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Gynoecium and fruit histology, structure and development in corky-warted representatives of Livistoninae (Trachycarpeae: Coryphoideae: Arecaceae)

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Representatives of three genera of Livistoninae (*Johannesteijsmannia*, *Licuala* and *Pholidocarpus*) develop corky-warted fruits in contrast to fruit with smooth surfaces in most other representatives of the 'apocarpous clade' of Arecaceae subfamily Coryphoideae. The present developmental study is focused on revealing the anatomical peculiarities of the fruit structure of corky-warted species of Livistoninae and tracing the development of their pericarp. Our study shows that the fruits of *Johannesteijsmannia, Licuala bintulensis* and *Pholidocarpus macrocarpus* are drupes of the *Rhapis* type and that the warts on their fruit surface originate soon after gynoecium pollination as the result of two or three developmental events: (1) suberinization of the exocarp cells; (2) progressive multiplication and growth of the outer zone of the mesocarp; and (3) cracking of the peripheral zone of the pericarp. The warts of taxa of Livistoninae develop at the mid stages of fruit organogenesis and are referred to as either alive, composed of parenchymatous cells and sclereids (species of *Licuala* and *Pholidocarpus*), or dead, consisting of cells with suberized walls and sclereids (*Johannesteijsmannia*). The nests of sclereids comprising the stone of *Johannesteijsmannia* spp. remain disunited during pericarp development until late developmental stages when the formation of a continuous sclerenchymatous layer occurs, which differs from other representatives of the 'apocarpous clade' of Coryphoideae with a continuous layer of sclereids persisting during fruit development (as in *L. bintulensis*). It is shown that the development of the stone within the drupes of the *Rhapis* type can be different even in closely related taxa. The recognition of the fruits of studied taxa of Livistoninae as drupes of the *Rhapis* type like the fruit of many other taxa of Trachycarpeae and the 'apocarpous clade' of Coryphoideae suggest this character as a synapomorphy for this tribe and the whole clade.

ADDITIONAL KEYWORDS: drupe of *Rhapis* type – exocarp – palms – pericarp cracking the organogenesis of the stone – pericarp histogenesis.

INTRODUCTION

Arecaceae subtribe Livistoninae comprise six genera of palms distributed in South-East Asia, Taiwan, Papuasia, northern, central and eastern Australia, New Caledonia and the New Hebrides and one species in South Arabia and the Horn of Africa (Dransfield, 1970; Moore, 1973; Dransfield & Uhl, 1983; Saw, 1997; Hodel & Pintaud, 1998; Rodd, 1998; Dransfield *et al.*, 2008; Henderson, 2009, 2016; Baker & Dransfield, 2016). *Johannesteijsmannia* H.E.Moore is sister to *Lanonia* A.J.Hend. & C.D.Bacon (a segregate of *Licuala* Wurmb *s.l.*) with moderate to strong support, and they in turn are sister to *Licuala*. *Livistona* R.Br., *Pholidocarpus* Blume and *Saribus* Blume (a segregate of *Livistona s.l.*) comprise another branch of sister genera in this subtribe (Dransfield *et al.*, 2008; Bacon & Baker, 2011; Henderson & Bacon, 2011; Bacon *et al.*, 2012; Baker & Dransfield, 2016). Previously, *Johannesteijsmannia*

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used to be placed as sister to *Licuala* (Uhl *et al.*, 1995) or *Pholidocarpus* with low support in Livistoninae (Asmussen *et al.*, 2006; Baker *et al.*, 2009), whereas *Livistona* was recognized as sister to the remaining genera of Livistoninae with lower support.

Livistoninae are nested in Trachycarpeae, a tribe comprising 19 genera of coryphoid palms (Arecaceae subfamily Coryphoideae) in total (Dransfield *et al.*, 2008; Baker & Dransfield, 2016). Trachycarpeae, with three other tribes (Phoeniceae, Sabaleae and Cryosophileae), comprise the 'apocarpous clade' of Coryphoideae, sister to the 'syncarpous clade', comprising tribes Chuniophoeniceae, Caryoteae, Corypheae and Borasseae (Dransfield *et al.*, 2008; Baker & Dransfield, 2016).

The representatives of three genera of pleonanthic understory palms in Livistoninae (all *Johannesteijsmannia* spp., most *Pholidocarpus* spp. and two *Licuala* spp.) develop corky-warted fruits. *Johannesteijsmannia* and *Pholidocarpus* are oligotypic genera of South-East Asian palms, whereas *Licuala* is a large genus broadly distributed in South-East Asia, Papuasia, north-eastern Australia and the New Hebrides. *Johannesteijsmannia* spp. are acaulescent or short-trunked palms with large diamond-shaped undivided leaves, rather short interfoliar inflorescences and infructescences of up to several dozens of large corky-warted fruits. *Pholidocarpus* includes robust single-stemmed tree palms with costapalmate leaves, interfoliar inflorescences and infructescences with several large corky-warted fruits (one species, *Pholidocarpus kingianus* (Becc.) Ridl., has smooth fruit). *Licuala* comprises small to moderate-sized acaulescent to one-stemmed or clustered pleonanthic palms with palmate (in many species undivided) leaves, shortened to elongated interfoliar inflorescences, and infructescences with few to many small fruits (usually fleshy to juicy, but corky-warted in *Licuala bintulensis* Becc. and *Licuala bruneiana* L.G.Saw). The common features of all taxa of Livistoninae are free ovaries and the stylodia fused into a common style. In most representatives of the subtribe, only one carpel develops into a fruit, in some taxa di- and trimerous fruits develop from time to time in addition to monomerous ones in the same infructescence (Hooker, 1883; Drude, 1887; Beccari, 1933; Morrow, 1965; Uhl & Moore, 1971; Moore, 1973; Uhl & Dransfield, 1987; Dransfield *et al.*, 1990; Dransfield & Uhl, 1998; Saw *et al.*, 2003; Dransfield *et al.*, 2008; Giddey *et al.*, 2009; Rudall *et al.*, 2011).

The flowers of four *Johannesteijsmannia* spp. are similar in structure and only differ slightly in the degree of fusion of the carpels (Dransfield, 1970). The flowers are built up on the basic trimerous symmetry of 3-3-6-3, and during their development the carpels originate as free crescent-shaped primordia which grow at the apex and produce three closely appressed vertical extensions, the stylodia. The epidermal cells of three separate carpels interdigitate at first, and then the tissues of the three separate carpels become joined as the result of meristematic activity (postgenitally fused). This connation first occurs at the tip of the carpels, forming a common style, and extends basipetally. The degree of connation varies from species to species and sometimes, within species, from flower to flower. Externally, the gynoecium appears to consist of a deeply three-grooved ovary and a common style. No nectaries are observed in the flowers of *Johannesteijsmannia* spp. (Dransfield, 1970). The mode of flower development in the studied *Licuala* spp. is generally similar to *Johannesteijsmannia* (Rudall *et al.*, 2011), whereas data on *Pholidocarpus* flower development are lacking. Two types of infralocular nectaries are described in two *Licuala* spp.: a short triradiate septal nectary located below the level of perianth insertion in *Licuala grandis* H.Wendl. (Rudall *et al.*, 2011); and a peculiar labyrinthine nectary in *Licuala peltata* Roxb. ex Buch.-Ham. (Stauffer *et al.*, 2009).

Two types of fruits are reported in the investigated representatives of the 'apocarpous clade' of Coryphoideae. The first one is the 'coryphoid fruit' type proposed by Murray (1973) (= the drupe of the *Rhapis* type with a putamen developing in the inner zone of the mesocarp and isolated from the endocarp by a few layers of thin-walled cells; Bobrov & Romanov, 2019). The second fruit type, revealed in the 'apocarpous clade' of Coryphoideae, usually referred to as the apocarpous berry [a berry of the *Schisandra* type according to Bobrov & Romanov (2019)], and is characterized by a fleshy or juicy pericarp and the presence of a continuous thin belt of sclereids located in the peripheral zone of the mesocarp (the belt is isolated from the exocarp by a few layers of thin-walled cells; Biradar & Mahabale, 1969; Landsberg, 1981; Danilova & Savchenko, 1985; Bobrov *et al.*, 2008b, unpublished data).

Based on the exomorphic features the dull (grey or chestnut-brown), mostly large corky-warted fruits in representatives of Livistoninae (*Johannesteijsmannia*, *Pholidocarpus* and *Licuala*) differ from the fruits with smooth surface in most other taxa of Coryphoideae, except some species of *Chelyocarpus* Dammer (Cryosophileae) with corky-warted fruits. One of the aims of the current study is to reveal the anatomical peculiarities of the fruit structure of corky-warted species of *Johannesteijsmannia*, *Licuala* and *Pholidocarpus* and to trace the development of pericarp structure during fruit ontogenesis. The corky-warted fruits of the three genera of Livistoninae could possibly be plesiomorphic in these three particular genera. Thus, in order to investigate further, the present investigation of representatives of the subtribe with corky-warted fruits was initiated. The study of pericarp histogenesis revealed the principal

structural and diagnostic characters that can be attributed to apomorphic and plesiomorphic states based on the latest phylogenetic scenarios.

Besides Coryphoideae taxa, corky-warted fruits are also found in Ceroxyloideae tribe Phytelepheae and in Arecoideae (*Manicaria* Gaertn., *Pelagodoxa* Becc., *Sommieria* Becc. and *Lemurophoenix* J.Dransf., Dransfield, 1970; Moore, 1973; Uhl & Dransfield, 1987; Chapin *et al.*, 2001; Dransfield *et al.*, 2008; Bobrov & Romanov, 2019). The irregularity of the presence of corky-warted fruits in palms highlights the need for an investigation of the histogenesis of their pericarp structure and comparison with each other and other fruit types in Arecaceae. The potential specific functions of the corky-warted fruits in palms are also of particular interest.

MATERIAL AND METHODS

Flowers and fruits of *Johannesteijsmannia* (Table 1) were studied at eight developmental stages (Table 2). Fresh flowers and fruits of *Johannesteijsmannia* fixed in FAA (formaldehyde:alcohol:acetic acid = 10%:50%:5% + 35% water) and stored in ethanol (70%) were used for this study. All materials were embedded in paraffin before sectioning. The histological investigation of gynoecium structure and fruit development in *Johannesteijsmannia* (Table 2) was carried out by J.D. in 1967–1969 as a part of his PhD thesis (Dransfield, 1970). The histology of the pericarp of *J. lanceolata* J.Dransf. and *L. bintulensis* Becc. at the late developmental stage and mature fruits of *J. altifrons* (Rchb.f. & Zoll.) H.E.Moore, *J. lanceolata*, *Licuala bintulensis* and *Pholidocarpus macrocarpus* Becc. have been recently studied by M.S.R. and A.V.F.CH.B (Tables 1 and 2). Transverse sections (TS) of flowers and fruiting carpels of *Johannesteijsmannia* spp., 20–30 μm thick made with a sliding microtome were treated by J.D. with safranin (to reveal the details of cell wall suberinization), or ruthenium red and toluidine blue (to reveal the details of cell wall structure) and the sections were preserved in Canada balsam. Sections of *J. altifrons*, *J. lanceolata*, *L. bintulensis* and *P. macrocarpus* fruits were treated by M.S.R. and A.V.F.CH.B. with phloroglucinol and hydrochloric acid (to reveal the lignification of cell walls in different topographical zones of the fruit wall), or stained with safranin (to reveal the details of cell wall suberinization), and preserved in glycerin (Prozina, 1960; O'Brien & McCully, 1981). All microtome sections were studied with a Leica DM1000 light microscope and a Zeiss Axio Imager A1 microscope. Photographs of the sections were made with a Canon EOS D6 digital camera, connected to the microscope via an adapter. The carpological terminology is used according to Bobrov and Romanov (2019) and the following indehiscent fruit types are discussed in the text below:

The drupe of the *Rhapis* type is an apocarpous fruit with a sclerenchymatous layer developing in the inner zone of the mesocarp and isolated from the endocarp by a few layers of thin-walled cells. The berry of the *Schisandra* type is an apocarpous fruit without a continuous sclerenchymatous layer in the pericarp. The pyrenarium of the *Butia* type is a coenocarpous fruit with a sclerenchymatous layer localized in the inner zone of the mesocarp and the endocarp. The

Table 1. Specimens of Livistoninae investigated

Johannesteijsmannia altifrons. Developmental stage 1, 3-8, *J. Dransfield, s.n.*, liquid-fixed material, Sungei Lalang Forest Reserve, Selangor, Malaysia, October, 1967;
Johannesteijsmannia altifrons. Developmental stage 8, *M.S. Romanov, A.V. Bobrov, # 1256*, Harold L. Lyon Arboretum, University of Hawaii, Hawaii, USA, liquid-fixed material, November, 18, 2008.
Johannesteijsmannia magnifica. Developmental stage 1-8, *J. Dransfield, s.n.*, liquid-fixed material, Sungei Lalang Forest Reserve, Selangor, Malaysia, November 1967 – September 1968.
Johannesteijsmannia lanceolata. Developmental stage 8, *J. Dransfield, s.n.*, liquid-fixed material, Sungei Lalang Virgin Jungle Reserve, Selangor, Malaysia, November 1967 – September 1968;
Johannesteijsmannia lanceolata. Developmental stage 7. *M.S. Romanov, A.V. Bobrov, # 1766*, Rimba Ilmu Botanical Garden, Malaysia, liquid-fixed material, December, 17, 2010.
Johannesteijsmannia perakensis. Developmental stage 8, *J. Dransfield, s.n.*, liquid-fixed material, Gunong Kledang, Perak, Malaysia, November 1967 – September 1968;
Johannesteijsmannia perakensis. Developmental stage 8, *M.S. Romanov, A.V. Bobrov, # 1594*, liquid-fixed material, Singapore Botanical Garden, Singapore, December, 22, 2009.
Licuala bintulensis. Developmental stages 4, 8, *M.S. Romanov, A.V. Bobrov, # 1604*, Singapore Botanic Gardens, Garden # 00/1139*A, Singapore, liquid-fixed material, December, 22, 2009.
Pholidocarpus macrocarpus. Developmental stage 8, *E.J.H. Corner, # A1*, L.H. Bailey Hortorium (BH), Cornell University, NY, USA

Table 2. The corky-warted species of Livistoninae and the fruit developmental stages studied

Species	Gynoecium (stage 1)	Young fruit (stage 2, 2 weeks after fertilization)	Young fruit (stage 3, 3 weeks after fertilization)	Mid-stage fruit (stage 4, 4 weeks after fertilization)	Mid-stage fruit (stage 5)	Mid-stage fruit (stage 6)	Late-stage fruit (stage 7)	Mature fruit (stage 8)
Johannesteijsmannia altifrons	+		+	+	+	+	+	+
Johannesteijsmannia lanceolata							+	+
Johannesteijsmannia magnifica	+	+	+	+	+	+	+	+
Johannesteijsmannia perakensis								+
Licuala bintulensis					+			+
Pholidocarpus macrocarpus								+

pyrenarium of the *Ilex* type is a coenocarpous fruit with a sclerenchymatous layer localized in the endocarp only.

Figure abbreviations

The following abbreviations are used in the manuscript: a = anther, en = endocarp, ex = exocarp, f = filament, fb = base of the filament, g = gynoecium, ie = inner epidermis, im = inner zone of the mesocarp, lc = locule, m = mesocarp, mm = middle zone of the mesocarp, mp = mesophyll, o = ovule, oe = outer epidermis, om = outer zone of the mesocarp, p = petal, pm = peripheral zone of the mesocarp, s = seed, se = sepal.

RESULTS

Gynoecium morphology and anatomy of *Johannesteijsmannia*

The gynoecium structure at the pre-anthesis stage (stage 1) in *Johannesteijsmannia magnifica* J.Dransf. and *J. altifrons* (Table 2) is similar. The gynoecium of *J. magnifica* comprises three conduplicate carpels with postgenitally fused ventral edges of the ovaries and stylodia fused in the distal part into a common style topped with slightly expanded punctiform stigmas (Fig. 1A). From this trimerous gynoecium of *Johannesteijsmannia* spp., the corky-warted fruits develop (Fig. 1A-M). The smooth ovaries are surrounded by an androecial ring composed of tangentially flattened filament bases (Fig. 2A). Three independent vascular bundles comprise a circle at the TS of the flower below the gynoecium base (Fig. 2B), each vascular bundle vascularizing its own carpel. There is one basal adaxially attached anatropous ovule per carpel, which fills in less than a half of the locule at its base (Fig. 2A, C). At the level of the ovule base the carpels are free on their lateral and abaxial side, whereas they are united on their adaxial side via a small knob of glandular thin-walled cells (Fig. 2C, D), the vestigial floral apex (it is not known whether this remnant has any function in the flower at anthesis). From the level of the middle of the ovule and up to the top of the ovary (Fig. 2E) all three carpels are united with their ventral edges and comprise a cylindrical air cavity in the centre (Fig. 2E-J). The distal part of the locule is free from the ovule and it protrudes into the stylodium as a narrow air canal (Fig. 2H-N). There is a zone of transition of the carpels into stylodia above the ovary level, and the stylodia represent three independent structures tightly attached to each other and contacting through the cuticle of their outer epidermis (Fig. 2L). A little higher, the stylodia become fused with each other and comprise a style (Fig. 2M-P) terminated with a stigma (Fig. 2Q, R). The three independent air canals of the stylodia fuse together in the style and the single air canal opens to the atmosphere at the stigma top (Fig. 2O-R).

In *J. altifrons*, the receptacle below the flower organ attachment level shows a circle of vascular bundles of the stamens and tepals surrounding the three bundles of the carpels (Fig. 3A). The trimerous gynoecium is surrounded by the base of the filaments ring fused with the tepals at the base (Fig. 3B-E). The carpels are free on the abaxial and lateral sides at the level of the ovule base and fused with each other in the centre by the ventral edges up to the base of the style (Fig. 3F-K). The fusion of the carpel ventral edges does not form an air canal in between the carpels as in *J. magnifica* gynoecium (Figs 2F-J, 3E-I). The style of *J. altifrons* comprises three fused stylodia of the carpels with three air canals at the stylodium base (Fig. 3L), which unite into a single triangular air canal

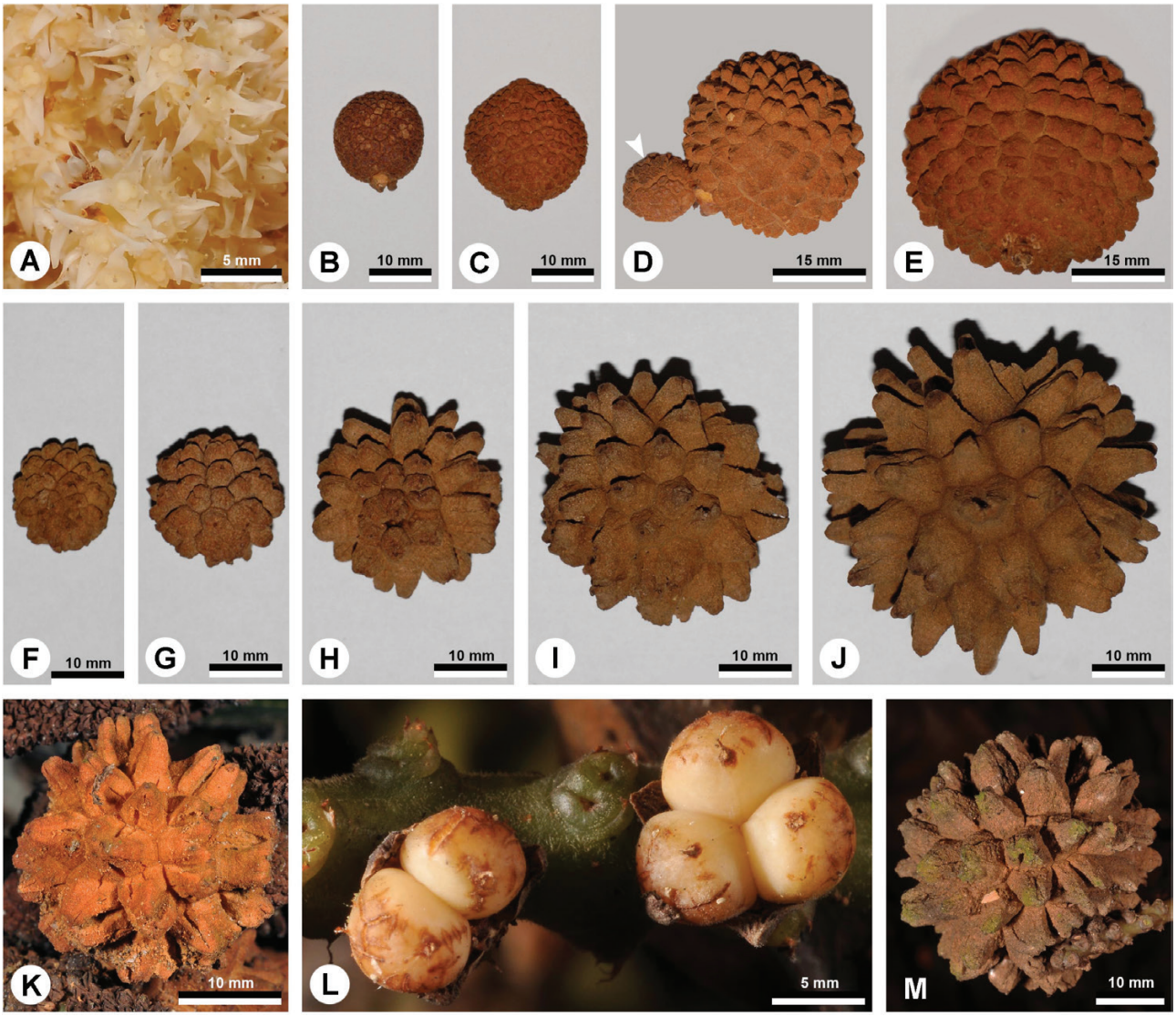

Figure 1. Gynoecium and fruit morphology of *Johannesteijsmannia* species. A-E, *J. magnifica*. A, gynoecium (stage 1). B, mid-stage fruit (stage 4). C, mid-stage fruit (stage 5). D, late-stage fruit (stage 7), note the undeveloped second fruiting carpel (arrow) at the base of the mature fruitlet. E, mature fruit (stage 8). F-J, *J. altifrons*. F, mid-stage fruit (stage 4). G, mid-stage fruit (stage 5). H, mid-stage fruit (stage 6). I, late-stage fruit (stage 7). J, mature fruit (stage 8). K, *J. lanceolata,* mid-stage fruit (stage 7). L, M, *J. perakensis*. L, young-stage fruit (stage 2). M, mature fruit (stage 8).

distally (Fig. 3M, N), which in turn transforms into a tubular canal in the distal part of the style (Fig. 3O).

Fruit morphology of *Johannesteijsmannia*

At the second and the third developmental stages the smooth pale fruiting carpel surface in *Johannesteijsmannia* spp. becomes broken up by discoloured cracks on the dorsal side (at the second stage, Fig. 1L), which (in comparison with ventral and lateral sides) has the priority in growth for a while, and then the carpels become covered with the cracks throughout (stage 3). By stage 4, the fruitlets become spherical, reddish-brown and covered with fine warts (Fig. 1B). Usually only one fruiting carpel of the three carpels comprising the gynoecium reaches maturity with two undeveloped carpels at the base (Fig. 1D), but double and trimerous fruits occur occasionally. Some double fruit may be the result of development of the carpels in two flowers of one partial cincinna.

During stages 5–8 the fruits enlarge proportionally, and the warts on their surface become more prominent. The fruits of *Johannesteijsmannia* spp. differ in number, size and shape of the warts; in *J. magnifica*

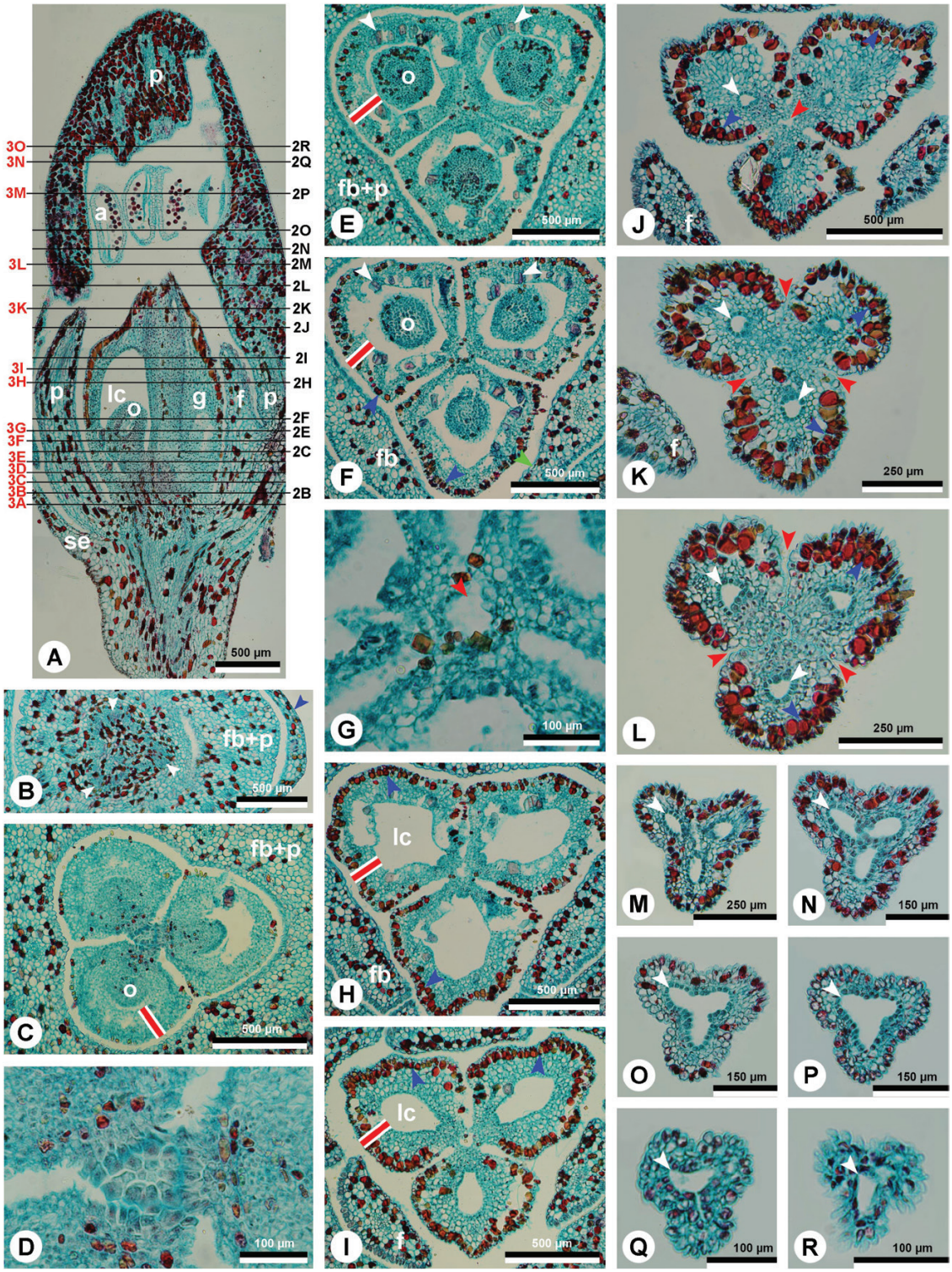

Figure 2. Gynoecium anatomy of *Johannesteijsmannia magnifica*, stage 1, the sections treated with toluidine blue. A, LS of the blossom, horizontal lines indicate the levels of TS of the same gynoecium illustrated on the further figures with numbers corresponding to the black symbols on the right for *J. magnifica* (2B-R) and red symbols on the left for *J. altifrons* (3A-O). B, TS through the base of the flower, note the disposition of carpellar vascular bundles (white arrows) and the sepal (blue arrow). C, D, TS of the gynoecium through the locule base, note the differentiation of the carpel wall (red line), and the

they are numerous, small, low and shaped like a volcano (Fig. 1C-E), whereas in three other species (*J. altifrons*, *J. lanceolata* and *Johannesteijsmannia perakensis* J.Dransf.) there are three to four times fewer warts than in *J. magnifica*, but they are three to four times taller, broader at the base and shaped like narrow cones (Fig. 1F-M). Cracking of *J. magnifica* is originally much finer than in *J. altifrons*, *J. lanceolata* and *J. perakensis*, and thus at maturity there are smaller warts in *J. magnifica* than in other species. The convexly warted fruits of *J. altifrons*, *J. lanceolata* and *J. perakensis* with the narrowly conical warts suggest more periclinal cell division in the corky zone than in the rather low, volcano-shaped warts of *J. magnifica*.

The histogenesis of the gynoecium wall and pericarp in *Johannesteijsmannia*

The histogenesis of fruit wall structure is described below at eight stages of fruit development in *J. magnifica* (Table 2). At the first developmental stage (pre-anthesis gynoecium stage), the carpellary wall comprises eight or nine layers of cells including one layer of smooth outer epidermis (thin-walled cells), six or seven layers of mesophyll and one layer of inner epidermis (thin-walled cells, Fig. 2A, C, E, F, H, I). The mesophyll mostly consists of pale thin-walled cells, but in the peripheral layers of the carpel wall there are numerous scattered tannin-containing thin-walled cells and solitary tannin-containing thick-walled sclereids. Tannin-containing cells are more abundant in the carpel walls at the level of the distal end of the ovary zone and in the stylodium, where they may form up to three peripheral layers of the mesophyll (Fig. 2A, F, H-P). The brachysclereids with thick lignified walls comprise an irregular layer of cells or represent scattered solitary cells located among the parenchymatous cells of two inner mesophyll layers (Fig. 4A, F). These stone cells are mostly located at the level of the ovule at the TS and around the base of the locules.

Differentiation of the carpel wall in *J. altifrons* is similar to *J. magnifica* and comprises ten to 13 cell layers (stage 1, Fig. 3E-H). Some differences are revealed in the structure of the mesophyll which consists of eight to 11 layers of rather small parencxhymatous cells; the cells of the peripheral layers contain tannins (Fig. 3G). On the adaxial faces, the carpels are papillose and individual cells interdigitate between epidermal cells of the next carpel (Fig. 3D-K). Large brachysclereids constitute almost a continuous sclerenchymatous layer in the inner mesophyll and can adjoin to the inner epidermis or be isolated from it by one or two layers of thin-walled cells of the inner mesophyll (Fig. 3G). There are numerous (eight to 13) small regularly disposed protovascular bundles in the centre of the mesophyll, which constitute a circle in the TS in *J. magnifica* and *J. altifrons* (Fig. 3E, G).

At the second stage of development of *J. magnifica* fruit (Fig. 4B-E), the outer and inner one-layered epidermis of the carpel transforms into a one-layered exocarp composed of tannin-containing cells (Fig. 4D) and a one-layered endocarp consisting of pale thin-walled cells correspondingly. On the dorsal side of the fruiting carpel the cellular walls of the exocarp become suberized and due to multiplication and expansion of the mesocarp cells the dorsal fruitlet surface become covered with some cracks. The mesocarp is differentiated into two zones of thin-walled cells (Fig. 4C), the peripheral zone composed of 30–35 layers of large isodiametric cells and the inner zone (ten to 12 layers) made up of small cells with scattered brachysclereids. There are numerous large spherical cells in the peripheral zone of the mesocarp, the walls of the outermost idioblast cells are insignificantly thickened. The cells of the inner layers of the mesocarp and the endocarp are elongated circumferentially. Numerous protovascular bundles are located in the inner part of the peripheral zone of the mesocarp (Fig. 4B, C).

At the third stage of fruiting carpel development, the principal changes occur in the peripheral zone of the mesocarp along the areas of the surface cracking (Fig. 4G-I). The walls of the exocarp cells become suberized all around the fruit and hence the epidermal cells die. Multiplication and expansion of the mesocarp cells causes irregular cracking of the dead exocarp layer

ovule base. D, close-up from (C), note the vestige floral apex in the centre. E, TS of the gynoecium through the centre of the ovule, note the differentiation of sclereids (arrows) in the carpel wall (red line). F, G, TS of the gynoecium through the top of the ovule, note the sclereids (white arrows), tannin cells layer (blue arrows) in the carpel wall (red line), and lateral fusion of the filaments (green arrow). G, close-up from (F), note the fusion of the carpels by their adaxial zones and the central cavity between them (arrow). H, I, TS of the gynoecium over the ovule, note tannin cells layer (arrows) in the carpel wall (red lines). J, TS of the gynoecium through the apical zone of the locule (white arrow), note the distal end of the central cavity (red arrow) and the tannin cells layer (blue arrows). K, L, TS of the gynoecium through the basal zone of the style, note the zones of contact (red arrows) of three independent stylodia with canals inside (white arrows) and tannin cells layer (blue arrows). M, N, TS through the middle zone of the style with three independent canals inside (arrows). O-R, TS through the distal part of the style with single style canals inside (arrows).

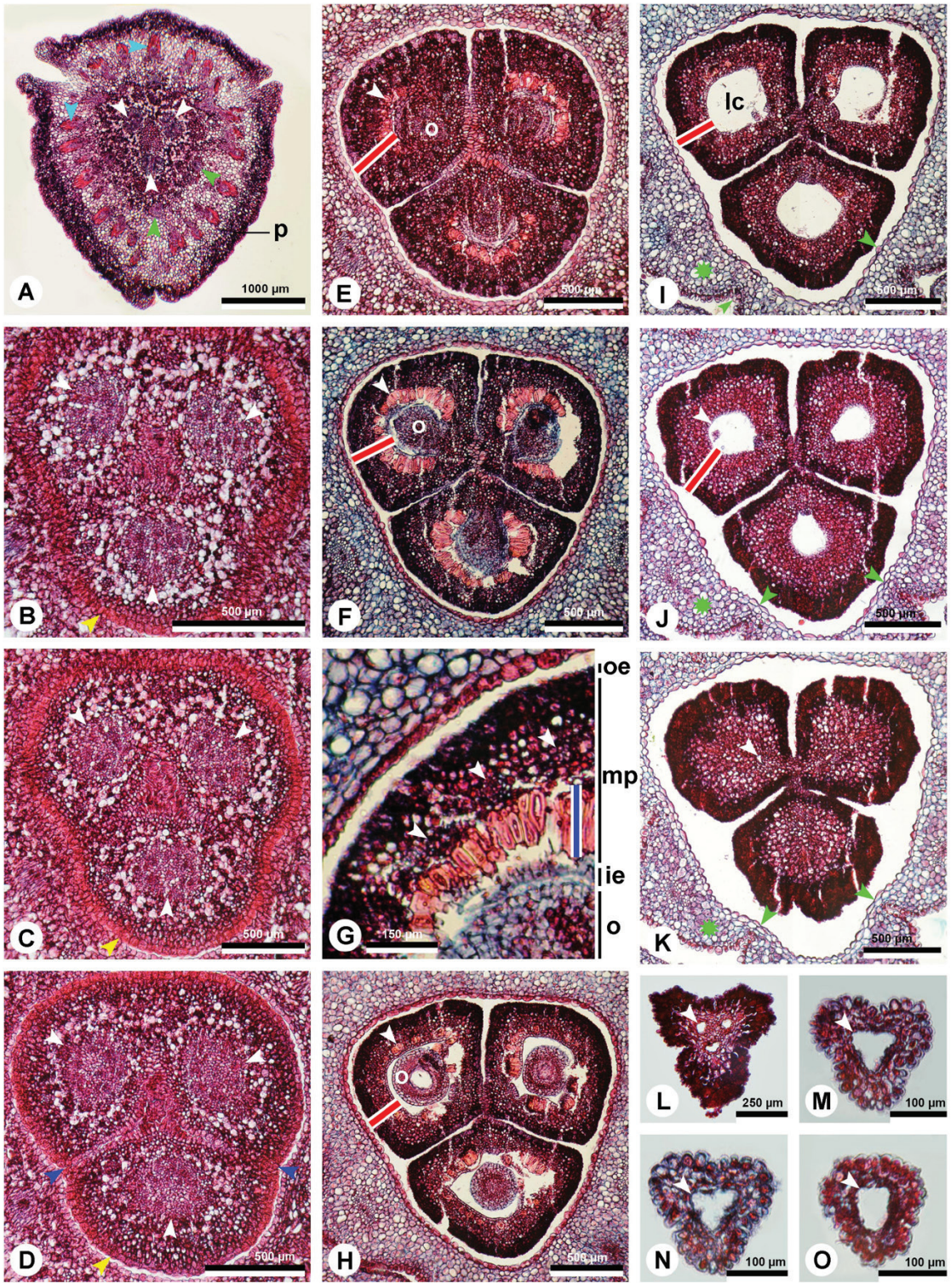

Figure 3. Gynoecium anatomy of *Johannesteijsmannia altifrons*, stage 1, the levels of TS of the gynoecium are indicated with the horizontal lines in Figure 2A and red symbols on the left indicate the figure number corresponding to the TS level. The sections are treated with safranin. A, TS through the base of the petals level, note the disposition of vascular bundles of petals (blue arrows), stamens (green arrows) and carpels (white arrows). B-D, TS of the gynoecium through its base, note the gynoecium outlines (yellow arrows), carpellar vascular bundles (white arrows) and the differentiation of the carpel borders (blue arrows). E, F, TS of the gynoecium through the locule base, note the differentiation of the sclereids (arrows) in

on all fruitlet surfaces. The peripheral zone of the mesocarp described for the previous developmental stage become differentiated into an outer corky zone composed of one to four layers of cells with thin suberized walls and scattered among them sclereids and tannin-containing cells (Fig. 4I) and the middle zone of the mesocarp represented by 22–27 layers of cells. Some of the large idioblast cells revealed earlier in the peripheral mesocarp zone transform into sclereids and now belong to the middle zone of the mesocarp. The cells of the inner zone of the mesocarp and the endocarp do not sufficiently transform. Generally, the anticlinal divisions of pericarp cells predominate over the periclinal ones in the mesocarp (Fig. 4H), and this further increases the tendency for the cracks to deepen.

Further differentiation of the pericarp continues at the fourth developmental stage (Fig. 4J-L). The exocarp is composed of the cells of the outer epidermis covered with a thin cuticle, and it is represented by isolated fragments persisting on the tops of the warts (Fig. 4K). The progressive multiplication and specialization of the cells of the mesocarp results in further differentiation of three topographic zones within it (Fig. 4J). The outer corky zone of the mesocarp comprises at this stage 20–25 layers of mostly thin-walled cells with suberized walls; about 30–40% of cells in this zone contain tannins (Fig. 4K). There are scattered large solitary or groups of two or three thin-walled (proto-)sclereids and sclereids with thickened walls in this zone. The corky zone of the mesocarp develops as the result of ongoing suberinization of its peripheral layers (followed by the consequent death of the cells) and multiplication of cell layers below. The middle mesocarp zone consists of 40–45 layers of thin-walled parenchymatous cells and numerous scattered (sometimes in groups of up to five cells) large (proto-)sclereids with poorly lignified walls and solitary tannin-containing cells (Fig. 4J). The inner zone of the mesocarp comprises ten to 12 layers of small parenchymatous cells with solitary scattered thick-walled brachysclereids and numerous large spherical cells located in the middle of this zone. The endocarp structure is similar to the previous stages (Fig. 4L).

At the fifth developmental stage, the exocarp fragments and outer layers of the mesocarp tend to be rubbed off (Fig. 4M, N). The thickness of the outer corky zone of the mesocarp increases up to 40 layers of cells with suberized walls and contains tannins (in more than 40% of cells, Fig. 4M). There are groups of up to seven to nine large thin-walled sclereids among the ground tissue of the outer mesocarp zone. The middle zone of the mesocarp consists of 55–60 layers of (Fig. 4M), and the inner zone of the mesocarp comprises *c*. 15 layers of small parenchymatous cells bordering the irregular belt of spherical sclereids with thickened walls in the centre of this zone (Fig. 4N). The belt of the sclereids is irregular and it is interrupted with inclusions of parenchymatous cells. The endocarp consists of a single layer of thin-walled rectangular cells (Fig. 4N).

By the sixth developmental stage, the histological structure of the mesocarp does not change significantly, whereas the thickness of its zones changes (Fig. 5A). The corky zone may reach 60 layers of cells, and there are numerous large thin-walled sclereids in it (mostly in small groups, Fig. 5A, B). The middle zone of the mesocarp comprises *c.* 35 layers of small thin-walled cells (including the tannin-containing ones) and scattered sclereids (Fig. 5A). The inner mesocarp zone comprises a discontinuous belt of brachysclereids with heavily thickened walls (Fig. 5C). The thin-walled cells of the endocarp are elongated circumferentially (Fig. 5C).

At the seventh developmental stage, the principal events of pericarp differentiation take place in the inner zone of the mesocarp (Fig. 5D-F). The thickness of this zone is 20–23 layers of cells, and it becomes differentiated into a discontinuous layer of brachysclereids in the centre surrounded by thin-walled cells. This layer comprises nests of brachysclereids, which are isolated from each other by undifferentiated thin-walled cells, which consequently differentiate into brachysclereids. These undifferentiated cells between the sclereids can divide periclinally. The sporadic differentiation of brachysclereids allows expansion of the seed chamber which occur through this and earlier developmental stages.

At the mature fruit stage (stage 8), the pericarp of *J. magnifica* reaches its final differentiation (Fig. 5G-I). The exocarp is rubbed off from the tops of the warts. The mesocarp is differentiated into an outer corky zone (up to 60 layers of cells in the warts), a middle parenchymatous zone (30–40 layers of cells) and an inner zone (*c*. 25 layers of cells) comprising the stone

the carpel wall (red lines), and the ovule base. G, close-up from (C), the gynoecium wall structure, note the vascular bundles (arrows) and the sclereid belt (blue line). H, TS of the gynoecium through the ovule distal zone, note the sclereids (arrow) and the carpel wall (red line). I, TS of the gynoecium over the ovule, note the carpel wall differentiation (red line) and basal lateral fusion (arrows) of the dilatated proximal part of the filaments (asterisk). J, K, TS of the gynoecium through the apical zone of the locule (white arrows), note the lateral fusion (green arrows) of the filaments (green asterisks). L, TS through the middle zone of the style with three independent canals inside (white arrow). M-O, TS through the distal part of the style with single style canals inside (white arrows).

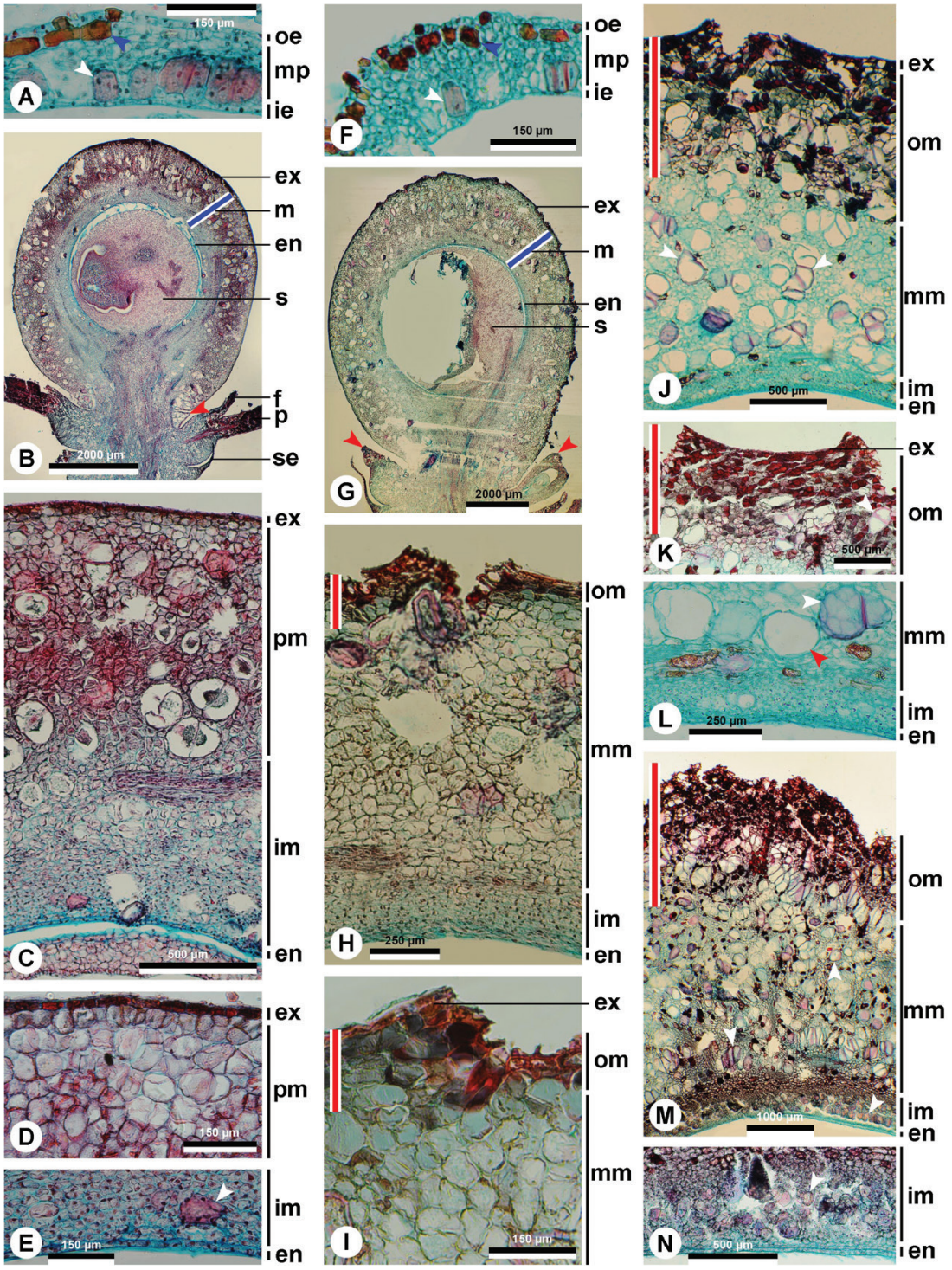

Figure 4. Carpel wall and pericarp histology of *Johannesteijsmannia magnifica* (stage 1–5), microtome longitudinal sections (A, B, G) and transverse sections (C-F, H-N), vertical red lines indicate the corky zone of the pericarp. The blue lines indicate mesocarp. The sections are treated with ruthenium red and toluidine blue. A, F, the carpel wall, stage 1, note the sclereids (white arrows) and tannin-containing cells (blue arrows) in the mesophyll. B-E, young fruit, stage 2. B, note the undeveloped carpel (red arrow). E, note the scattered sclereids (white arrow) in the mesocarp. G-I, young fruit, stage 3. G, note the undeveloped carpels (red arrows). J-L, mid-stage fruit, stage 4, note the scattered sclereids (white arrows) in the mesocarp and the large spherical cells (red arrow). M, N, mid-stage fruit, stage 5, note the scattered sclereids (white arrows) in the mesocarp.

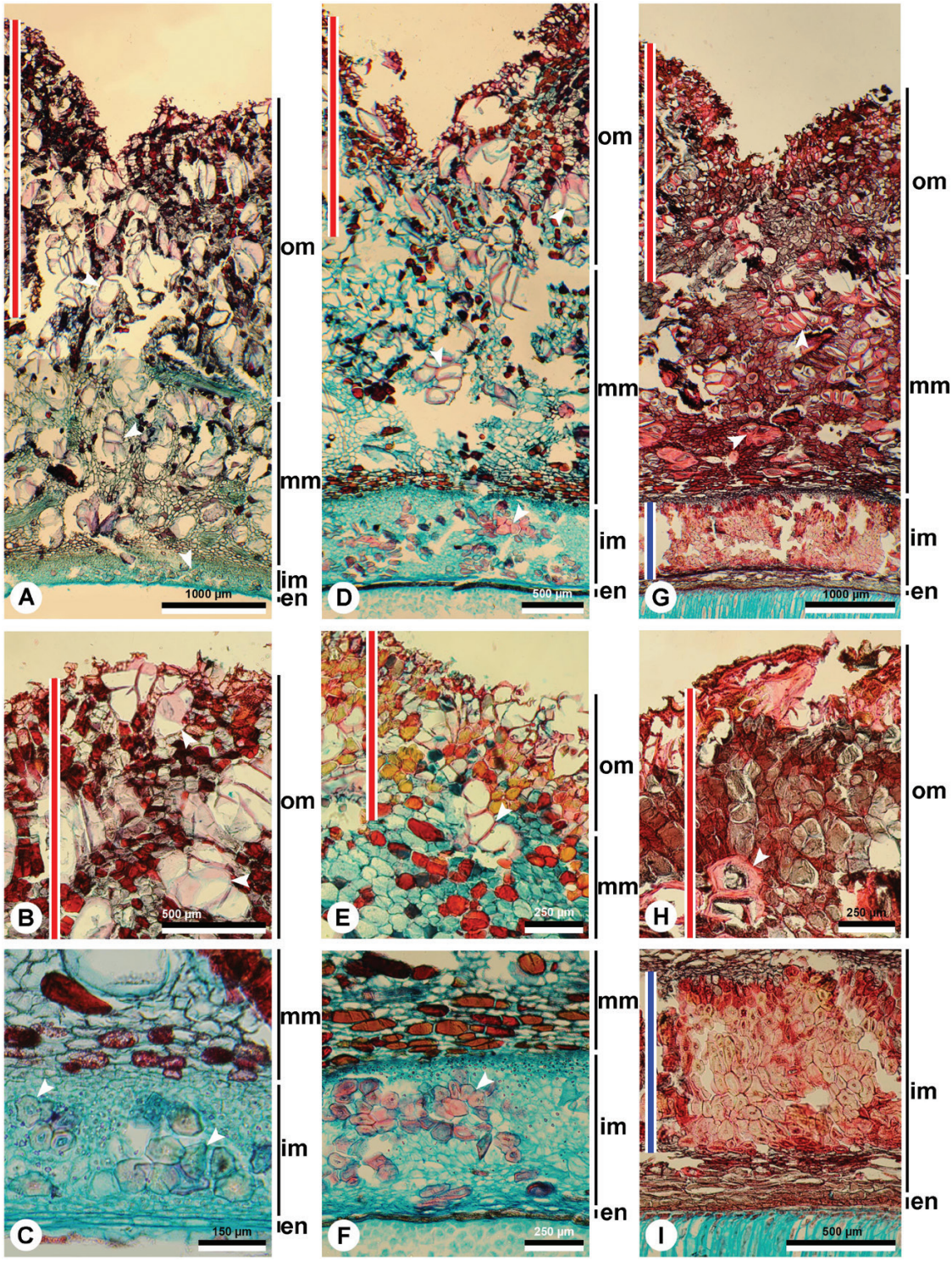

Figure 5. Pericarp histology of *Johannesteijsmannia magnifica*, stages 6–8, microtome transverse sections (vertical red lines indicate the corky zone, vertical blue lines indicate the putamen). The sections are treated with ruthenium red and toluidine blue. A-C, mid-stage fruit, stage 6, note the scattered sclereids (white arrows) in the mesocarp. D-F, late-stage fruit, stage 7, note the scattered sclereids (white arrows) in the mesocarp. G-I, mature fruit, stage 8, note the scattered sclereids (white arrows) in the mesocarp.

of the fruit. The corky zone of the mesocarp consists of three types of cells: the cells with thin suberized walls, tannin-containing cells and medium- and large-sized sclereids with varying thicknesses of walls (Fig. 5G, H). The middle zone of the mesocarp comprises small thin-walled cells (including the tannin-containing ones) and scattered sclereids or groups of several sclereids with different thicknesses of walls. The inner zone of the mesocarp comprises a continuous belt of 12–15 layers of brachysclereids with heavily thickened and lignified walls with prominent pits (Fig. 5I). At the point of attachment of the seed the sclereid belt is thicker (up to 50 cell layers in thickness). There are five to seven layers of mostly small thin-walled cells inside and outside of the sclereid belt. The innermost parenchymatous cells of the mesocarp as well as the thin-walled cells comprising the single layer of the endocarp are elongated circumferentially (Fig. 5I).

The structure of the pericarp of *J. lanceolata* at the late stage of development (stage 7) and in mature fruits of *J. altifrons*, *J. lanceolata* and *J. perakensis* is generally similar to *J. magnifica* (Fig. 5G-I, 6A-F). The thickness of the outer corky zone of the mesocarp exceeds 200 layers of cells in the warts of all *Johannesteijsmannia* spp. except *J. magnifica*, the middle parenchymatous zone of the mesocarp consists of 60–80 layers of cells and includes scattered sclereids and the inner zone consists of 25–30 layers of cells (Fig. 6A, B). In contrast to *J. magnifica*, the cells of the outer corky zone of the mesocarp have mostly thin lignified walls in *J. altifrons* and *J. lanceolata* fruits (Fig. 6C, D). The thickness of the continuous belt of brachysclereids in the inner mesocarp zone is comparable in *J. altifrons* and *J. magnifica* (*c*. 12–15 layers of brachysclereids). In *J. lanceolata*, at the seventh developmental stage, the sclereid belt comprises eight to ten layers of the brachysclereids and the belt is discontinuous (the sclereid nests are interspaced with parenchymatous cells, as shown for the same stage of *J. magnifica*, Fig. 6E, F). The cells of the single layer of the endocarp in all studied species of *Johannesteijsmannia* are elongated circumferentially (Fig. 6F). There are numerous vascular bundles in the middle zone of the mesocarp in mature fruits of all studied *Johannesteijsmannia* spp.

Fruit morphology and pericarp histology of *Pholidocarpus macrocarpus*

The spherically ellipsoidal fruits of *P. macrocarpus* are the largest of the representatives of the 'apocarpous clade' of Coryphoideae, reaching *c.* 6 × 9 cm (Fig. 7A). The fruits are typically monomerous, they develop from one of three carpels (two carpels remain undeveloped). The fruits are covered with rather flat broad warts each looking like a volcano with a flat top (Fig. 7A, B). The pericarp reaches 25–35 mm in thickness on the different sides of the fruit and is composed of 150–230 layers of cells of different sizes (Fig. 7C-I). Both the exocarp and the endocarp are represented by single layers of cells of the outer epidermis covered with a thick cuticle and thin-walled tannin-containing cells of the inner epidermis correspondingly (Fig. 7E, F, G, I). The mesocarp is differentiated into three conspicuous zones: the outer corky, the middle coriaceous and the inner zone differentiated into a strong sclerenchymatous stone with a thick fleshy subzone outside it and a mostly parenchymatous subzone inside (Fig. 7C-I). The peripheral layers of the corky zone comprise the warts of the fruit (15–20 layers of angular parenchymatous mostly tannin-containing cells with poorly thickened and selectively lignified walls and scattered among them sclereids with thick walls). The middle zone of the mesocarp comprises 90–130 layers of thin-walled cells with scattered among them solitary sclereids or small nests of sclereids and fibrovascular bundles (Fig. 7E-G). About 30–50% of thin-walled cells of the coriaceous zone of the mesocarp are tangentially elongated. The fleshy subzone of the inner zone of the mesocarp is the thickest one, it comprises *c*. 70–75% of the pericarp and is composed of 15–30 layers of thin-walled extremely radially elongated cells (1–2 mm in length) interspaced with radial bundles of fibre-like sclereids. Both types of cells are connected with the sclerenchymatous stone and rise from the stone like the rays from a light source. The stone of the mesocarp is composed of *c.* 50 layers of brachysclereids varying in shape and size; there are concentric nests consisting of 40–50 large sclereids among the ground tissue composed of smaller brachysclereids (Fig. 7G, H). Inside the stone there are 12–17 layers of tannin-containing cells with poorly thickened unlignified walls and tannin-containing sclereids scattered among them (Fig. 7G, I). These cells represent the innermost layers of the mesocarp.

Fruit morphology and pericarp histology of *Licuala bintulensis*

The spherical fruits of *L. bintulensis* reach 8–10 mm in diameter at maturity and are covered with warts in the shape of a volcano with a flat top (Fig. 7J, K). The fruits are usually monomerous and develop from one of three carpels of the gynoecium; they are subtended by three sepals and attached to the short thickened pedicel. Pericarp structure was studied at the middle developmental stage and at maturity (Table 2). The mature pericarp is about 43–57 layers thick, the exocarp is represented by a single layer of small epidermal cells with unevenly thickened partly lignified walls covered with a thick cuticle, a multi-layered mesocarp and an endocarp composed of a single layer of pale thin-walled cells (Fig. 7L-N). The mesocarp is differentiated

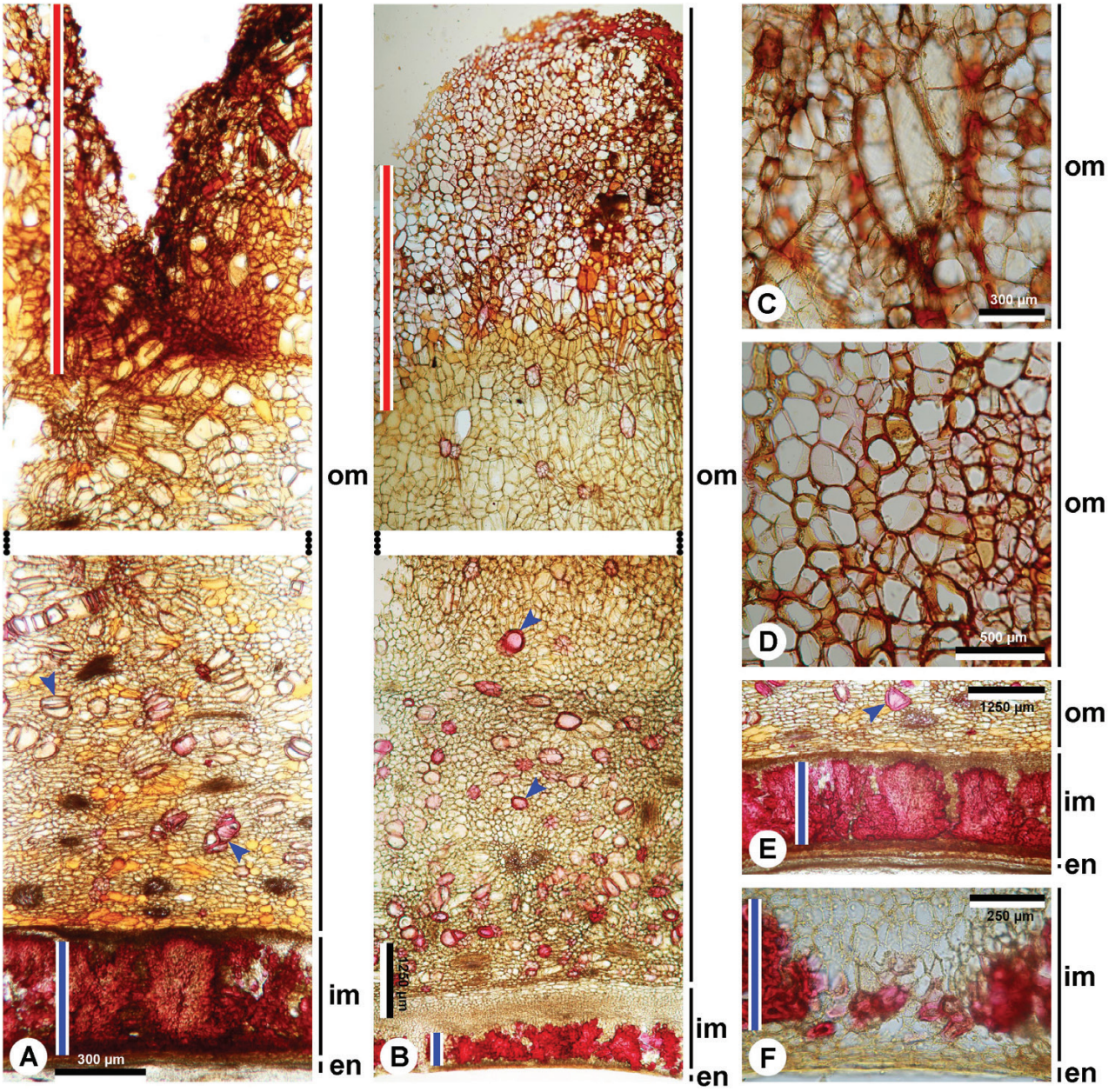

Figure 6. Pericarp histology of *Johannesteijsmannia* species, microtome transverse sections (vertical red lines indicate the corky zone, vertical blue lines indicate the putamen, blue arrows point to sclereids). The sections are treated with phloroglucinol and hydrochloric acid. A, C, E, *J. altifrons*, mature fruit, stage 8. B, D, F, *J. lanceolata*, mid-stage fruit, stage 7. A, B, TS of the pericarp. C, D, close-up of the corky zone of the pericarp. E, F, the putamen structure.

into three zones: the outer corky one composed of up to eight layers of thin-walled tannin-containing cells varying in size and outline (some cells have lignified walls but lack the suberinization), the middle zone represented by 32–37 layers of parenchymatous cells with numerous scattered spherical mostly large tannin-containing cells and fibrovascular bundles and the inner zone composed of thin-walled cells with a sclerenchymatous stone in its centre (Fig. 7L). The stone consists of three to five layers of sclereids with different thicknesses of lignified wall; there are both cells with heavily thickened walls (brachysclereids) and thin-walled sclereids (Fig. 7N). As far as it is seen from the studied developmental stages, during pericarp development the stone represents a continuous belt of sclereids, which separate as the fruit enlarges and the resulting spaces are filled by the enlargement of adjacent parenchyma cells which subsequently become

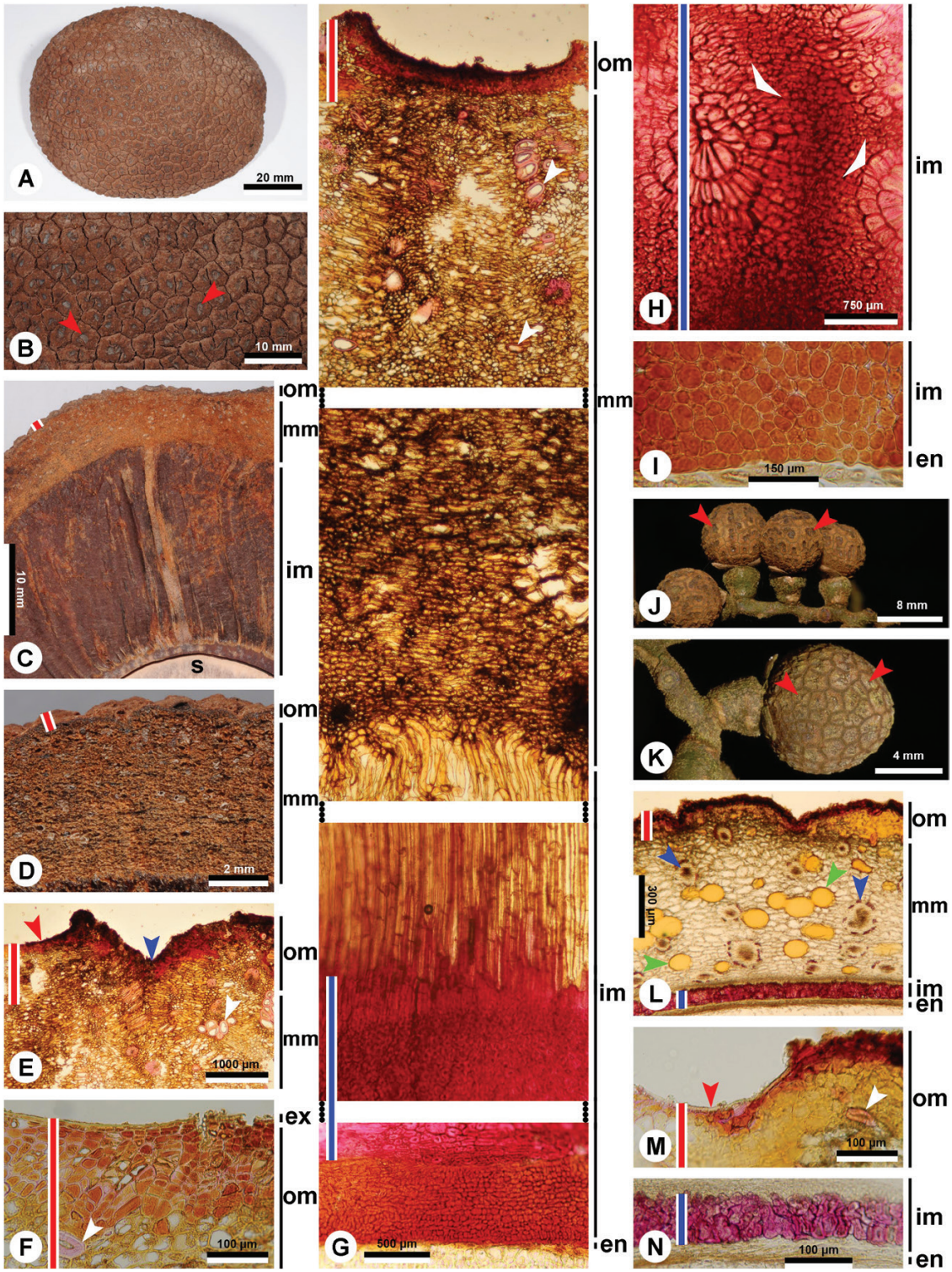

Figure 7. Fruit morphology and pericarp histology of *P. macrocarpus* and *L. bintulensis* (vertical red lines indicate the corky zone, vertical blue lines indicate the putamen). The sections are treated with phloroglucinol and hydrochloric acid. A-I, *P. macrocarpus*, stage 8. A, fruit morphology. B, close-up of the fruit surface, note the fragments of the exocarp (red arrows). C, a fragment of the fruit section. D, close-up from Figure 7C, the peripheral zone of the pericarp. E, F, histological structure of the peripheral zone of the pericarp, note a fragment of the exocarp (red arrow), the crack in the fruit surface (blue arrow) and the sclereids (white arrows). G, TS of the pericarp, note the sclereids in the middle mesocarp zone (white arrows). H,

sclerified. Three to five layers of thin-walled pale cells inside of the stone comprise the innermost part of the mesocarp and adjoin to the endocarp.

DISCUSSION

The structure and development of the pericarp in corky-warted fruits of Livistoninae

The present developmental carpological study of *Johannesteijsmannia* shows the principal events in the development of fruitlets and details of their pericarp structure (Table 3). At the early developmental stages (stages 2, 3), the walls of the exocarp cells become suberized, die off and soon the smooth pale fruiting carpel surface becomes broken up by discoloured cracks due to cell multiplication in the subdermal layers. By the middle stages of fruit development, the exocarp becomes rubbed off from the tops of the warts and cannot be seen in mature fruits. During the middle stages of fruit development (stages 4–6), the differentiation and growth of the corky zone of the mesocarp takes place, while the stone of the fruit begins its fast differentiation in the inner zone of the mesocarp only at the late developmental stage (stage 7). The mature fruit shows differentiation of the massive thick corky-warted zone (the outer zone of the mesocarp) composed of cells with suberized or lignified walls on the periphery of the fruit and a stone composed of brachysclereids in the inner zone of the mesocarp. Both the exocarp and the endocarp play a minor role in pericarp formation apart from early suberinization of the outer epidermis which causes cracking of the pericarp due to internal pressure of multiplying subdermal layers of cells. Early exocarp suberinization seems to be the key event in the origin of warts in *Johannesteijsmannia*, a kind of trigger for the beginning of cracks in the surface of the young fruiting carpels.

Similar to the situation in *Johannesteijsmannia*, both the exocarp and endocarp play minor roles in the formation of the pericarp of *L. bintulensis* and *P. macrocarpus*. Differing from *Johannesteijsmannia*, the exocarp fragments in *L. bintulensis* and *P. macrocarpus* persist on the tops of the warts until maturity. The pericarp of *L. bintulensis* comprises 43–57 layers of cells which is thinner than in *Johannesteijsmannia* species (the pericarp may reach 300 layers thick in the warts) and in *P. macrocarpus* (the pericarp is 150–230 cellular layers thick). The thick pericarp of the large fruits of *P. macrocarpus* (25–35 mm) is comparable with the thickest pericarps in representatives of Borasseae (*Borassodendron* Becc., *Borassus* L. and *Lodoicea* Comm. ex DC.; Coryphoideae) (Romanov *et al.*, 2011), *Nypa fruticans* Wurmb (Nypoideae) (Bobrov *et al.*, 2012; Matsunaga & Smith, 2021) and *Cocos nucifera* L. (Arecoideae) (Winton, 1901; Lloyd, 1910; Juliano, 1926). The unique character of *P. macrocarpus* among tax of Livistoninae is the differentiation of the thin-walled radially elongated cells interspaced with radial fibre bundles comprising a thick subzone of the mesocarp (*c.* 70–75% of pericarp thickness). In three other *Pholidocarpus* spp. [*Pholidocarpus ihur* (Giseke) Blume, *Pholidocarpus mucronatus* Becc. and *Pholidocarpus sumatranus* Becc.], the same radial fibre bundles rise from the stone in the pericarp, whereas in *Pholidocarpus majadum* Becc. and *Pholidocarpus kingianus* such radial fibre bundles do not develop (Dransfield, 1970; unpublished data). It should be added that *P. kingianaus* is unique in the genus in having smooth fruit. A similar structure of the fleshy zone of the mesocarp, but composed of thin-walled radially elongated cells only is described for *Bismarckia nobilis* Hildebrandt & H.Wendl. (Romanov *et al.*, 2011: fig. 4A). At all developmental stages in the pericarp of the studied species of *Johannesteijsmannia*, *Licuala and Pholidocarpus*, the vascular bundles of the fruit are located in the middle zone of the mesocarp and do not participate either in construction of the warts or in the stone structure. The corky zone of the pericarp, as well as the inner zone of the mesocarp in which the stone is differentiated, lack vascular bundles.

The structure of warts in fruits of *Johannesteijsmannia*, *Licuala* and *Pholidocarpus*

The thickness and structure of the corky zone is different in *Johannesteijsmannia* spp., *L. bintulensis* and *P. macrocarpus*. The outer corky zone of *Johannesteijsmannia* spp. consists of 60–200 layers of three types of cells: cells with thin suberized walls, tannin-containing cells and medium- and large-sized

close-up of a fragment of the sclerenchymatous stone, note the concentric nests of sclereids (white arrows). I, the histology of the innermost layers of the pericarp. J-N, *L. bintulensis.* J, K, fruit morphology, note the fragments of the exocarp (red arrows) on the tops of the warts. J, the young fruits, stage 5. K, the mature fruit, stage 8. L-M, TS of the pericarp, stage 8. L, the pericarp histology with numerous scattered tannin-containing cells (green arrows) and vascular bundles (blue arrows). M, close-up of the peripheral zone of the pericarp, note a fragment of the exocarp (red arrow) and a sclereid (white arrow). N, close-up of the inner zone of the mesocarp with a continuous sclereid belt.

Table 3. The principal events in the organogenesis of fruits and histogenesis of the pericarp of *Johannesteijsmannia* (the most important characters of the selected developmental stages are in bold)

Stage	Morphology	Pericarp histology and anatomy
Gynoecium (stage 1)	– The ventral edges of the trimerous gynoecium composed of free carpels are fused – The single basal adaxially attached anatropous ovule fills in less than a half of the locule	**– Presence of an irregular layer of brachysclereids in the inner mesophyll layers** – Abundant tannin-containing cells in the peripheral layers of the mesophyll at the level of the distal end of the ovary zone and in the stylodium
Young fruit (stage 2, 2 weeks after fertilization)	**– The smooth pale fruiting carpel surface becomes broken up by discoloured cracks on the dorsal side**	**– The cell walls of the exocarp become suberized on the dorsal side of the fruiting carpel (and the epidermal cells die)** – The mesocarp becomes differentiated into two zones: the peripheral zone and the inner zone
Young fruit (stage 3, 3 weeks after fertilization)	**– The fruiting carpel surface becomes broken up by discoloured cracks on the lateral and ventral sides (in addition to the dorsal side)**	– The cell walls of the exocarp become suberized on the lateral and ventral sides **– The mesocarp becomes differentiated into three zones: the thin outer corky zone and the thick middle and inner zones**
Mid-stage fruit (stage 4, 4–5 weeks after fertilization)	– Two of three fruiting carpels become reduced and only the last carpel continues further development **– The fruiting carpels become spherical, reddish-brown in colour and covered with fine warts**	– The exocarp is represented by isolated fragments persisting on the tops of the warts – The mesocarp comprises three zones, comparable in thickness **– The corky zone consists of thin-walled cells with suberized walls, tannin-containing cells and sclereids**
Mid-stage fruit (stage 5)	– Proportional growth of the spherical fruiting carpels and the warts on their surface	– The exocarp is rubbed off from the tops of the warts
Mid-stage fruit (stage 6)	– The number of warts on fruitlet's surface remains unchanged from the fourth developmental stage to the mature fruit stage	**– The thickness of the corky zone gradually increases as the result of specialization of the cells on the periphery of the middle zone, in which thickness become reduced**
Late-stage fruit (stage 7)		**– The specialization of the thin-walled cells in the centre of inner mesocarp zone results in origin of a discontinuous belt composed of large nests of brachysclereids isolated from each other by zones of unspecialized thin-walled cells**
Mature fruit (stage 8)		– The corky zone of the mesocarp consists of three types of cells: cells with thin suberized walls, tannin-containing cells and sclereids with varying thicknesses of walls – The inner mesocarp zone comprises the stone of the fruit, a continuous belt of 12-15 layers of brachysclereids with heavily thickened and lignified walls (due to thickening and total sclerenchymatization of the walls in all cells in this zone) – The endocarp consists of thin-walled cells elongated circumferentially (similar to all previous developmental stages)

sclereids with varying thicknesses of walls, whereas in *L. bintulensis* it consists of eight layers of thin-walled tannin-containing cells varying in size and outline (some cells have lignified walls but lack suberinization), and in *P. macrocarpus* with 15–20 layers of cells of angular parenchymatous mostly tannin-containing

cells with poorly thickened and selectively lignified walls and scattered among them sclereids with thick walls. So, the fruit warts of *Johannesteijsmannia* are composed of dead cells with suberized or lignified walls, whereas in *L. bintulensis* and *P. macrocarpus* the warts are mostly composed of living cells, and they are topped with the exocarp at maturity. In this way, the warts of Livistoninae taxa can be referred to as alive (not suberized) or to dead (suberized) types. The number of warts on the fruit surface of studied taxa at maturity is related to the time of cracking and the texture of the peripheral zone of the pericarp (the smaller the fruit is when cracking takes place, the fewer the number of warts at maturity). It is probably the varying combinations of these two factors and the varying numbers of periclinal cell divisions which account for the appearance of the mature fruit.

The cracking of the fruit is due to differential growth in the peripheral layers of the fruit. In *Johannesteijsmannia*, this differential growth may be explained in two ways: (1) the epidermis becomes suberized and hence dies and then is ripped apart as the lower layer expands or (2) the epidermal periclinal cell divisions cannot keep pace with the divisions in the lower layers, cracking occurs and cork production occurs as a wound response. In *J. magnifica* and *J. altifrons*, in sections of the young fruiting carpels the epidermis shows suberinization of the cell walls, so it seems likely that the initiation of the cracks results from the suberinization of the epidermis and its consequent death. Once the cracks are formed suberinization of the outer layers of the pericarp is massive, and the cracks deepen as the fruit expands and the superficial layers are unable to divide further. Anticlinal divisions of the pericarp cells predominate over periclinal divisions, and this further increases the tendency for the cracks to deepen. As far as in *L. bintulensis* and *P. macrocarpus*, the suberinization of the cell walls of the exocarp is not revealed, the origin of the warts may be caused by the pressure of the subdermal cell layers which multiply more quickly than the exocarp cells and pericarp cracking occurs. As the structure of the warts is rather different in all studied genera of Livistoninae it is supposed that they originated independently in *Johannesteijsmannia*, *Licuala* and *Pholidocarpus*.

A COMPARISON OF CORKY-WARTED FRUIT STRUCTURE IN CORYPHOIDEAE, CEROXYLOIDEAE AND ARECOIDEAE

Corky-warted fruits are observed in unrelated clades in three of five subfamilies of Arecaceae. The structure of fruits of *Chelyocarpus* (Cryosophileae: Coryphoideae) has not been studied yet. Preliminary data for *Phytelephas* Ruiz & Pav. (Ceroxyloideae) and *Pelagodoxa* (Arecoideae) show that their fruits can be attributed to pyrenariums of the *Butia* type with the pyrenes composed of the inner mesocarp zone and the endocarp (typical for many Arecoideae: Essig, 1977, 1999, 2002; Essig & Young, 1979; Essig *et al.*, 1999, 2001; Essig & Hernandez, 2002; Essig & Litten, 2004) with complex anatomical structure of the peripheral zone of the mesocarp (Dransfield, 1970; Chapin *et al.*, 2001; Bobrov & Romanov, 2019). The fruits of *Manicaria* and *Sommieria* are preliminarily referred to as pyrenariums of the *Ilex* type (with the pyrene composed of the endocarp only; Bobrov & Romanov, 2019). Data on fruit structure in *Lemurophoenix* are lacking. The differences in fruit structure in the subfamilies of the Arecaceae suggest differences in their histogenesis and independent origin of the corky warts. Therefore, the presence of corky-warted fruits in different clades of Arecaceae probably represents an example of parallel evolution, i.e. it is homoplasious.

THE FUNCTION AND EVOLUTIONARY SIGNIFICANCE OF THE CORKY-WARTED FRUITS

The presence of corky-warted fruits in some taxa of Livistoninae may be explained by (1) a protective adaptation (Corner, 1966; Imkhatitzkaya, 1985), (2) an adaptation to dispersal (Ridley, 1930), (3) a functionless 'archaism' (the warty pericarp may be a relic without any function at the present time, but with some functional significance in the past) (van der Pijl, 1982), (4) a functionless incidental result (a 'by-product') of the fruit developmental process. As the immature fruit of *Johannesteijsmannia* are often found chewed the protective function of the warts seems unlikely (Dransfield, 1970). The corky zone of *Johannesteijsmannia* fruits is impervious to water and may prevent rotting of the fruit, but actually the rotting of the immature fruits, which develop at the ground level covered with debris is quite common in the wild (Dransfield, 1970). The presence of corky-warted fruits, which develop in the air in *P. macrocarpus* and *L. bintulensis*, and both occupying the same habitats as *Johannesteijsmannia* can also not be explained. The fact that the fruits of *Johannesteijsmannia* are often found with the pericarp chewed, suggests, perhaps, that the warts render the fruits in some way acceptable to animals and represent an adaptation to animal dispersal. On the other hand, the warts may be non-adaptive at the present day or in the past. The possession of corky warts in *Johannesteijsmannia*, *Licuala* and *Pholidocarpus* (and other palm genera) may represent an ancient character of little significance to the plants at the present time. Despite the similar structure and differentiation of the pericarp in all three studied coryphoid palm genera with corky-warted fruits, the structure of their warts is different. This is also the case of the corky-warted

genera of Arecoideae and Ceroxyloideae; as far as is known, the structure of the warts is specific for each genus, and the only common feature of all corky-warted fruits in palms is the cracking of the pericarp surface and fragmented exocarp since the early/middle developmental stages and its poor preservation or absence in mature fruit is due to its rubbing off during fruit growth. This fact supports the treatment of the warts as the incidental result of the early events of fruit development resulting in the cracking of the pericarp. In addition to Arecaceae, monospermous corky-warted fruits have been reported for *Litchi chinensis* Sonn. and *Paranephelium macrophyllum* King (Sapindaceae, Davis, 1984; Underhill & Critchley, 1992), but their structure differs by the presence of outer epidermis (the exocarp) on all outer surfaces of the fruits; the exocarp covers the surface of the warts and the warts do not originate as the result of cracking of the exocarp. The warts of corky-warted fruits in representatives of Sapindaceae originate as the result of programmed development and they cannot be recognized as a by-product of the fruit developmental process, but rather represent a protective adaptation of the fruits.

The structure and development of the fruit stone in representatives of the 'apocarpous clade' of Coryphoideae

Based on the differentiation of the pericarp (particularly the differentiation of the stone in the inner zone of the mesocarp), fruits of the studied species of Livistoninae are recognized as drupes of the *Rhapis* type with a complex external protective corky zone. The structure of their mature pericarp (except for the corky zone) resembles that of a number of genera of Coryphoideae with drupes of the *Rhapis* type (*Chamaerops* L., *Coccothrinax* Sarg., *Livistona*, *Pritchardia* Seem. & H.Wendl., *Rhapidophyllum* H.Wendl. & Drude, *Rhapis* L.f. ex Aiton, *Serenoa* Hook.f., *Trachycarpus* H.Wendl., *Thrinax* L.f. ex Sw., *Washingtonia* H.Wendl.) (Murray, 1973; Danilova & Savchenko, 1985; Bobrov & Romanov, 2007; Bobrov *et al.*, 2008a, b; Bobrov & Romanov, 2019; unpublished data). The recognition of the fruits of the studied taxa of Livistoninae as drupes of the *Rhapis* type, which is also revealed in other representatives of Trachycarpeae and the 'apocarpous clade' of Coryphoideae, suggest this character as a synapomorphy of this tribe and the whole clade. The thickness of the stone in mature fruits of all studied representatives of Livistoninae with corky-warted fruits varies. In *Johannesteijsmannia*, the stone thickness reaches (eight) 12–15 layers of brachysclereids, whereas in *L. bintulensis* it consists of three to five layers and in *P. macrocarpus* *c*. 50 layers. The number of brachysclereid layers (about three to five layers of sclereids of the stone) and general differentiation of the pericarp in *L. bintulensis* is comparable with other investigated species from the 'apocarpous clade' of Coryphoideae in which the drupes of the *Rhapis* type are revealed (Murray, 1973; Danilova & Savchenko, 1985; unpublished data). The thickness of the stone in *Johannesteijsmannia* exceeds the average thickness of the putamen in this clade of palms by two to four times. Furthermore, in *P. macrocarpus*, the thickness of the stone reaches 50 layers of brachysclereids, which is about 15–20 times thicker than in a typical drupe of the *Rhapis* type of a representative of the 'apocarpous clade' of Coryphoideae. The thickness of the stone of *P. macrocarpus* is comparable with the pyrenes in the representatives of borassoid palms, but differs from them in structure (brachysclereids vs. fibre-like sclereids, Romanov *et al.*, 2011).

According to Murray (1973), during development of the 'coryphoid fruit type' the stone of the fruiting carpel (the inner zone of the mesocarp) tends to comprise a continuous belt of sclereids, which tend to separate as the fruit enlarges and the resulting spaces are filled by the enlargement of adjacent parenchyma cells and subsequently become sclerified. In this manner the sclereid belt is maintained as a continuous layer throughout the development of the fruit. The present study revealed that the development of the stone in the inner zone of the mesocarp of *L. bintulensis* proceeds in the same mode as in other species of coryphoid palms with smooth fruits including one species of *Livistona*, *Livistona chinensis* (Jacq.) R.Br. ex Mart. (Murray, 1973). The mode of stone development in *Johannesteijsmannia* spp. is different: before fruit maturity the sclerenchymatous belt located in the inner zone of the mesocarp comprises scattered brachysclereids or nests of brachysclereids separated by thin-walled cells which continuously transform into brachysclereids. Only by fruit maturity do the last undifferentiated thin-walled cells differentiate into brachysclereids, a continuous sclerenchymatous layer (the stone) of the fruit originate, and maximum rigidity and hardness of the stone reached. Thus, the second mode of origination of the stone of the drupe of the *Rhapis* type in palms is revealed in the present research in *Johannesteijsmannia*. The cardinal difference of the two modes of the stone development lies in the presence of a continuous sclerenchymatous layer in the inner mesocarp since the early or middle developmental stage in cases of most studied taxa of the 'apocarpous clade' of Coryphoideae in contrast to development of a discontinuous layer of sclereids or the nests of sclereids separated by the parenchymatous cells in *Johannesteijsmannia* and formation of the continuous sclerenchymatous layer in its fruits only by fruit maturity.

CONCLUSION

The present carpological research has shown that the fruits of all studied corky-warted taxa of Livistoninae are drupes of the *Rhapis* type with a complex structure to the external protective corky zone. The warts of *Johannesteijsmannia*, *L. bintulensis* and *P. macrocarpus* originate on the fruiting carpel surface soon after gynoecium pollination as the result of (1) early suberinization of the exocarp cells, and/or (2) progressive multiplication and growth of the outer zone of the mesocarp, and/or (3) cracking of the peripheral zone of the pericarps. By maturity the exocarp is rubbed off from the tops of the warts (*Johannesteijsmannia*) or persists on them (*Pholidocarpus* spp. and *L. bintulensis*). Both *P. macrocarpus* and *L. bintulensis* differ from *Johannesteijsmannia* spp. in lacking suberized cells in their fruit warts. The corky warts on the fruit surface of taxa of Livistoninae probably represent the result of specific pericarp developmental processes starting with the early suberinization and consequent death of the exocarp cells in *Johannesteijsmannia* or originate due to the pressure of the subdermal layers of cells on the epidermal of cells of the exocarp in *P. macrocarpus* and *L. bintulensis*. The present study reveals that the stone of the drupe of the *Rhapis* type in palms can originate in two different modes, as in *Licuala* and other studied taxa of the 'apocarpous clade' of Coryphoideae or as described here for *Johannesteijsmannia*. Thus, the development of the stone within the same morphogenetic fruit type can be different in some important details of histogenesis even in closely related taxa.

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