Verhuellia is a segregate lineage in Piperaceae: more evidence from flower, fruit and pollen morphology, anatomy and development

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• Background and Aims The perianthless Piperales, i.e. Saururaceae and Piperaceae, have simple reduced flowers strikingly different from the other families of the order (e.g. Aristolochiaceae). Recent molecular phylogenies proved Verhuellia to be the first branch in Piperaceae, making it a promising subject to study the detailed structure and development of the flowers. Based on recently collected material, the first detailed study since 1872 was conducted with respect to morphology, anatomy and development of the inflorescence, pollen ultrastructure and fruit anatomy.

• Methods Original scanning electron microscopy (SEM), transmission electron microscopy (TEM) and light microscopy (LM) observations on Verhuellia lunaria were compared with those of Piperaceae, Saururaceae and fossils.

• Key Results The inflorescence is an indeterminate spike with sessile flowers, each in the axil of a bract, developing in acropetal, helical succession. Flowers consist of two (occasionally three) stamens with basified tetrasporangiate anthers and latrorse dehiscence by a longitudinal slit. The gynoecium lacks a style but has 3–4 stigma branches and a single, basal orthotropous and unitegmic ovule. The fruit is a drupe with large multicellular epidermal protuberances. The pollen is very small, inaperturate and areolate, with hemispherical microechinulate exine elements.

• Conclusions Despite the superficial similarities with different genera of Piperaceae and Saururaceae, the segregate position of Verhuellia revealed by molecular phylogenetics is supported by morphological, developmental and anatomical data presented here. Unitegmic ovules and inaperturate pollen, which are synapomorphies for the genus Peperomia, are also present in Verhuellia.

Key words: Verhuellia lunaria, Piperales, Peperomia, Appomattoxia ancirophora, floral development, floral anatomy, fruit morphology, pollen morphology, unitegmic ovule, inaperturate pollen.

INTRODUCTION

Verhuellia is a Piperaceae genus of three species, known from very few collections and localities on Cuba and Hispaniola (Haiti and Dominican Republic). It is a small, herbaceous, saxicolous perennial with monopodial architecture, whorled leaves and perianthless flowers organized in solitary lateral spikes (Fig. 1). These flowers, each subtended by a bract, consist of a gynoecium with three to four stigmas and two tetrasporangiate stamens. With the exception of Schmitz (1872a, b), who only described some morphological characters based on herbarium specimens, detailed morphological, anatomical or developmental studies have never been conducted. This is probably due to the very limited number of collections and its absence from living collections and also to the presumed close relationship with the genus Peperomia.

The absence of some floral organs in the flowers of perianthless Piperales has recently re-enhanced the focus on the representatives of the sister families Saururaceae and Piperaceae, presenting them as an interesting model for studying floral development and evolution (Jaramillo and Kramer, 2007; Arias and Williams, 2008; Madrid and Friedman, 2009). Furthermore, several phylogenetic studies in the order Piperales are now available as a robust framework to interpret character evolution (Wanke et al., 2007a, b). With the exception of the two Piperaceae genera Verhuellia and Manekia, floral characters of nearly all genera have been extensively studied in earlier years (Tucker, 1975, 1976, 1979, 1980, 1981, 1982a, b, 1985; Liang and Tucker, 1989, 1990, 1995; Tucker et al., 1993; Igersheim and Endress, 1998; Lei and Liang, 1998, 1999). In addition, Smith and Stockey (2007) studied the pollen of the four Saururaceae genera. Detailed observations on the fruit morphology in this family are lacking. In Piperaeae, pollen and fruit development have not been studied extensively despite the large number of species.

Verhuellia was generally considered to be part of or at least closely related to Peperomia (e.g. Tebbs, 1993), due to its

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superficial similarities with this genus. However, Wanke et al. (2007b) recently showed that Verhuellia is sister to all other Piperaceae, a position formerly thought to be occupied by Zippelia and Manekia (Jaramillo et al., 2004; Wanke et al., 2007a). As a consequence, the traditional sub-division of the family Piperaceae into Piperoideae and Peperomioideae has been revised, resulting in three subfamilies congruent with the clades recovered by Wanke et al. (2007b); Verhuellioideae (with Verhuellia); Zippelioideae (with Zippelia and Manekia) and Piperioideae (with Piper and Peperomia) (Samain et al., 2008). Figure 2 shows a summary cladogram of the perianthless Piperales.

Given the hypothesis that Verhuellia is sister to the four other Piperaceae genera (Wanke et al., 2007b), intermediate characters between Piperaceae and Saururaceae could be expected. This is supported by the fact that Zippelia begentifolia, placed in the next branching clade in Piperaceae (Jaramillo et al., 2004; Wanke et al., 2007a, b), also shows some similarities to Saururaceae (Igersheim and Endress, 1998). Tucker et al. (1993) and Liang and Tucker (1995) consider these characters as plesiomorphic. Zippelia has sometimes been considered a member of Saururaceae (e.g. Blume, 1830; Wu and Wang, 1957; Heywood, 1993) whereas it has never been questioned that Verhuellia belongs to Piperaceae.

The aims of this study are: (a) to present a detailed study on morphology, anatomy and development of the Verhuellia inflorescence, flower, pollen and fruit based on fresh material to overcome the lack of knowledge on this genus; and (b) to discuss the floral ontogenetic findings in the light of its new position in the family Piperaceae and in relation to available data of other perianthless Piperales, including the presumed fossil relative Appomattoxia ancistrophora.

MATERIALS AND METHODS

Plants of Verhuellia lunaria (Ham.) C.DC., the type species of the genus, originating from the Dominican Republic were cultivated in the Botanical Gardens of Ghent University and Dresden Technical University. Inflorescences, flowers and fruits of all stages were collected and preserved. Voucher specimens are deposited in the herbaria of Ghent University (GENT) and Dresden Technical University (DR).

Material for scanning electron microscopy (SEM) was prepared by fixation in Kew mix (53 % industrial methyated spirit, 37 % water, 5 % formaldehyde solution and 5 % glycerol), followed by dehydration and critical point drying with carbon dioxide in a BAL-TEC CPD 030 critical-point dryer. Subsequently, samples were fixed to aluminium stubs (Plano GmbH, Lünen, Germany) using a carbon adhesive tape (LEIT-TABS, Plano GmbH) and sputter-coated with gold (20 nm) under an argon atmosphere using an EMITECH K550 sputter-coater. Images were obtained with a LEO 420 scanning electron microscope at acceleration voltage of 15 kV.

Light microscopy (LM) samples were prepared in 70 % ethanol and subsequently gradually transferred to 100 % ethanol. The samples were then transferred to LR White Resin, hard grade (London Resin Company Ltd, Reading, UK) in a graded LR White Resin/ethanol series using solutions of 25/75, 50/50, 75/25, 100/0 resin/100 % ethanol for at least 5 h each. Subsequently, the samples were placed in a closed capsule filled with fresh resin and hardened at 60 °C for 48 h. Sections of 2 μm were made with a rotation microtome (Microm HM360, Walldorf, Germany) and subsequently stained with 0.1 % toluidine blue. The stained sections were fixed on microscopy slides using Eukitt© quick hardening mounting medium (Fluka Chemie GmbH, Buchs, Switzerland). Observations were done with a light microscope (Leitz Dialux 20, Van Hopplynus, Brussels, Belgium) equipped with a camera (PixeLINK PL-B622CF, Ottawa, Canada) with specially developed software (Microscopica v1.3, Orbicule, Leuven, Belgium).

For TEM pictures, pollen of V. lunaria was fixed in 50 % alcohol, embedded in Agar Low Viscosity Resin (Agar Scientific Ltd, Stansted, Essex, UK); ultrathin sections were post-stained using uranyl acetate–lead citrate, KMnO4 and the Thiry reaction for polysaccharides. Images were obtained with a Zeiss EM-900 transmission electron microscope. For SEM pictures, fixed V. lunaria pollen was rehydrated, critical-point dried and sputter-coated. Images were obtained with a JEOL JSM 6390 scanning electron microscope.
SEM and LM pictures were also taken from accessions of the genus *Peperomia* for comparison with *Verhuellia* morphology. This material was taken from the living collection of the Botanical Garden of Ghent University.

**RESULTS**

The inflorescence is initiated in the axil of one of the 3–5 whorled leaves on the stem (Figs 3A and 4A, B). Its primordium is surrounded by large, multicellular trichomes (Figs 4A, B and 5C). The inflorescence is an indeterminate spike with sessile flowers on a filiform axis (Fig. 4C). The inflorescence apex produces bracts in acropetal, helical succession (Figs 3A, 4A and 5A–C). Each flower primordium appears in the axil of a peltate bract, which originated considerably earlier (Figs 3A, 4A, 5A–D and 10F). The bract develops large trichomes at its margin, which are especially conspicuous during the young stages of the bract (Figs 3A, 4A, 5B, C and 6A). Subsequently, two lateral stamen primordia are formed, followed by the gynoecium, which appears as an annular structure (Figs 5D and 6A, B). Meanwhile, the bract increases in size, overarching the developing flower (Figs 3A, 4A and 5A–C).

Next, the stigma primordia become apparent on the upgrowing ovary wall (Figs 6A, B and 8A–C), followed by the differentiation of each stamen into a short filament and a basifixed anther (Fig. 6A). The anthers are tetrasporangiate (Figs 6A–D and 7A–E). The lateral stamens develop simultaneously. Exceptionally, a third stamen is formed adaxially (Fig. 6B). In immature stamens, the connective ends in a bulge in between the two thecae (Fig. 6A, B). This bulge consists of one large gland (Fig. 7E). At maturity, the stamen is T-shaped as the connective grows out laterally between the pollen sacs in each theca, forming a broad, oblate, saddle-shaped connection between the thecae (Figs 6C, D and 7A–C). The remnant of the apical bulge, which was prominent in immature stamens, is still visible in mature stamens (Fig. 7B, C). The anthers dehisce latrorsely with a longitudinal slit (Fig. 7B, C). After dehiscence, the anther wall recures (Fig. 7B, C). Shortly after dehiscence, the stamens are shed.

On the annular young gynoecium (Figs 6B and 8A) generally four stigma primordia develop: one abaxial, one adaxial and two lateral stigmas (Fig. 8B–D). Occasionally, only three stigma branches are present (Figs 6D and 8E). This variation in stigma number can occur within a single inflorescence. As long as the ovary is open, no ovule development is visible (Fig. 8B, C). The ovule is unitegmic, orthotropous and basal (Fig. 10A–C). The micropyle is situated at the apex of the ovule (Fig. 10C, D). The stigma branches become papillate in later development (Figs 7A–C and 8F). The fruit develops into a drupe with a relatively thin mesocarp and a stony endocarp and large multicellular protuberances on its wall (Figs 9C–F and 10E, F). The drupe is situated on a short gynophore, which slightly elongates during maturation of the fruit (Fig. 10A, B, F).

The pollen grains are shed as very small spherical monads only approx. 8–10 μm in diameter (Fig. 11A, B). Pollen is inaperturate and areolate with evenly distributed hemispherical microechinate exine elements (Fig. 11A–D). These exine elements are separated by narrow channels, forming a negative reticulum (Fig. 11C, D).
Fig. 4. SEM images of inflorescence development in *Verhuellia lunaria*. (A) Young spike with developing floral bracts with young flowers in their axils (one young flower with its subtending bract is circled). A young leaf develops next to the spike. Multicellular trichomes (arrowed) surround the spike. The older leaves on the main stem are removed. (B) View of a stem node with two older leaves still present and the immature spike (encircled) in the axil of the removed leaf. Multicellular trichomes (arrowed) surround the spike. (C) Lateral view of the maturing spike shortly before anther dehiscence. The peltate bracts partially cover the nearly mature anthers which, in turn, cover the young gynoecia. FL, foliage leaf; *, apex of the spike.

Fig. 5. SEM images of inflorescence and floral development in *Verhuellia lunaria*. (A) Apical view of the spike apex and bract and young flowers in successive stages of development. (B) Lateral view of the spike apex with developing bracts partially covering flowers at successive stages of development. In the most developed floral primordia visible, two stamen primordia and the gynoecium primordium are present. On the margins of the bracts, trichomes are present. (C) Lateral view of a spike at the same developmental stage as in B. The youngest flowers and bracts are circled. Multicellular trichomes are present at the base of the spike. (D) Apical view of a developing flower with two lateral stamens and the gynoecium primordium (floral bract removed). a, anther; B, bract; F; flower; f, filament; fa, floral apex; s, stamen; *, apex of the spike.
pollen expansion, these channels become conspicuously broader, and in such cases one or more apertural regions are mimicked (Fig. 11B, C). However, the absence of any intine thickening in ultrathin sections demonstrates the absence of apertures (Fig. 12A, B). The pollen wall consists of an ektexine (a massive and prominent tectum with very low columellae and a thin foot layer), an evenly thin endexine and an evenly thin intine (Fig. 12A, B). Extremely small very inconspicuous orbicules have been observed (not shown).

**DISCUSSION**

Wanke et al. (2007b), based on molecular data, showed that *Verhuellia* and *Peperomia* are not closely related, and this is supported by this study. Our morphological, anatomical and ontogenetic observations of flower, pollen and fruit in *V. lunaria* show that the genus is a clearly distinct lineage within Piperaceae and perianthless Piperales. An overview of important characters for Piperaceae and Saururaceae is given in Table 1.

The acropetal, helical succession of the initiation of bracts subtending the flowers in *Verhuellia* is comparable with the inflorescence development of all other Piperaceae studied. As in all other Piperaceae, there is a shift in position of the floral primordia with respect to their subtending bract as well as a considerable plastochron between the initiation of the bract and the floral primordium (Tucker, 1980, 1982a, b; Liang and Tucker, 1995). In contrast, Saururaceae have a so-called common floral and bract primordium, which is a synapomorphy for the family (Tucker, 1975, 1981, 1985; Liang and Tucker, 1989). In many Piperaceae and Saururaceae investigated, trichomes around or on the developing and mature spikes as well as on the bracts and gynoecia occur in a wide range of shapes and sizes (unicellular, multicellular, pearl glands, etc.) (e.g. Tucker, 1975, 1976, 1982a, b; Fig. 11A, B). The function of these trichomes remains unknown. However, in his study of *Peperomia magnoliifolia*, Vogel (1998) suggested that the bract trichomes attract pollinators through secretion of small quantities of sugars.

All *Verhuellia* flowers observed are bisexual. However, the stamens are shed relatively soon after anthesis, which could explain why de Candolle (1866) reported ‘female’ flowers at the base of the inflorescence (see also Schmitz, 1872a).

In addition, de Candolle (1866) described hermaphrodite flowers in the middle of the inflorescence and male flowers at the apex. However, this may be based on a misinterpretation as the flowers develop in acropetal succession: the stamens are already shed in the basal ‘female flowers’, while they still cover the small, developing gynoecia in the distal ‘male flowers’. The arrangement of floral organs, number and order of initiation are highly variable among the perianthless Piperales investigated (see all abovementioned references, especially the floral diagrams in Tucker et al., 1993; Liang and Tucker, 1995, and the ancestral state reconstruction in Wanke et al., 2007b). Nevertheless, these characters are usually constant within Piperaceae and more or less variable...
**FIG. 7.** SEM and LM images of floral development in *Verhuellia lunaria*. (A–C) SEM images of stamens at anthesis from early (A) to late (C). One entire stamen is encircled. (A) Flower with its subtending bract before anther dehiscence. The gynoecium has four receptive stigmas. (B) Flower with its subtending bract after anther dehiscence. The anther wall has recurved, exposing the tapetum. The connective is broadening and flattening. (C) Flower with bract after anther dehiscence and with four withering stigmas. The connective forms a broad, oblate, saddle-shaped connection between the thecae. (D) Transverse section through an anther showing the four pollen sacs. (E) Longitudinal section through a flower and inflorescence axis; in each stamen, one of the pollen sacs is visible. The connective ends in a bulge in between the two thecae and this bulge contains a single large oil cell. co, connective tissue; f, filament; ps, pollen sac.

**FIG. 8.** SEM images of gynoecium development in *Verhuellia lunaria*. (A) The adaxial and abaxial stigma branches start to develop from the annular gynoecium base (circled). (B) Beginning closure of the ovary (encircled). The adaxial stigma branch develops first, followed by the two lateral ones. The abaxial stigma branch is the last to develop. The four stigma branches are arrowed. (C) Ovary closed. The three most developed stigma branches are arrowed. (D) All four stigma branches are distinct. (E) Gynoecium with three stigmas at the same stage as in (D). (F) Mature gynoecium with four recurved receptive stigmas with a papillose surface. One multicellular protuberance is arrowed. sg, stigma branch.
FIG. 9. SEM images of fruit development in *Verhuellia lunaria*. (A) Lateral view of immature fruit with bract; the four papillate stigmas are still visible (circled) and each stigma branch is decurrent on the fruit wall as a longitudinal rim. The initially single-celled wart-like protuberances on the fruit wall have started to divide (arrowed). (B) Lateral view of immature fruit with four papillate stigmas (circled) and bract, seen from the adaxial side. One protuberance is arrowed. (C) Mature fruit with three stigmas and three rims. The fruit is still attached to the inflorescence axis and the multicellular protuberances on the fruit wall are fully developed. (D) Mature fruit, fallen from the inflorescence axis. (E) Detail of a single-celled fruit wall protuberance. (F) Detail of a mature protuberance after cell division.

FIG. 10. LM images of sections through developing gynoecium and fruit of *Verhuellia lunaria*. The stamens are shed. (A) Longitudinal section through the mature gynoecium; two stigmatic branches are visible. The micropylar area is arrowed. The gynoecium stands on a short gynophore on the axis of the inflorescence (in transverse section). (B) Longitudinal section through the ripening fruit with the single ovule. The gynophore has elongated and the fruit wall protuberances are fully developed (arrowed). (C) Detail of a ripening fruit showing the single central ovule with micropyle formed by the single integument. (D) Detail of the micropylar area (micropyle arrowed), showing the single integument and the apical part of the nucellus. (E) Transversal section through mature fruit, with the single ovule. One of the multicellular protuberances on the fruit wall is arrowed. (F) Maturing fruit in the axil of the bract. Large glandular cells are present in the bract and the stigmatic area. The arrow points to the micropyle. B, bract; gp, gynophore; mp, micropyle; o, ovule; ov, ovary; nc, nucellus; sg, stigma; st, stigma branch; tg, integument.
in Saururaceae. However, being a member of Piperaceae, *Verhuellia* proves to be an exception to this rule as (a) the number of stamens and stigma branches can vary between 2 and 3, and 3 and 4, respectively (Fig. 6A–D); (b) the initiation sequence of the stigma branches is variable (Fig. 8B vs. C); and (c) in the case of three stigma branches, their position is not fixed (Figs. 6D vs. 8E). In contrast, in *Gymnotheca* (Saururaceae), the number of stigma branches and stamens can also vary but it usually is the abaxial stigmatic branch that is missing. In addition, the abaxial median stamen is also usually absent in these flowers (Liang and Tucker, 1989).

Despite this aberrant floral development in *Verhuellia*, the zygomorphic symmetry of its flowers is similar to that of all other perianthless Piperales (Figs 7B, C and 11A, B). The genus also shares the pairwise initiation of stamens and stigma branches and the simultaneous development of each pair of organs with most other Piperaceae and Saururaceae (Fig. 5D, although the two stamens in *Peperomia* as well as the second stamen pair in some *Piper* sometimes show disparity in size throughout development, Fig. 13B; Tucker 1980, 1982). The two stamens arise at the same position as the first two stamens in *Piper* and the only two stamens in *Peperomia* and a few other *Piper* species (Figs 7A–C and

**Fig. 11.** SEM images of pollen morphology of *Verhuellia lunaria*. (A) Pollen grain with evenly distributed isolated microechinate exine elements. Note that the channels are uniformly very narrow. (B) Pollen grain with artificially broad channels between the microechinate elements, mimicking a furrow-like aperture. (C) Detail of the pollen surface, with microechinate exine elements and a channel mimicking an aperture. (D) Detail of the pollen surface with a very narrow channel.

**Fig. 12.** TEM images of pollen ultrastructure of *Verhuellia lunaria*. (A) Pollen wall stratification: with ektexine (prominent tectum, short columellae, thin foot layer), meandering highly electron-dense endexine and medium-dense, evenly thin intine. (B) Pollen in cross-section. The intine (this is the layer below the highly electron-dense endexine) is meandering, however never distinctly thickened, thus indicating an inaperturate condition. cm, columella; en, endexine; fl, foot layer; in, intine; T, tectum.
The development of a flower with three stamens has not been observed, as only one mature flower with three stamens was captured (Fig. 6B). However, it can be assumed that this third adaxial stamen in the median sagittal plane does not develop exactly at the same time as the two lateral stamens, but slightly later, similar to the third stamen in *Houttuynia* (Saururaceae; Tucker, 1981) and in the dioecious species of *Piper* (Lei and Liang, 1998).
Liang, 1998), which occupies the same position in the flower. Similar to most Piper species, the anthers of Verhuellia are tetrasporangiate and dehisce latrrosely with longitudinal slits, whereas Peperomia anthers are bisporangiate and open extrorsely with longitudinal slits (Fig. 7B, C vs. Fig. 13A–D). The laterally extended connective is not present in Peperomia (Fig. 11C, D).

In Verhuellia, as well as in Zippelia, Piper and Saururaceae, the gynoeicum begins development as an annular structure that could be the result of a congenital fusion of several carpels. Another possible explanation could be a complete ontogenetic reorganization resulting in an ontogenetically acarpellate gynoeicum primordium. In both interpretations, the meristems from which the stigma branches develop can be considered to be carpel tips. In the second interpretation, the stigma primordia then can be seen as remnants of congenitally fused carpels. The upper part of the carpels can still be seen in the three or four rims on the fruit (Fig. 9A–D). In Peperomia, the ovary also originates from an annular primordium, but this can be understood as the ascidiate base of a single carpel (Fig. 13B; Tucker, 1980).

Similar to all other Piperaceae, the gynoeicum is superior and unilocular with a single basal ovule (Igersheim and Endress, 1998). As in all other perianthless Piperales studied, the ovule is orthotropous. The ovules of Verhuellia and Peperomia are characterized by a single integument, in contrast to the other representatives of the family which are bitectic (Igersheim and Endress, 1998). This can result in the formulation of two hypotheses about the origin and evolution of unitegmy and bitecty in Piperaceae: (1) The ovule of ancestral Piperales was unitegmic and bitectic evolved in the common ancestor of Zippelioideae and in Piper and underwent a reversal to unitegmy in Peperomia or (2) ancestral Piperales were characterized by bitectic ovules and unitegmy evolved independently in Verhuellia and Peperomia. Given that the outgroups to Piperales are bitectic, hypothesis 2, which assumes two origins of unitegmic ovules (two steps), is clearly more parsimonious than hypothesis 1, which requires one origin of unitegmic ovules at the base of the perianthless Piperales, followed by a reversal to bitecty in the common ancestor of Zippelioideae and Piperoideae, and another reversal to unitegmy in Peperomia (three steps).

In Verhuellia, a young gynoeicum shows some superficial similarities to that of Zippelia and Saururus, including the presence of four stigma branches and the verrucose surface of the outer wall (Fig. 9A–D). However, the protuberances on the ovary of Zippelia at anthesis develop into glochidiate hairs (Liang and Tucker, 1995), whereas the ovary of Saururus is characterized by warty ridges (Tucker, 1976), showing no similarity to the multicellular protuberances on the ovary wall of Verhuellia. Another character shared by Verhuellia, Zippelia and Saururus is the four recurved free papillate stigmas.

Large multicellular protuberances on the fruit wall of Verhuellia were also observed by Schmitz (1872b) and are unique for the genus. The fruit wall of many Peperomia species, in contrast, is also provided with protuberances, but these are sticky and unicellular papillae. It can be supposed that the protuberances have a function in epizoochory, but histochemical studies and detailed field observations are required to give conclusive statements about this.

Pollen of Verhuellia resembles Peperomia pollen in being inaperturate and very small. However, Verhuellia pollen is distinctively characterized by its ornamentation: evenly distributed, uniquely formed exine elements consisting of more-or-less hemispherical microechinate aggregates (Fig. 11A–D, resembling mace-heads, the medieval cutting weapons). Pollen of Peperomia is characterized by a less conspicuous ornamentation with unevenly shaped verrucate with few, tiny microechini (Fig. 14A, B). The inaperturate pollen of Verhuellia and Peperomia contrasts with the sulcate pollen in other perianthless Piperales (see Table 1). Remarkably, pollen of the genus Aristolochia, another member of the Piperales, is also inaperturate, whereas the pollen of its closest relatives are (mono)sulcate (Asaroidae, Hydnoraceae and Lactoris). Hence, parallel to the origin and evolution of unitegmy in Piperaceae, two hypotheses about the occurrence of inaperturate pollen in Piperales can be made: (1) Ancestral Piperales pollen was inaperturate and the various aperturate morphologies have evolved in the different groups and (2) ancestral Piperales had a particular type of aperturate pollen and inaperturate pollen and the various aperturate pollen types developed in the different Piperales lineages. The interpretation of their evolution is somewhat uncertain because of uncertainty on the positions of Lactoris and Hydnoraceae, but, given the sulcate condition in Canellales,

![Fig. 14. SEM images of pollen morphology of Peperomia rubella. (A) Pollen grain with verrucate ornamentation; the verrucae are covered with few, tiny microechini. (B) Close-up the of pollen surface. Note the different size of verrucae.](https://academic.oup.com/aob/article-abstract/105/5/677/179683/686)
Saururaceae and most Piperaceae, hypothesis 2 is probably much more parsimonious than loss of the sulcus at the base of Piperales followed by several reappearances within the order. In basal magnoliids inaperturate pollen is infrequent, however not rare; beside the mentioned examples it is found also in, for example, Laurales (Furness et al., 2002). Apart from the difference in pollen ornamentation, Verhuellia also contrasts with Peperomia in the presence of oribules. Very inconspicuous oribules have been observed on the tapetum of Verhuellia whereas oribules are not known from Peperomia.

Finally, the very distinct ornamentation of Verhuellia pollen allows comparison with presumed fossil members of Piperales. According to Friis et al. (2006), Appomattoxoa anciestropha is most closely related to members of Piperales. However, Doyle and Endress (2010) state in a phylogenetic study of a morphological data set for extant basal angiosperms to assess the relative parsimony of placements of early angiosperm fossils that Appomattoxoa is more parsimoniously placed near Chloranthaceae or Amborella than in Piperales. Smith and Stockey (2007) already showed that Appomattoxoa pollen differs from Saururaceae pollen. Pollen of *A. anciestropha* also differs from *Verhuellia* pollen in dimension, aperture condition, pollen wall stratification and pollen ornamentation (Friis et al., 1995). It is much larger (16–19 μm), it is sulcate (*Verhuellia* pollen is inaperturate), it has a thick foot layer and a continuous tectum (*Verhuellia* shows a thin foot layer and a strictly discontinuous tectum) and its ornamentation is verrucose–rugulate and microechinate.

To conclude, observations on the morphology, anatomy and development of inflorescence, flower, pollen and fruit of *V. lunaria* confirm that the genus is a clearly distinct lineage within Piperales. It does not show any intermediate characters between Saururaceae and Piperales and there are only superficial similarities with any other specific group within perianthless Piperales. The unitegmic ovule and the inaperturate pollen of Verhuellia and *Peperomia* appear to be either an independent parallel evolution or a reversal to the ancestral state. *Appomattoxoa anciestropha* pollen is different from the pollen of *Verhuellia*.

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**LITERATURE CITED**


