Comparative analysis of floral secretory structures in selected species of Orchidaceae Juss. and Apocynaceae Juss. attracting flies (Diptera)

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Diverse floral features and structures have developed as a consequence of pollinator pressure. Flowers pollinated by flies (Diptera) mimic food sources or oviposition sites of flies by using dark floral colours and shape or texture of flowers. Sapromyophilous flowers produce no nectar for flower-visiting insects. The scent of rotting fruit, carcasses, or excrement, emitted by the osmophores, often located on motile elements of the perianth, is the flower's main attractant.

This dissertation describes sapromyophilic species from two unrelated families: Apocynaceae and Orchidaceae. They share a series of flower adaptations resulting from convergence. The formation of pollinia (coherent masses of pollen grains) or the formation of new organs in synorganization process: gynostemium in Orchidaceae and gynostegium in Apocynaceae [1, 2] are considered the most important adaptations. Orchidaceae species, like Apocynaceae (according to the APG IV system), are pollinated mainly by flies (Diptera), which played a role in the early radiation of angiosperms [3, 4]. For the research presented in this doctoral dissertation, species from both families were selected: *Bulbophyllum echinolabium* J.J. Sm., *B. levanae* Ames and *B. nymphopolitanum* Kraenzl. from section *Lepidorhiza* Schltr. (Orchidaceae) and *Echidnopsis cereiformis* Hook.f. and *Stapelia scitula* L.C. Leach (Apocynaceae). So far, a few species from the studied families have been analyzed histochemically, micromorphologically and ultrastructurally. Only one sapromiophilous species was found to secrete nectar [5], but this has not been verified yet. Published comprehensive investigations of the fly-pollinated species, including the analysis of secretory structures, secretion method and type of secreted substances are lacking. An extensive comparative analysis of different sapromiophilic species would provide a better understanding of plant adaptations to pollination by Diptera.

The purpose of my doctoral dissertation was to analyze features of floral secretory tissue in selected fly-pollinated species of Orchidaceae and Apocynaceae.

The specific objectives of this dissertation include:

1. Characteristics of floral secretory structures and attractants for the potential pollinators (macro-, micromorphological, ultrastructural analysis).

2. Histochemical analysis: to determine what type of substances are secreted: sugars, proteins, lipids, pectic acids / mucilages, dichlorophenols.
3. Analysis of chemical composition of the emitted scent and characterization of the floral scent profile of selected species.
4. Comparison of similarities and differences in floral secretory tissue among the selected species from two fly-pollinated families.

Studies on *Bulbophyllum* species from the section *Lepidorhiza* showed that the secretory structures are located within the labellum and lateral sepals (tepals of the outer whorl) [**6, 7**]. There were differences in the type of secretory structure (nectaries or osmophores) and the nature of secretion, even among species within one section. In *B. levanae* and *B. nymphopolitanum* the apices of lateral sepals functioned as osmophores. In turn, in *B. echinolabium* the apical part of the lip (epichile) emitted an intense smell of decomposing flesh acting as an osmophore, while a nectary was present in the basal part of the labellum (hypochile). In Asclepiadoideae, osmophores were observed in corolla lobes [**8**]. A profusion of proteins was noted in the flowers of species of the families studied which is probably connected with the unpleasant scent emitted by the flowers imitating the decomposition of carrion. Waxes gathered on the surface of the epidermis together with cuticular striations cause the brilliance of floral tepals, thus imitating the surface of carrion or open flesh wounds attracting flies. Histochemical analysis of *B. echinolabium* floral secretory tissue together with chemical studies of the labellum secretions [**7**] confirmed the presence of nectar. This is additional evidence that sapromiophilous species can produce nectar.

Both families share many anatomical and ultrastructural features of the floral secretory tissue. Both the adaxial epidermal cells of the labellum in Orchidaceae and the corona lobes in Apocynaceae showed typical features of secretory activity. The periplasmic spaces were present in the adaxial epidermal cells of lips, between the cell wall and the protoplast, of all investigated species, although they differed in sizes among species [**6, 7**]. In *B. nymphopolitanum*, the spaces were rather small, while the secretion gathered under the cuticle layer on the surface of the adaxial epidermis. In turn, in *B. levanae* the exudation is secreted through micro-channels in the cuticle. The combination of periplasmic space and microchannels has not previously been recorded in the *Bulbophyllum*. The presence of periplasmic spaces in the adaxial epidermal cells of the corona lobes of

Apocynaceae species were also observed [**8**], which proves the existence of similarities in sapromiophilous species of both families. The formation of periplasmic spaces is probably associated with a granulocrine type of secretion. The secreted material is transported via vesicles outside the protoplast, where it gathers in the periplasmic spaces underneath the cell wall and then, as a result of pressure increase in the protoplast, is secreted outside [9].

Floral mimicry includes both olfactory clues and colour of flowers. There may also be a correlation of olfactory and visual effects [10, 11]. Succulents of the Apocynaceae subfamily Asclepiadoideae emit the scent of decaying meat, fish, fruit and other organic substances, as well as excrements and urine. Jürgens and co-authors [11] distinguished four types of odour mimicry, depending on the dominant fragrance compound. The aim of the present studies was to verify whether similar fragrance profiles and correlations can be found in the Orchidaceae. Therefore, floral scent of *B. echinolabium* was analysed chemically [**7**]. The presence of chemical compounds that are attractants of flies has been demonstrated. Some of these compounds were also noted in the smell of ox carcasses, urine and faeces [12**,** 13]. That proves the olfactory mimicry, which, together with visual cues such as colour and size of flowers and the glittering wax on the tepal surface imitating flesh wounds, ensures pollination success in *B. echinolabium.*

Likewise, comparative analysis of different species from one genus could be used in taxonomic research. Morphological and anatomical studies of *B. levanae* and *B. nymphopolitanum* showed that, despite many similarities, there are significant differences in the morphometry of tepals and micromorphological and ultrastructural features of flowers of both species [**6**].

In conclusion, the presented series of publications broadens the knowledge of fly-pollinated species and enables a better understanding of the flower-pollinator relationships. The conducted studies allowed the characterization of the secretory tissue of sapromiophilic flowers and definition of the types of secreted substances. The results presented, together with the literature studied, indicate a number of similarities between the species from within one family. Furthermore, they constitute the preliminary studies for the wider comparative analysis between two phylogenetically distant families of angiosperms.

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