Labellum and gynostemium micromorphology in *Polystachya* (Orchidaceae)

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Abstract

Polystachya is a large, pantropical orchid genus of 200 species, most of which occur as epiphytes in sub-saharan Africa. The three-lobed labellum of most *Polystachya* species possesses a fleshy callus and various types of trichomes and papillae. In this paper, we present the results of micromorphological studies on the labellum of 20 species, representing eight of the 13 sections in the genus, using scanning electron microscopy (SEM). Our results show the scale of infrageneric diversity of trichomes and papillae relative to the sampled sections. They also demonstrate the taxonomic value of labellar micromorphology at the sectional level. The study revealed seven types of papillae and five types of trichomes (uni- and multi-cellular) in *Polystachya*, some of which, are described here for the first time. Clavate trichomes are the most common and are present in 60% of the species studied. Moniliform trichomes mainly occur in sect. *Polystachya* and are strongly characteristic of the section. Pseudopollen is formed by fragmentation of moniliform trichomes or the detachment of other trichomes as bicellular units. We provide, for the first time, evidence for the detachment of the terminal cells of capitate trichomes.

Keywords Floral micromorphology · Orchidaceae · *Polystachya* · Taxonomy · Trichomes

Introduction

Polystachya Hook., in the family Orchidaceae, comprises almost 200 species of pantropical distribution. However, most of these species (93%) occur in sub-saharan Africa and Madagascar, and on neighbouring islands. Whereas the majority of the species has a more or less restricted

distribution, over 80 species are narrow endemics known only from a few populations. The genus is almost entirely epiphytic, although rarely, some species may occur as lithophytes or grow terrestrially. Members of this genus are sympodial, often producing pseudobulbs that vary greatly in form, the inflorescence arising terminally from the apex of mature pseudobulbs. The characteristic feature of *Polystachya* is the non-resupinate flower resulting in the uppermost position of the labellum and frequently, the partial fusion of the two lateral sepals to the long column-foot, thus forming a pouch-like mentum at the top of the flower. The last comprehensive taxonomic revision of the genus (Mytnik-Ejsmont 2011) resulted in the recognition of thirteen sections. Apart from the nominal section whose members are pantropical, all sections are confined to Africa and Madagascar, two sections being endemic to Madagascar and the islands of an Indian Ocean (Mytnik-Ejsmont 2011).

Historically, despite their great morphological diversity, the adaxial labellar trichomes of *Polystachya* have usually been referred to as pseudopollen. The term, used for the first time by Janse (1886), usually referred to farinaceous hairs that often resembled free, powdery pollen-attracting potential insect visitors. We now know, however that in

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Orchidaceae, not all farinaceous putative pseudopollen contains food reserves, and that not all food-hairs are necessarily farinaceous (Vogel 1978; Davies et al. 2013). Furthermore, pseudopollen lacking food reserves still has the capacity to attract pollinators (Vogel 1978; Kjellsson and Rasmussen 1987; Davies et al. 2002; Davies and Turner 2004). Reproductive biology and pollination strategy have been studied for some groups of epidendroids, e.g. *Maxillaria* (Davies et al. 2004; Davies et al. 2013), *Psilochilus* (Pansarin and Amaral 2008), *Stanhopea* (Pansarin and Amaral 2009), *Epidendrum* (Pansarin and Pansarin 2014), *Gomesa* (Pansarin et al. 2017), *Bulbophyllum* (Stpiczyńska et al. 2015, 2018), Catasetinae (Pansarin and Maciel 2017) and *Heterotaxis* (Krahl et al. 2019). Flowers of *Polystachya* display a remarkable range of labellar trichomes (Davies et al. 2002, Davies in Kull et al. 2009, Pansarin and Amaral 2006). Typical pseudopollen, as occurs in *Maxillaria* Ruiz & Pav., is formed by the fragmentation of uniseriate, moniliform trichomes into rounded, elliptic or lemon-shaped individual component cells. They appear as yellow-white farinaceous powder on the labellum. It is noteworthy, however that in some species of *Maxillaria*, such hairs do not always contain food material and still attract pollinators (Davies et al. 2013). In *Polystachya*, moniliform labellar hairs of identical morphology to those of *Maxillaria*, are known mainly from members of sect. *Polystachya*. Food-hairs, occurring in most other members of this genus, are uniseriate, one- to four-celled, often with subclavate to clavate terminal cells, or are bristle-like, or fusiform, sometimes tapered at the apex, the cells

being much longer than wide (Davies et al. 2002). Pansarin and Amaral (2006) indicated the collection of pseudopollen in two species of *Polystachya*, *P. concreta* and *P. foliosa* (referred to as *P. estrellensis* in Pansarin and Amaral 2006, though in our opinion, these taxa are conspecific), together with the list of pollinators. The main difference between the two types of labellar trichomes is that food-hairs generally remain intact, whereas pseudopollen is formed by fragmentation of the trichome into individual cells or into short chains of cells. These results are summarised in Table 1. It is significant that flowers producing pseudopollen tend to lack nectar (Van der Pijl and Dodson 1969). According to Davies et al. (2002), the types of trichomes found in *Polystachya* generally have limited taxonomic value, with the exception of moniliform, pseudopollen-forming trichomes which seem to occur only in a single section, namely, *Polystachya* sect. *Polystachya*. The great diversity of labellar hairs found elsewhere in the genus, whether or not they contain food reserves, probably reflects pollinator specificity.

Although nectar is the most common food-reward amongst members of the Orchidaceae (Van der Pijl and Dodson 1969; Arditti 1992; Dressler 1993), it is lacking in *Polystachya*. Instead, members of this genus produce various types of food-hair (including pseudopollen) (Davies et al. 2002; Pansarin and Amaral 2006) and are mainly pollinated by halictid bees (Halictidae), such as *Lasioglossum emirnense*, *L. nitididorsatum* and *Dialictus* aff. *creberrius* (Dodson and Frymire 1961; Dodson 1962; Goss 1977; Dressler 1993; Pettersson and Nilsson 1993), as well as

Table 1 Types of labellar trichomes occurring in *Polystachya* (after Davies et al. 2002)

Section	Species	Trichome type (number of cells)
<i>Calluniflorae</i> Kraenzl	<i>Polystachya woosnami</i> Rendle	Bristle-like (1–4)
<i>Caulescentes</i> Kraenzl	<i>Polystachya bennettiana</i> Rchb.f	Clavate (2–4)
	<i>Polystachya caloglossa</i> Rchb.f	Clavate (2–4)
	<i>Polystachya cultriformis</i> (Thouars) Sprengel	Clavate (1–2)
<i>Cultriformes</i> Kraenzl	<i>Polystachya cf. cultriformis</i> (Thouars) Sprengel	Clavate (1–2)
	<i>Polystachya maculata</i> P.J.Cribb	Clavate (2–4)
	<i>Polystachya campyloglossa</i> Rolfe	Cylindrical (1–3)
	<i>Polystachya lawrenceana</i> Kraenzl	Clavate (2–4)
<i>Humiles</i> Summerh	<i>Polystachya cf. ottoniana</i> Rchb.f	Clavate (2–4)
	<i>Polystachya piersii</i> P. J.Cribb	Clavate (1–2)
	<i>Polystachya pubescens</i> (Lindl.) Rchb.f	Bristle-like but with clavate apices (1–3)
	<i>Polystachya aff. villosa</i> Rolfe	Clavate (1)
<i>Polychaete</i> P.J.Cribb	<i>Polystachya elegans</i> Rchb.f	Bristle-like (1–4)
	<i>Polystachya seticaulis</i> Rendle	?
<i>Polystachya</i>	<i>Polystachya concreta</i> (Jacq.) Garay & H.R.Sweet	Moniliform (of various lengths)
	<i>Polystachya foliosa</i> (Hook.f.) Rchb.f	Moniliform (of various lengths)
	<i>Polystachya tessellata</i> Lindl	Moniliform (of various lengths)
<i>Superpositae</i> Kraenzl	<i>Polystachya eurygnatha</i> Summerh	Clavate (2–4)
	<i>Polystachya spatella</i> Kraenzl	Bristle-like (1–3)

representatives of Apidae (*Melipona* sp., *Plebeya droryana*, *Tetragonisca angustula*, *Trigona spinipes* and *Paratetrapedia* aff. *fervida*) (Pansarin and Amaral 2006).

Histochemical analyses conducted by Davies et al. (2002) revealed that the main food substance found in the food-hairs of *Polystachya* was protein. Lipids were invariably absent, whereas starch was often, but not always, detectable. This protein was distributed throughout the cytoplasm and the relative intensity of the xanthoproteic reaction suggested that elevated levels of aromatic amino acids were usually present. Although easily and frequently used for the detection of aromatic amino acids, the xanthoproteic test is not specific for this class of compound, forming a coloured nitroso reaction product with a range of compounds containing an active benzene ring. Consequently, Davies et al. (2002) have recently recommended using the more specific stain Coomassie Blue to test for the presence of protein. According to the xanthoproteic test (Davies et al. 2002), in *Polystachya*, both atrichotamous labellar epidermal cells and the mucilage secreted onto the adaxial surface of the labellum (e.g. *Polystachya campyloglossa* Rolfe) were also often rich in protein. Numerous small plastids containing starch were also present in the labellar trichomes of some species (e.g. *Polystachya foliosa* (Hook.f.) Rchb.f.), whereas in others (e.g. *Polystachya pubescens*) starch was present in atrichomatous adaxial epidermal cells of the labellum, but it was absent from the trichomes, (Davies et al. 2002). Many angiosperms have flowers that produce fragrance and nutritious, free, powdery pollen. Such flowers, therefore, both attract and are pollinated by pollen-collecting bees (Reis et al. 2004). Thus, the presence of food-hairs and fragrance in *Polystachya* would indicate a similar pollination strategy.

Labellar trichomes are very common in *Polystachya* and show a great diversity of form and function. With few exceptions, the first SEM analysis for this genus (Davies et al. 2002), demonstrated that particular types of trichome were ubiquitous, and thus of limited value in taxonomy, as their distribution did not coincide with the classification accepted at the time. Thus, the aim of the present study is to build upon the foundation laid by Davies et al. (2002) and to extend it to include additional species, as well as to study the micromorphology of the gynostemium relative to the latest infrageneric classification of *Polystachya* proposed by Mytnik-Ejsmont (2011). Comparative studies of this kind would enable further assessment of the potential taxonomic value of these remarkable epidermal structures.

Materials and methods

Labella and gynostemium of twenty species of *Polystachya*, representing eight sections occurring in Africa, Madagascar and Central America (Mexico), were examined by

means of scanning electron microscopy. The material used in the study, preserved in Kew Mixture (70% ETOH, 29% water and 1% glycerol), was obtained from the collection of the Department of Plant Taxonomy and Nature Conservation at the University of Gdansk, Poland (Table 2). The taxonomy of *Polystachya* follows that proposed by Mytnik-Ejsmont (2011), and the authorities for plant names follow Brummit and Powell (1992).

Fresh plant material was firstly fixed so as to avoid decomposition and distortion of tissue (Karcz 2009a), and in order to maintain its integrity relative to that of living tissue (Wróbel et al. 2005). This was achieved by using a buffered aqueous solution of two different fixatives, namely 12.5% (w/v) paraformaldehyde (PFA) and 2.5% (v/v) glutaraldehyde (GA) at pH 7. Labellar material was fixed at 4 °C for 24 h. Spirit-preserved material did not require fixation, merely transferring to the next stage. Both fixed and spirit-preserved plant material were then dehydrated using a graded ethanol series (Wróbel et al. 2005) and subjected to conventional critical-point drying using liquid carbon dioxide (CO₂), as described by Karcz (2009b). The material was subsequently secured to stubs before coating with gold (Karcz 1996). Finally, the specimens were examined using a Philips XL30 (FEI) scanning electron microscope at an accelerating voltage of 5 or 15 kV.

Results

The micromorphology

The results for labellar trichomes and papillae generally closely resemble those previously reported by Davies et al. (2002), as follows:

- Diversity of form. Apart from multicellular, cylindrical trichomes, few other types are described here.
- Clavate trichomes are the most common and are present in 55% of the species studied here.
- With few exceptions, those species bearing moniliform trichomes are mainly restricted to sect. *Polystachya*.
- Pseudopollen is formed by fragmentation of moniliform trichomes or the detachment of trichomes as bicellular units. Here, we also provide, for the first time, evidence of detachment of terminal cells of clavate trichomes.
- Cells of moniliform trichomes contain protein and some starch (with few exceptions not repeated here but will form the basis of a future study).
- Secreted material is often deposited on the labellar surface, and this may form a film that obfuscates labellar topography.

Table 2 List of species of *Polystachya* used in this study (the order of the taxa follows the Mytnik-Ejsmont systematic approach to Polystachyinae (2011))

Section	Species	Origin	Accession number
<i>Calluniflorae</i> Kraenzl	<i>Polystachya adansoniae</i> Rchb.f	Africa	p-2729
	<i>Polystachya coriscensis</i> Rchb.f	Africa	p-2724
<i>Galeatae</i> Mytnik	<i>Polystachya galeata</i> Rchb.f	Africa	p-1008
	<i>Polystachya nyanzensis</i> Rendle	Africa	p-2728
<i>Caulescentes</i> Kraenzl	<i>Polystachya laxiflora</i> Lindl	Africa	p-2361
	<i>Polystachya bennettiana</i> Rchb.f	Africa	4248
	<i>Polystachya albescens</i> subsp. <i>imbricata</i> (Rolfe) Summerh	Africa	p-2736
	<i>Polystachya thomensis</i> Summerh	Africa	p-1630
<i>Polystachya</i> Hook.	<i>Polystachya concreta</i> (Jacq.) Garay & H.R.Sweet	Africa	p-2734
	<i>Polystachya paniculata</i> (Sw.) Rolfe	Africa	85PTGA378
	<i>Polystachya lineata</i> Rchb.f	Central America	4226
	<i>Polystachya odorata</i> Lindl	Africa	p-2725
<i>Superpositae</i> Kraenzl	<i>Polystachya bamendae</i> Szlachetko, Baranow & Mytnik	Africa	4227
	<i>Polystachya simplex</i> Rendle	Africa	p-2727
<i>Humiles</i> Summerh	<i>Polystachya bella</i> Summerh	Africa	p-1345
	<i>Polystachya pubescens</i> (Lindl.) Rchb.f	Madagascar	p-1395
<i>Cultriformes</i> Kraenzl	<i>Polystachya cultriformis</i> (Thouars) Sprengel	Africa	p-1815
	<i>Polystachya maculata</i> P.J.Cribb	Africa	p-1823
	<i>Polystachya vulcanica</i> Kraenzl	Africa	p-1479
<i>Pseudomonophyllae</i> Mytnik	<i>Polystachya rosea</i> Ridl	Madagascar	8871

Classification of epidermal papillae/trichomes

A great diversity of epidermal structures was present on the labellum and gynostemium of *Polystachya*. Based on the work of Davies et al. (2002), we recognized two main groups: Papillae (unicellular protuberances of various length that are usually wider at the base) and trichomes (unicellular or multicellular structures of diverse shape, with a narrow point of insertion and often with a differently shaped apex). It must, however, be acknowledged that such classification is largely used for convenience since in many orchids, the morphology of labellar papillae and trichomes often intergrade, and in such cases, trichomes probably develop from papillae. Fourteen different types of papillae/trichomes were found on the labella and gynostemium of the species investigated in the present paper. These are shown below (Figs. 1, 2).

Owing to the varying density of papillae/trichomes on different regions of the labellum, the latter was divided into distinct areas and the relative densities (Fig. 3) of these epidermal structures were recorded diagrammatically for each of the species investigated, as follows (Fig. 4).

Trichomes in *Polystachya* are generally uniseriate, one- to four-celled, often with differently shaped (subclavate to clavate) terminal cells. The complete range of labellar micromorphology for all studied species of *Polystachya* is represented in Table 3 and in Fig. 5. Detailed descriptions

of each species investigated are presented below according to sections.

Polystachya Hook. sect. *Calluniflorae* Kraenzl

Polystachya adansoniae Rchb.f

The labellar trichomes are cylindrical (Fig. 6a, Online Resource 1a, b) and two- to four-celled; apices often conical and tapering. The trichomes present on the proximal part of the labellum consist of two cells, whereas those occurring on the central part of the labellum consist of two, three or four cells (Online Resource 1c-f). The trichomes are not frequent, but are more densely arranged centrally. The labellum margin lacks trichomes and the labellum lacks obvious papillae.

Polystachya coriscensis Rchb.f

The labellum is densely clothed with cylindrical trichomes centrally (Fig. 6b, Online Resource 2a), occurring less densely towards the lateral lobes. The trichomes consist of one or two cells (Online Resource 2b, c). The distal part of the labellum (epichile) bears obpyriform to hemispherical papillae (Online Resource 2d).

Fig. 1 The types of papillae found on the labella and gynostemium of the *Polystachya* species investigated in the present paper

Fig. 2 The types of trichomes found on the labella and gynostemium of the *Polystachya* species investigated in the present paper

Polystachya Hook. sect. *Galeatae* Mytnik

Polystachya galeata Rchb.f

The labellum is clothed with papillae and multicellular trichomes (Fig. 6c). The trichomes are clavate and comprise two or three cells. Proximally, the central part bears very dense, short, conical papillae (Online Resource 3a, b). Individual, shortly conical papillae with rounded apices occur on the margins of the proximal part of the labellum (Online

Fig. 3 Relative density of trichomes and papillae on labella and gynostemium of *Polystachya*

Degree	Trichomes	Papillae	Characterization	Description as used
4			Density of trichomes/papillae is so great that their proximal parts are not visible	Very dense
3			6-15 trichomes/papillae per 0.01 mm ²	Dense
2			3-5 trichomes/papillae per 0.01 mm ²	Rare, small number
1			Individual, 1-2 trichomes/papillae per 0.01 mm ²	Single, individual
0			No trichomes / papillae	No trichomes / papillae

Fig. 4 Terminology for parts of the labellum used in the Results section

Resource 3c). Margins of the central, fleshy ridge bear very dense obpyriform to hemispherical papillae (Online Resource 3d–f). The epichile is also ridged and bears clavate (club-like), 2–3-celled trichomes and very dense conical and obpyriform to hemispherical papillae (Online Resource 3 g, h). Clavate, two-celled, sparse trichomes occur on the gynostemium (Online Resource 3i, j). Individual papillae occur on the column-foot (Online Resource 3j).

Polystachya nyanzensis Rendle

The labellum of *Polystachya nyanzensis* is papillose with sparse, clavate trichomes (Fig. 6d, Online Resource 4a). The proximal trichomes comprise two or three cells (Online Resource 4b–d), whereas those on the central and distal parts of the labellum are two-celled (Online Resource 4e). Many conical, obpyriform and hemispherical papillae occur along the mid-nerve (Online Resource 4f, g). The epichile is

rugose. Clavate, two- or three-celled trichomes occur on the column-foot (Online Resource 4 h).

Polystachya Hook. sect. *Caulescentes* Kraenzl

Polystachya laxiflora Lindl

The labellum of *Polystachya laxiflora* is clothed with short, clavate (club-like), two-celled (Fig. 6e, Online Resource 5a–d) trichomes and conical, obpyriform, shortly hemispherical or villiform, unicellular papillae (Online Resource 5e–g). Conical and obpyriform to shortly hemispherical papillae occur proximally (Online Resource 5a). Conical papillae are replaced by villiform papillae on the distal part of the labellum (Online Resource 5f). The gynostemium is clothed with two-celled, cylindrical and clavate trichomes arranged along the lateral margins and at the apex of the column (Online Resource 5 h–j). The dorsal sepal is clothed adaxially with clavate trichomes

Table 3 Types of labellar structures found in all *Polystachya* species studied (present results appear in bold, the reminder are those of Davies et al. (2002))

Section	Species	Trichome type (number of cells)	Papillae
<i>Calluniflorae</i> Kraenzl	<i>Polystachya adansoniae</i> Rchb.f	Cylindrical (2–4)	Obpyriform to hemispherical
	<i>Polystachya coriscensis</i> Rchb.f	Cylindrical (1–2)	
	<i>Polystachya woosnami</i> Rendle	Bristle-like (1–4)	
<i>Galeatae</i> Mytnik	<i>Polystachya galeata</i> Rolfe	Clavate (2–3)	Conical and obpyriform to hemispherical
	<i>Polystachya nyanzensis</i> Rendle	Clavate (2–3)	Conical, obpyriform and hemispherical
<i>Caulescentes</i> Kraenzl	<i>Polystachya laxiflora</i> Lindl	Clavate (2)	Conical, obpyriform, shortly hemispherical or villiform, unicellular
	<i>Polystachya bennettiana</i> Rchb.f	Clavate (2–4) cylindrical (1)	Obpyriform to hemispherical to spherical
	<i>Polystachya albescens</i> subsp. <i>imbri-cata</i> (Rolfe) Summerh	Clavate (2–4)	hemispherical
	<i>Polystachya thomensis</i> Summerh	Moniliform (2–5)	Villiform, obpyriform to hemispherical to spherical
	<i>Polystachya caloglossa</i> Rchb.f	Clavate (2–4)	
<i>Polystachya</i> Hook.	<i>Polystachya concreta</i> (Jacq.) Garay & H.R.Sweet	Moniliform (2–6) cylindrical (1–2)	Obpyriform to hemispherical or almost spherical
	<i>Polystachya odorata</i> Lindl	Moniliform	Obpyriform to hemispherical
	<i>Polystachya lineata</i> Rchb.f	Cylindrical (1) moniliform–clavate (1–5)	Obpyriform to hemispherical
	<i>Polystachya paniculata</i> (Sw.) Rolfe	Clavate (2) cylindrical (1)	Obpyriform
	<i>Polystachya foliosa</i> (Hook.f.) Rchb.f	Moniliform (of various lengths)	
	<i>Polystachya tessellata</i> Lindl	moniliform (of various lengths)	
	<i>Polystachya simplex</i> Rendle	Columnar or cylindrical (1) clavate (2)	Obpyriform to hemispherical
<i>Superpositae</i> Kraenzl	<i>Polystachya bamendae</i> Szlachetko, Baranow & Mytnik	Clavate (1–2) cylindrical (1)	Villiform, conical and obpyriform
	<i>Polystachya eurygnatha</i> Summerh	Clavate (2–4)	
	<i>Polystachya spatella</i> Kraenzl	Bristle-like (1–3)	
	<i>Polystachya bella</i> Summerh	Cylindrical and clavate (1) clavate (2)	Conical, obpyriform, villiform and hemispherical
<i>Humiles</i> Summerh	<i>Polystachya pubescens</i> (Lindl.) Rchb.f	Bristle-like but with clavate apices (1–3) cylindrical (1–3)	
	<i>Polystachya lawrenceana</i> Kraenzl	Clavate (2–4)	
	<i>Polystachya cf. ottoniana</i> Rchb.f	Clavate (2–4)	
	<i>Polystachya piersii</i> P.J.Cribb	Clavate (1–2)	
	<i>Polystachya aff. villosa</i> Rolfe	Clavate (1)	
	<i>Polystachya cultriformis</i> (Thouars) Sprengel	Clavate (1–2)	Conical to obpyriform or hemispherical
	<i>Polystachya maculata</i> P.J.Cribb	Clavate (2–4)	
<i>Cultriformes</i> Kraenzl	<i>Polystachya vulcanica</i> Kraenzl	Absent	Conical, obpyriform and hemispherical
	<i>Polystachya campyloglossa</i> Rolfe	Cylindrical (1–3)	
<i>Pseudomonophyllae</i> Mytnik	<i>Polystachya rosea</i> Ridl	Clavate and moniliform (1–5)	Villiform, hemispherical, conical and obpyriform
<i>Polychaete</i> P.J.Cribb	<i>Polystachya elegans</i> Rchb.f	Bristle-like (1–4)	

Fig. 5 Sections (including subsections) of *Polystachya*, showing the types of trichome present

Section	Species	Trichome type	Figure
<i>Calluniflorae</i> Kraenzl.	<i>Polystachya adansoniae</i> Rchb.f.	cylindrical
	<i>Polystachya coriscensis</i> Rchb.f.	cylindrical
<i>Galeatae</i> Mytnik	<i>Polystachya galeata</i> Rolfe	clavate
	<i>Polystachya nyanzensis</i> Rendle	clavate

consisting of an elongate stalk cell and an inflated terminal cell, together with conical and hemispherical papillae (Online Resource 5 k).

***Polystachya bennettiana* Rchb.f**

The labellum of *Polystachya bennettiana* bears obpyriform to hemispherical to spherical papillae and sparse, cylindrical trichomes (Fig. 6f, Online Resource 6a). The trichomes

comprise one or two cells with tapering apices (Online Resource 6b-d). Distally, the labellum is rugose, and the labellar margin glabrous (Online Resource 6e, f). Centrally, residues of secretion were present (Online Resource 6 g, h). Cells of the labellum are elongate and striate proximally, but more isodiametric and papillose distally. The central part of the column, just beneath the stigma, bears unicellular, cylindrical trichomes (Online Resource 6i, j).

Fig. 5 (continued)

<i>Caulescentes</i> Kraenzl.	<i>Polystachya laxiflora</i> Lindl. (subect. <i>Caulescentes</i>)	clavate
	<i>Polystachya bennettiana</i> Rchb.f. (subect. <i>Caulescentes</i>)	clavate cylindrical
	<i>Polystachya albescens</i> ssp. <i>imbricata</i> (Rolfe) Summerh. (subect. <i>Caulescentes</i>)	clavate

***Polystachya albescens* subsp. *imbricata* (Rolfe) Summerh**

The labellum of *Polystachya albescens* subsp. *imbricata* is characterised by the presence of 2–4-celled, clavate trichomes and hemispherical papillae (Fig. 6g, Online Resource 7a). Proximally, the trichomes are two- to four-celled (Online Resource 7b, c), but centrally and distally, two-celled (Online Resource 7e, g). They occur mainly on the margins of the fleshy callus, but are only sparsely distributed on the central part of the callus (Online Resource 7d). Hemispherical papillae occur on the distal part of the

labellum (Online Resource 7f). The trichomes are very densely distributed on the epichile, and have rounded terminal cells (Online Resource 7 g, h). Similar, 2-celled trichomes occur on the column, but their terminal cells tend to have conical bases and more pointed apices (Online Resource 7i–k).

***Polystachya thomensis* Summerh**

The labellum of *Polystachya thomensis* is characterised by multicellular, moniliform trichomes (Fig. 6h, Online Resource

Fig. 5 (continued)

	<i>Polystachya thomensis</i> Summerh. (subsect. <i>Rhodopterae</i> Mytnik)	moniliform
<i>Polystachya</i>	<i>Polystachya concreta</i> (Jacq.) Garay & H.R.Sweet	moniliform cylindrical
	<i>Polystachya odorata</i> Lindl.	moniliform
	<i>Polystachya lineata</i> Rchb.f.	moniliform-clavate

8a, b) comprising spherical to columnar cells (Online Resource 8c). The proximal part of the labellum is very densely clothed with two- to five-celled moniliform trichomes (Online Resource 8d) forming a wide band that recedes centrally (Online Resource 8e). Some trichomes occur on the callus (Online Resource 8f-h) and, together with villiform papillae (Online Resource 8i), densely clothe the margins of the lateral

lobes of the labellum and the area beneath the callus (Online Resource 8 h). The relative density of trichomes, however, diminishes towards the rugose epichile (Online Resource 8j, k) and the labellar papillae may be obpyriform to hemispherical to almost spherical.

The column-foot bears individual, short trichomes and hemispherical papillae (Online Resource 8 l).

Fig. 5 (continued)

	<i>Polystachya paniculata</i> (Sw.) Rolfe (sect. <i>Caulescentes</i> ?)	clavate cylindrical
<i>Superpositae</i> Kraenzl.	<i>Polystachya simplex</i> Rendle	clavate columnar or cylindrical
	<i>Polystachya bamendae</i> Szlachetko, Baranow & Mytnik	clavate cylindrical

Polystachya* Hook. sect. *Polystachya***Polystachya concreta* (Jacq.) Garay & H.R.Sweet**

The labellum of *Polystachya concreta* bears two types of trichomes (Fig. 6i). Unicellular, cylindrical, striate trichomes (together with occasional bicellular clavate trichomes) occur densely on the proximal part of the labellum (Online Resource 9a–d), whereas small, cylindrical

trichomes occur sparsely on the callus (Online Resource 9e, f), and moniliform, two- to six-celled trichomes form a cushion along the fleshy ridge (Online Resource 9 g, h). The latter is very densely distributed on the central part of the labellum (Online Resource 9i), becoming reduced in number towards the epichile, where they comprise three to seven cells. Here also occur one- to two-celled cylindrical trichomes, papillae tend to be obpyriform to hemispherical or almost spherical (Online Resource 9j–l).

Fig. 5 (continued)

<i>Humiles</i> Summerh.	<i>Polystachya bella</i> Summerh.	clavate cylindrical
	<i>Polystachya pubescens</i> (Lindl.) Rchb.f.	cylindrical
<i>Cultriformes</i> Kraenzl.	<i>Polystachya cultriformis</i> (Thouars) Sprengel (subsect. <i>Cultriformes</i>)	clavate
	<i>Polystachya maculata</i> P.J.Cribb (subsect. <i>Cultriformes</i>)	clavate

***Polystachya odorata* Lindl**

The proximal part of the labellum of *Polystachya odorata* is clothed with shortly moniliform trichomes (Fig. 6j, Online Resource 10a) with a highly striate cuticle (Online Resource 10b, c). In most moniliform trichomes, the terminal cell is inflated (Online Resource 10d, e). No trichomes occur on the callus (Online Resource 10f). Obpyriform to hemispherical papillae are present on the epichile (Online Resource 10 g) and these are highly striate (Online Resource 10 h).

***Polystachya lineata* Rchb.f**

The labellum of *Polystachya lineata* is characterised by the presence of very dense, unicellular and cylindrical or multicellular, moniliform-clavate trichomes (Fig. 6k, Online Resource 11a, b). The moniliform-clavate trichomes consist of one to five cells (Online Resource 11c). The basal cells of moniliform trichomes are elongate and stalk-like, and the terminal cells tend to become detached (Online Resource 11d–g). No trichomes occur on the labellum margins (Online Resource 11a, h), and both the epichile and

Fig. 5 (continued)

	<i>Polystachya vulcanica</i> Kraenzl. (subsect. <i>Gracilentae</i> Mytnik)	absent
<i>Pseudomonophyllae</i> Mytnik	<i>Polystachya rosea</i> Ridl. (sect. <i>Polystachya</i> ?)	clavate moniliform

margins comprise obpyriform to hemispherical papillae (Online Resource 11 h). The gynostemium is glabrous.

***Polystachya paniculata* (Sw.) Rolfe**

The labellum of *Polystachya paniculata* is rarely clothed with unicellular, cylindrical trichomes. Bicellular, clavate trichomes and individual obpyriform papillae both rarely occur only on the proximal part of the labellum (Fig. 6l, Online Resource 12a–d). The epidermal cells are slightly convex, isodiametric and have a smooth or slightly striate cuticle (Online Resource 12e). The gynostemium lacks trichomes (Online Resource 12f).

***Polystachya* Hook. sect. *Superpositae* Kraenzl**

***Polystachya simplex* Rendle**

Individual, unicellular, columnar or cylindrical trichomes and bicellular, clavate trichomes occur on the labellum of *Polystachya simplex* (Fig. 6m, Online Resource 13a). Bicellular, clavate trichomes, are found predominantly on the proximal and central parts of the labellum (Online Resource 13b–d). The epichile is rugose and clothed with both types of trichome (Online Resource 13e–g). Atrichomatous cells of the labellar epidermis are isodiametric and slightly convex and striate, whereas the papillae may be obpyriform to hemispherical and striate. Scattered, two-celled, clavate or unicellular, cylindrical trichomes occur on the column-foot (Online Resource 13 h), and again, papillae here are both isodiametric and striate.

***Polystachya bamendae* Szlachetko, Baranow & Mytnik**

Proximally, the labellum of *Polystachya bamendae* bears clavate and unicellular or bicellular trichomes (Fig. 6n, Online Resource 14a, b). Individual, clavate trichomes occur scattered on the callus, which is located on the proximal part of the labellum (Online Resource 14c). The central part of the labellum bears a cushion of very dense, unicellular, villiform papillae, whereas the margins bear conical papillae (Online Resource 14d, e). The rugose epichile bears scattered, individual, unicellular, cylindrical trichomes and bicellular, clavate trichomes, as well as conical and obpyriform papillae (Online Resource 14f, g). Residues of secretion or detritus seemingly occur on the labellum (Online Resource 14 h). Adaxial epidermal cells of the labellum are generally isodiametric, except on the central part of the callus, where they are elongate (Online Resource 14c). The column-foot is densely clothed with villiform or conical papillae, with pointed or rounded apices and unicellular, cylindrical trichomes (Online Resource 14i–k).

***Polystachya* Hook. sect. *Humiles* Summerh**

***Polystachya bella* Summerh**

The labellum of *Polystachya bella* is clothed with both papillae and trichomes (Fig. 6o, Online Resource 15a). The former structures occur proximally and are mainly obpyriform (occasionally hemispherical), whereas the trichomes are cylindrical to clavate and 1-celled, or clavate and bicellular

Fig. 6 Relative density of trichomes and papillae on the labellum of *Polystachya*: **a** *P. adansoniae*, **b** *P. coriscensis*, **c** *P. galeata*, **d** *P. nyanzensis*, **e** *P. laxiflora*, **f** *P. bennettiana*, **g** *P. albescens* subsp.

imbricata, **h** *P. thomensis*, **i** *P. concreta*, **j** *P. odorata*, **k** *P. lineata*, **l** *P. paniculata*, **m** *P. simplex*, **n** *P. bamendae*, **o** *P. bella*, **p** *P. pubescens*, **q** *P. cultriformis*, **r** *P. maculata*, **s** *P. vulcanica*, **t** *P. rosea*

(Online Resource 15b). The callus has similar papillae and scattered, clavate trichomes (Online Resource 15c). A film of secreted material coats the callus, partly obfuscating the papillae (Online Resource 15d). The margins of the hypochile bear conical, obpyriform, villiform and hemispherical papillae that also become coated with a film of secreted material (Online Resource 15e–g). The epichile is fleshy, slightly rugose and glabrous (Online Resource 15 h). Both trichomes and papillae are moderately striate. The gynostemium is glabrous.

Polystachya pubescens (Lindl.) Rchb.f

The labellum of *Polystachya pubescens* bears one type of trichome only and no obvious papillae (Fig. 6p, Online

Resource 16a). Trichomes are present mainly on the lateral lobes, but also extend onto the mid-lobe of the labellum (Online Resource 16a–d). They are one- to three-celled and cylindrical, and often have fusiform terminal cells. (Online Resource 16d). There are no trichomes on the proximal and distal parts of the labellum (Online Resource 16e). Trichomes are also absent from the gynostemium. Individual, cylindrical, unicellular or few-celled trichomes are also present on the adaxial surfaces of petals and lateral sepals (Online Resource 16f–h).

Polystachya* Hook. sect. *Cultriformes* Kraenzl**Polystachya cultriformis* (Thouars) Sprengel**

The labellum of *Polystachya cultriformis* is clothed with papillae and clavate trichomes. These trichomes are present over the entire surface of the labellum (Fig. 6q, Online Resource 17a), although those on the callus are rather sparser, often occurring singly (Online Resource 17b). Those present on the proximal and central parts of the labellum consist of one or two cells (Online Resource 17c, d), whereas trichomes present on the epichile are one-celled (Online Resource 17e, f). A film of secretory residue coats the proximal part of the callus and partly obfuscates the labellar topography (Online Resource 17 g).

Papillae are present on the margins of the proximal part of the labellum. They are conical to obpyriform or hemispherical (Online Resource 17 h, i), and occasional columnar trichomes are present. The callus lacks papillae, although they may occur on the central part of the labellum. Conical papillae occur on the epichile (Online Resource 17f). The column bears scant one- to two-celled trichomes, and the epidermal cells are strongly striate (Online Resource 17j).

***Polystachya maculata* P.J.Cribb**

The labellum of *Polystachya maculata* mainly bears clavate trichomes (Fig. 6r, Online Resource 18a). They are densely distributed on the proximal part of the labellum, but less so on the central part (Online Resource 18a–c). Scattered, clavate or columnar trichomes occur on the callus (Online Resource 18d, e). The trichomes comprise two to four cells, and the terminal cell is inflated (Online Resource 18f). Trichomes are absent from the rugose epichile (Online Resource 18 g). The labellum lacks papillae and the epidermal cells are isodiametric and striate (Online Resource 18 h, i). The gynostemium is glabrous.

***Polystachya vulcanica* Kraenzl**

Polystachya vulcanica has a glabrous labellum. The proximal cells are elongate and faintly striate (Fig. 6s, Online Resource 19a, b). Cells of the central part of the labellum are slightly papillose and similarly striate (Online Resource 19c–e). Conical, obpyriform and hemispherical papillae occur on the epichile (Online Resource 19f, g). The gynostemium is glabrous (Online Resource 19 h).

Polystachya* Hook. sect. *Pseudomonophyllae* Mytnik**Polystachya rosea* Ridl**

The labellum of *Polystachya rosea* is densely hirsute (Fig. 6t, Online Resource 20a). Clavate and moniliform, one- to five-celled trichomes are very densely distributed proximally on the labellum, together with villiform papillae (Online Resource 20b, c). Conical and obpyriform papillae are present at the margins of the labellum, together with occasional, shortly villiform papillae (Online Resource 20d). The terminal cells of the clavate trichomes tend to become detached (Online Resource 20e). The callus is very densely clothed with moniliform trichomes. Trichomes on the central part of the labellum and along the callus are moniliform, comprising one to five cells (Online Resource 20f). Moniliform, 1–5-celled trichomes, together with villiform and hemispherical papillae also occur on the epichile, and stages in the transition of papillae to trichomes are visible here (Online Resource 20 g, h). The column-foot is also papillose, with conical and obpyriform papillae (Online Resource 20i). Individual, two-celled clavate trichomes are scattered along the column-foot (Online Resource 20j) and also occur on the adaxial surface of the petals (Online Resource 20 k).

Discussion

As well as confirming previous results, the present investigation extends the work of Davies and colleagues in a number of ways. Firstly, it considers the labellar micromorphology of a further fifteen species. Secondly, it analyses the distribution of these various epidermal structures on the labellar surface and attempts to relate distribution to function. However, perhaps its most important contribution is that for the first time, it is possible to relate the labellar micromorphology of *Polystachya* to a robust, taxonomic classification (Mytnik-Ejsmont 2011). Although Kraenzlin (1926) had subdivided the genus on morphological grounds into 12 sections, and a further five sections had subsequently been added (Summerhayes 1942, 1947; Brenan 1954; Cribb 1978; Podzorski and Cribb 1979), when Davies et al. (2002) had their work published, still no satisfactory infrageneric classification existed, making it difficult to relate micromorphology to taxonomy in a meaningful way. Since then, a monograph and new classification for *Polystachya* has been published (Mytnik-Ejsmont 2011), thus providing a useful framework for such comparisons. Each of the eight sections represented is discussed below.

***Polystachya* Hook. sect. *Calluniflorae* Kraenzl**

Three species of this section were examined, namely *Polystachya adansoniae* and *P. coriscensis* in this study, and *P. woosnamii* by Davies et al. (2002). In all cases, the labella bear multicellular, 1–4-celled, cylindrical trichomes with tapering apices, described by Davies et al. (2002) as ‘bristle-like’. These trichomes occur most densely on the central part of the labellum and on the lateral lobes. Despite their similarity, these trichomes may vary in the number of cells they contain, the labellum of *P. woosnamii* also bearing clavate trichomes.

All three species possess similar types of trichome distributed in a similar manner on the labellum, therefore this may have taxonomic value.

***Polystachya* Hook. sect. *Galeatae* Mytnik**

Species representing section *Galeatae* were, until 2011 (Mytnik-Ejsmont 2011), assigned to section *Cultriformes* (sensu Kraenzlin 1926) owing to their possession of unifoliate stems. Results of molecular analyses (Russell et al. 2010a, b a,b; Mytnik-Ejsmont 2011), however, revealed that section *Cultriformes* sensu Kraenzlin (1926) is polyphyletic and that the unifoliate shoot is a homoplasious character. Consequently, plants formerly assigned to *Cultriformes* were placed in three sections, one of which is section *Galeatae*. Labella of *P. galeata* and *P. nyanzensis* share the same type of labellar papillae and the labella of both species are densely clothed with clavate, 2–3-celled trichomes. Their gynostemium bear 2-celled, cylindrical trichomes. Since the labellar trichomes of two representatives of each of the sections *Galeatae* and *Cultriformes* subsect. *Cultriformes* (see below) show no significant differences, labellar micromorphology here appears to have little taxonomic value.

***Polystachya* Hook. sect. *Caulescentes* Kraenzl**

Four of the five species studied (*P. caloglossa*, *P. bennettiana*, *P. laxiflora* and *P. albescens* subsp. *imbricata*) represent the nominal subsection, whereas *P. thomensis* represents the subsection *Rhodopterae* Mytnik. Differences in the type of labellar indumentum are visible at the subsectional level, two different types of trichomes are present in the subsections. Labella of representatives of subsection *Caulescentes* are mainly clothed with clavate, 2–4-celled trichomes, although less dense, cylindrical, unicellular trichomes are sometimes present (*P. bennettiana*). Proximally, the labellum of *P. thomensis* bears 1–5-celled moniliform trichomes. Hitherto, moniliform trichomes had been recorded only for representatives of section *Polystachya* (Davies et al. 2002),

but are observed here in a representative of another section for the first time.

Polystachya* Hook. sect. *Polystachya

Five species of this section were examined, namely, *P. concreta*, *P. foliosa* (Davies et al. 2002), *P. odorata*, *P. lineata* (from Mexico) and *P. paniculata*. The taxonomic position of the latter species is the subject of many discussions among taxonomists, with *P. paniculata* being assigned to the nominal section or to the section *Caulescentes* based on its set of unique characters. The labella of the first four species, which are undoubtedly members of the nominal section, are densely clothed with moniliform, pseudopollen-forming hairs, whereas the gynostemium is glabrous. These multicellular trichomes are detachable and are likely to be collected by insects (Pansarin and Amaral 2006). Probably, the cylindrical trichomes present on the labellum of *P. concreta* and *P. lineata* represent what remain of moniliform trichomes, following fragmentation. The pollination biology of pseudopollen-producing species is still poorly known. Goss (1977) describing the pollination of *P. flavescens* claims that nectar is lacking when pseudopollen is present. Dressler (1993) suggested that the main system of pollination in *Polystachya* involves the rewarding of small bees with pseudopollen. Pansarin and Amaral (2006) studied the pollination biology of *P. concreta* and *P. foliosa* (syn. *P. estrellensis*) and observed the collection of pseudopollen from the lip of the flowers. However, the occurrence of pseudopollen produced by fragmentation of moniliform hairs appears largely to be restricted to representatives of sections *Polystachya* (excluding *P. paniculata*), *Pseudomonophyllae* (*P. rosea*) and *Caulescentes* (*P. thomensis*, see discussion above).

The labellar micromorphology of *Polystachya paniculata* differs from that of all other representatives of sect. *Polystachya* investigated to date in that moniliform hairs are lacking, with individual, clavate and cylindrical 1- to 2-celled trichomes scattered throughout the proximal part of the labellum. It is worth noting that hitherto, the taxonomic status of *P. paniculata* has caused problems, with some authors considering it to be a member of sect. *Caulescentes* (Kraenzlin 1926; Cribb 1984; la Croix and la Croix 1997) and others, a member of section *Polystachya* (Piers 1968; Mytnik-Ejsmont 2011). The labellar micromorphology of *P. paniculata* are similar to that of members of *Caulescentes* (clavate, 2-celled), but not the section *Polystachya* (moniliform). This is interesting and may be worth further taxonomic investigation. Apart from this problematic species, moniliform trichomes are characteristic of members of the nominal section and have taxonomic value.

***Polystachya* Hook. sect. *Superpositae* Kraenzl**

Four species were examined, *P. eurygnatha*, *P. spatella* (Davies et al. 2002), *P. simplex* and *P. bamendae*. All the species are characterised by the presence of clavate and cylindrical 1–4 celled trichomes on the labellum. Cylindrical trichomes also occur on the column-foot. In addition, the lateral lobes of the labellum and the epichile of *P. bamendae* possess villiform papillae.

***Polystachya* Hook. sect. *Humiles* Summerh**

Both cylindrical and clavate labellar trichomes were present in representatives of this section. Of the species investigated, *P. pubescens* is characterised by its possession of cylindrical, 1–3 celled trichomes only, and *P. bella* has unicellular, cylindrical or 1–2-celled clavate trichomes. Similarly, *P. aff. villosa* has 1-celled, clavate trichomes, whereas *P. campyloglossa*, *P. lawrenceana* and *P. cf. ottoniana* produce 2–4-celled clavate trichomes. The diversity of trichome structure found in sect. *Humiles* indicates that labellar micromorphology has limited taxonomic value here.

***Polystachya* Hook. sect. *Cultriformes* Kraenzl**

Representatives of two subsections were studied, *P. cultriformis* and *P. maculata*, representing the nominal subsection, and *P. vulcanica*, representing the subsection *Gracilentae* Mytnik. This study confirmed the presence of clavate, two- to four-celled trichomes on the labella of *P. cultriformis* and *P. maculata*, as described by Davies et al. (2002). In addition, unique columnar trichomes were observed on the callus of the latter species. Study of the gynostemium revealed the presence of cylindrical 2-celled trichomes. The labellum of *P. vulcanica*, however, differed from that of all the other species studied in that it lacked trichomes and only the epichile was papillose. This may be characteristic of all representatives of this subsection, and therefore, further studies are required to ascertain its taxonomic value. Representatives of both subsections clearly differ: *Gracilentae* are relatively tall and slender plants lacking a labellar callus, whereas those of subsection *Cultriformes* are robust plants possessing a labellar callus.

***Polystachya* Hook. sect. *Pseudomonophyllae* Mytnik**

Polystachya rosea (endemic to Madagascar, Comoros and Seychelles) is often confused with *P. concreta* (sect. *Polystachya*), but these species differ in a number of ways, including the shape and size of leaves, flower size, colour and scent, and labellum structure. The first pollination event recorded for this species was that observed by Pettersson

and Nilsson (1993). According to these authors, the labellum lacks pseudopollen and pollination occurs as a result of deception achieved by extensive variation in flower colour. Moreover, the absence of any fragrance and pseudopollen is possibly an evolutionary consequence of partial fitness dependence on male bee pollinators. Our results, however, clearly show that pseudopollen is present on the labellum of this species, the latter bearing two types of trichome, clavate and moniliform, and both showing a tendency to fragment (Online Resource 20e). According to the results of phylogenetic studies (Mytnik-Ejsmont 2011), *P. rosea* is in the same clade of *P. concreta* and other representatives of the nominal section (with moniliform trichomes).

Labellar trichomes play an important role, providing tactile and sometimes olfactory stimuli, as well as containing a range of floral pigments, thereby guiding the pollinator and thus facilitating pollination. Furthermore, papillae are able to concentrate solar energy and provide a sufficiently warm environment on the surface of the flower for optimal insect activity. Conversely, reflection of heat by papillae also helps maintain an optimal temperature, and reflection of light (especially in orchid species possessing a speculum) helps attract potential pollinators, whereas its reflection by epidermal striae helps maintain the ‘brightness’ of the flower, further aiding the pollination process (Davies et al. 2006). Some labellar papillae and trichomes also contain food materials that both attract and reward pollinators, and thus reinforce pollinator behaviour (Davies and Turner 2004). The presence, diversity and location of such trichomes are also important in plant identification and plant taxonomy (Davis and Heywood 1963). However, caution needs to be exercised when considering such characters, as in some cases, they may have no bearing whatsoever on taxonomy, but simply reflect pollinator specificity and the pollination strategy employed (Davies and Turner 2004). As such, floral micromorphology characters should be used in taxonomy only in conjunction with other more reliable characters. For example, the labella of all members of sect. *Polystachya* hitherto studied, with the sole exception of *P. paniculata* (a taxonomically problematic species), bear moniliform trichomes, but all are united in their general gross morphology. Therefore, should *P. paniculata* be removed from this section? This poses a taxonomic conundrum since some authors (Kraenzlin 1926; Cribb 1984; La Croix and la Croix 1997) place *P. paniculata* in section *Caulescentes* (its members are characterised by clustered, reed-like stems), whereas others (Piers 1968; Mytnik-Ejsmont 2011) assign it to section *Polystachya* on account of its distinctive pseudobulbs. However, *P. paniculata* is a distinct species, even for the nominal section, owing to its flower colour and entire lip, a combination of characters which is perhaps rather unique to the genus as a whole, but in particular to section *Polystachya*. What is more remarkable is that molecular studies (Mytnik-Ejsmont

2011) also confirm its unique status. The topology of trees based on Bayesian and parsimony analysis of combined data shows that *Polystachya paniculata* (together with *P. steudneri*) is not part of the clade representing section *Polystachya*, but forms an isolated clade sister to other clades (Mytnik-Ejsmont 2011, page 47, the strict consensus of the 108 most parsimonious trees in combined plastid (*rpl16-trnK*, *rpl16*, *rpl32-trnL*) analysis for the subtribe Polystachyinae). Since micromorphology data for the labellum raises further doubts, one is perhaps justified in raising this unique species to sectional rank.

The labella of *Polystachya thomensis* and *P. rosea* also possess moniliform hairs and there is sufficient justification for assigning the former species to sect. *Caulescentes*, based on gross morphology and molecular analyses (Mytnik-Ejsmont 2011, page 42, the trees from the Bayesian and parsimony analyses, clade III). Conversely, the latter species, assigned to section *Pseudomonophyllae* by Mytnik-Ejsmont (2011) is problematic, since based on the results of molecular analyses (Mytnik-Ejsmont 2011, page 42, the trees from the Bayesian and parsimony analyses, clade I), it belongs to the same clade as does *P. concreta* and other representatives of the nominal section *Polystachya* (which also possess moniliform trichomes similar to those present of *P. rosea*). Therefore, separating species currently assigned to sect. *Pseudomonophyllae* from those of sect. *Polystachya*, can no longer perhaps be justified and consequently, further studies on *Pseudomonophyllae* are needed.

Similarly, *Polystachya vulcanica* differs from all other species of sect. *Cultriformes* investigated in that its labellum is entirely devoid of trichomes. However, this species is the only representative of the subsection *Gracilentae* investigated, the other two species studied representing subsection *Cultriformes*. Representatives of the former subsection are relatively tall and slender plants lacking a labellar callus, whereas those of the latter are robust plants with a labellar callus. Differences between the labellar micromorphology of *P. vulcanica* and that of members of other species of sect. *Cultriformes* may also be taxonomically significant at sub-sectional level.

Conclusions

The study revealed seven types of papillae and five types of trichomes (uni- and multi-cellular) in *Polystachya*, some of which are described here for the first time. Clavate trichomes are the most common and are present in 60% of the species studied. Moniliform trichomes mainly occur in the sect. *Polystachya* and are strongly characteristic of the section. Pseudopollen is formed by fragmentation of moniliform

trichomes or the detachment of other trichomes as bicellular units. We also provide, for the first time, an evidence for the detachment of the terminal cells of capitate trichomes.

This study endorses the seminal work of Davies and co-workers (Davies et al. 2002), but also raises some further important questions of its own regarding the infrageneric classification of *Polystachya*, its pollination and the evolution of its labellar characters. These include: how did the various types of labellar trichome observed here evolve? Can we relate the different types of papillae and trichomes to particular roles? Clearly, when it comes to understanding *Polystachya*, we have only just scraped the surface.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This chapter does not contain any studies involving human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Information on Electronic Supplementary Material

Online Resource 1. Labellum of *Polystachya adansoniae*, **a–b** cylindrical, multicellular trichomes on proximal part of labellum, **c–f** central part of labellum with 2–4-celled trichomes with tapering, pointed apices.

Online Resource 2. Labellum of *Polystachya coriscensis*, **a–c** central part with cylindrical 1- to 2-celled trichomes, **d** obpyriform to hemispherical papillae on the epichile. Note the presence of cuticular striae.

Online Resource 3. Labellum and gynostemium of *Polystachya galeata*, **a–b** proximal part of labellum with conical papillae and clavate, 2–3-celled trichomes, **c** proximal part of labellum with developing papillae and a clavate trichome, **d–f** central part of the labellum (the ridge), **g–h** rugose epichile with clavate trichomes and conical and obpyriform to hemispherical papillae, **i** the gynostemium with sparse trichomes and papillae, **j** the column-foot with individual clavate trichomes and papillae.

Online Resource 4. Labellum and gynostemium of *Polystachya nyanzensis*, **a–d** proximal and central part of the labellum with clavate, multicellular trichomes, **e–g** central and distal part of the labellum with

conical and obpyriform to hemispherical papillae and two-celled trichomes, **h** column-foot with sparse clavate, two-celled trichomes.

Online Resource 5. Labellum, gynostemium and dorsal sepal of *Polystachya laxiflora*, **a-b** proximal part of the labellum with conical and obpyriform to shortly hemispherical papillae and 2-celled, clavate trichomes, **c-d** central part of the labellum with 2-celled, clavate trichomes and obpyriform papillae, **e-g** distal part of the labellum with clavate trichomes and conical and villiform papillae, **h-j** gynostemium with cylindrical and clavate trichomes, **k** adaxial surface of dorsal sepal with, conical papillae and clavate trichomes. Note the cuticular striae (**i-j**) and that there is some evidence of cuticular blistering and possible secretion on the surface of the terminal cell of certain trichomes (**d** and **i**).

Online Resource 6. Labellum and gynostemium of *Polystachya benettiana*, **a** labellum, **b-d** cylindrical, one- or two-celled trichomes, **e-f** ridged distal part of labellum with unicellular trichomes and obpyriform to hemispherical or spherical papillae, **g-h** labellar surface showing residues of secreted material, **i-j** gynostemium with unicellular, cylindrical trichomes beneath the stigma.

Online Resource 7. Labellum and gynostemium of *Polystachya albescentis* subsp. *imbricata*, **a** labellum, **b-c** proximal part of labellum with clavate, 2- to 4-celled trichomes and obpyriform papillae, **d-e** labellar callus, **f** labellum epichile with hemispherical and obpyriform papillae, **g-h** epichile with trichomes, **i-k** trichomes on the column with more elongate terminal cells.

Online Resource 8. The labellum and gynostemium of *Polystachya thomensis*, **a** labellum, **b-e** moniliform trichomes (**d-e**) occur on the proximal part of the labellum, **f-h** detail of the labellar callus, **i** callus comprising obpyriform to hemispherical to almost spherical papillae, and margin of lateral lobe of labellum with villiform papillae and moniliform trichomes, **j-k** epichile of labellum with obpyriform to hemispherical papillae and moniliform trichomes, **l** column-foot with hemispherical papillae and trichomes with distinct heads.

Online Resource 9. Labellum and adaxial surface of dorsal sepal of *Polystachya concreta*, **a** entire flower, **b** labellum, **c-d** proximal part of the labellum with cylindrical trichomes, **e-f** callus with cylindrical and capitate trichomes, **g-h** central part of the labellum with moniliform trichomes along the callus, **j-l** epichile of labellum with moniliform and cylindrical trichomes, together with obpyriform to hemispherical papillae.

Online Resource 10. Labellum of *Polystachya odorata*, **a** entire labellum, **b-c** trichomes, **d-e** striate moniliform trichomes with inflated terminal cells, **f** labellum callus lacking trichomes, **g** distal part of the labellum with obpyriform to hemispherical papillae, **h** labellar papillae showing cuticular striations.

Online Resource 11. Labellum of *Polystachya lineata*, **a** labellum, **b-f** unicellular, cylindrical trichomes and multicellular trichomes, the latter comprising chains of up to five cells, **g** stage in fragmentation of trichome, **h** view of central and distal part of labellum.

Online Resource 12. Labellum and gynostemium of *Polystachya paniculata*, **a-d** proximal part of the labellum with obpyriform papillae and 2-celled, clavate and unicellular cylindrical trichomes, **e** epidermal cells of the labellum with smooth cuticle, **f** details of glabrous gynostemium.

Online Resource 13. Labellum and gynostemium of *Polystachya simplex*, **a** entire labellum, **b-d** bicellular, clavate trichomes on proximal and central part of the labellum, **e-g** rugose epichile of labellum with, unicellular, cylindrical trichomes and striate obpyriform, hemispherical or almost spherical papillae, **h** column-foot with clavate and cylindrical trichomes.

Online Resource 14. Labellum and gynostemium of *Polystachya bamendae*, **a** entire labellum, **b** proximal part of labellum with clavate trichomes, **c** callus with scant clavate trichomes, **d-e** central part of the labellum with conical and villiform papillae, **f-g** epichile with clavate trichomes and conical, obpyriform and villiform papillae, **h** residues of

secretion or detritus on epichile, **i-k** column-foot with shortly villiform and conical papillae with pointed or rounded apices.

Online Resource 15. Labellum of *Polystachya bella*, **a** entire labellum, **b** the proximal part of the labellum bears mainly obpyriform papillae together with clavate and cylindrical, trichomes, **c** callus with scattered, clavate trichomes and mainly obpyriform papillae, **d** callus surface with overlying secretion obfuscating its topography, **e-g** margin of hypochile with conical, villiform, obpyriform and hemispherical papillae, often with striate cuticle. The overlying film of secreted material is initially deposited between individual cells, **h** moderately rugose epichile.

Online Resource 16. Labellum of *Polystachya pubescens*, **a** entire labellum, **b-d** one- to three-celled cylindrical trichomes on labellum margins, **e** glabrous epichile of labellum, **f-g** adaxial surface of lateral sepal with unicellular or few-celled, cylindrical trichomes, **h** similar trichomes also occur on the petals.

Online Resource 17. Labellum and gynostemium of *Polystachya cultiformis*, **a** entire labellum, **b** callus with bicellular, clavate trichomes, **c-d** central part of labellum with clavate trichomes, **e-f** epichile with two-celled trichomes and conical papillae, **g** proximal part of labellum with film of secretion partly obfuscating topography, **h-i** margin of proximal part of labellum base with conical and obpyriform papillae, and ellipsoid trichomes, **j** gynostemium with bicellular trichomes and striate epidermal cells.

Online Resource 18. Labellum of *Polystachya maculata*, **a** entire labellum, **b** proximal part of labellum with multicellular, clavate trichomes, **c** general view of callus, **d** detail of callus with clavate trichomes, **e** callus with columnar trichome, **f** detail of clavate trichome with inflated terminal cell, **g** general view of rugose epichile, **h-i** atrichomatous cells of labellum with striate cuticle.

Online Resource 19. Labellum of *Polystachya vulcanica*, **a-b** proximal part of labellum with elongate cells, **c-d** central and distal part of labellum with isodiametric cells and obpyriform and hemispherical papillae, **f-g** epichile with obpyriform and conical papillae, **h** general view of glabrous gynostemium.

Online Resource 20. Labellum, gynostemium and petals of *Polystachya rosea*, **a** entire labellum, **b-c** proximal part of labellum clothed with clavate and moniliform trichomes, **d** proximal part of labellum with conical, obpyriform and villiform papillae, **e** trichomes on proximal part of the labellum showing fragmentation and detachment of terminal cells. Note that such detachment of terminal cells may result in the formation of “unicellular, cylindrical hairs”. These, however, can be distinguished from intact cylindrical hairs by their truncated appearance and the flattened outer tangential wall (the original cross-wall of the intact bicellular trichome) with its associated, round scar (point of attachment of terminal cell), **f** details of central part of labellum, **g-h** epichile with obpyriform and villiform papillae, together with moniliform trichomes, **i** column-foot with conical and obpyriform papillae, **j** clavate trichome on ventral column surface, **k** a similar clavate trichome on adaxial surface of petal.

References

- Arditti J (1992) Fundamentals of orchid biology. Wiley-Interscience, New York
- Brenan JPM (1954) Plants collected by the Vernay Nyasaland expedition of 1946. Mem New York Bot Gard 9:1–132
- Brummitt RK, Powell CE (1992) Authors of Plant Names: a list of authors of scientific names of plants, with recommended standard forms of their names, including abbreviations. Kew, England
- Cribb PJ (1978) Studies in the genus *Polystachya* (Orchidaceae) in Africa. Kew Bull 32:743–766

- Cribb PJ (1984) Orchidaceae. In: Polhill RM (ed) Flora of tropical East Africa. Balkema, Rotterdam, pp 237–412
- Davies KL (2009) Food-hair form and diversification in orchids. In: Kull X, Arditti TJ, Wong SM (eds) Orchid biology: reviews and perspectives. Springer, Berlin, pp 159–184
- Davies KL, Roberts DL, Turner MP (2002) Pseudopollen and food-hair diversity in *Polystachya* Hook. (Orchidaceae). *Ann Bot (Oxford)* 90:477–484
- Davies KL, Stpiczyńska M, Turner MP (2006) A rudimentary labellar speculum in *Cymbidium lowianum* (Rchb.f.) Rchb.f. and *Cymbidium devonianum* Paxton (Orchidaceae). *Ann Bot (Oxford)* 97:975–984
- Davies KL, Stpiczynska M, Kaminska M (2013) Dual deceit in pseudopollen-producing *Maxillaria* s.s. (Orchidaceae: Maxillariinae). *Bot J Linn Soc* 173:744–763
- Davies KL, Turner MP (2004) Morphology of floral papillae in *Maxillaria* Ruiz and Pav. (Orchidaceae). *Ann Bot (Oxford)* 93:75–86
- Davis PH, Heywood VH (1963) Principles of Angiosperm Taxonomy. Van Nostrand, Princeton
- Dodson CH, Frymire GP (1961) Natural pollination of orchids. *Missouri Bot Gard Bull* 49:133–139
- Dodson CH (1962) The importance of pollination in the evolution of the orchids of tropical America. *Amer Orchid Soc Bull* 31:525–735
- Dressler RL (1993) Phylogeny and classification of the orchid family. Timber Press, Portland
- Goss GJ (1977) The reproductive biology of the epiphytic orchids of Florida 6. *Polystachya flavescens* (Lindl.) J.J. Smith *Amer Orchid Soc Bull* 46:990–994
- Janse JM (1886) Imitirte pollenkorner bei *Maxillaria* sp. *Ber Deutsch Bot Ges* 4:277–283
- Karcz J (2009a) Mikroskopia elektronowa skaningowa w biologii. Uniwersytet Śląski, Katowice
- Karcz J (2009b) Etapy przygotowania preparatów do badań w mikroskopie skaningowym. Uniwersytet Śląski, Katowice
- Karcz J (1996) Skaningowy mikroskop elektronowy w badaniach karpologicznych. *Wiadom Bot* 40(3/4):55–65
- Kjellsson G, Rasmussen FN (1987) Does the pollination of *Dendrobium unicum* Seidenf. involve pseudopollen? *Orchidee* 38:183–187
- Kraenzlin F (1926) Monographie der Gattung *Polystachya* Hook. *Repert Spec Nov Regni Veg Beih* 39:1–136
- Krahl AH, Holanda ASS, Krahl DRP, Martucci MEP, Gobbo-Neto L, Webber AC, Pansarin ER (2019) Study of the reproductive biology of an Amazonian *Heterotaxis* (Orchidaceae) demonstrates the collection of resin-like material by stingless bees. *Pl Syst Evol* 305:281–291
- La Croix IF, la Croix E (1997) African orchids in the wild and in cultivation. Timber Press, Portland
- Mytnik-Ejsmont J (2011) A monograph of the subtribe Polystachyinae Schltr. (Orchidaceae). Wydawnictwo Uniwersytetu Gdańskiego, Gdańsk
- Pansarin ER, Amaral MCE (2006) Reproductive biology and pollination of two species of *Polystachya* Hook. in southeastern Brazil: evidence of in Polystachyae (Orchidaceae). *Revista Brasil Bot* 29:423–432
- Pansarin ER, Amaral MCE (2008) Pollen and nectar as a reward in the basal epidendroid *Psilochilus modestus* (Orchidaceae: Triphoreae): A study of floral morphology, reproductive biology and pollination strategy. *Flora* 203:474–483
- Pansarin ER, Alves-dos-Santos I, Pansarin LM (2017) Comparative reproductive biology and pollinator specificity among sympatric *Gomesa* (Orchidaceae: Oncidiinae). *Pl Biol* 19:147–155. <https://doi.org/10.1111/plb.12525>
- Pansarin ER, Pansarin LM (2014) Reproductive biology of *Epidendrum tridactylum* (Orchidaceae: Epidendroideae): a reward-producing species and its deceptive flowers. *Pl Syst Evol* 300:321–328. <https://doi.org/10.1007/s00606-013-0884-9>
- Pansarin ER, da Silva UF, Amaral MCE (2009) Reproductive biology and pollination of southeastern Brazilian *Stanhopea* Frost ex Hook. (Orchidaceae). *Flora* 204:238–249. <https://doi.org/10.1016/j.flora.2008.01.014>
- Pansarin ER, Maciel AA (2017) Evolution of pollination systems involving edible trichomes in orchids. *AoB PLANTS*. <https://doi.org/10.1093/aobpla/plx033>
- Pettersson B, Nilsson LA (1993) Floral variation and deceit pollination in *Polystachya rosea* (Orchidaceae) on an inselberg in Madagascar. *Opera Bot* 121:237–245
- Piers F (1968) Orchids of East Africa, 2nd edn. Cramer, Lehre
- Podzorski AC, Cribb PJ (1979) A revision of *Polystachya* sect. *Cultriformes* (Orchidaceae). *Kew Bull* 34:147–186
- Reis MG, Pansarin ER, da Silva UF, Amaral MCE, Marsaioli AJ (2004) Pollinator attraction devices (floral fragrances) of some Brazilian orchids. *Arkivoc* 6:103–111
- Russell A, Samuel R, Rupp B, Barfuss MHJ, Safran M, Besendorfer V, Chase MW (2010) Phylogenetics and cytology of a pan-tropical orchid genus *Polystachya* (Polystachyinae, Vandaeae, Orchidaceae): evidence from plastid DNA sequence data. *Taxon* 59:389–404
- Russell A, Samuel R, Klejna V, Barfuss MHJ, Rupp B, Chase MW (2010) Reticulate evolution in diploid and tetraploid species of *Polystachya* (Orchidaceae) as shown by plastid DNA sequences and low-copy nuclear genes. *Ann Bot (Oxford)* 106:37–56
- Stpiczyńska M, Davies KL, Kamińska M (2015) Diverse labellar secretions in African *Bulbophyllum* (Orchidaceae: Bulbophyllinae) sections *Ptiloglossum*, *Oreonastes* and *Megaclinium*. *Bot J Linn Soc* 179:266–287
- Stpiczyńska M, Plachno BJ, Davies KL (2018) Nectar and oleiferous trichomes as floral attractants in *Bulbophyllum saltatorium* Lindl. (Orchidaceae). *Protoplasma* 255:565–574
- Summerhayes VS (1942) African Orchids: XII. *Bot Mus Leaf* 10:257–299
- Summerhayes VS (1947) African orchids: XVII. *Kew Bull* 2:123–133
- Van der Cingel NA (2001) An atlas of orchid pollination: America, Africa, Asia and Australia. A.A Balkema Publishers, Rotterdam
- Van der Pijl L, Dodson CH (1969) Orchid flowers: their pollination and evolution. University of Miami Press, Coral Gables
- Vogel S (1978) Evolutionary shifts from reward to deception in pollen flowers. In: Richards AJ (ed) The pollination of flower by insects, linnean society symposium series. Academic Press, London, pp 89–96
- Wróbel B, Zienkiewicz K, Smoliński D, Niedojadło J, Świdziński M (2005) Podstawy mikroskopii elektronowej. Skrypt dla studentów biologii, Uniwersytet Mikołaja Kopernika, Toruń

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