Comparative micromorphology and anatomy of seeds and endocarps of selected Primulaceae and their systematic implications

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Abstract
Seed and endocarp micromorphology and anatomy of 16 taxa from the genera Ardisia, Geissanthus, Stylogyne, Myrsine (Myrsinoideae), and Jacquinia (Theophrastoideae) were examined using stereo- and scanning electron microscopy and discussed in the light of the current phylogenetic framework. In all species, the ornamentation of the seed surface was reticulate, tuberculate, or tuberculate-colliculate with several differences concerning the cell outline and anticlinal cell wall boundaries. For seeds of almost all Myrsinoideae species, one-layered seed coat devoid of rhomboid or prismatic crystals was characteristic, while seeds of J. armillaris had a two-layered seed coat with prismatic crystals. The one-layered seed coat in Myrsinoideae may be considered a synapomorphy of this subfamily. The endosperm tissue in seeds of Myrsinoideae was differentiated into two types. Seeds of Ardisia and Geissanthus species were characterized by ‘pitted’ endosperm, while in seeds of Myrsine species both the ‘pitted’ endosperm and endosperm with evenly thickened cell walls were present. In seeds of Theophrastoideae, the endosperm was ‘pitted.’ Our results confirmed that the concave hilum area is characteristic of subglobose seeds of Myrsinoideae. The ruminate endosperm was present in all the examined Myrsine species, but it was absent in Ardisia crenata, Geissanthus ambiguus, and Stylogyne pauciflora seeds. Thus, the ruminate endosperm is not the feature clearly distinguishing the seeds of Myrsinoideae and Theophrastoideae. Endocarps of Myrsinoideae vary in terms of their morphology and anatomy. The variation within the primary and secondary sculpture of their inner surface and the presence of stomata in endocarps of particular species may have systematic implications.

Keywords Ardisia · Geissanthus · Jacquinia Myrsine · Ruminate endosperm · Seed coat · Stylogyne

Introduction
Primulaceae (Order Ericales) is a monophyletic family with four subfamilies (Maesoideae, Myrsinoideae, Primuloideae, and Theophrastoideae) that comprises 2590 species widely distributed worldwide (APG IV 2016). This current circumscription was due to exhaustive morphological (Anderberg and Ståhl 1995) and molecular surveys (Anderberg et al. 1998, 2000; Källersjö et al. 2000; Schönenberger et al. 2005) that helped to clarify the boundaries in the previously called ‘primuloid group,’ that were formed by the currently excluded families Maesaceae, Myrsinaceae, Primulaceae, and Theophrastaceae.

Despite the unquestionable monophyly of the family, many generic and species boundaries still lack delimitation. In this sense, morphology, wood, and leaf anatomy and several different types of secretory tissues are used as highly informative taxonomic characteristics to distinguish particular genera, subfamilies, or clades within Primulaceae.
Such features are also helpful in phylogenetic analyses based on mixed molecular, morphological, and anatomical data (Källersjö et al. 2000; Källersjö and Ståhl 2003; Lens et al. 2005; Luna et al. 2013, 2014, 2017, 2018, 2019). Although fruits and seeds of Primulaceae species, including both herbaceous (Primuloideae and Myrsinoideae) and woody plants (Myrsinoideae and Theophrastoideae), have been examined according to their development, morphology, and anatomy, the obtained results were rather ambiguous regarding systematics and phylogeny of the studied plants (Otegui et al. 1998b; Otegui and Cocucci 1999; Anderberg et al. 2007; Oh et al. 2008; Morozowska et al. 2011).

Otegui et al. (1998a, b, 1999a, b), who performed studies on secretory structures, seed structure, and endosperm development of *Myrsine laetevirens*, confirmed the rumination of the seeds and the type of the ‘pitted’ endosperm cell walls to be typical for the examined species. The ruminations, assumed to be typical for the large subglobose seeds of Myrsinoideae, were at first thought to be caused by the activity of the seed coat or the endosperm itself during later stages of seed development (Corner 1976; Anderberg and Ståhl 1995). However, Otegui et al. (1998a) showed that in *M. laetevirens* seeds the indentations responsible for their lobed outline are formed from crystals secreted by the placenta, which surrounds the developing seed. The same authors found that in the ovules of *M. laetevirens*, the groups of secretory cells in the epidermis of the placenta secrete pure rapanone, the main constituent of seed crystals. They also suggested that a similar situation could be expected in other genera of Myrsinoideae.

The seeds of Primulaceae are differentiated based on their morphology and anatomy. According to Anderberg and Ståhl (1995) and Anderberg and Kelso (1996), two types of seeds were described within Primulaceae (previously called ‘primuloid clade’). Most genera of the former families Theophrastoideae and Myrsinaceae were supposed to have rather large, subglobose seeds with a thin testa devoid of crystals and a ‘pitted’ endosperm (very thick endosperm cell walls with irregular thickenings). The main differences between these two families concerned the concave hilum area and a more or less ruminate endosperm observed in Myrsinaceae seeds. In turn, almost all of the former Primulaceae and the genus *Maesa* were characterized by having small angular seeds with a thick, usually distinctly two-layered testa with rhomboid crystals and smooth, evenly thickened endosperm cell walls (Anderberg and Ståhl 1995).

Although it is well known that fruit and seed morphology and anatomy are of considerable importance in plant taxonomy, data gathered on Theophrastoideae and Myrsinaceae are rather limited and most of the available information concerns the herbaceous taxa (Oh et al. 2008; Morozowska et al. 2011). Oh et al. (2008), who examined seed morphology in the genus *Lysimachia* and related taxa, found that the seed coat structure provides potentially synapomorphic character states for various subclades of *Lysimachia*. Morozowska et al. (2011) examined several herbaceous species of Primulioideae, Myrsinoideae, and Theophrastoideae and provided new and important data concerning seed shape and size, seed coat sculpture, thickness and structure, the presence of oxalate crystals in testa, and the endosperm structure. According to the cited authors, most of the described characters were poorly correlated with major clades; however, some of them were recognized as potentially informative characters, which may support molecular evidence in resolving unclear relationships between species, genera, or subfamilies of Primulaceae.

The present study is a continuation in a series of papers that started with Morozowska et al. (2011) discussing seed and endocarp morpho-anatomy in some Primulaceae herbaceous species. In the present work, we examined fruit and seed morphology and anatomy in several woody Myrsinoideae and Theophrastoideae species to provide additional information on their reproductive structures that are still little known in Primulaceae. The taxonomic importance and systematic implications of the results are discussed in a phylogenetic framework. Furthermore, we provided a compilation of the earlier and presently obtained results to discuss our findings in a broader context regarding the entire Primulaceae family.

**Materials and methods**

The material studied comprises woody representatives of two subfamilies: the pantropical Myrsinoideae and the neotropical Theophrastoideae from Primulaceae. We analyzed plants found in a variety of habitats. Thirteen of the examined species represent Brazilian flora (Carrijo et al. 2012; Freitas and Kinoshita 2015; Freitas et al. 2017), while *Myrsine wightiana* is native to India and is also distributed in Sri Lanka (Sasidharan n.d.), the *Myrsine africana* native range comprises ES Africa, the Arabian Peninsula, China, and the Azores, while the native range of *Ardisia sieboldii* is SE China and Temperate E Asia (WFO 2018).

Morphological and anatomical fruit and seed characters of nine *Myrsine*, four *Ardisia*, one *Stylogyne*, and one *Geissanthus* species representing Myrsinoideae, as well as *Jacquinia armillaris* of Theophrastoideae, were examined by stereomicroscopy and scanning electron microscopy (SEM) using plant material obtained from various herbarium collections (Table 1). Seed coat thickness was measured on the longitudinal and cross-sections of ten seeds from each species based on scanning electron microscope images. Before cutting, fruits and seeds were rehydrated for 24 h in water, brushed clean, rinsed in distilled water (two times for 10 min), and sectioned. After sectioning, the hand cuttings...
were dried using an acetone series of 30%, 50%, 70%, 90%, and 100%, three times for 6 min in each. For SEM examinations, seeds and fruits as well as their cuttings were gold-sputtered and examined under a Zeiss EVO 40 electron microscope at 8-15 kV depending on the species. The terminology used to describe fruit and seed ultrastructure follows Barthlott (1981) and Barthlott et al. (1998).

### Phylogeny and character optimization

Phylogenetic relationships were inferred from rbcL sequences from Genbank accessions (see Supplementary Electronic Material) of 12 Primulaceae genera, representing the four subfamilies, Diospyros virginiana (Ebenaceae) and Manilkara zapota (Sapotaceae) were selected as outgroups, as both families are sister groups of Primulaceae (Schönberger et al. 2005).

A heuristic search was performed with 5000 repetitions and 100 trees per replication using TBR swapping, based on the character optimization method ACCTRAN (accelerated transformation optimization; Farris 1970, Swofford and Maddison 1987), with unordered characters of equal weight and the retention of multiple most parsimonious trees (MAXTREE) using maximum parsimony in PAUP* version 4.0b10 for Windows (Swofford 2002). Bootstrap analysis was performed with a random addition sequence of taxa, using TBR for 1000 replicates (Felsenstein 1985) to provide node support values.

To trace character evolution, 11 were plotted using parsimony optimization on one of the most parsimonious trees obtained from the phylogenetic analysis using the Mesquite software (Maddison and Maddison 2018) with parsimony optimization. Additional anatomical data on Androsace, Primula, Soldanella (Primuloideae), Samolus (Theophrastoideae), Cyclamen, and Lysimachia (Myrsinoideae) were taken from the literature (Morozowska et al. 2011). The selected characters and their states are presented in Table 2, while the character states of the studied Primulaceae genera are shown in Table 3.
Sclerenchyma cells were characterized by their fleshy drupe fruits with one subglobose seed inside, while in *Jacquinia armillaris* the described indentations were rather small (Fig. 5j), while seeds of *A. sieboldii* lacked such indentations and they were smooth in the outline (Table 5; Fig. 3g, m, o). Under the stereomicroscope, these indentations were orange (Fig. 3b, d, e, h, i, j), while in SEM they were filled with numerous crystals (Fig. 5c, d, 6h, i, k, Online Resource 2). In seeds of *A. sieboldii*, the described indentations were rather small (Fig. 5j), while seeds of *A. crenata*, *G. ambiguus*, and *Stylogyne pauciflora* lacked such indentations and they were smooth in the outline (Table 5; Fig. 3g, m, o).

Seeds of all Myrsinoideae were covered by a strongly wrinkled, compressed, membranous tissue of remnants of the placenta (Figs. 3d, g, l, m, o, 4d, i, 5b, c, h, 6e, Online Resource 2). On the inner surface of placenta remnants groups of secretory cells were observed in seeds of *A. solanacea* (Fig. 4e).

Microsculpturing of Myrsinoideae seeds was either reticulate (Fig. 5e, 6a, f, n), tuberculate (4f, 5i), or tuberculate-colliculate (6j). The testa surface was often wrinkled, and thus the outline of the epidermal cells was not always clearly visible; however, the surface testa cells in seeds of *A. crenata*, *A. solanacea*, *A. humilis*, *M. africana*, *M. coriacea*, and *M. lineata* were polygonal in outline with straight, raised anticlinal cell wall boundaries and concave outer periclinal walls (Figs. 4a, 5e, 6a, f,n). In *G. ambiguus* and *A. sieboldii*, the outer periclinal walls were convex (Fig. 4f, 5i). The seed coat in all *Ardisia*, *Myrsine*, and *G. ambiguus* was one-layered, undifferentiated, very thin (approx. 2.5–6.95 µm and 6.45–9.09 µm, respectively), and devoid of crystals (Table 5, Fig. 6b, d, l).

### Results

All the examined woody Myrsinoideae species representing the genera *Ardisia*, *Myrsine*, *Geissanthus*, and *Stylogyne* were characterized by their fleshy drupe fruits with one subglobose seed inside, while in *Jacquinia armillaris* from Theophrastoideae fruits are berries with 3-4 subglobose seeds. Stony endocarps of drupes in all the examined Myrsinoideae species are longitudinally ribbed with vascular elements present in the strands of tissues running in the meridian orientation on the endocarp surface (Fig. 1a). Endocarps of Myrsinoideae were composed of 1–4(5) layers of very thick-walled isodiametric or slightly elongated sclerenchyma cells with numerous pits (Table 4). In the endocarps of most examined species sclerenchyma cells were radially orientated, whereas in the endocarps of *Ardisia sieboldii*, *Myrsine venosa*, and *M. guianensis* they were also tangentially orientated (Figs. 1d, 2c, k). Sclerenchyma cells were characterized by the lamellar structure of their secondary cell walls (Fig. 2a), whereas in *A. humilis* endocarps many rhomboid or prismatic crystals were present (Fig. 1f). Endocarps of all the examined *Ardisia* species, *Geissanthus ambiguus*, and *Stylogyne pauciflora* were composed of only one layer of sclerenchyma, while endocarps of *Myrsine* most often consisted of 2–4(5) sclerenchyma layers (Figs. 1b, d, f, h, k, 2a, c, e, g, i, k, m, o, t).

The cellular pattern on the endocarp surface was reticulate or reticulate-striated. The cells on the inner surface of endocarps in all the examined species were often polygonal, either equal- or elongate-sided, with straight or acute endings, or they were more or less rounded in their outline. Anticlinal cell wall boundaries were straight, raised, or depressed, while the periclinal walls were flat, concave, or convex. The secondary sculpture of the periclinal walls was micro-reticulate, verrucose, foveate, or foveolate (Table 4; Fig. 2b, d, f, h, j, l, n, p, u). Additionally, in the endocarps of *Ardisia humilis*, *Geissanthus ambiguus*, and *Stylogyne pauciflora*, numerous stomata were observed on the inner surface of the endocarp (Fig. 1g, j, l).

### Table 2 Seed and endocarp selected characters and character states

| Character                                | Character states                                                                 |
|------------------------------------------|---------------------------------------------------------------------------------|
| Secondary sculpture of the endocarp      | 0—smooth; 1—micro-reticulate; 2—foveolate; 3—verrucose; 4—striate; 5—absent    |
| Hilum shape                              | 0—depressed; 1—narrow; 2—narrowly elliptic; 3—elliptic; 4—circular             |
| Seed coat cellular pattern               | 0—reticulate; 1—tuberculate; 2—weakly reticulate; 3—poroid-foveolate; 4—vesiculose |
| Endosperm cell walls                     | 0—evenly thickened, smooth; 1—unevenly thickened, pitted (= with hollows); 2—thin, paper-like; 3—with helical thickenings; 4—strongly undulate |
| Number of sclerenchyma cell layers in the endocarp | 0—1; 1—2; 2—3; 3—4; 4—5; 5—absent                                              |
| Differentiation of the endosperm into layers | 0—absent; 1—present                                                            |
| Seed coat structure                      | 0—1 layered; 1—2 layered                                                        |
| Stomata in the inner surface of the endocarp | 0—absent; 1—present                                                             |
| Seed shape                               | 0—subglobose; 1—sectoroid; 2—polyhedral; 3—ovoid                              |
| Calcium oxalate crystals in the seed coat | 0—absent; 1—present                                                             |
| Cellular pattern of the endocarp inner surface | 0—reticulate; 1—reticulate-striated; 0—absent                                   |

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The endosperm of *Ardisia*, *M. africana*, *M. parvula*, and in *G. ambiguus* was composed of cells with very thick and ‘pitted’ cell walls (Table 5, Fig. 4b, c, h, 5 g, k, l, 6b, c, Online Resource 2). In the endosperm of *A. crenata* also smooth cell walls were observed. Additionally, the endosperm of these species, except *A. crenata* and *A. sieboldii* was distinctly differentiated into the outer and the inner parts. Both parts differed according to the cell size and shape, and the cell wall thickness. The outer part of the endosperm consisted of two–three layers of square or rectangular cells with smooth and thin walls, while in the inner part of the endosperm, the cells were elongated, thick-walled, and ‘pitted’ (Figs. 4b, c, 5f, g, 6b, c, Online Resource 2). In *A. solanacea*, *M. parvula*, and *Geissanthus ambiguus*, the ‘pitted’ type of endosperm was not as distinct, but still noticeable (Fig. 4c, h, Online Resource 2). In *M. lineata* and *M. guianensis*, the endosperm cell walls were papery thin, sometimes slightly undulate (Fig. 6o); additionally, in *M. guianensis*, circular or helical thickenings were present on the inner surfaces of the cell walls (Table 5). In the five other examined *Myrsine*, the endosperm consisted of cells with smooth and evenly thickened walls, and no differentiation in the endosperm was observed (Table 5, Fig. 6l, m, Online Resource 2).

*Jacquinia armillaris* (Theophrastoideae) berries contained few subglobose seeds with a slightly depressed hilum, and they were entirely surrounded by the placental pulp (Fig. 3p). The exocarp was thin and brittle. The mesocarp and endocarp consisted of several layers of thin-walled, parenchymatic cells. The inner surface of the endocarp was micro-reticulate. The epidermal cells were rounded in outline with straight, raised anticlinal cell wall boundaries and flat outer periclinal walls. The cuticle of these periclinal walls was irregularly striated. Additionally, stomata were present in cells of the inner epidermis of the endocarp (Fig. 7e, h). Testa surface was micro-reticulate and the seed coat was double-layered, approx. 11.20 μm in thickness. The outer periclinal walls of the exotesta were flattened or slightly concave, while the anticlinal cell wall boundaries were straight and raised. The mesotesta consisted of several layers of crushed cells with rhomboid or prismatic crystals (Table 5). The tegmen was composed of a solid tissue approx. 1.8 μm in thickness (Fig. 7c, d). The ‘pitted’ endosperm of *Jacquinia armillaris* seeds was copious and hard, composed of bony cells. Two–three layers of the outer endosperm consisted of cells with smooth and thin walls (Fig. 7a, b).

### Table 3: Seeds and endocarp character states of 12 Primulaceae genera

| Taxon/Character | Hilum shape | Seed coat cellular pattern | Secondary sculpture of the endocarp | Number of sclerenchyma layers in the endocarp | Differentiation of the endosperm into layers | Cell wall thickness in the endocarp | Calcium oxalate crystals in the seed coat | Stomata in the inner surface of the endocarp | Seed shape | Number of sclerenchyma layers in the endocarp | Endosperm cell walls | Secondary sculpture of the endocarp | Hilum shape | Seed coat cellular pattern |
|-----------------|-------------|---------------------------|-----------------------------------|-----------------------------------------------|---------------------------------------------|--------------------------------------|-----------------------------------------|------------------------------------------|-------------|-----------------------------------------------|---------------------|--------------------------------------|-------------------|---------------------------|
| Jacquinia       | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Stamolus        | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Androsace       | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Primula-Dio     | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Soldanella      | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Cyclamen        | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Lysimachia      | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Mysore           | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Stylogyne       | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Geissanthus     | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |
| Arcturus        | 0           | 1                         | 0.1                               | 0.1                                           | 0.1                                         | 0.1                                  | 0.1                                    | 0.1                                      | 0.1          | 0.1                                           | 0.1                 | 0.1                                  | 0.1               | 0.1                        |

### Phylogenetic analysis

From 1332 base pair characters in the *rbcL* sequences, 1078 were constant, 150 were parsimony-uninformative, and 104 were parsimony-informative characters. The parsimony
analysis resulted in a single tree with 408 steps. The consistency index was 0.74, the retention index was 0.56, and the homoplasy index was 0.26. Sequence variation in rbcL supports the monophyly of Primulaceae (100% bootstrap value) and resolves the four subfamilies (Fig. 8a).

Character optimization

Most of the seed, endosperm, and endocarp characters show some homoplasy (Figs. 9, 10). A depressed hilum was found in Myrsinoideae species and Jacquinia’ (Theophrastoideae) (Fig. 8b). The tuberculate seed coat is shared by both Primuloideae and Myrsinoideae (Fig. 8c). A poroid alveolate seed coat and endosperm cell walls with a helical thickening appear to be apomorphies of Lysimachia (Fig. 8c, d). The differentiation of the endosperm in layers is seen in Ardisia, Myrsine, and Geisanthus from Myrsinoideae, as well as Jacquinia from Theophrastoideae (Fig. 9a).

Sectoroid seeds were found in Primuloideae and Lysimachia (Myrsinoideae), polyhedral seeds evolved multiple times and are found in Samolus (Theophrastoideae), Androsace (Primuloideae), and Lysimachia (Myrsinoideae) (Fig. 9b). Ovoid seeds are probably an apomorphy of Primula (Primuloideae) (Fig. 9b). The one-layered seed coat is probably a synapomorphy for the monophyletic clade formed by Myrsine, Stylogyne, Geisanthus, and Ardisia from Myrsinoideae (Fig. 9c). The absence of calcium oxalate crystals is a feature shared by both Primula (Primuloideae) and the monophyletic clade formed by Myrsine, Stylogyne, Geisanthus, and Ardisia (Myrsinoideae) (Fig. 9d).

A striate endocarp appeared as an apomorphy for Jacquinia (Theophrastoideae), while the foveolate ornamentation of the endocarp was found only in the woody Myrsinoideae (Fig. 10a). In the monophyletic clade formed by Myrsine, Stylogyne, Geisanthus, and Ardisia, the number of sclerenchyma cell layers in the endocarp ranges from 1 to 5, as observed in Myrsine (Fig. 10b).

Fig. 1 SEM micrographs of endocarp morphology and anatomy (cross-sections) of Ardisia, Geisanthus, and Stylogyne. a-c Ardisia crenata, a outer surface, b one layer of almost equally dimensional sclerenchyma cells with numerous pits, c inner surface with reticulate cellular pattern, raised anticalinal cell wall boundaries, flat periclinal walls; d, e A. sieboldii, d one layer of elongated sclerenchyma cells and one-three layers of tangentially orientated cells (arrows), e inner surface with reticulate cellular pattern, raised anticalinal cell wall boundaries, concave periclinal walls, micro-reticulate/foveolate secondary sculpture; f, g A. humilis, f one layer of strongly elongated sclerenchyma cells with numerous prismatic crystals, g inner surface with reticulate-striate cellular pattern, raised anticalinal cell wall boundaries, convex periclinal walls, and stomata; h-j Geisanthus ambiguus, h one layer of slightly elongated sclerenchyma cells with numerous pits, i inner surface with reticulate cellular pattern, thick and raised anticalinal cell wall boundaries, concave periclinal walls, foveolate secondary sculpture, j inner surface with stomata; k, l Stylogyne pauciflora, k one layer of endocarp sclerenchyma cells with remnants of stomata, l inner surface with verrucose secondary sculpture and numerous stomata deeply sank into the endocarps surface.
The presence of stomata in the inner surface of the endocarp is a feature shared by the monophyletic clade formed by *Ardisia* and *Stylogyne*, and they have evolved independently in *Jacquinia* (Fig. 10c). The reticulated pattern in the inner surface of the endocarp was found in the woody Myrsinoideae and *Jacquinia* (Theophrastoideae) (Fig. 10d).

### Discussion

The obtained results proved that seeds of woody Myrsinoideae and Theophrastoideae differed to some extent according to the type of hilum, the seed outline, the presence or absence of crystals in the seed coat, the testa structure, and its thickness. The results concerning the depressed hilum found in all the examined species did not support the previous statement of Anderberg and Ståhl (1995) that a concave hilum distinguishes most of the Myrsinoideae and Theophrastoideae. According to Morozowska et al. (2011), in herbaceous Myrsinoideae, a concave hilum was not always observed. The membranous remnants of the placenta surrounding the matured seeds of Myrsinoideae are in agreement with the earlier findings according to which the ovules of Myrsinoideae are more or less deeply immersed in the placenta (Anderberg and Ståhl 1995; Otegui and Cocucci 1999; Ma and Saunders 2003). Our results supported also the suggestion of Wanntorp et al. (2012) that the ovule embeddedness in the placental column appears to be a synapomorphy for Myrsinoideae. With reference to Theophrastoideae, some different opinions concerning the embeddedness of the ovules in the placenta are known. Caris et al. (2000) and Ma and Saunders (2003) proved that the ovules were not embedded in the placenta. According to Anderberg and Ståhl (1995), the mature seeds were more or less surrounded by the placental pulp and that was confirmed in the present work according to *Jacquinia armillaris*.

Regarding the seed outline, the ruminate seeds may be considered a synapomorphy for *Myrsine*. In the examined *Ardisia* and *Geissanthus* species, the ruminate seeds were typical only in some of them, and no ruminations were observed in *Jacquinia armillaris*. Earlier such seed indentations were found in *M. laetevirens* by Otegui et al. (1998a), who performed structural and complementary chemical studies on specialized secretory structures producing benzoquinones in *M. laetevirens* and proved that the crystals found inside the indentations contain rapanone as the main constituent. Otegui et al. (1998a) suggested that crystals of hydroxybenzoquinones on the seed surface might serve as inhibitors of germination.

Among the widely distributed mineral depositions in plants, calcium oxalate (CaC$_2$O$_4$) is one of the most common salts. It has been observed in most plant tissues and organs as an intracellular or extracellular deposit. A great diversity of their potential functions and morphology has been described. The crystals found in cell walls were

### Table 4 Micromorphological and anatomical characters of endocarps in Myrsinoideae and Theophrastoideae species examined

| Species            | No of sclerenchyma cell layers | Inner surface                                                                 | Secondary sculpture                                                                 |
|--------------------|-------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| *Ardisia crenata*  | 1                             | Polygonal/raised/flat                                                        | Smooth                                                                           |
| *Ardisia humilis*  | 1 (with crystals)              | Polygonal/raised/flat                                                        | Smooth                                                                           |
| *Ardisia sieboldii*| 1                             | Polygonal/raised/concave                                                     | Micro-reticulate/foveolate                                                      |
| *Ardisia solanacea*| 1                             | Polygonal/raised/flat                                                        | Smooth                                                                           |
| *Myrsine africana* | 2–3                           | Polygonal/raised/flat                                                        | Smooth                                                                           |
| *Myrsine coriacea* | 3–4                           | Polygonal/raised/flat                                                        | Verrucose                                                                        |
| *Myrsine gardneriana* | 1–2                       | Polygonal/raised/concave                                                     | Foveolate                                                                        |
| *Myrsine guianensis* | 3–4                         | Polygonal/pressed/flat                                                       | Foveolate                                                                        |
| *Myrsine lineata*  | 2–3                           | Polygonal/raised/concave                                                     | Micro-reticulate/foveolate                                                      |
| *Myrsine parvula*  | 2                             | Polygonal/raised/flat                                                        | Smooth                                                                           |
| *Myrsine umbellata*| 3                             | Rounded/pressed/convex                                                        | Verrucose                                                                        |
| *Myrsine venosa*   | 3                             | Polygonal/pressed/flat d                                                     | Foveate                                                                          |
| *Myrsine wightiana*| 4–5                           | Polygonal/pressed/flat                                                        | Smooth                                                                           |
| *Geissanthus ambiguus* | 1                         | Polygonal/raised/concave                                                     | Foveolate                                                                        |
| *Stylogyne pauciflora* | –                          | Rounded/flat/concave                                                         | Verrucose                                                                        |
| *Jacquinia armillaris* | –                          | Polygonal-rounded/raised/flat                                                 | Striate                                                                           |
Fig. 2 SEM micrographs of endocarp inner surface morphology and anatomy (cross-sections) of *Myrsine*. a, b *Myrsine parvula*, a two layers of slightly elongated sclerenchyma cells with numerous pits and lamellar structure of sclerenchyma secondary cell walls (arrow), b reticulate cellular pattern, raised anticlinal cell wall boundaries, flat periclinal walls; c, d *M. venosa*, c three layers of almost equally dimensional sclerenchyma cells orientated either radially or tangentially (arrows), d reticulate cellular pattern, depressed anticlinal cell wall boundaries, flat periclinal walls, foveate secondary sculpture; e, f *M. lineata*, e two–three layers of more or less elongated sclerenchyma cells with numerous pits, f reticulate cellular pattern, raised anticlinal cell wall boundaries, concave periclinal walls, micro-reticulate/foveolate secondary sculpture; g, h *M. coriacea*, g three-four layers of slightly elongated or equally dimensional sclerenchyma cells with numerous pits, h reticulate cellular pattern of elongated cells, raised anticlinal cell wall boundaries, flat periclinal walls, verrucose secondary sculpture; i, j *M. unbellata*, i three layers of slightly elongated sclerenchyma cells with numerous pits; j reticulate cellular pattern, depressed anticlinal cell wall boundaries, convex periclinal walls, verrucose secondary sculpture; k, l *M. guianensis*, k three-four layers of slightly elongated sclerenchyma cells of radial or sometimes tangential orientation (arrows), with numerous pits, l reticulate cellular pattern, depressed anticlinal cell wall boundaries, flat periclinal walls, foveolate secondary sculpture; m, n *M. africana*, m two–three layers of almost equally dimensional sclerenchyma cells, n reticulate cellular pattern, raised anticlinal cell wall boundaries, flat periclinal walls; o, p *M. wightiana*, o four-five layers of more or less equally dimensional sclerenchyma cells orientated radially and tangentially, p reticulate-striated cellular pattern, raised anticlinal cell wall boundaries, flat periclinal walls; r–u *M. gardneriana*, r fragment of fruit and seed cross-section with seed indentation, endosperm tissue, remnants of the placenta, endocarp and mesocarp, s crystals in seed indentation, enlargement of r, t one layer of elongated sclerenchyma cells with numerous pits, u inner surface with reticulate-striated cellular pattern, raised anticlinal cell wall boundaries, concave periclinal walls, foveolate secondary sculpture.
rhombohedral or prismatic (Franceschi and Horner 1980; Franceschi and Nakata 2005). The presence of oxalate crystals on the surface of the inner seed coat layer was described by Oh et al. (2008) in Lysimachia fordiana, Anagallis minima, and A. arvensis seeds. Morozowska et al. (2011) showed that oxalate crystals were present both in angular and subglobose seeds of Primulaceae. The occurrence of prismatic crystals in Jacquinia armillaris confirms the presence of crystals in subglobose seeds.

The presented results proved that the examined woody Myrsinoideae had a one-layered seed coat, which might suggest that their ovules were unitegmic. In the earlier studies, however, bitemgic ovules were described both in woody and in herbaceous Myrsinoideae (Corner 1976; Morozowska et al. 2011). With reference to Theophrastoideae, the presence of the seed coat differentiated into two layers may confirm the occurrence of bitemgic ovules in J. armillaris (Corner 1976; Anderberg et al. 2002).

Fig. 3 Stereo microscope images of fruits, endocarps, and seeds of Ardisia, Myrsine, Geissanthus, Stylogyne, and Jacquinia. a, b Ardisia humilis, a globose endocarp with longitudinally arranged vessel elements present on the surface, b seed partly covered by endocarp, with surface indentations filled with orange crystals and concave hilum (arrow); c–e A. solanacea, c globose endocarps with surface longitudinally striated by vessel elements, d seed with surface indentations filled with orange crystals and remnants of the placenta, e seed with depressed hilum (arrow), seed indentations, endosperm, and embryo in the middle (cross-section); f, g A. crenata, f seed in the partly opened endocarp with longitudinally arranged vessel elements on the surface, g seed with depressed hilum (arrow), endosperm, embryo, and remnants of the placenta on the surface (longitudinal section); h Myrsine venosa seed with depressed hilum (arrow), seed indentations filled with orange crystals, endosperm, and embryo in the middle (cross-section); i M. parvula endocarp and seed with orange surface indentation, endosperm, and embryo visible (cross-section); j M. wightiana seed with concave hilum area (arrow) and numerous seed indentations filled with orange crystals; k–m Geissanthus ambigua, k globose dry fruits, l globose seeds with elliptic, depressed hilum (arrow), partly covered by remnants of the placenta, m seed with concave hilum area (arrow), endosperm and remnants of the placenta (cross-section); n, o Stylogyne pauciflora, n globose dry fruits, o globose seed with elliptic, depressed hilum (arrow), partly covered by remnants of the placenta; p Jacquinia armillaris seeds surrounded by the placenta pulp.
Primulaceae are predominantly characterized by possessing from several to many tenuinucellate, usually anatropous, bitegmic ovules in which the micropyle is formed from both integuments (Anderberg and Ståhl 1995; Källersjö et al. 2000; Anderberg et al. 2002; Stevens 2001 onwards; Wanntorp et al. 2012). However, Otegui and Maldonado (1998) and Otegui et al. (1998b) showed that Myrsine laetivirens (Myrsinoidae) has unitegmic ovules, the seed coat develops from a single integument, and it lacks a mechanical layer and consists of the outer epidermis and several underlying collapsed layers. In an earlier study, Anderberg and Ståhl (1995) reported that unitegmic ovules are also typical of herbaceous Cyclamen (Primuloidae), while earlier only the genus Aegiceras (Myrsinoidae) was described as having unitegmic ovules (Corner 1976; Philipson 1974). According to Corner (1976), Myrsinaceae (including Theophrastaceae) and Primulaceae have both bitegmic and unitegmic ovules, whereas among Myrsinaceae the ‘uni-integument’ ovules are typical only of the genus Aegiceras Gaertn. In the opinion of Corner (1976), the existence of pairs of taxa with bitegmic-unitegmic ovules suggests that there have been parallel lines of unitegmic evolution within dicotyledons. The same author concluded that unitegmic ovules were derived from bitegmic ones, thus unitegmy is clearly secondary and polyphyletic. Considering the literature data, the suggestion concerning the presence of the unitegmic ovules in all Myrsinoideae species examined in the presented work needs to be confirmed in detailed embryological and anatomical studies based on extensive sampling of Myrsinoideae species.

Fig. 4 SEM micrographs of seed cross- and longitudinal sections of Ardisia solanacea and Geissanthus ambiguous; a–e A. solanacea, a fragment of seed with surface indentation (cross-section), b fragment of seed with outer (evenly thickened cell walls) and inner (‘pitted’) parts of endosperm (cross-section), c ‘pitted’ inner part of endosperm (longitudinal section), d outer surface of the placenta remnants, e inner surface of the placenta remnants with groups of secretory cells; f–i G. ambiguous, f tuberculate seed coat surface pattern, g fragment of seed with outer and inner parts of endosperm (longitudinal section), h slightly ‘pitted’ endosperm, enlargement of g, i membranous remnants of placenta.
The seed coat sculpture observed on seeds of all the examined species was not specific at the genus or subfamily level; however, some differences concerning primary sculpture were species-specific and may be of taxonomic importance.

Besides the seed morphology and the testa structure, the endosperm tissue was considered in our work. The
‘pitted’ endosperm type was accepted by Anderberg and Ståhl (1995) as typical of the large subglobose seeds in most woody Myrsinoideae and Theophrastoideae genera, but the same authors also confirmed that Myrsine species, e.g., *M. africana*, are an exception to that rule, as they have an endosperm tissue with evenly thickened smooth cell walls. The presented results are in agreement with these findings with reference to all the examined *Ardisia*, *Geissanthus*, and *Jacquinia* species. However, surprisingly in seeds of *M. africana*, the ‘pitted’ type of the endosperm was present, similarly as in seeds of *M. laetevirens* examined by Otegui et al. (1998b). The same authors suggested that when the cell walls are thicker, the pits may be more conspicuous and that may be why some other *Myrsine* species have endosperm cells with smooth cell walls. In *M. africana*.

Table 5  Seed morphological and anatomical characters of the Myrsinoideae and Theophrastoideae species examined

| Species                | Hilum shape/seed outline | Seed coat                                                                 | Outer pericylinal walls | Structure** | Endosperm   | Differentiation** |
|------------------------|--------------------------|--------------------------------------------------------------------------|--------------------------|-------------|-------------|------------------|
| *Ardisia crenata*      | Depressed/smooth         | Polygonal/raised, straight                                              | Flat/little concave     | 1/−         | Evenly thickened-‘pitted’ cell walls | –                |
| *Ardisia humilis*      | Depressed/ruminate       | Polygonal/raised, straight or undulate                                   | Concave                  | 1/−         | ‘Pitted’    | +                |
| *Ardisia sieboldii*    | Depressed/ruminate       | Polygonal/depressed, straight or curved                                   | Convex                   | 1 –         | ‘Pitted’    | –                |
| *Ardisia solanacea*    | Depressed/ruminate       | Polygonal/straight                                                       | Concave                  | 1/−         | ‘Pitted’    | +                |
| *Myrsine africana*     | Depressed/ruminate       | Polygonal/raised, straight                                               | Concave                  | 1/−         | ‘Pitted’    | +                |
| *Myrsine coriacea*     | Depressed/ruminate       | Polygonal/raised, straight                                               | Flat                     | 1/−         | Smooth evenly thickened cell walls | –                |
| *Myrsine gardneriana*  | Depressed/ruminate       | Polygonal/raised, straight                                               | Convex                   | 1/−         | Smooth evenly thickened cell walls | –                |
| *Myrsine guianensis*   | Depressed/ruminate       | Polygonal/raised, straight                                               | Flat with secondary striations | 1/−     | Papery thin cell walls with helical thickenings | –                |
| *Myrsine lineata*      | Depressed/ruminate       | Polygonal/raised, straight                                               | Concave                  | 1/−         | Papery thin cell walls | –                |
| *Myrsine parvula*      | Depressed/ruminate       | Rounded/depressed, straight or curved                                    | Convex                   | 1/−         | ‘Pitted’    | +                |
| *Myrsine umbellata*    | Depressed/ruminate       | −                                                                         | −                        | 1/−         | Smooth evenly thickened cell walls | –                |
| *Myrsine venosa*       | Depressed/ruminate       | Rounded/depressed, straight or curved                                     | Convex                   | 1/−         | Smooth evenly thickened cell walls | –                |
| *Myrsine wightiana*    | Depressed/ruminate       | Three-, tetragonal/raised, straight or curved                             | Concave                  | 1/−         | Smooth evenly thickened cell walls | –                |
| *Geissanthus ambiguus* | Depressed/smooth         | Rounded/depressed, straight or curved                                    | Convex                   | 1/−         | ‘Pitted’    | +                |
| *Stylogyne pauciflora* | Depressed/smooth         | −                                                                         | −                        | 1/−         | −           | −                |
| *Jacquinia armillaris* | Depressed/Smooth         | Raised/straight                                                           | Flat/little concave      | 2+          | ‘Pitted’    | +                |

*Seed coat structure either: one-layered = 1; or two-layered = 2; **Differentiation of an endosperm tissue into the outer and inner regions: (+) presence/ (−) absence*
africana seeds, the endosperm cell walls were very thick and maybe for that reason the pits were so clearly visible and the obtained results differed from those of Anderberg and Ståhl (1995). Other differences observed in the endosperm tissue concerned the presence of cells with papery thin cell walls, sometimes with helical thickenings on their inner surface. Such type of an endosperm was present in seeds of M. lineata or M. guianensis. A similar type of the endosperm was already described by Morozowska et al. (2011) in seeds of the herbaceous Lysimachia nemorum (Myrsinoideae). Furthermore, the heterogeneity of endosperm observed in some Myrsine was similar to the differentiation of the endosperm found in M. laetevirens by Otegui et al. (1998b), who additionally confirmed the deposition of different storage materials in different parts of the endosperm.

All the described differences concerning the endosperm seem to suggest that seeds of Myrsine species are not as uniform in terms of tissue type (‘pitted’ vs. ‘with evenly thickened and smooth cell walls’) as it was thought previously. However, to verify this assumption, seeds of more Myrsine species need to be examined.

To date, endocarp morphology and anatomy in Myrsinoideae and Theophrastoideae have not been extensively studied (Morozowska et al. 2013; Otegui et al. 1998b), thus most of the presented results are new to science. The most important findings concern the microornamentation pattern found on the inner surface of the endocarp. It was reticulate for all of the examined Myrsinoideae; however, differences concerning the details of the primary and secondary sculpture in particular species are taxonomically important and may be useful in the identification of living and fossil plant materials. The presence of stomata observed on the inner surface of endocarps in Ardisia humilis, Geissanthus ambigus, Stylogyne pauciflora, and Jacquinia armillaris were not described before. The occurrence of stomata in fruits or seeds, especially in places where the light has either very limited or no access at all, was rarely described (Rugenstein and Lersten 1981; Paiva et al. 2006; Zielinski and Tomaszewski 2010). With reference to Paiva et al. (2006), stomata present on seeds may be involved in water intake during the first phase of seed germination. According to Zielinski and Tomaszewski (2010), who described the presence of stomata on the pericarp of roses, a similar explanation is difficult to accept as the pericarp is thick and the cells are strongly lignified. The endocarps of Myrsinoideae also consist of thick-walled and strongly lignified cells, while the observed stomata, similarly as those in Jacquinia fruits, were located in places with no access to light. In that case, the main function of stomata concerning their participation in photosynthesis, transpiration, and gas exchange is difficult to explain and the role of the observed stomata needs further studies. Despite the lack of an unambiguous explanation of stomata function, their presence in the endocarps of Myrsinoideae and Theophrastoideae is taxonomically important.

In conclusion, few new characters concerning the fruit and seed morphology of Myrsinoideae and Theophrastoideae were described. With reference to endocarps, the variation within the primary and secondary sculpture of their inner surface and the presence of stomata in some species may have systematic implications. The one-layered seed coat

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**Fig. 7** SEM micrographs of Jacquinia armillaris, a–d seed cross-sections; e–h endocarp morphology. a Fragment of seed with endosperm tissue differentiated into outer and inner parts and remnants of the placenta on the surface, b ‘pitted’ endosperm, enlargement of a, c prismatic crystals (arrows) on the seed coat surface, d two-layered seed coat with rhomboid crystal (arrow), e reticulate cellular pattern on pericarp inner surface with stomata (arrows), f enlargement of e, g single cell with raised anticlinal cell wall boundaries and secondary cuticle striations, enlargement of f, h stomata, enlargement of e.
observed in all examined Myrsinoideae species may be a synapomorphy of that subfamily. The systematic value of the 'pitted' endosperm previously accepted as the feature typical for subglobose seeds of all Myrsinoideae and Theophrastoideae was not confirmed. Similarly, the selective occurrence of the ruminate endosperm in examined Myrsinoideae suggests that this feature does not differentiate completely subfamilies Myrsinoideae and Theophrastoideae. Here, we presented the optimization of seed and endocarp characters for the first time and discussed them in a broad context. However, further morphological and anatomical studies encompassing more species from other genera of Primulaceae, especially Maesoideae, are needed to understand the importance of these characters to the family. In this sense,
we hope that the results of our future studies, together with the results of monographic treatment of the genus *Myrsine* undertaken by Otegui et al. (1998b), will provide more insight into the phylogeny of that large and heterogeneous family.
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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals Not applicable.

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Information on Electronic Supplementary Material

Online Resource 1. Genebank accessions.
Online Resource 2. SEM micrographs of seed cross- and longitudinal sections of Myrsine parvula, M. guianensis, M. venosa, and M. wightiana.

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