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Fruit ontogeny of *Garcinia gardneriana* (Planch. & Triana) Zappi (Clusiaceae)[1]

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RESUMO

(Ontogênese do fruto de *Garcinia gardneriana* (Planch. & Triana) Zappi (Clusiaceae)). *Garcinia gardneriana* (Clusiaceae) é árvore de pequeno a médio porte frequente na Planície de Inundação do Alto Rio Paraná e de importância na alimentação das populações ribeirinhas, sendo consumida *in natura* e na forma de sucos e doces. O objetivo do presente estudo foi descrever aspectos morfológicos e estruturais dos frutos e sementes dessa espécie como contribuição à classificação do tipo de fruto e da porção carnosa e comestível que envolve as sementes. Ovários e frutos, em diferentes estádios de desenvolvimento foram coletados de cinco espécimes de uma população localizada na ilha do Aurélio, rio Baía (MS, BR) e processados segundo técnicas usuais em anatomia vegetal. O fruto é bacóide, esférico, de coloração amarelo-alaranjado. A casca é lisa e coriácea, constituída pelo exocarpo lignificado. O endocarpo é derivado da atividade de um meristema adaxial, cujas células alongam-se radialmente. A polpa tem coloração branca, comestível, de sabor doce, de origem principalmente endocárpica, mas é constituída também por parte do mesocarpo esponjoso, cujos ductos perdem a funcionalidade. Os óvulos anátropos e unitegumentados originam sementes também anátropas, exalbuminosas, com testa colapsada. O embrião é hipocotilar. Os caracteres aqui descritos de maneira geral são comuns a Clusioideae-Garcinieae e Symphonieae.

Palavras-chave: anatomia, Guttiferae, morfologia, pericarpo, semente

ABSTRACT

(Fruit ontogeny of *Garcinia gardneriana* (Planch. & Triana) Zappi (Clusiaceae)). *Garcinia gardneriana* (Clusiaceae) is a small to medium-sized tree that usually occurs on the floodplains of the Paraná River and it is an important food source for the local inhabitants. The fruit itself is consumed *in natura*, and juices and sweets are made from it. The purpose of this study was to describe morphological and structural aspects of fruits and seeds of this species in order to classify the fruit type and the pulpy layer which involves the seeds. The material analyzed consisted of ovary and fruits in different stages of development, collected from five plants from Aurélio Island, Baía River (MS, BR) and the description was done according to standard procedures in anatomy. The fruit is a yellowish-orange spherical berry ("bacóide" type). The smooth, coriaceous skin consists of the lignified exocarp. The endocarp cell layers are derived from the activity of an adaxial meristem that undergoes radial elongation. The edible sweet white pulp is formed by the endocarp, as well as the spongy mesocarp, in which the secretory ducts lose their function. The unitegmic anatropous ovules develop into anatropous exalbuminous seeds with a collapsed and undifferentiated testa. The embryo is hypocotylar. The features here described generally occur in Clusioideae-Garcinieae and Symphonieae.

Key words: anatomy, Guttiferae, morphology, pericarp, seed

Introduction

Garcinia L. (Clusiaceae – Clusioideae - Garcinieae) is a pantropical genus comprising approximately 500 species, most of which are found in the Indo-Malaysian region. The Neotropical species were often included in the *Rheedia* L genus, although lately they have been considered a synonym of *Garcinia* (Bittrich 2003).

Garcinia gardneriana (Planch. & Triana) Zapp, known as "abricó-do-mato", "bacupari", "pacori", "bacopari" (Berg 1979) or "limãozinho", is consumed by humans living on the floodplain of the Alto Paraná River (Porto Rico, Paraná state, Brazil) in the form of juice or sweets, or *in natura* (Pagotto & Souza, unpublished data). This species is widely distributed from the states of Amazônia to Rio Grande do Sul and it is found in the Atlantic Forest, the transition areas of Atlantic

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Forest-*restinga* (shore vegetation), the high plains forest, as well as in the riparian forest. It has an arboreal or shrubby habit, growing up to 10m tall, flowers from August to January, and produces fruits from November until February. The fruits are commonly eaten by the Brown Capuchin Monkey and "cutia" rodents (Bittrich 2003).

Vegetative and floral characters have significant diagnostic value in the Clusiaceae, mainly at the generic and species level, and can be utilized to delimit subfamilies and tribes when combined with characteristics of the fruits and seeds (Engler 1888; Brandza 1908; Guillaumin 1910; Engler & Keller 1925; Hutchinson 1959; Cronquist 1981; Heywood 1985; Takhtajan 1997). Detailed ontogenic studies of the fruits and seeds of Clusiaceae, however, have showed controversies in terms of the classification of the fruits, as well as doubts about the position of the species in the subfamilies and tribes as discussed by Mourão & Beltrati (1995ab; 2000; 2001) and have thus not contributed significantly to recent discussions about infrafamily relationships (Gustafsson *et al.* 2002; Stevens 2007a,b, 2008).

Corner (1976) pointed out that there is significant structural diversity among the seeds of the different species of this family and noted that all of the genera and most of the species needed to be more closely examined, mainly due to the difficulty in obtaining seeds in different stages of development. This author described the seeds of *Garcinia mangostana* L., *Garcinia xanthochymus* Hook f., and *Garcinia* sp, and noted that they seemed to be superficially quite similar and therefore required more detailed examination, although many differences were noted in terms of the pericarp structure, particularly the endocarp.

In addition, relevant aspects regarding the differences between these species is the origin of the structures that aid their dispersal. Pijl (1982) reported that the pulp and the sarcotesta of *Garcinia* and *Symphonia* L.f. formed an edible layer. However, in a study of the seeds and germination of *Garcinia mangostana*, Sprecher (1919) noted that the pulp arises only from the endocarp (to which the seed integument is attached), being very similar to *Platonia insignis* Mart. and *Mammea americana* L. as reported by Mourão & Beltrati (1995ab; 2000).

This work presents data concerning the ontogenesis of the pericarp and seed coat of *Garcinia gardneriana* that broadens our knowledge of these organs in the Clusiaceae, and contributes to ecological and taxonomic studies of the group, mainly in terms of subfamily and tribe classification.

Materials and methods

Ovaries and fruits of *Garcinia gardneriana* in different stages of development were collected from five specimens of a population growing on Aurélio Island in the Baía River (22°41'S x 53°14' W) within the floodplain of the Alto Paraná River, Paraná state, Brazil. Reference samples were incorporated into the Universidade Estadual de Maringá herbarium (HUEM) (registration number 14504).

Morphological and anatomical studies were carried out using both fresh and fixed materials. The fruits were measured and separated into size classes characterizing their development stages, identical to those used in describing seed development. The samples used for morphological and anatomical studies were fixed in FAA 50 (Johansen 1940) and subsequently conserved in 70% ethanol (Jensen 1962).

Samples used for anatomical studies of the pericarp and developing seeds were analyzed in transversal, longitudinal, and paradermal sections that were cut either freehand or using a rotary microtome. Two different techniques were used in order to obtain permanent slides. In the first, they were prepared by dehydrating the fixed samples in an ethanol series followed by a xylene series, embedding in paraffin (Sass 1951), stained with safranin and Astra Blue (Gerlach 1969). In a second approach, metacrylate (Leica®) was used for embedding and preparation of the permanent slides, following the instructions of the manufacturer, and these sections were stained with 0.05% toluidine Blue in pH 4.7 acetate buffer (O'Brien *et al.* 1964). In both techniques the slides were mounted in Permount. Histochemical tests were performed to detect different substances: phloroglucinol with hydrochloric acid to detect lignin (Sass 1951); Sudan IV to detect lipidic substances; Lugol for starch detection; iron chlorate with sodium carbonate to detect phenolic compounds (Johansen 1940).

The morphology illustrations of the seeds were made using a *camera lucida* apparatus adapted to a Leica Wild M32 stereomicroscope. Pericarp and seed anatomy was documented with photomicrographs obtained by using a digital camera coupled to an Olympus BX 50 optical microscope and processed with Image Pro-Plus version 4.0 software (Media Cybernetics®).

A pachymeter was used to measure fruit and seed length and diameter, and fruit-type classification was performed according to Barroso *et al.* (1999). The terminology to define the pericarp layers was based on Roth (1977); the nomenclature used to describe the seeds was that of Corner (1976); and the infrafamily classification used in the discussion section is that of Stevens (2007a,b; 2008).

Results

Fruit development

The superior ovary in *Garcinia gardneriana* is tricarpelar, trilocular at its base and median region (Fig. 1), becoming unilocular near the apex. There is only one ovule per locule, which is erect, with axial placentation (Fig. 1, 15).

The outer ovarian epidermis is uniseriate and composed of cubical cells with periclinal external cell walls that are somewhat thickened and covered by a cuticle. The mesophyll is multi-stratified and parenchymal and it is divided into two regions, a larger external region that is penetrated mainly longitudinally, by numerous secretory ducts and collateral vascular bundles, both of which are ramified, with the diameter of the ducts increasing gradually from the periphery towards the center. An internal region, which has fewer layers and no secretory ducts, is delimited by vascular bundles with larger diameter (Fig. 2-5). Crystalliferous idioblasts containing druses can be observed dispersed throughout the ovarian mesophyll. The inner epidermis that delimits the locule is also uniseriate and composed of cubical cells with thin cell walls. An adaxial meristem was observed within the first subepidermal layer and cell divisions can be observed throughout the entire ovarian mesophyll (Fig. 5).

Increasing numbers of cell layers were observed in the immature fruit, measuring 3.0 cm in length by 2.5 cm in diameter, as well as significant cellular expansion. Alterations occur in the exocarp and in the subepidermal layers of the adaxial epidermis during this phase, and their component cells have radial elongation (Fig. 6-7).

The developed but still immature fruits and the mature fruits measure 4.0 cm in length by 3.8 cm in diameter. In these phases there is accentuated thickening of the anticlinal and outer periclinal cell walls of the exocarp cells, with subsequent lignification. In the mesocarp, a tangential elongation in the first five or so layers was observed and in the mature fruit these layers were slightly collapsed (Fig. 6, 8, 10). The ducts lost their functionality and in the remaining mesocarp cells, where starch is found, the formation of lisogenic spaces can be observed (Fig. 12-14). The completely differentiated endocarp adheres to the seminal tegument and it consists of the unaltered inner epidermis of the ovary, the radially elongated cells derived from adaxial meristem activity and of cells layers externally surrounded by the vascular bundle with greater caliber (Fig. 5, 7, 9, 11-14).

The fruit of *Garcinia gardneriana* is a berry, generally single-seeded, and slightly rostrate due to the persistence of a short style. The fruit is initially dark green, becoming yellowish-green and finally orange-tinted as it matures. The fruit skin is smooth and coriaceous, being formed by the exocarp. The pulp is white, edible with a sweet flavor, and is mainly derived from the endocarp, although it includes part of the spongy mesocarp in which the ducts have lost their functionality. The shape of the fruit can vary from oblate to oblong even on the same plant, although more detailed morphometric studies would be needed to quantify this variation.

Seed development

The ovules are anatropous with a single multiplicative integument with 18-20 layers (Fig. 15-19) and an internal and papilose meristematic epidermis, constituting the endothelium (Fig. 20). The nucellus is elongated distally, filling more than half of the length of the micropylar channel, and it is composed in large part by two cell layers, although the numbers of cell layers increases from the base to the apex (Fig. 15-19).

A procambial strand runs through the very short, thick funicle and through the rapheal region in the direction of the chalaza, and post-chalaza extensions of these strands ramifying throughout the peripheral region of the single integument (Fig. 15-19).

The immature seed structure in initial phase is characterized by a thickening of the testa to approximately 26 layers (Fig. 21-22); reabsorption of the nucellus; formation of the nuclear endosperm (Fig. 22-23); a papilose inner epidermis more evident than in the ovule (Fig. 22-23); and differentiation of the amphicribral vascular bundles in the testa (Fig. 21-22). A disorganization of the more internal layers of the testa and the initiation of the collapse of the other layers was observed in a posterior phase in which the embryo occupies the entire seminal cavity and there were no visible remains of the endosperm (Fig. 24).

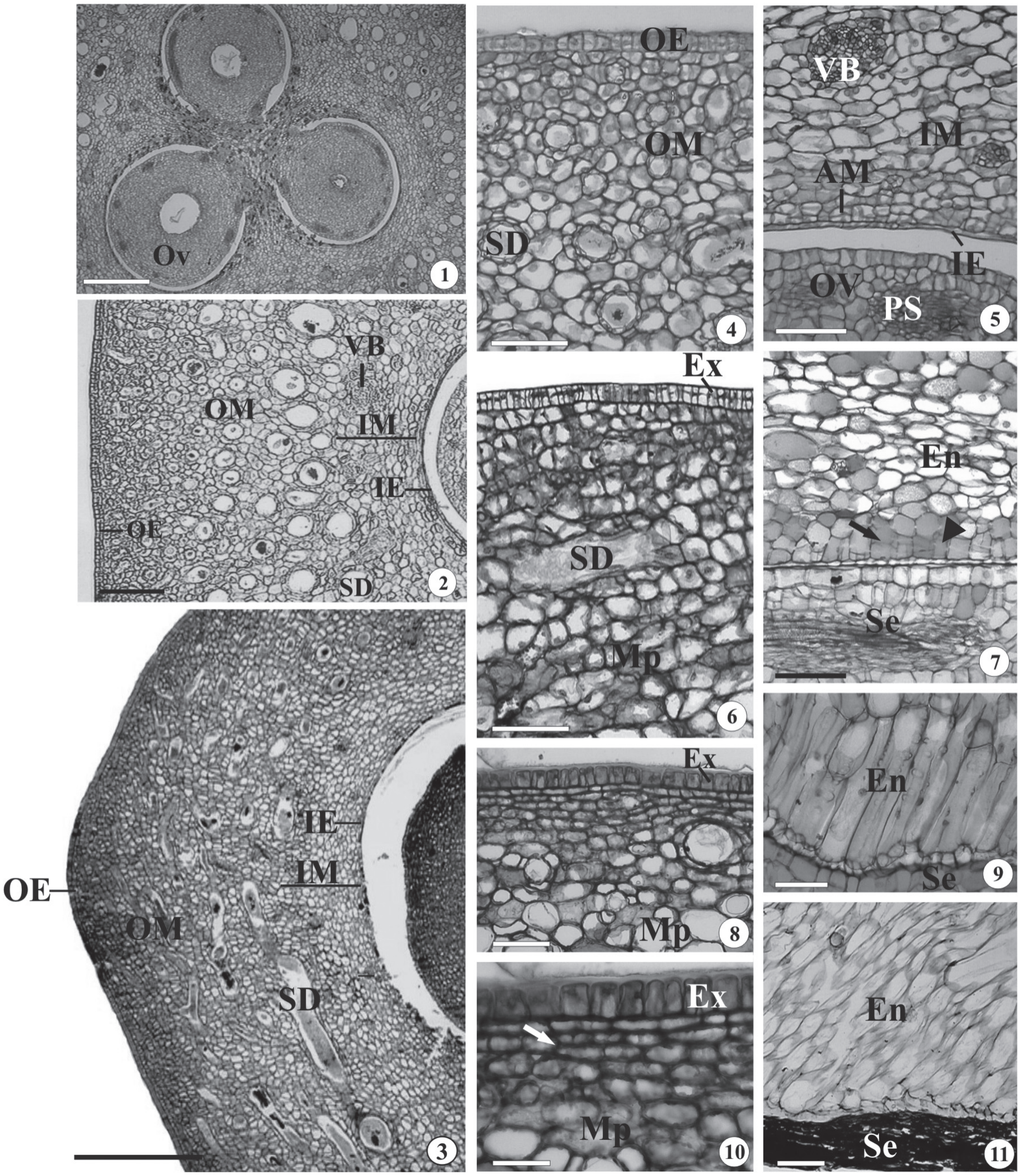
The mature seed is anatropous, exalbuminous and takes on an ellipsoidal form in lateral view (Fig. 26). However, in frontal or hilar view it is more or less angular. It has a coriaceous seed coat that is completely collapsed and lignified (Fig. 25). After removing the pulp, the seed cover was brown, vascularized, with a circular and protuberant hilum that is more lightly colored than the rest of the integument, with an indistinct micropyle (Fig. 26).

The embryo is composed of a long, thick hypocotyl-radicule axis, with vestigial cotyledons. The cotyledons, which are much reduced in size, are indistinct, and are characterized externally by a lateral grove in the extreme upper portion of the hypocotyl-radicule axis located opposite to the slightly protuberant radicule (Fig. 27-28). The embryonic axis is composed mostly of fundamental meristem whose cells are rich in starch and that is longitudinally penetrated by many secretory ducts and by a central procambial cylinder (Fig. 29-31).

Discussion

Engler (1888) described the Garcinieae ovary as unilocular with one ovule with parietal placentation. However, Corner (1976) reported that the number of locules in the ovary of *Garcinia* varied from 2 to 16 with one erect ovule per locule, as was observed in the present study. The characteristics described for the ovary of *Garcinia gardneriana* in the present study agree with those mentioned by Bittrich (2003), except that this author also reported bicarpelar ovaries.

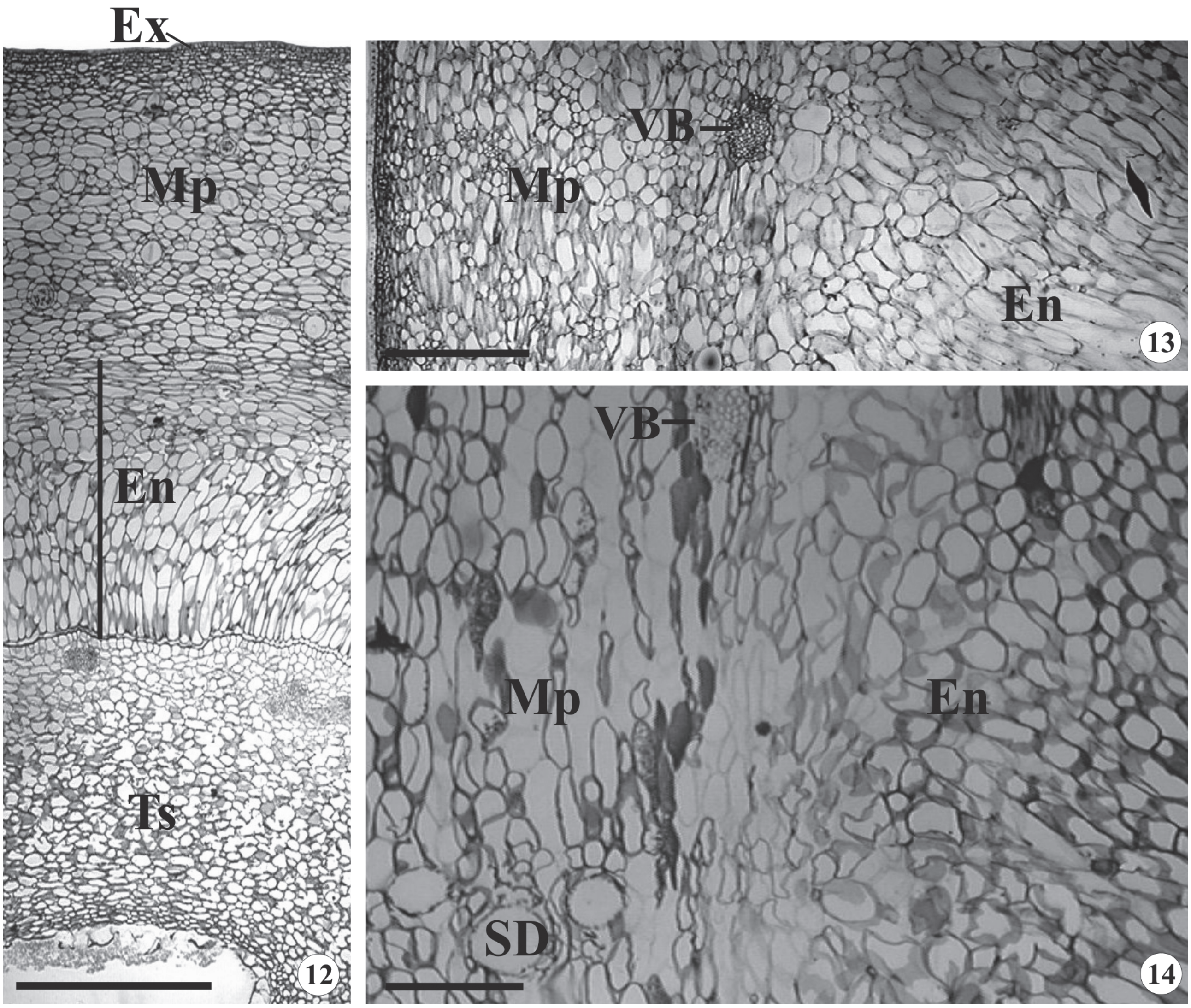
The anatomical modifications observed during pericarp development in *Garcinia gardneriana* were similar to those described by a number of authors for other species of Clusiaceae, such as *Symphonia clusioides* Baker (Clusioideae



Figures 1-11. *Garcinia gardneriana* (Planch. & Triana) Zappi. Ovaries and pericarp development in transverse (1-2, 4-5, 8-11) and longitudinal (3, 6-7) sections. 1-3: Central region of the ovary and overall aspect of the ovary wall; 4-5: Ovary (note the outer mesophyll with secretory ducts and in the inner mesophyll the subepidermal adaxial meristem; 6-9: Immature fruit (adaxial meristem) - arrow; derived cell undergoing a radial elongation – arrow point); 10-11: Mature fruit (note in mesocarp the collapse of the first cell layers – arrow). AM = adaxial meristem, En = endocarp, Ex = exocarp, IE = inner epidermis, IM = inner ovarian mesophyll, Mp = mesocarp, OE = outer epidermis, OM = outer ovarian mesophyll, Ov = ovule, PS = procambial strand, SD = secretory duct, Se = seed, VB = vascular bundle (Bar scales: 500 µm (1); 250 µm (2-3, 10-11); 100 µm (4-9)).

- Symphonieae), *Rheedia calcicola* Jum. & H. Perr. and *Tsimatimia pervillei* Jum. & H. Perr. (Clusioideae - Garcinieae) described by Cordemoy (1911), especially in terms of the last two species, which are included in the same tribe as the species in the present study. According to Cordemoy (1911) the earlier interpretations that the pulp surrounding the seeds of the species in this tribe represents an aril were erroneous. This author affirmed that the pulp in Garcinieae fruits originates from a meristematic generative zone in the deep layers of the pericarp subepidermal to the locules of the ovary, as was also observed in *G. gardneriana* in the present research. It is interesting to note that Rao & Kothagoda (1984) described the pulp of *Garcinia mangostana* as an aril even though they did not accompany the ontogenesis of the fruit.

Sprecher (1919), Corner (1976), and Rao & Kothagoda (1984) published similar descriptions for the pericarp of *Garcinia mangostana*, as did Corner (1976) for *Garcinia xanthochymus*. *G. mangostana* differed from the species

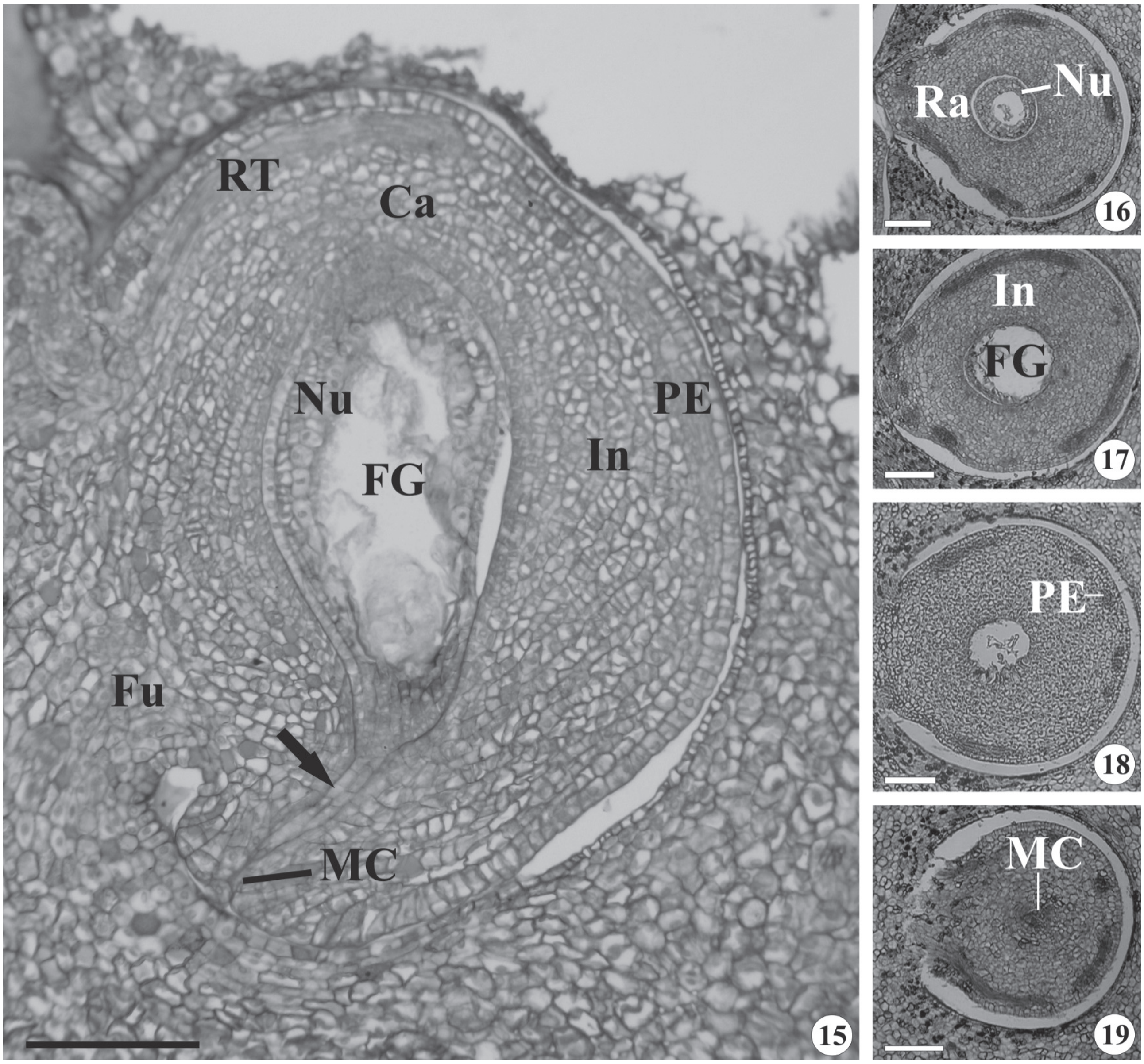


Figures 12-14. *Garcinia gardneriana* (Planch. & Triana) Zappi. Immature (12) and mature (13-14) fruits in transverse sections. 12: Pericarp and seed; 13: Exocarp, mesocarp and endocarp; 14: Mesocarp and endocarp, demonstrating spongy tissues. En = endocarp, Ex = exocarp, Mp = mesocarp, SD = secretory duct, Ts = testa, VB = vascular bundle (Bar scales: 400 µm (12-13); 200 µm (14)).

studied here in having a suberized exocarp, an outer collenchymal and sclerotic mesocarp, and an endocarp made up of cells with thick walls that are lignified in some regions. The endocarp of *G. mangostana* also originated in the same manner as that of *Garcinia gardneriana*, although Sprecher (1919) and Corner (1976) noted divisions in the inner epidermis of the species that they studied and the presence of secretory ducts in that region. The endocarp in *G. xanthochymus* is collenchymal. A pericarpal structure similar to that of *G. mangostana* was described by Corner (1976) for the fruits of *Garcinia* sp.

There seems to be general uniformity in the structure of the ovary walls of Clusiaceae, mainly the presence of two distinct regions in the ovarian mesophyll and the external parenchymal region being longitudinally crossed by vascular bundles and ramified secretory ducts, while the internal region is also parenchymal but without any ducts. However, modifications in this structure occur during the development of the fruits, whether they are capsules or berries. This anatomical aspect was very clear, even without complete ontogenic studies in the older descriptions such as those of Cordemoy (1911), Sprecher (1919), Rao & Kothagoda (1984), and Corner (1976), as well as in the more detailed studies undertaken by Mourão & Beltrati (1995a,b; 2000; 2001), Mourão & Marzinek (2009), and Campana *et al.* (2010) as well as in the present work.

It is interesting to note that Mourão & Beltrati (1995a; 2000) described the development of the pericarp, and especially the endocarp of the berry fruits of *Platonia insignis* (Clusioideae - Symphonieae) and *Mammea americana* (Kielmeyeroideae - Calophylleae), as being similar to that of *Garcinia gardneriana*. However, *M. americana* presents a peridermal exocarp and the pulp that surrounds the seeds in the two species is purely endocarpic



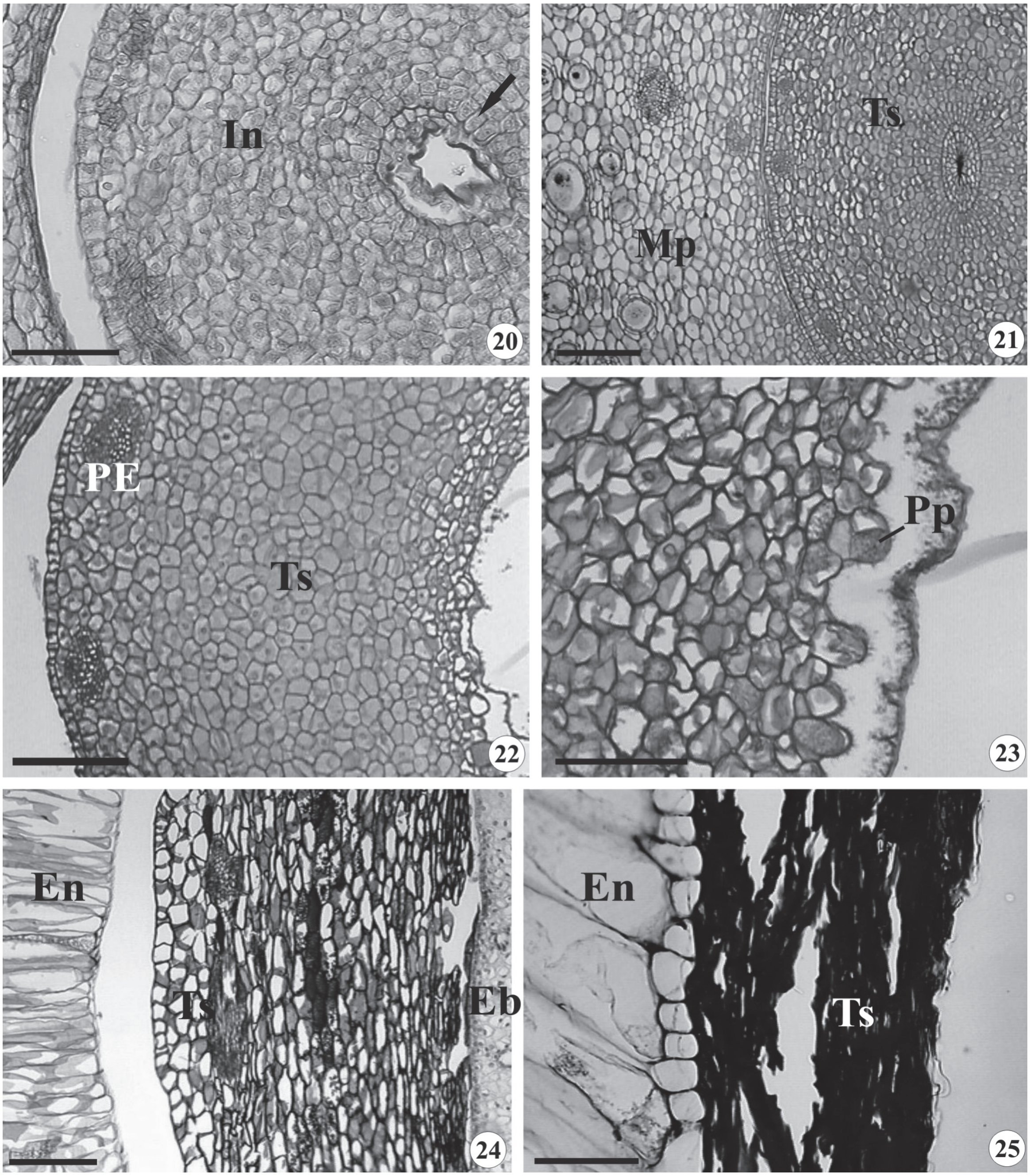
Figures 15-19. *Garcinia gardneriana* (Planch. & Triana) Zappi. Ovules in longitudinal (15) and transverse (16-19) sections. 15: Overall aspect (note the nucellus distally elongated filling part of the micropylar channel - arrow); 16-19: Basal (16), median (17) and apical (18) regions of the nucellus and micropylar region (19). Ca = chalaza, FG = female gametophyte, Fu = funicle, In = integument, MC = micropylar channel, Nu = nucellus, PE = post-chalazal extension of the rapheal vascular trace, Ra = raphe, RT = rapheal trace (Bar scales: 100 μm (15); 250 μm (16-19)).

in origin. The present study confirmed the participation of the layers that constitute the inner mesophyll in the constitution of the fruit pulp of *G. gardeneriana*.

Cordemoy (1911) and Corner (1976) described the seeds of *Tsimatimia pervillei* (Clusioideae - Garcinieae) as being surrounded by a pulpy endocarp and mesocarp. Likewise, Mourão & Beltrati (2001) indicated that the numerous seeds of *Vismia guianensis* (Aubl.) Choisy (Hypericaceae - Vismeae) are embedded in the reddish pulp that is derived in large part from the mesocarp, which has secretory ducts that lose their functionality, and the endocarp, which is derived from the adaxial meristem. Those descriptions are similar to those of the present study.

Barroso *et al.* (1999) characterized the fruits and seeds within the subfamilies and tribes of the Clusiaceae, classifying the fruits of the Moronobeoideae (Clusioideae – Symphonieae, *Platonia insignis*) and Clusioideae-Garciniae as "bacóides", those of Hypericaceae - Vismieae (*Vismia* Vand.) as "bacóides campomanesoídio", and those of Calophylloideae (Kielmeyeroideae – Calophylleae, *Calophyllum* L.) and Moronobeoideae (Clusioideae – Symphonieae, *Symphonia*) as "bacóide bacáceo". Mourão & Beltrati (2001), however, affirmed that the fruit of *Vismia guianensis* (Hypericoideae – Vismieae) was more appropriately described as the "bacóide-bacídio" type.

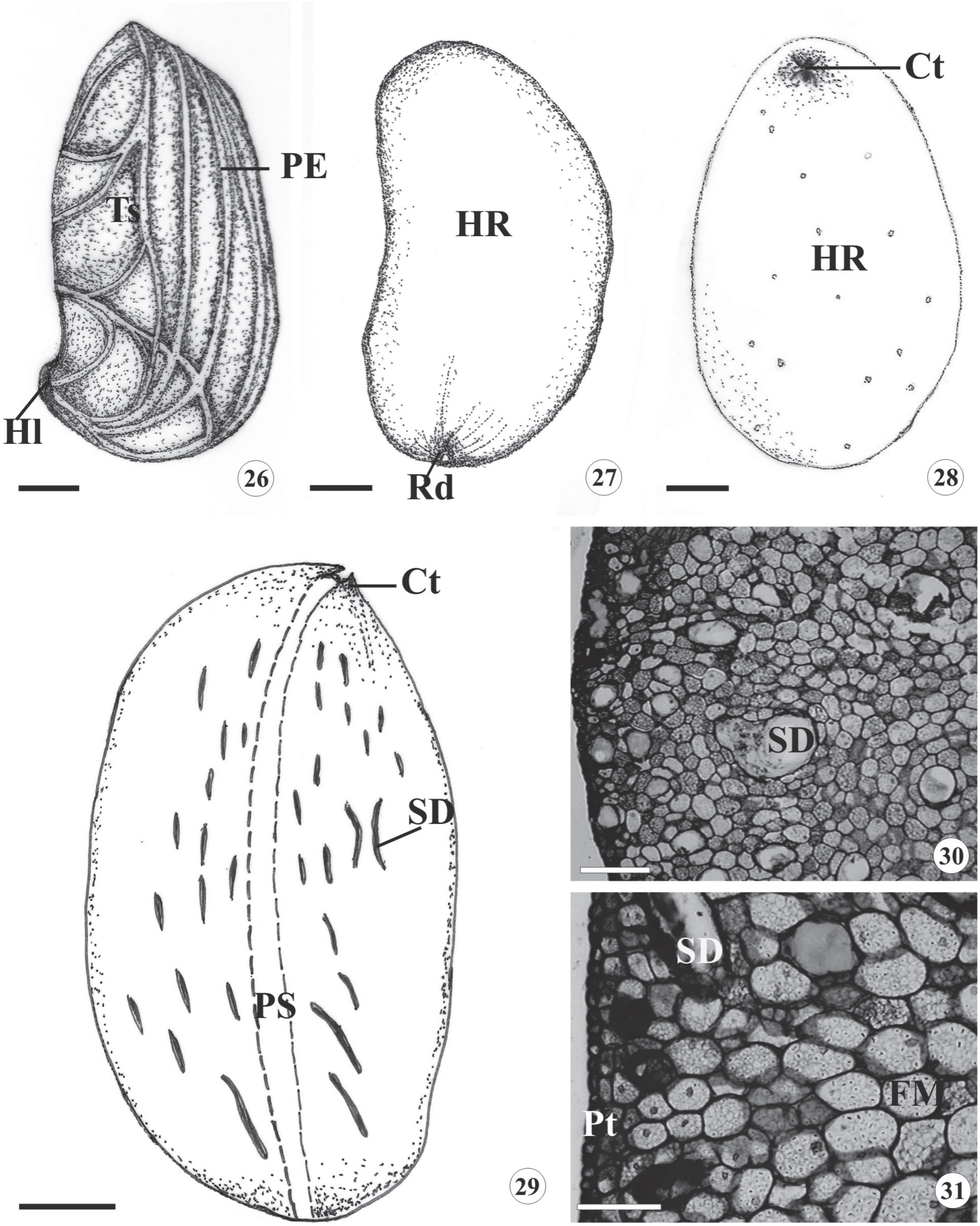
By the definition of Spjut (1994), the fruit of the species



Figures 20-25. *Garcinia gardneriana* (Planch. & Triana) Zappi. Development of the seed coat in transverse section. 20: Ovule (note the meristematic epidermis constituting the endothelium – arrow); 21: Part of the pericarp, testa and nucellus of the very young seed; 22-23: Immature seed in a posterior phase shown in figure 21 (note in figure 23 the papillose inner epidermis of the testa in detail); 24: Endocarp elongated cells and immature seed evidencing the beginning of the testa collapse and the embryo occupying all seminal cavity; 25: Mature seed (note the endocarp adhered to the collapsed testa). Eb = embryo, En = endocarp, In = integument, Mp = mesocarp, PE = post-chalazal extension of the rapheal vascular bundle, Pp = papillae, RB = rapheal vascular bundle, Ts = testa) (Bar scales: 100 μm (20, 23, 25); 250 μm (21, 22, 24)).

studied here is a berry, while according to the definition of Barroso *et al.* (1999) this fruit belongs to the "bacóide" type, which is characteristic of Clusieae, *Rheedia*, and *Garcinia*. These latter authors also affirm that the fruits of *Rheedia* are "bacóide campomanesoídeo", of the subtype with a fleshy pericarp and a central cavity filled with a uniform pulpy tissue whose central portion contains thin locules arranged radially around the seeds which are generally present in small numbers, similar to the *Garcinia gardneriana* in the present work.

Mammea americana (Kielmeyeroideae – Calophylleae) described by Mourão & Beltrati (2000) and *Garcinia gardneriana* present unitegmic ovules contrary to what was observed by Corner (1976) in Clusiaceae. Judging by the illustrations of *Garcinia* prepared by Corner (1976), what this author interpreted as the internal tegument corresponds to the nucellus described in the present study for *G. gardneriana*.



Figures 26-31. *Garcinia gardneriana* (Planch. & Triana) Zappi. Mature seed. 26: General aspect; 27-28: Overall aspect of the embryo in rapheal and anti-rapheal views respectively (note in figure 28 the minute cotyledons); 29: Embryo in longitudinal section, evidencing the procambial strand and minute cotyledons; 30-31: Details of transverse sections of the embryo (note the starch grains in the fundamental meristem cells - arrow). Ct = cotyledon, FM = fundamental meristem, Hl = hilum, HR = hypocotyl-radicle axis, PE = post-chalazal extension of the rapheal vascular bundle, PS = procambial strand, Pt = protoderm, Rd = radicle, SD = secretory duct, Ts = testa (Bar scales: 0,25 cm (26-29); 200 µm (30); 100 µm (31)).

In the illustrations prepared by Sprecher (1919) of *Garcinia mangostana* there seems to be a very thin nucellus layer next to the biseriate internal tegument. Similarly, the embryological studies of Lim (1984) who examined the same species clearly indicated the presence of an internal tegument in the ovule primordium, although there are doubts concerning descriptions of the presence of this layer in the mature ovule. Future studies examining the ontogenesis of the ovule in Clusiaceae and in *Garcinia gardneriana* may be able to clarify the question of whether the occurrence of unitegmic ovules represents an ontogenetic or a phylogenetic reduction.

The presence of an endothelium in ovules of Clusiaceae and Hypericaceae was mentioned in *Hypericum patulum*, *Hypericum mysorense* (Rao 1957), *Clusia* sp. (Corner 1976), *Platonia insignis* (Mourão & Beltrati 1995a), *Vismia guianensis* (Mourão & Beltrati 2001) and *Clusia parviflora* Humb. & Bonpl. ex Willd. (Mourão & Marzinek 2009) and it is the inner epidermis of the inner integument in all species mentioned. In *Garcinia gardneriana* the endothelium is located in inner epidermis of the single integument and it is most similar to this layer described by Mourão & Beltrati (1995a) for *P. insignis* in the inner integument.

A nuclear endosperm was described in embryological studies of Hypericaceae-Hypericeae by Swamy (1946) and Rao (1957) and, according to Delay & Mangenot (1960) and Corner (1976) this structure occurs in all of the Clusiaceae. Lim (1984) described the endosperm of *Garcinia mangostana* (Clusioideae-Garcinieae) as initially being nuclear, but evolving to be cellular, and then being consumed during the development of the embryo. Only future studies that closely examine the endosperm and embryological development in *Garcinia gardneriana* will be able to confirm this sequence of events.

Corner (1976) described non-specialized testa and tegmen by Garcinieae, however for *Allanblackia floribunda* Oliv., also in this tribe, the cells of the endotesta infiltrate among the exotegmic cells and this latter layer becomes very large, columnar, and lignified, similar to that described in *Platonia insignis* (Clusioideae - Symphonieae) by Mourão & Beltrati (1995a). It is interesting to note that in an earlier taxonomic treatment by Stevens (2007a), both *Allanblackia* Oliv. and *Platonia* Mart. were included in Moronobeae.

The anatropous seeds of *Mammea americana* (Kielmeyeroideae - Calophylleae) described by Mourão & Beltrati (2000) develop like those of *Garcinia gardneriana* from anatropous unitegmic ovules, but in the former species the seeds have a distinct testa and the embryo is conferruminate.

Corner (1976) pointed out that the rapheal bundle in the Clusiaceae may or may not emit post-chalazal extensions. Among the genera that have seeds showing post-chalazal vascularization are *Calophyllum* (Kielmeyeroideae - Calophylleae), *Garcinia*, *Septogarcinia* Kostern (Clusioideae - Garcinieae), *Tovomitopsis* Planch. & Triana (Clusioideae - Clusieae) and *Pentadesma* Sabine (Clusioideae - Symphonieae). Mourão & Beltrati (1995a, 2000) described post-chalazal ramifications of the rapheal bundle in *Platonia insignis* (Clusioideae - Symphonieae) and *Mammea americana* (Kielmeyeroideae - Calophylleae), as was observed in the present work, and these authors also pointed out the great morphological similarity between the seeds of *P. insignis* and *Garcinia gardneriana*.

Corner (1976) suggested that the ancestral seeds of the Clusiaceae were arillate with an exotegmen, but in some arillate seeds such as *Tovomitopsis* (Clusioideae – Clusieae) the exotegmen is undifferentiated, and that this state seems to lead to the undifferentiated seeds of the Clusioideae - Garciniae. Another interesting observation raised by this author is that *Septogarcinia* (Garcinieae), which has a capsule fruit, has seeds that are covered by a fleshy endocarp and represents an intermediary condition between the Clusieae and the Garcinieae, demonstrating that this modification of the endocarp appeared before the development of berry fruits in the family. This same author also noted the structural similarity between *Garcinia atroviridis* Griff. ex T. Anderson and *Septogarcinia*, in which *G. atroviridis* suggests an indehiscent *Septogarcinia*.

There is significant uniformity among all the genera of the Clusioideae in relation to the occurrence of a hypocotylar embryo (Brandza 1908; Guillaumin 1910; Sprecher 1919; Delay & Mangenot 1960; Corner 1976; Mourão & Beltrati 1995b; Barroso *et al.* 1999).

Corner (1976) went on to suggest that Clusieae and Garcinieae are also related due to their hypocotylar embryo, even though they differ in germination patterns, for the seedlings of Clusieae species are phanerocotylar while those of the Garcinieae are cryptocotylar. Stevens (2007a), however, observed that some species of *Garcinia* in the section *Macrostigma* germinate in the same way as *Clusia*.

Stevens (1976) emphasized that all of the tribes and subtribes in the Clusiaceae exhibit various characteristics considered ancestral, as well as others considered derived. Molecular analyses (Gustafsson *et al.* 2002; Notis 2004) combined with morphological data have resolved the positions of a number of genera in the family, as well as in the subfamilies and tribes. The analysis of Gustafsson *et al.* (2002) indicated that Clusioideae is monophyletic and comprises Clusieae, Symphonieae, and Garcinieae. Notis (2004) utilized multigenic analyses and the evolution of the characteristics of the fruits and seeds among others of Kielmeyeroideae to confirm the monophyly of the tribes within this subfamily and affirms that this type of fruit is a homoplastic characteristic in the Clusiaceae. However, by combining the characters of the fruits and seeds that are considered ancestral or derived in older phylogenetic classification systems to the placement observed in systems resulting from molecular analyses such as those performed by Stevens (2007a), the following conclusions can be made: the Kielmeyeroideae (*Kielmeyera* Mart.) and Clusioideae – Clusieae present ancestral characteristics such as dehiscent fruits (capsules), arillate and exotegmic seeds, and embryos with cotyledons varying from distinct to very reduced. The Clusioideae-Garcinieae and Symphonieae present characteristics that are considered derived, including indehiscent fruits (berries or drupes), seeds without an aril, tegument little differentiated or mesotesta, and hypocotylar embryos.

It is also important to note that the use of fruit and seed characters in establishing relationships in recent phylogenetic treatments of the family is still somewhat precarious due to the fact that ontogenic studies have been undertaken with only a limited number of species - leaving doubts as to the correct classification of the fruits and structures enveloping the seeds, as well as the integument layers of the seeds.

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