Structural and histochemical characterization of the osmophores in corollas of Asteraceae (tribes Onoserideae and Famatinantheae)

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ABSTRACT
Osmophores are engaged in scent production and differ from other secretory structures by their product, site, duration, and anatomical structure. Whereas osmophores have been well-documented in flowers of several families, they are barely mentioned in the Asteraceae. The aims of this study are to: 1) determine the occurrence of osmophores in corollas of 39 species of the tribe Onoserideae and the only species of Famatinantheae with histochemical methods; and 2) analyze the morphology and structure of osmophores in these groups. Histochemical and histological techniques revealed osmophores in the marginal and in the central corollas of *Famatinanthus* Ariza & S.E. Freire and *Plazia* Ruiz & Pav. at the apex and margin of the corolla lobes, and at the sinuses. Osmophores were confirmed following positive staining reactions for TIOFH and TIOFH3 (Neutral Red, Oil Red O, and Iodine-Potassium-Iodide); the samples reacted negatively for Benedict's test for detecting reducing sugars. The osmophores found in this study are constituted uniquely by a papillose or non-papillose epidermis, or they extend into the mesophyll forming scent glands. In both cases, they are associated to stomata and starch grains. The corollas of the other species of Onoserideae did not react positively for osmophores.

KEY WORDS
Anatomy,
Asteraceae,
Compositae,
histochemistry,
osmophore,
scent production,
secretory structures.

RÉSUMÉ
Caractérisation structurale et histochimique des osmophores dans les corolles d'Asteraceae (tribus des Onoserideae et des Famatinantheae).
Les osmophores participent à la production d'odeurs et se différencient des autres structures de sécrétion par leur produit, leur localisation, leur durée et leur structure anatomique. Alors que les osmophores ont été bien documentés dans les fleurs de plusieurs familles, ils sont à peine mentionnés dans les Asteraceae. Les objectifs de cette étude sont les suivants : 1) déterminer la présence d'osmophores dans les corolles de 39 espèces de la tribu des Onoserideae et de la seule espèce de Famatinantheae en utilisant des méthodes histochimiques ; et 2) analyser la morphologie et la structure des osmophores dans ces groupes. Les techniques histochimiques et histologiques ont révélé la présence d'osmophores dans les corolles marginales et centrales de *Famatinanthus* Ariza & S.E. Freire and *Plazia* Ruiz & Pav. au sommet et au bord des lobes de la corolle et aux sinus. Les osmophores ont été confirmés à la suite de réactions de coloration positives pour TIOFH et TIOFH3 (Rouge Neutre, Oil Red O et Iodure de Potassium Iodé) ; les échantillons ont réagi négativement au test de Benedict pour la détection des sucres réducteurs. Les osmophores trouvés dans cette étude sont constitués uniquement d'un épiderme papilleux ou non, ou s'étendent dans le mésophylle formant les glandes odorantes. Dans les deux cas, ils sont associés à des stomates et à des grains d'amidon. Les corolles des autres espèces d'Onoserideae n'ont pas réagi positivement pour les osmophores.

MOTS CLÉS
Anatomie,
Asteraceae,
Compositae,
histochimie,
osmophore,
production de parfum,
structures de sécrétion.

INTRODUCTION

Osmophores parts are engaged in scent production and differ from other secretory structures (e.g., hydathodes, nectaries, resin ducts) by their product, site, duration, and anatomical structure, and they are commonly found in certain floral and inflorescence parts (Stern *et al.* 1987; Vogel 1990; Effmert *et al.* 2005; Wiemer *et al.* 2009). The osmophoric function may be fulfilled only by the epidermis (glandular epithelium), sometimes papillose or with trichomes, or the glandular structure may also extend into the mesophyll tissue forming scent glands (Vogel 1990).

The fragrant substances secreted by osmophores are mainly lipophilic, volatile, low terpenes, which are the primary constituents of the essential oils. They occur in the form of minute droplets in the cytoplasm of the epidermal and neighboring parenchyma cells. At an appropriate temperature, the droplets diffuse in gaseous form from the cytoplasm through the cell wall and cuticle to the outside. With the diffusion of droplets, new ones are constantly produced. Below the epidermis the cells are filled with starch grains which, used as a source of energy, reveal the secretory activity of the osmophores (Fahn 1979).

Osmophores have been well-documented and analyzed in flowers of members of Orchidaceae (e.g., Stern *et al.* 1987; Wiemer *et al.* 2009; Cabral de Melo *et al.* 2010; Kowalkowska *et al.* 2015), and also Araceae (e.g., Méndez & Obeso 1992; Singh *et al.* 1996; Hadacek & Weber 2002), Aristolochiaceae (Vogel 1990), Asclepiadaceae (Vogel 1990; Aliscioni *et al.* 2017), and Solanaceae (Sazima *et al.* 1993; Cocucci 1996). These structures are mentioned in other families as part of pollination studies, for example in Amaryllidaceae (Dobson *et al.* 1997), Lecythidaceae (Ormond *et al.* 1981), Lentibulariaceae (Plachno *et al.* 2017), Rutaceae (Rodrigues Marques *et al.* 2015), and Xanthorrhoeaceae (Bernhardt 1995).

In Asteraceae, floral parts are not regarded as being particularly fragrant (Lane 1996), which could be the reason why osmophores are barely mentioned in the family. Field observations and herbarium label information, however, show that many members of this cosmopolitan family have, or are described with, fragrant flowers. Detailed studies of osmophores in Asteraceae are lacking; they are only briefly mentioned, for example, as a complement of floral pollination studies. In a study of reproductive strategies of *Mikania glomerata* Spreng. and *M. hirsutissima* DC. (Eupatorieae), Eiterer (1965) rejects the presence of osmophores in these taxa arguing that the odor is produced by the pollen grains, the anthers, and the stigmatic lines of the styles. Combas *et al.* (1999), in a floral biology study, localize osmophores in the veins, margins and base of the neutral corollas of *Tithonia diversifolia* (Hemsl.) A. Gray (Heliantheae). Santos de Castro *et al.* (2015) indicate that the odor in florets of *Moquiniastrum oligocephalum* (Gardner) G. Sancho (Gochnatieae) is due to osmophores located in the corolla lobes, styles and anther apices. Melo Santos *et al.* (2016) mention osmophores in *Tridax procumbens* L. (Heliantheae) with an unclear location, although indicating petals and 'sepals' as possible scent-producing sites.

During a routine light microscope examination of florets of *Plazia daphnoides* Wedd. (Asteraceae, Onoserideae), our attention was drawn to the presence of some unusual structures in the corolla. This led us to investigate if these structures were in fact osmophores, and if they were also present in other members of Onoserideae. This mainly Andean tribe is part of the subfamily Mutisioideae, one of the major early branching clades of the family tree, and contains seven genera: *Aphyllocladus* Wedd. (four species), *Gypothamnium* Phil. (one species), *Lycoseris* Cass. (11 species), *Onoseris* Willd. (32 species), *Paquirea* Panero & S.E. Freire (one species), *Plazia* Ruiz & Pav. (four species) and *Urmenetea* Phil. (one species) (Katinas *et al.* 2009; Panero & Freire 2013; Katinas & Funk 2020). Onoserideae is characteristic by its herbaceous or shrubby species, sometimes dioecious, commonly with

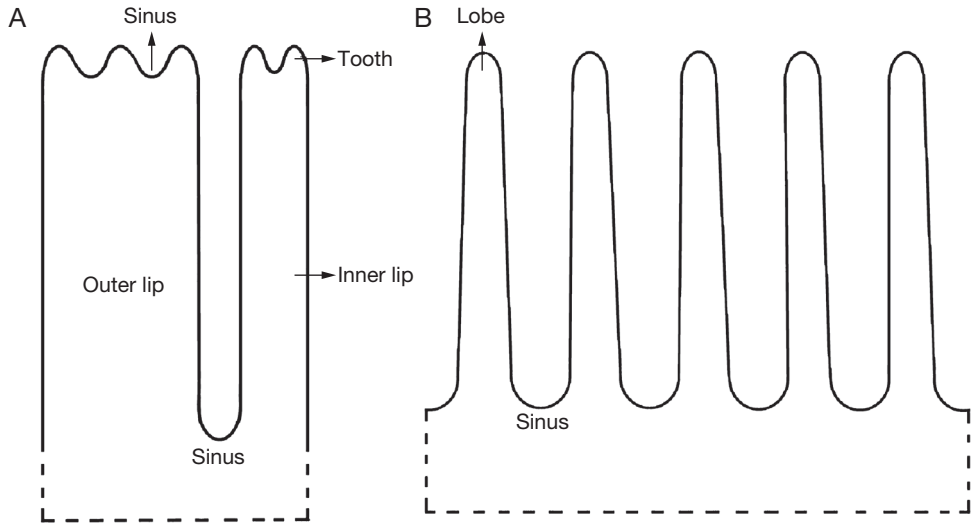


Fig. 1. — Corolla parts mentioned in this contribution: **A**, opened bilabiate, marginal, corolla; **B**, opened, tubular, central corolla.

solitary or few heterogamous capitula and dimorphic florets, rarely with discoid capitula (e.g., species of *Plazia* and *Onoseris*; Sancho 2004; Dillon & Luebert 2014). The marginal corollas are usually bilabiate (3 + 2) with an outer 3-cleft lip and an inner 2-cleft lip (Fig. 1A), occasionally pseudobilabiate (3+1; 4+1) or true ray (3 + 0), and the central corollas are more commonly tubular-funnelform and 5-lobate (Fig. 1B) or 5-dentate (0 + 5). The species *Aphyllocladus decussatus* Hieron. was accommodated in the new genus *Famatinanthus* Ariza & S.E. Freire with its own monotypic tribe Famatinantheae and subfamily Famatinanthoideae (Panero *et al.* 2014). Because it was previously related to Onoserideae, we also analyzed *Famatinanthus decussatus* in this study.

There is little evidence in the literature and in the herbarium labels of florets fragrance in Onoserideae and Famatinantheae. Cabrera (1978: 581) mentions that florets of *Plazia daphnoides* have a soft fragrance, and florets of some species of *Onoseris* (under the name *Centroclinium*) are described as fragrant by Hooker (1831: 3115).

The lack of detailed studies of scent-producing structures in the family Asteraceae led us to the present work. This study aims to: 1) determine the occurrence of osmophores in corollas of members of the tribes Onoserideae and Famatinantheae with histochemical methods; and 2) analyze the morphology and structure of osmophores in these groups.

MATERIAL AND METHODS

Material

This study is based on herbarium material of six genera of Onoserideae (Mutisioideae), i.e., *Aphyllocladus*, *Gypothamnium*, *Lycoseris*, *Onoseris*, *Plazia*, *Urmenetea* and 39 of its 54 species, and the only genus and species of Famatinantheae (Famatinanthoideae), *Famatinanthus decussatus*. Material of *Paquirea* was not available. A list of specimens analyzed is provided in Appendix S1, available as Supplementary Material to this paper. Up to five mature marginal and central florets of each species were dissected and the opened corollas were analyzed. The corolla parts mentioned in this contribution are displayed in the Fig. 1. Papillae size (length and width in micrometers) are values of more than 50 measurements.

Histological tecnhiques

For anatomical observations, such as venation, stomata or certain tissues, corollas were rehydrated, treated with a clearing process, and fixed in FAA (formalin: glacial acetic acid: ethanol 70%). Transverse and longitudinal sections of corolla samples were obtained with a rotary microtome HM 315R using conventional methods for paraffin infiltration and embedding (Johansen 1940). Serial sections were made at 5-10 µm and histological samples were stained with Safranin 2 %, Safranin-Fast Green 0.5 % (Johansen 1940), Safranin-Astra Blue 0.5 % (Srebotnik & Messner 1994), Nile Blue 0.05 % (Cain 1947), or left unstained, and mounted in Canada balsam.

Histochemical techniques

The flowers were reconstituted by immersion in water using an oven at 30°C for 24-72 h, fixed with FAA, washed twice with sterilized distilled water, and discolored with Sodium hypochlorite (NaClO), 15-30%, 2-8 h. The reactions were performed and temporary or semipermanent slides were made using as montage medium glycerin (90%) and gelatin-glycerin. Free hand transections were performed in some samples and mounted in glycerin.

For histochemical analyses we followed the technique for the identification of osmophores in flowers of herbarium material (TIOFH and TIOFH3; Hernández & Katinas 2019), which tests for the presence of osmophores in herbarium material using Neutral Red (NR, 5 g in 100 mL of distilled water, dissolved 0.1% when used), which stains vacuoles and cell walls of the potential osmophoric areas (Johansen 1940), Oil Red O (ORO, 0.5 g in 100 mL ethyl alcohol 80°), which tests for lipophilic substances (Proescher 1927), and

Iodine-Potassium-Iodide (IKI) for detecting starch granules (Johansen 1940). With this technique the cells, papillae, and trichomes of the osmophoric area stain dark red to orange-red (NR, ORO), either with well- defined droplets or staining the whole cytoplasm and cell walls. The starch grains in or nearby the osmophoric area stain dark blue-violet to black. Fresh flowers of *Narcissus tazetta* L. were used to test for the NR histochemical reaction, and transversal cuts of herbarium florets of *Jacaranda mimosifolia* (petals, stamens, staminodes) and *Narcissus tazetta* (crown, tepals) were used to test for NR and ORO. The results were compared with the reactions for the species of Asteraceae here studied.

Benedict's test (Korwar *et al.* 2010) was employed to check for the presence for reducing sugars, such as glucose, in the analyzed structures to dismiss the possibility that they could be nectariferous structures. After depositing a drop of the reagent, slides were flamed 3-4 times; the formation of orange-red color in the cells indicates a positive reaction for reducing sugars (Korwar *et al.* 2010).

Results were examined by means of Leitz SM Lux and Nikon Eclipse E200 light microscopes. Photomicrographs and measurements were taken with Moticam 2300 attached to the eyepiece microscope and software Motic Image Plus 2.0, and Nikon Coolpix S10 camera. We produced a total of 265 slides for the anatomical observations and histochemical tests.

RESULTS

Our general results are displayed in Table 1 and Figure 2. We detected osmophores in *Famatinanthus* (*F. decussatus*) and *Plazia* (*P. cheiranthifolia* with discoid capitula and *P. daphnoides* with radiate capitula), which were positive to TIOFH and TIOFH3 (NR, ORO and IKI) and negative to Benedict's test, indicating that these areas do not produce reducing sugars (such as those released by nectaries). The corollas of the other species of Onoserideae did not react positively for osmophores.

Osmophores

The osmophores found here are: 1) constituted by a glandular epithelium (osmophoric epidermis), usually papillose (osmophoric papillae); or 2) constituting scent or osmophoric glands, with histologically distinguishable cell layers. In both cases, they are associated to stomata and starch grains. Starch grains are minute and can be either isolated or aggregated in clusters.

The osmophoric papillae in the marginal and in the central corollas of *Famatinanthus* and *Plazia* (Table 1; Fig. 2A-D) may be arranged in clusters on the adaxial side of the sinuses between corolla lobes (Fig. 3A-C), at the corolla apex (Fig. 3D) or there are sparse, solitary papillae at the lip and lobe margins (Fig. 3E-G). The sinus location of the osmophoric area differs slightly in the two genera. In the marginal corollas of *Famatinanthus* (Fig. 2A), the osmophores are located between the outer and the inner lip, and also below the sinuses forming an arch. In the marginal corollas of *Plazia daphnoides* (Fig. 2C), the osmophores are placed between each tooth and also at the tooth apex of the outer lip. The central corollas of *Famatinanthus and Plazia* show osmophoric papillae at the apex of the lobes, at the sinuses between lobes, at the margin of the lobes, and scattered below the sinuses between the lobes (Fig. 2B, D). Occasionally, *Famatinanthus* shows osmophoric papillae at the base of each lobe.

The osmophoric papillae are apically globose and narrow at the base (Fig. 3C), cylindrical (Fig. 3H) or finger-like reaching the hair-like type (Fig. 3I). The length of the papillae ranges from 13.3 to 69.6 µm and the width ranges from 10.2 to 43.9 µm. They have a striate thick cuticle, a thick cell wall of cutinized cellulose, and an ample lumen filled with oil drops (Fig. 3H, I). The striations form deep channels over the surface of the papillae (Fig. 3C), although it cannot be discarded that it could be an artifact produced by the dehydration process. In frontal view the papillae have polyhedral shape with straight or slightly wavy anticlinal walls.

At the apex of the tubular-funnelform corollas of *Plazia* and *Famatinanthus* and at the apex of the outer lip of the bilabiate corollas of *Plazia daphnoides*, the osmophores are conspicuous structures constituting osmophoric glands. They are rounded in shape, bulging above the corolla surface in *Famatinanthus* (Fig. 4A, B) whereas the osmophores are flatter in *Plazia* (Fig. 4C, D). The external evidence of the osmophores is correlated with an underlying specific tissue arrangement, which has the same characteristics in both genera (Fig. 4E-H): 1) an outer layer formed by a papillose epidermis with a thick external periclinal cell walls and a striate cuticle and thin internal cell walls. The papillae are generally gathered in the abaxial side (the face opposite to the gynoecium and androecium) of the corolla apex whereas the adaxial side (facing the gynoecium and androecium) is covered by an epidermis with rectangular cells in transection; the papillae and the other epidermal cells contain abundant oil drops; 2) the center of the osmophore is constituted by a secreting parenchyma of isodiametric cells, with oil drops and starch grains. These cells have thin walls and lie side by side without noticeable intercellular spaces among them. The TIOFH3 revealed the secreting activity of the osmophore, noticeable by the red oil drops in papillae, epidermal cells, and parenchyma tissue (Fig. 4I, J). The vascular tissue is represented by collateral bundles, and corresponds to the junction area of the two marginal veins of each corolla lobe or lip (Fig. 5A); and 3) the parenchyma tissue below the glandular area also contains starch grains which react positively to IKI (Fig. 5B, E). A well-developed ventilation system was found here, constituted by large substomatal chambers (Fig. 5C) associated to numerous stomata (Fig. 5D-G), whose guard cells are also positive for IKI (Fig. 5G). Stomata are commonly located on the abaxial side of the corolla, between the osmophoric papillae and also below the glandular area.

Non-osmophoric epidermis

The corollas of the remaining genera analyzed, i.e., *Aphyllocladus*, *Gypothamnium*, *Lycoseris*, *Onoseris*, and *Urmenetea* (Table 1; Fig. 2), have some epidermal cells and papillae with

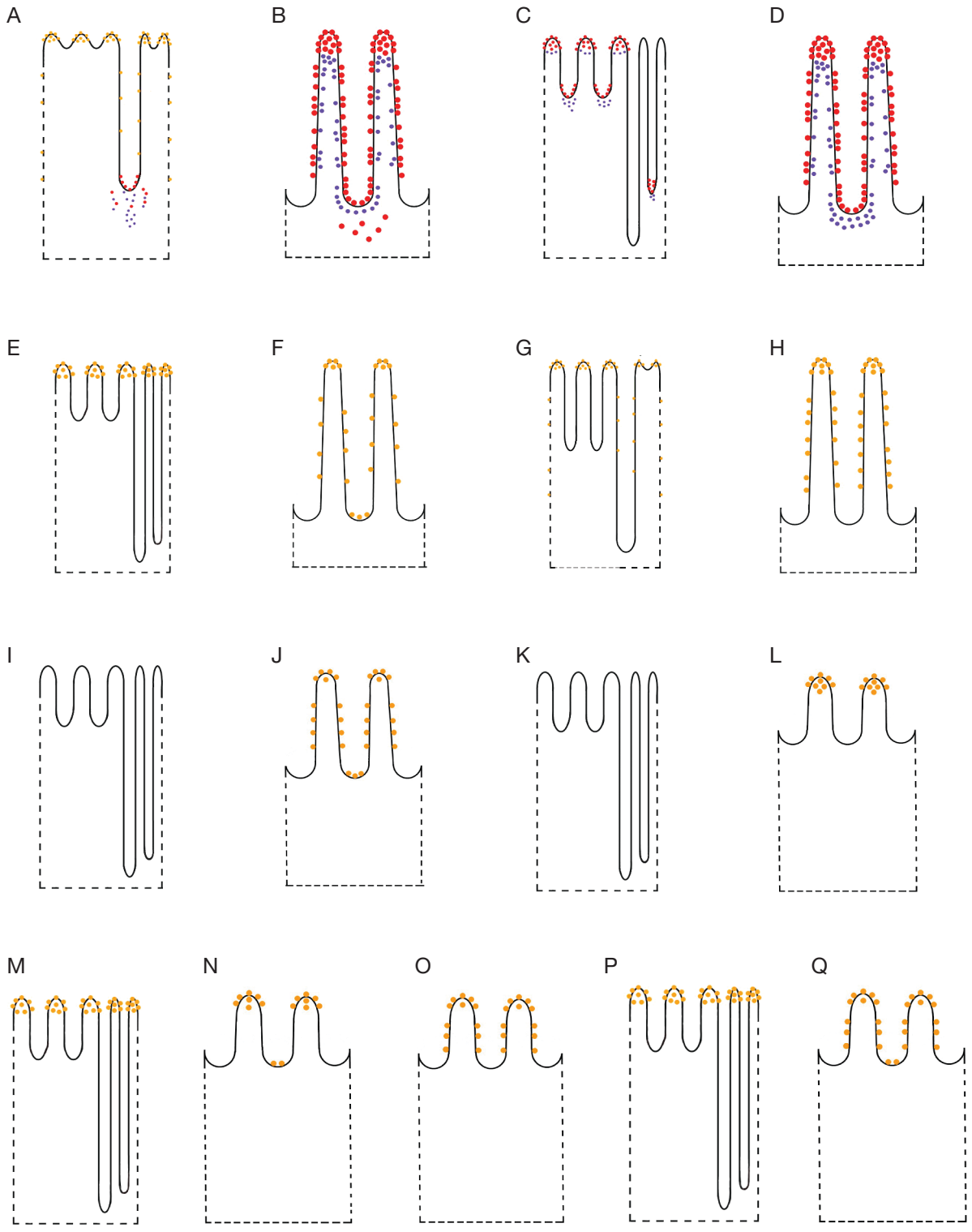


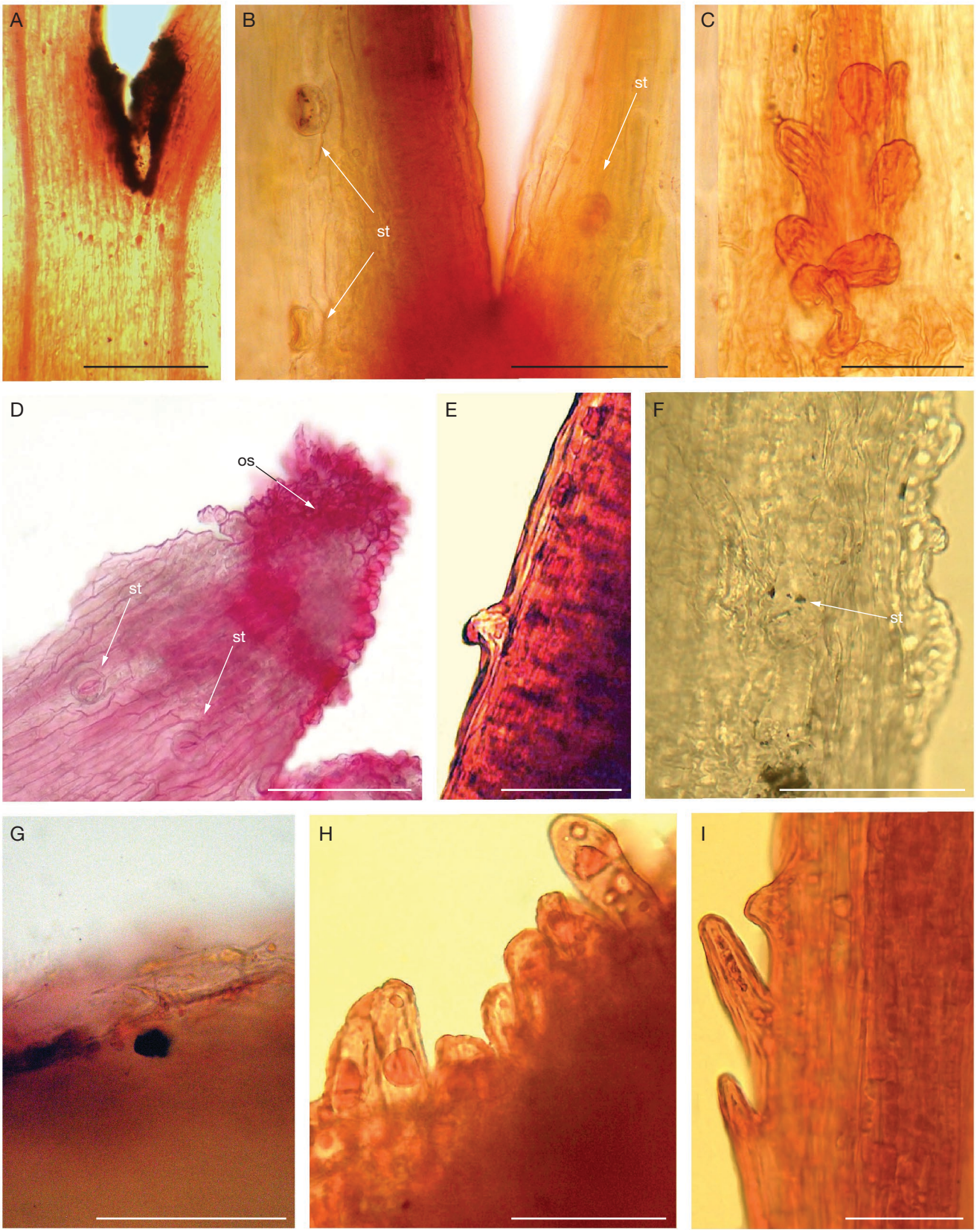
Fig. 2. — Diagrams showing the location of osmophores and non-osmophoric papillae in marginal, bilabiate corollas (**A**, **C**, **E**, **G**, **I**, **K**, **M**, **P**), in the central tubular corollas (**B**, **D**, **F**, **H**, **J**, **L**, **N**, **Q**), and in all tubular corollas (**O**) (only two of the five lobes are shown) of Famatinantheae and selected Onoserideae: **A**-**D**, osmophores; **A**, **B**, *Famatinanthus decussatus* (Hieron.) Ariza & S.E. Freire; **C**, **D**, *Plazia daphnoides* Wedd.; **E**-**Q**, non-osmophoric papillae; **E**, **F**, *Aphyllocladus spartioides* Wedd.; **G**, **H**, *Gypothamnium pinifolium* Phil.; **I**, **J**, *Lycoseris trinervis* (D. Don) S.F. Blake; **K**, **L**, *Onoseris gnaphalioides* Muschl.; **M**, **N**, *Onoseris drakeana* André; **O**, *Onoseris onoseroides* (Kunth) B.L. Robinson; **P**, **Q**, *Urmenetea atacamensis* Phil. **Dotted line**, area of corolla incision; **Red**, osmophores; **blue**, starch; **yellow**, non-osmophoric papillae.

lipophilic substances, but the epidermis and the underlying layers are negative to IKI for detecting starch, and have not associated stomata. There is the possibility that these tissues have run out of starch or that starch is produced in a subsequent flower stage. However, the lack of nearby stomata makes us to presume that these are non-osmophoric areas.

TABLE 1. — Type of corollas (Egeröd & Ståhl 1991; Sancho 2004; Katinas *et al.* 2008) with location of osmophores and non-osmophoric papillae in species of Onoserideae and Famatinantheae (Asteraceae). N.o. = non-osmophoric. For further details see Appendix S1, available as Supplementary Material to this paper.

Species	Marginal corolla: type of corolla and location of osmophores and N.o. papillae	Central corolla: type of corolla and location of osmophores and n.o. papillae
Aphyllocladus denticulatus	Bilabiate. N.o. papillae absent	Tubular-funnelform, deeply five-lobed. N.o. papillae: sinuses, lobe margin, lobe apex
A. sanmartinianus	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, deeply five-lobed. N.o. papillae: sinuses, lobe apex
A. spartioides	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, deeply five-lobed. N.o. papillae: sinuses, lobe margin, lobe apex
Famatinanthus decussatus	Bilabiate. Osmophores: sinuses between lips, below sinuses. N.o. papillae: tooth apex, lip margin	Tubular-funnelform, deeply five-lobed. Osmophores: sinuses, below sinuses, lobe margin, lobe apex, occasionally at the lobe base
Gypothamnium pinifolium	Bilabiate. N.o. papillae: lip margin, tooth apex	Tubular-funnelform, deeply five-lobed. N.o. papillae: lobe margin, lobe apex
Lycoseris eggersii	Bilabiate, pseudobilabiate, or true ray 3-dentate. N.o. papillae absent	Tubular-bilabiate, tubular-funnelform, shallowly five-lobed. N.o. papillae: sinuses, lobe margin, lobe apex
L. trinervis	True ray 3-dentate. N.o. papillae absent	Tubular-funnelform shallowly five-lobed. N.o. papillae: sinuses, lobe margin, lobe apex
L. triplinervia	Bilabiate, pseudobilabiate, or true ray 3-dentate. N.o. papillae absent	Tubular-bilabiate, tubular-funnelform, shallowly five-lobed. N.o.papillae: sinuses, lobe margin, lobe apex
Onoseris acerifolia	Pseudobilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. alata	Bilabiate. N.o. papillae absent	Tubular-funnelform, shallowly five-lobed. Papillae: sinuses, lobe apex
O. albicans	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: lobe apex, occasionally in sinuses
O. amplexicaulis	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. annua	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. brasiliensis	Pseudobilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: lobe apex
O. castelneana	Bilabiate. N.o.papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: lobe apex
O. chrysactinioides	Bilabiate. N.o. apillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. costaricensis	Florets absent	Sub-pseudobilabiate, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. cummingii	Bilabiate. N.o. papillae: tooth apex	Sub-pseudobilabiate, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. donnell-smithii	Florets absent	Tubular-funnelform, shallowly five-lobed. N.o. papillae: lobe apex
O. drakeana	Pseudobilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. gnaphalioides	Bilabiate. N.o. papillae absent	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. hastata	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: lobe margin, lobe apex
O. hyssopifolia	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. linearifolia	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. lopezii	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. macbridei	Bilabiate. Papillae absent	Bisexual, corolla tubular-funnelform shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. minima	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. odorata	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: lobe apex
O. onoseroides	Florets absent	Sub-pseudobilabiate, unequally, shallowly five-lobed. N.o. papillae: lobe margin, lobe apex, occasionally in sinuses
O. peruviana	Pseudobilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: lobe apex
O. purpurea	Pseudobilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: lobe apex
O. sagitatta	Pseudobilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. salicifolia	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, unequally, shallowly five-lobed. N.o. papillae: sinuses, lobe margin, lobe apex
O. silvatica	Pseudobilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. speciosa	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
O. werberbaueri	Bilabiate. N.o. papillae: tooth apex	Tubular-funnelform, shallowly five-lobed. N.o. papillae: sinuses, lobe apex
Plazia cheiranthifolia	Florets absent	Tubular-subcampanulate, deeply five-lobed. Osmophores: sinuses, below sinuses, lobe margin, lobe apex
P. daphnoides	Bilabiate. Osmophores: sinuses between teeth, tooth apex	Tubular-subcampanulate, deeply five-lobed. Osmophores: sinuses, below sinuses, lobe margin, lobe apex
Urmenetea atacamensis	Bilabiate. N.o. papillae: tooth apex	Tubular-bilabiate, shallowly five-lobed. N.o. papillae: sinuses, lobe apex, lobe margin

FIG. 3. — Osmophoric epidermis in *Famatinanthus* and *Plazia*: **A**, sinus between the outer and inner lips of the marginal corolla of *Famatinanthus decussatus* (Hieron.) Ariza & S.E. Freire, note the strong positive reaction for NR; **B**-**F**, *Plazia daphnoides* Wedd.; **B**, sinus between two lobes of the central corolla showing numerous stomata (**arrows**) (TIOFH3); **C**, sinus between two lobes of the central corolla showing the reddish cluster of osmophoric papillae (ORO); **D**, abaxial view of one tooth of the marginal corolla showing the osmophore and two stomata (Safranin); **E**, papilla in the margin of one lobe of the central corolla, note the reddish



secreting drop under the cuticle (ORO); **F**, papillae in the margin of a central corolla lobe, showing starch content as dark dots (IKI); **G**, lobe margin in the central corolla of *Famatinanthus decussatus* (Hieron.) Ariza & S.E. Freire, showing a cluster of starch grains below the epidermis (TIOFH3); **H**, **I**, *Plazia daphnoides* Wedd.; **H**, papillae at the lobe apex of the central corolla, note the lipophilic content (ORO); **I**, papillae at the lobe margin of the central corolla, note the thick cell wall and the striate cuticle (ORO). Abbreviations: **OS**, osmophore; **SM**, starch mass; **ST**, stoma. **A**, **G**, *Funk & Bonifacino 13233* (LP); **B**, *Ruthsatz s.n.* (LP s.n.); **C**, *Hunziker & Caso 6154* (LP); **D**, *Cabrera 8331* (LP); **E**, **I**, *Rauh P423* (LP); **F**, **H**, *Zöllner 4902* (LP). Scale bars: A, 150 µm; B, F, G, 100 µm; C, E, H, I, 50 µm; D, 380 µm.

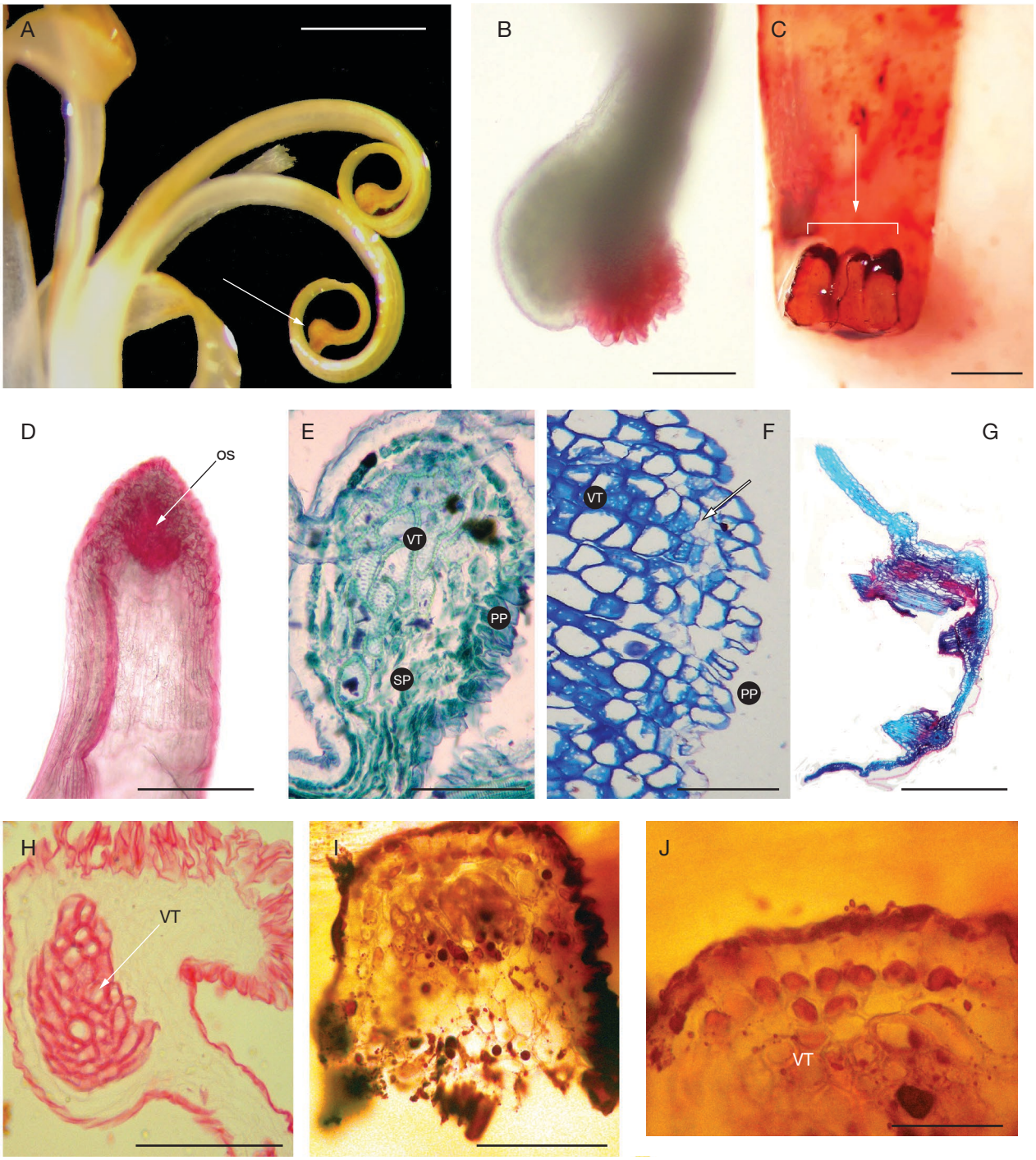


Fig. 4. — Osmophoric glands in *Famatinanthus* and *Plazia*: **A**, **B**, *Famatinanthus decussatus* (Hieron.) Ariza & S.E. Freire; **A**, central corolla showing osmophores at the apex of the lobes (stereomicroscopic image); **B**, apex of one lobe of the central corolla showing the reddish osmophore (Safranin); **C**, **D**, *Plazia daphnoides* Wedd.; **C**, apex of the 3-dentate outer lip of the marginal corolla showing the osmophores with a strong positive reaction for NR (stereomicroscopic image); **D**, apex of one lobe of the central corolla showing the osmophore (Safranin); **E**, longitudinal section of one lobe of the central corolla of *Famatinanthus decussatus*, showing the apical gland and the tissues (Safranin-Fast Green); **F**-**H**, *Plazia daphnoides*; **F**, longitudinal section of one lobe of the central corolla, showing part of the apical gland, note the vascular tissue mainly constituted by xylem vessels and some phloem elements (**arrow**) (Nile blue); **G**, transection at the apex of the outer lip of the marginal corolla, note the three osmophoric glands protruding on the surface (Safranin- Astra Blue); **H**, longitudinal section of one lobe apex of the central corolla, showing the vascular tissue of the osmophoric gland (Safranin); **I**, **J**, *Famatinanthus decussatus*; **I**, transection of the osmophoric gland, note the reddish color of the papillae and the parenchyma showing the secretory function of both tissues (TIOFH3); **J**, detail of the previous figure (TIOFH3), note the red content of the cells and the vascular tissue. Abbreviations: **OS**, osmophore; **PP**, papillose epidermis; **SP**, secreting parenchyma; **VT**, vascular tissue. **A**, **B**, **E**, **I**, **J**, *Funk & Bonifacino 13233* (LP); **C**, **G**, *Ruthsatz s.n.* (LP s.n.); **D**, *Cabrera 8331* (LP); **F**, **H**, *Rauh P423* (LP). Scale bars: A, C, 500 µm; B, E, I, J, 120 µm; D, 0.25 mm; F, 50 µm; G, 250 µm; H, 110 µm; J, 60 µm.

Most of the species have, in general, the corolla limb papillate, with the papillae more conspicuous at the lobes and lips margin, at the sinuses, and at the apex of the corolla (Fig. 2E-H, J, L-Q). In general, the papillae are sparsely distributed at the lobes and lips margin and at the sinuses level, whereas they are usually in clusters at the corolla apex (Fig. 6A-E). The

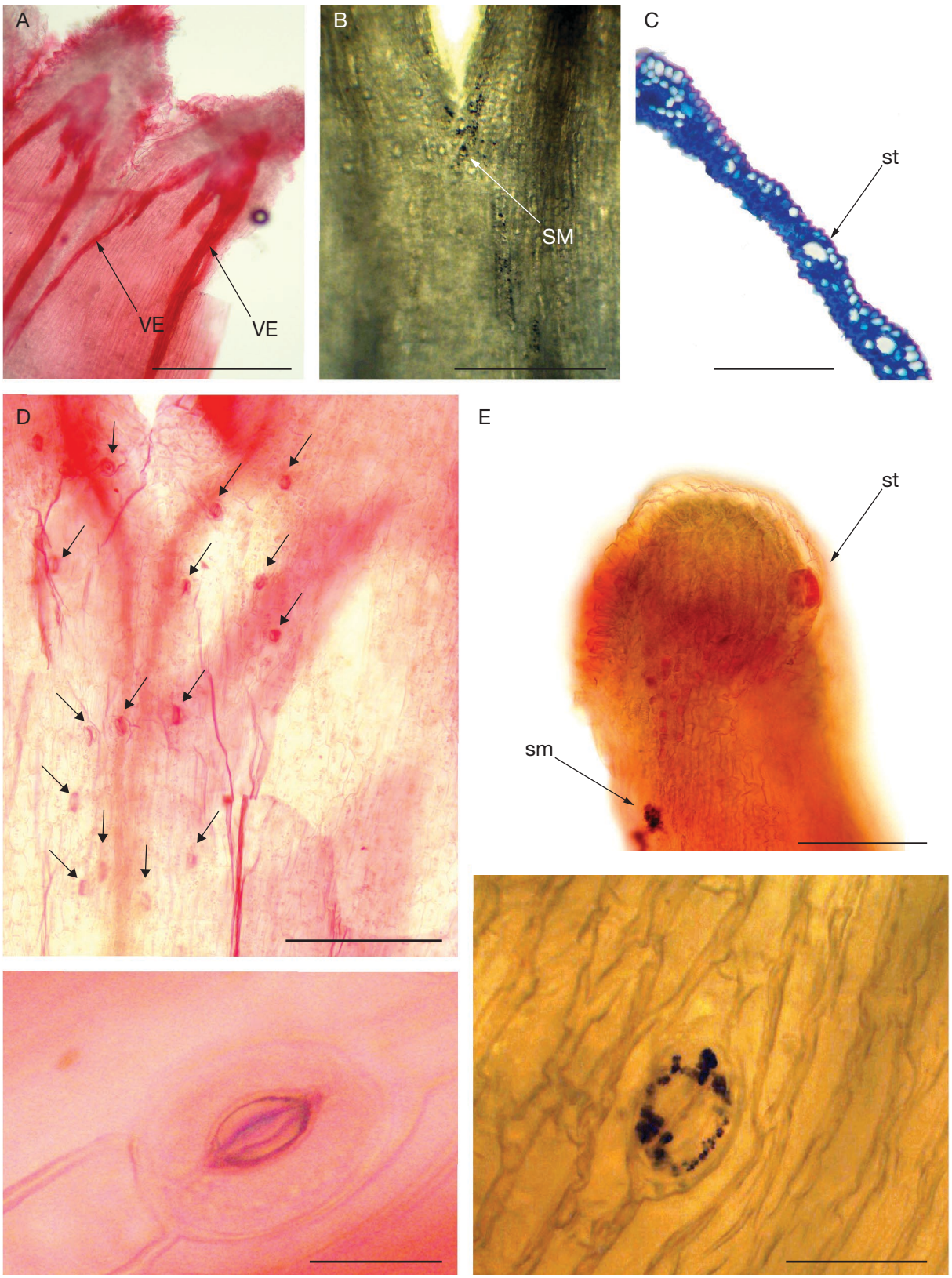


Fig. 5. — Osmophore elements in *Famatinanthus* and *Plazia*: **A**, two teeth of the marginal corolla showing the venation in *Plazia daphnoides* Wedd., note the two veins at each tooth margin joining at the apex (Safranin); **B**, sinus between the outer and the inner lips of the marginal corolla in *Famatinanthus decussatus* (Hieron.) Ariza & S.E. Freire showing starch grains (IKI); **C**, **D**, *Plazia daphnoides*; **C**, transection of the marginal corolla showing stomata with large substomatal chambers (Safranin-Astra Blue); **D**, abaxial surface view of one sinus of the central corolla, showing numerous stomata (**arrows**) near the osmophoric area (Safranin); **E**, apex of one lobe of the central corolla in *Famatinanthus decusssatus*, showing one large stomata and a cluster of starch grains below (TIOFH3); **F**, **G**, *Plazia daphnoides*; **F**, detail of one corolla stoma at the sinus area (Safranin); **G**, detail of one corolla stoma showing starch content as dark dots (IKI). Abbreviations: **SM**, starch mass; **ST**, stomata; **VE**, vein. **A**, **D**, **F**, *Ruthsatz s.n.* (LP s.n.); **B**, **E**, *Funk & Bonifacino 13233* (LP); **C**, *Rauh P423* (LP); **G**, *Zöllner 4902* (LP). Scale bars: A, 750 µm; B, 150 µm; C, E, 125 µm; D, 200 µm; F, 23 µm; G, 50 µm.

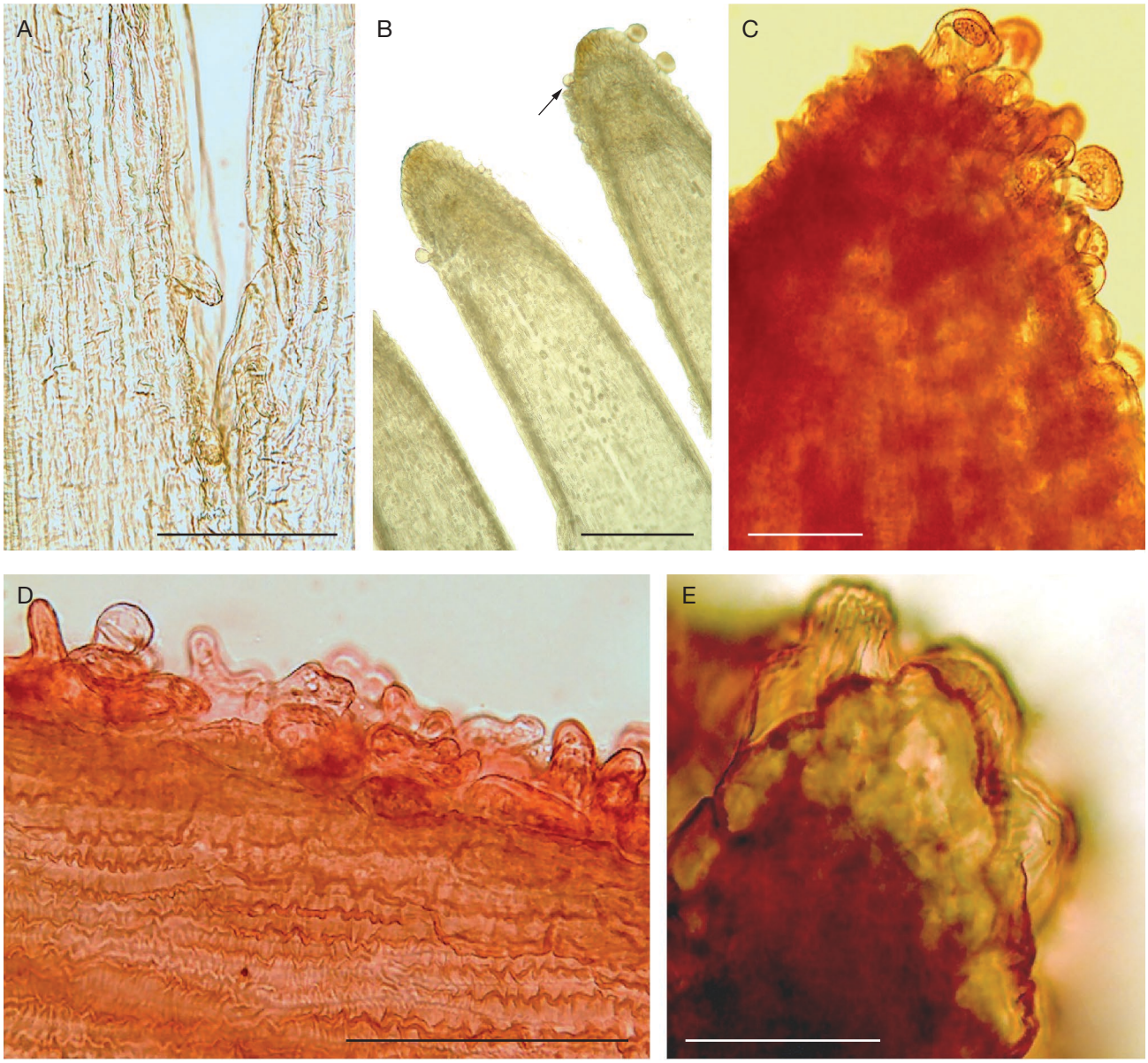


Fig. 6. — Non-osmophoric papillae in genera of Onoserideae: **A**, sinus between two lobes of the central corolla in *Aphyllocladus denticulatus* Hieron., showing papillae with negative reaction for starch test (IKI); **B**, two lobes of the central corolla in *Gypothamnium pinifolium* Phil., with apical and marginal papillae, several of them secreting drops (**arrow**) and negatively reacting for starch test (IKI); **C**, lobe of the central corolla in *Lycoseris trinervis* (D. Don) S.F. Blake, note the papillae with lipophilic content (ORO); **D**, tooth margin of the marginal corolla in *Onoseris hastata* Wedd., note the papillae with lipophilic content (ORO); **E**, lobe apex of the central corolla in *Urmenetea atacamensis* Phil., note the short, rounded papillae with striate cuticle (NR). **A**, *Ricardi 3674* (LP); **B**, *Roig Juñent 22* (LP); **C**, *Sagástegui 6867* (LP); **D**, *Fabris 6157* (LP); **E**, *Cabrera et al. 22543* (LP). Scale bars: A, 100 µm; B, 250 µm; C-E, 50 µm.

papillae are lacking in the marginal corollas of *Aphyllocladus denticulatus*, *Lycoseris*, and some species of *Onoseris* (Fig. 2I, K). The morphology of these papillae is similar to that of the osmophoric papillae.

DISCUSSION

This is the first comprehensive study that describes in detail osmophores in the plant family Asteraceae. Osmophores are found in the marginal and central corollas of the capitula of *Famatinanthus* (Famatinantheae) and *Plazia* (Onoserideae). In these taxa the osmophores are either exclusively constituted by the epidermal tissue (osmophoric epidermis, i.e., epidermal cells and papillae) or they have the scent-gland characteristics described by Vogel (1990), resulted positive to TIOFH and TIOFH3 (which include the staining of starch), are negative to Benedict's test, and have associated abundant stomata. The other species analyzed have cells with lipophilic content but they lack nearby starch grains and stomata, and therefore are not considered here as osmophores. The presence of lipophilic materials, such as terpenes, fats, waxes and flavonoid aglycones, is common in plants and fulfills multiple functions (Fahn 2000).

The fragrance of flowers mentioned by some authors in species of *Onoseris* and *Plazia* (Hooker 1831; Cabrera 1978) is not correlated with our findings, because we found osmophores in *Plazia* but not in the analyzed species of *Onoseris*. This could be only the tip of what turns out to be a complex issue, regarding the place and time of flower fragrance emission. According to Vogel (1990) even when sometimes the fragrance is not perceived by humans, odor structures could be present in the corollas. The minimum perceptibility of flower odor by pollinators is often far below than that for mankind either because of their chemical structure or because their concentration is too low for our perception. Hernández & Katinas (2019) found osmophores in the labelum of the unscented flowers of *Canna indica* L. (Cannaceae). In addition, some flowers parts other than osmophores may emit fragrance, as illustrated for example by Balao *et al.* (2011) who reported scented nectar in flowers of one species of *Dianthus* L. (Caryophyllaceae). This suggests that: 1) the lack of human scent perception does not always mean that the flower lacks osmophores, and 2) the scent perception could be linked to the presence of osmophores but also to the secretion of other flower structures, such as nectaries. Another issue to be taken in account is the floral stage in which the fragrance is emitted and how this process changes throughout the floral development. For example, Hernández & Katinas (2019) found that immature, unscented florets of *Lantana camara* L. (Verbenaceae) and *Sagittaria montevidensis* Cham. & Schltdl. (Alismataceae), reacted positively to IKI but not to NR and ORO, showing that even when these flowers have osmophores at a mature stage, the secretion substances (and therefore the fragrance) were not present at the bud stage yet.

Some of the osmophores described here have the characteristic elements mentioned by Vogel (1990) for scent glands: a positive chemical reaction for RN and IKI, histologically different cell layers, an elaborate ventilation system constituted in this case by numerous, sometimes large, stomata with large substomatal chambers, and a highly developed vascular tissue in the area of secretion (Fig. 4E, F, H). The correlation between the dense vascular supply and the glandular function of osmophores was attributed by Vogel (1990) to the phloematic elements capacity for transporting organic materials. Besides, during the fragrance synthesis the osmophoric cells require numerous stomata as respiratory openings due to the oxidative degradation of the reserve material (as starch) (Vogel 1990). In *Famatinanthus* and *Plazia*, we found that the guard cells of the stomata have frequently abundant starch grains (Fig. 5G). Although starch may typically accumulate in the underlying layers of the epidermal cells or close to the scent glands, the stomatal starch could serve as another source of energy in the process of scent emission.

Regarding the mode of emission in the studied genera, we observed that the fragrance material as droplets reaches the exterior through the epidermal cells and papillae membrane, and trespasses the cell wall, accumulates under the cuticle (Fig. 3E) and then they volatilize. The clusters of papillae in the osmophores, thus, would increase the epidermal surface area to accomplish this function. Despite the fact that papillae are apparently the main route for volatile secretions, we cannot rule out the possibility that stomata opening and closure could help in odor emission, as observed in some members of Orchidaceae (Cabral de Melo *et al.* 2010).

The osmophores of *Famatinanthus* and *Plazia* are relatively smaller and less cell-layered than those of some species of Araceae, Aristolochiaceae, Asclepiadaceae, and Orchidaceae, as illustrated by Vogel (1990). It could be the case that the members of other families, mostly with large and solitary flowers, require major scent-producing structures to attract pollinators. Comparatively, the smaller flowers of Asteraceae, but gathered in capituliform inflorescences, may reach the same results in fragrance production with smaller and less complex osmophores.

From a taxonomical point of view, the presence of osmophores in *Famatinanthus decussatus* would be thus another feature that separates this species from the species of *Aphyllocladus*, the genus from which *Famatinanthus* was segregated and where osmophores were not found. The fact that the osmophores appear in different clades of the family phylogenetic tree, such as Famatinantheae and Onoserideae, suggests that these structures may have evolved independently in Asteraceae.

Flower fragrance is one of the specific traits of the different pollination syndromes because, for example, insects are sensitive to odor but birds have little or no sense of smell. Some studies (Galetto 1995; Mendonça & dos Anjos 2006; Katinas *et al.* 2009; Sancho & Freire 2009; Stuessy *et al.* 2009; Funk & Roque 2011; Vogel 2015) demonstrated that ornithophily is in fact concentrated in the early branching clades of Asteraceae, where Famatinantheae and Onoserideae belong, without disregarding the role of bees as pollinators in these groups. For future research, it would be interesting to test if there is a higher development of osmophores in more recently branched clades of the family (e.g., subfamily Asteroideae) in comparison with that of the basal branches, due to an adaptive shift between pollinator types. For example, corollas with stomata were described in genera of Eupatorieae (King & Robinson 1987; Grossi & Katinas 2013) but their potential connection with the presence of osmophores has not been investigated yet. Shifts from hummingbird specific to a generalized pollination system might have occurred in Asteraceae as happened in other families, such as Euphorbiaceae (Armbruster & Baldwin 1998) related to historical biogeographic dispersal processes to new areas where the specific pollinators are absent. A full understanding of the processes underlying the evolution of pollination syndromes requires knowledge of whether the traits that constitute a syndrome can confer higher fitness (relative to the ancestral condition) independently of each other, or whether the adaptive advantage depends on joint variation in floral features (Fenster *et al.* 2004).

It is expected that a broader analysis of the morphology and distribution of osmophores within the Asteraceae, may be together with the study of other structures such as nectaries, hold promise for additional insight in the interpretation of the evolution of floral pollination syndromes in this family.

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