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Seed anatomy and dormancy class of 14 species from the Andean montane forests of Colombia

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Research Paper

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Abstract

Seed dormancy is a key trait used around the world to help understand the ecological dynamics of plant species, however, in some ecosystems such as the Andean forests of Colombia, the identification of dormancy class remains poorly known. To address this, we described the morphology, anatomy and dormancy class of the seeds and fruits of 14 species using microtome sections, light microscopy and scanning electron microscopy. Five native pioneer species showed Morphophysiological Dormancy, three Physical Dormancy + Physiological Dormancy and two Physical Dormancy, whereas two invasive species showed Physiological Dormancy. Only the species of the genus *Cecropia* had seeds with PY + PD that was promoted by the anatomical structure of the achenes. Of the analysed species, 85% showed dormancy and followed a pattern like other tropical montane ecosystems in the world. The anatomy of achenes and development of seed dormancy play important roles in the capacity of *Cecropia* species to contribute to the natural regeneration of Andean ecosystems. Additionally, seed dormancy may promote the high invasiveness of *Thunbergia alata* and *Ulex europaeus* in the Andean forests of the Central Cordillera of Colombia.

Introduction

The emergence of the ovule in vascular plants is the method by which vegetation cover today is dominated and structured by seed plants (Linkies et al., 2010). The seed is one of the most notable evolutionary novelties in the plant kingdom. It is an efficient propagule with structures that enclose and protect an embryo, allowing the embryo to develop and survive in environments that are adverse for many seedless plants (Niklas, 2008). Morphological and anatomical features of the tissues that make up the seeds, as well as climatic and spatial variations, are fundamental in the control of germination and the development of seed dormancy (Vázquez-Yanes and Orozco-Segovia, 1993; Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014; Geneve et al., 2018). However, the morpho-anatomy of seeds is a poorly studied field compared with other types of plant structures such as stems and leaves (Moles and Westoby, 2006).

The ability of seeds to remain viable in the soil for a given period has been related to the size of the seed, the density of seeds produced by the mother plant and the development of seed dormancy (Thompson et al., 1993; Gioria et al., 2020). Seed dormancy is probably an evolutionary characteristic that allows seeds to avoid adverse conditions for seedling establishment (Kollmann 2008; Baskin and Baskin 2014), which is related to the morphological and anatomical traits of seeds (Forbis et al., 2002; Baskin and Baskin, 2014). Goebel (1898) was the first to identify the morphology of the embryo as one of the important factors in the dormancy and germination of seeds when he found a group of plants with seeds that contained embryos in them that needed a period after maturation to germinate. Years later, Martin (1946) incorporated a description of the embryonic morphology of 1400 genera of Angiosperms, introducing a classification of the types of embryos, and considering their shape and development. Baskin and Baskin (2007) implemented adjustments to this classification and highlighted the importance of some types of embryos, such as rudimentary and underdeveloped ones, in the development of morphological and morphophysiological dormancy (MPD) in seeds.

One of the anatomical features that most predominantly determines the interaction of seeds with the external environment is the seed coat. Corner (1976) introduced a useful conceptual framework for the anatomical description of the integuments that cover the seed (testa and tegmen), highlighting a wide plasticity in the shape of the cells and the different mechanical restrictions formed by the cell layers that make up the seeds and form the integuments. The functional value of these cell layers has been widely related to the impermeability of the seeds, since they possibly form part of a mechanical barrier that limits the entry of water into the seeds, preventing cell elongation of the embryo and, consequently, the development of physical dormancy (Sautu et al., 2007; Baskin and Baskin, 2014; Geneve et al., 2018).

Baskin and Baskin (2014) developed a hierarchical classification system that includes five classes of seed dormancy: Physiological Dormancy (PD), Morphological Dormancy (MD), Physical Dormancy (PY), Morphophysiological Dormancy (MPD) and Physiophysical Dormancy (PY + PD). Seed dormancy has been widely used as a functional trait that allows a better approximation of the ecology of seed germination in both temperate and tropical zones (Baskin and Baskin, 2005; Carta et al., 2013, 2016; de Souza et al., 2015; Chia et al., 2016), using dormancy as one of the characteristics that most prominently influences the functional dynamics of seeds (Baskin and Baskin, 2014; Kildisheva et al., 2020).

Several studies of this type have been carried out in the Neotropics, mainly in life zones corresponding to tropical humid forest and tropical dry forest (Sautu, 2004; de Souza et al., 2015; Escobar et al., 2018). However, in Andean montane forests, identified as sites of high species diversity (Myers et al., 2000) knowledge about life-history traits of native pioneer and invasive species in relation to the dormancy, germination and morpho-anatomical characteristics of the seeds is limited.

Pioneer plants are species in which seed germination and seedling establishment occur in disturbed areas of ecosystems, where there is high light exposure (Swaine and Whitmore, 1988). In addition, their seeds can develop dormancy and form seed banks in the soils of the ecosystems; seed banks are of great importance (Swaine and Whitmore, 1988; Zalamea et al., 2018). However, many of the native pioneer species of the Andean forests, such as *Piper aduncum* L. and *Cecropia peltata* L., have a high invasive expression in other ecosystems of the world (Rogers and Hartemink, 2000; Putri et al., 2021). These species may be pioneering invasive species due to morphological and ecological characteristics that enable them to behave in this way (Fehmi et al., 2021).

The Andean montane forests of the altiplano of eastern Antioquia represent some of the few fragments that persist in the Andean zone of Colombia. They have high floristic diversity that continues to be under pressure from anthropic effects and biological invasion of exotic species (Rubiano et al., 2017). Due to the importance of these forest remnants, efforts aiming at their protection and recovery are essential for the maintenance of natural dynamics and for the reduction of factors that affect the ecosystem services they offer (Gómez-Salazar et al., 2016). Such efforts require extensive knowledge about the plasticity, adaptation and evolution of vegetation cover that must include a deep understanding of seed traits and function (Saatkamp et al., 2019).

This paper presents the first detailed analysis of seed dormancy for 12 native pioneer and two invasive species in Andean ecosystems of Colombia, with the aim of describing the seed and achene morphology, anatomy and dormancy classes that predominate in native pioneer and invasive species. Seed dormancy is described for the first time in the genera *Cecropia* and *Chrysochlamys*, and the occurrence of PY + PD in the family Urticaceae is reported. In addition, this study seeks to describe the main morphological and anatomical traits of seeds associated with dormancy classes and to discuss ecological aspects of dormancy in native pioneer species compared with invasive species in Andean ecosystems.

Materials and methods

Study area

The study was carried out in remnants of Andean montane forests in the highlands of eastern Antioquia, with an elevation between 2150 and 2300 m asl, in the village of La Rivera in the municipality of El Carmen de Viboral, Antioquia (6°5′24.73″N, 75°18′4.71″W) and the village of Fátima in the municipality of La Ceja, Antioquia (5°57′36.82″N, 75°25′50.95″W) (Fig. 1), both located on the eastern slope of the Cordillera Central, southeast of the department of Antioquia, Colombia. These areas are classified as lower montane moist forest, according to the classification system of Holdridge (1967). They have slopes greater than 25% with a relief that varies from slightly undulating to very steep.

Collection of fruits and seeds

We took random walks through the interior of the entire forested area of the ecosystem in search of ripe fruits and seeds. Simultaneously, a floristic inventory was carried out, where vegetative and reproductive structures were collected that were useful for the taxonomic determination of the species. For each species, about 150–200 fruits with seeds were collected, trying to collect fruits from different specimens belonging to the populations of each species found within the ecosystem. Both fruits and seeds were stored in paper bags at room temperature and sown no later than 7 d after collection in order to avoid the loss of viability of some seeds (Baskin and Baskin, 2014). The specimens from the floristic inventory were deposited in the reference collection of the herbarium of the Universidad Católica de Oriente (HUCO).

Preparation of anatomical samples

Mature seeds of each species and achenes of *Cecropia* were fixed in a mixture of formalin, ethanol and acetic acid (FAA) for 24 h at 6°C (Johansen, 1940). The seeds were subsequently softened in 10% ethylenediamine for 6 h at 70°C (Carlquist, 1982) and dehydrated in ethanol series (50, 60, 70, 80, 90, and 95%). After dehydration, they were rinsed in HistoChoice® twice for 12 h each and subsequently embedded in a solution composed of HistoChoice® and Paraplast® paraffin 2:1 for 2 h, then in a 1:2 solution for 2 h, and finally three series of 12 h in 100% Paraplast® (Ruzin, 1999). Sections were made in different cutting planes, 5–7 μm thick, using a Leica RM2125 rotary microtome with disposable blades. Staining was done with Safranin-Alcian Blue (Fasga) (Tolivia and Tolivia, 1987) for structural differentiation of primary and secondary walls. Sections were observed through an Olympus CX31 optical microscope.

Scanning electron microscopy

To carry out the morphological descriptions with scanning electron microscopy (SEM), seeds of the different species were fixed and dehydrated in 100% methanol for 48 h (Talbot and White, 2013). Subsequently, they were dried to a critical point with a SAMDRI®-795 desiccator. Samples were mounted on double-sided conductive carbon tape and plated with gold in a DENTON VACUUM DESK IV ionizer for 8 min. Micrographic observations and recordings were made with a TESCAN® scanning electron microscope.

Morpho-anatomical analysis

In each seed or fruit, morphological characteristics corresponding to the seed coat or pericarp (such as the surface and shape) were measured and described. At the anatomical level, characteristics related to the number of integuments, shape of the cells that

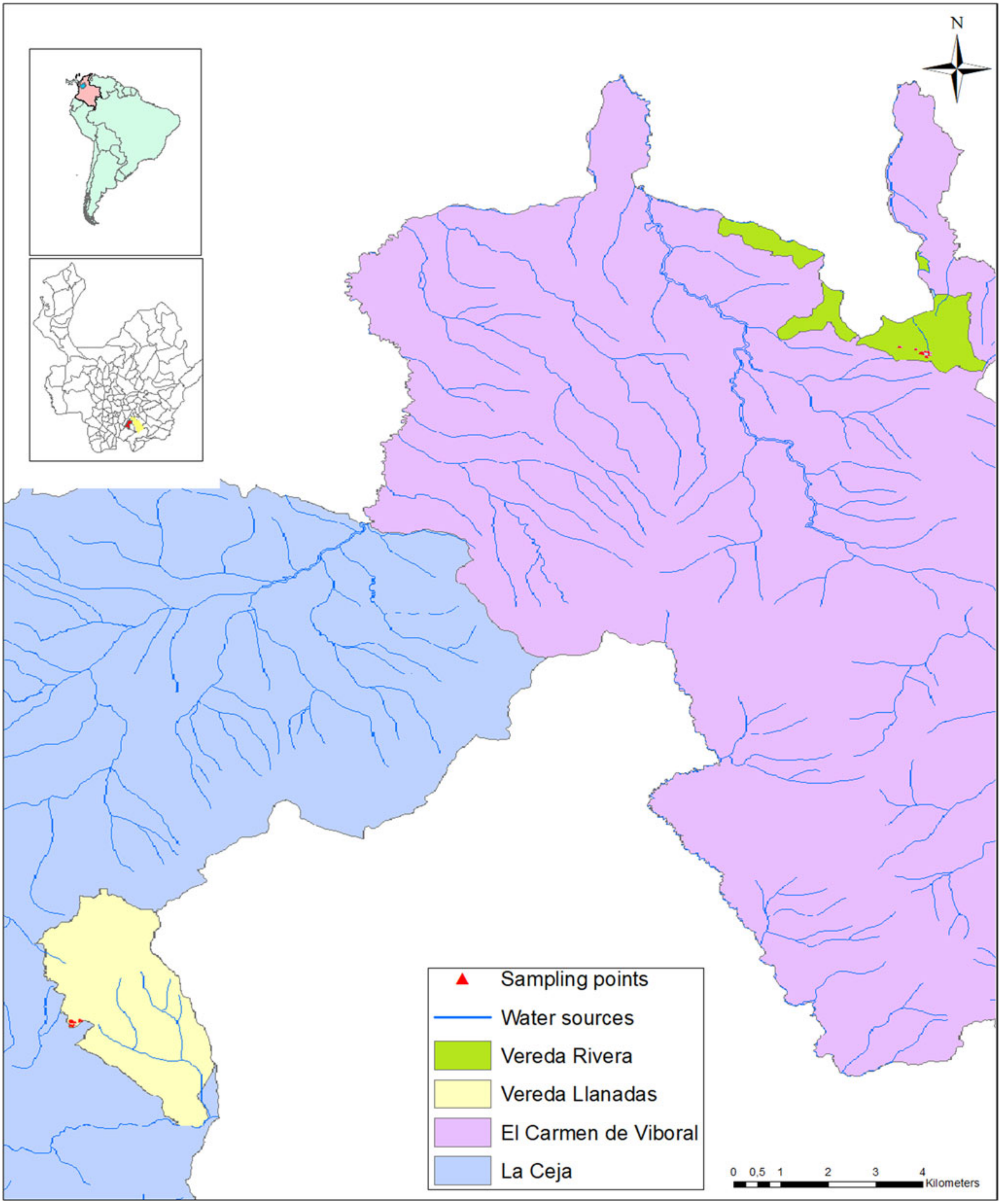

Figure 1. Spatial location of the study areas in the municipalities of El Carmen de Viboral and La Ceja, Antioquia.

compose them, stratification and shape of the cell layers were examined. In addition, the cell layers of the pericarp of *Cecropia* fruits were described. Cell orientation and quantitative characteristics such as the thickness of cell layers composing each seed coat tegument and embryo length in each seed were quantified. In the case of achenes, the thickness of each cell layer that makes up the pericarp was measured using ImageJ2 version 2.3.0 (Schneider et al., 2012). The terminology used in the descriptions was based on Martin (1946), Murley (1951), Corner (1976), Wilkinson (1979), Barthlott (1981), Stearn (1983) and Font Quer (2001).

Germination tests

The sowing and germination of the collected material was carried out in a nursery located in the municipality of Rionegro, Antioquia at 2080 m asl. Three repetitions per species were prepared and a maximum of 50 seeds or fruits per repetition were sown using coconut fiber as a substrate. Watering was constant, we applied two to three irrigations a day with a duration of 2 min, using a nebulizer. Seed germination progress was monitored weekly for 5 months. The total germination time and the mean germination time (MGT) were calculated as a measure of dormancy following the methodology proposed by Sautu et al. (2006).

Assignment of seed dormancy classes

Seeds were considered dormant if their MGT exceeded 30 d without any pre-treatment. In addition, anatomical features of the seeds were used for the classification of dormancy classes into dormant seeds. The species were grouped into those with dormant seeds and those with non-dormant seeds. Following Baskin and Baskin (2014), dormant seeds were separated into five classes: (1) Physical Dormancy (PY), seeds with impermeable coats and fully developed but non-dormant embryo (germination occurs in the following 30 d after scarification); (2) Morphological Dormancy (MD), seeds with an underdeveloped embryo and with a MGT ≤ 30 d; (3) Morphophysiological Dormancy (MPD); seeds with undeveloped embryo and a MGT > 30 d; (4) Physiological Dormancy (PD), those that had water-permeable coats, a fully developed embryo and a MGT > 30 d and (5) Physiophysical Dormancy (PY + PD), seeds with impermeable coats and physiological dormancy.

Seed permeability to water was determined by an inhibition test. For the species *S. saponaria* and *U. europaeus*, 15 non-scarified seeds and 15 seeds mechanically scarified with sandpaper were used. Three replicates per treatment were made for each species. The mass of the seeds was quantified and subsequently placed in Petri dishes with filter paper saturated with water. During 8 h, the increase in mass was quantified at intervals of 1 h and the percentage increase in fresh mass was calculated using the equation: $\%M_s = [(M_i - M_0)/M_0] \times 100$, where M_0 and M_1 represent the fresh mass value at times T_1 and T_2. The progression of the percentage increase in mass over time was analysed using linear and non-linear regressions. All analyses were undertaken with Past version 4.07 software (Hammer et al., 2001).

Results

In this study, 14 species belonging to 11 genera and 8 families of Angiosperms were analysed (Table 1). Of these, 12 were native and 2 were commonly reported as invasive in Andean ecosystems (Portilla-Yela, 2019; Quijano-abril et al., 2021). Of the native species, five had morphophysiological dormancy (MPD), three physiophysical dormancy (PY + PD), two physical dormancy (PY) and two did not show dormancy (ND). The two invasive species presented physiological dormancy (PD).

Anatomical description of seeds with MPD

The seeds with MPD exhibited shapes ranging from ellipsoid, reniform, subglobose-turbinate or slightly orbicular-ovoid with a length of 0.94 to 10.96 mm and 0.66 to 4.10 mm wide, respectively, with the presence of a sarcotesta or a funicular red crenulated aril (Figs 2C and 3B). *Piper* species had seed coats with a

Table 1. Families and species analysed, with the type of embryo and the class of dormancy that the seeds showed.

Family	Species	Origen	Type of embryo	MGT ± SE	% Germination	Dormancy
Acanthaceae	*Thunbergia alata* Bojer ex Sims.	Invasive	Bent	19.61 ± 15.56	100 ± 2.83	PD
Clusiaceae	*Clusia multiflora* Kunth.	Native	Lineal	8.11 ± 0.91	100 ± 5.65	ND
Clusiaceae	*Chrysochlamys colombiana* (Cuatrec.) Cuatrec.	Native	Lineal (underdeveloped)	49.22 ± 0.87	40.67 ± 6.11	MPD
Fabaceae	*Abarema lehmannii* (Britton & Rose ex Britton & Killip) Barneby & J.W. Grimes.	Native	Investing	8.57 ± 2.24	100 ± 3.45	ND
Fabaceae	*Erythrina rubrinervia* Kunth.	Native	Bent	31.36 ± 9.79	63.32 ± 2.34	PY
Fabaceae	*Ulex europaeus* L.	Invasive	Bent	31.68 ± 2.65	72.23 ± 11.31	PD
Papavearaceae	*Bocconia frutescens* L.	Native	Rudimentary	66.32 ± 21.55	34.21 ± 11.34	MPD
Piperaceae	*Piper aduncum* L.	Native	Broad	47.64 ± 10.07	60.76 ± 5.11	MPD
Piperaceae	*Piper daniel-gonzalezii* Trel.	Native	Broad	43.41 ± 12.60	39.45 ± 12.03	MPD
Primulaceae	*Myrsine coriacea* (Sw.) R. Br. ex Roem. & Schult.	Native	Undifferentiated	41.83 ± 10.92	38.93 ± 6.18	MPD
Sapindaceae	*Sapindus saponaria* L.	Native	Folded	30.06 ± 10.32	89.76 ± 2.04	PY
Urticaceae	*Cecropia angustifolia* Trécul.	Native	Spatulate	39.35 ± 7.36	37.21 ± 15.42	PY + PD
Urticaceae	*Cecropia peltata* L.	Native	Spatulate	63.73 ± 6	46.59 ± 6.56	PY + PD
Urticaceae	*Cecropia telealba* Cuatrec.	Native	Spatulate	46.21 ± 9.59	36.23 ± 12.46	PY + PD

MGT, mean germination time; SE, standard error.

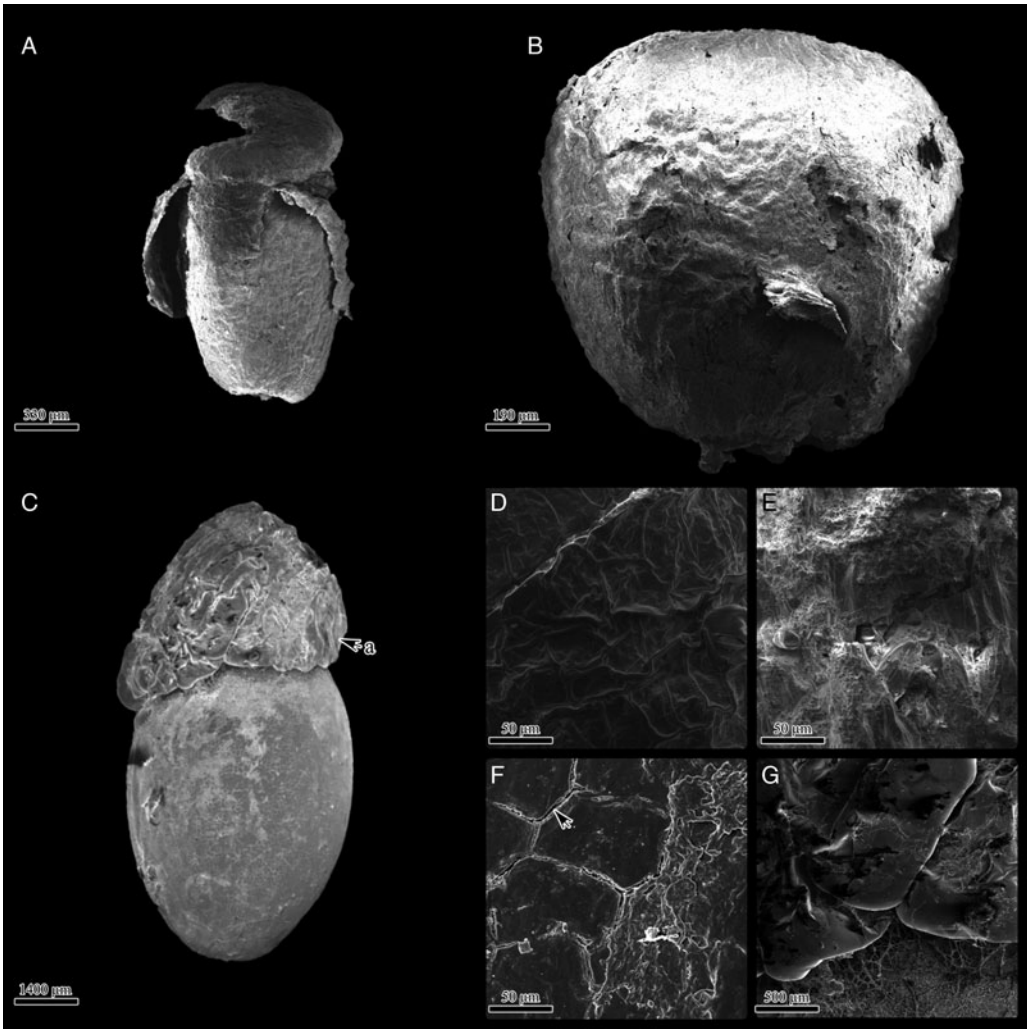

Figure 2. Micrographs representing seed morphology with MPD. (A,D) *Piper aduncum*. (B,E) *Piper daniel-gonzalezii*. (A,B), subglobose – turbinate seeds. (D,E) Seeds with ruminate surface. (C,F,G) *Bocconia frutescens*. (C) Ellipsoid seed with funicular aril. (F,G) Seeds with areolate surface, clefts between cell junctions and crenulate aril. a, aril. The arrow indicates the clefts between the cells.

ruminate surface (Fig. 2D, E). *Bocconia frutescens* L. had a seed coat with an areolate outer surface with clefts between cell junctions (Fig. 2F), as did *Chrysochlamys colombiana* (Cuatrec.) Cuatrec. with a colliculate surface (Fig. 3F), while *Myrsine coriacea* (Sw.) R. Br. ex Roem. & Schult. exhibited a lined surface (Fig. 3D).

The seed coat in these species is composed of two integuments (Corner, 1976). The external integument may be attached to the internal integument forming a membrane of cuboidal cells 0.02 to 0.04 mm thick with abundant accumulations of tannins as exhibited by individuals of the genus *Piper* (Figs 4T and 6C) or differentiated. When the external integument differentiates into cellular layers, it may be composed of a 0.11 mm sclerotic exotesta, with longitudinally elongated cells and a 0.18 mm endotesta, formed by collapsed cells with scattered accumulations of tannins, as exhibited in *M. coriacea* (Fig. 4B). In *B. fructescens*, the external integument is made up of a 0.03 mm uniestratified exotesta, composed of tabular cells with thick walls, a flattened cell mesotesta and an endotesta with 0.16 mm cuboid cells accumulating waxy, brown substances (Fig. 4H), while in *C. colombiana*, the external integument was modified forming a differentiated sarcotesta in an upper and lower epidermis, made up of cuboid, isodiametric cells and a multiplying mesophyll 0.04 mm thick, composed of 10 to 11 layers of flattened cells that were radially elongated (Fig. 4N).

The internal integument may form a thick-walled cuboid cell layer with abundant accumulations of tannins as in *M. coriacea* (Fig. 4B, C), restricted to the micropyle and with a fibriform exotegmen with a thick cell wall and accumulation of substances, waxy substances in the cell lumen, a mesotegmen consisting of conspicuously perforated, thick-walled cells with a slightly flattened appearance, and an endotegmen with perforated, thin-walled, tabular cells as in *B. fructescens* (Fig. 4I, J), or exhibiting a uniseriate, discontinuous upper epidermis, a multiplicative mesophyll composed of 10 to 11 layers of flattened and radially elongated cells and a sclerotic endotegmen, with cuboidal, lignified cells with oblong lumen and thick wall with abundant perforations as in *C. colombiana* (Fig. 4O). The seeds with MPD have an endosperm that occupies almost the entire volume of the seed (except in *C. colombiana*). This endosperm is composed of parenchyma cells with thick walls (Figs 4A, G, S and 6A). Undifferentiated embryos (Fig. 4A, E), rudimentary (Fig. 4G, L), broad (Figs 4U and 6B) or an underdeveloped linear embryo, hypocotylar occupy almost the entire internal area of the seed with poorly developed

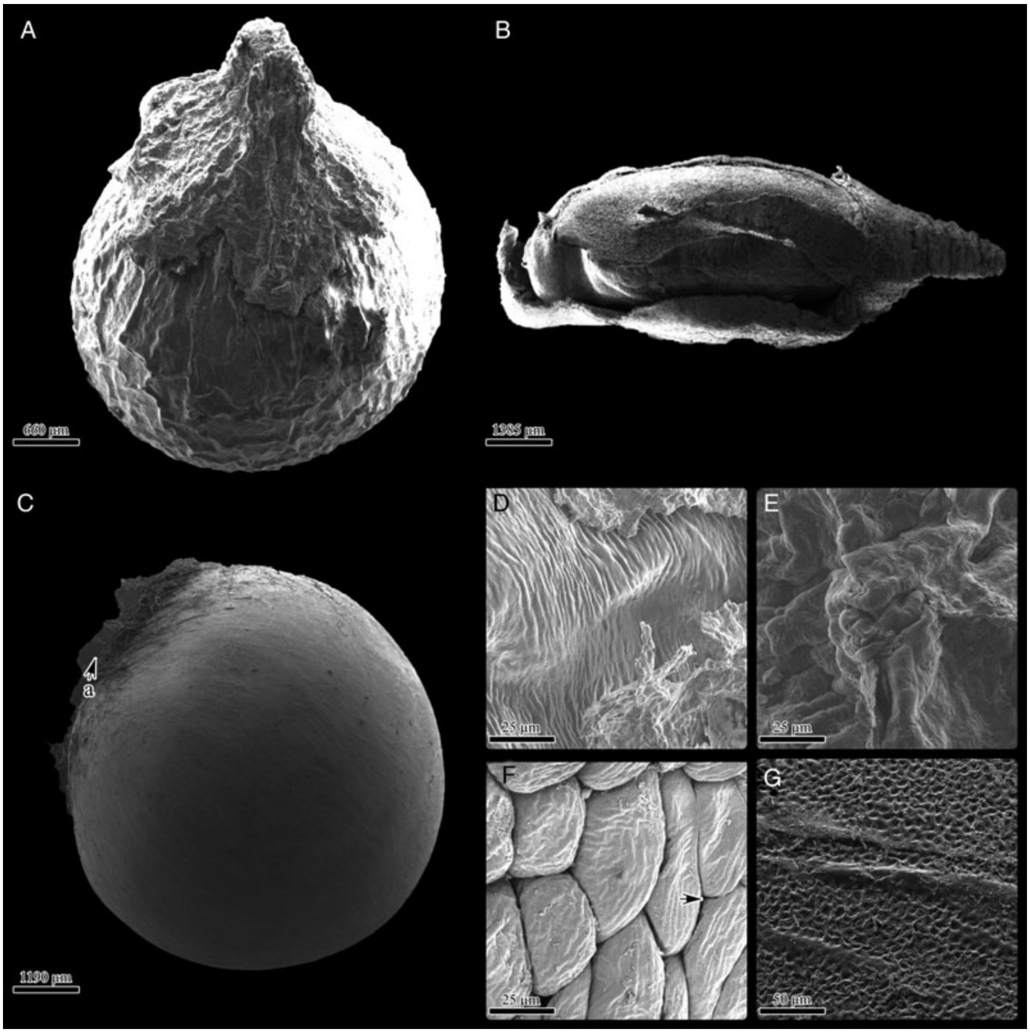

Figure 3. Micrographs representing seed morphology with MPD and PY. (A,D,E) *Myrsine coriacea*. (A) orbicular – ovoid seeds. (D,E) Seed with lineate surface. (B,F) *Chrysochlamys colombiana*. (B) Reniform seeds. (F) Seeds with colliculate surface and clefts between cells. (C,G) *Sapindus saponaria*. (C) Subglobose seeds. (G) Seeds with reticulate – foveolate surface. a, aril. The arrow indicates the clefts between the cells.

dimorphic cotyledons and abundant secretory ducts of waxy substances (Fig. 4M, P, Q, R).

Anatomical description of seeds with PY

Seeds are subglobose or reniform with a reticulate-foveolate surface as in *Sapindus saponaria* L. (Fig. 3C, G) or glebulate as in *Erythrina rubrinervia* Kunth. (Fig. 5A, C). The seeds of *E. rubrinervia* have a subcentral, oblong-ovate hilum with a central cleft (Fig. 5D), while those of *S. saponaria* exhibit a white cottony micropylar aril, arranged laterally (Fig. 3C). Seeds with impermeable seed coat (Fig. 10A, B) are derived from two integuments: the external integument is composed of a layer of cells arranged in a palisade with a thick and lignified cell wall as in *S. saponaria* (Fig. 8R, T) and with equidistant clefts in the external cell wall as in *E. rubrinervia* (Fig. 6F, G). An endotesta with a multiple layer of cells arranged in a palisade, thick-walled and with abundant accumulations of idioblasts (Fig. 8U) or as a multiple layer of radially elongated, thin-walled column of cells (Fig. 6E). Internal integument reduced to a layer of cuboidal cells, alternated with an extension of the chalaza that surrounds ¾ of the embryo forming a pachychalaza of cuboidal cells with abundant accumulations of tannins (Fig. 8Q, U) (Corner, 1976); or it is differentiated into two cell layers, an exotegmen composed of thin-walled parenchyma cells and an endotegmen composed of cuboidal to slightly tabular cells (Fig. 6E). The embryo is folded (Fig. 8Q) or bent fully developed with incumbent cotyledons (Fig. 6D).

Anatomical description of seeds with PD

These are reniform (Fig. 6H) or subglobose seeds with the presence of a micropylar aril as in *Ulex europaeus* L. (Fig. 6L, N). The seed coat is derived from an integument reduced to two layers of cells, an outer layer composed of prismatic cells and a layer of tabular cells with accumulations of tannins (Fig. 6I, J) or it is derived from two integuments as in *U. europaeus* with an outer integument made up of a permeable exotesta (Fig. 10C and D), composed of palisade cells, an endotesta with thick-walled osteosclereids and a collapsed inner integument (Fig. 6M, O). A fully developed bent embryo with endosperm composed of lignified wall cells and storage cotyledons as in *Thunbergia alata* Bojer ex Sims (Fig. 6H, K) or incumbent in *U. europaeus* (Fig. 6L).

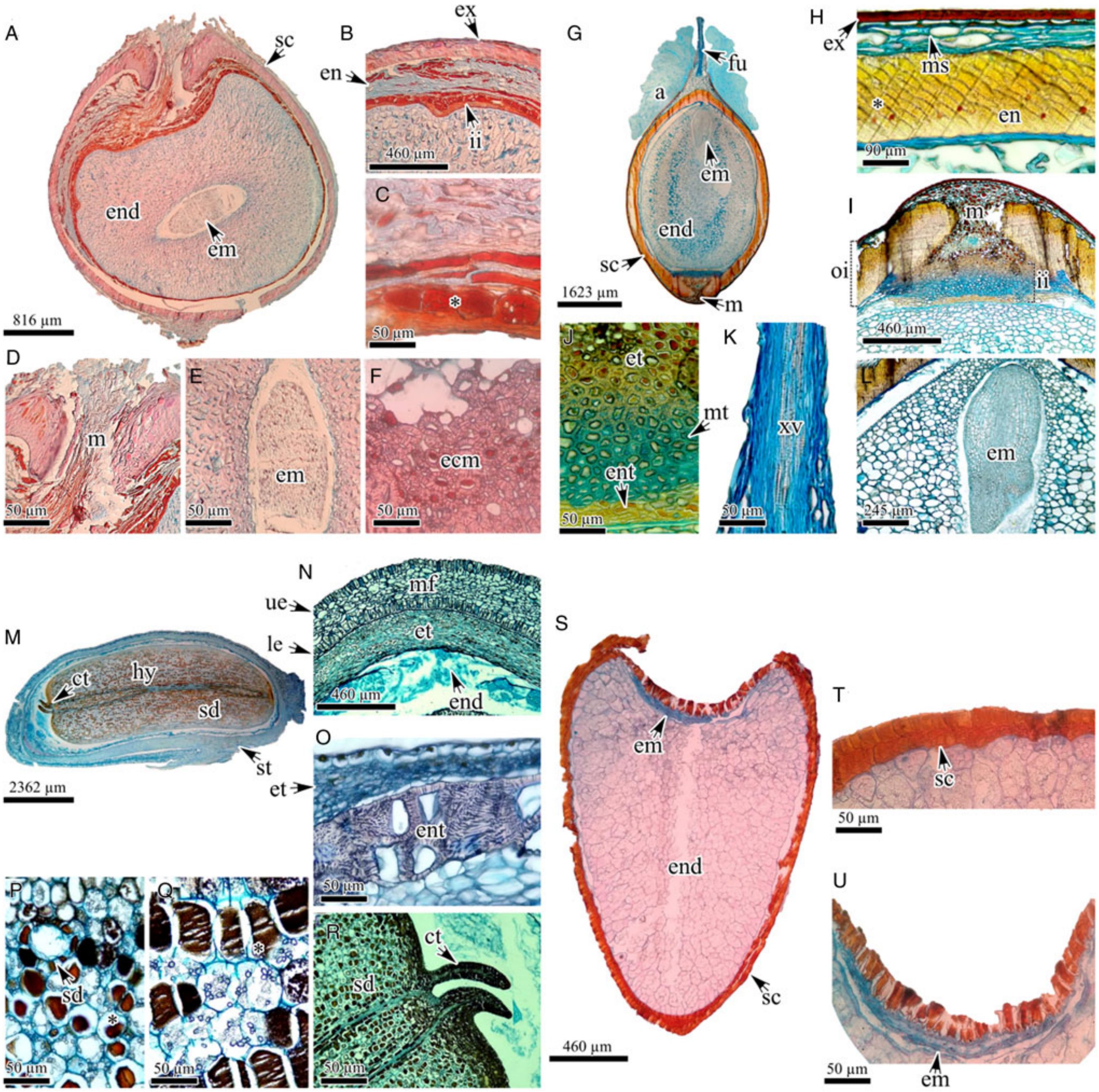

Figure 4. Comparative anatomy of seeds with MPD. (A–F) *Myrsine coriacea*. (A,E) Rigid endosperm and undifferentiated embryo. (B,C) Seed coat with sclerotic exotesta, collapsed endotesta and internal integument with tannin deposits. (D,F) Micropyle composed of lignified sclerenchyma cells. (G–L) *Bocconia frutescens*. (G,L) Funicular aril, presence of parenchymal endosperm and rudimentary embryo. (H–J) Seed coat with uniestratified exotesta and endotesta accumulating waxy substances, fibriform exotegmen, exotegmen and endotegmen with cells with perforated walls. (K) Xylem vessels in the funicle. (M–R) *Chrysochlamys colombiana*. (M,R) Hypocotylar embryo with dimorphic cotyledons. (N,O) Sarcotesta with upper and lower epidermis and sclerotic endotegmen. (P,Q) Secretory cavities of different size and shape located in the embryo. (S–U) *Piper daniel-gonzalezii*. (S,U) Wide embryo and rigid, parenchymal endosperm. (T) Seed coat with accumulation of tannins. a, aril; ct, cotyledons; ecm, sclerenchyma; en, endotesta; end, endosperm; ent, endotegmen; em, embryo; et, exotegmen; ex, exotesta; fu, funicle; hy, hypocotyl; ii, internal integument; le, lower epidermis; m, micropyle; mf, mesophyll; ms, mesotesta; mt, mesotegmen; oi, external integument; sc, seed coat; sd, secretory cavities; ue, upper epidermis; xv, xylem vessels. * Show sites of accumulation of substances.

Anatomical description of fruits and seeds with PY + PD

The seeds with PY + PD dormancy belong to the analysed individuals of the *Cecropia* genus. In the species of this genus, the seeds are attached to the fruit, forming ellipsoid-ovoid achenes (Fig. 7A–C), with a length of ~1.84 mm and a width of ~0.75 mm with a rugose to ruminate surface (Fig. 7D–G). Conspicuous pedicel scar, with helically thick-walled, clustered xylem vessels as in *C. peltata* and *Cecropia angustifolia* Trécul. (Fig. 8E, J, N). Pericarp is ~0.25 mm thick and differentiated into exocarp, mesocarp and endocarp (Fig. 8A, G, M). Exocarp is made up of two layers of mucilaginous cells of ~0.13 mm with thin wavy walls and the presence of dispersed tannins in some of the cells, as in *C. angustifolia* (Fig. 8I). Mesocarp with

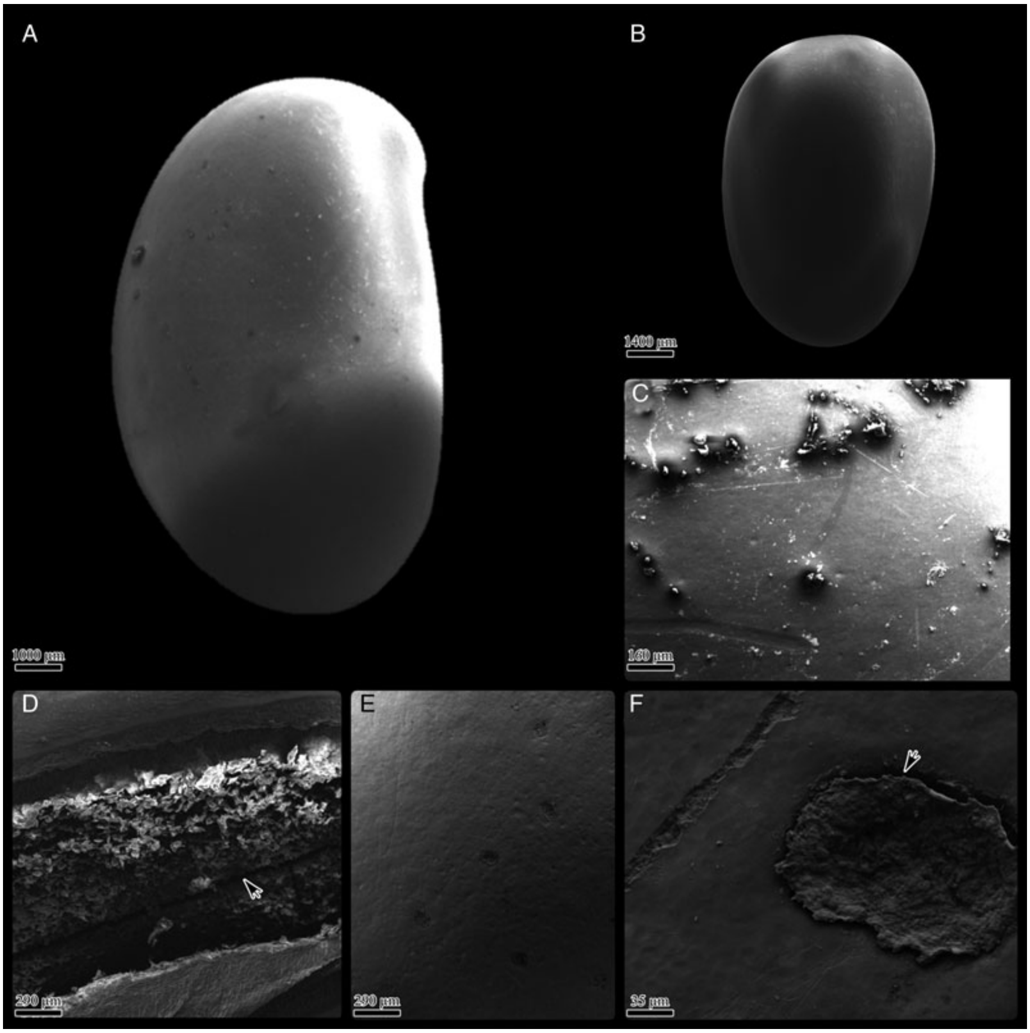

Figure 5. Micrographs representing the morphology of seeds with PY and with ND. (A,C,D) *Erythrina rubrinervia*. (A) Reniform seeds, (C) glebulate surface, (D) subcentral hilum with a central cleft. (B,E,F) *Abarema lehmannii*. (B) Oval seeds, (E,F) seeds with smooth surface and funicle with a marginal cleft. The arrows point at the clefts.

two to three layers of cells of ~0.10 mm, differentiated into two zones, one of tabular cells irregularly filled with tannins and another layer of lignified thick-walled macrosclereids that are grouped below the pedicel scar (Fig. 8B, G, N). Endocarp composed of one to six layers of macrosclereids of ~0.08 mm, radially elongated in a palisade, cells with oblong lumen, narrow, rounded at the apices and with thick and lignified cell walls (Fig. 8C, K, O). Seed composed of a seed coat derived from two integuments (Lobova et al., 2003) reduced to a ~0.14 mm thick membrane and one to two layers of cuboidal cells with the presence of tannins (Fig. 8F, L, P). Spatulate and developed embryo of ~0.87 mm (Fig. 8A, G, M).

Description of seeds without dormancy (ND)

Oval seeds have a smooth surface, with a sclerotic funicle showing clefts on the margin (Figs 5B, E, F and 9J) or obovate to slightly reniform with the presence of an aril that covers the entire seed as in *Clusia multiflora* Kunth. (Fig. 9A). The seed coat is derived from two integuments that may be made up of an external integument composed of an exotesta with cuboid cells with a thick external wall and abundant accumulations of waxy substances and an endotesta with three to four layers of collapsed cells and with the presence of crystals of calcium oxalate, and an internal integument reduced to a flattened cell membrane acquiring an almost lamellar shape (Fig. 9C, D) or by an external integument with an exotesta composed of discontinuous, palisaded cells with a thick cell wall, an endotesta made up of a layer of more or less collapsed, thick-walled cells, and an internal integument consisting of a layer of thick-walled parenchyma cells (Fig. 9G). Investing embryo developed with conduplicate cotyledons (Fig. 9F, H, I) or linear embryo developed with abundant substance-secreting cavities (Fig. 9A, B, E).

Discussion

Ecological conservation and restoration must be supported by detailed studies on functional traits of seeds that condition germination and the development of seed dormancy (Saatkamp et al., 2019; Kildisheva et al., 2020). It is necessary to include studies on anatomical traits related to the ecology of seed germination and seed dormancy of native and invasive species within the strategies that are generated for the protection and recovery of Andean

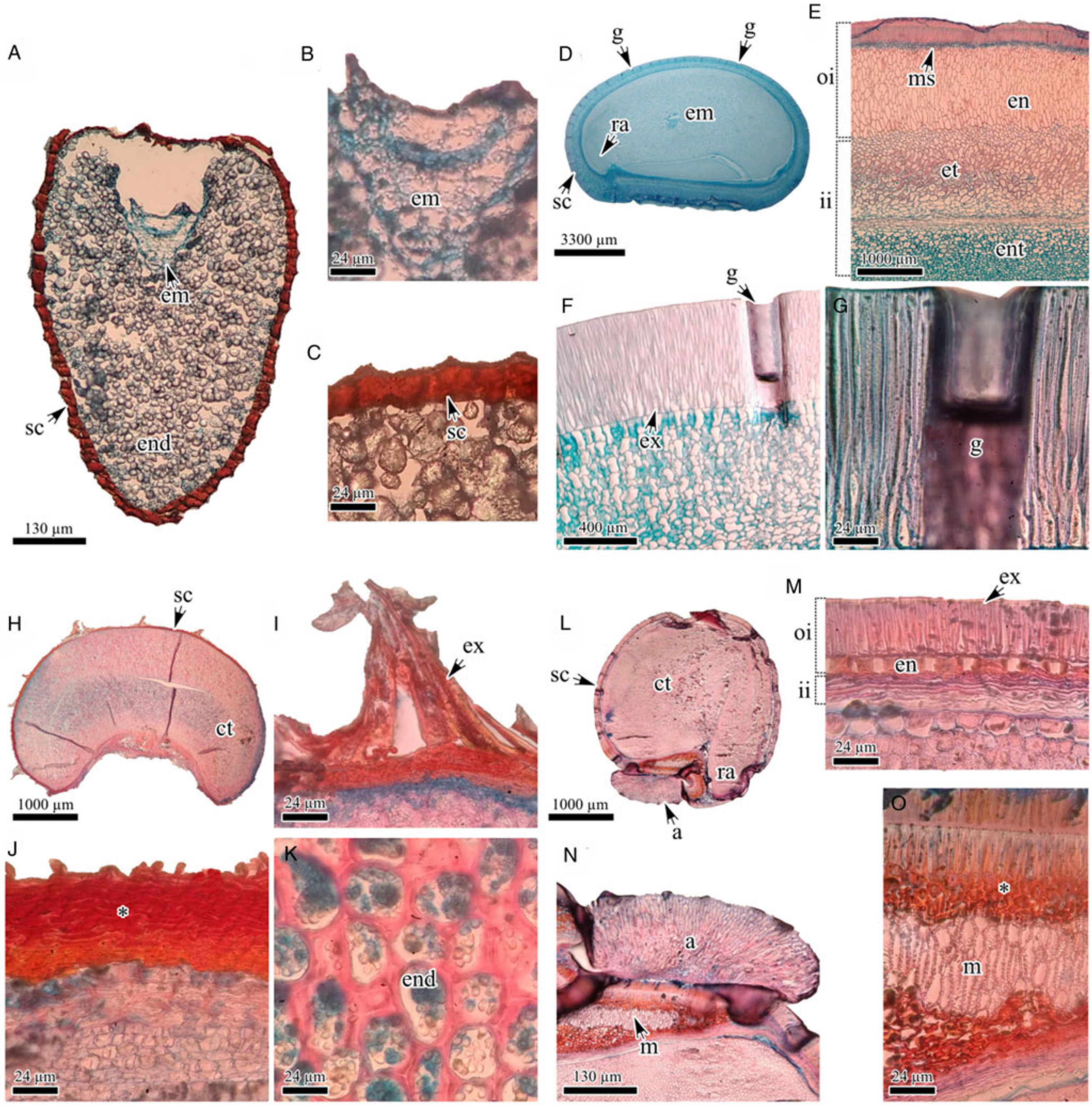

Figure 6. Comparative anatomy of seeds with MPD, PY and PD. (A–C) *Piper aduncum*. (A,B) Large embryo and parenchymal endosperm. (C) Seed coat reduced to a cell membrane with abundant tannin deposits. (D–G) *Erythrina rubrinervia*. (D) Fully developed bent embryo. (E) Seed coat composed of a palisade exotesta with equidistant clefts, an endotesta of columnar cells, exotegmen composed of thin-walled parenchyma cells and an endotegmen composed of cuboidal to slightly tabular cells. (F,G) Palisade exotesta with occluded clefts. (H–K) *Thunbergia alata*. (H,K) Bent, developed embryo with endosperm composed of cells with a lignified wall. (I,J) Seed coat derived from an integument reduced to two layers of cells, an outer layer composed of prismatic cells and a layer of tabular cells with accumulations of tannins. (L–O) *Ulex europaeus*. Bent embryo, developed with incumbent cotyledons. (M,O) External integument made up of a permeable exotesta, composed of palisade cells, an endotesta with thick-walled osteosclereids and a collapsed internal integument. (N) Micropylar aril. a, aril; ct, cotyledons; en, endotesta; end, endosperm; ent, endotegmen; em, embryo; et, exotegmen; ex, exotesta; g, gap; ii, internal integument; m, micropyle; ms, mesotesta; oi, external integument; ra, radicle; sc, seed coat. *indicating sites of accumulation of substances.

ecosystems. Our results indicate that ~85% of the analysed species produce dormant seeds; ~36% displayed MPD, ~21% PY + PD, ~14% PY and ~14% PD (Table 1). This phenomenon is consistent with dormancy data reported for tropical montane forests (Baskin and Baskin, 2014; Athugala et al., 2021). In the present study, the proportion of seeds with PY + PD is higher compared with current records of dormancy in tropical montane ecosystems, in which only 5% of the species have seeds that can develop PY + PD (Baskin and Baskin, 2014).

A recent study carried out in a tropical montane forest of Sri Lanka with the aim of profiling the predominant dormancy classes in these ecosystems and contrasting it with previous studies carried out in tropical montane forests, does not report the development of PY + PD in any of the 80 species analysed

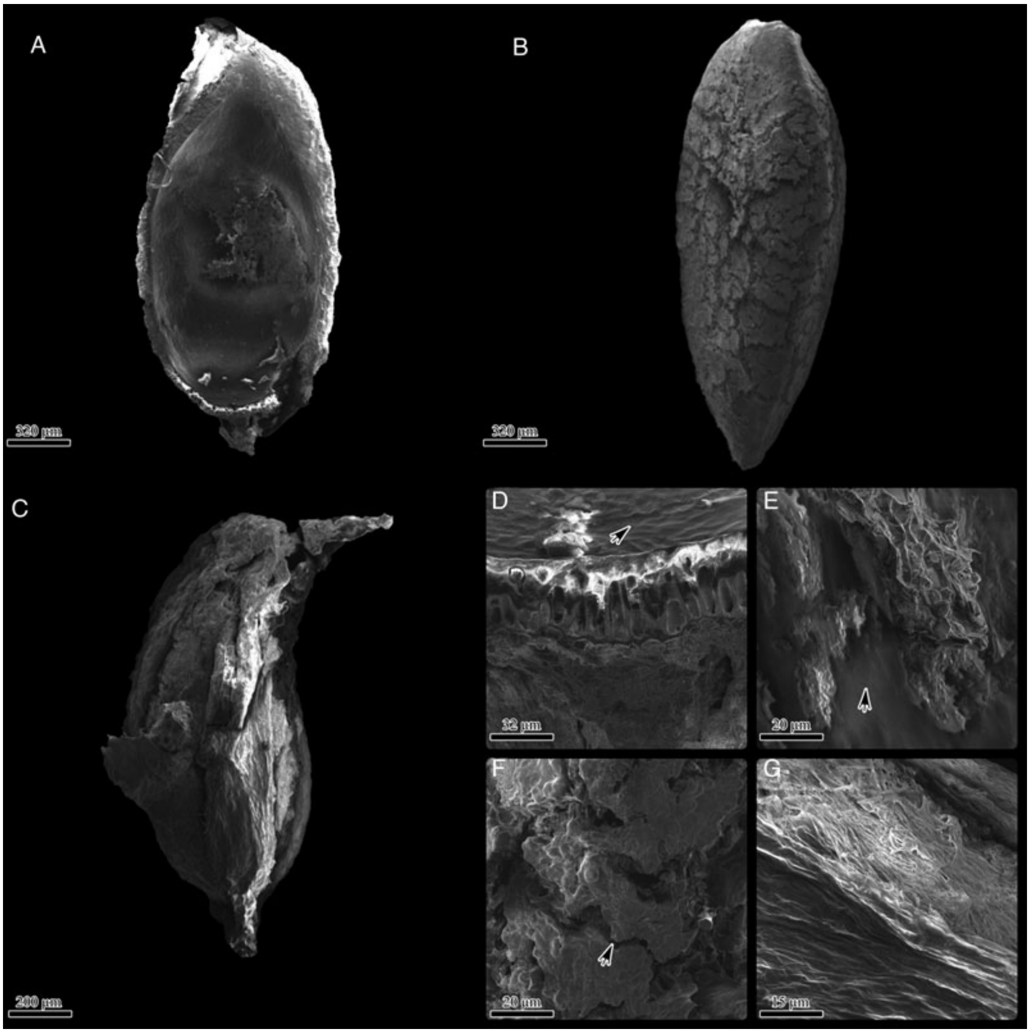

Figure 7. Micrographs showing morphological details of the achenes of the *Cecropia* species analysed. (A,D,E) *Cecropia angustifolia*. (B,F) *Cecropia telealba*. (C,G) *Cecropia peltata*. (A–C) Ellipsoid – ovoid achenes. (D–G) Achenes with ruminate to slightly rugose surface with a mucilaginous layer covering them heterogeneously. Arrows indicate spaces in the achenes that are not covered by mucilaginous cells.

(Athugala et al., 2021). The difference between the documented dormancy profiles in Asian montane forests and Andean montane forests may be due to climatic conditions and the species diversity of each ecosystem. Climate may promote the development of different mechanisms related to dormancy classes, because the abiotic conditions of each ecosystem may condition germination and the formation of dormancies such as physiological or combined dormancy (Baskin and Baskin, 2014). In montane forests of the Andes, the diversity of species is higher compared to montane forests of the paleotropics (Raven, 1976; van der Hammen, 2000). This diversity is mainly due to the geological events that gave rise to the Andes mountain ranges that led to vicariance, dispersion, as well as climatic variations that could influence the radiation of lineages with restricted distribution and single morphophysiological characteristics (van der Hammen, 1974; Gentry, 1982; Noguera-Urbano, 2016).

Functional role of physiophysical dormancy (PY + PD) in Cecropia *species*

Our results report the presence of PY + PD in Urticaceae that supports the hypothesis proposed by Willis et al. (2014), that assumes a specific pattern of dormancy classes in Angiosperms, where different lineages of the Rosid clade have developed PY + PD in parallel. Of the 14 species studied, only those of the genus *Cecropia* presented PY + PD. *Cecropia* is a neotropical lineage that seems to have radiated in parallel with the first uplift of the Andes (Franco-Rosselli and Berg, 1997), and its species have a wide distribution in neotropical ecosystems, playing a fundamental role in regeneration processes in disturbed areas (Lobova et al., 2003; Treiber et al., 2016). Studies indicate that the achenes of different *Cecropia* can germinate after 4 or 5 years of remaining dormant in the forest litter (Holthuijzen and Boerboom, 1982). The results of this study indicate that dormancy in *Cecropia* is a trait that develops mainly due to the structural design of the fruits, given that the pericarp in the species analysed is made up of a mucilaginous layer that covers the achenes and can regulate seed germination in environments with little water availability (Fahn and Werker, 1972). In addition to this, the presence of a mesocarp with abundant accumulations of tannins and an endocarp with lignified cells arranged in a palisade confer impermeability to the diaspores (Lobova et al., 2003); also to constituting a mechanical barrier that limits the flow of water and gases from the environment to the seeds, it allows the development of PY + PD.

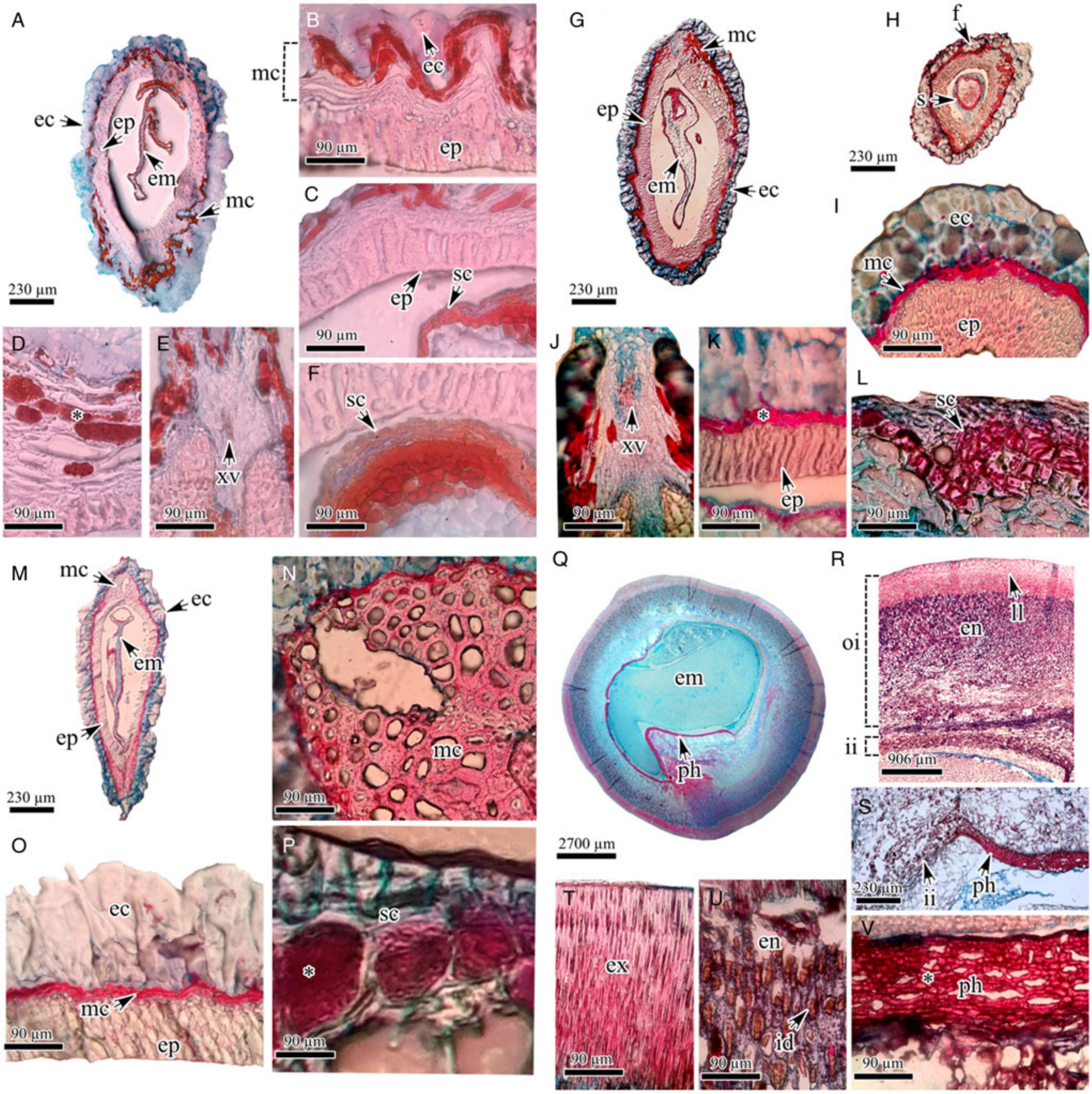

Figure 8. Anatomy of fruits and seeds with PY + PD and PY dormancy. (A–F) *Cecropia peltata*. Spatulate embryo, developed with physiological dormancy. (B–D) Pericarp made up of a layer of mucilaginous cells, a mesocarp with cells filled with tannin and a layer of lignified cells, and an endocarp with cells arranged in a palisade, thick-walled and lignified. (E) Conspicuous pedicel scar with presence of helically thickened xylem vessels. (F) Seed coat composed of two layers of cells, an outer collapsed one and an inner one with cuboid cells saturated with tannins. (G–L) *Cecropia angustifolia*. (G,H) Achenes with ellipsoid seed and spatulate embryo, developed. (I–K) Pericarp made up of an exocarp of mucilaginous cells with scattered tannins, a mesocarp with abundant accumulations of tannins, which projects to the pedicel scar that has conspicuous xylem vessels with helical thickening of the cell wall and an endocarp in palisade. (L) Seed coat with thick-walled cuboid cells accumulating tannins. (M–P) *Cecropia telealba*. (M) Achenes with developed spatulate embryo. (N,O) Exocarp with mucilaginous cells, mesocarp composed of a layer of lignified thick-walled sclerenchyma cells that reach the insertion site of the fruit at the infructescence and a thin layer of cells with deposits of tannins and an endocarp with cells in palisade. (P) Seed coat composed of cuboidal cells saturated with tannins. (Q–V) *Sapindus saponaria*. (Q) Seed with folded, developed embryo and incumbent cotyledons. (R–V) Palisade exotesta with a light line, endotesta with abundant accumulations of internal idioblasts and integument alternating with a pachychalaza with saturation of tannins in its cells. ec, exocarp; em, embryo; en, endotesta; ep, endocarp; ex, exotesta; f, fruit; id, idioblasts; ii, internal integument; ll, light line; mc, mesocarp; oi, external integument; ph, paquicalaza; s, seed; sc, seed coat; xv, xylem vessels. * Shows sites of accumulation of substances.

Different authors have proposed a possible mechanism of dormancy in *Cecropia* seeds that requires specific light conditions to stimulate germination (Vázquez-Yanes and Orozco-Segovia, 1986; de Souza and Válio, 2001), that is consistent with the presence of PD in *Cecropia*, since PD is a class of dormancy that occurs mainly in response to abiotic factors such as light

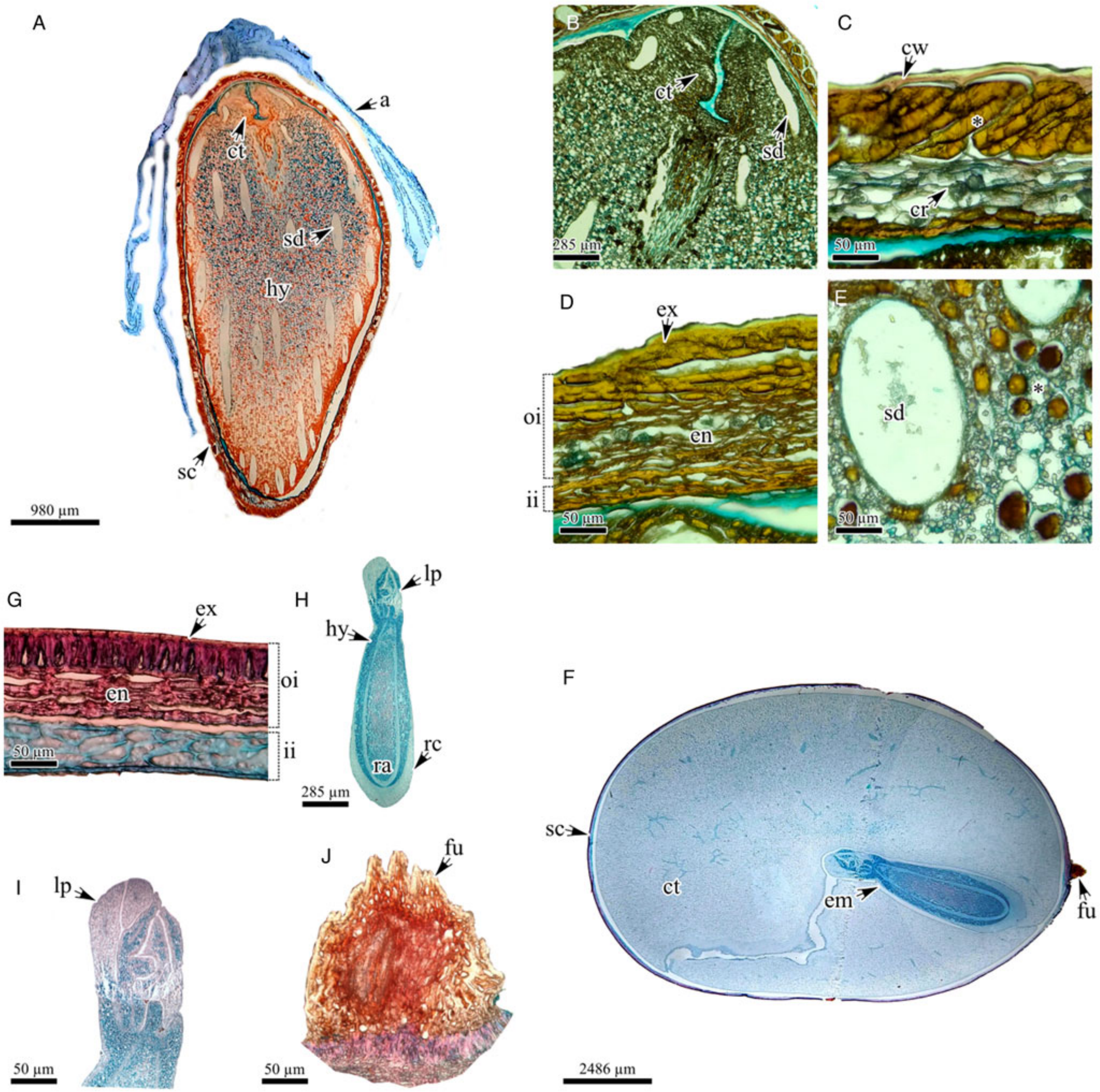

Figure 9. Comparative anatomy of seeds with ND. (A–E) *Clusia multiflora*. (A) Seeds with an aril that completely covers them and a linear embryo, developed with abundant secretory cavities. (B,E) Cotyledons and secretory cavities present in the embryo. (C,D) Exotesta of cuboid cells with abundant concentrations of waxy substances, collapsed endotesta with presence of calcium oxalate crystals and an internal integument reduced to a cell membrane. (E) Secretory cavities. (F–J) *Abarema lehmannii*. (F,H,I,J) Seeds with sclerotic funicle and folded embryo, developed with conduplicate cotyledons. (G) Exotesta with palisade seminal cover, with thick-walled cells and collapsed endotesta. a, aryl; cr, calcium oxalate crystals; ct, cotyledons; cw, cell wall; en, endotesta; end, endosperm; em, embryo; ex, exotesta; fu, funicle; hy, hypocotyl; ii, internal integument; lp, leaf primordium; oi, outer integument; ra, radicle; rc, calyptra; sc, seed coat; sd, secretory cavities; xv, xylem vessels. * Show sites of accumulation of substances.

variations, temperature and humidity (Baskin and Baskin, 2014). The efficient germination of *Cecropia* seeds may be related to the light stimuli caused by modifying the plant cover because of anthropogenic alterations. In addition, the ability of the species of this genus to colonise new environments being the main pioneer trees in most neotropical ecosystems (Treiber et al., 2016), may be related to the development of PY + PD and with the set of anatomical characteristics of the fruits, allowing them to persist for long periods of time stored in the litter of the forests until the conditions are suitable for the establishment of the seedlings.

Another important aspect in the efficient colonisation of *Cecropia* species in montane forests has to do with the ecological relationships of these plants with different species of bats that are key in the dispersal of their seeds (Vázquez-Yanes and Orozco-Segovia, 1986). The passage of the fruits through the digestive tract of these mammals stimulates the germination of the seeds

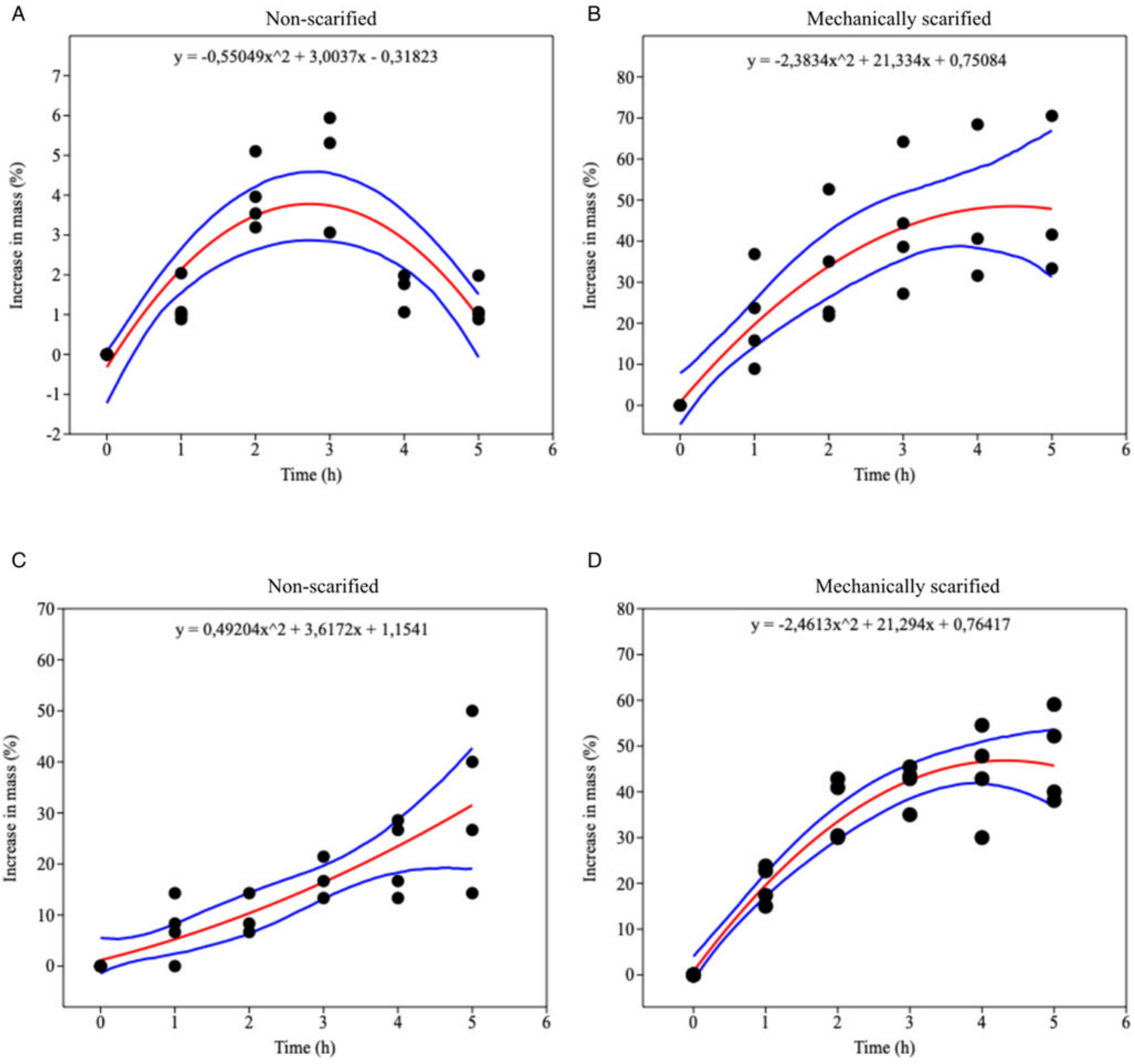

Figure 10. Permeability tests on seeds with PY and PD. (A,B) *Sapindus saponaria* seeds. (C,D) *Ulex europaeus* seeds. Blue lines indicate 95% confidence intervals.

by disintegrating the cell layers of the pericarp and promoting the breaking of dormancy (Lobova et al., 2003; Carvalho et al., 2017). This phenomenon could explain why many seeds germinate quickly under natural conditions, while others can remain dormant for a long time. In some cases, the only thing that the dispersers consume are the residues of the perianth, without affecting the tissues that constitute the pericarp; therefore, the scarification product of animal digestion is not homogeneous (Lobova et al., 2003).

Relationship between the development of dormancy and the secretion of substances by seeds with MPD

The seeds with MPD were characterised by having undifferentiated, rudimentary, broad or linear underdeveloped embryos, with a MGT greater than 30 d, rigid endosperms (except in *C. colombiana*) and seed coats with accumulation of tannins or waxy substances. One of the distinctive characteristics of seeds with MPD is a water-permeable seed coat (Baskin and Baskin, 2014), however, the accumulation of hydrophobic substances in different layers of the integuments (Fig. 4) may play an important role in the seed coat and regulation of the gaseous exchange with the environment. This forms a barrier that conditions the physiological development of the embryo by limiting the flow of environmental signals that stimulate its growth and subsequent germination (Baskin and Baskin, 2004; Da Silva et al., 2007).

Veldman et al. (2007) suggest that the secondary metabolites present in the seeds of *B. fructescens* constitute a chemical defence against pathogens, facilitating their persistence in the litter of the forests and forming seed banks. Similarly, tannins protect diaspores against pathogens (Roth, 1987), additionally contributing to the impermeability of the seed coat (Rangaswamy and Nandakumar, 1985), thus regulating germination by making it difficult for gas flow and decomposition of seed coats in the soil and causing germination to be delayed (Kigel, 1995).

Physical dormancy and mechanisms that promote the entry of water into the seeds

The seeds of species with PY were characterised by a thick seed coat made up of a palisade exotesta, an endotesta with abundant accumulation of idioblasts, a pachychalaza with tannin depositions, and a light line that separates the exotesta from the rest of the cells, as in *S. saponaria*. The light line has been identified as the main barrier to water penetration in seeds of some species of the Fabaceae family with PY, due to the fact that it is a structure with accumulation of waxes that confers impermeability to the seed (Janská et al., 2019). It is possible that dormancy in species of the Sapindaceae family such as *S. saponaria* is a product of the impermeability generated by the light line and the accumulation of idioblasts in the endotesta.

Among the mechanisms that can contribute to the rupture of PY, the Type I, II and III water gap complexes stand out (Gama-Arachchige et al., 2013; Geneve et al., 2018). In the anatomical and micrographic sections made of *E. rubrinervia* seeds, the presence of a hilar cleft and different clefts at the external margin of the exotesta could be observed, this being consistent with Type I water gaps (Figs. 5D, 6F and G). Said cracks are the product of narrow, linear openings, generally occluded, composed of modified elongated palisade cells that allow the flow of water from the environment into the interior of the seeds, stimulating the breaking of dormancy and subsequent germination (Gama-Arachchige et al., 2013).

On the other hand, the development of physiological dormancy may be an evolutionary trait that favours the colonisation of invasive species in the Andean forests of Colombia. The presence of PD in the seeds is related to a high rate of speciation and could promote the colonisation of new environments, since it allows the species to adapt the moment of germination to different climatic regimes (Donohue et al., 2005; Willis et al., 2014). This capacity has been previously identified in *U. europaeus* seeds, for which the presence of physical dormancy has been suggested (Udo et al., 2017). However, in this study, we found that the seeds of *U. europaeus* had a large non-dormant fraction, as there was also significant seed coat impermeability in the seed lot (Fig. 10C, D), combined with a dormant fraction that took some time to germinate (Table 1). Whether this represents slow imbibition, or a form of PD requires further exploration.

Conclusions

In conclusion, dormancy in the Andean Forest species analysed follows a pattern like that recorded in other tropical montane ecosystems around the world. In the case of the achenes of the *Cecropia* species, the development of PY + PD is regulated by a physical and chemical barrier of the pericarp that limits not only the flow of water and gases, but also the physiological development of the embryo. Both the dormancy and the anatomical features of *Cecropia* achenes are essential for this lineage to be important in the natural regeneration of Andean montane forests. The deposition of substances in the cell layers of the seed coat is a trait that may be associated with the limitation of the flow of environmental signals that stimulate the breaking of dormancy in seeds with MPD of the studied species that developed this class of dormancy. In the seeds of *S. saponaria*, the presence of a thick seed coat, provided with a light line and abundant accumulations of idioblasts in the endotesta, may be associated with the impermeability of the seeds and the development of PY. While the hilar clefts and the margin of the seed coat are associated with the Type I water gap mechanism that allow water ingress and subsequent PY rupture in *E. rubrinervia*. Finally, dormancy is a characteristic that could be related to the high biological invasion of *T. alata* and *U. europaeus* in the Andean forests of the Central Cordillera of Colombia.

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