

## Floral function in relation to floral structure in two *Periploca* species (Periplocoideae) Apocynaceae

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**Abstract:** This study comprises an investigation into the floral function in relation to the floral structure of *Periploca aphylla* and *P. angustifolia*, using light and scanning electron microscopy. Both species display the following floral structures: style-head bearing pollen translators, shelf-like nectaries, staminal feet, nectar-collecting troughs, trisegmented corolline corona lobes and corolla lobes, and anthers and nectaries covered by unicellular hairs. Lateral segments of adjacent corona lobes are fused with half of the adaxial base of the adjacent corolla lobes, creating openings through which pollinators can reach the nectar-collecting troughs in the corolla base. Flowers are rotate with spreading corolla lobes and exposed gynostegium, thus sorting in the open-access fly pollination system. The following features are useful to distinguish the flowers of the two species: corolla color, presence or absence of long unicellular hairs and white spots size on the adaxial surface of corolla lobes, translator length, pollen tetrads color, and presence or absence of linear tetrads. It seems that the floral modifications of the two *Periploca* species serve to optimize pollination efficiency by attracting a wide number of pollinators, thus enhancing the pollination success of plants.

**Key words:** Corolline corona, nectar trough, open-access pollination, pollen tetrads, shelf-like nectaries, spatulate translators, staminal feet

### 1. Introduction

The subfamily Periplocoideae (Apocynaceae) comprises ca. 195 species belonging to 33 genera with most of the genera containing only a few species (Ionta and Judd, 2007). The largest genera are *Raphionacme* Harv. (37 species), *Cryptolepis* R.Br. (27 species), *Pentopetia* Decne. (21 species) (Klackenberg, 1999), and *Periploca* L. (14 species) (Venter, 1997). *Periploca* was described by Linnaeus (1753) and is the type genus of the subfamily Periplocoideae. *Periploca* species are limited to the Old World in their distribution in both Africa and Asia, from the Cape Verde Islands and the Canary Islands to North China and from the Balkan Peninsula to Malawi in East Africa (Browicz, 1966; Verhoeven and Venter, 1994; Venter, 1997). This genus exhibits a wide range of growth habit from forest climber to scrambling or erect shrub. *Periploca aphylla* Decne is an erect leafless shrub (2–4 m high) with distribution from Egypt and the Arabian Peninsula to Afghanistan (Venter, 1997). *Periploca angustifolia* Labill. is an erect leafy shrub (0.5–1.5 m high) with distribution in northern Africa, southern Europe, and Syria.

The Periplocoideae flowers are bisexual, actinomorphic, pentamerous (except for the bicarpellate gynoecium), semi-epigynous, and fleshy (Venter and Verhoeven, 1997, 2001). These small flowers are complex in structure, exhibiting a high degree of synorganization between androecia and gynoecia, typical for derived members of Apocynaceae *s.l.* These flowers are characterized by their unique, spatulate pollen translators, which assist in pollen transfer (Safwat, 1962; Schick, 1982; Verhoeven and Venter, 2001). The relatively soft translators consist of a spoon-shaped receptacle onto which pollen is shed at anthesis, an adhesive disc that attaches to the pollinator, and a stipe (stalk) connecting the two (Verhoeven and Venter, 1997; Endress and Bruyns, 2000; Endress, 2004). Schick (1982) introduced the term “scutellum” for the solid part of the adhesive disc, which proved to be very useful. Ontogeny of the translators revealed that the scutellum developed first and the stipe became attached to the disc before the adhesive substance was added to the lower side of the scutellum or the stipe elongated to connect the adhesive disc to the receptacle (Kunze, 1993). Venter and Verhoeven (1997) used the floral structures

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in a tribal classification of Periplocoideae: corolla form, exposure of the gynostegium from the corolla, and the position of coronas and stamens in the corolla tube. There is a wide range of corolla shapes, diversification of corolline coronas, varying degrees of fusion between coronas and stamens, and differences in the position of floral organs on the corolla tube (Venter and Verhoeven, 2001). Verhoeven and Venter (2001) distinguished the Periplocoideae by the presence of pollen arranged in tetrads or pollinia that are shed onto spoon-shaped translators with a soft, sticky, amorphous viscidium. Verhoeven and Venter (1994) found that the Periplocoideae pollen tetrads were rhomboidal, decussate, or tetragonal in arrangement and with 4–6 pores at the junction area of adjacent grains.

*Periploca* was distinguished as a genus by its filiform corona lobes and hairy anthers (Linnaeus, 1754) or the distinctness of the corolla tube, position and shape of the corona lobes, and presence or absence of hairs on the stamens (Brown, 1810; Blume, 1828). *Periploca* is further more characterized by the following structures: shallow corolla tube, exposed gynostegium, presence of flap-like coronal segments on the corolla lobes, and hairy anthers (Browicz, 1966). Diptera pollination is probably the norm in some *Periploca* species (Schick, 1982). Jonkers (1993) observed *Polistes watti* (Hymenoptera: Vespidae) and Diptera: Calliphoridae visiting *Periploca aphylla* flowers in Oman but he did not record actual removal or insertion of pollinia. Ollerton and Liede (1997) reported that *Polistes* wasps were known to visit *Periploca aphylla* in Oman but they had never been observed carrying translators. Pisciotta et al. (2011) identified fifteen species within nine families of Diptera as pollinators of *Periploca angustifolia*.

Floral and extrafloral nectaries in plants favor pollination and defense against herbivory (Almeida et al., 2012). Taxonomic revisions and pollen studies have been published on *Periploca* species by Browicz (1966), Verhoeven and Venter (1994), and Venter (1997). However, the morphological variations and significance of nectaries in pollination have not been analyzed so far. Nectar and pollen are the main floral rewards for pollinators (Nocentini et al., 2012). Moreover, organization of the internal floral part has remained virtually unexplored in the genus *Periploca*. This present study examined the floral structures in *P. aphylla* and *P. angustifolia* in relation to functional aspects. This study also investigated how flower structure might help to guide insects into the flowers, with particular emphasis on the nectar position. The observed data are discussed and compared with those of previous studies on the subfamily Periplocoideae (Kunze, 1990, 1991; Endress and Bruyns, 2000; Venter and Verhoeven, 2001; Pisciotta et al., 2011).

## 2. Materials and methods

### 2.1. Plant collection

*Periploca aphylla* Decne. was collected from Umm Kibath, Sharm El Sheikh, Egypt, 27.6.2009, Heneidak s.n. *Periploca angustifolia* Labill. was collected from Wadi Salufa, El-Salûm, Egypt, 12.6.2009, Heneidak s.n. The voucher specimens are deposited in the Suez University Herbarium.

### 2.2. Electron microscopy

For the scanning electron microscopic (SEM) study, flowers were fixed in glutaraldehyde 2.5% and dehydrated through an alcohol series from 70% to 100% ethanol using an automatic tissue processor (Leica EM TP). These flowers were then dried using a carbon dioxide critical point drier (Tousimis Audosamdri-815), and thereafter dissected to reveal their organs, mounted onto stubs, and coated with a gold sputter coater (SPI-Module). Specimens were examined and photographed with a JEOL JSM-5500LV SEM at 13–18 kV.

For examination of pollen tetrads by SEM, mature and unopened buds were dissected under a microscope (Olympus type BH-2). Pollen tetrads were prepared for study using acetolysis (Erdtman, 1960). Acetolysed pollen tetrads were pipetted onto specimen stubs and sputter-coated with a gold sputter coater (SPI-Module). Pollen tetrads were examined and photographed with a JEOL JSM-5500LV SEM at 13–18 kV.

### 2.3. Light microscopy

For the light microscopy (LM) study, flowers were fixed and preserved in FAA, and then transferred to 70% ethanol and embedded in Paraplast Plus wax (Johansen, 1940). Using a rotary microtome, serial sections (10–20 µm) were obtained and double stained, using crystal violet (2% in 50% ethanol) and erythrosin (1% in absolute ethanol and clove oil) (Corgan and Widmoyer, 1971). The stained sections were examined and photographed on a Carl Zeiss Axiostar Plus microscope fitted with a Canon (Pc1200 PowerShot A641) digital camera at the Zoology Department, Faculty of Science, Suez Canal University.

Semipermanent pollen preparations were made by releasing the pollen tetrads from mature anthers on a slide, and diluted glycerin was added. Pollen tetrads were investigated using the light microscope, length and width were determined in micrometers (µm) with the aid of a calibrated ocular micrometer (measurements averages based on ten tetrads for each species), and photographed on a Carl Zeiss Axiostar Plus microscope fitted with a Canon (Pc1200 PowerShot A641) digital camera.

### 2.4. Terminology

The term corolline corona was proposed by Liede and Kunze (1993). The term gynostegium is used for the compound structure of the androecium and the style-head (Bookman, 1981). The terms style-head and staminal foot are used according to Endress and Bruyns (2000).

### 3. Results

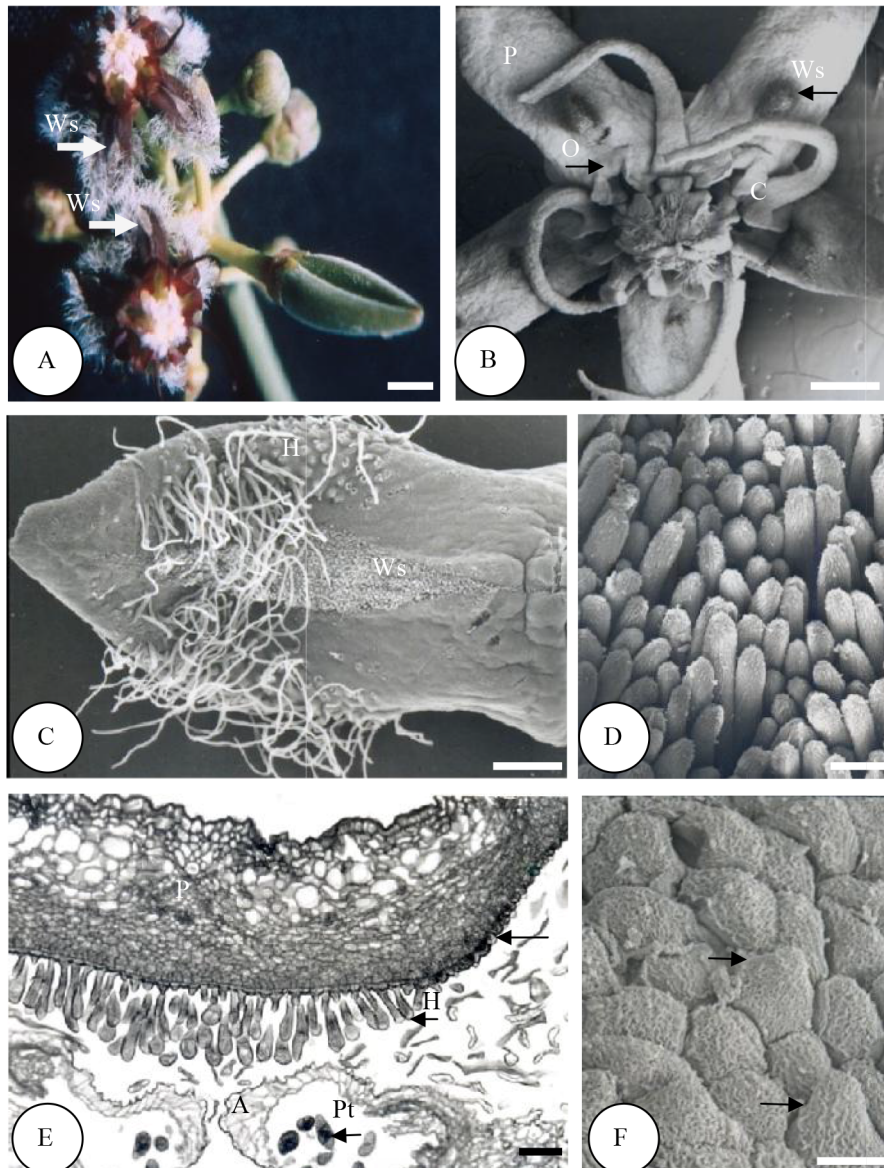
#### 3.1. Inflorescences and flowers

Inflorescences of *P. aphylla* are carried on 1.2–2.5 cm long cymes of 3–15(–20) flowers (Figure 1A). These flowers are rotate, pentamerous, actinomorphic, bisexual, dark purple, 10–15 mm in diameter, and fragrant (Figure 1A). Inflorescences of *P. angustifolia* are carried on 3–12-mm-long cymes with peduncles bearing 2–9(–15) flowers. The flower structure is similar to that of *P. aphylla* except that

the corollas are violet to maroon and ca. 9 mm in diameter (Figure 1B).

#### 3.2. Corolla

In both species, the corolla tube is short and shallow,  $\pm 2$  mm long. Corolla lobes of *P. aphylla* are 3–6  $\times$  2.5–3 mm, dark purple, oblong-ovate, obtuse, entire, fleshy and leather-like (Figure 1A). Adaxially, the corolla lobes are densely covered with long white unicellular hairs along their margins, from middle to apex (Figure 1A). However,



**Figure 1.** SEM micrographs and LM micrograph of inflorescences, flower, and petal of two *Periploca* species. **A-** Inflorescence of *P. aphylla*. **B-** Top view of *P. angustifolia* flower showing rotate flower. **C-** Adaxial view of corolla lobe showing dense long white unicellular hairs and white spot. **D-** Unicellular clavate papillae of white spot on corolla lobe. **E-** Cross section through a bud showing structure of white spot and papillose surface (arrow). **F-** Adaxial papillose surface of corolla lobe (arrows). Scale bars: A = 2 mm; B = 1 mm; C = 500  $\mu$ m; D = 25  $\mu$ m; E = 50  $\mu$ m; F = 17  $\mu$ m. A = anther; C = corona lobe; H = hairs; O = opening between corona lobes; P = petal; Pt = pollen tetrads; Ws = white spot.

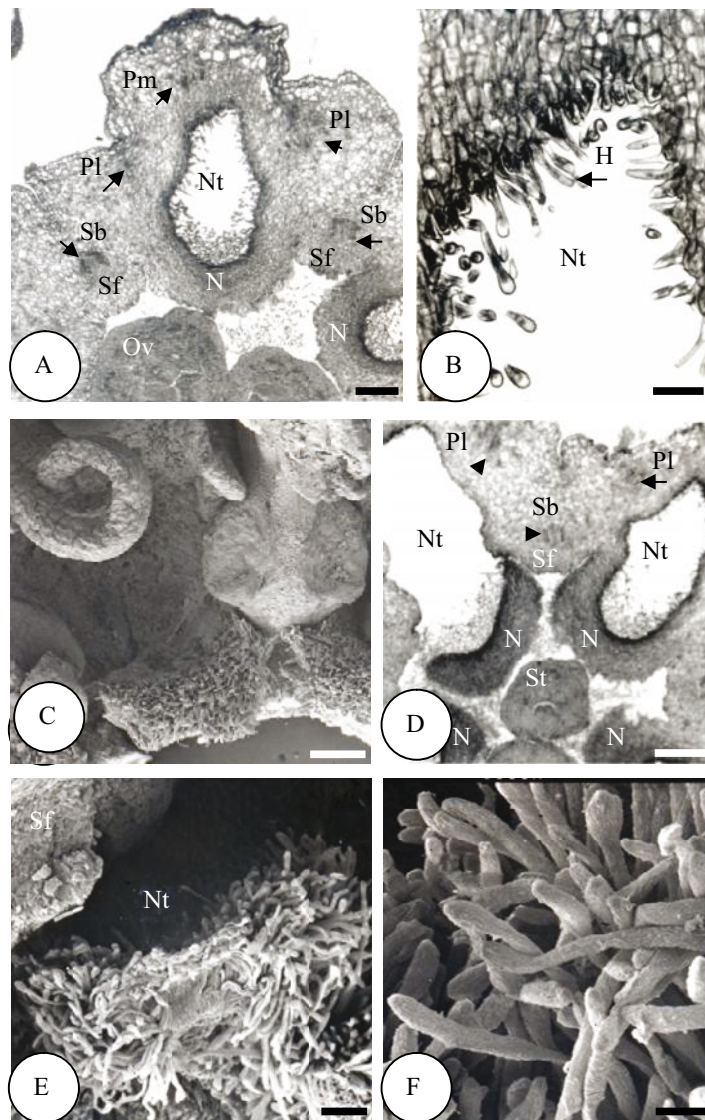
corolla lobes of *P. angustifolia* are ca. 3.5 mm long with glabrous margin (Figure 1B) and are violet to maroon contrasting with greenish yellow margins adaxially. There is a characteristic narrowly elliptic white spot of clavate unicellular papillae 2-3 × 1 mm on the middle part of the corolla lobes of *P. aphylla* (Figures 1C and 1D). Similar spots of 1-1.5 × 0.5 mm are present on the corolla lobes of *P. angustifolia* (Figure 1B). Adaxial surfaces of corolla lobes are densely papillose in both *Periploca* species (Figures 1E and 1F).

### 3.3. Corolla-androecium tube

In both species, the corolla and androecium are fused at the base and form the corolla-androecium tube after the separation of the ovary (Figures 2A-2C).

### 3.4. Floral nectaries

Characteristics of the floral nectaries of both species are similar. There are five troughs in between the staminal feet for collecting the nectar (Figures 2A and 2B). Sides of these troughs are covered by unicellular hairs (Figures 2A and 2B). Each trough is bordered by a nectary, a fleshy



**Figure 2.** LM micrographs and SEM micrographs of *Periploca aphylla*. **A-** Part of the corolla-androecium tube with nectar-collecting trough in cross section. **B-** Sides of this trough covering by unicellular hairs in cross section. **C-** Shelf-like nectary alternating with the swollen fleshy staminal feet and bordering the nectar-collecting trough. **D-** Shelf-like nectaries encircling the two styles in cross section. **E-** Shelf-like nectary densely covering by hairs. **F-** Unicellular hairs covering shelf-like nectary. Scale bars: A, C = 250 µm; B = 100 µm; D = 500 µm; E = 67 µm; F = 20 µm. H = hairs; N = shelf-like nectary; Nt = nectar-collecting trough; Ov = ovary; Pl = petal marginal bundle; Pm = petal median bundle; Sb = staminal bundle; Sf = staminal foot; St = style.

staminal foot, and the corolla tube (Figures 2C and 2D). The five nectaries alternate with the staminal feet (Figures 2C-2E), are shelf-like,  $\pm 0.3$  mm long, and densely covered by unicellular hair (Figures 2C-2F). These nectaries encircle the upper part of the ovaries and the styles (Figures 2D and 4D).

**3.5. Corona lobes**

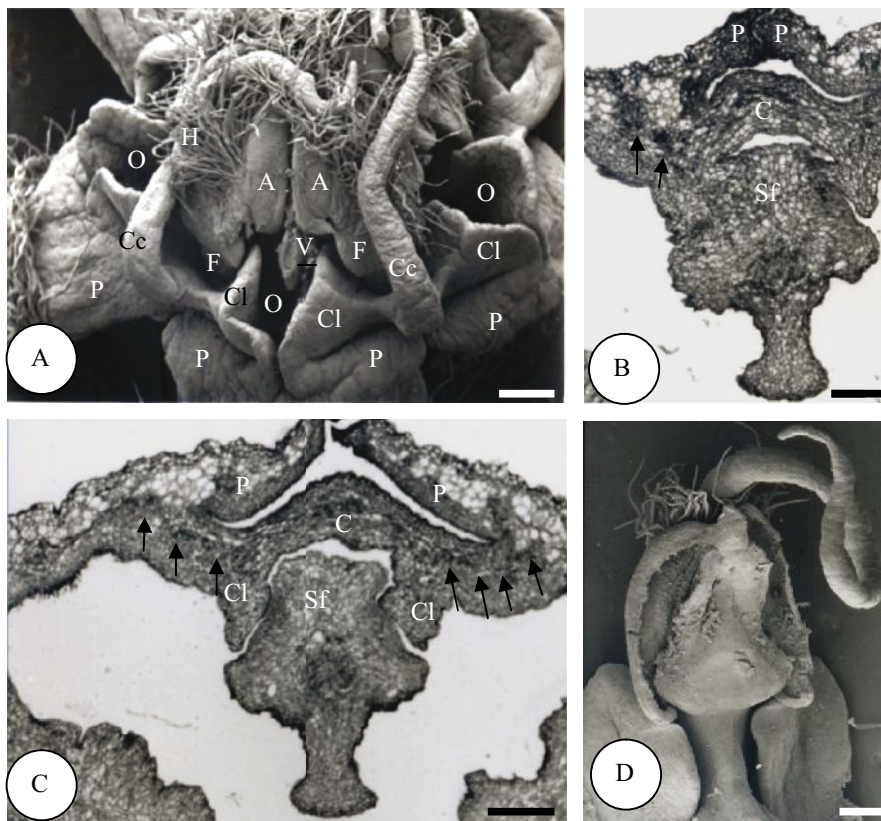
In both *P. aphylla* and *P. angustifolia*, the corona consists of five trisegmented lobes representing outgrowths of the inner face of the corolla tube. The corona lobes alternate with the corolla lobes, arising slightly above the stamens and the nectar collecting troughs (Figures 3A and 3B). The corona lobes receive vascular supply from the petal marginal bundles (Figures 3B and 3C). The corona lobes are laterally fused to the adjacent corolla lobe bases (Figures 3A and 3C).

Corona lobes of *P. aphylla* are dark purple or violet, slightly fleshy, glabrous, and exerted from the corolla mouth. These lobes are trisegmented with two lateral

deltoid-ovoid segments of  $2-3 \times 2-3$  mm and a robust central filiform segment 6-8 mm long (Figures 3A and 3D). The central segment is simple and twisted over the anthers (Figures 3A and 3D). The lateral segments of the corona lobes fit tightly around the stamens, leaving a small entrance opening through which a visiting pollinator is guided to the basal nectar-collecting troughs in the lower corolla-androecium tube (Figures 3A, 3C, and 3D). In *P. angustifolia*, the coronal lobes are violet to maroon, minutely puberulent, and somewhat shorter than the corolla lobes. These lobes have two lateral segments of  $1-1.5 \times 0.3-0.5$  mm and a central coronal segment 7-8 mm long (Figures 1B).

**3.6. Gynostegium**

The anthers connive over the style-head with their connective apices touching and their bases fused to the margin of the style-head, thus forming the gynostegium (Figure 4A). The gynostegium is completely exposed and positioned above the corolla tube mouth (Figure 3A).



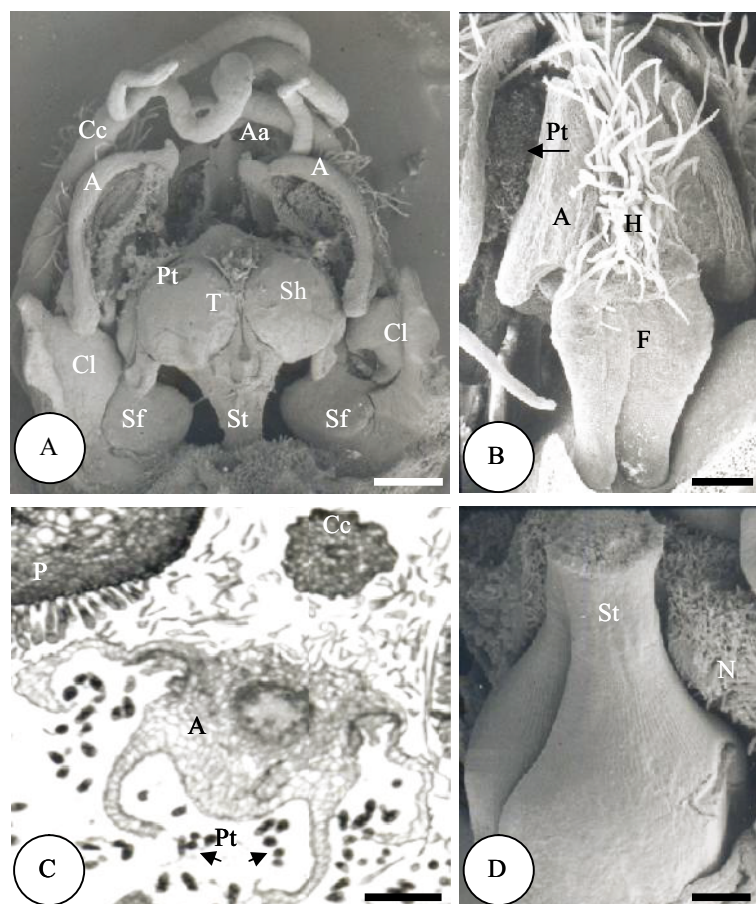
**Figure 3.** SEM micrographs and LM micrographs of *Periploca aphylla*. **A-** Lateral segments of corona lobes fused with half of the adaxial base of the adjacent corolla lobes. **B-** A cross section through a corona lobe having vascular supply from the adjacent petal marginal bundles (arrows), and fused with the staminal feet only at the base. **C-** Lateral segments of corona lobes fused with the adaxial base of the adjacent corolla lobes. **D-** Ventral view of stamen fused to the corona lobe, lateral segments of corona lobe fitting tightly around it, and a filiform central segment of corona lobe twisted over the anther. Scale bars: A = 500  $\mu$ m; B, C = 150  $\mu$ m; D = 250  $\mu$ m. A = anther; Aa = anther apical appendage; C = corona lobe; Cc = central segment of corona lobe; Cl = lateral segment of corona lobe; F = filament; H = hairs; O = opening between each two lateral segments of adjacent corona lobes; P = petal; Sf = staminal foot; V = viscidium.

In both *Periploca* species, the androecium has five stamens inserted at the base, which alternate with the corolla lobes. Each stamen has a more or less cylindrical, short filament beneath the anther (Figure 4B). Filaments are  $1-1.5 \times 1$  mm, free, arising on swollen spherical-elliptically thickened staminal feet around the styles (Figure 4A) and are fused to the lateral segments of the coronal lobes (Figure 3D). The staminal feet are derived from the stamen and vascularized by staminal bundles, not corolline in origin (Figures 3B and 3C). The anthers are  $2 \times 1$  mm, closely pressed together and touching each other over the style-head (Figures 3A, 4A, and 4B). The anthers, furthermore, each have a cluster of long unicellular hairs on the central abaxial side (Figures 3A and 4B). An anther consists of two thecae, each with two locules, which open laterally with slits (Figure 4C), no guide rails or lignified tissue on the anther margins, but the connective has a short membranous apical appendage (Figures 3D and 4A).

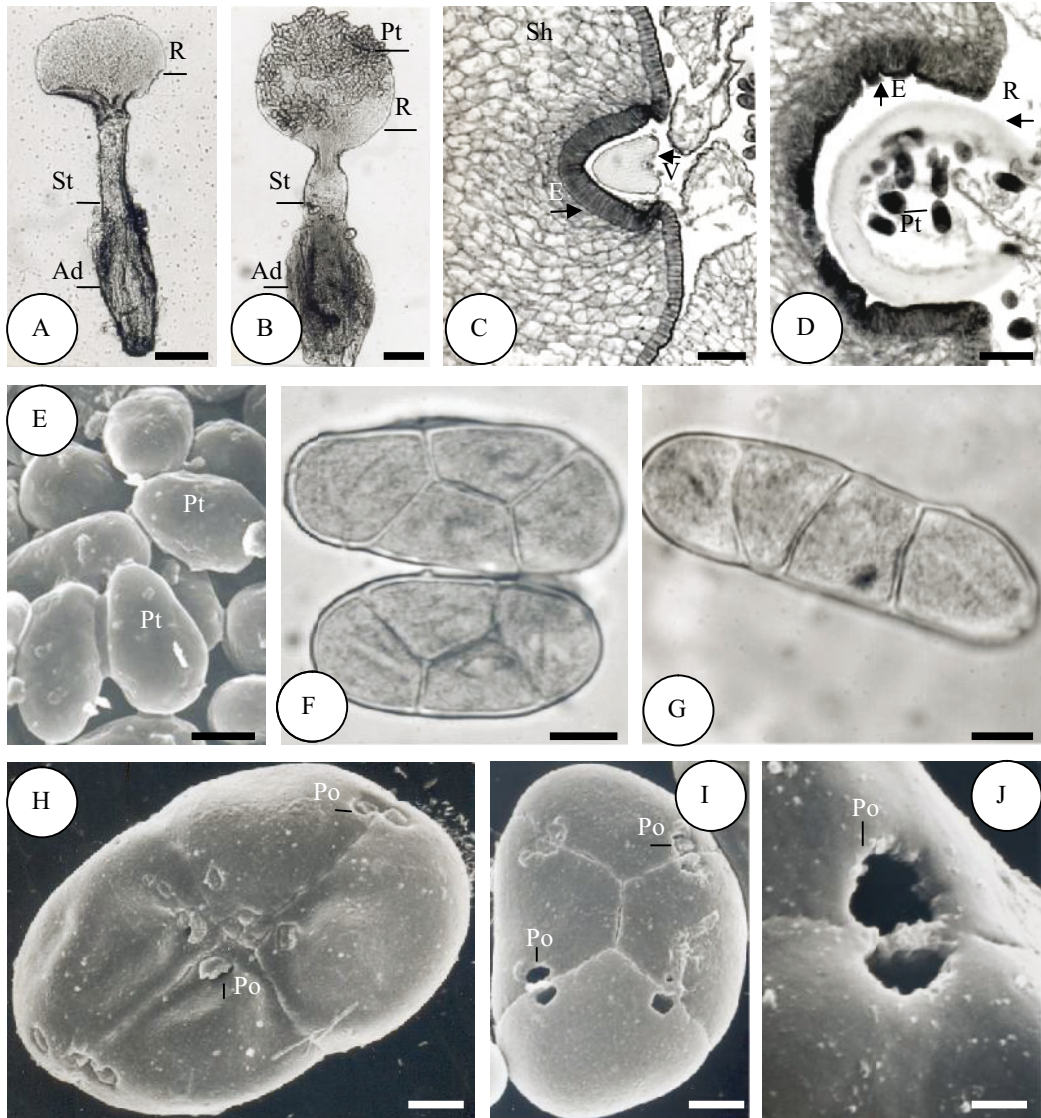
In both species the gynoecium consists of two apocarpous, semi-inferior ovaries with two styles that fuse terminally into a style-head. The ovaries of *P. angustifolia* are ca.  $1 \times 0.5$  mm and glabrous (Figure 4D), its two styles are short, ca.  $0.7 \text{ mm} \times 0.3 \text{ mm}$  and a  $\pm 0.5 \times 1$  mm dilated style-head (Figure 4D). Gynoecia of *P. aphylla* have  $1-1.5 \times 0.5$  mm ovaries,  $1-1.5 \times 0.3$  mm styles, and a style-head of  $\pm 1 \times 2$  mm that is pentagonal, fleshy, and broadly ovoid with an acute apex (Figure 4A). There are five lateral grooves in the style-head of both species, alternating with the anthers (Figure 4A).

### 3.7. Translators and pollen tetrads

In both species there are five relatively soft spatulate translators embedded in the lateral grooves of the style-head,  $1.5-1.7$  mm long in *P. aphylla* (Figure 3A) and  $\pm 1$  mm long in *P. angustifolia* (Figure 5A). Each translator is a complex pollen carrier, and consists of a basal, soft, narrow adhesive disc; a distinct stipe; and a suborbicular



**Figure 4.** SEM micrographs and LM micrograph of *P. aphylla* and *P. angustifolia*. **A-** Gynostegium, stamens with swollen staminal feet fused to corona lobes, style-head with translators in their lateral grooves, and pollen tetrads deposited from the anthers on the translator in *P. aphylla*. **B-** Each stamen consisting of short filament below anther in *P. aphylla*. **C-** Cross section of anther consisting of two thecae with four locules in *P. aphylla*. **D-** Shelf-like nectaries around the upper parts of the two ovaries and styles in *P. angustifolia*. Scale bars: A = 500  $\mu\text{m}$ ; B = 250  $\mu\text{m}$ ; C = 50  $\mu\text{m}$ ; D = 125  $\mu\text{m}$ . A = anther; Aa = anther apical appendage; Cc = central segment of corona lobe; Cl = lateral segment of corona lobe; F = filament; H = hairs; N = shelf-like nectary; Sf = staminal foot; P = petal; Pt = pollen tetrads; Sh = style-head; St = style; T = translator.



**Figure 5.** LM micrographs and SEM micrographs of translators and pollen tetrads of two *Periploca* species. **A, B-** Translator structure in *P. angustifolia* and *P. aphylla*, respectively. **C-** Special glandular epithelial cells secreting a stipe at the lateral groove on the style-head in *P. aphylla*. **D-** Special glandular epithelial cells secreting a receptacle at the top of the lateral groove of the style-head in *P. aphylla*. **E-** Pollen tetrad grouped together in *P. aphylla*. **F-** Rhomboidal pollen tetrad in *P. aphylla*. **G-** Linear tetrad in *P. aphylla*. **H-** Decussate pollen tetrad in *P. angustifolia*. **I-** Rhomboidal pollen tetrad with equal adjacent pores in *P. aphylla*. **J-** Uneven pore edge in two adjacent pollen grains in *P. angustifolia*. Scale bars: A, B = 173  $\mu$ m; C, D = 50  $\mu$ m; E = 17  $\mu$ m; F = 9  $\mu$ m, G = 6  $\mu$ m; H = 5  $\mu$ m; I = 7  $\mu$ m; J = 2  $\mu$ m. Ad = Adhesive disc; E = epithelial cells; Po = germination pore; Pt = pollen tetrads; R = receptacle; Sh = style-head; St = stipe; T = translator.

apical receptacle (Figures 5A and 5B). These translators are secreted on the upper surface of the style-head from hardened secretions of special epithelial cells, in positions that alternate with the anthers surrounding the style-head (Figures 5C and 5D). The adhesive disc is secreted by special epithelial cells at the base of the lateral groove on the style-head (Figure 5C). This adhesive disc is positioned directly above the opening created by the lateral segments

of two adjacent corona lobes (Figure 3A) in order for the translator to attach itself to a pollinator attempting to reach the nectar in the nectar-collecting trough, and the translator with its load of pollen tetrads can thus be transported to the next flower visited. The pollen tetrads from each two adjacent anther thecae become deposited onto the sticky concave opposite receptacle at anthesis (Figure 4A), where they adhere to the receptacle loosely.

Pollen development in *P. aphylla* and *P. angustifolia* is of the simultaneous type. The pollen grains of *P. aphylla* are united in bright yellow  $44\text{--}65 \times 25\text{--}35 \mu\text{m}$  tetrads (Figure 5E). These grains have rhomboidal (Figures 5F and 5I), linear (Figure 5G), or decussate (Figure 5H) arrangement in the tetrads, which have smooth exines. Pollen tetrads of *P. angustifolia* are  $39\text{--}62 \times 20\text{--}35 \mu\text{m}$  in size, free, pale yellow with rhomboidal or decussate arrangement without any linear tetrads (Figure 5H). Germination pores are 4 to 6, round to elliptical, restricted to the junction area of adjacent grains (Figures 5H–5J) and equal (Figure 5I) or unequal in size (Figure 5J). These pores are sometimes covered with a thin layer of exine material (Figures 5H and 5I).

### 3.8. Open-access fly pollination system

The flowers of *P. aphylla* and *P. angustifolia* are rotate with indistinct short corolla tubes, spreading corolla lobes, and exposed gynostegium, and thus of the open-access configuration and adapted to insect pollination. The pollinator can alight freely and has ready access to the nectar in the troughs in the lower corolla-androecium tube and to the translators in the lateral grooves of the style head (Figures 3A and 4A). There is also no morphological mechanism that may prevent a pollinator from leaving the flower. The long unicellular hairs and white spots seem to direct insects along the corolla lobes down to the openings created by the lateral segments of the adjacent corona lobes directly above the nectar-collecting troughs, which are situated just below the pollen translators (Figures 1B and 3A), thus attracting pollinators towards a position for pollen transfer.

### 4. Discussion

Our observations reveal that the flowers of *P. aphylla* and *P. angustifolia* are highly complex in structure with many modifications to enhance visits of pollinators for nectar (Table). The corolla-androecium tube and the normal corolla tube as reported in studied *Periploca* species can be differentiated to the lower and upper corolla tubes as discussed by previous authors (Endress and Bruyns, 2000; Venter and Verhoeven, 2001; Kunze, 2005). The lower corolla tube consists of the congenitally fused corolla, stamens, and nectaries (Venter and Verhoeven, 2001). The upper corolla tube is formed of just the corolla (Nishino, 1982; Sennblad et al., 1998; Endress and Bruyns, 2000). The present study showed that the corolla-androecium tube in both species is densely hairy and not glabrous as mentioned by Venter and Verhoeven (2001 (in the lower corolla tube of the Periplocoideae species).

The present observations showed that the adaxial surfaces of the corolla lobes are densely covered by unicellular hairs in *P. aphylla* or glabrous in *P. angustifolia*. Venter (1997) also distinguished series *Periploca* (*P.*

*aphylla*) from series *Laevigatae* (*P. angustifolia*) according to adaxially villous margins or glabrous corolla lobes, respectively. There are unique feature of adaxial corolla lobes in both studied *Periploca* species showing a central white papillose spot, as reported by Ionta and Judd (2007) in nine *Periploca* species. The white spots on the surface of the petals may be considered as possible outer nectaries (Vogel, 1977; Schick, 1982). Adaxial surfaces of the corolla lobes are papillose in the two examined *Periploca* species. Apart from *Periploca*, Venter and Verhoeven (2001) also noted papillose adaxial surfaces on the corolla lobes in *Omphalogonus* and *Phyllanthera*.

The corona lobes for both *Periploca* species are corolline and well marked by the presence of swollen staminal feet fused only at the base. The originally free petal margins are considered a prerequisite for the formation of corolline coronas in the staminal sectors of the tube (Kunze, 2005). The corolline corona is the oldest form of a corona and widespread in the subfamily Periplocoideae, where they diversify considerably (Liede and Kunze, 1993). The basic structure of the corona is often used to delimit genera, whereas differences in shape and color constitute the characters most frequently used for species delimitation. Corona lobes are basally trisegmented in both examined *Periploca* species, as delimited by Venter (1997) for the two sections in *Periploca* by their presence (section *Periploca*) or their absence (section *Monocoronata*). The function of the corona in both *Periploca* species is to attract and guide pollinators past the gynostegium to the nectar in the lower corolla-androecium tubes. In passing the gynostegium, a translator may adhere to the pollinator and thus may carry pollen on the translator receptacle to another flower where the pollen may rub off on the stigmatic surface of the style-head. The corona serves as guidance of insects into proper position for pollen removal and insertion by mechanically positioning the proboscides (Kunze, 1990, 1991; Endress and Bruyns, 2000), and optical cues to pollinators (Endress, 1994).

The present study clarifies that the staminal feet are part of the stamen and are not corolline in origin. Endress and Bruyns (2000) found staminal feet at the base of the stamen, but could not differentiate clearly between tissues derived from the stamen and those that are corolline in origin. The anther bases of both *Periploca* species are fused to the lateral sides of the style-head forming the gynostegium. In the Periplocoideae, the anthers are clearly postgenitally fused to the style head (Endress and Bruyns, 2000). The anthers also in the two studied species have hairy outer sides, which have been used as a diagnostic character for *Periploca*, *Ectadium*, and *Pentopetia* (Verhoeven and Venter, 2001).

The translators play a fundamental role in pollination by attaching to the insect by means of an adhesive disc



**Table.** Useful characters distinguish between the flowers of the two *Periploca* species.

Floral character	<i>P. aphylla</i>	<i>P. angustifolia</i>
<u>Inflorescence:</u>		
Length	12–25 mm long	3–12 mm long
No. of flowers	3–15(-20)	2–9(-15)
Flower diameter	10–15 mm	c. 9 mm
<u>Corolla lobe:</u>		
Length	3–6 mm	c. 3.5 mm
Color	dark purple	violet to maroon
Margin texture	covered with long white unicellular hairs	glabrous
Margin color	dark purple	greenish yellow
<u>White spot:</u>		
Size	2–3 × 1 mm	1–1.5 × 0.5 mm
Shape	narrowly elliptic	Small circular
<u>Corona lobes:</u>		
Color	dark purple or violet	violet to maroon
Size of two lateral segments	2–3 × 2–3 mm	1–1.5 × 0.3–0.5
Length of a central segment	6–8 mm	7–8 mm
Texture	glabrous	minutely puberulent
Ovary length	1–1.5 mm	c. 1 mm
Style length	1–1.5	c. 0.7 mm
Style-head size	±1 × 2 mm	±0.5 × 1 mm
Translator length	1.5–1.7 mm	±1 mm
<u>Pollen grains:</u>		
Size	44–65 × 25–35 µm	39–62 × 20–35 µm
Color	bright yellow	pale yellow
Shape	rhomboidal, linear, or decussate	rhomboidal or decussate

(Zito and Sajeve 2012). The spatulate translators in the two examined *Periploca* species comprise a basal adhesive disc, a stipe, and a broad apical receptacle secreted by special epithelial cells of the lateral grooves on the style-head. The upper part of the adhesive disc and the base of the stipe are regarded as central elements of the Periplocoid translator, being homologous with the corpusculum of the Asclepiadoideae and Secamonoideae (Kunze, 1993). The homology of these two structures is supported by their secretion in the same region of the style-head (Endress, 2001). Pisciotta et al. (2011) observed *Chrysomya albiceps* inserting their heads in the openings leading to the nectar

troughs, extracting translators, bringing them attached to their mouthparts to other flowers, and escaping without them.

*Periploca aphylla* can be distinguished from *P. angustifolia* by the presence of linear tetrads as also noted by Verhoeven and Venter (1994), and by clustered bright yellow tetrads as recorded by Verhoeven and Venter (1994) in *P. acuminata* Rahman & Wilcock and *P. laevigata* Aiton. Schill and Jäckel (1978) stated that the translator forms, the configurations of the tetrads, and the apertures give clear indications for a classification of the genera within the Periplocoideae. Dane (2000) found that the pollen

tetrads of *Periploca graeca* L. germinated within the anther loculi with a germination ratio of 90% and formed 1–4 pollen tubes per tetrad.

It is clear that nectar is secreted by shelf-like nectaries in the two *Periploca* species studied. These shelf-like nectaries develop together with the adjacent, swollen spongy staminal feet and the nectar-collecting troughs in the corolla-androecium tube. Endress and Bruyns (2000) also recorded shelf-like nectaries in seven Periplocoideae genera (*Cryptolepis*, *Cryptostegia*, *Ectadium*, *Maclaudia*, *Omphalogonus*, *Stomatostemma*, and *Telectadium*). Nectar has a great role in flower pollination by offering a reward to pollinators visiting the flower (Bookman, 1981; Kevan et al., 1989). Zito et al. (2013) isolated 16 essential oil compounds from *P. angustifolia* flowers, among them semiochemicals that attract the pollinators on flowers. These pollinators use the volatile compounds of the flowers to find pollen or nectar (Zito and Sajeve, 2012). The flowers of the two examined *Periploca* species are the same as those of the fly pollinated species belonging to the open-access fly pollination system as reported before in some other *Periploca* species by Schick (1982) and Ollerton and Liede (1997). Open-access fly pollinated flowers are flat to campanulate (Bruyns, 2000). Therefore, the tribe Periploceae contains the only genus (*Periploca*) that has some fly pollinated species, though other genera (e.g., *Cryptostegia*) are moth pollinated (Ollerton and Liede, 1997). This pattern of a mixture of strictly fly pollinated and nonfly pollinated species occurring within the same clade recurs throughout the family (Ollerton and Liede, 1997). Only Lepidoptera (hawkmoths) and Diptera are recorded as legitimate pollinators in the Periplocoideae, but this is based on scant information and the actual range of pollinators may be much broader. Recently, Pisciotta et al. (2011) confirmed that *Periploca angustifolia* is fly pollinated and has a broader spectrum of pollinators, with 17 species of Diptera involved. These authors revealed that *P. angustifolia* showed functional specialization to attract Diptera, but also had ecological generalization as they relied on several taxa for success pollination. Kunze (1991) found that removal and insertion of pollinaria by

the insect pollinator is aided by various structural devices such as hairs or corona elements guiding the proboscis towards the entrance of the anther slit. It is already reported that all the morphological and functional floral specializations in the two *Periploca* species attract and cover a wide number of pollinators with a similar biology (Ollerton and Liede, 1997; Pisciotta et al., 2011; Zito et al., 2013).

Our results lead us to conclude that the flowers in both *Periploca* species show specific adaptations for ensuring successful pollination. In this respect, the following modifications are noticeable: (i) dark purple corolla lobes with the contrast of the bright white gynostegium in the flower center (*Periploca aphylla*), violet to maroon corolla lobes contrasting with their greenish yellow margins (*Periploca angustifolia*), (ii) characteristic white spots on the corolla lobes, which may orient visiting insects, (iii) long hairs on the adaxial face of the corolla lobes (*Periploca aphylla*) and on the anthers, which may act as visual attractants, especially if they move in the breeze, (iv) rotate flowers with spreading corolla lobes and exposed gynostegium presenting free access to a wide range of visiting insects, (v) long central filiform segments of corona lobes lie over the back of the anthers, providing additional mechanical support to the anthers and guidance to a pollinator, (vi) lateral segments of adjacent corona lobes fitting tightly around stamens and very close to each other, restrict pollinators to the opening leading to the nectar troughs in the lower corolla-androecium tubes, (vii) hairs covering the shelf-like nectaries, (viii) fragrant flowers. All of these floral modifications may serve principally to guide pollinators to the nectar-collecting troughs and to the translator on the style-head. Therefore, the ability to attract many species of pollinators may enhance the chance of being pollinated and ensure reproduction.

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