

Notes on the self-pollination of *Dendrobium biflorum* (G. Forst.) Sw. (Orchidales, Dendrobiinae)

Agnieszka K. Kowalkowska^{1*}, Hanna B. Margońska²

¹ Department of Plant Cytology and Embryology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

² Department of Plant Taxonomy and Nature Conservation, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

Abstract

The conditions favouring self-pollination are determined for a species of *Dendrobium* Sw. A survey of gynostemium micromorphology in collected flowers of *Dendrobium biflorum* revealed two stages of a unique form of autogamy: on germination of pollen tetrads, pollen tubes reached the stigmatic region without the pollen being displaced from the anther locules. This is the first time for this type of autogamy to be reported in *Dendrobium biflorum*. Pollen grains germinated directly from the locules, bypassing the stigma or falling or sliding down onto the stigmatic surface. The pollen tubes were long and reached the stylar canal. On The Society Islands, differences in exposure of the habitats to weather conditions and altitude gradients influence both composition and form of the vegetation, as well as the occurrence of pollinators. The form of autogamy described here may be the usual method of pollination found on The Society Islands (and even the whole of French Polynesia), where suitable pollinators are absent and/or growth conditions stressful.

Keywords: autogamy, *Dendrobium biflorum*, gynostemium micromorphology, Orchidaceae, The Society Islands

Introduction

Members of the Orchidaceae are well-known for their complex flowers. A particular type of floral morphology, colour pattern, fragrance or reward, all result in the attraction of a specific pollinator, and thus, the tendency for autogamy (automatic self-pollination) is reduced [1–3]. Nevertheless, in populations living in habitats where the occurrence of potential pollinators is low, autogamy is the preferred method of pollination and, in fact, is a rather common phenomenon within the family [3,4]. Autogamy is estimated to occur in approximately 3% of orchid species [2], or even as much as 5–20% [5]. In pollinator-free environments and under stressful growing conditions, the facultative process of self-pollination occurs at the end of anthesis (with the ability for cross-pollination maintained in flower fitness) [6]. Self-pollination mechanisms have been confirmed for two species of *Dendrobium* Sw. [5]. In *D. chryseum* Rolfe and *D. mastersianum* F. Muell. & Kraenzl, entire pollinia, owing to the close proximity of anther and stigma, were observed to fall or slide down onto the stigma, thus resulting in self-pollination.

Owing to different, recently proposed, taxonomic approaches to the genus *Dendrobium* and the publication of a second, unwarranted, heterotypic description of the species *D. biflorum*, we present our taxonomic treatment of the genus and describe the specimens studied, so as to avoid future errors in identification. *Dendrobium* Sw. [nom cons. Nova Acta Regiae Soc Sci Upsal 1799;6:82. Generitype: *Dendrobium moniliforme* (L.) Sw. typ. cons.] is a large genus containing ca. 600 species, widely distributed in subtropical and tropical regions of Asia and Australia to New Zealand and Oceania. The plants are epiphytic, lithophytic to rarely terrestrial, small to large, forming colonies of various sizes. Their shoots are cane-like or pseudobulbous, few- to multinodal, erect to pendent, whereas leaves are coriaceous or fleshy, conduplicate, sessile or petiolate. The inflorescence may be lateral, axillary to pseudoterminal, racemose to paniculate, with single to many flowers, which are small to medium-sized, often showy, resupinate or not. Lateral sepals are basally adnate to the column foot, forming a distinct mentum. Lip is usually 3-lobed or entire, spurless, mobile or not, attached to the apex of the column foot, embracing the gynostemium, whereas the disc is furnished with calli, ridges, lamellae or other appendages. The column part is prominent, longer than anther, column foot usually very long, sometimes longer than the column part, massive. Stigma is semi-ellipsoid, oblong to semi-ovoid, deeply concave. Rostellum is very short, incumbent, truncate, thickened along the margins. Viscidium is single, narrow, detachable, multilayered or absent. Staminal nodes have usually massive, wing-like form. Clinandrium is apical, rather voluminous to relatively obscure, entire to 3-lobed. Anther is incumbent, mobile, ellipsoid or ovoid, 2-chambered. Connective is narrow, but very thick, papillate

* Corresponding author. Email: dokakow@ug.edu.pl

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or not, forming a roof in front over the chambers. Pollinia are 4 in 2 pairs, clavate or oblong, apically distinctly attenuate. Caudicles are absent.

Dendrobium biflorum (G. Forst., Fl Ins Austr. 1786;60) Sw., Nova Acta Regiae Soc Sci Upsal. 1799;6:84, non *Dendrobium biflorum* A. Rich, Ess Fl N Zel. 1832;167 (= *Dendrobium cunninghami* Lindl., Bot Reg. 1835;21: sub t. 1756, non *Dendrobium cunninghami* Steud, Nomencl Bot. 2nd ed. 1840;1:490, nom. illeg.) is one of the representatives of the genus [section *Grastidium* (Bl.) J. J. Sm.]. The species is rather widely distributed. It occurs in New Guinea and neighbouring islands, in Oceania from Solomon Islands, Vanuatu, Fiji, Samoa, New Caledonia to The Society Islands. At The Society Islands it was recorded from Maupiti, Bora Bora, Huahine, Tahaa, Raiatea, Moorea, Mehetia and Tahiti, where the type-specimens were collected (G. Forster f. 169 LECTO-K!, ISOLECTO-G!). In most habitats *D. biflorum* is medium-sized to large, (20)50–160 cm tall, with rhizome branched, rooted and scaled at nodes. Shoot is 0.2–0.6 cm in diameter, erect to pendent according to size, age and habitat, elongate, cane-like, multi-nodal, lignified, leafy at apical part. The leaves are numerous, distichously

arranged, with blades 5–10(17) cm long, 0.4–0.8 cm wide, thin. Inflorescences are axillary, a few at any given time, always 2-flowered with peduncle strongly contracted. Flowers up to ca. 3 cm in diameter when spread, short-lived, white (Fig. 1b,d), becoming creamy-white when older. All tepals are thin and delicate, nearly translucent, lustrous, somewhat similar in general outline. Lip is 3-lobed, spirally recurved at the apex, base tinged with pale pink, lateral lobes with dark and deep brown-red to purple-red margins, lateral fimbriae of the mid-lobe tinged or blotched brown-red to purple-red. Hypochile is more fleshy, base constricted and adnate to the apex of the column foot, its lateral lobes oblique, oblong-triangular to semi-ovate, obtuse to subacute, with anterior margins usually entire, posterior margins erose. Middle lobe is narrowly triangular, attenuate and filiform at the apex, with lateral margins strongly and deeply undulate, erose, crenulate to fimbriate. Its central surface has ca. 3 rows of papillae, whereas the disc is ornamented with oblong, flattened thickening, usually vanishing at the base of the middle lobe. Gynostemium morphology of *Dendrobium biflorum* exhibited features characteristic of cross-pollination. Gynostemium (Fig. 2a,b) is 0.15–0.2 cm

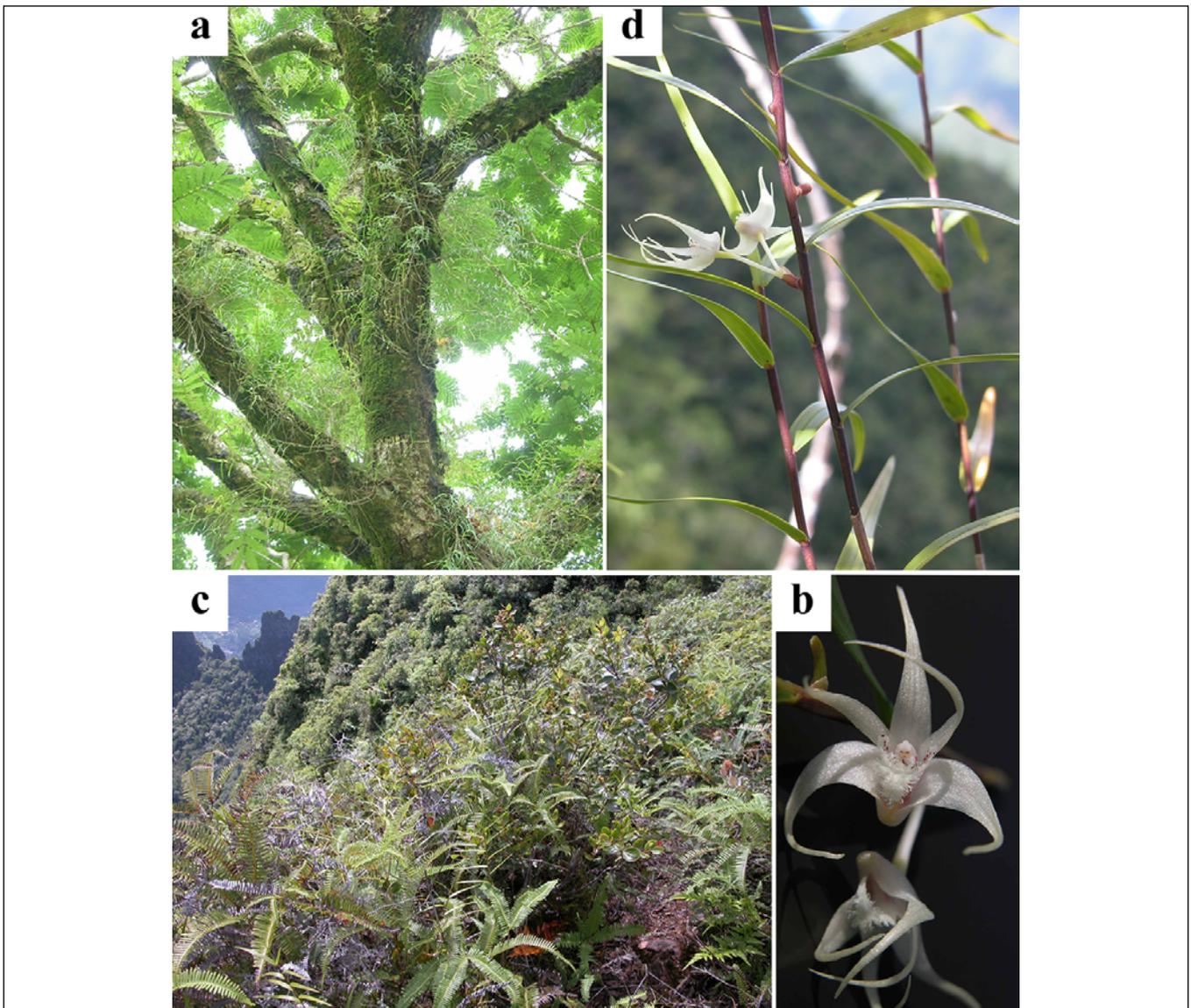


Fig. 1 *Dendrobium biflorum* (G. Forst.) Sw. **a** Epiphytic plants as found in most typical lowland habitats, forming large, dense clusters and colonies on tree trunks and branches. **b** Flowers of lowland plants (Tahiti, Mahape Valley, 2007.09.07). **c** Terrestrial plants (much smaller, erect and more robust) from highland habitats. **d** Flowers of highland plants (Moorea, Mount Rotui, 2007.10.22). Phot. H. B. Margońska.

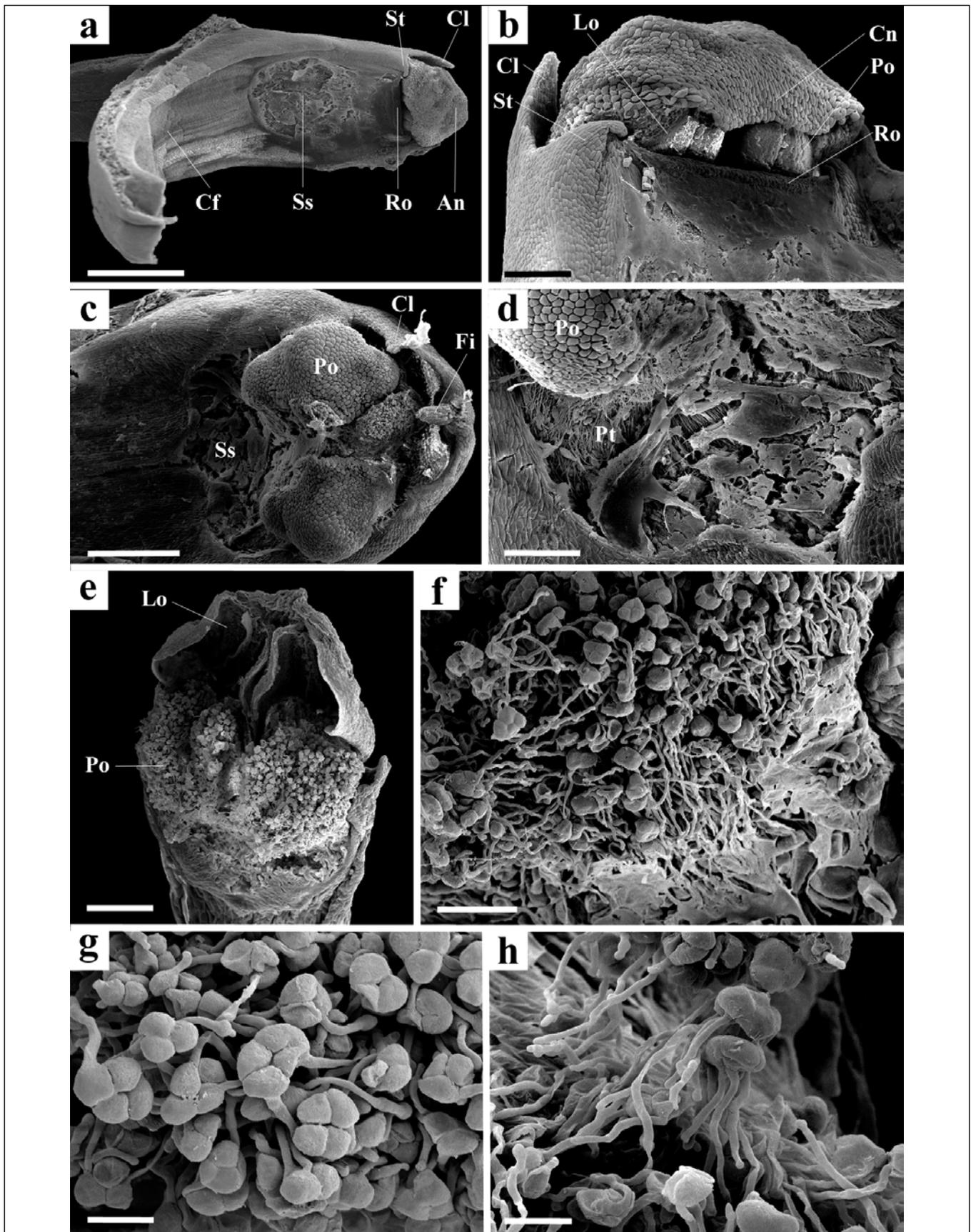


Fig. 2 SEM images showing gynostemium morphology of *Dendrobium biflorum* (G. Forst.) Sw. **a** Anterior view of gynostemium of typical flower (without autogamy) derived from a lowland plant (Fig. 1a,b). **b** Detail of anther with four pollinia. **c** Anterior view of gynostemium of self-pollinated flower from Moorea, Belvedere Plateau. **d** Detail of **c** showing long pollen tubes growing directly from locules (without releasing pollen) towards the stigma. **e** Gynostemium of flower of highland plant (Moorea, Mount Rotui) in advanced stage of autogamy. **f** Detail of pollinia (from **e**) showing germinating pollen grains growing towards the stigmatic surface. **g,h** Germination of pollen grains in tetrads (detail of **f**). An – anther; Cf – column foot; Cn – connective; Fi – filament; Lo – locules; Po – pollinium; Pt – pollen tubes; Ro – rostellum; St – staminodium; Ss – stigmatic surface. Scale bars: **a** 1 mm; **b,d,e** 200 µm; **c** 500 µm; **f** 50 µm; **g,h** 20 µm. Phot. A. K. Kowalkowska.

long, erect, with column foot longer than the column part. The stigma is semi-ellipsoid, deeply concave, distinctly limbate with erect rim, with secretion. The rostellum is very short, incumbent, truncate, thickened along the margins. The staminodes are rather obscure, subparallel to the column, oblong, wing-like, larger and more massive closer to the stigma, distally attenuate. The clinandrium is apical, rather distinct and 3-lobed. The filament is narrow and incorporated into distal part of clinandrium. The anther is incumbent in natural position, mobile, semi-ovoid, 2-chambered. Both locules are well separated by the connective. The connective is rather narrow, but thick and glabrous, forming a short and wide roof in front over the locules. Pollinia are 4 in 2 pairs, clavate, apically distinctly attenuate. The pollen grains are arranged in tetrads.

Amongst the numerous flower specimens of *D. biflorum* collected, we found several examples of a unique form of autogamy: the pollen tetrads germinated to the stigmatic region without being displaced from the anther locules. In this article, we compare the morphological features of the gynostemium in typical flowers of the species with those of three selected specimens showing different stages of self-pollination. We also discuss reasons for this particular type of autogamy.

Material and methods

A study of the taxonomy of the orchid flora of The Society Islands (French Polynesia) by Margońska, resulted in the analysis of more than 1500 herbarium and spirit-preserved specimens deposited in over 20 collections. The field observations [over 300 specimens studied, records included in Archivum Orchidialium (Arch. Orch.)] and preserved samples (deposited as part of UGDA-HBM) are the result of two scientific expeditions in 2007 and 2009 to The Society Islands (Tahiti, Mo'orea, Rai'atea, Taha'a and Bora Bora). We have chosen three specimens of *D. biflorum* for micromorphological studies and to demonstrate the various stages of autogamy found in this species (Tab. 1). Plant material was fixed in 2.5% glutaraldehyde (GA) and 2.5% paraformaldehyde (PFA) in 0.05 M cacodylate buffer (pH = 7.0) at room temperature. For scanning electron microscopy (SEM), the samples, following dehydration in an ethanol series, were subjected to critical-point drying using liquid CO₂, coated with gold and observed using a Philips XL-30 SEM at an accelerating voltage of 15 kV. For light microscopy (LM), the plant material, following fixation and dehydration in ethanol, was embedded in epoxy resin (Technovit) and stained with 0.05% toluidine blue O (TBO) in 1% aqueous sodium tetraborate solution for 1 min at 60°C on

a hot plate [7,8]. Samples were prepared in accordance with procedures described previously [9–11].

Results

Micromorphology

SEM and LM studies revealed floral dissimilarities between plants derived from lowland and highland habitats. Flowers from the lowland part of the Mahape Valley, Tahiti (Fig. 1a,b, Fig. 2a,b), were observed to have developed fully in typical manner. The four pollinia were hidden beneath the anther cap. The stigma was copiously coated with secretion and germination of pollen tetrads was not observed.

Samples gathered from Mo'orea, the Belvedere Plateau (Fig. 2c,d) and the highest crest of Mount Rotui (Fig. 1c,d, Fig. 2c,d) displayed two different stages of self-pollination. In the first set of samples (Belvedere Plateau; Fig. 2c,d), the long pollen tubes germinated directly from the locules, without the release of pollen from the anther, and passed through the copious stigmatic exudate towards the stigma. In the second set of samples (from the crest of Mount Rotui; Fig. 1c,d), more advanced autogamous stages were observed: the anther thecae (locules) were nearly empty and the pollen tetrads had slid down slightly towards the stigmatic surface (Fig. 2e). All pollen grains had germinated (including each component of a tetrad, Fig. 2f–h), their pollen tubes growing towards the stigmatic surface (Fig. 2f, Fig. 3a). Further LM observations of longitudinal sections of the column (Fig. 3c,d) demonstrated that pollen grains germinated after sliding down onto the stigmatic surface and also directly from locules, bypassing the stigma. The pollen tubes grew (Fig. 3a,b) and reached the stylar canal (Fig. 3c,d).

Discussion

A similar autogamous pollination mechanism, where flowers display both autogamous and allogamous traits, occurs in *Epipactis microphylla* (Ehrh.) Sw. [6]. Morphological features of the gynostemium related to the mechanisms of cross-pollination are barely noticeable: a rostellum is present, but very short; it is coated with adhesive substance and is not easily detached from the stigma. Conversely, a few autogamous traits are also displayed. A distinct anther is supported by a short, narrow filament, the clinandrium is poorly developed and pollen grains remain packed in tetrads, even after sliding down to the stigma. We agree with Bonatti et al. [6], who claimed that the

Tab. 1 Samples used for micromorphological studies (deposited in UGDA-HBM) and the results of SEM observations.

Species name	Place and date of collection	Collector and voucher number	Observation of autogamy
<i>Dendrobium biflorum</i> (G. Forst.) Sw.	Tahiti, Mahape Valley, 2007.09.07.	Margońska H. B. 7TAHMahapeVal20092007 07aa	germination of pollen tetrads not observed
<i>Dendrobium biflorum</i> (G. Forst.) Sw.	Mo'orea, Belvedere Plateau 2007.10.12.	Margońska H. B. 21MREABelved 212102007 03a-c	pollen tetrads germinated directly from locules by passing stigma
<i>Dendrobium biflorum</i> (G. Forst.) Sw.	Mo'orea, Mount Rotui, 2007.10.22.	Margońska H. B. 29MREAMtRotui 22102007 04ff	pollen tetrads slightly slid down towards the stigma, mainly germinated from locules bypassing stigma, excluding some pollen tubes emerged from stigmatic surface

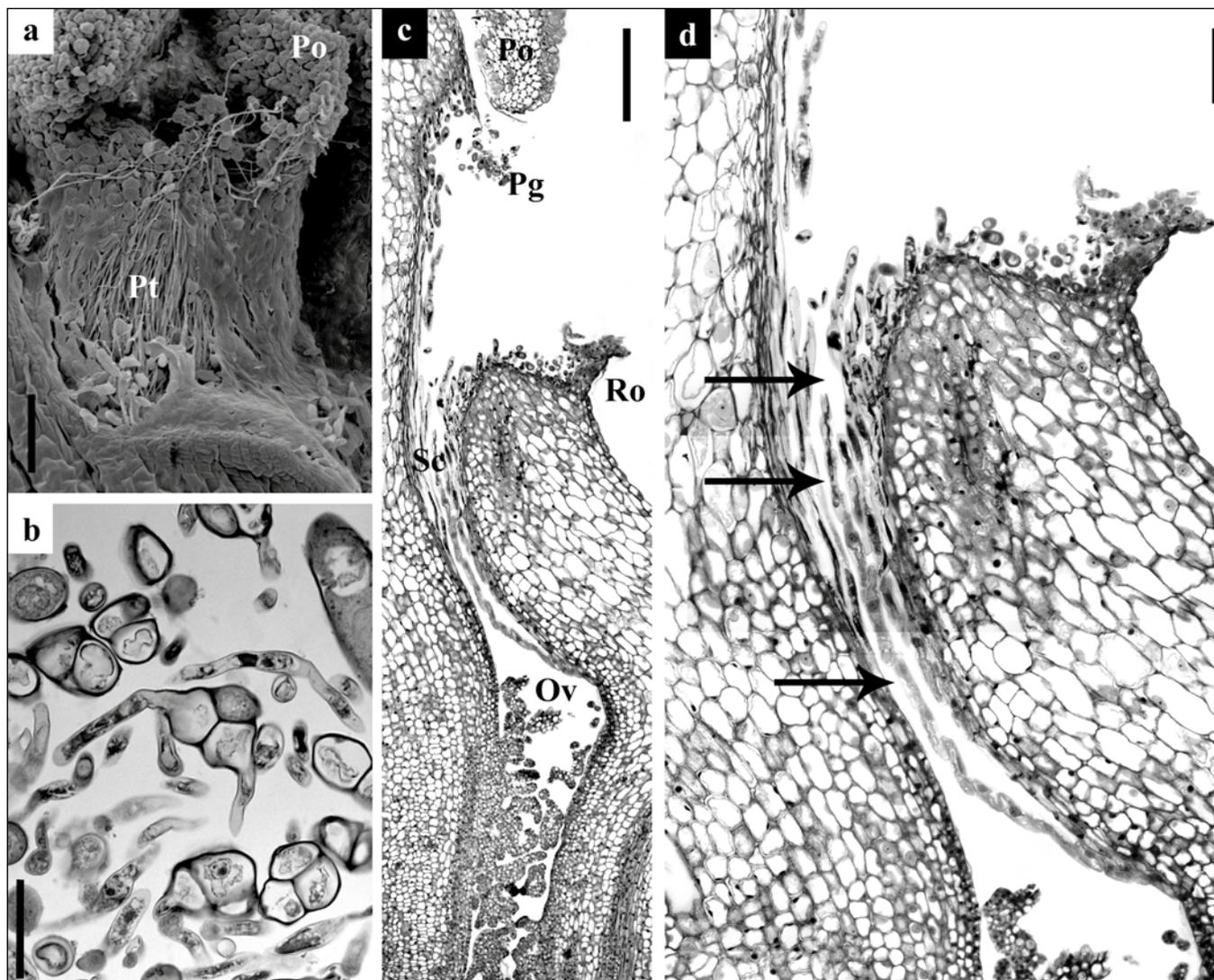


Fig. 3 *Dendrobium biflorum* (Moorea, Mount Rotui). **a** Anterior view of gynostemium showing long pollen tubes growing directly from locules (SEM). **b** Germinated pollen grains (LM). **c** Longitudinal section of the column, stylar canal and ovary. The pollen tubes have elongated and reached the stylar canal (LM). **d** Detail of stylar canal (arrowheads) showing pollen tubes growing towards the ovules (LM). Ov – ovary; Po – pollinium; Pg – pollen grains; Pt – pollen tubes; Ro – rostellum; Sc – stylar canal. Scale bars: **a–d** 100 μ m; **b** 25 μ m; **c** 250 μ m. Phot. A. K. Kowalkowska.

adhesive rostellum and the stigmatic exudates, located along the upper border restraining the clinandrium cavity, seem to favour both the retention of released pollen from the locules and growth of the pollen-tube. The extension of pollen tubes in *D. biflorum* was observed both in locules and also after the pollen had slid down slightly onto the stigmatic surface. In flowers of *E. microphylla* (cleistogamous and also chasmogamous species) pollen germinated inside the anther, transiently or totally bypassing contact with the receptive stigma [6,12–16]. Bonatti et al. [6] maintained that in cleistogamous and self-pollinated flowers, pollen tubes emerging from pollen grains retained in the locules were growing beneath the stigmatic surface layer. In *D. biflorum*, we also observed the presence of developing pollen tubes beneath the stigmatic exudate (Fig. 2d, Fig. 3a). Margońska, however, did not observe cleistogamous forms during field work.

A characteristic feature of many Polynesian islands is the enormous diversity of plants to be found there, variation in the floras of individual islands resulting from differences in the degree of exposure to weather conditions. On the humid, windward sides of volcanic islands, especially, tropical

phytocenosis occur, whereas on dry, windward slopes, xeromorphic, low forest and scrub prevail and become grass and/or fern vegetation.

The composition and form of vegetation on The Society Islands depends not only on its exposure to seasonal winds and rains, but also on altitude gradients. In most habitats of The Society Islands, *D. biflorum* is recognized as a rather common and abundant plant, growing between 40–1600 m. This epiphytic orchid often forms very large and dense clusters and colonies on tree trunks and branches, usually directly on bark, but always in sunny situations (Fig. 1a,b). In wetter, somewhat colder conditions, at higher elevations, for example cloud forests, they grow in deep moss-covers with many other epiphytic plants, including other orchids. At the highest altitude range for *D. biflorum*, this species grows terrestrially (Fig. 1c,d). It is worth noting that when the habitat changes markedly, even though the flowers remain identical, the plants are much smaller, erect, and robust.

Ridges of Mount Rotui (especially the North side) and its peak, have rather extreme climate conditions (although not unique in Polynesian islands). Each day, until about noon,

they are exposed to full sun, resulting in distinctly increased temperatures and aridity. During the afternoon, clouds increasingly gather around the mountain peak, especially from the Western windward side, because winds are forced upward by mountain masses. During the day, the temperature is still high, but humidity increases. Later, this rises as vapour and condenses as it makes contact with high level, colder air. Thus, heavy rains are frequent. Throughout the dry season, as well as an obvious reduction in humidity, a significant increase in temperature is observed. During the rainy season, rainfalls are more frequent, and may even last all day and night [17]. In addition, throughout all this time, the habitats of *D. biflorum* are subject to distinctly windy conditions. Not only do these climatic conditions make it difficult for many plants (lower and more xeromorphic character of the phytocenosis), but they make it especially difficult for pollinators to live in these locations. The unique kinds of autogamy recorded here for *D. biflorum* may be the result of limited opportunity for insect pollination.

Margońska (during the scientific expeditions of 2007 and 2009) and Jean-Yves Meyer, Jean-François Butaud and Jacq Fred (personal communication) did not record observing pollinators at these flowers. Instead, Margońska observed only occasional visits by ants and spiders, but these did not precipitate pollination.

Self-pollination may be the norm in *Dendrobium biflorum*, not only for The Society Islands, but perhaps for the whole of French Polynesia. The specific climatic conditions described above are typical for most of the islands of the Archipelagos, and these result in pollinator-free environments and/or stressful growing conditions. Further research into Pacific Island floras would perhaps help us better explain the occurrence of autogamy in species that are usually cross-pollinated.

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References

1. Darwin C. The various contrivances by which orchids are fertilized by insects. London: John Murray; 1890.
2. Pijl L, Dodson CH. Orchid flowers: their pollination and evolution. Coral Gables FL: University of Miami Press; 1966.
3. Dressler RL. Phylogeny and classification of the orchid family. Portland OR: Dioscorides Press; 1993.
4. Tremblay RL, Ackerman JD, Zimmerman JK, Calvo RN. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biol J Linn Soc.* 2005;84(1):1–54. <http://dx.doi.org/10.1111/j.1095-8312.2004.00400.x>
5. Catling PM. Auto-pollination in the Orchidaceae. In: Arditti J, editor. *Orchid biology: reviews and perspectives*. Portland OR: Timber Press; 1990. p. 121–158.
6. Bonatti PM, Sgarbi E, Del Prete C. Gynostemium micromorphology and pollination in *Epipactis microphylla* (Orchidaceae). *J Plant Res.* 2006;119(5):431–437. <http://dx.doi.org/10.1007/s10265-006-0001-z>
7. Feder N, O'Brien TP. Plant microtechnique: some principles and new methods. *Am J Bot.* 1968;55(1):123. <http://dx.doi.org/10.2307/2440500>
8. Ruzin SE. *Plant microtechnique and microscopy*. New York: Oxford University Press; 1999.
9. Kowalkowska AK, Margońska HB. Diversity of labellar micromorphological structures in selected species of Malaxidinae (Orchidales). *Acta Soc Bot Pol.* 2009;78(2):141–150.
10. Kowalkowska AK, Margońska HB, Koziaradzka-Kiszkurno M. Comparative anatomy of the lip spur and additional lateral sepal spurs in a three-spurred form (*F. fumeauxiana*) of *Anacamptis pyramidalis*. *Acta Biol Cracov Ser Bot.* 2010;52(1):13–18. <http://dx.doi.org/10.2478/v10182-010-0002-z>
11. Kowalkowska AK, Margońska HB, Koziaradzka-Kiszkurno M, Bohdanowicz J. Studies on the ultrastructure of a three-spurred fumeauxiana form of *Anacamptis pyramidalis*. *Plant Syst Evol.* 2012;298:1025–1035. <http://dx.doi.org/10.1007/s00606-012-0611-y>
12. Anderson WR. Cryptic self-fertilization in the Malpighiaceae. *Science.* 1980;207(4433):892–893. <http://dx.doi.org/10.1126/science.207.4433.892>
13. Lord EM. Cleistogamy: a tool for the study of floral morphogenesis, function and evolution. *Bot Rev.* 1981;47(4):421–449. <http://dx.doi.org/10.1007/BF02860538>
14. Pacini E, Franchi GG. Germination of pollen inside anthers in some non-cleistogamous species. *Caryologia.* 1982;35:205–215.
15. Mayers AM, Lord EM. Comparative flower development in the cleistogamous species *Viola odorata*. III. A histological study. *Bot Gaz.* 1984;145(1):83. <http://dx.doi.org/10.1086/337430>
16. Caiola MG, Brandizzi F, Canini A. *Hermodactylus tuberosus* L. (Iridaceae) pollen organisation before and after anther dehiscence. *Plant Biosyst.* 2000;134(3):353–364. <http://dx.doi.org/10.1080/11263500012331350505>
17. Margońska HB, Szlachetko DL. *Orchidaceae of Tahiti (Polynesie Française)*. Gdańsk: Gdańsk University Press; 2010.