

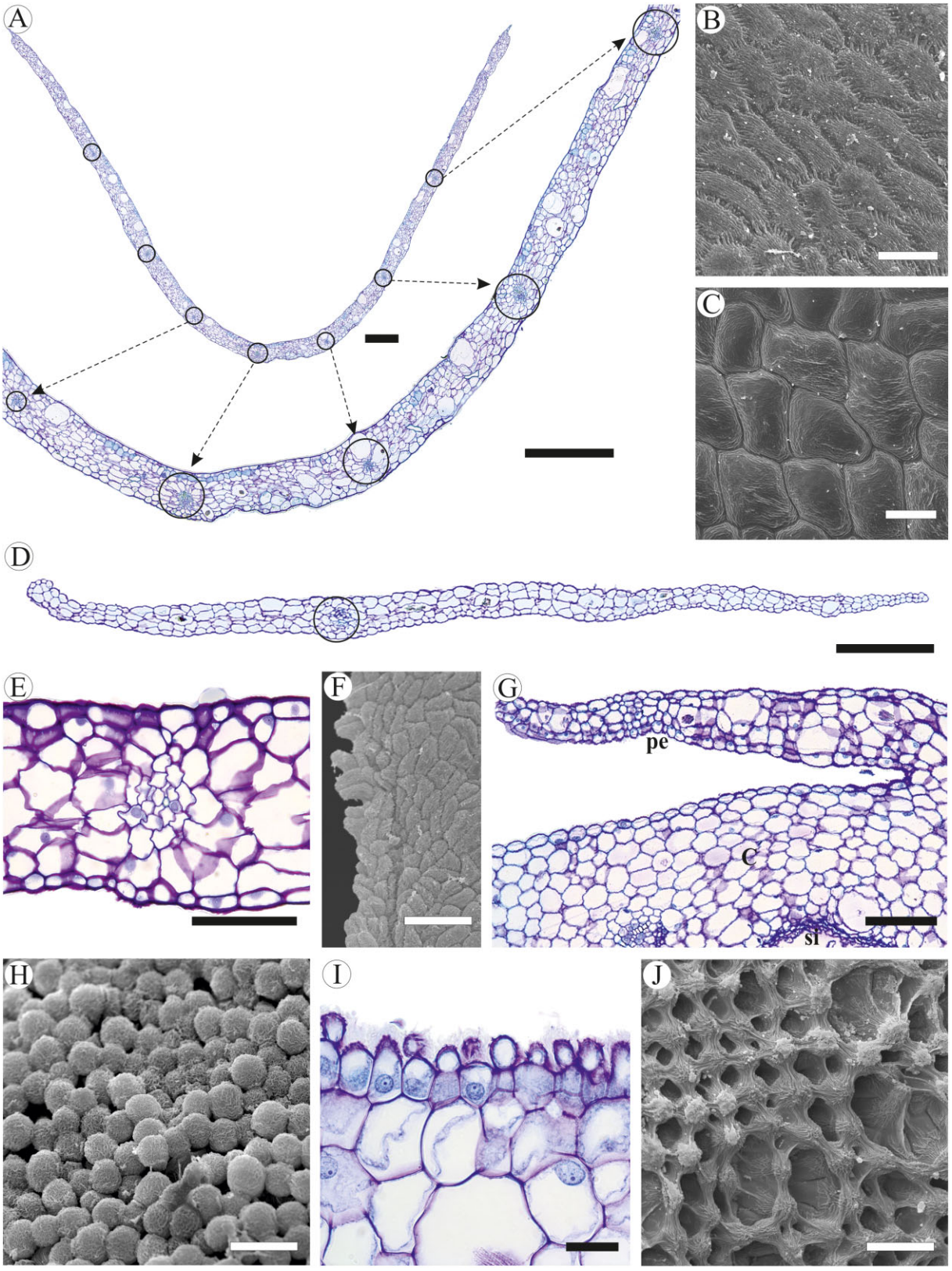

Figure 4. See caption on next page.

Figure 4. Anatomy and micromorphology of the lateral sepal, petal and mid third of the labellum of *Bulbophyllum* section *Napelli*. A, lateral sepal. B–G, petal. H–J, labellum. B, C, F, H, J, scanning electron microscope photomicrographs. A, E, G, I, transverse sections. A, transverse section (TS) of a lateral sepal with homogeneous mesophyll and featuring seven collateral vascular bundles, *Bulbophyllum malachadenia.* B, adaxial epidermis with striate ornamentation and irregular-shaped cells, *B. malachadenia.* C, abaxial epidermis with striate ornamentation and irregular-shaped cells, *Bulbophyllum granulosum.* D, TS of a petal with homogeneous mesophyll, epidermal cells with convex outer periclinal walls, and a single vascular bundle, *Bulbophyllum regnellii*. E, detail of a TS of a petal showing a collateral vascular bundle and epidermal cells with convex outer periclinal walls, *Bulbophyllum campos-portoi*. F, detail of the papillose margin of the petal of *B. granulosum*. G, TS of the nonvascularized petal where it fuses with the gynostemium, *B. granulosum*. H, adaxial view of the labellum showing unicellular trichomes with striate surface, *B. granulosum*. I, detail of a TS of the labellum showing the adaxial epidermal cells and unicellular trichomes. J, adaxial view of the labellum showing unicellular trichomes connected by 'bridges', *Bulbophyllum atropurpureum*. C, column; pe, petal; si, stigma. The circles indicate the vascular bundles. Scale bars = 500 μm in (A) and (D); 40 μm in (B), (C, (F), (H) and (J); 100 μm in (E) and (K); 200 μm in (G).

inner epidermis lining the loculus is composed of elliptic cells with convex outer periclinal walls (Fig. 7H). The ovary mesophyll is homogeneous with regular parenchyma and idioblasts either containing raphides or idioblasts with cellulose helical wall thickenings (Figs 6J, 7G, H); there is a single collateral vascular bundle per lobe (Fig. 7G, H).

Iridescence was observed on the floral surfaces of fresh flowers of *B. campos-portoi* and *B. malachadenia* during dissection under a stereomicroscope; in *B. atropurpureum* and *B. granulosum*, iridescence was also observed but only after critical-point drying. In none of the aforementioned cases did digital photography provide adequate documentation. A survey of the most significant floral characteristics and their variation between species is provided in Table 3.

DISCUSSION

The present study describes several floral micromorphological and anatomical characteristics that are shared by most of the studied species of *B.* section *Napelli*. The distinguishing features are the presence of multicellular glandular trichomes and stomata on the abaxial surface of the sepals, reduced petals with papillose margins, and a single collateral vascular bundle that is sometimes lacking, an entire labellum with a striate surface, epidermis with unicellular trichomes or papillae, a secretory sulcus adaxially and abaxial keel, two pollinia with smooth tetrads, and mesophyll with both crystalliferous and thickened idioblasts in all of the floral parts.

Our findings better characterize *B*. section *Napelli* as grouped according to Smidt *et al*. (2011), even though we have studied only six species, and Smidt *et al*. (2011) used molecular markers to study nine species. Prior to these studies, the most recent treatment of the group was that of Pabst & Dungs (1975), in which the species that are currently assigned to *B.* section *Napelli* were scattered among informal alliances: *B*. section *Bulbophyllaria – Bulbophyllum micropetaliforme* alliance, *B*. section *Didactyle – B. glutinosum* alliance, and *B*. section *Micrantha – B. micranthum* alliance. In addition, we found 25 characteristics for which states vary between species, and these are thus useful for distinguishing between taxa (Table 3).

A feature of four of the studied species (*B. malachadenia*, *B. campos-portoi*, *B. atropurpureum*, and *B. granulosum*) is petal reduction resulting in lack of vascular bundles in the petals of two species of these species, namely *B. atropurpureum* and *B. granulosum*. Such reduction has been reported for most species of this section, with the exception of *B. regnellii* and *B. napelli* (Smidt *et al*., 2011). Showy petals are part of the visual features causing effective pollination, and specific elaborations of petals are often triggered by the type of pollinators that evolved with the flowers (Ronse de Craene, 2010); this applies in particular to the labellum in Orchidaceae. However, reduction of the other two petals is not uncommon in Orchidaceae and this is reported for other *Bulbophyllum* spp. (Vermeulen, 1982), as well as several genera of Pleurothallidinae (Karremans *et al*., 2013; Kolanowska, 2013), being associated with the dominant petaloidy of the sepals in *Stelis* Sw.and other allied genera (Karremans *et al*., 2013). Because the petals of species of *Bulbophyllum* section *Napelli* are mostly reduced, the floral display is composed mainly of sepals and possibly the labellum. The sepal, petal, and labellum of most of the studied species have epidermal cells with ornamented outer walls and, often, some degree of striation, as shown by SEM of transverse sections.

Although such striation patterns are usually considered to be the result of cuticle deposition process and growth pressures generated by the cell wall (Kourounioti *et al*., 2013), in *B*. section *Napelli*, we

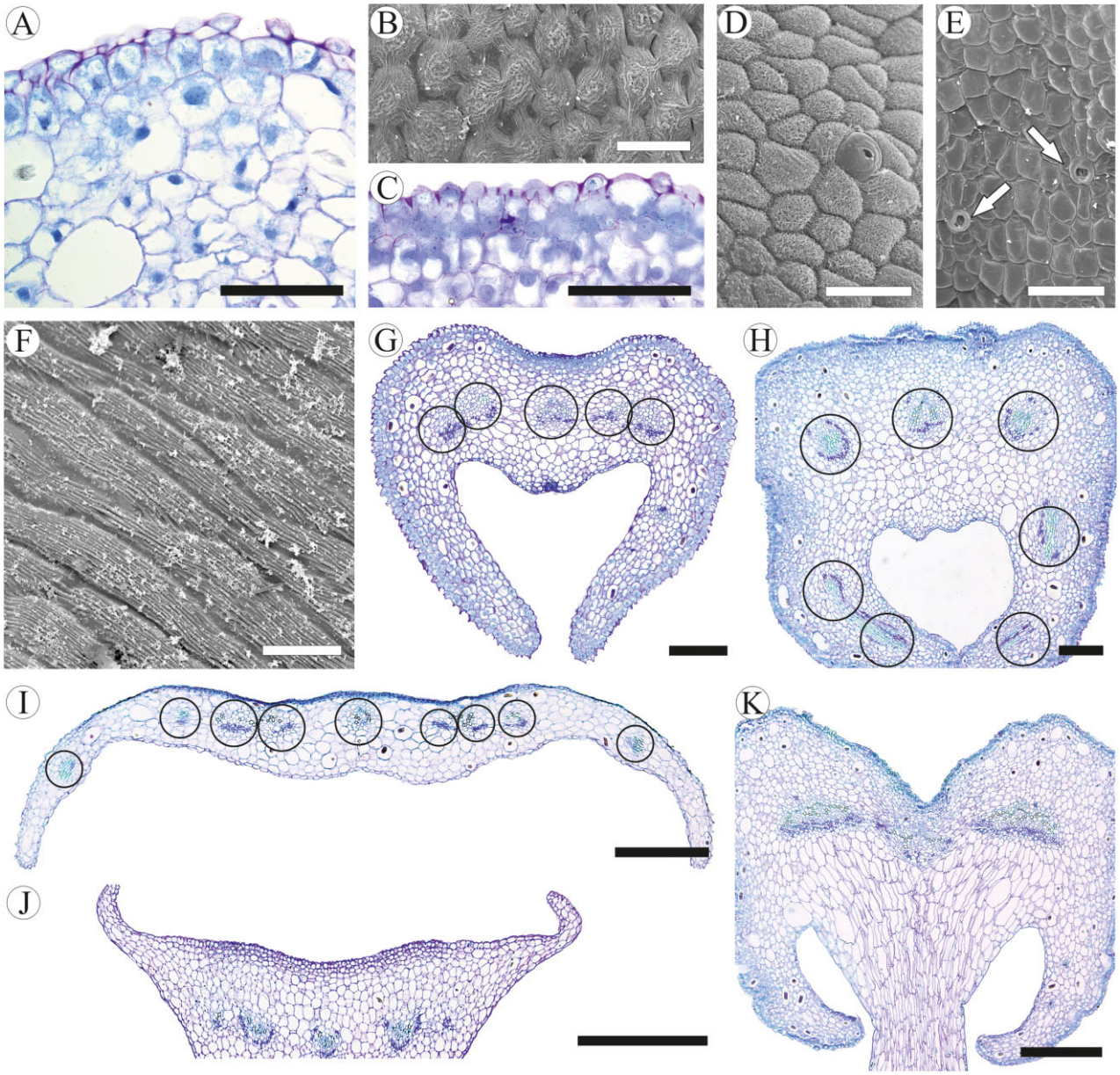

Figure 5. Anatomy and micromorphology of the labellum of *Bulbophyllum* section *Napelli*. A–I, mid third. J, K, proximal third. A, C, G–K, transverse sections. B, D, E, F, scanning electron microscope photomicrographs. A, detail of a transverse section (TS) of the labellum showing unicellular trichomes connected by 'bridges', *Bulbophyllum campos-portoi*. B, adaxial view of the labellum showing epidermal cells apparently papillose, *Bulbophyllum malachadenia*. C, detail of a TS of the labellum of *B. malachadenia* with epidermal cells with different sizes and heights, *B. malachadenia.* D, abaxial view of the labellar middle third with striate epidermal cells and stomata (arrows), *Bulbophyllum atropurpureum*. E, abaxial view of the labellar middle third without conspicuous ornamentation, irregular-shaped epidermal cells, and stomata (arrows), *Bulbophyllum napelli*. F, abaxial view of the labellar middle third with striate ornamentation and longitudinally elongate epidermal cells, *B. malachadenia.* G, TS of the labellar middle third with homogeneous mesophyll and five collateral vascular bundles, *B. atropurpureum*. H, TS of the labellar middle third with homogeneous mesophyll and seven collateral vascular bundles, *B. malachadenia*. I, TS of the labellar middle third with homogeneous mesophyll and seven collateral vascular bundles, *B. napelli.* J, TS of the labellar proximal third showing an adaxial secretory sulcus, a homogeneous mesophyll and five collateral vascular bundles, *Bulbophyllum regnellii*. K, TS of the labellar proximal third showing a deep adaxial secretory sulcus, a homogeneous mesophyll and five collateral vascular bundles, *B. malachadenia.* The circles indicate the vascular bundles. Scale bars = 100 μm in (A) and (E); 40 μm in (B–D) and (F); 500 μm in (G) and (I–K); 250 μm in (H).

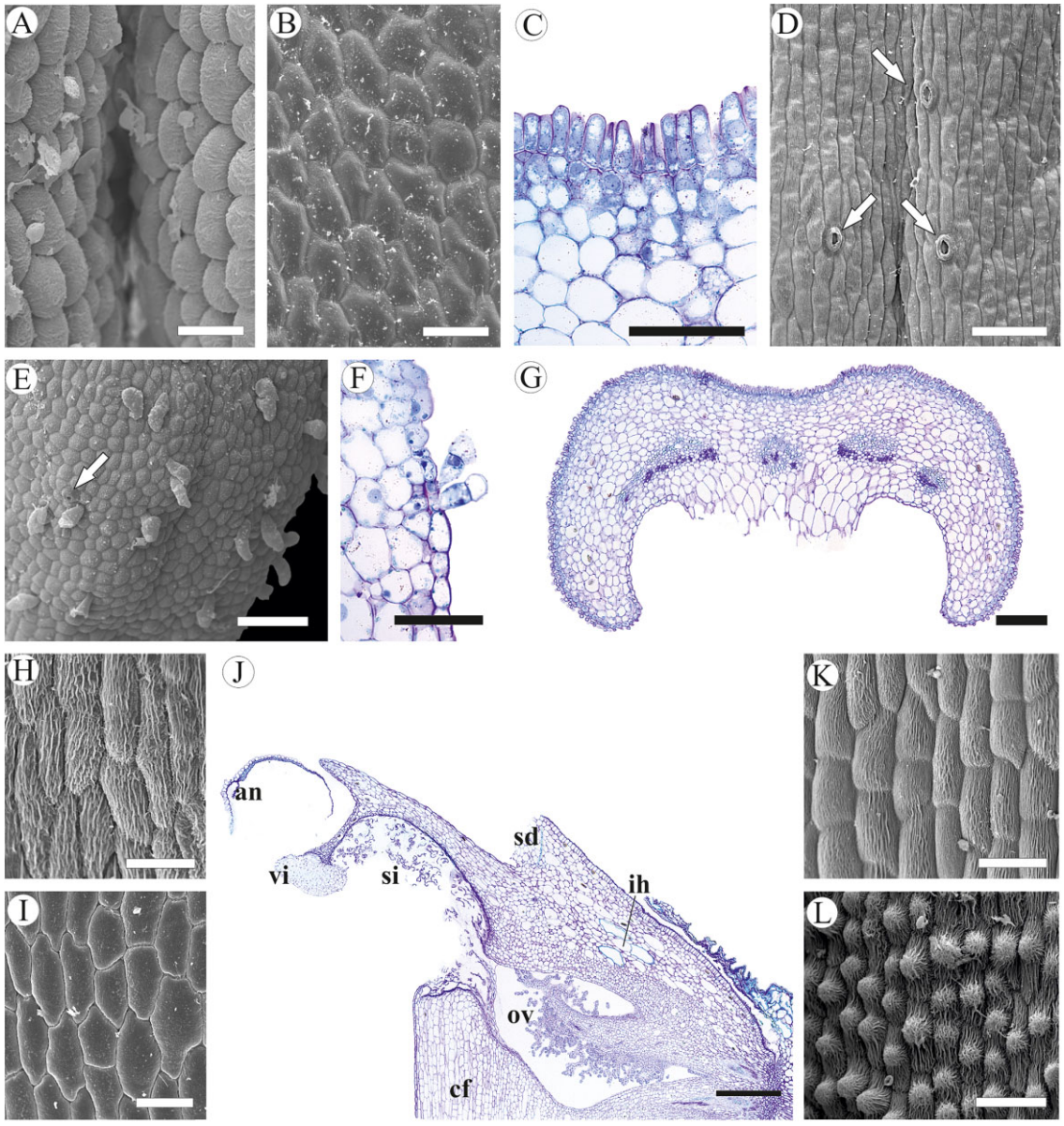

Figure 6. Micromorphology and anatomy of the labellum and column of *Bulbophyllum* section *Napelli*. A, B, D, E, H, I, K, L, scanning electron microscope photomicrographs. C, F, G, transverse sections. J, longitudinal section. A, detail of the adaxial secretory sulcus with isodiametric epidermal cells, *Bulbophyllum malachadenia.* B, detail of the adaxial secretory sulcus with irregular-shaped epidermal cells, *Bulbophyllum napelli.* C, transverse section (TS) of the secretory sulcus showing papillose epidermal cells, *Bulbophyllum campos-portoi*. D, detail of the abaxial keel showing longitudinally elongate epidermal cells and stomata (arrows), *B. napelli*. E, detail of the abaxial keel showing mostly irregular-shaped epidermal cells, stomata (arrow) and multicellular glandular trichomes, *B. campos-portoi*. F, TS of the abaxial keel showing two multicellular glandular trichomes, *Bulbophyllum atropurpureum*. G, TS of the labellar proximal third showing an adaxial secretory sulcus, homogeneous mesophyll and seven collateral vascular bundles, *Bulbophyllum granulosum*. H, gynostemium surface showing longitudinally elongate epidermal cells with striate ornamentation, *Bulbophyllum regnellii*. I, gynostemium surface showing longitudinally elongate epidermal cells without conspicuous ornamentation, *B. malachadenia*. J, LS of the gynostemium composed of regular parenchyma with both idioblasts with raphides and with cellulose helical wall thickenings, *B. napelli*. K, ventral view of the column-foot showing longitudinally elongate epidermal cells with striate ornamentation, *B. regnellii*. L, ventral view of the column-foot showing longitudinally elongate papillose epidermal cells with striate ornamentation, *B. atropurpureum.* an, anther; cf, column-foot; ih, idioblast with helical wall thickenings; ov, ovary; sd, dorsal sepal; si, stigma; vi, viscidium. Scale bars = 40 μm in (A), (B), (H), (I), (K), and (L); 100 μm in (C–F); 200 μm in (G); 500 μm in (J).

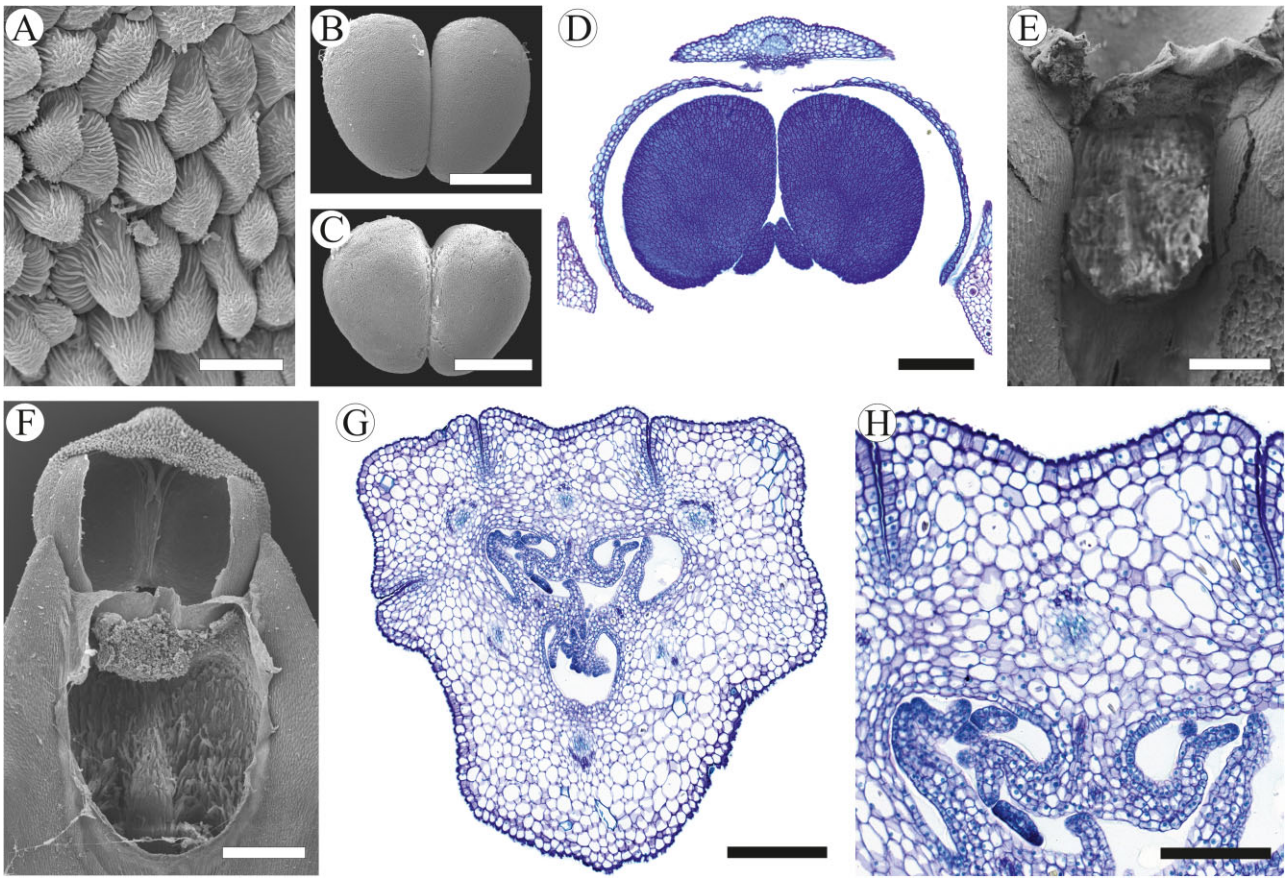

Figure 7. Micromorphology of anther and stigma and anatomy of pollinia and ovary of *Bulbophyllum* section *Napelli*. A–C, E, F, scanning electron microscope photomicrographs. D, G, H, transverse sections. A, detail of papillose epidermal cells of the anther with striate ornamentation, *Bulbophyllum granulosum*. B, dorsal view of the pollinia of *Bulbophyllum atropurpureum*. C, ventral view of the pollinia of *B. campos-portoi*. D, transverse section (TS) of an anther showing four pollinia, *B. atropurpureum.* E, detail of the narrow stigma of *Bulbophyllum malachadenia*. F, detail of the wide stigma of *B. campos-portoi.* G, TS of the ovary with homogeneous mesophyll and one vascular bundle per lobe, *B. granulosum*. H, detail of a TS of the ovary showing the outer epidermis composed of columnar cells and the inner one composed of elliptic cells, *B. granulosum*. Scale bars: 40 μm in (A); 400 μm in (B) and (C); 500 μm in (D–G); 200 μm in (H).

have demonstrated that these patterns are primarily determined by the cell wall itself. Furthermore, we consider that those striation patterns may function as diffraction gratings. Such structures create iridescence by changing the perceived hue of a surface relative to the angle of observation in at least in ten angiosperm families, and may act as cues for pollinators, as shown by experiments with bees (Whitney *et al*., 2009). Sometimes, iridescence is visible to human eyes only when the pigment colour is separated from the petal structure (Whitney *et al*., 2009) and, in the case of species of *B*. section *Napelli*, iridescence was observed when preparing samples for SEM after critical-point drying and consequent removal of pigments. In addition to the presence of striation on the flower surface, convex or papillose epidermal cells and idioblasts containing raphides were common in the studied species. These cells are considered to act as lenses that focus light into pigment-containing vacuoles, thus enhancing colour saturation (Kay, Daoud & Stirton, 1981; Gorton & Vogelmann, 1996; Whitney *et al*., 2011), and the crystals may have a light-gathering and reflecting function (Franceschi & Horner, 1980; Franceschi, 2001). Therefore, it is reasonable to suggest that these cells are perhaps, with the striate floral surfaces, involved in pollinator attraction in *B*. section *Napelli*; this is probably related to an epiphytic mode of life in forest habitats. Further evidence is provided by *B*. section *Didactyle* (Nunes *et al*., 2014) and *B*. section *Micranthae* (E.L.P Nunes, P.M. Rabelo, E.C. Smidt, T. Stützel and A.I. Coan, unpublished data), which show more variability both in terms of plant habit and habitat. Although iridescence has been correlated to

Table 3. List of the variable characteristics between species of *Bulbophyllum* section *Napelli*

Floral part	Character		*Bulbophyllum malachadenia*	*Bulbophyllum napelli*	*Bulbophyllum campos-portoi*	*Bulbophyllum granulosum*	*Bulbophyllum atropurpureum*	*Bulbophyllum regnellii*
Dorsal Sepal	Ad	Surface ornamentation on pw	**Longitudinally striate**	Irregular	Granular	Striate	Irregular	Striate
		Cell shape front view	**Longitudinally elongate**	Transversely elongate	Irregular	Transv!Ersely elongate	Irregular	Transversely elongate
	Ab	Surface ornamentation on pw	**Longitudinally striate**	Striate	Irregular	Irregular	Irregular	Irregular
	Margin	Margin cell type	Smooth	Smooth	Smooth	Smooth	**Papillose**	Smooth
	Mesophyll	Vascular Bundles (middle third)	**5**	**4**	3	**1**	3	3
Lateral Sepal	Ad	Surface ornamentation on pw	Smooth	Smooth	Smooth	Striate	Smooth	Striate
		Cell shape front view	**Transversely elongate**	Irregular	Irregular	Irregular	Irregular	**Longitudinally elongate**
	Mesophyll	Vascular Bundles (middle third)	**7**	3	3	2	3	2
Petal	Ad	Surface ornamentation on aw	Present	Absent	Present	Present	Present	Present
		External periclinal wall shape	Convex	**Flat**	Convex	Convex	Convex	Convex
	Ab	External periclinal wall shape	Convex	**Flat**	Convex	Convex	Convex	Convex
	Mesophyll	Vascular Bundles	1	1	1	0	0	1
Labellum: mid third	Ab	Surface ornamentation on pw	Striate	**Smooth**	Striate	Striate	Striate	Striate
		Surface ornamentation on aw	Present	Absent	Present	Present	Present	Present
	Mesophyll	Vascular Bundles	7	**9**	5	7	5	5
Labellum: proximal third	Ad	Secretory sulcus shape	**Deep**	Shallow	Shallow	Shallow	Shallow	Shallow
		Secretory sulcus periclinal wall	Convex	Convex	**Papillose**	Convex	Convex	Convex
	Ab	Surface ornamentation on pw	Striate	**Smooth**	Striate	Striate	Striate	Striate
		Surface ornamentation on aw	Present	Absent	Present	Present	Present	Present
		Keel	**Very conspicuous**	**Inconspicuous**	Conspicuous	Conspicuous	Conspicuous	Conspicuous
Gynostemium	Epidermis	Surface ornamentation on pw	**Smooth**	Striate	Striate	Striate	Striate	Striate
	Teeth	Size	**Elongate**	Short	Short	Short	Short	**Inconspicuous**
Column Foot	Epidermis	External periclinal wall shape	Convex	Convex	Convex	Convex	**Papillose**	Convex
Anther	Epidermis	External periclinal wall shape	**Convex**	Papillose	Papillose	Papillose	Papillose	Papillose
Carpel	Stigma	shape	**Longitudinally elongate**	Transversely elongate	Transversely elongate	Transversely elongate	Transversely elongate	Transversely elongate

States of a characteristic that are exclusive to a single species are shown in bold. Ad, adaxial surface; Ab, abaxial surface; aw, anticlinal walls; pw, periclinal walls.

the leaves of plants associated with shaded habitats (Graham, Lee & Norstog, 1993; Feild, Franks & Sage, 2003), to our knowledge, no information is available for flowers and how iridescence relates to fly pollination.

Two of the species that were investigated in the present study do not exhibit petal reduction (or perhaps show a secondary increase in size): *B. napelli* and *B. regnellii*. Although Verola (2002) reported that *B. regnellii* emitted a sweet cinnamon scent and was visited only by a species of Halictidae (Hymenoptera), pollination did not occur; *B. glutinosum* (closely related to *B. campos-portoi* investigated in the present study) emitted an odour resembling decomposing plant matter and was pollinated by female Tachinidae flies via a mechanism that is similar to that described by Ridley (1890) for the Asian *B. macranthum* Lindl. Considering the different morphology present in this pair of species [reduced petals and prominent column teeth in *B. glutinosum* (closely related to the *B. campos-portoi* investigated in the present study) and enlarged petals and reduced teeth in *B. regnellii*], we might hypothesize a trade-off in the function of petals and column teeth in *B. regnellii* and *B. napelli*. This is most likely related to a change in pollinator and the mechanical support necessary for the flower to accommodate the movement of the pollinator within it and to facilitate pollinarium removal by the insect.

In three of the studied species (*B. campos-portoi*, *B. atropurpureum*, and *B. granulosum*), the labellar epidermis possesses a layer in which the cells can appear joined, resulting in the formation of an extensive system of intercellular spaces (in *B. campos-portoi* and *B. atropurpureum*). Because the interlocking of epidermal cells provides mechanical strength and acts as a barrier that is almost totally impermeable to water and protects against herbivory (Glover, 2000), it is unlikely that this external layer is the only component of the epidermis. Thus, we interpret this layer to be an indumentum of unicellular trichomes that form 'bridges' between adjoining cells (in *B. atropurpureum* and *B. campos-portoi*) and this results in the labellar surface adopting a reticulate appearance. These trichomes apparently function as osmophores based on certain evidence: the usually unpleasant odour that occurs during collection (personal observation), positive reactions to neutral red tests *in vivo* and under ultraviolet light and Sudan III (Table 2), and the presence of dense cytoplasm and a relatively large nucleus. A somewhat similar epidermal arrangement, although lacking bridges between trichomes, was observed in the orchid *Cyclopogon elatus* (Sw.) Schltr. (Wiemer *et al*., 2009). Developmental studies are now necessary if aim to understand epidermal formation in species of *B.* section *Napelli* and, in particular, the formation of the bridges between trichomes and their phylogenetic significance, especially because these occur in closely related species (Smidt *et al*., 2011).

Most of the species that were investigated in the present study showed a well-differentiated sulcus adaxially on the proximal portion of the labellum. Histochemical tests, dense cytoplasm, a prominent nucleus, and the presence of secretion on collected flowers all indicate that the sulcus cells have a secretory function and that it possibly functions as a nectary. Previously, the presence of nectar has been reported near the labellum base for *B. glutinosum* and *B. regnellii* (Verola, 2002; Teixeira *et al*., 2004), although no histological evidence for a nectary could be found (Teixeira *et al*., 2004). We are of the opinion that there is sufficient evidence to consider the labellum sulcus of the studied species, including *B. regnellii*, as the best candidate for the nectar production site, although this remains to be confirmed.

A characteristic of all of the studied species is the presence of only two pollinia in the mature pollinarium, identified for the first time in the present study, whereas the basic number for *Bulbophyllum* and Orchidaceae is four, in agreement with the four pollen sacs that are usually found in angiosperms (Dressler, 1993). This character is a promising apomorphy for this group but needs verifying for other Neotropical sections of the genus. Secondary reduction or increase in the number of pollinia is common and widespread in Orchidaceae (Dressler, 1993), including Pleurothallidinae, which are also predominantly fly pollinated (Dressler, 1993). According to Freudenstein & Rasmussen (1996), in Epidendroideae, there are two archesporial zones that result in four or eight pollinia per anther solely by septation, whereas the lack of septation results in the formation of two pollinia. For *B.* section *Napelli*, the ontogenetic process leading to the formation of two pollinia remains unclear. However, because *B. atropurpureum* occasionally has two additional, central but reduced (vestigial) pollinia, the early fusion of four pollen sacs cannot be ruled out.

Bulbophyllum section *Napelli* shows some unique features, such as the labellar epidermis and pollinarium composed of two pollinia, which differ from reports for other species from the Neotropics (present study; Teixeira *et al*., 2004; Nunes *et al*., 2014) or elsewhere (Davies & Stpiczyńska, 2014). However, a labellar secretory sulcus or cavity appears to be a common feature for the genus (Teixeira *et al*., 2004; Davies & Stpiczyńska, 2014; Nunes *et al*., 2014), regardless of the substance secreted [protein-rich mucilage in *Bulbophyllum* section *Racemosae* (Davies & Stpiczyńska, 2014) or oil and sugar in Neotropical species (Teixeira *et al*., 2004; Nunes *et al*., 2014)].

Importantly, given the high number of species in this genus, our current knowledge of the flowers of *Bulbophyllum* is preliminary and much remains to be discovered.

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