



SEM observations on seeds of some herbaceous *Phyllanthus* L. species (Phyllanthaceae)

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RESUMO: “Observações de sementes de algumas espécies herbáceas de *Phyllanthus* L. (Phyllanthaceae) em MEV”. Seis espécies herbáceas de *Phyllanthus* L. (*P. tenellus* Roxb., *P. niruri* L., *P. stipulatus* (Raf.) Webster, *P. urinaria* L., *P. caroliniensis* Walt. e *P. amarus* Schum. & Thonn.) mencionadas como medicinais apresentam dificuldades na identificação. Com o objetivo de auxiliar neste problema e determinar diferenças entre as espécies, o padrão de ornamentação das sementes foi estudado utilizando técnicas de microscopia eletrônica de varredura (MEV). As sementes apresentaram ornamentação distinta, sendo observadas as superfícies laterais, dorsais e ventrais. As espécies puderam ser separadas em três tipos de ornamentação, que demonstraram importância como caractere diagnóstico para a sua separação.

Unitermos: *Phyllanthus*, Phyllanthaceae, ornamentação da superfície da semente, microscopia eletrônica de varredura (MEV).

ABSTRACT: Six medicinal herbaceous *Phyllanthus* L. (Phyllanthaceae) species, *P. tenellus* Roxb., *P. niruri* L., *P. stipulatus* (Raf.) Webster, *P. urinaria* L., *P. caroliniensis* Walt., and *P. amarus* Schum. & Thonn. are difficult to distinguish. In order to solve this problem, the ornamentation of the seed surfaces was analyzed by SEM (scanning electron microscopy). Differences between lateral, dorsal and ventral surfaces were observed. The seeds were cut into sections at different angles, exposing testa sculptures. Three types of surface ornamentation patterns were observed and they showed to be valuable diagnostic.

Keywords: *Phyllanthus*, Phyllanthaceae, seed ornamentation, scanning electron microscopy (SEM).

INTRODUCTION

The genus *Phyllanthus* L. has a pan-tropical distribution, with over 800 described species of trees, shrubs, and herbs (Webster; Carpenter, 2002). According to Judd et al. (2002), APG II (2003), and Samuel et al. (2005) *Phyllanthus* genus should be now reported as belonging to the Phyllanthaceae family, instead to the former subfamily Phyllanthoideae of the Euphorbiaceae. *Phyllanthus* species are largely used in Brazilian traditional or folk medicine as a diuretic and are also indicated in the treatment of jaundice, hepatitis, kidney stones and diabetes (Oliveira; Saito, 1987/1989; Calixto et al., 1997; Calixto et al., 1998).

This complex genus is considered to have at least ten subgenera (Webster, 1967). It is difficult to identify the species correctly, such as the case of *P. niruri* and *P. amarus* (Unander et al., 1990; 1991; 1992), and

to establish which of the species has useful medicinal properties with the available ethno-botanical and ethno-pharmacological data. In herbaceous *Phyllanthus*, floral characters have limited utility for the identification of species and supra-specific taxa, due to the small size of the flowers and the relative narrow range of variation in number and shape of parts, but it is unquestionable that the mean size and the ornamentation pattern of seeds tend to be the best diagnostic (Webster, 1970; Silva; Sales, 2004). Based on a literature revision, the present work focuses on some medicinal herbaceous *Phyllanthus* species occurring in Brazil known as “quebra-pedra” (Smith; Downs, 1959; Smith et al., 1988; Falcão et al., 2005; Barbosa-Filho et al., 2005) in order to study the surface ornamentation patterns of *P. amarus* Schum. & Thonn., *P. caroliniensis* Walt., *P. niruri* L., *P. stipulatus* (Raf.) Webster, *P. tenellus* Roxb., and *P. urinaria* L. seeds. The infra-generic classification of the six *Phyllanthus*

Table 1. Infrageneric classification of six herbaceous *Phyllanthus* species, according to Webster (1967; 1970; 2002) focused on seed SEM analyses.

Subgenera	Section	Subsection	Species
<i>Isocladius</i> Webster	<i>Loxopodium</i> Webster (1967; 1970)		<i>P. caroliniensis</i> Walt.
<i>Kirganelia</i> (Juss.) Webster	<i>Pentandra</i> Webster (1967; 1970)		<i>P. tenellus</i> Roxb.
<i>Phyllanthus</i>	<i>Phyllanthus</i> (1967; 1970; 2002)	<i>Niruri</i> Webster (1967; 1970; 2002)	<i>P. niruri</i> L.
		<i>Swartziani</i> Webster (1967; 1970; 2002)	<i>P. amarus</i> Schum. & Thonn.
		(1967; 2002)	<i>P. stipulatus</i> (Raf.) Webster
	<i>Urinaria</i> Webster (1967; 1970)		<i>P. urinaria</i> L.

species listed below (Table 1) is based on Webster (1967; 1970; 2002).

Chen and Wu (1997) compared SEM photographs of pollen grains and seed surface ornamentation of herbaceous *Phyllanthus* species in Taiwan. They studied *P. amarus* Schum. & Thonn., *P. tenellus* Roxb., *P. urinaria* L. subsp. *nudicarpus* Rossign. & Haic., reviewed in this paper, and other five species. Also Silva and Sales (2004) studied some species with a taxonomic approach, with short seed descriptions and illustrations.

MATERIAL AND METHODS

Seeds of *Phyllanthus amarus*, *P. caroliniensis*, *P. niruri*, *P. tenellus*, and *P. urinaria* were kindly provided by the experimental station CPQBA-UNICAMP (courtesy of Prof. Dr. Pedro Melillo de Magalhães, Campinas-SP, Brazil), and were also collected from the field at different locations in Brazil. The seeds of *P. stipulatus* were collected from the field. The vouchers are deposited at ICN herbarium, UFRGS (Brazil) (Table 2). Five mature seeds of each species were air dried and selected for the exposure of ventral, lateral, and dorsal views, and divided into sections to verify ornamentation pattern on longitudinal or transversal view (Chen; Wu, 1997). They were mounted on stubs with double-sided carbon adhesive tape. The specimens were gold coated (20-25 nm), post-coated with carbon on ion sputter coater (Bal-Tec SCD 005), and observed under XL-30 Philips Scanning Electron Microscope at 15 kV accelerating voltage. The terminology used for surface patterns was based on Barthlott (1990) and Barthlott et al. (1998) and adapted when needed. In order to determine the

terminology of ornamentation pattern of *P. amarus* and *P. stipulatus*, some sculptures on transversal sections mounted on stubs were broken. The SEM images showed a massive core. According Barthlott *et al.* (1998), the best word for this structure is "rodlet".

RESULTS AND DISCUSSION

The six studied species of Brazilian herbaceous *Phyllanthus*, all seeds were wedge-shaped (trigonus) (Burger; Huft, 1995), and two seeds were present in each locus of the capsular fruits. Through micromorphologic examination of each seed coat, three types of rib disposition on dorsal and ventral face were recognized. The seed descriptions and illustrations of Silva and Sales (2004) for *P. amarus*, *P. caroliniensis*, *P. niruri*, *P. stipulatus* and *P. tenellus* can not be compared with the SEM micromorphology described in Table 2 because of the lower magnification used.

The seed coat on dorsal face of the first type group presented longitudinal ribs with transversal finger-shaped rodlets. In *P. amarus* (Table 2; Figs 1-6), the seed coat had 6-9 dorsal regular longitudinal ribs, where the rodlet end forms the ribs. The same pattern was recognized in *P. stipulatus* (Table 2, Figs 7-13), but the finger-shaped rodlets were irregularly arranged in different sizes, forming 11-15 irregular dorsal longitudinal ribs, with spaces between the fingers-shaped rodlets. The lateral and ventral faces results are on Table 2, and the dates (with Figs. 1-6) showed that the seed ornamentation is consistent with Chen and Wu (1997) studies to *P. amarus*.

In the second type group, the seed coat on

Table 2. Micromorphology of seeds of some herbaceous *Phyllanthus* species.

Taxa <i>Phyllanthus</i> L.	Seed ornamentation		
	dorsal	lateral	Ventral
<i>P. amarus</i> Schum. & Thonn. (ICN 123978; ICN 123979; ICN 123980; ICN 123981) *	Regular longitudinal ribs regular finger-shape transversal rodlets	Regular or irregular concentric C-shape ribs, regular longitudinal finger-shape rodlets	Regular or irregular concentric C-shape ribs, regular longitudinal finger-shape rodlets
<i>P. caroliniensis</i> Walt. (ICN 123971; ICN 123972; ICN 123973) *	Stellate verrucose aligned on a regular longitudinal ribs, with crusts	Stellate verrucose regular or irregular concentric C- shape alignment, with crusts	Stellate verrucose aligned on a regular or irregular concentric C- shape ribs, with crusts
<i>P. niruri</i> L. (ICN 123950; ICN 123951; ICN 123952; ICN 123953; ICN 123955) *	Stellate verrucose aligned on a regular or irregular longitudinal ribs, with crusts	Stellate verrucose regular concentric C-shape alignment, with crusts	Stellate verrucose aligned on a regular concentric C-shape ribs, with crusts
<i>P. stipulatus</i> (Raf.) Webster (ICN 123974; ICN 123975; ICN 123976) *	Regular or irregular longitudinal ribs, regular finger-shape, transversal rodlets with spaces	Regular or irregular concentric C- shape ribs regular longitudinal finger-shaped rodlets	Regular or irregular concentric C- shape ribs, regular longitudinal finger-shaped rodlets
<i>P. tenellus</i> Roxb. (ICN 123964; ICN 123965; ICN 123966) *	Stellate verrucose aligned on a irregular or regular longitudinal ribs, with crusts	Verrucose irregular or regular concentric C- shape alignment, with crusts	Verrucose aligned on a irregular or regular concentric C-shape ribs, with crusts
<i>P. urinaria</i> L. (ICN 123970) *	Transversal ribs, microverrucose on ribs, crusts on valleys	Asymmetrical longitudinal ribs, microverrucose on ribs, crusts on valleys	Asymmetrical longitudinal ribs, microverrucose on ribs, crusts on valleys

* Herbarium ICN (UFRGS, Porto Alegre, Brazil)

dorsal face presented longitudinal ribs formed by stellate verrucose ornamentation in longitudinal arrangement. In *P. caroliniensis* (Table 2, Figs 14-20), the seed coat presented a regular pattern of about 10 longitudinal ribs, and the verrucose coat was lost. The verrucose coat and the crust were easily detachable on dorsal and lateral faces. *P. niruri* (Table 2) presented regular longitudinal and concentric ribs on dorsal and lateral faces (Figs 23-26). However, in some seeds, these ribs were occasionally irregular (Figs 21, 22). *P. tenellus* (Table 2, Figs 27-32) usually had irregular longitudinal and concentric alignment ribs, with less prominent verrucose ornamentation, which look like a fried egg (Figs 29 and 30), particularly on the lateral face.

The third type was represented by *P. urinaria* (Table 2, Figs 33-38). The seed coat showed transversal ribs on the dorsal face, and longitudinal ribs on the lateral face. This is a typical shape, which was already mentioned by Chen and Wu (1997), bearing symmetrical or asymmetrical ornamentation.

Webster (1956) mentioned a curious and unique kind of seed ornamentation in *P. urinaria*, as well as the presence of deep pits on the sides of transverse ridges on

radial walls. Dot-shaped microverrucose ornamentation was found on the ribs, and crust waxes on dorsal and ventral faces (Figs 34-36). They appear as a deep pit due of their black color. Crusts were mainly found in valleys. The transversal section of all seeds shows pits on the seed coat, and in *P. stipulatus* (Fig 39), it is easy to see several pits inside the cell wall.

A partial relationship between Webster's (1956; 1967; 1970) infra-generic classification and the three types of ornamentation observed can be implied. The first type of seed ornamentation was found in three distinct subgenera (*Isocladus*, *Kirganelia* and *Phyllanthus*). However, in the last subgenus (*Phyllanthus*), three types of ornamentation for three species studied were observed, showing the importance and the need of studies on more species of the genus.

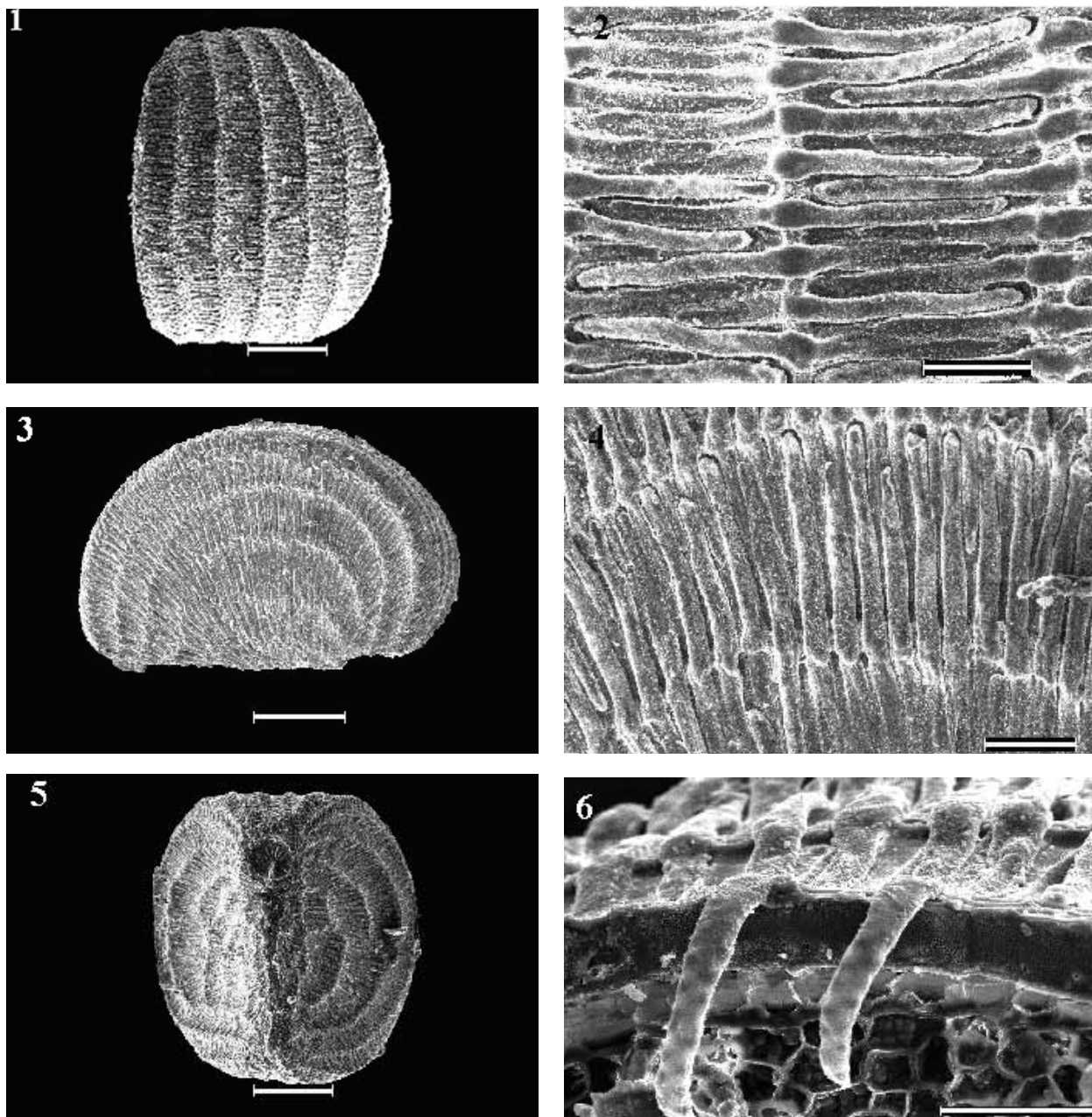
There is considerable micromorphological diversity among seeds of herbaceous *Phyllanthus* species. Our observations reinforce the results of previous studies. A new image on seed of the pits in the seed coat, never published before, was taken. These results, as well as those of Chen and Wu (1997), emphasize the importance of SEM in seeds studies, and underscore the need of

more extensive use of this technique in taxonomic classifications in Phyllanthaceae.

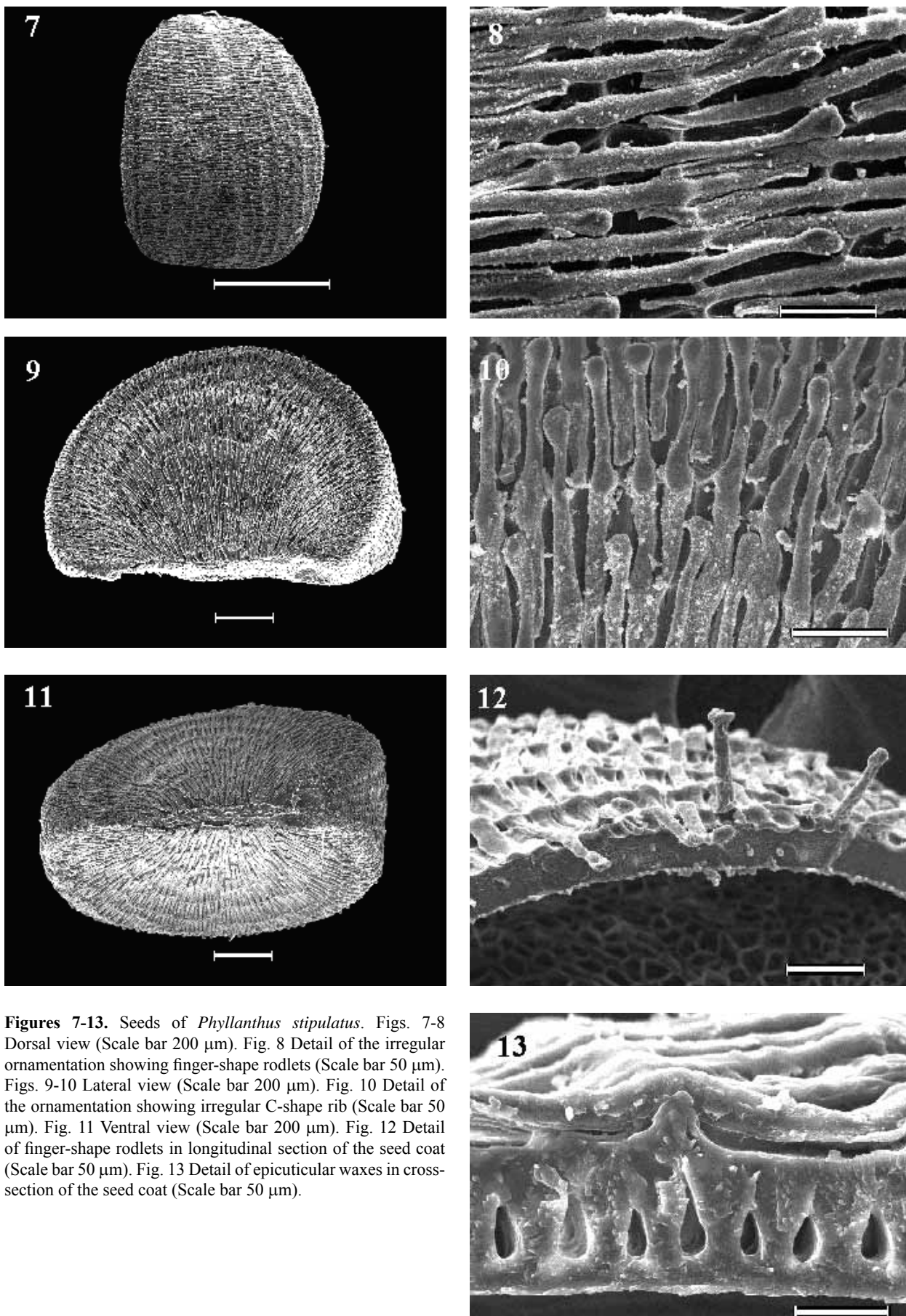
Phyllanthus niruri and *P. tenellus* were published in the Brazilian Pharmacopoeia (F. Bras., 2004). The data found in this study allows the differentiation of these herbaceous species from others, and can be used as a diagnostic trait in the assessment of the authenticity of plant raw materials.

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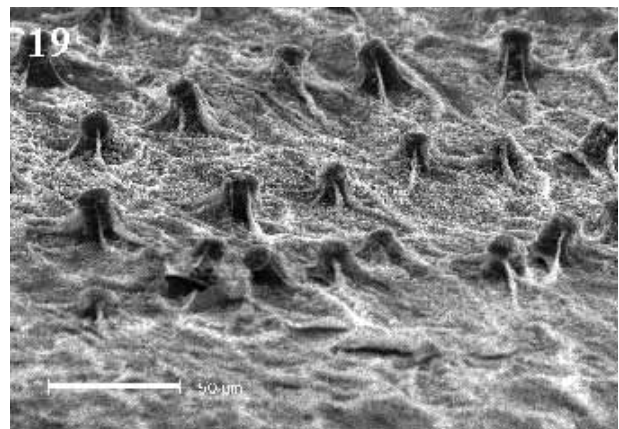
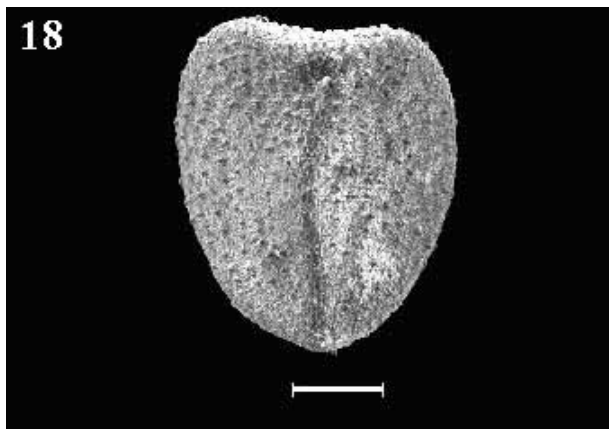
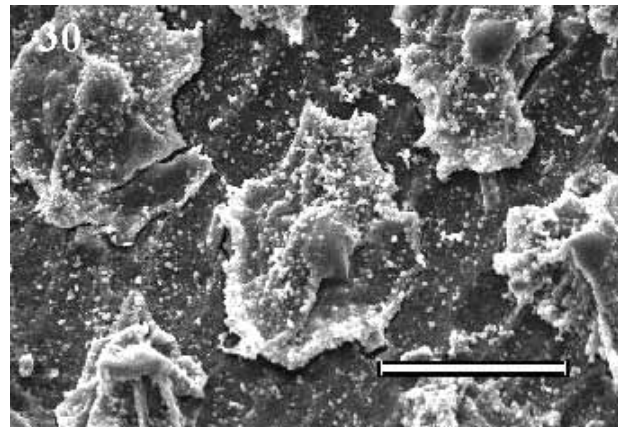
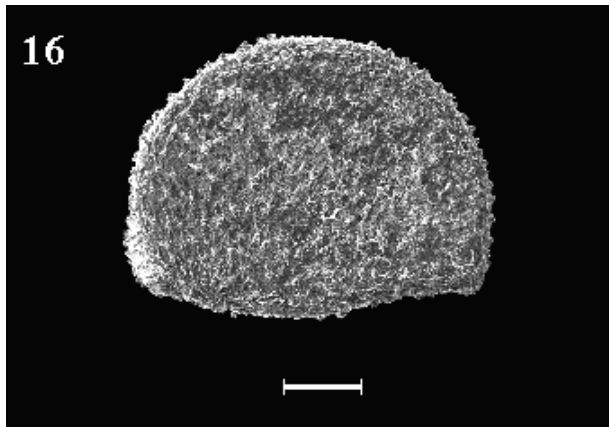
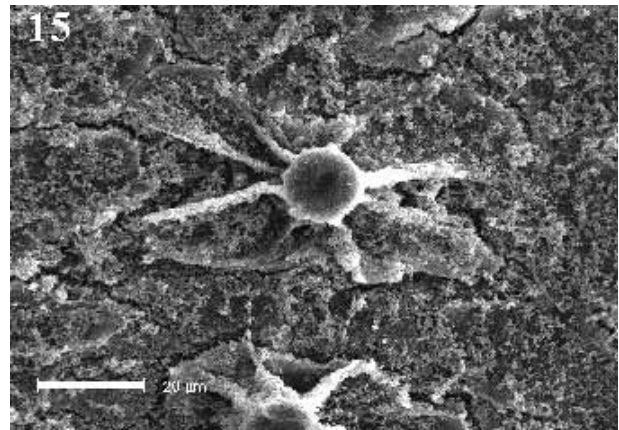
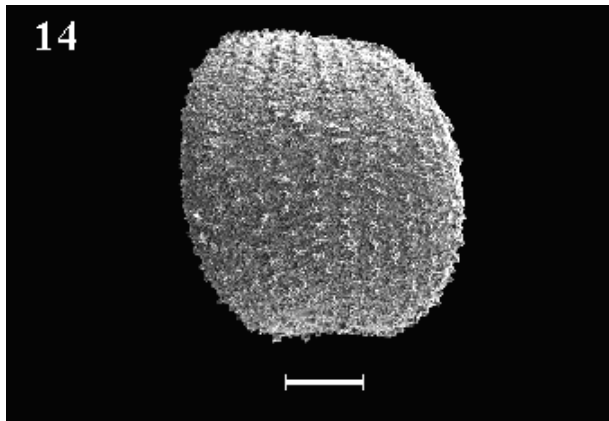
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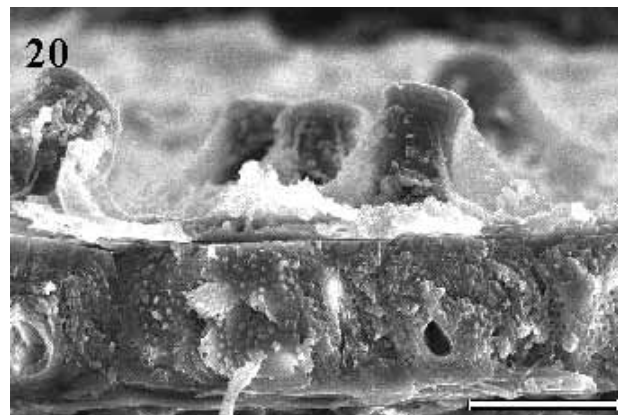
Figures 1-6. Seeds of *Phyllanthus amarus*. Figs. 1-2 Dorsal view (Scale bar 200 μ m). Fig. 2 Detail of the ornamentation showing finger-shape rodlets forming ribs (Scale bar 50 μ m). Figs. 3-4 Lateral view (Scale bar 200 μ m). Fig. 4 Detail of the ornamentation on lateral face (Scale bar 50 μ m). Fig. 5 Ventral view (Scale bar 200 μ m). Fig. 6 Longitudinal section showing epicuticular waxes of rodlets type (Scale bar 50 μ m).

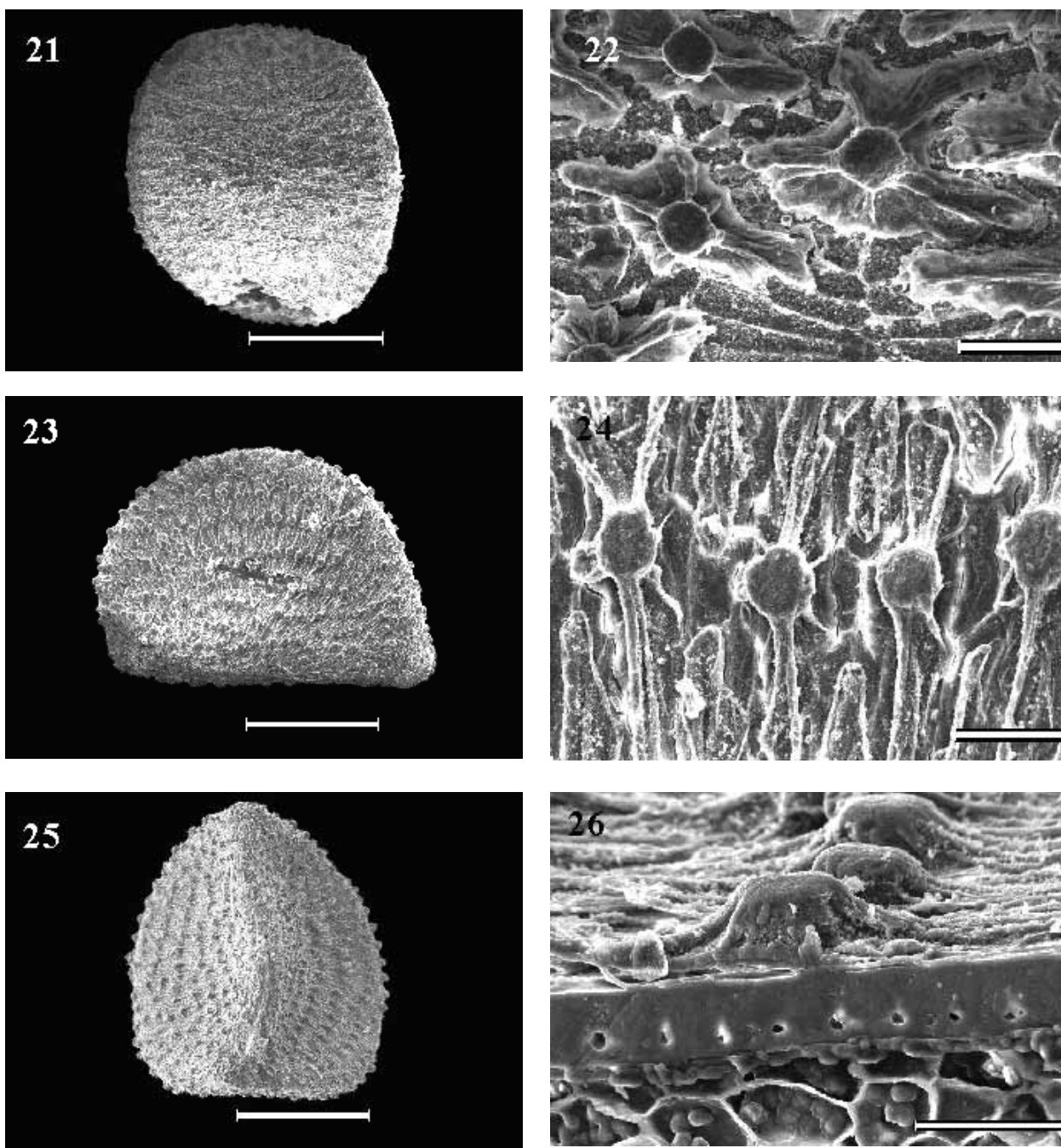


Figures 7-13. Seeds of *Phyllanthus stipulatus*. Figs. 7-8 Dorsal view (Scale bar 200 μm). Fig. 8 Detail of the irregular ornamentation showing finger-shape rodlets (Scale bar 50 μm). Figs. 9-10 Lateral view (Scale bar 200 μm). Fig. 10 Detail of the ornamentation showing irregular C-shape rib (Scale bar 50 μm). Fig. 11 Ventral view (Scale bar 200 μm). Fig. 12 Detail of finger-shape rodlets in longitudinal section of the seed coat (Scale bar 50 μm). Fig. 13 Detail of epicuticular waxes in cross-section of the seed coat (Scale bar 50 μm).

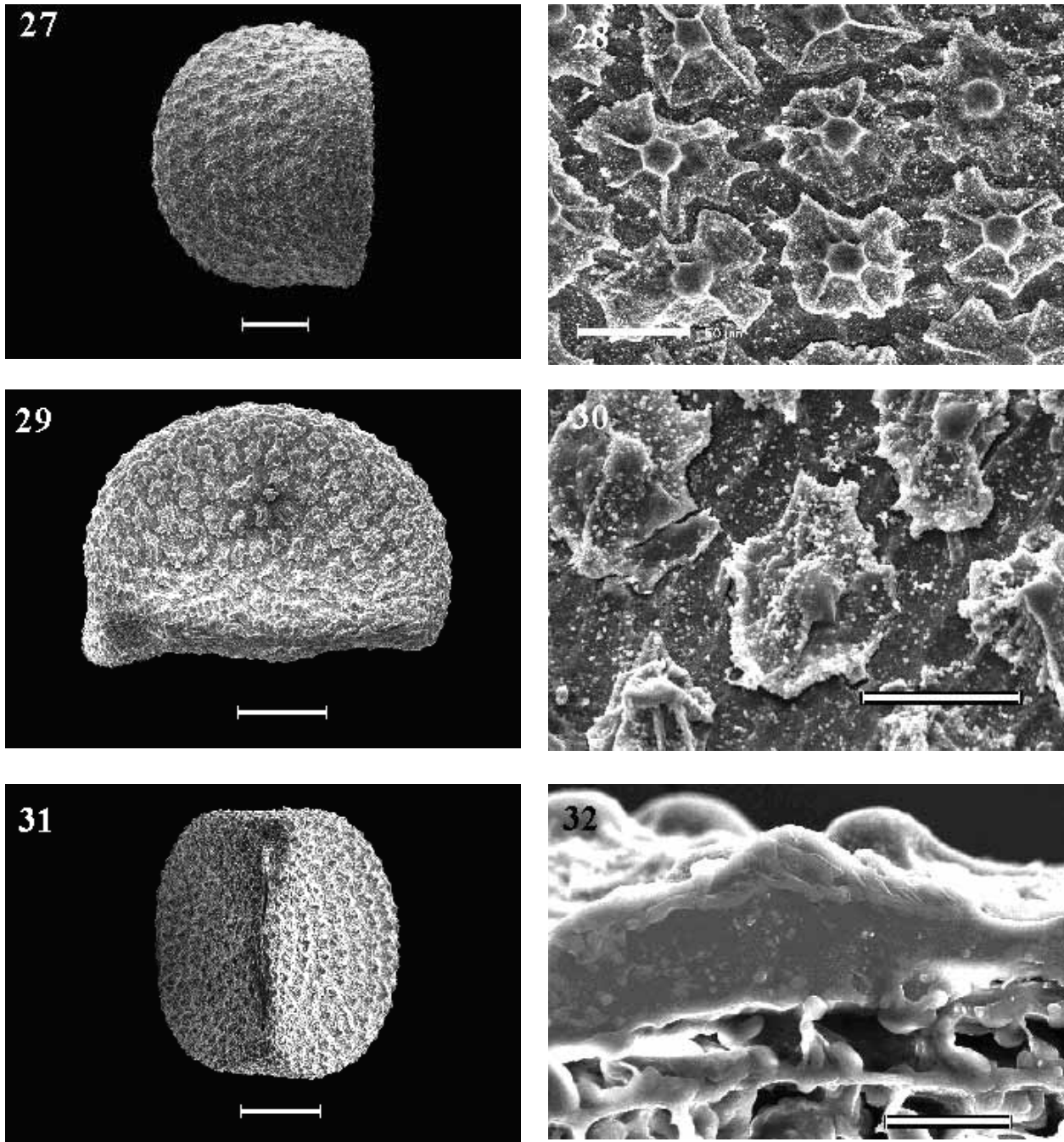


Figures 14-20. Seeds of *Phyllanthus caroliniensis*. Figs. 14-15 Dorsal view (Scale bar 200 µm). Fig. 15 Detail of the stellate verrucose ornamentation (Scale bar 50 µm). Figs. 16-17 Lateral view (Scale bar 200 µm). Fig. 17 Detail of irregular ornamentation showing stellate verrucose forms with crusts (Scale bar 50 µm). Fig. 18 Ventral view. Note regular concentric C-shape alignment of stellate verrucose (Scale bar 200 µm). Fig. 19 Detail of stellate verrucose ornamentation on ventral view (Scale bar 50 µm). Fig. 20 Detail of stellate verrucose ornamentation in cross-section of seed coat (Scale bar 20 µm).

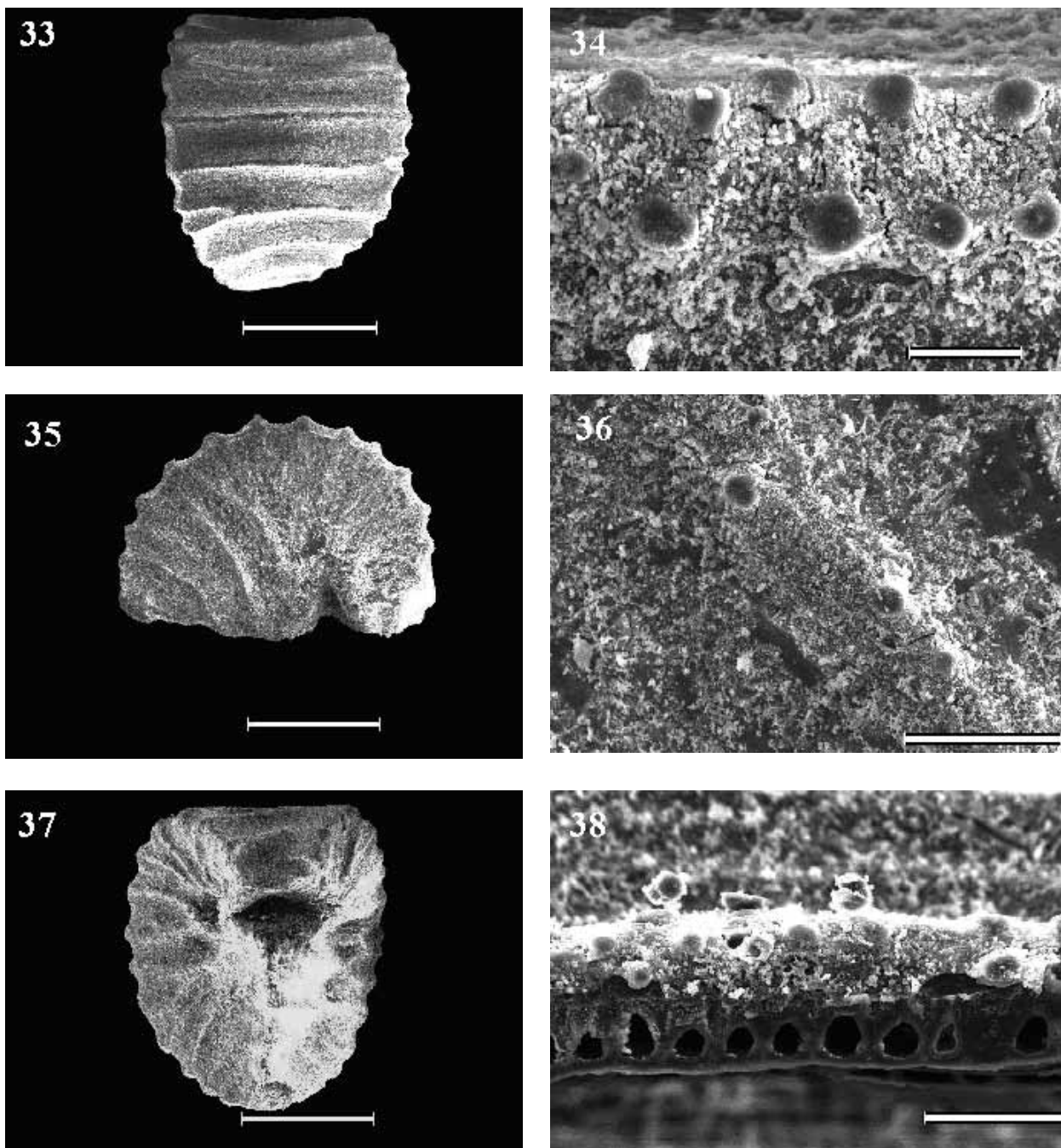




Figures 21-26. Seeds of *Phyllanthus niruri*. Figs. 21-22 Dorsal view (Scale bar 500 µm). Fig. 22 Detail of the stellate verrucose ornamentation (Scale bar 50 µm). Figs. 23-24 Lateral view (Scale bar 500 µm). Fig. 24 Detail of regular stellate verrucose alignment (Scale bar 50 µm). Fig. 25 Ventral view. Note regular concentric C-shape alignment of stellate verrucose ornamentation (Scale bar 500 µm). Fig. 26 Detail of stellate verrucose ornamentation in cross-section of seed coat (Scale bar 50 µm).



Figures 27-32. Seeds of *Phyllanthus tenellus*. Figs. 27-28 Dorsal view (Scale bar 200 μm). Fig. 28 Detail of the stellate verrucose ornamentation (Scale bar 50 μm). Figs. 29-30 Lateral view (Scale bar 200 μm). Fig. 30 Detail of verrucose ornamentation on dorsal face (Scale bar 50 μm). Fig. 31 Ventral view. Note regular concentric C-shape alignment of the verrucose ornamentation (Scale bar 200 μm). Fig. 32 Detail of verrucose ornamentation in cross-section of seed coat (Scale bar 50 μm).



Figures 33-38. Seeds of *Phyllanthus urinaria*. Figs. 33-34 Dorsal view (Scale bar 500 μm). Fig. 34 Detail of the ribs showing crusts and microverrucose ornamentation (Scale bar 50 μm). Figs. 35-36 Lateral view (Scale bar 500 μm). Fig. 36 Detail of longitudinal rib with crusts and microverrucose ornamentation (Scale bar 50 μm). Fig. 37 Ventral view. Note asymmetrical distribution of the ribs (Scale bar 500 μm). Fig. 38 Detail of epicuticular waxes in cross-section of seed coat (Scale bar 50 μm).

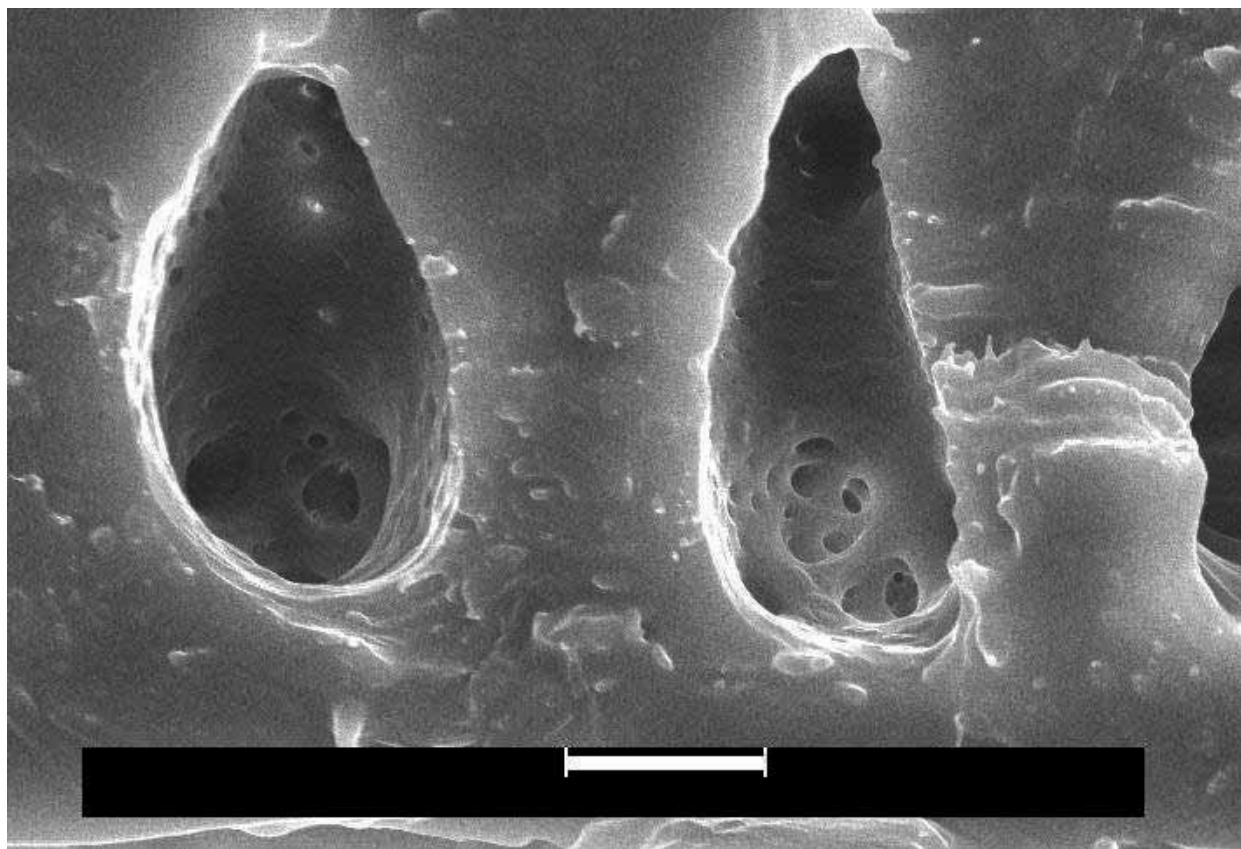


Figure 39. Cross-section of *P. stipulatus* seed. Note pits in the cell wall of the seed coat (Scale bar 5 μ m).

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