

Development and morphology of the fruit and seed of the hemiparasite genus *Jodina* (Cervantesiaceae)

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Abstract

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The monotypic genus *Jodina* is endemic to central and southeastern South America, common in forests and scrubland environments. The fruit type in *Jodina* is controversial since it has been described at different stages of development. The main objective of this work was to analyze the morphology of the fruit of *J. rhombifolia* during its maturation to attain a consensus about its type. Also characteristics of seed development and anatomy were analyzed in detail. Material was processed according to conventional techniques for LM and SEM studies. The fruit of *J. rhombifolia* is a pseudodrupe since the ovary is half-inferior; the fleshy layer of the pericarp is constituted by an increase of the nectary disk whereas the stony layer is represented by the mesocarp. Participation of the nectary disk as part of the fruit wall has not been mentioned previously. The seed is “naked”—the integument disintegrates during development—and the resulting structure is a pyrene. The interpretations made by other authors on the fruit of *Jodina* are also discussed.

Keywords: Anatomy, *Jodina rhombifolia*, nectary disk, pseudodrupe, pyrene.

Resumen

Luna, M.L., Giudice, G.E., Grossi, M.A. & Gutiérrez, D.G. 2017. Desarrollo y morfología del fruto y la semilla del género hemiparásito *Jodina* (Cervantesiaceae). *Anales Jard. Bot. Madrid* 74(1): e051.

El género monotípico *Jodina* es endémico del centro y sureste de América central, común en bosques y ambientes de matorral. El tipo de fruto en *Jodina* es controvertido, ya que se lo ha descrito en diferentes etapas de su desarrollo. El principal objetivo de este trabajo fue analizar la morfología del fruto de *J. rhombifolia* durante su maduración para alcanzar un consenso sobre su tipo. Asimismo, se analizaron en detalle diferentes características del desarrollo y anatomía de la semilla. El material se estudió de acuerdo con las técnicas convencionales utilizadas en estudios con MO y MEB. El fruto de *J. rhombifolia* es una pseudodrupa, dado que el ovario es semiinfero; la capa carnosa del pericarpio está constituida por un engrosamiento del disco y la capa dura está representada por el mesocarpio. La participación del disco como parte de la pared del fruto no se ha mencionado anteriormente. La semilla es “desnuda”—el tegumento se desintegra durante el desarrollo— y la estructura resultante es un pireno. Se discuten asimismo las interpretaciones realizadas por otros autores acerca del fruto de *Jodina*.

Palabras clave: anatomía, disco glandular, *Jodina rhombifolia*, pireno, pseudodrupa.

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INTRODUCTION

According to the more recent phylogeny inferred from DNA sequences, Cervantesiaceae Nickrent & Der includes 8 genera that formerly belonged to Santalaceae R. Br. s.l. (Der & Nickrent, 2008; Nickrent & al., 2010). However, the classical concept of Santales R. Br. ex Bercht. & J. Presl and its families based on morphology (v.gr., Cronquist, 1981; Takhtajan, 1997) are followed by other researchers (v.gr., Kuijt & Hansen, 2015). Cervantesiaceae is formed by hemiparasitic trees or shrubs with thorns, bisexual or unisexual flowers, solitary or grouped in inflorescence, 4-5-merous campanulate or urceolate corollas, hairs on the petals that are opposite to and in equal number with the stamens, and a nectary disk often with lobes alternating between the petals (Nickrent & al., 2010).

Currently this family is formed by genera *Acanthosyris* (Eichler) Griseb., *Cervantesia* Ruiz & Pav., *Jodina* Hook. & Arn. ex Meisn., *Okoubaka* Pellegrin & Normand, *Pilgerina* Z.S. Rogers & al., *Pyrularia* Michx., *Scleropyrum* Arn., and *Staufferia* Z.S. Rogers & al., all them mainly from

temple and Andean South America, tropical Africa and Madagascar, temple and tropical Asia reaching Malesia, and one species from the southeastern North America. It was claimed that the intergeneric relationship within this family was totally resolved, and was composed of two monophyletic groups. One of them, the *Cervantesia* group, includes all South American genera, *Acanthosyris*, *Cervantesia*, and *Jodina*. These genera show shared features such as an unilocular ovary with a twisted placental column, although the position of the ovary varies, being half-inferior —*Acanthosyris*—, superior —*Cervantesia*—, or inferior —*Jodina*— (Nickrent & al., 2010).

Among them, *Jodina* is the southernmost genus, endemic to central and southeastern South America, where it grows from Bolivia, extreme southern Brazil, Uruguay, and Argentina, reaching there its southernmost distribution range, approximately in the north of Colorado River in Buenos Aires and La Pampa Provinces (Luna & De la Sota, 2003; Dettke & Caires, 2016). This is a monotypic genus with the species *J. rhombifolia* (Hook. & Arn.)

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Reiss., called “sombra de toro” or “peje”. *Jodina rhombifolia* is a tree up to 8 m tall, with rhombic and glabrous leaves, and 3 spines on the free ends (Luna & de la Sota, 2003; Kuijt & Hansen, 2015). One of its more interesting characteristics is to be a hemiparasite plant, having been encountered haustorial connections with roots of *Celtis tala* Gillies ex Planch. and *Scutia buxifolia* Reissek (Luna & Giudice, 2005). In relation to the reproductive features, *Jodina* has bisexual flowers, a nectary disk that alternates with the petals, and a fleshy fruit with a single seed (Kuijt & Hansen, 2015).

The fruits of Cervantesiaceae and Santalaceae s.l. were mentioned in general terms as drupaceous with a stony pit (Der & Nickrent, 2008; Rogers & al., 2008) or as true drupes (Nickrent & al., 2010; Kuijt & Hansen, 2015). With regard to *Jodina*, different types of fruit have been described. Following Dawson (1944) and Rodríguez Mattos (1967), the fruit of *J. rhombifolia* is a drupaceous capsule because the “tepals” are deciduous at maturity. Then, Bhatnagar & Sabharwal (1969) defined this fruit as a pseudodrupe, since the stony layer of the fruit wall is the mesocarp. According to Kuijt & Hansen (2015), *Jodina* has a drupe-like fruit with a stony endocarp, and a fleshy rugose exocarp that detaches into 5 parts at maturity. The drupe has been broadly defined as an indehiscent, singled seeded fruit with a fleshy-stony pericarp, being the stony portion constituted by the endocarp (Roth, 1977, 1987). Transitional types may be found, giving rise to concepts such as pseudodrupe or drupaceous fruit for those with fleshy exocarp and undifferentiated endocarp, or with stony mesocarp (Spjut, 1994). On the development and anatomy of *J. rhombifolia* seed, the only published works were those of Bhatnagar & Sabharwal (1966, 1969), which emphasized on endospermogenesis and the early stages of embryo development.

Because the type of fruit is an important feature used in Systematics, and the available information on the development and structure of the fruit of *Jodina* is scarce and controversial, the main objective of this work was to study its fruit structure and seed morphology at different developmental stages.

MATERIAL Y METHODS

Flowers, fruits and seeds of *J. rhombifolia* were collected at Partido de Magdalena —35° 11' S, 57° 17' W, north-eastern Buenos Aires Province, Argentina— from May to December during the years 2011-2013. Thus, different stages of fruit and seed development were covered, from immature fruits with greenish persistent petals enclosing the pericarp and developing seed —about 3-4 months after anthesis—, to more mature stages of fruit and seed formation, with the reddish persistent petals or when they were detached exposing the white fleshy covering —5-6 months after anthesis—. Ripe seeds, considered at this stage once the fleshy covering has disorganized —near 7 months after anthesis—, were also collected. The number of trees sampled during the three years was around 60. The voucher specimens were deposited at herbarium LP —ARGENTINA. Buenos Aires, Pdo Magdalena, Estancia San Isidro, 23-V-2011, *M.J. Luna 114 & al.*; ibidem, 7-IX-2011, *M.J. Luna 135 & al.*; ibidem, 16-VII-2012, *M.J. Luna*

142 & al.; ibidem, 21-X-2012, *M.J. Luna 150 & al.*; ibidem, 15-XII-2012, *M.J. Luna 154 & al.*; ibidem, 5-VI-2013, *M.J. Luna 161 & al.*; ibidem, 14-VIII-2013, *M.J. Luna 167 & al.*

For light microscopy analyses (LM), flowers, fruits, and seeds at different stages of development were fixed in formaldehyde-acetic acid-alcohol, dehydrated through an ethanol series and embedded in Paraplast. Sections (8-10 µm thick) were double stained with safranin-fast green (Johansen, 1940).

Other staining procedures were used, such as iodine-potassium iodide for amyloids (Kooiman, 1960), periodic acid-Schiff (PAS) for carbohydrates (O'Brien & McCully, 1981), and the following fluorochromes: Nile blue for neutral lipids and 8-anilino-1-naphthalene-sulphonic acid for proteins —for both: BV filter, main length wave 463 µm— (Fulcher, 1982). Observations were made in a Nikon Microphot-FX epifluorescence microscope.

For scanning electron microscopy —SEM—, flowers were fixed in formalin: propionic acid: ethyl alcohol —0.5:0.5:9—, dehydrated in ethanol-acetone series and critical point dried using CO₂ (Sancho & Otegui, 2000). The seeds were analyzed under SEM without pre-treatment. In both cases, materials were mounted on double-sided tape on aluminum stubs, sputter coating with gold-palladium and examined with a Jeol JSM-T100 microscope.

Crude protein in endosperm was determined preliminarily by the Macro-Kjeldahl method whereas fats were calculated by continuous extraction with Soxhlet extractor, using benzene as solvent (Bullock & Moore, 1992).

RESULTS

The previous descriptions reported in the literature for the fruit type of *J. rhombifolia* are summarized in Table 1.

Morphology of the flower

The flowers were bisexual and monochlamydeous, tetramerous or pentamerous —1:1— (Fig. 1a). Following Nickrent & al. (2010) and Kuijt & Hansen (2015), we assumed the single floral whorl as the corolla. Stamens were opposite and epipetalous; the nectary disc showed lobes that alternated with the petals (Fig. 1a, b). The number of lobes of the nectary disk was 4 or 5, in coincidence with the number of petals, and they were not vascularized. The ovary was half-inferior, tricarpeal, and unilocular. The twisted vascularized placenta bore 2 ovules which were anatropous and unitegmic (Fig. 1b, d). Parts of the corolla and nectary disk underwent changes in connection with the fruit development (Table 2).

Morphology and development of the fruit

During the development of the fruit, petals were persistent and increased in size, becoming reddish as the fruit was ripening. Some 50 days after anthesis —DAA— (Table 2), the developing pericarp with its typical 3 layers —epicarp, mesocarp, and endocarp— was observed beneath the petals (Fig. 2a). At this stage, all of the layers underwent cell divisions. Early stages of the endosperm development were detected in one of the ovules, showing the haustorial and cellular portions. At the apical zone of the fruit, the epicarp

Table 1. *Jodina rhombifolia* fruit type, origin of fruit layers, and comments according to different authors.

	Fruit type	Origin of the fleshy layer	Origin of the stony layer	Comments
Dawson (1944)	Drupaceous capsule	Probably epicarp + endocarp	Endocarp	The “tepals” are modified when the fruit ripens, and they are the 5 (or 4) deciduous segments at maturity.
Bhatnagar & Sabharwal (1966, 1969)	Pseudodrupe	Parenchymatic epicarp	Mesocarp	The perianth is persistent. The fruit wall is composed by parenchymatous epicarp and endocarp, and by sclerenchymatic mesocarp.
Rodriguez Mattos (1967)	Drupaceous capsule	Probably epicarp + endocarp	Endocarp	The fruit is divided into 5 parts when ripe, which correspond to the “tepals”.
Nickrent & al. (2010)	Drupe	“Inner exocarp”	Mesocarp	The five perianth valves extend basipetally upon fruiting. Then, the “outer exocarp” dehisces.
Kuijt & Hansen (2015)	Drupe-like	Exocarp	Endocarp	The exocarp divides into 5 longitudinal parts separating at maturity.

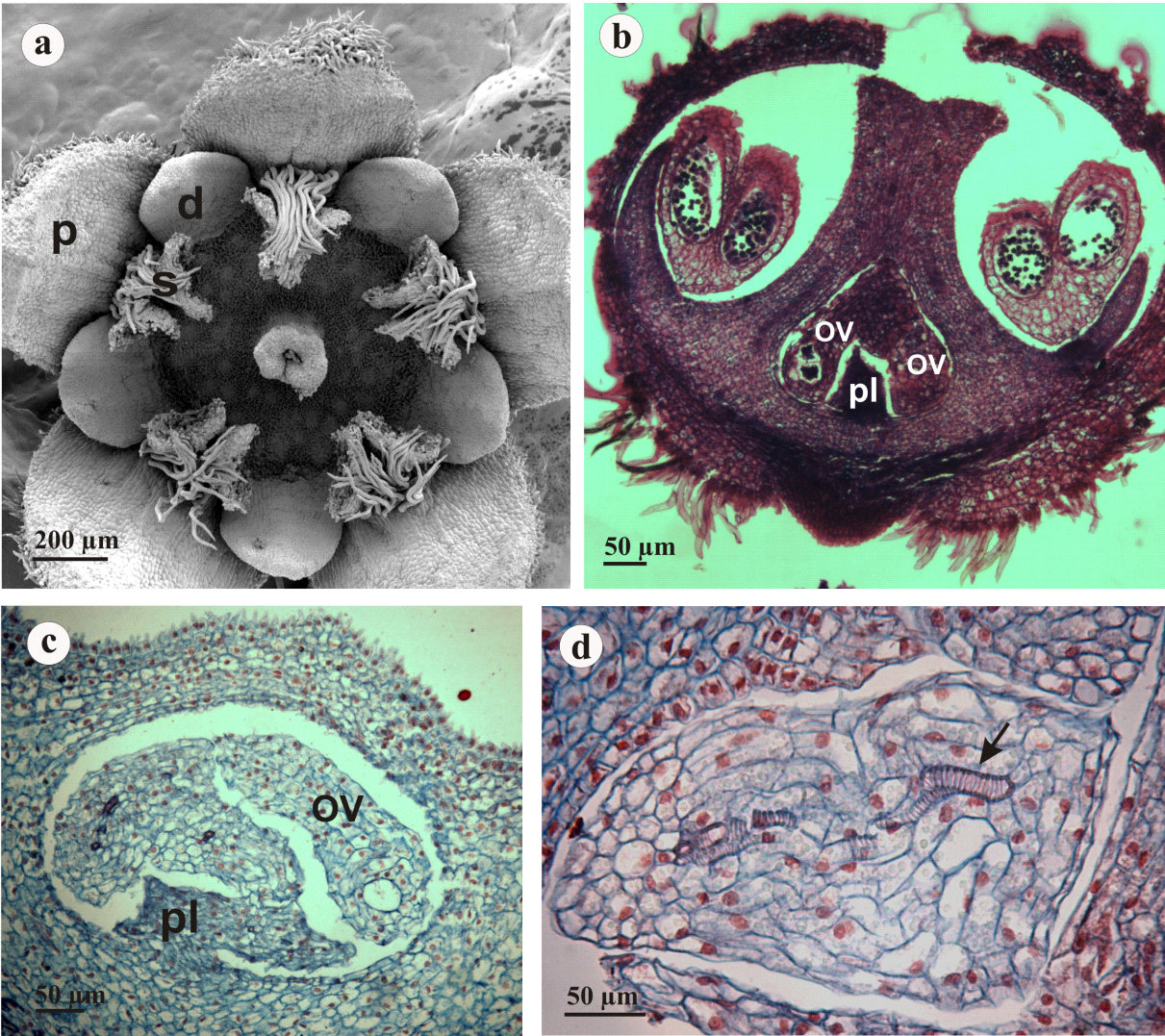


Fig. 1. Flower structure: **a**, scanning electron micrograph of a flower with 5 petals, the 5 lobes of the nectary disk alternating with them, the stamens epipetalous, bearing hairs behind them; **b**, longitudinal section showing half-inferior position of the ovary, the placenta bearing 2 ovules; **c**, detail of twisted and vascularized placenta; **d**, magnification of tracheids (arrow) in placenta. [Abbreviations: petal (p), nectary disk (d), stamen (st), stigma (s), ovule (ov), placenta (pl)].

Table 2. Changes recorded in the structures and tissues involved in *Jodina rhombifolia* fruit and seed development.

	40-50 DAA	70 DAA	90 DAA	150-180 DAA
Petals	Greenish, almost completely enclosing the developing fruit.	Greenish. They have grown by successive mitosis and enclosed completely the fruit.	Greenish, somewhat wrinkled. They continue to increase in size with the growth of the fruit.	Reddish and wrinkled. Then detached.
Epicarp	Made up of the epidermis and 3-4 layers of parenchyma cells.	As in the previous stage. Cell divisions are registered.	Cell divisions are stopped.	Disintegrated.
Mesocarp	Consisting of 4-5 layers of parenchyma cells with abundant plastids with starch grains.	Cell divisions are still observed.	Sclerification begins in the apical zone and continues towards the basal portion. Rhombic crystals of calcium oxalate present.	Completely sclerified.
Endocarp	Vascularized. Consisting of 22 to 30 layers of parenchyma cells containing starch grains and rhombic crystals of calcium oxalate.	Starts to be consumed by the haustorial endosperm. Cell divisions are observed.	Continues to be consumed by the haustorial endosperm.	Reduced to a few papyraceous layers.
Nectary Disk	Undergoes mitotic divisions.	Continues growing by mitotic divisions.	Continues to grow and gives rise to the fleshy layer.	Disorganizes and completely exposes the mesocarp = seed coat.
Cellular portion of endosperm	Cell divisions are registered.	Consisting of a greater number of cells than in the previous stage. Cell divisions are observed.	Cell divisions continue.	With thin cell walls. Cells containing lipids, protein bodies and starch grains.
Haustrorial portion of endosperm	Present, with starch grains. Appears consuming the placenta, which is almost completely disintegrated.	It has consumed completely the placenta and the ovule integument, then going towards the endocarp.	Consuming the endocarp, which appears almost completely disintegrated.	Absent.
Embryo	Not observed.	Globular stage, with starch grains.	Heart-shape and torpedo-shape stages.	Embryo incurved, with starch grains in all tissues.

included the epidermis and 3-4 layers of parenchyma cells (Fig. 2b). Following inward, the mesocarp was constituted by 4-5 layers of parenchyma cells compactly arranged. More inwards the endocarp appeared, which was vascularized and consisted of 20-30 layers of parenchyma cells with abundant starch grains and calcium oxalate crystals. Towards the middle zone of the fruit, the epicarp merged with the nectary disk, which had grown by successive mitosis (Fig. 2c).

Near 70 DAA (Table 2), the 3 layers of the pericarp still underwent cell divisions. The haustorial portion of the endosperm directed towards the endocarp and started to consume their reserves (Fig. 2d, e). About 90 DAA (Table 2) cell divisions stopped in both epicarp and mesocarp, while they continued to occur in the endocarp. At this point, the innermost layers of the endocarp were still digested by the haustorial portion of the endosperm (Fig. 2f). Sclerification of the mesocarp began to occur when endosperm was mostly cellular, and it took place from the apical to the basal zone of the fruit (Fig. 2g, h). This process was accompanied by deposition of calcium oxalate crystals (Fig. 2i).

The fruit reached the mature stage about 150 DAA. As the fruit was ripening, the persistent petals became reddish and wrinkled (Fig. 3a). Beneath them the nectary disk underwent changes forming a whitish, fleshy, sweet taste covering which almost completely surrounded the pericarp except at the apical zone (Fig. 3b). This fleshy layer was composed up of 10-15 layers of parenchyma cells.

Approximately 180 DAA (Table 2), the petals were released and the epicarp disintegrated (Fig. 3c). The fleshy covering surrounded the stony and smooth mesocarp that remained exposed at the apical zone of the fruit (Fig. 3d). Depending on the number of lobes of the nectary disk, the fleshy layer of the fruit consisted of 4 or 5 segments. The mature fruit was near 7×9 mm in size.

Morphology and development of the seed

Approximately 40 DAA, endosperm development was recorded in only one ovule—the other ovule disorganized—. It comprised the haustorial portion, that remained unicellular during development, and the cellular portion which underwent mitotic divisions (Fig. 4a).

At near 50 DAA (Table 2) the haustorial portion branched and primarily consumed the placenta and then the integument of the ovule, meanwhile the cellular portion continued under cell divisions (Fig. 4b). Because the integument was completely disintegrated in this process, the seed was considered naked. About 70 DAA (Table 2), both the placenta and the integument of the ovule were completely consumed by the haustorial portion of the endosperm. The cellular portion of the endosperm continued under mitotic divisions increasing its volume (Fig. 4c). At this step, it was recorded the globular stage of pro-embryo with starch contents (Fig. 4d). Near 130 DAA (Table 2), the endosperm was completely cellular (Fig. 4e). Its thin-walled cells, PAS positive, accumulated starch grains, lipids, and protein bodies (Fig. 4e, f).

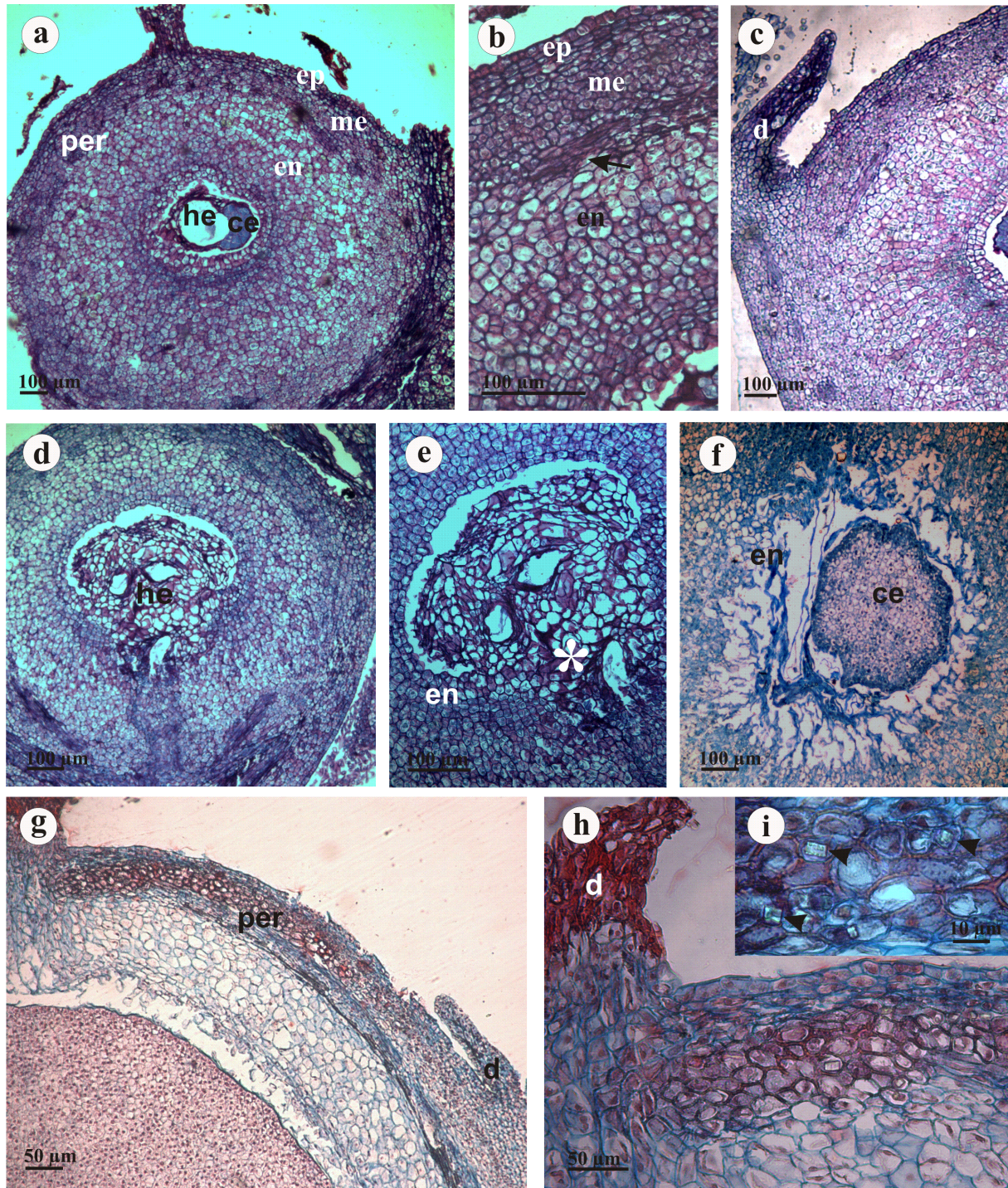


Fig. 2. Different stages of fruit wall development: **a**, earlier stage of fruit wall development where epicarp, mesocarp and endocarp are observed, the cellular and haustorial portions of the endosperm are also detected; **b**, detail of the different layers of the fruit wall, the endocarp is vascularized (arrow); **c**, portion of the fruit wall showing that the nectary disk is merged with the pericarp and accompanies its development; **d**, more advanced stage in which the haustorial portion of the endosperm begins to consume the endocarp; **e**, detail of haustorial endosperm (asterisk) moving towards the endocarp; **f**, somewhat later increased disorganization of the endocarp is observed; **g**, step of fruit wall development in which sclerification of the mesocarp is recorded, at this moment the endosperm is almost completely cellular; **h**, a more detailed view of stony mesocarp; **i**, magnification of mesocarp showing deposit of crystals (arrowheads). [Abbreviations: pericarp (per), epicarp (ep), mesocarp (me), endocarp (en), nectary disk lobe (d), haustorial endosperm (he), cellular endosperm (ce)].

Along with the development of the endosperm (Fig. 5a), the heart-shaped and torpedo-shaped pro-embryo stages were observed. The embryo was incurved and the cotyledons showed abundant chloroplasts and starch grains (Fig. 5c, d). Xylem elements—tracheids—were observed

frequently in the endosperm, next to the mature embryo (Fig. 5e, f). Near 180 DAA (Table 2), the “seed coat” was constituted by the remains of endocarp, which had become papyraceous, together with the stony mesocarp (Fig. 5g).

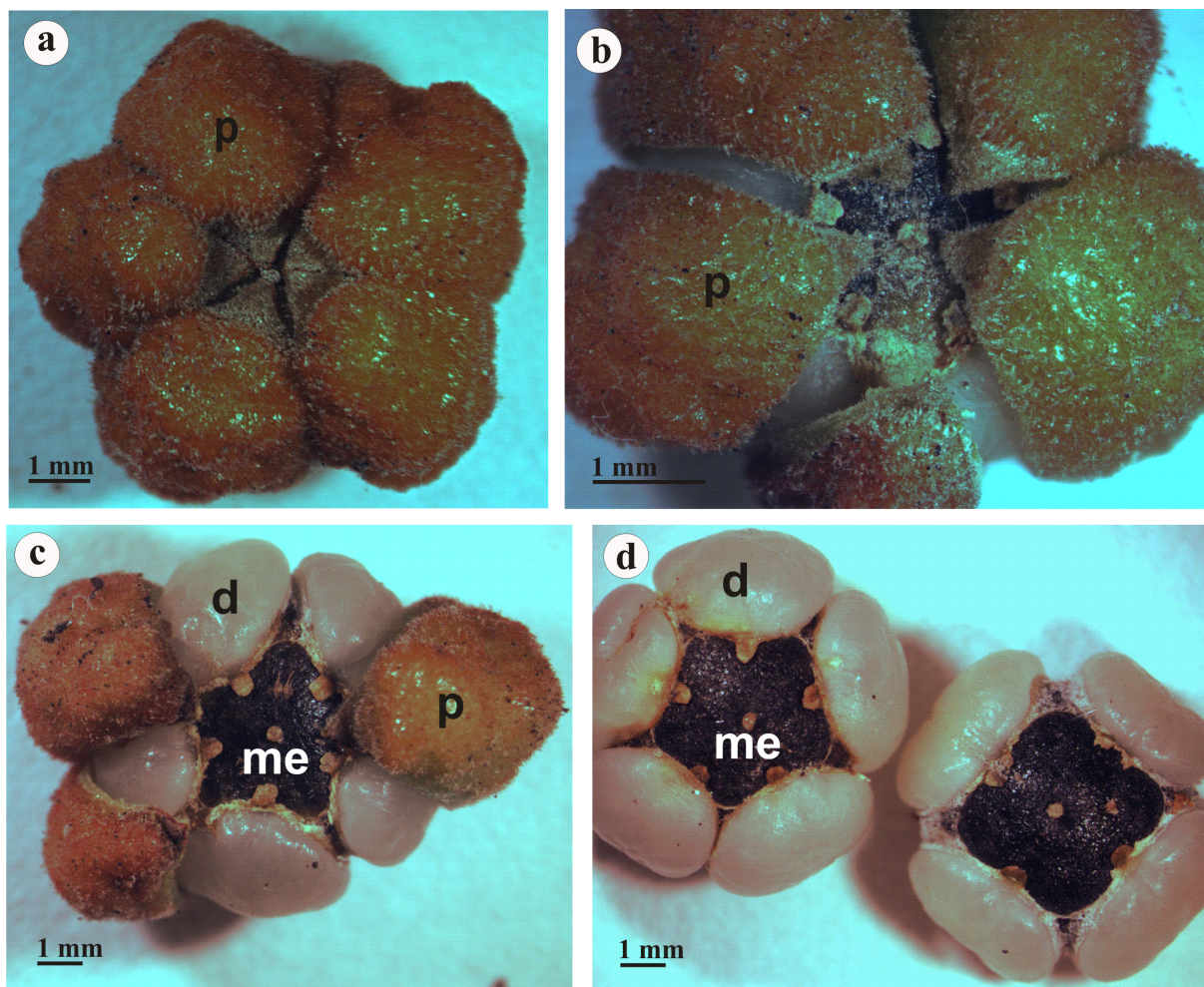


Fig. 3. Macroscopic views of fruits at various steps of maturation: **a**, fruit with persistent reddish petals; **b**, after a while the petals start to wrinkle and separate, exposing the modified nectary disk; **c**, in a more advanced stage of maturity the petals begin to come off, leaving the disc completely exposed; **d**, in a late stage of ripening the fruit wall is constituted by the disc plus the stony mesocarp, the image reveals that the nectary disk may have 4-5 lobes. [Abbreviations: petal (p), nectary disk (nd), nectary disk (d), mesocarp (me)].

The pyrene —i.e., seed plus mesocarp— was subspheroidal, with somewhat flattened poles, and ca. 5×7 mm in size (Fig. 5h). Raw protein content in the endosperm was ca. 20%, while the lipids reached ca. 68%.

DISCUSSION

The fruit of *Jodina* is a pseudodrupe, since the fruit wall is constituted by the persistent reddish petals, along with the modified nectary disk —“white fleshy layer”— plus the sclerified mesocarp. Actually, this “white fleshy layer” of the fruit, which corresponds to the modified nectary disk, has not been mentioned previously for this genus. The development of the nectary disk after fertilization and its persistence in the mature fruit has been cited only for *Quinchamalium* Molina (Johri & Agarwal, 1965), a genus of Schoepfiaceae Blume (*sensu* Nickrent & al., 2010), a basal family of the Santalales. However the authors have not considered the modified nectary disk when described the fruit wall of *Quinchamalium chilense* Lam. Following Spuji (1994), pseudodrupes also include fruits with “accessory structures” —like the nectary disk—, as part of the fruit wall.

Description of the fruit at different stages of development leads to its classification in different types. In this sense, the fruit of *Jodina* has been described in a less mature stage when petals have not yet fallen off, or in a more mature stage after their detachment (Table 1). Bhatnagar & Sabharwal (1966) also classified the fruit of *Jodina* as a pseudodrupe, but in this case considering the persistent perianth as the fleshy layer. Furthermore, Heide-Jørgensen (2008) described as 5 carpels the modified and persistent reddish petals of *J. rhombifolia* —as already mentioned, the gynoecium is tricarpeal.

Taking into account the Cervantesiaceae, Der & Nickrent (2008) and Nickrent & al. (2010) mentioned that in *Jodina* the “outer exocarp” dehisces leaving exposed a white fleshy inner “exocarp”, whereas in other genera, such as *Cervantesia* and *Staufferia*, the 5 perianth valves remain on the mature fruit. As mentioned above, the white inner “exocarp” corresponds to the modified nectary disk. Detailed studies about fruit wall development are not recorded in the other related genera —*Cervantesia* and *Staufferia*—. In the case of *Acanthosyris*, Nee (1996) stated that the fleshy layer corresponded to the mesocarp, but without conducting developmental studies. Given what

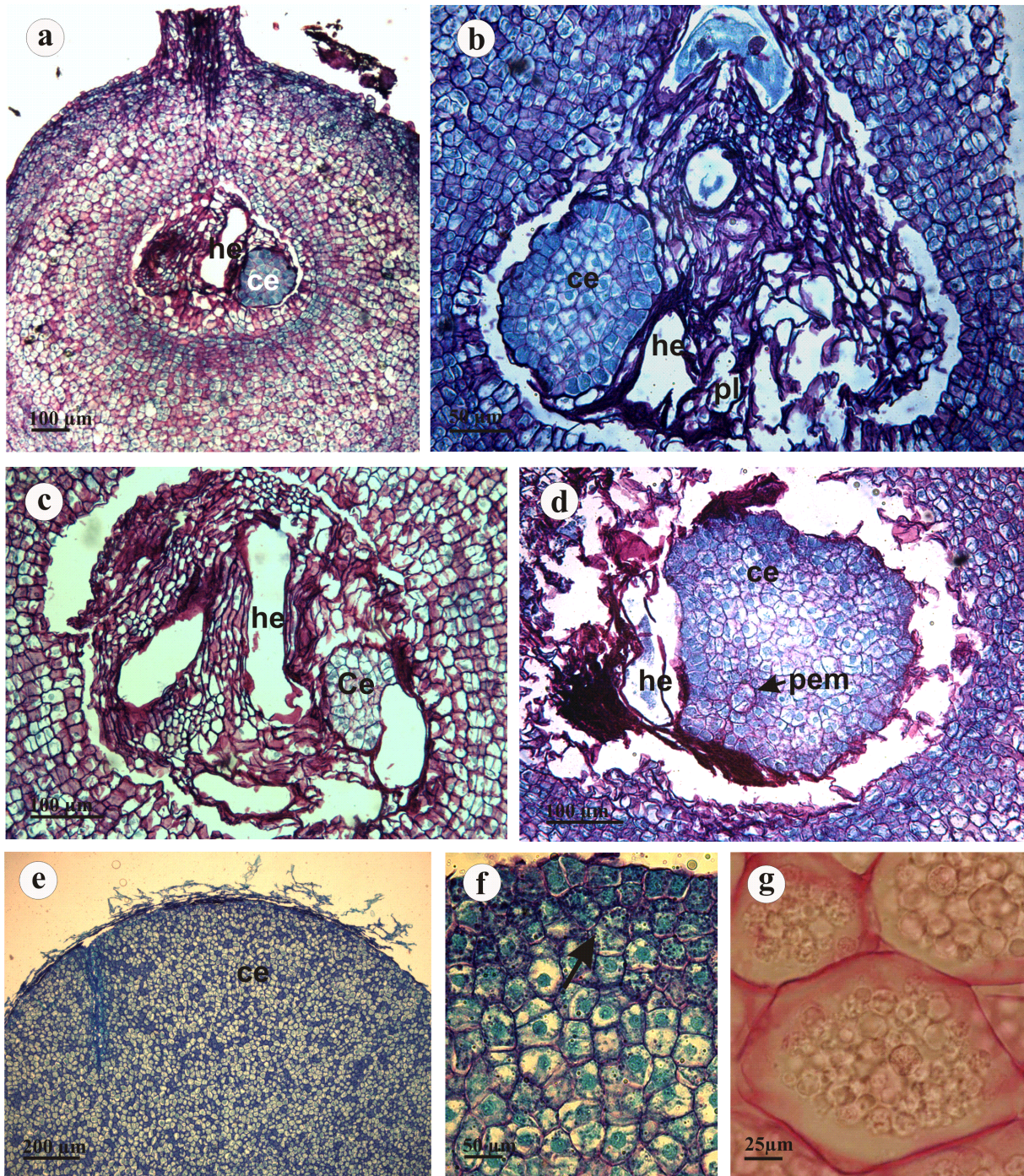


Fig. 4. Different stages of endosperm development: **a**, early stage showing the haustorial and cellular portions of endosperm in one ovule; **b**, branching of the haustorial portion and placenta almost completely consumed; **c**, a more advanced stage in which the haustorial endosperm consumes also the endocarp, the cellular portion has undergone relatively few mitotic divisions; **d**, later the increase in cellular endosperm occurs and the globular stage of pro-embryo is detected; **e**, section of seed showing endosperm totally cellular and rests of endocarp; **f**, detail of starch grains in the outer layers of the endosperm (arrow); **g**, deposits of lipids in endosperm cells. [Abbreviations: haustorial endosperm (he), cellular endosperm (ce), placenta (pl), pro-embryo (pem)].

was found in the present work a re-analysis of fruit development in the other genera of Cervantesiaceae will provide additional information for their systematic relationships.

With respect to the development of the seed, and specifically for endospermogenesis, we registered different developmental stages of the cellular and haustorial portions of the endosperm, as described previously (Bhatnagar &

Sabharwal, 1966, 1969). Since the ovule integument was consumed by the haustorial portion of the endosperm, the resulting seed became naked and the resulting structure should be called pyrene —i.e., mesocarp plus the enclosed seed—. As for reserves contained in the endosperm, we detected protein bodies and starch grains, besides lipids previously described (Bhatnagar & Sabharwal, 1969).

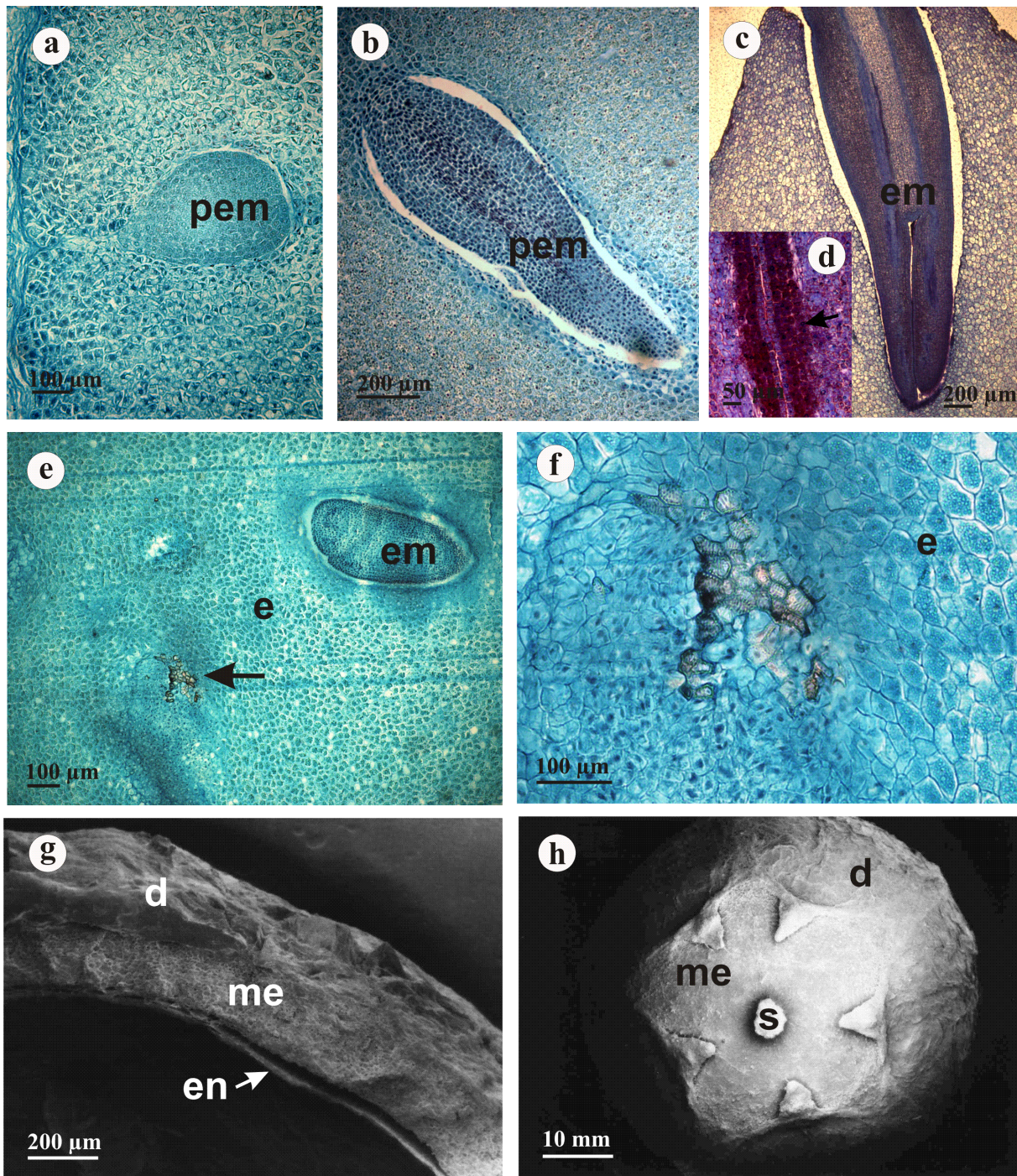


Fig. 5. Different stages of embryo development: **a**, early heart-shaped pro-embryo; **b**, torped-shaped pro-embryo; **c**, embryo with accumulation of starch grains in the cotyledons; **d**, detail of starch grains in palisade parenchyma (arrow); **e**, tracheary cells (arrow) detected in the endosperm, near the embryo; **f**, magnification of the tracheids occurring in the endosperm; **g**, detail of “seed coat”; **h**, of a mature pyrene. [Abbreviations: rests of nectary disk (d), stony mesocarp (me), papyraceous endocarp (en), stigma (s)].

Protein content of the seeds is similar to that found in *Santalum acuminatum* (R. Br.) A. DC. —Santalaceae s.str., Lott & Buttrose (1978)—. Chemical studies within Cervantesiaceae would contribute to clarify more aspects of endosperm development and the seed reserves present in the genera of this family.

As a corollary, information about the fruit type —fleshy or dry— may help to understand evolutionary

and ecological aspects in angiosperms. Since the kind of fruit has been associated with different habitats (Lorts & al., 2008), the misinterpretations of fruit types may lead to erroneous ecological or evolutionary associations. As the fruits of the Santalaceae s.l. have been interpreted as dry or fleshy by the different authors, studies like the one here presented will be fundamental to clarify such assumptions.

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