Comparative floral structure and systematics in Apodanthaceae (Rafflesiales)

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Abstract. Comparative studies on floral morphology, anatomy, and histology were performed to identify shared features of the genera of Apodanthaceae (Rafflesiales): *Apodanthes*, *Pilostyles*, and *Berlinianche*. *Berlinianche* was studied for the first time in detail and its affinity to Apodanthaceae was confirmed. It has a previously undescribed hair cushion on the inner perianth organs and inaperturate pollen. Shared features of members of Apodanthaceae are: unisexual flowers; three (or four) alternating di-/tetra- or tri-/hexamerous whorls of scales of which the inner one or two correspond to a perianth; a synandrium with pollen sacs typically arranged in two rings; opening by a dehiscence line between the two rings of pollen sacs; large vesicular hairs above the synandrium; a gynoecium with four united carpels; inferior and unilocular ovaries with four parietal placentae, ovules tenuinucellate, anatropous with two well developed integuments, oriented in various directions; a nectary disk. Apodanthaceae share some special structural features with Malvales.

Key words: Apodanthaceae, *Apodanthes*, *Berlinianche*, eudicots, Malvales, parasitic plants, *Pilostyles*, Rafflesiales.

Introduction

Apodanthaceae (Rafflesiales) are endoparasitic, achlorophyllous herbs. Their vegetative endophyte, often compared to a fungal mycelium, resides in the host, as in other Rafflesiales. The aerial portions of the plant consist of flowering shoots, each with a single flower, which burst out of the host's cortex during development. Flowers are less then a centimetre across. Apodanthaceae contain three genera: *Apodanthes* with one or several species in the Neotropics, *Pilostyles* with about 20 species in the Neotropics, Mediterranean southwestern Asia, and subtropical southwestern Australia, and *Berlinianche* with two species in subtropical eastern Africa (Kuijt 1969, Meijer 1993). The reproductive organs of these parasites are preceded by three or four series of scales. *Apodanthes* is known to parasitize Flacourtiaceae (now in Salicaceae; Chase et al. 2002, APG II 2003), such as *Casearia* (Gomez 1983, Eliasson 1994) and *Xylosma* (Gentry 1993), and representatives of Burseraceae and Meliaceae (Gomez 1983).

Pilostyles parasitizes a wide range of Fabaceae (de Vattimo 1971), and *Berlinianche* is restricted to Amherstieae of Fabaceae (Verdcourt 1998).

Most traditional classifications placed Rafflesiales in or near Aristolochiales (Melchior 1964, Hutchinson 1969, Takhtajan et al. 1985, Takhtajan 1997). In the absence of molecular data, Rafflesiales were placed as an unresolved group together with several magnoliid families at the base of the angiosperms (APG 1998). Later, Hydnoraceae were found to be related to Piperales (Nickrent et al. 2002) and distant from the other Rafflesiales. In the APG II (2003) classification, Rafflesiales (without Hydnoraceae) are assigned to a group of taxa of uncertain position among angiosperms. In a molecular study based on a three gene analysis (but only nuclear SSU rDNA sequences for Rafflesiales), Rafflesiales were found to be monophyletic and were placed in eudicots near Malvales (Nickrent 2002). Phylogenetic relationships of Apodanthaceae relative to other families of Rafflesiales has been addressed in two molecular studies (Blarer et al. 2000 and unpublished results). Previous studies of floral morphological and anatomical features of Apodanthaceae were only fragmentary, not comparative and without use of serial microtome sections or the SEM. Taxa thus studied include: *Pilostyles* (Guillemin 1834, Solms-Laubach 1874b, as *Apodanthes* in Robinson 1891, Endriss 1902, Harms 1935, Kummerow 1962, Rutherford 1970), *Berlinianche* (as *Pilostyles*: Solms-Laubach 1874b, Harms 1935) and *Apodanthes* (Harms 1935; de Vattimo 1955, 1956, 1971, 1978; Gentry 1973). In the majority of these studies, neither serial microtome sections nor the SEM were utilized. Only in the study of ovules and seeds in *Apodanthes* and *Pilostyles* and ovules in *Berlinianche* by Bouman and Meijer (1994) was SEM used.

The objective of this study is to compare the floral structure of the three genera of Apodanthaceae and to discuss relationships of the genera and the position of Apodanthaceae in Rafflesiales.

Materials and methods

The following species were studied:

Apodanthes caseariae Poit.: D. L. Nickrent 3007; along trail opposite bus stop and housing development, ca. 1.0 km S of Rio General, Costa Rica, buds and open flowers, male and female, parasitic on *Casearia* sp. (Salicaceae).

Pilostyles thurberi A. Gray: D. L. Nickrent 2993; near Benjamin, Texas, USA, buds and open flowers, male and female, parasitic on *Dalea frutescens* A. Gray (Fabaceae).

Berlinianche aethiopica (Welw.) Vattimo-Gil: A. Blarer 192 (male), 193 (female); near Harare, Zimbabwe, buds and open flowers, parasitic on *Julbernardia globiflora* (Benth.) Troupin (Fabaceae).

Male and female flowers at anthesis (postanthetic female flowers of *Pilostyles*) and buds shortly before anthesis, when available, were fixed in FAA and stored in 70% ethanol or shock-frozen in liquid nitrogen and stored at −80 °C. They were investigated with light microscopy (LM) and scanning electron microscopy (SEM). Specimens were embedded in Kulzer's Technovit 2-hydroethyl methacrylate or paraplast (Igersheim 1993, Igersheim and Cichocki 1996) and sectioned with a Microm HM 335 rotary microtome and conventional microtome knife (grade D); both transverse and longitudinal sections were with a few exceptions cut at 5 μm. Sections were stained with ruthenium red and toluidine blue (Weber and Igersheim 1994) and mounted in Histomount on glass slides. Some materials (especially male flowers of *Apodanthes*) were difficult to section with either paraffin or paraplast embedding due to heavily tanniferous tissues. Standard specimen preparation procedures were used for osmium tetroxide impregnated samples for SEM studies. Vouchers and slides are deposited at the Institute of Systematic Botany of the University of Zürich (Z), Switzerland.

Results

Androecium and gynoecium are surrounded by a number of sterile organs. As it is uncertain whether they are all perianth organs (tepals, or sepals and petals) or whether the outer ones are bracts (and thus not floral organs), we refer to them as "scales" in the

Results, before we give an interpretation in the Discussion.

Scales of *Berlinianche aethiopica*. Anthetic flowers are globose or ellipsoid, firm, red, and odourless (Fig. 1A). The scales are arranged in several series. They can be interpreted as three whorls (see Discussion). Scale number changes from three in the outer whorl to six in the other whorls. Of the six scales of the middle whorl, three alternate with the scales of the outer whorl, and three are in front of those of the outer whorl. The six scales of the inner whorl alternate with those of the middle whorl (Fig. 10A). We did not find variation in scale number except in the inner whorl, which is sometimes heptamerous (Fig. 5). Aestivation in all whorls is imbricate: in the outer whorl it is sometimes contort; in the middle whorl three scales cover the other three, the former are those that alternate with those of the outer whorl; in the inner whorl, aestivation is sometimes contort. However, aestivation in the outer and inner whorl is somewhat irregular and there is no predominant pattern (Fig. 10A). The scales are free. They are broadly ovate to elliptic, rounded at the apex, have thin margins (one to two cell layers thick), and are persistent. The scales of the inner whorl differ from the other ones in their thicker insertion area. In addition, they are covered with elongate, one- to four-celled, uniseriate hairs adaxially at the base. The scales of the three whorls are inserted at different levels. The inner whorl is inserted at the level of the nectary, the outer one close to the base of the floral shoot. A sinusoid, six-angled, thick nectary disk surrounding the reproductive organs is shaped by the bases of the inner scales (Figs. 2, 4).

In the outer two whorls, vascular bundles are not visible. In the scales of the inner whorl, transverse sections show multiple small vascular bundles in the lower third of their length. However, small vascular bundles are found at the insertion sites of all whorls. The vascular bundles are thin and few-celled in TS (Figs. 2, 4).

Stomata could not be found in the scales but are present on the nectary disk. The scales are rich in tanniferous tissue. Neither cells with starch or oxalate crystals, nor oil and mucilage cells were found.

Reproductive structures in male flowers of *Berlinianche aethiopica*. The androecium consists of a tubular synandrium, which surrounds the sterile gynoecium. Anthers and filaments are not differentiated. The synandrium contains two rings of c. 15 extrorse pollen sacs each. The upper ring has a slightly smaller diameter. The number of pollen sacs may differ slightly in the two rings and they are neither strictly superposed nor alternating with each other. Opening is by a continuous dehiscence line between the two rings of pollen sacs. In the
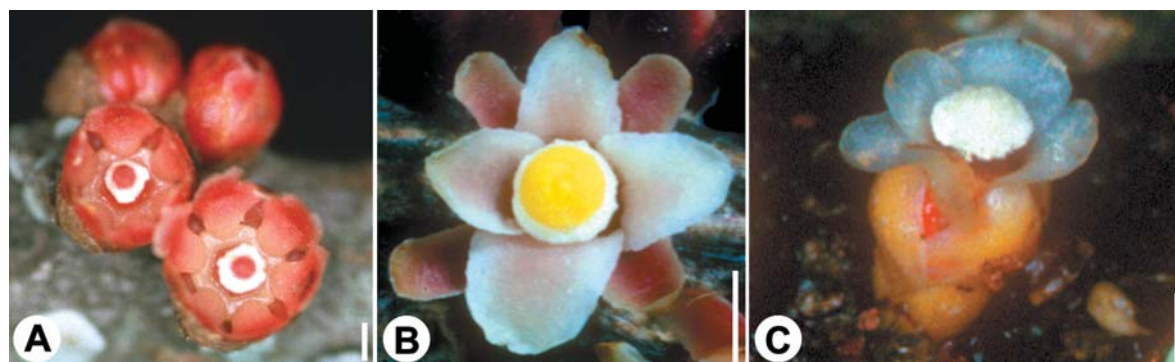

Fig. 1. Flowers of Apodanthaceae. **A** *Berlinianche aethiopica*, male (A. Blarer 192; photo by same). **B** *Pilostyles thurberi*, male (D. L. Nickrent 2293; photo by K. Robertson). **C** *Apodanthes caseariae*, male (D. L. Nickrent 3007; photo by same). Scale bars c. 1 mm
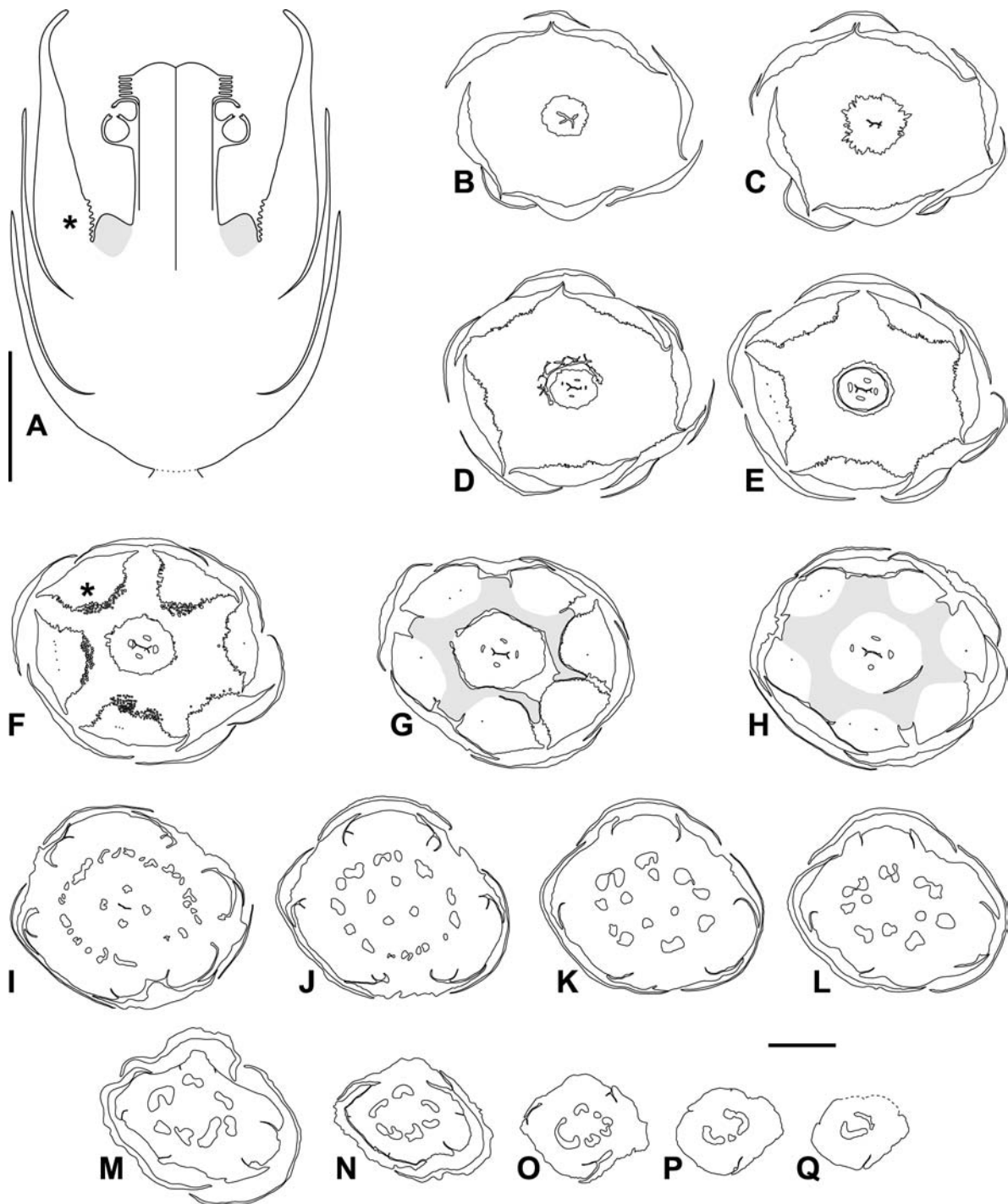
Fig. 2. *Berlinianche aethiopica,* anthetic male flowers with open pollen sacs. **A** Schematic LS. **B–Q** TS series. **B** Level of stylar head. **C** Level of vesicular hairs above androecium. **D** Level of pollen sacs. **E** Level of free staminal tube. **F** Level of hair cushion on inner perianth organs. **G–H** Level of nectary. **I** lower part of stylar canal. **J–Q** Below stylar canal. Morphological surfaces, thick lines; vascular bundles, thin lines; nectary, shaded; junction to haustorium, dotted line (in A); asterisks, hair cushion. Scale bars = 1 mm
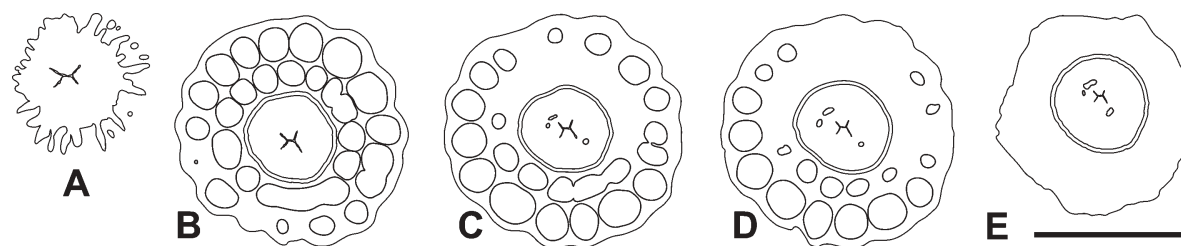
Fig. 3. *Berlinianche aethiopica,* preanthetic male flower with closed pollen sacs. TS series. **A** Level of vesicular hairs above androecium. **B–D** Level of pollen sacs. **E** Below level of pollen sacs, androecium free from gynoecium. Morphological surfaces, thick lines; vascular bundles and pollen sacs, thin lines. Scale bar = 1 mm

upper part of the sterile gynoecium, topographically immediately above the pollen sacs, there is a fringe of large, elongate vesicular cells with narrow tips, arranged in about five series. A dome-shaped region without vesicular cells forms the apex of the gynoecium (Fig. 11A). The stylar canal is similar to that in female flowers, but narrower and shorter (Fig. 2). It extends down to the level of the insertion site of the inner whorl of scales (Fig. 2). There is no rudimentary locule (Fig. 2). Pollen grains are single, small (diameter c. 10 μm), variable in form (spheroidal to subspheroidal), psilate, and have no apparent apertures (Fig. 12G).

Small vascular bundles at the insertion level of the organs of the inner whorl become larger and more distinguishable as c. 10–12 discrete vascular bundles toward the base of the flower shoot. In anthetic flowers, vascular bundles cannot be recognized in the androecium. However, in the sterile gynoecium, four vascular bundles are present. They converge (sometimes two of them join towards the base of the gynoecium resulting in three bundles) with the above mentioned c. 10–12 discrete vascular bundles between the insertion level of the middle and outer whorl of scales. The number of united vascular bundles decreases and they converge towards the insertion level of the outer whorl into c. 6 and towards the flower base to c. 4 bundles. At the transition to the haustorium, vascular bundles are no longer distinguishable. Below the nectary small vascular bundles can be seen, which are scattered in a circle that has a diameter greater than the base of androecium and within the insertion site of the inner whorl of scales (Fig. 2).

In the androecium, there is less tannine than in other flower parts. In the sterile gynoecium, the mesophyll contains more tannine than epidermis and vascular bundles.

Reproductive structures in female flowers of Berlinianche aethiopica. There are no rudiments of an androecium. The gynoecium is completely syncarpous with a conical style and an inferior ovary. As seen from the internal structure it is probably tetracarpellate and it is completely symplicate. The stigma is not broader than the style, it is hemispherical without separate stigmatic lobes and is almost completely covered with elongate unicellular papillae, each with a rounded apex, which are heavily secretory (Fig. 13A, D). A stylar canal, which is 'H'-shaped in TS, leads from the stigma into the ovary locule and forms the pollen tube transmitting tract (PTTT) (Fig. 4). Thus, a compitum is present from the stigma to the ovary locule. At the transition to the locule the stylar canal widens abruptly (Fig. 4). The locule has its base at the level of the insertion site of the outer whorl (Fig. 4). The inner surface of the ovary including the ovules is covered with a secretion. There are four unevenly distributed, simple, parietal, more or less protruding placentae. Each placenta bears c. 15–20 ovules, which are oriented in various directions. They are bitegmic, tenuinucellar and anatropous (Fig. 13G, J). Ovules in anthetic flowers are found in the state of meiosis of the megaspore mother cell. The outer integument is slightly shorter than the inner;
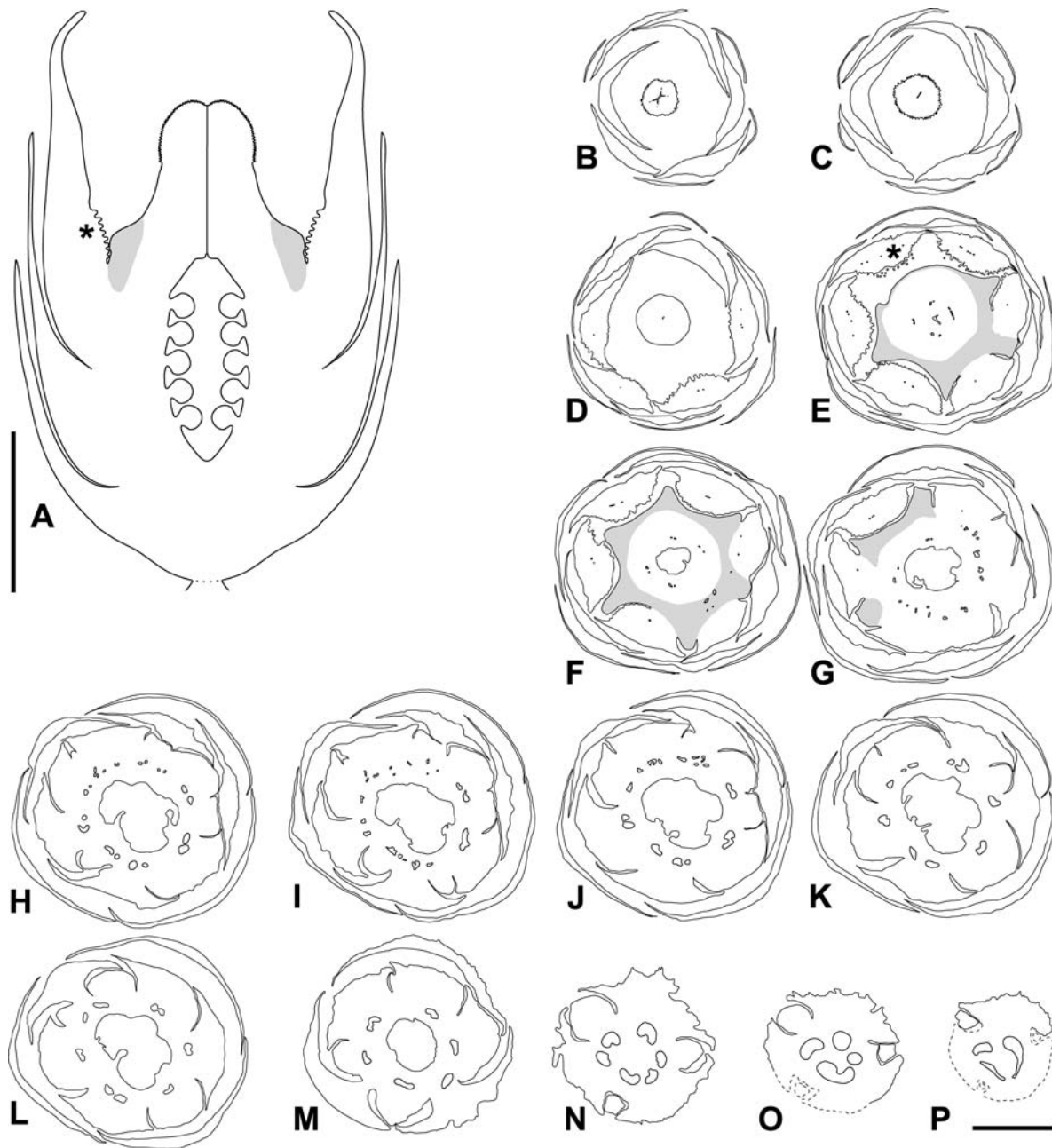
Fig. 4. *Berlinianche aethiopica,* anthetic female flowers. **A** Schematic LS. **B–P** TS series of regular hexamerous female flower. **B** Upper level of stylar head. **C** Lower level of stylar head. **D** Level of style. **E** Upper part of ovary, level of hair cushion. **E–G** Level of nectary. **F–M** Level of ovary. **N–P** Floral base. Morphological surfaces, thick lines; vascular bundles, thin lines; nectary, shaded; junction to haustorium, dotted line (in A); asterisks, hair cushion. Scale bars = 1 mm

both are distinctly developed, although a micropyle is not present (Fig. 13M); an endothelium is not differentiated. The inner integument is two cell layers thick; the outer one is one or two cell layers thick. In the funiculus, the subepidermal tissue has large intercellular spaces (but not at the insertion site of the funiculus).
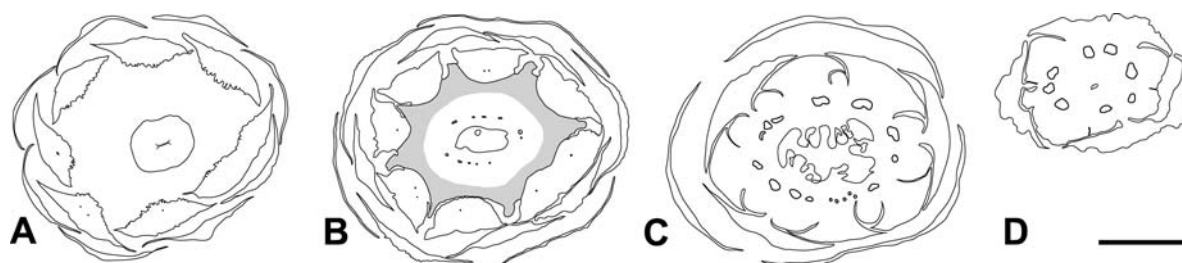
Fig. 5. *Berlinianche aethiopica,* irregularly heptamerous female flower. TS series. **A** Level of style. **B** Level of nectary. **C** Mid-level of ovary. **D** Lower level of ovary. Morphological surfaces, thick lines; vascular bundles, thin lines; nectary, shaded. Scale bar = 1 mm

Vasculature is poorly developed without any larger vascular bundles. Small vascular bundles of variable number are present in the style. They extend downwards and form about eight distinguishable bundles at the base of the style. They continue downwards along the ovary, and at midlength of the ovary there are c. 14 vascular bundles. No vascular bundles can be associated with the placentae. There are no vestiges of vascular bundles leading to the ovules. Towards the base of the ovary, the number of vascular bundles decreases, converging into eight and finally only four. At the transition to the haustorium, vascular bundles are no longer distinguishable (Fig. 4).

In the style, an outer circular region of mesophyll contains more tannins than the epidermis and the inner circular region that includes the vascular bundles and the PTTT.

Scales of *Pilostyles thurberi*. Anthetic flowers are globular or ovoid, soft, red to brown (Fig. 1B). The scales are arranged in three alternating tetramerous whorls. In 20 flowers studied we found one pentamerous variation. Aestivation in the outer and middle whorl is open, in the inner whorl, it is imbricate, sometimes contort (with irregularities), or most often, two scales cover two others (Fig. 10B). The scales are free. They are broadly ovate, rounded at the apex, have thin margins (one to two cell layers thick), and are persistent. The inner whorl is lighter (white) than the others. The scales of the three whorls are inserted at different levels. The outer whorl is close to the base of the floral shoot, the inner

one is inserted at the level of the nectary disk (Figs. 6, 7).

In the scales, an irregular number of thin and few-celled vascular bundles are present.

Stomata could not be found on the scales but are present on the nectary. All floral organs are rich in tanniferous tissue. Cells with starch or oxalate crystals, and oil and mucilage cells were not found.

Reproductive structures in male flowers of *Pilostyles thurberi*. The androecium consists of a tubular synandrium, which surrounds the sterile gynoecium. Anthers and filaments are not formed (Fig. 11B). The synandrium contains three rings of c. 18 extrorse pollen sacs each. All rings have a similar diameter. The number of pollen sacs per ring is only more or less the same and they do not strictly alternate with each other. In the upper part of the sterile gynoecium, topographically immediately above the pollen sacs, there is a fringe of large, elongate vesicular cells with narrow tips arranged in c. 3 series. Some of them seem to have ruptured at anthesis. A dome-shaped region without vesicular cells forms the apex of the sterile gynoecium. The stylar canal is similar to that in female flowers but narrower and shorter (Fig. 6). It extends down to the level of the lower vesicular cells (Fig. 6). There is no rudimentary locule (Fig. 6). Pollen grains are similar as in *Apodanthes* but are slightly triangular as seen from the pole (Fig. 12H).

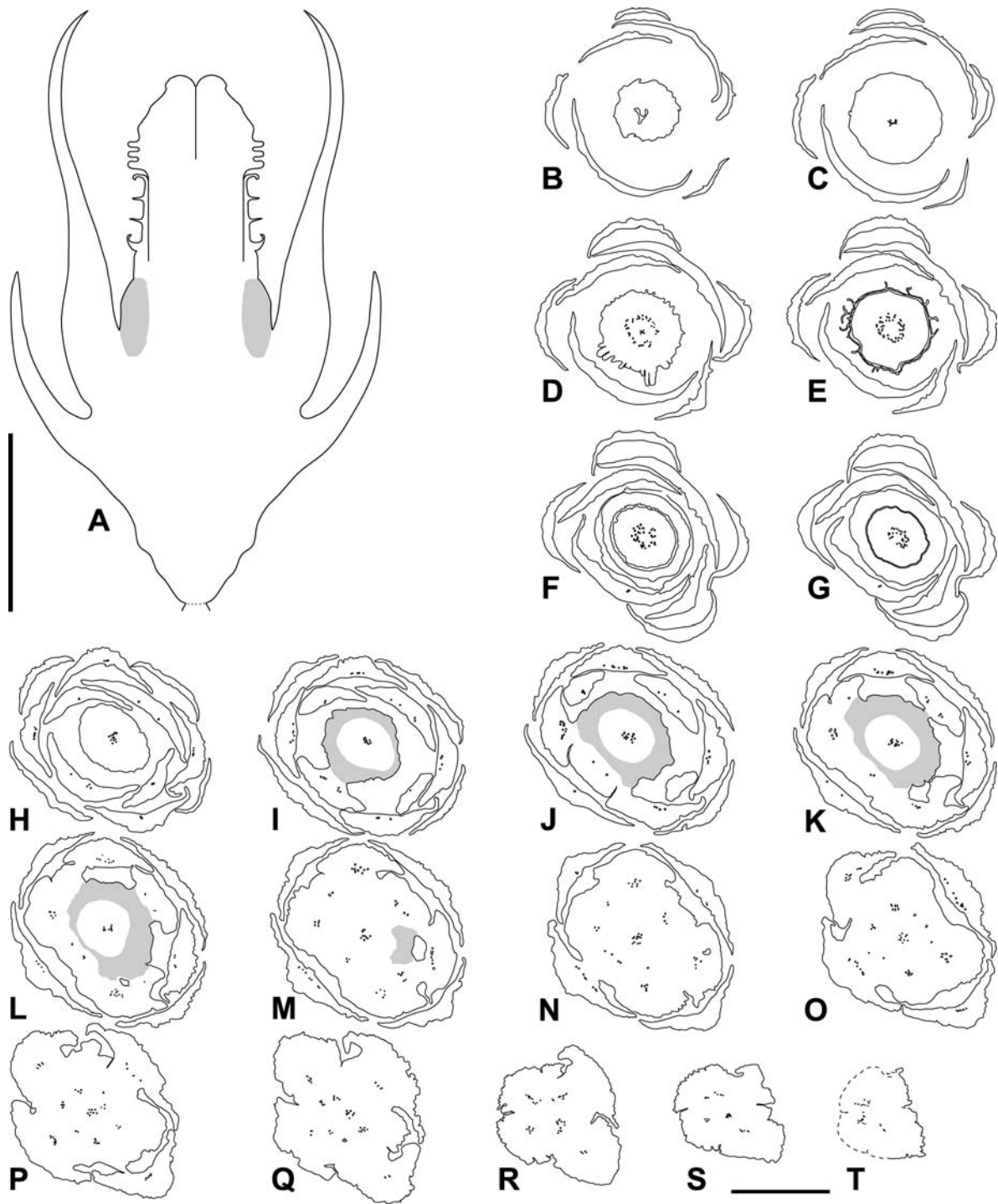In anthetic flowers, no vestiges of vascular bundles can be recognized in the androecium.

Fig. 6. *Pilostyles thurberi*, anthetic male flowers with open anthers. **A** Schematic LS. **B–T** TS series. **B** Upper level of stylar head. **C** Lower level of stylar head. **D** Level of vesicular hears above androecium, lower level of stylar canal. **E** Level of pollen sacs. **F** Upper level of staminal tube. **G** Lower level of staminal tube. **H** Upper level of androecium fused to gynoecium. **I–M** Level of nectary. **N–R** Below nectary. **S–T** Floral base. Morphological surfaces, thick lines; vascular bundles, thin lines; nectary, shaded; junction to haustorium, dotted line (in A). Scale bars = 1 mm

Fig. 7. *Pilostyles thurberi*, female flowers. **A** Schematic LS, anthetic. **B–V** TS series, post-anthetic. **B** Level of stylar head. **C–H** Level of nectary. **C** Style. **D–N** Level of ovary. **D** Upper level of ovary. **N** Lower level of ovary. **O–V** Floral base. Morphological surfaces, thick lines; vascular bundles, thin lines; nectary, shaded; junction to haustorium, dotted line (in A). Scale bars = 1 mm
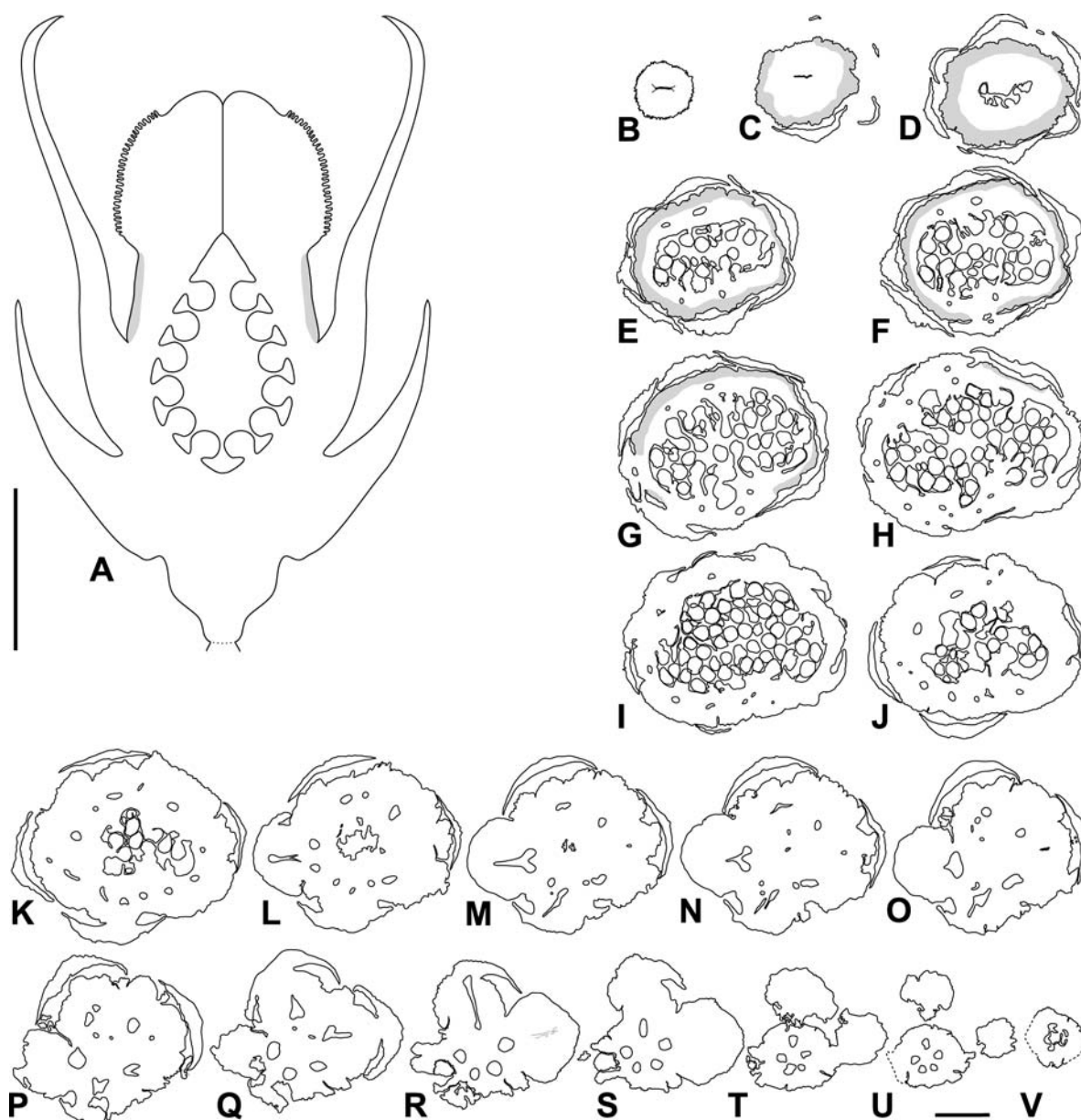
In the style, small vascular bundles of variable number are present and circle the centre of the style. Towards the level of the nectary, they become more centred in the flower axis. Some of them converge with each other while still in the style. Median bundles of the scales become visible at their respective insertion sites.

Towards the base, vascular bundles converge and fuse with each other. They gradually diminish until they disappear at the base (Fig. 6).

Tanniferous tissue is evenly distributed in the flower; it is more sparse than in *Berlinianche*.

Reproductive structures in female flowers of *Pilostyles thurberi*. There are no rudiments of an androecium. The gynoecium is completely syncarpous with a conical style and an inferior ovary, based on the internal structure it is probably tetracarpellate. The stigma is conical with a larger diameter than the style and topped with a smaller hemispherical rounded apex. There are four slightly separated stigmatic lobes. Except for the hemispherical apex, the conical stigma is covered with elongate unicellular papillae with a rounded apex; it is heavily secretory (Fig. 13E). A stylar canal, which is flattened in TS, leads from the stigma into the ovary locule and forms the PTTT (Fig. 7). Thus a compitum is present from the stigma to the ovary locule. The stylar canal widens gradually towards the locule (Fig. 7). The locule has its base at the level of the insertion site of the outer scales (Fig. 7). The unilocular ovary is completely symplicate. The inner surface of the gynoecium, including the ovules, is covered with a secretion. There are four unevenly distributed, slightly protruding-diffuse parietal placentae. Each placenta bears c. 30 to 50 ovules, which are oriented in various directions (Fig. 13H, K). They are bitegmic, tenuinucellar and anatropous (Fig. 13N). Both integuments are well developed, a micropyle is formed by both integuments in this postanthetic stage. In the funiculus the subepidermal tissue has large intercellular spaces (but not at the insertion site of the funiculus).

In the style small vascular bundles of variable number are present, which become larger toward the base. Four dorsal bundles and four synlateral bundles, which serve the placentae, are present in the upper part of the ovary. There are no traces of vascular bundles leading to the ovules. At the insertion site of the scales there are vascular bundles that can be associated with the organs of the scales. Below the base of the locule the number of vascular bundles decreases by convergence to four. At the transition to the haustorium, vascular bundles are no longer distinguishable

(Fig. 7). Tanniferous tissue is distributed as in male flowers.

Scales of *Apodanthes caseariae*. Anthetic flowers are ellipsoid, firm, cream and yellow to orange (Fig. 1C). The scales are arranged in several series. They can be interpreted as three whorls (but see Discussion). The outer whorl is dimerous, the others tetramerous (Fig. 10C). Of the four scales of the middle whorl, two alternate with the scales of the outer whorl, and two are in front of them. The four scales of the inner whorl alternate with those of the middle whorl (Fig. 10C). We did not find variation in organ number. Aestivation is imbricate: in the outer whorl, one scale covers the other; in the middle whorl, there is an outer and an inner, as well as two intermediate organs, which are those that alternate with the scales of the outer whorl; aestivation in the inner whorl is either the same as in the middle whorl or contort (Fig. 10C). The scales of the inner and outer whorls are free; those of the middle whorl are basally united. All scales are elliptic, rounded at the apex, and have thin margins (one to two cell layers thick). The scales of the inner whorl are narrower at the base, lighter (white), and are caducous; all others are persistent. The scales of the outer whorl are much smaller. The scales of the three whorls are inserted at different levels: the outer close to the base of the aerial part of the floral shoot, the middle ones at the lower level of the nectary, and the inner ones at the medium level of the nectary. A sinusoid, four-angled nectary disk (thicker in male flowers than in female ones) surrounding the reproductive organs is shaped by the bases of the middle and inner scales. The middle ones are situated at the flanks, the inner ones in the sinuses of the nectary (Figs. 8, 9).

In the scales, vascular bundles are as in *Berlinianche* and in *Pilostyles* in TS (Fig. 9).

Stomata could not be found on the scales but are present in the nectary. All floral organs are rich in tanniferous tissue. Cells with starch or oxalate crystals, and oil and mucilage cells were not found.
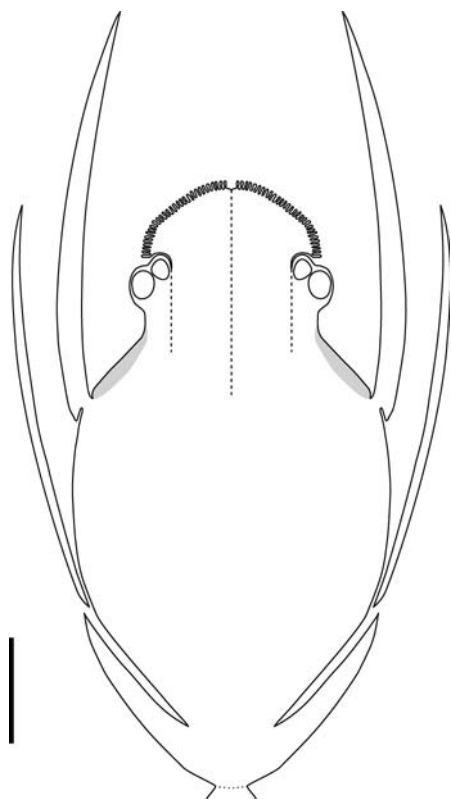
Fig. 8. *Apodanthes caseariae,* anthetic male flower with closed anthers. Schematic LS. Morphological surfaces, thick lines; pollen sacs, thin lines; nectary, shaded; presumed internal surface of sterile gynoecium and presumed surface of androecium adjacent to gynoecium, interrupted lines; junction to haustorium, dotted line. Scale bar = 1 mm

Reproductive structures in male flowers of *Apodanthes caseariae*. The androecium consists of a tubular synandrium, which surrounds the sterile gynoecium. Anthers and filaments are not formed. The synandrium contains two rings of c. 20 extrorse pollen sacs each. The upper ring has a slightly smaller diameter. The number of pollen sacs may be slightly different in the two rings and they do not strictly alternate with each other. In the upper part of the sterile gynoecium, topographically immediately above the pollen sacs, large, elongate vesicular cells with narrow tips cover almost the entire top (Fig. 11C). An examination of the inner morphological surface of the sterile gynoecium could not be conducted. Pollen grains are single, similar in size, and psilate as in *Berlinianche* but are spheroidal, somewhat flattened, and tricolpate (Fig. 12I). Nectary tissue covers the basal area of the androecium.

Anatomical studies could not be conducted for male *Apodanthes* flowers because of difficulties in obtaining serial transverse microtome sections.

The sterile gynoecium is heavily tanniferous; the distribution of tanniferous tissue in the other parts of the flower is similar as in *Berlinianche*.

Reproductive structures in female flowers of *Apodanthes caseariae*. There are no rudiments of an androecium. The gynoecium is completely syncarpous with a conical style and an inferior ovary. As seen from the internal structure it is probably tetracarpellate and is completely symplicate. The stigma is not broader than the style, it is hemispherical without separate stigmatic lobes and is almost completely covered with elongate unicellular papillae, which are irregular ramified; it is secretory (Fig. 13C, F). A stylar canal, which is irregularly 'H'-shaped in TS and postgenitally fused, leads from the stigma into the ovary locule and forms the PTTT (Fig. 9). Thus, a compitum is present from the stigma to the ovary locule. The stylar canal widens gradually towards the locule. The locule has its base above the level of the insertion site of the outer whorl of scales. The inner surface of the ovary, including the ovules, is covered with a secretion. There are four unevenly distributed protruding-diffuse parietal placentae. In one flower studied, one of the placentae splits in two at about mid-length of the locule, resulting in five unequal placentae in the lower part of the locule. Each placenta bears c. 90 ovules, which are oriented in various directions (Fig. 13I, L). Ovules are as in *Berlinianche*: bitegmic, tenuinucellar and anatropous (Fig. 13O). Nectary tissue covers the basal area of the free part of the gynoecium.

Small vascular bundles of variable number are present in the upper part of the style and become larger toward the base. About eight
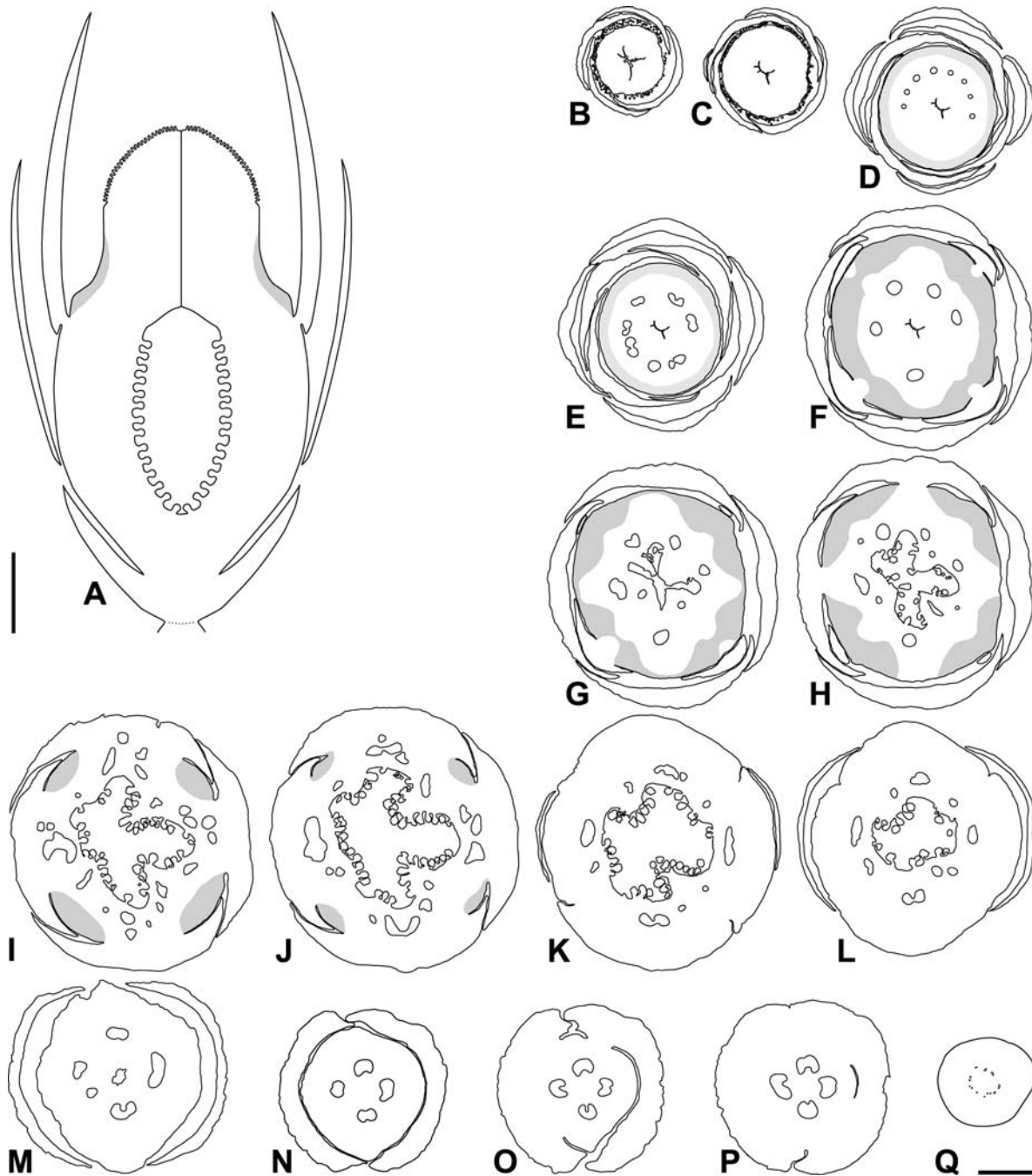
Fig. 9. *Apodanthes caseariae,* Anthetic female flowers. **A** Schematic LS. **B–Q** TS series. **B** Upper level of stylar head. **C** Lower level of stylar head. **D** Level of style. **D–J** Level of nectary. **F–M** Level of ovary. **F** Upper level of ovary. **M** Lower level of ovary. **N–P** Floral base. Morphological surfaces, thick lines; vascular bundles, thin lines; nectary, shaded; projected scale of inner whorl, interrupted lines; junction to haustorium, dotted line (in A). Scale bars = 1 mm

are found at mid-length of the style. Further down, they become fewer, and form four dorsal bundles. In addition, four synlateral bundles, which serve the placentae, are present at mid-length of the ovary, some of them may extend upwards to the base of style. There are
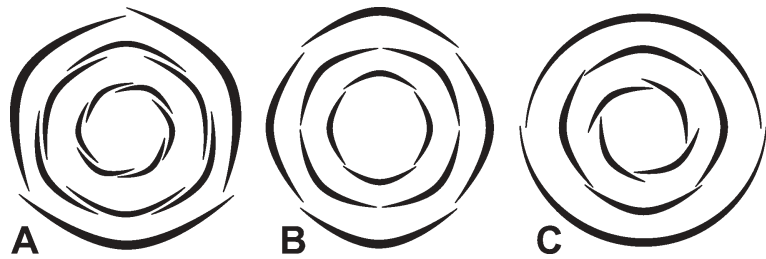
Fig. 10. Whorls of scales and aestivation. **A** *Berlinianche aethiopica*, **B** *Pilostyles thurberi*, **C** *Apodanthes caseariae*.
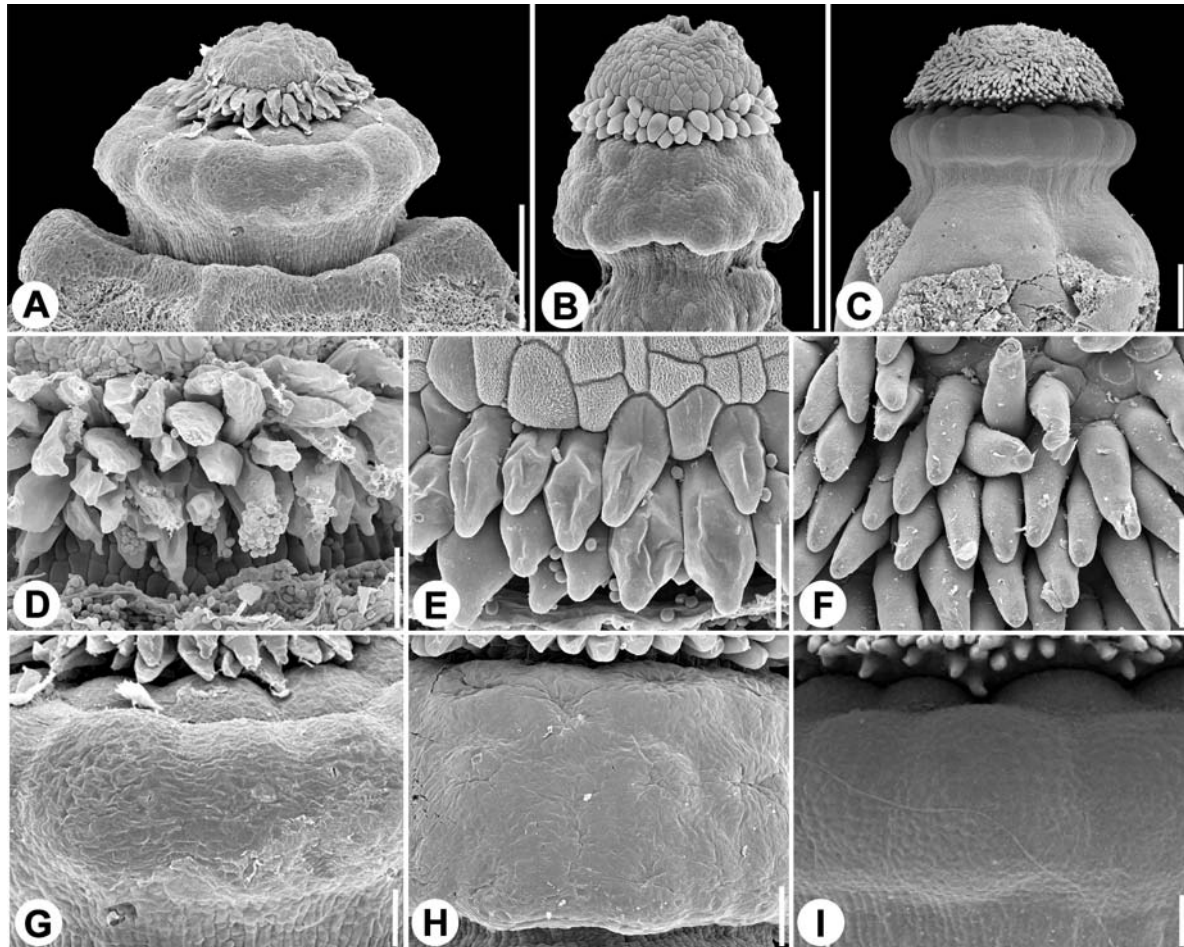

Fig. 11. Male flowers, perianth removed. **A–C** Floral centre from the side. **D–F** Vesicular hairs. **G–I** Partial view of rings with closed pollen sacs. **A, D, G** *Berlinianche aethiopica*. **B, E, H** *Pilostyles thurberi*. **C, F, I** *Apodanthes caseariae*. Scale bars = 0.5 mm in A–C; 0.1 mm in D–I

no traces of vascular bundles leading to the ovules. In the inferior part of the ovary, there are in addition, c. 8 vascular bundles, which can be associated with the scales of the middle whorl. Towards the base of the locule, the number of vascular bundles decreases: they converge into four large ones. At the transition to the haustorium, vascular bundles are no longer distinguishable (Fig. 9).

The style is less tanniferous than in male flowers; otherwise tanniferous tissue distribution is similar.
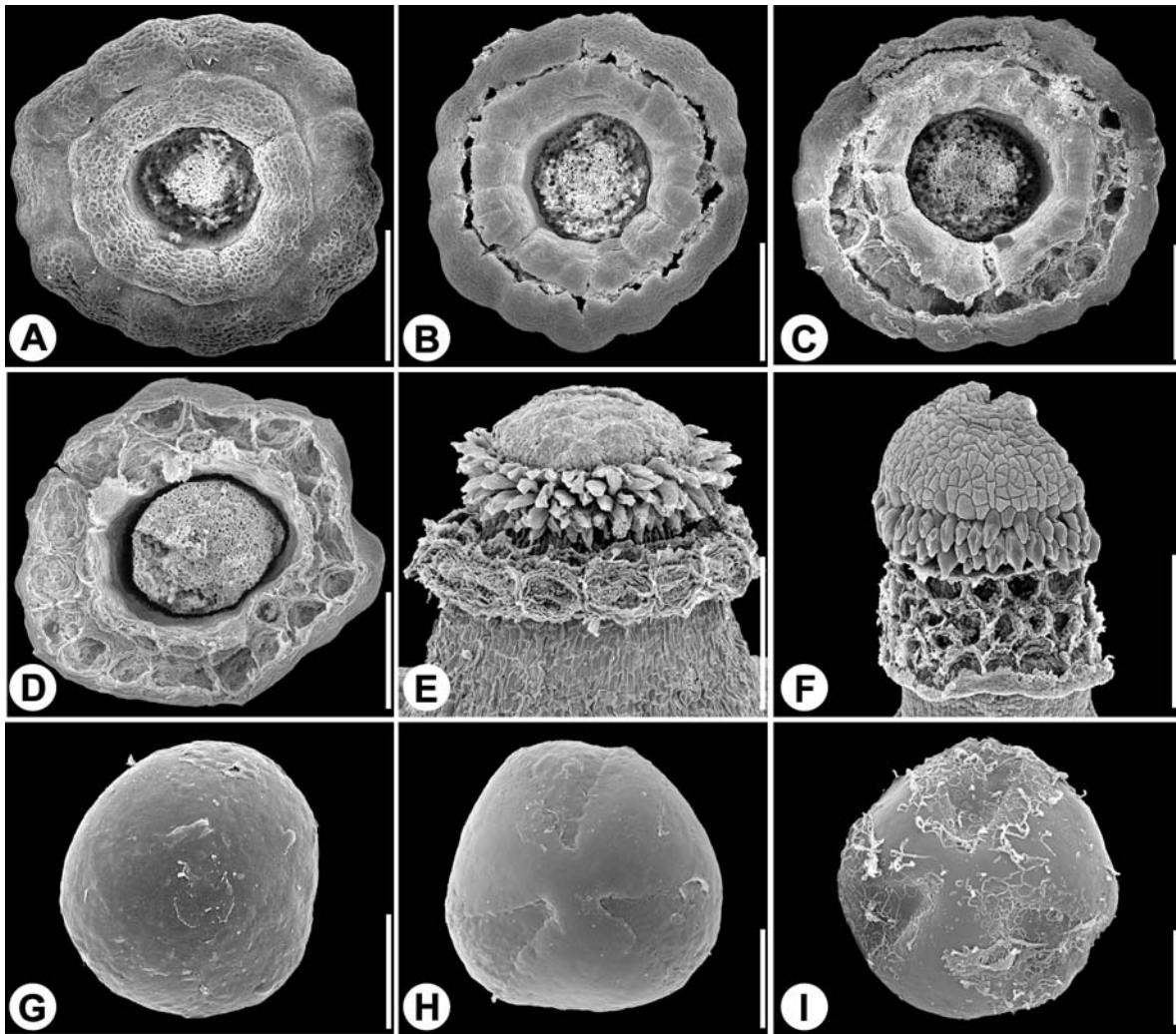
Fig. 12. Synandria and pollen grains. **A–D** *Berlinianche aethiopica*, synandria in different stages of opening, seen from above, stylar head removed. **E–F** Synandria with open pollen sacs, seen from the side. **E** *Berlinianche aethiopica*. **F** *Pilostyles thurberi*. **G–I** Pollen grains. **G** *Berlinianche aethiopica*. **H** *Pilostyles thurberi*. **I** *Apodanthes caseariae*. Scale bars = 0.5 mm in A–F; 5 µm in G–I

Discussion

Scale whorl identity and aestivation. In most Apodanthaceae the reproductive organs of each flower are preceded by three or four whorls (series) of scales, beginning close to the narrow attachment to the host (Fig. 14). Since there are no conspicuous differences in the scales of different whorls at the first sight, the question arises whether the inner one or two series of scales or all series are homologous to a perianth as in other core eudicots flowers. In other Rafflesiales there are more scales, and

Fig. 13. Female flowers, perianth removed. **A–C** Free part of gynoecium. **D–F** Stigmatic papillae. **G–I** ovary TS. **J–L** ovary LS. **M–O** Ovules (M, O anthetic, N postanthetic). **A, D, G, J, M** *Berlinianche aethiopica*. **B, E, H, K, N** *Pilostyles thurberi*. **C, F, I, L, O** *Apodanthes caseariae*. Scale bars = 0.5 mm in A–C, G–L; 0.01 mm in D–F; 0.05 mm in M–O
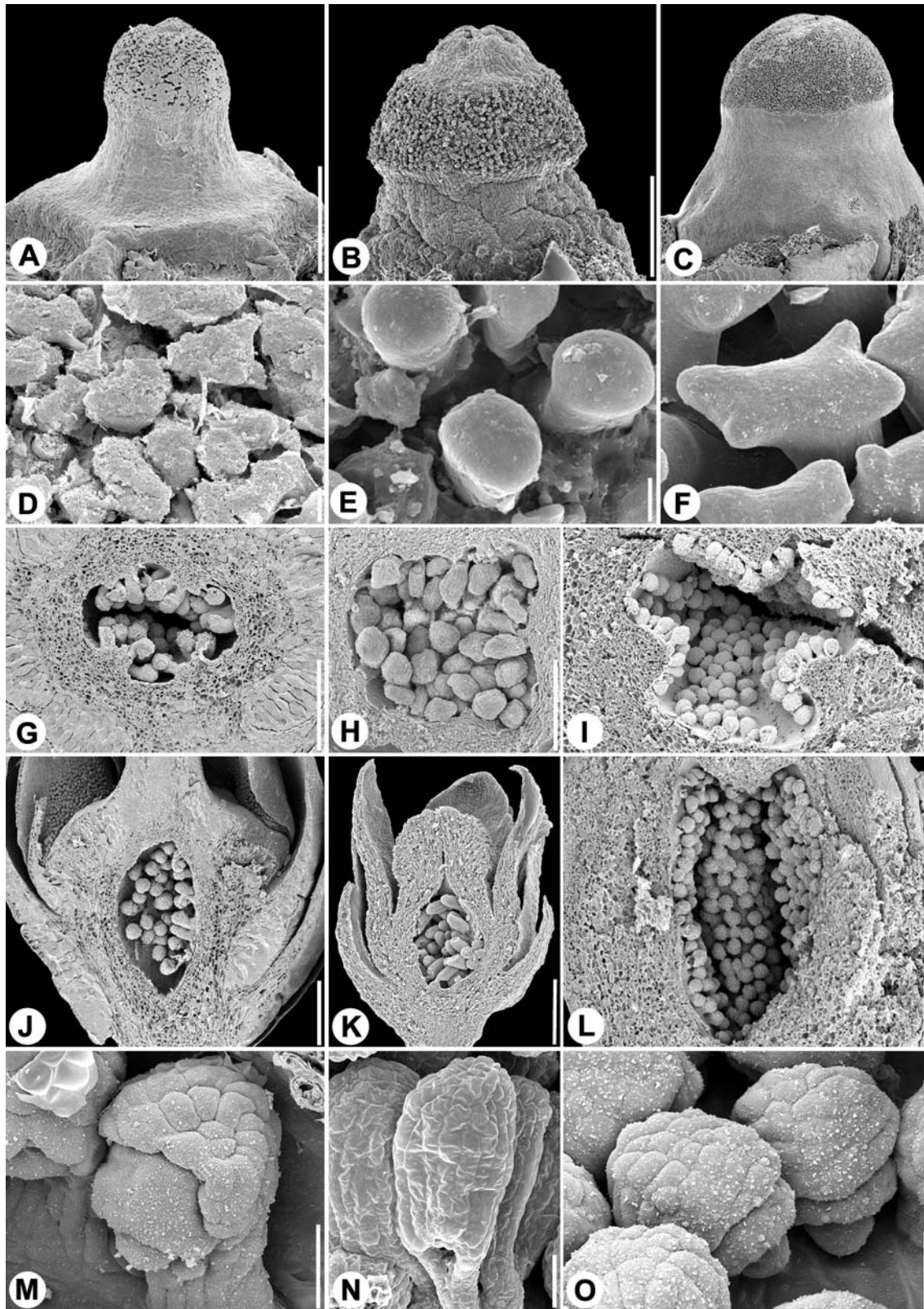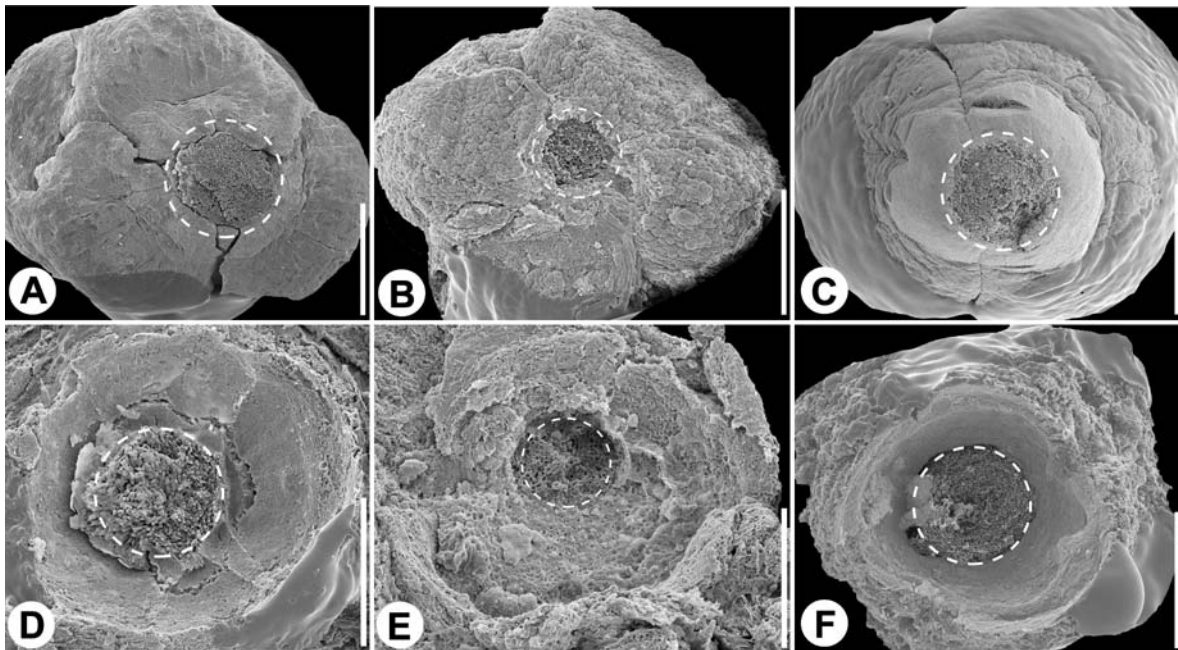
Fig. 14. Connection site of the parasite corm/flower with the host. **A–C** Parasite seen from beneath. **D–F** Host with cup-like structure and attachment area of parasite. **A, D** *Berlinianche aethiopica*. **B, E** *Pilostyles thurberi*. **C, F** *Apodanthes caseariae*. Attachment areas, white circle. Scale bars = 1 mm

the outer ones are clearly not part of the flower, especially if they precede an inflorescence, and not a single flower (e.g. Harms 1935). Thus, comparatively, the presence of more than two whorls in Apodanthaceae suggests that at least the outer whorl is not part of a perianth. Thus, the question must be asked whether there is a perianth, and if there is one, whether it consists of one or two series of organs. In all three genera, on closer inspection, the scales of the inner whorl are distinctively different from those of the other whorls. They are narrower in *Apodanthes* and thicker in *Berlinianche* than in the preceding whorls and have a lighter colour in *Apodanthes* and *Pilostyles*. Only in *Apodanthes* are the scales of the outer whorl much smaller than the others and the middle whorl has basally united organs.

Another problem with the interpretation of the perianth is that the delimitation of the whorls is somewhat ambiguous. When they are counted starting with the inner whorl, *Berlinianche* has three whorls of which the inner and middle ones have six scales each and the outer one has three scales (3, 6, 6). However, when viewed in the other direction, one is inclined to distinguish four whorls: three trimerous whorls and the fourth hexamerous, alternating with the six scales of the two preceding whorls (3, 3, 3, 6). Thus, the outer whorl is clearly trimerous and the inner one hexamerous, but the intermediate region is more difficult to interpret. This is similar in *Pilostyles*, in which the respective numbers are (4, 4, 4) versus (2, 2, 4, 4). Here, the inner whorls are clearly tetramerous. *Apodanthes* is even more puzzling, because both interpretations lead to a difficulty. If regarded as three whorls (2, 4, 4), regular alternation of organs would be lacking from the first to the second whorl. If regarded as four whorls (2, 2, 2, 4), the aestivation of the second and third whorl would not be as expected. Nevertheless, the outer whorl is clearly dimerous and the inner is tetramerous.

Aestivation of the scales is imbricate but somewhat variable. Unexpected irregularities

of aestivation (such as in *Apodanthes*) and variations in aestivation may be explained by a relatively late overlapping of the scales, which are narrow in early stages and then may be influenced by irregularities in the bark of the host or pressure by neighbouring flowers. In *Berlinianche holtzii* (Engl.) de Vattimo (*Pilostyles holtzii*), all whorls seem to be contort (Engler 1912).

Furthermore, however the whorls are described, there is a change to the double number of scales in the inner whorl(s) (except for *Pilostyles*, with 4, 4, 4). This is not an uncommon phenomenon in flowers. It can be formally viewed as the presence of double organ positions, as described, e.g., by Endress (1987, 1994) for perianth organs or stamens. In *Apodanthes* and *Berlinianche*, the scales between the outer and inner whorl, and in *Pilostyles,* the outer scales, can be assigned to one or two whorls. In *Apodanthes*, the four scales of this ambiguous zone are slightly united and much larger than those of the outer whorl. In all genera, the scales of the ambiguous zone are closer to each other than to those of other whorls. We thus assign the scales of the ambiguous zone to one whorl, and assume that all three genera have just three whorls.

If one interprets the floral shoot of Apodanthaceae as having three whorls of scales (bracts preceding the floral organs and an isomerous calyx and corolla), this arrangment is consistent with a position of the family among the eudicots (Nickrent 2002). Support for this hypothesis could be the fact that in *Apodanthes* the organs of the inner whorl have narrow bases and are deciduous, as common for petals, whereas the organs of the middle whorl have broad, united bases and are persistent as is common for sepals.

Another hypothesis that can be advanced is that the distinctive organs of the inner whorl represent sepals as in those Malvales without petals. The outer two whorls would then be interpreted as bracts preceding the floral organs. In *Berlinianche*, organs of the inner whorl are covered adaxially at the base with densely packed elongate one- to four-celled uniseriate hairs adaxially at the base (in male flowers, this hair carpet is somewhat more pronounced than in female ones). These hair carpets resemble the floral nectaries on sepals in many Malvales (Vogel 2000). However, there are two caveats: (1) in a few Malvales these hair carpets are not on sepals but on petals or stamens (Vogel 2000), and (2) in *Berlinianche* the hair carpets most probably do not function as nectarines. Evidence supporting the latter contention is that the flowers have a disk nectary like other Apodanthaceae, that these hairs are highly vacuolized and that the area below the hairs is not supplied by vasculature, thus indicating that the hairs are not secretory. These hairs may function as a nectar cover, a reservoir for nectar, or may be instrumental in flower opening. The nectary disk in male flowers is more conspicuous than in female flowers because it is more set apart from the inner floral parts by a circular furrow, whereas there is no furrow in female flowers. Endriss (1902) assigns a functional role in flower opening to the 'annulus' (nectary disk) in *Pilostyles ingae* (Karsten) Hook. f. He assumes that the growing 'annulus' pushes the organs outwards, more so in male flowers than in female flowers because the male flowers have a larger 'annulus'.

There is a general pattern in the number of whorls of scales and merism in flowers in all three genera. Generally, all three genera have three whorls; *Apodanthes* is commonly dimerous and tetramerous, *Pilostyles* tetramerous, and *Berlinianche* trimerous and hexamerous. However, there are some cases deviating from the general pattern of organ number. Pentamery, which one expects in core eudicots, also occurs but is not predominant.

Pilostyles haussknechtii Boiss. has two alternating hexamerous whorls of scales (Harms 1935). In *Pilostyles hamiltonii* C. Gardner, the 8–12 organs are in two whorls (Dell 1984). In *P. collina* Dell, the total number of organs is 12–15 and four to five in each of the three whorls (Dell 1984). The same may be

true for *P. hamiltonii* in which Harms (1935) found three pentamerous whorls.

In *Berlinianche holtzii*, the middle whorl was described as having five scales and the inner one three (Engler 1912, 1915; Peter 1932; Harms 1935). As the type material has been destroyed (see Verdcourt 1998), the original data can no longer be verified. It is not impossible that the normal number of scales in the middle whorl is six instead of five and thus, the outer and inner whorls would be trimerous whereas the middle whorl would be hexamerous as in other species (see also Harms 1935). In *Apodanthes tribracteata* Rusby, three organs in the outer whorl were described (Rusby 1920). This may be based on an incorrect interpretation of a split organ, which we also observed in our material.

In the remaining Rafflesiales perianth organs can be determined more easily than in Apodanthaceae. The number of perianth parts in *Rhizanthes* is typically (14–15-)16, which seem to be arranged in one whorl (Bänziger and Hansen 2000), in *Rafflesia* it is commonly five arranged in one whorl, and in *Sapria* ten arranged in two whorls (Harms 1935). In Cytinaceae, *Cytinus* has four to six and *Bdallophyton* has five to nine perianth parts in one whorl (Harms 1935). Mitrastemonaceae seem to have four at least partially united perianth parts in one whorl (Watanabe 1937a). The inner perianth whorl of all other families of Rafflesiales may be homologous to the inner whorl of Apodanthaceae.

Androecium. The androecium in Apodanthaceae consists of a congenitally united tube surrounding the sterile gynoecium. Vascular bundles are not differentiated in the androecium at anthesis. The androecium deviates from a normal angiosperm androecium with tetrasporangiate, dithecal stamens. Individual stamens and thecae cannot be distinguished. Pollen sacs are not distinctly organized in pairs. However, the dehiscence line between the two rings of pollen sacs results in the joint opening of two adjacent (irregularly superposed) pollen sacs close to the septum between the two rings. Thus, a LS of

the two rings resembles a TS of a theca of a normal anther. The dehiscence line widens gradually (see also Endriss 1902, Kummerow 1962, Kuijt 1969). Even before dehiscence, some pollen sacs of the same ring were observed to be confluent (Fig. 3). A probable explanation is that the septa between the pollen sacs had partly dissolved, as Endriss (1902) had observed for *Pilostyles ingae*. A tapetum is present and an endothecium is absent as reported earlier for *Pilostyles* (Endriss 1902, Kummerow 1962, Rutherford 1970). Similar athecal androecia can also be found in Mitrastemonaceae and Rafflesiaceae s. str. among Rafflesiales but are rare in other angiosperms (some Viscaceae and *Polyporandra* (Icacinaceae), Endress and Stumpf 1990). Cytinaceae are the only Rafflesiales with normal dithecal anthers. Malvaceae are the only other angiosperm group that also shows the entire gamut between taxa with normal stamens, with stamens only slightly deviating from the common pattern (van Heel 1966, Endress and Stumpf 1990) to athecal androecia (von Balthazar and Nyffeler 2002). A comparative study of androecial diversity in Rafflesiales and Malvaceae may help in understanding the evolution of extreme forms.

Reports of other authors on variation in the androecium may be added. In *Apodanthes* pollen sacs are always arranged in two rings (de Vattimo 1956, 1971, this study), in *Berlinianche*, in one or two rings (Welwitsch 1869, Solms-Laubach 1901, Engler 1912, de Vattimo 1971, this study), in *Pilostyles*, in two to four rings (Rose 1909, Harms 1935, Kummerow 1962, de Vattimo 1971, Dell 1984, Meijer 1997, this study). In Rafflesiaceae s. str. the androecium consists of a ring of anthers with two (*Rhizanthes*, *Sapria*) to several (*Rafflesia*) microsporangia (Harms 1935). In *Sapria* each of the 20 anthers opens by a single pore (Solms-Laubach 1889, Bänziger and Hansen 1997), in *Rhizanthes* each of the 40–50 anthers opens by two superimposed pores, and in *Rafflesia* each of the c. 50 anthers opens by one pore (Griffith 1845; Solms-Laubach 1889, 1901; Harms 1935; Endress and Stumpf 1990). Endriss (1902)

hypothesizes that in *Pilostyles ingae*, which has two rings of pollen sacs, the maximal and normal number of pollen sacs per ring is 20 and as four pollen sacs may belong to one original anther, a ring would be derived from five anthers. As the number of pollen sacs is often less than 20 (e. g. Endriss 1902, this study) he assumes that some pollen sacs were lost. We question this because flowers in Apodanthaceae are mostly tetramerous or hexamerous. Thus, with this interpretation, one would expect either about 16 pollen sacs per ring for *Pilostyles* and *Apodanthes* or about 24 pollen sacs for *Berlinianche* but we found c. 20 pollen sacs for *Pilostyles* and *Apodanthes* and c. 15 for *Berlinianche*. Solms-Laubach (1901), Kummerow (1962) and Rutherford (1970) homologize single pollen sacs with entire anthers in *Pilostyles*.

Pollen of *Apodanthes* is tricolpate (Roubik and Moreno 1991, this study) as was also found in *Pilostyles* (Kummerow 1962, Takhtajan et al. 1985). *Berlinianche*, which was investigated here for the first time, has inaperturate pollen. The tricolpate pollen strongly indicates a position of Apodanthaceae in eudicots as suggested by Nickrent (2002) based on molecular studies. Pollen of Mitrastemonaceae is triporate (Takhtajan et al. 1985). In Cytinaceae, *Cytinus* pollen is two-, or three-, or four-porate, which may be regarded as derived compared with the tricolpate pollen of *Bdallophyton* (Takhtajan et al. 1985). Pollen of Rafflesiaceae s. str., however, is reported to be monoporate by Takhtajan (1985). If Rafflesiales are correctly placed in eudicots, it would be interesting to know how this monoporate pollen may be derived from tricolpate pollen. However, according to our own unpublished data on *Rafflesia*, *Rhizanthes*, and *Sapria*, pollen of Rafflesiaceae s. str. is inaperturate (see also Erdtman 1952) and thus could also be derived from tricolpate forms.

Gynoecium. The female flowers of Apodanthaceae are more cone-like and broader than the male ones. They share a tetracarpellate, completely syncarpous gynoecium and a semi-inferior, unilocular ovary with four more or less protruding parietal placentae. The secretory stigma has unicellular papillae, which are found on the whole apical platform in *Apodanthes* and in *Berlinianche* but are lacking in the centre in *Pilostyles*. The four placentae are slightly protruding into the locule. According to some authors such as Pulle (1909), Rose (1909), and Kuijt (1969), the whole inner surface of the ovary is covered with ovules in some species of *Apodanthes* and *Pilostyles*. We question this, as it may be a wrong interpretation of hand sections. This was clearly not the case in our material, which was studied using serial microtome section and SEM.

Ovules are oriented in various directions. They are tenuinucellate (Bouman and Meijer 1994, this study) and anatropous (Karsten 1856, 1858; Solms-Laubach 1901; Harms 1935; Kummerow 1962; Rutherford 1970; Bouman and Meijer 1994; this study). There are two well developed integuments (Solms-Laubach 1874a, Endriss 1902, Rutherford 1970, Bouman and Meijer 1994, this study), in contrast to other Rafflesiales, in which the outer integument is more reduced (Bouman and Meijer 1994, Igersheim and Endress 1998). The inner integument is one or two cell layers thick, the outer one is one cell layer thick (Endriss 1902, Rutherford 1970, Bouman and Meijer 1994, this study). In *Pilostyles*, the micropyle is formed by two integuments (Bouman and Meijer 1986, this study) or by the inner one (Rutherford 1970).

In male flowers, a sterile gynoecium with a stylar canal but without a locule is present. It is almost completely fused with the androecium in *Pilostyles* (Guillemin 1834, Endriss 1902, Kummerow 1962, Rutherford 1970, this study) and in *Apodanthes* (de Vattimo 1956, this study) or partially free from it in *Berlinianche* (Welwitsch 1869, Engler 1912, de Vattimo 1955, this study).

Ring of vesicular hairs. In male flowers, in the upper part of the sterile gynoecium, topographically immediately above the pollen sacs, there is a fringe of large, elongate vesicular cells with narrow tips. Some of them

seem to have ruptured at anthesis in the fixed material. This was also observed by Endriss (1902) in 'old' flowers of *Pilostyles ingae* in which the tip of these cells was torn. He assumed that the content of these cells might glue the pollen together. A comparison with similar hairs in some other angiosperms suggests that they may release an adhesive for pollen transport when touched (Vogel 1981, 2002; Endress et al. 1998)

Nectary. A nectary disk, as present in Apodanthaceae, is typical for core eudicots. Rutherford (1970) and Dell and Burbidge (1981) have described nectary disks for *Pilostyles*. Endriss (1902), although supposing a main function of the disk in flower opening in *Pilostyles ingae* (see above), assumed that the stomata on the disk release nectar or mucilage. In Rafflesiaceae s. str., *Rafflesia* (Beaman et al. 1988) and *Sapria* (Hans Bänziger, pers. comm.) seem to have no nectaries. However, *Rhizanthes* has a nectary on the distal part of the perianth organs (Bänziger 1995, Bänziger and Hansen 2000), but this is likely not homologous to nectaries surrounding the gynoecium. In Cytinaceae, *Cytinus* (Hayek 1912, Igersheim and Endress 1998) has a nectary disk (*Bdallophyton* is unstudied). Mitrastemonaceae have nectar-releasing stomata on the ovary and at the base of the androecium and upper scales (Watanabe 1937b). Although most Malvaceae have hair nectaries on perianth parts (Vogel 2000), disk nectaries are also present in a few Malvales such as in Thymelaeoideae (Herber 2002), Muntingiaceae (Bayer et al. 1998, Bayer 2002b), Sphaerosepalaceae (Bayer 2002e), and Sarcolaenaceae (Bayer 2002d). In addition, a number of families either have no nectaries or detailed information is lacking, such as Bixaceae (Poppendieck 2002a), Cistaceae (Arrington and Kubitzki 2002), Cochlospermaceae (Poppendieck 2002b), Diegodendraceae (Bayer 2002a,) Dipterocarpaceae (Ashton 2002), Neuradaceae (Bayer 2002c), and many Thymelaeaceae (Herber 2002).

Possible pollinators of *Berlinianche aethiopica* are dipters (*Drosophila*, Sciaridae, Psych-odidae, Cecidomyiidae), thrips (Thriphidae), and beetles (Curculionidae). These insects were collected from *Berlinianche* flowers by D. Plowes and were identified by G. Bächli. A suggested pollinator for *Pilostyles hamiltonii* in Australia is a small, unidentified, native wasp (Dell and Burbidge 1981). Solitary bees are reported to be pollinators for *Pilostyles berterii* (Kummerow 1962). To our knowledge, there are no reports on pollinators for *Apodanthes*.

Conclusions

The present studies support close relationships among *Apodanthes*, *Pilostyles*, and *Berlinianche*. Shared features of all three genera are: three alternating whorls of scales in flowering shoots of both genders; pollen sacs arranged in two rings; large, elongate vesicular cells with narrow tips above the pollen sacs in male flowers; pollen tube transmitting tissue one cell layer thick; gynoecium of four completely united carpels; inferior and unilocular ovaries with four parietal placentae; ovules oriented in various directions, tenuinucellate, anatropous, with two well developed integuments; inner integument two cell layers thick; outer integument one or two cell layers thick; single, psilate, small pollen grains (diameter c. 10 μm). Shared features between *Apodanthes* and *Berlinianche* are: doubling of the number of scales from the outer to the middle whorl; isomerous middle and inner whorl; in female flowers, stigmatic papillae covering the whole apical platform; style not narrower than the stigmatic region. Shared features between *Apodanthes* and *Pilostyles* are: di-/tetramerous whorls of scales; lacking hair-cushion on the upper side of the organs of the inner whorl; sterile gynoecium in male flowers almost completely fused to the androecium; less conspicuous disk nectary than in *Berlinianche*; tricolpate pollen. Shared features between *Berlinianche* and *Pilostyles* are: parasites of Fabaceae; perianth organs of the inner whorl with broad insertion and caducous; distribution of hairs in a ring in male flowers; in female flowers, simple stigmatic unicellular

papillae; placentae less protruding and bearing fewer ovules than in *Apodanthes*.

Berlinianche and *Pilostyles* were regarded as one genus and distinct from *Apodanthes*, mainly because of the persistence and the type of the insertion site of the perianth organs of the inner whorl (e. g. Harms 1935). However, *Berlinianche* is distinct from *Pilostyles* in that it is tri- and hexamerous (but there is also a hexamerous *Pilostyles* species) and it possesses unique hair cushions on the perianth organs of the inner whorl. *Berlinianche* is more limited in the range of hosts among Fabaceae than *Pilostyles*. Their shared host family, Fabaceae, suggests an especially close relationship between those two genera as suggested in a molecular study (Blarer et al. 2000).

Shared features of Apodanthaceae with eudicots and especially Malvales are: pollen tricolpate (restricted to eudicots); nectary disk (in many core eudicots); androecial tube (in some Malvaceae, such as Malvoideae and Bombacoideae); trend from normal stamens to synandria without anthers and thecae (in Malvaceae); carpet of hairs on perianth organs (in many Malvaceae); flowers tri- to hexamerous (in Thymelaeaceae); placenta parietal (in Cistaceae). Thus, a position of Rafflesiales in eudicots and close to Malvales, as suggested by a molecular study (Nickrent 2002) may not be out of place with regard to structural features.

References

APG (The Angiosperm Phylogeny Group) (1998) An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard. 85: 531–553.

APG II (2003) An update of the Angiosperm Phylogeny Group classification for the order and families of flowering plants: APG II. Bot. J. Linn. Soc. 141: 399–436.

Arrington J. M., Kubitzki K. (2002) Cistaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 62–71.

Ashton P. S. (2002) Dipterocarpaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 182–197.

Bänziger H. (1995) Ecological, morphological and taxonomic studies on Thailand's fifth species of Rafflesiaceae: *Rhizanthes zippelii* (Blume) Spach. Nat. Hist. Bull. Siam Soc. 43: 337–365.

Bänziger H., Hansen B. (1997) Unmasking the identity of *Sapria poilanei* Gagnepain emend., and description of *Sapria ram* sp.n. (Rafflesiaceae). Nat. Hist. Bull. Siam Soc. 45: 149–170.

Bänziger H., Hansen B. (2000) A new taxonomic revision of a deceptive flower, *Rhizanthes* Dumortier (Rafflesiaceae). Nat. Hist. Bull. Siam Soc. 48: 117–143.

Bayer C. (2002a) Diegodendraceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 175–177.

Bayer C. (2002b) Muntingiaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 315–319.

Bayer C. (2002c) Neuradaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 325–328.

Bayer C. (2002d) Sarcolaenaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 345–352.

Bayer C. (2002e) Sphaerosepalaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 359–362.

Bayer C., Chase M. W., Fay M. F. (1998) Muntingiaceae, a new family of dicotyledons with malvalean affinities. Taxon 47: 37–42.

Beaman R. S., Decker P. J., Beaman J. H. (1988) Pollination of *Rafflesia* (Rafflesiaceae). Amer. J. Bot. 75: 1148–1162.

Blarer A., Nickrent D. L., Bänziger H., Endress P. K., Qiu Y. -L. (2000) Phylogenetic relationships among genera of the parasitic family Rafflesia-

ceae s.lat. based on nuclear ITS and SSU rDNA, mitochondrial LSU and SSU rDNA, *atp*1, and *mat*R sequences. Amer. J. Bot. 87 (6, Suppl.): 503.

Bouman F., Meijer W. (1986) Comparative seed morphology in Rafflesiaceae. Acta Bot. Neerl. 35: 521–521.

Bouman F., Meijer W. (1994) Comparative structure of ovules and seeds in Rafflesiaceae. Plant Syst. Evol. 193: 187–212.

Chase M. W., Zmarzty S., Lledò M. D., Wurdack K. J., Swensen S. M., Fay M. F. (2002) When in doubt, put it in Flacourtiaceae: a molecular phylogenetic analysis based on plastid *rbc*L DNA sequences. Kew Bull. 57: 141–181.

de Vattimo I. (1955) Notice sur la tribu Apodantheae R. Br. (Rafflesiaceae). Taxon 4: 211–212.

de Vattimo I. (1956) Notes on *Apodanthes caseariae* Poit. and *Pilostyles calliandrae* (Gardn.) R. Br. (Rafflesiaceae-Apodantheae). Not. Syst. 15: 225–229.

de Vattimo I. (1971) Contribuição ao conhecimento da tribu Apodantheae R. Br. Parte 1 – Conspecto das espécies (Rafflesiaceae). Rodriguésia 26: 37–62.

de Vattimo I. (1978) Uma nova espécie de *Apodanthes* Poit. (Rafflesiaceae). Rodriguésia 29: 269–306.

Dell B. D. (1984) Rafflesiaceae. In: George A. S. (ed.) Flora of Australia. Australian Government Publishing Service, Canberra, pp. 147–150.

Dell B. D., Burbidge A. H. (1981) Notes on the biology of *Pilostyles* (Rafflesiaceae) in Western Australia. W. Austral. Herb. Res. Notes 5: 71–79.

Eliasson U. H. (1994) Rafflesiaceae. In: Harling G., Andersson L. (eds.) Flora of Ecuador, vol. 59. Council of Nordic Publications in Botany, Copenhagen, pp. 45–48.

Endress M. E., Igersheim A., Garnock-Jones P. (1998) Sexual dimorphism and secondary pollen presentation in *Geniostoma ligustrifolium* (Geniostomaceae). Amer. J. Bot. 85 (6, Suppl.): 126.

Endress P. K. (1987) Floral phyllotaxis and floral evolution. Bot. Jahrb. Syst. 108: 417–438.

Endress P. K. (1994) Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge.

Endress P. K., Stumpf S. (1990) Non-tetrasporangiate stamens in the angiosperms: structure, systematic distribution and evolutionary aspects. Bot. Jahrb. Syst. 112: 193–240.

Endriss W. (1902) Monographie von *Pilostyles ingae* (Karst.) (*Pilostyles ulei* Solms-Laub.). Flora 91: 209–236.

Engler A. (1912) Rafflesiaceae Africanae. Bot. Jahrb. Syst. 46: 293.

Engler A. (1915) Rafflesiaceae. In: Engler A., Drude O. (eds.) Die Vegetation der Erde, IX. Die Pflanzenwelt Afrikas insbesondere seiner tropischen Gebiete, III. Band, 1. Heft. Engelmann, Leipzig 114.

Erdtman G. (1952) Pollen morphology and plants taxonomy. Angiosperms. Almqvisit and Wiksell, Stockholm.

Gentry A. H. (1973) Rafflesiaceae. Flora of Panama. Ann. Missouri Bot. Gard. 60: 17–21.

Gentry A. H. (1993) A field guide to the families and genera of woody plants of northwest South America (Colombia, Ecuador, Peru), with supplementary notes on herbaceous taxa. Conservation International, Washington, DC.

Gomez L. D. (1983) Rafflesiaceae. In: Burger W. C. (ed.) Flora of Costa Rica. Fieldiana 13: 89–91.

Griffith W. (1845) On the root parasites referred by authors to *Rhizantheae*; and on various plants related to them. Trans. Linn. Soc. Lond. 19: 303–347.

Guillemin M. (1834) Mémoire sur le *Pilostyles*, nouveau genre de la famille des Rafflesiacées. Ann. Sci. Nat., Bot., Sér. 2, 2: 19–25.

Harms H. (1935) Rafflesiaceae. In: Engler A., Prantl K. (eds.) Die natürlichen Pflanzenfamilien (ed. 2), 16b. Engelmann, Leipzig, pp. 243–281.

Hayek A. (1912) Über die Blütenbiologie von *Cytinus hypocystis* L. Österr. Bot. Z. 62: 238–240.

Herber B. E. (2002) Thymelaeaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 373–396.

Hutchinson J. (1969) Evolution and phylogeny of flowering plants: Dicotyledons. Academic Press, London.

Igersheim A. (1993) The character states of the Caribbean monotypic endemic *Strumpfia* (Rubiaceae). Nord. J. Bot. 13: 545–559.

Igersheim A., Cichocki O. (1996) A simple method for microtome sectioning of prehistoric charcoal specimens, embedded in 2-hydroxyethyl methacrylate (HEMA). Rev. Palaeobot. Palynol. 92: 389–393.

Igersheim A., Endress P. K. (1998) Gynoecium diversity and systematics of the paleoherbs. Bot. J. Linn. Soc. 127: 289–370.

Karsten H. (1856) Plantae Columbianae. Linnaea 28: 387–462.

Karsten H. (1858) Über die Stellung einiger Familien parasitischer Pflanzen im natürlichen System. 2 - Über einige Rafflesiaceen. Nova Acta Acad. Caes. Leop. Carol. 26: 884–923.

Kuijt J. (1969) The biology of parasitic flowering plants. University of California Press, Berkeley.

Kummerow J. (1962) Pilostyles berterii Guill., eine wenig bekannte Rafflesiaceae in Mittelchile. Z. Bot. 50: 321–337.

Meijer W. (1993) Rafflesiaceae. In: Kubitzki K., Rohwer J. G., Bittrich V. (eds.) The families and genera of vascular plants. II. Flowering plants. Dicotyledons. Magnoliid, Hamamelid and Carophyllid families. Springer, Berlin, pp. 557–563.

Meijer W. (1997) Rafflesiaceae. In: Kalkman C., Kirkup D. W., Nooteboom H. P., Stevens P. F., de Wilde W. J. J. O. (eds.) Flora Malesiana, vol. 13. Rijksherbarium, Leiden, pp. 1–42.

Melchior H. (1964) Aristolochiales. In: Melchior H. (ed.) A. Engler's Syllabus der Pflanzenfamilien (ed. 14), 2. Borntraeger, Berlin, pp. 151–156.

Nickrent D. L. (2002) Orígenes filogenéticos de las plantas parásitas. In: López-Sáez J. A., Catalán P., Sáez L. (eds.) Plantas parásitas de la Península Ibérica e Islas Baleares. Mundi-Prensa Libros, S. A., Madrid, 29–56.

Nickrent D. L., Blarer A., Qiu Y.-L., Soltis D. E., Soltis P. E., Zanis M. (2002) Molecular data place Hydnoraceae with Aristolochiaceae. Amer. J. Bot. 89: 1809–1817.

Peter A. (1932) Flora von Deutsch-Ostafrika. Feddes Rep. Beih. 40, 2: 1–272.

Poppendieck H.-H. (2002a) Bixaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 33–35.

Poppendieck H.-H. (2002b) Cochlospermaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. IV. Springer, Berlin, pp. 71–74.

Pulle A. A. (1909) Rafflesiaceae. Rec. Trav. Bot. Néerl. 6: 259–261.

Robinson B. L. (1891) Two undescribed species of Apodanthes. Bot. Gaz. (Crawfordsville) 16: 82–84.

Roubik D. W., Moreno J. E. (1991) Pollen spores of Barro Colorado Islands Monographs in systematic Botany, No, 36, Missouri Botanical Garden, St. Louis, Missouri, pp. 1–268.

Rose J. N. (1909) Studies of Mexican and Central American plants—No. 6. Contributions from the National Herbarium, vol. 12, part 7. Government Printing Office, Washington, pp. 262–265.

Rusby H. H. (1920) Descriptions of three hundred new species of South American plants. Rusby, New York.

Rutherford R. J. (1970) The anatomy and cytology of Pilostyles thurberi Gray (Rafflesiaceae). Aliso 7: 263–288.

Solms-Laubach H. (1874a) Über den Bau der Samen in den Familien der Rafflesiaceae und Hydnoraceae. Bot. Zeitg. 32: 337–342, 353–358, 369–374, 385–389.

Solms-Laubach H. (1874b) Über den Thallus von Pilostyles haussknechtii. Bot. Zeitg. 32: 49–59.

Solms-Laubach H. (1889) Rafflesiaceae. In: Engler A., Prantl K. (eds.) Die natürlichen Pflanzenfamilien, III, 1. Engelmann, Leipzig, pp. 274–282.

Solms-Laubach H. (1901) Rafflesiaceae. In: Engler A. (ed.) Das Pflanzenreich, IV, 75. Engelmann, Leipzig, pp. 1–19.

Takhtajan A. L. (1997) Diversity and classification of flowering plants. Columbia University Press, New York.

Takhtajan A. L., Meyer N., Kosenko R., Kosenko V. N. (1985) Pollen morphology and classification in Rafflesiaceae s.l. Bot. Zhurn. (Moscow and Leningrad) 70: 153–162.

van Heel W. A. (1966) Morphology of the androecium in Malvales. Blumea 13: 177–394.

Verdcourt B. (1998) Rafflesiaceae. In: Beentje H. J. (ed.) Flora of tropical East Africa. Balkema, Rotterdam.

Vogel S. (1981) Die Klebstoffhaare an den Antheren von Cyclanthera pedata (Cucurbitaceae). Plant Syst. Evol. 137: 291–316.

Vogel S. (2000) The floral nectaries of Malvaceae sensu lato – a conspectus. Kurtziana 28: 155–171.

Vogel S. (2002) Extra-tapetal pollen adhesives: Where they occur and how they function. In: Schönenberger J., von Balthazar M., Matthews M. (eds.) Flowers: Diversity, Development & Evolution. Institute of Systematic Botany, University of Zurich, Zurich, 48.

von Balthazar M., Nyffeler R. (2002) The peculiar androecium of Cullenia (Durioneae, Malvaceae s.l.). In: Schönenberger J., von Balthazar M.,

Matthews M. (eds.) Flowers: Diversity, Development & Evolution. Institute of Systematic Botany, University of Zurich, Zurich, 89.

Watanabe K. (1937a) Morphologisch-biologische Studien über die Gattung *Mitrastemon*, 1. J. Jap. Bot. 12: 603–618.

Watanabe K. (1937b) Morphologisch-biologische Studien über die Gattung *Mitrastemon*, 5. J. Jap. Bot. 13: 14–24.

Weber M., Igersheim A. (1994) Pollen buds in *Ophiorrhiza* (Rubiaceae) and their role in pollenkitt release. Bot. Acta 107: 257–262.

Welwitsch F. M. J. (1869) Sertum Angolense. Trans. Linn. Soc. Lond. 27: 1–94.

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