Using Scanning electron microscopy to establish the floral micromorphology of the genus Restrepia (Orchidaceae) and the potential consequences for pollination

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Restrepia *(Figure 1)* is a small Pleurothallid genus, comprising 61 species, 44 of which have been discovered since 1970. These species are indigenous to Central and South America, where their montane forest habitats are under increasing pressure from changes in land use. Pleurothallids are the largest group of fly-pollinated orchids, their pollinators being the small Dipteran species common at the high elevations where they grow. With increasingly fragmented habitats and dwindling plant numbers the pollination systems of these obligate out-breeding genera may no longer function efficiently which may potentially lead to their extinction.

A study of the breeding system of the genus [1] showed that Restrepia exhibited a gametophytically controlled self-incompatibility (SI) breeding system in which, although self-pollination resulted in capsule set, only empty testae formed. A subsequent study [2] performed an investigation of the floral structures in the genus Restrepia, using cryo-scanning electron microscopy (Cryo-SEM) in order to formulate a pollination hypothesis for the genus and to establish any links between the breeding system and micromorphology. The advantage in using Cryo-SEM was to preserve transient surface details, such as any cellular exudates, which would be lost using older methodologies.

Materials and methods

The plants used came from the collection of H. Millner. All plants were greenhouse grown (minimum night temperature = 58°F/15°C and day length = 14 hours).

Scanning electron microscopy was carried out at the Centre for Electron Microscopy, University of Birmingham, United Kingdom. Specimens were mounted onto a Cryo Stage (Quorum PolarPrep S2000 Cryo Transfer System, Quorum Technologies, Lewes, East Sussex, UK), and were then rapidly frozen using liquid nitrogen to a temperature of -180°C and sputter coated with platinum. The Cryo Stage allows rapid freezing which results in improved sample integrity with fewer ice crystals. This produces images which are more 'true to life'. The specimens of dorsal sepal osmophores, labellum, cirrhi and calli were examined under a FEI XL30 FEG ESEM, FEI UK Ltd, Cambridge, UK, and the images processed in Photoshop.

Pollination hypothesis. The fly (typically a small dipteran species) is attracted to the flower *(Figure 1)* from a distance by scent produced by the osmophores *(Figures 2 and 3)* and locates the flower by a combination of sight and 'scent trails' from the osmophores (Figure 2). After landing on the synsepal/labellum, the conical papillae present provide 'grip' for the fly together with tactile and olfactory cues which guide it along the labellum. The cells of the epichile (lower labellum) produce waxes and oils *(Figure 4)* which the fly can sense via its proboscis or other organs. The fly then progresses along the isthmus on to the hypochile (upper labellum), guided/lured by the structural optical effects of the calli. As the fly progresses along the labellum, the surface of the hypochile becomes smoother and steeper. This makes further progress more difficult, and it is at this point where the fly is positioned/trapped between the cirrhi and beneath the column (Figure 5). Pollination is then brought about by pollinia being deposited on to the stigmatic surface or pollinia from the column becoming attached to the fly. The fly is then able to leave the flower having performed its role in the pollination of the flower, although unrewarded.

Discussion

Non-nectar rewarding myophily or fly-pollination is thought to prevent self-pollination by discouraging the return of the pollinator, which reduces the likelihood of self-pollination and promotes out-breeding [1]. However, for endangered species, such as Restrepia and other Pleurothallids, with dwindling populations the incidence of self-pollination will increase due to pollinators having fewer available flowers to visit. Populations may then be no longer be self-sustaining through seed production [1].

Restrepia were found to exhibit a gametophytically controlled SI breeding system with self-pollination which results in capsule set and the formation of empty testae, both of which represent a waste of resources and lack of reproductive success for wild plants [1]. It is vital, therefore, for them to avoid self-pollination. Their breeding systems and pollination mechanisms, of necessity, should work in tandem in order to prevent this. Through this morphological study [2] Restrepia was shown to represent a non-nectar rewarding and 'deceptive' orchid genus and that these mechanisms operate in conjunction with its breeding system to reduce self-pollination.

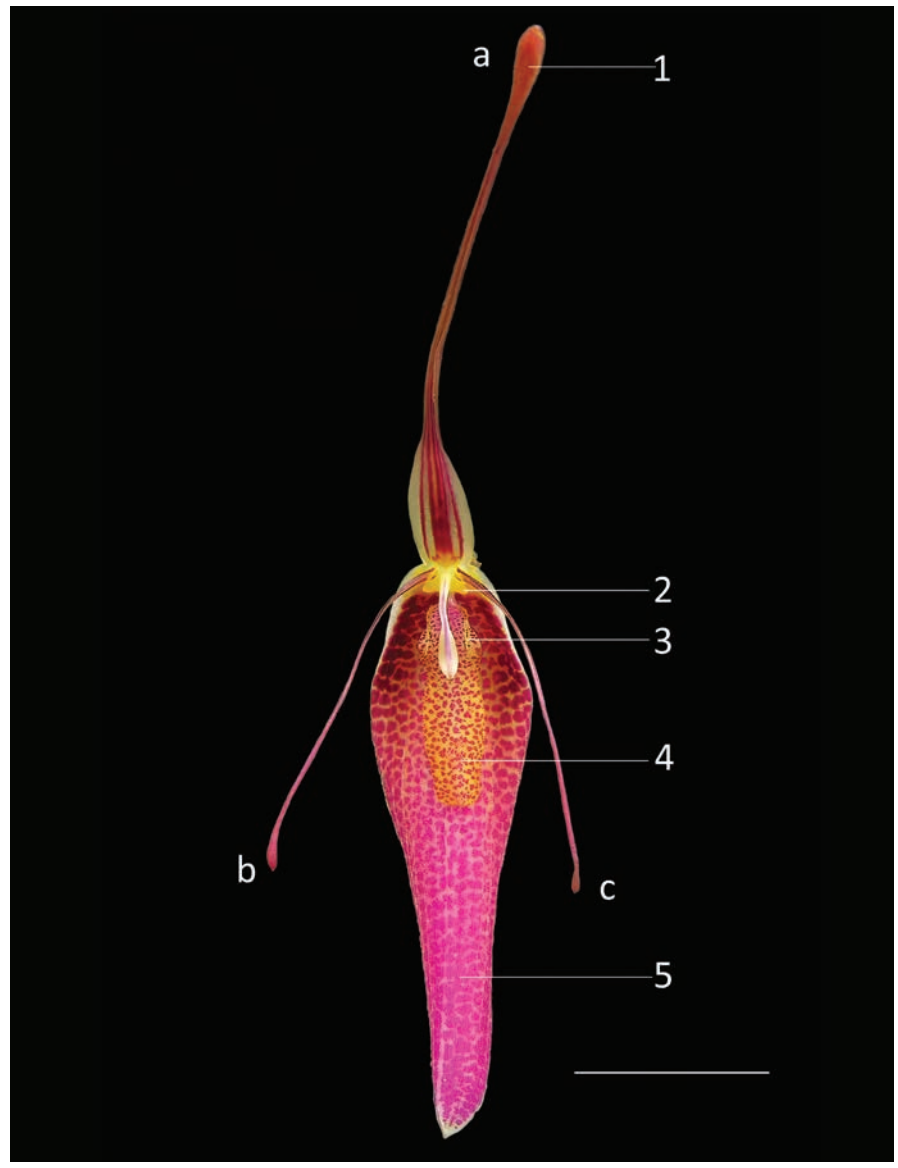

Figure 1. Structure of a typical Restrepia flower (Restrepia guttulata). Scale bar = I cm

(1) the dorsal sepal and position of osmophores, (2) the calli, (3) the cirrhi, (4) the labellum, or lip, (5) the synsepal (joined sepals) and (a,b,c) the triangular arrangement of the apical osmophores of the dorsal sepal and lateral petals

Features studied using scanning electron microscopy: (1) dorsal sepal osmophores, (2) calli, (3) cirrhi and (4) labellum.

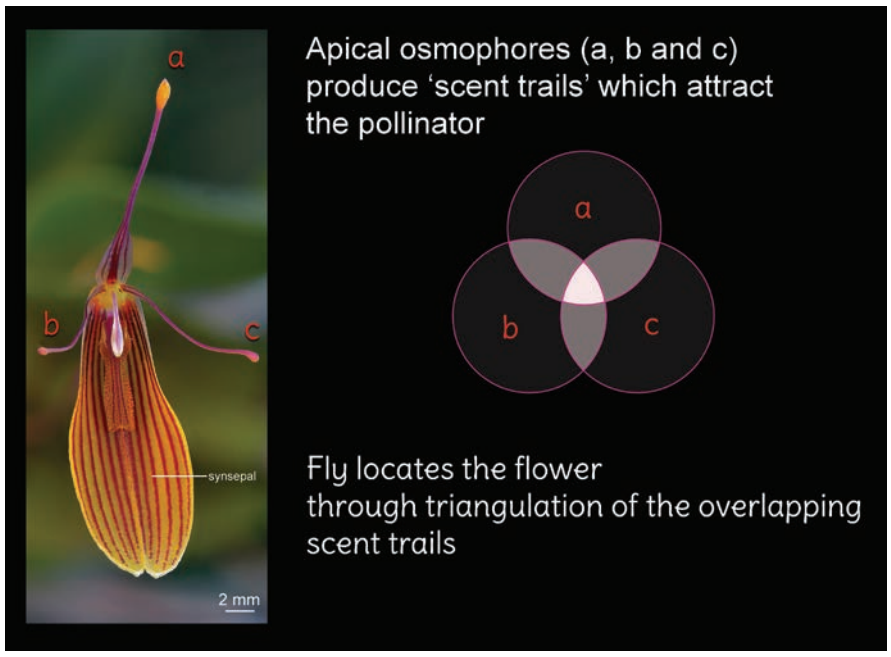

Figure 2. The triangular arrangement of the apical osmophores of the dorsal sepal and lateral petals

(a,b and c) illustrate the triangular arrangement of the osmophores. When the pattern of the scent trails is presented as a Venn diagram, their possible overlapping pattern may be observed. The strongest scent will be in the centre where the three trails overlap and so act as a kind of scent triangulation location mechanism enabling the fly firstly to locate the flower and secondly to guide it onto the labellum.

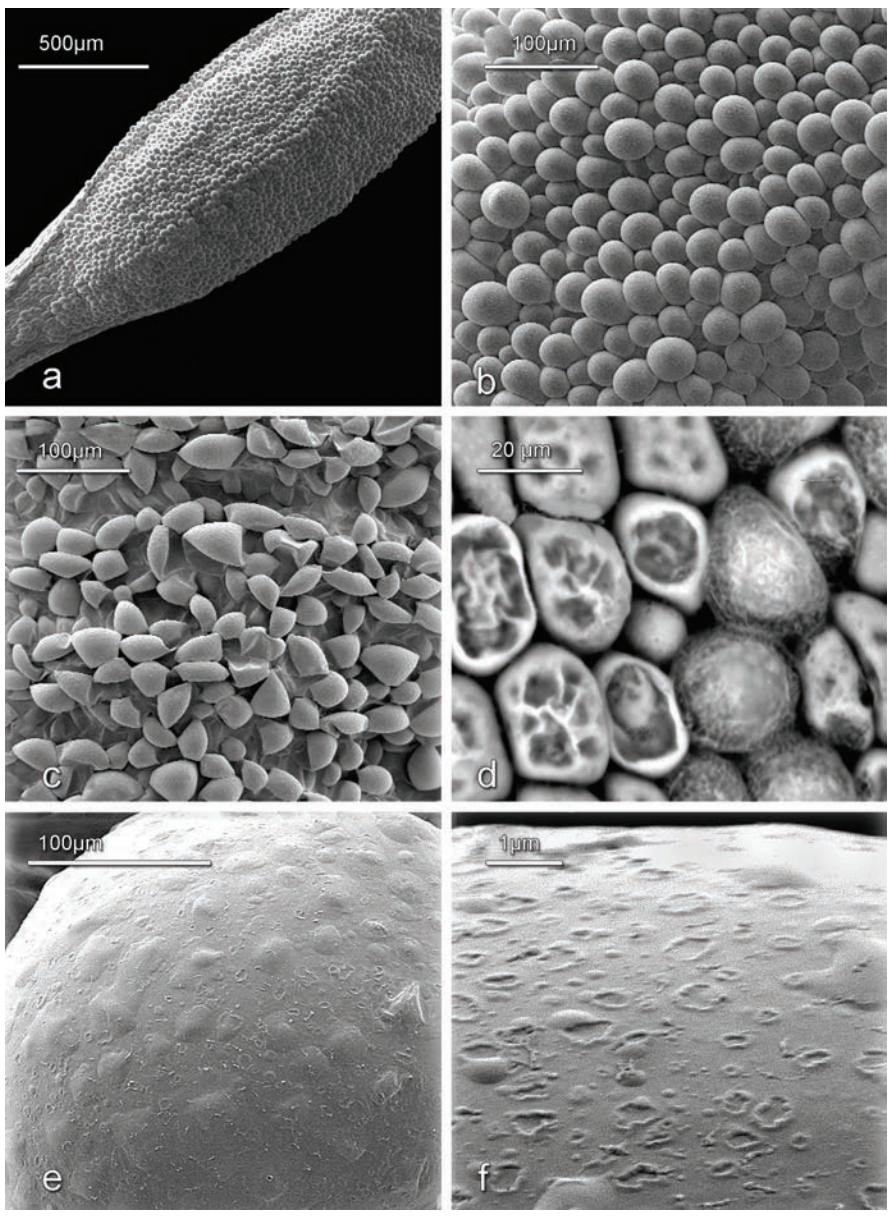

Figure 3. Osmophore (scent gland) structure

(a) osmophores of dorsal sepal (b) at anthesis, with no vesicles visible, (c) after 24 to 48 hours, the osmophores have begun to shrink and collapse, having emitted their volatile scents and (d) after 48 hours the osmophores have shrunk, the top surfaces show indentations. (e,f) details of the vesicles of the osmophores between anthesis and 48 hours, (e) the vesicles containing the scents may be observed as 'blisters' on the surface of the osmophore and (f) some of the vesicles have ruptured, the scents have been released, the osmophore will now begin to collapse as in (b) and (c).

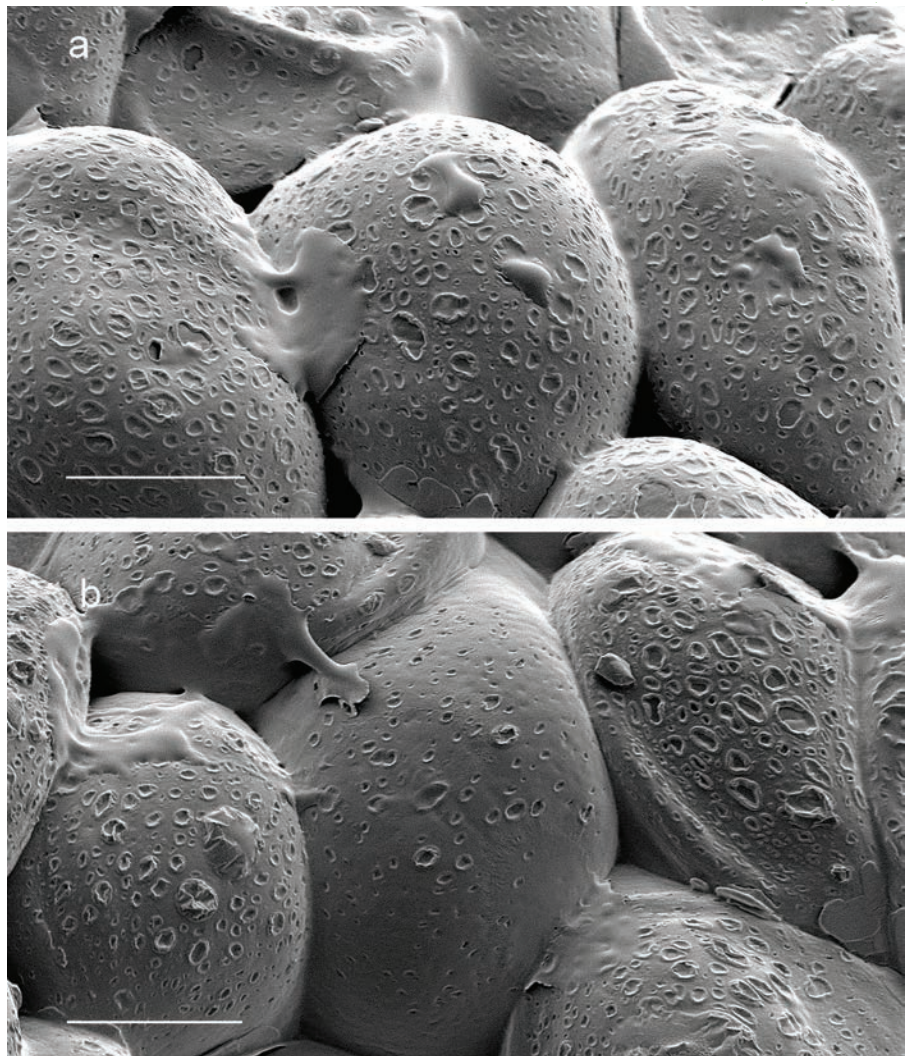

Figure 4. Cellular surface structure. One advantage of the SEM method over those used previously [3] is that it preserves the surface details of cells. This had the result that it was possible to observe cellular secretions/exudates (a, b) and thereby identify secretory parts of the Restrepia flower.

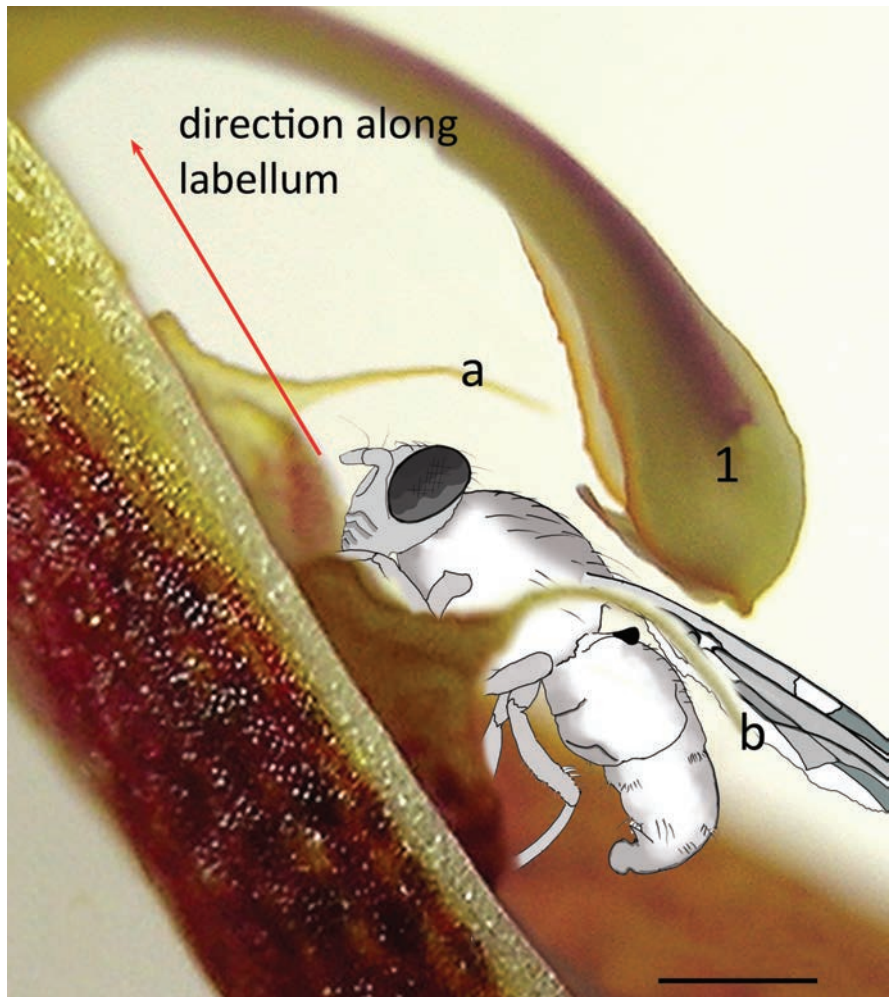

Figure 5. Position of the fly between the cirrhi. The cirrhi act to trap the fly beneath the column and so it is forced to pass between them. Any movement there by the fly will bring about pollination as the stigmatic surface is then directly above it. Although these orchids are not considered to be species specific for pollination, these mechanisms will only work for flies that are the right 'size' and proportion. Too small or too large and pollination cannot occur.

References

1. Millner H.J; McCrea A. R. and Baldwin T.C. (2015). An investigation of self-incompatibility within the genus Restrepia. American Journal of Botany, 102 (3): 487 -494.
2. Millner H.J. and Baldwin T.C. (2016). Floral micromorphology of the genus Restrepia (Orchidaceae) and potential consequences for pollination. Flora, 225:10 - 19.
3. Pridgeon, A. and Stern W.L. (1983). Ultrastructure of osmophores in Restrepia (Orchidaceae). American Journal of Botany 70(8):1233-1243.