



Ecological and evolutionary aspects of seed coat and seedling development in *Juncus tenuis* (Juncaceae, Poales)

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Abstract

This work aimed to understand ecological and evolutionary aspects of *Juncus tenuis* (Juncaceae) by analyzing its seed coat and seedling development. Mature seeds were germinated under controlled conditions and analyzed every 24 h for 60 days. Seeds and seedlings at different developmental stages were analyzed using a stereomicroscope, light microscopy, and scanning electron microscopy. The seed coat consists of the endotegmen, endotesta, and exotesta. The endotegmen is tanniferous, and the exotesta is mucilaginous. The germination percentage was low (28%), a result that may be related to specific light, humidity, and temperature requirements to overcome dormancy. Germination begins with the disruption of the seed coat and the emergence of a phaneromer, followed by the development of the collar rhizoids and the primary root. The anatomical analyses show that the formation of shoot and root apical meristems occurs only after the elongation of the phaneromer. The anatomy of the seedlings at early development is described for the first time for the family. The phaneromeric cotyledon is shared by Juncaceae and Thurniaceae and is most likely the ancestral condition in the cyperid clade (Poales). The presence of a mucilaginous exotesta is probably related to seed protection, dispersal, and germination.

Keywords Cotyledon · Germination · Mucilaginous exotesta · Phaneromer · Seed coat

Introduction

Juncaceae is one of the families of the order Poales, integrating the cyperid clade with Thurniaceae and Cyperaceae (Linder and Rudall 2005; Givnish et al. 2010, 2018; Bouchenak-Khelladi et al. 2014). Juncaceae is relatively small, including about 440 species widely distributed throughout the temperate and subtropical regions, but with few representatives occurring in the tropics (Kirschner et al. 2002a, b, c). The family is divided into eight genera, as follows: *Juncus* L. and *Luzula* DC., distributed

worldwide; *Distichia* Nees & Meyen, *Oxychloë* Phil. and *Patosia* Buchenau, restricted to South America; *Marsippospermum* Desv. and *Rostkovia* Desv., which occur in South America and New Zealand (Kirschner et al. 2002a, b, c), and *Oreojuncus* Záv. Drábk. & Kirschner, occurring from Europe to Siberia and from Greenland to the United States (Drábková and Kirschner 2013).

Juncus is the largest genus in the family, with approximately 300 species (Kirschner et al. 2002b, c), which are usually perennial, rhizomatous herbs. Their fruits are loculicidal capsules, with numerous small, often reticulated seeds (Balslev 1996, 1998). The gynoecium consists of three united carpels, and placentation is parietal, on intruding septa; the ovules are anatropous and bitegmic, with each integument consisting of two cell layers (Balslev 1996, 1998; Oriani et al. 2012).

Juncus tenuis Willd. is native in North America but introduced to many areas and established in numerous regions over extratropical regions of both hemispheres (Kirschner et al. 2002c). The individuals grow in different environments, in exposed or shaded sites in soils ranging from sand to clay, under moist or drier conditions, on natural or disturbed areas (Kirschner et al. 2002c). Therefore, it is a

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key-species for studies on seed germination and seedling development.

The reproduction of *Juncus tenuis* and its dispersal were studied by Salisbury (1974), who reported the presence of a mucilaginous layer on wet seeds. According to Salisbury (1974), the dispersal of *J. tenuis* is facilitated by the ability of the seed coat to swell and become sticky, easily adhering to the dispersers. However, other dispersal strategies are also present in *Juncus*. Balslev (1998) proposed that the species of *Juncus* may have different dispersal strategies, including anemochory and/or hydrochory. The swelling of the testa upon moistening in some species appears to cause capsule dehiscence (Balslev 1998).

According to Zaman (1950), who studied the embryology of *Juncus prismatocarpus* R.Br. and *J. effusus* L., the seed coat is composed of endotegmen and endotesta; the outer layer of both outer and inner integuments is absorbed during the seed development. The exotesta becomes glandular at the micropylar end, and the endotegmen cells exhibit thickened inner walls and a red color content (Zaman 1950).

Although several studies have addressed the seedling development of monocots, few have focused on species of Juncaceae and its seedling anatomy remained unknown. Of the few papers found in the literature, Tillich (1995) describes the cotyledon of some species of *Juncus* and *Luzula*, which consists of an elongated, assimilating, and unifacial hyperphyll with a small distal haustorium, and a bifacial hypophyll forming a ventrally closed sheath. Tillich (2007) describes the morphology of the seedlings of *J. maritimus* Lam., *Luzula nivea* (L.) DC., *Marsippospermum reichii* Buch., and *Rostkovia magellanica* Hook, reporting the formation of a phaneromeric cotyledon and a short hypocotyl in *Juncus* and *Luzula*, whereas the hypocotyl is long in species of *Marsippospermum* and *Rostkovia*.

Tillich (2007) emphasizes that studies on seedling development in monocots are important to identify characters with taxonomic and phylogenetic value. These studies are of particular interest for Poales since species of this order have different cotyledon types. Tillich (2007) proposed a hypothesis for the cotyledon evolution in Poales, stating that the compact type would be the ancestral cotyledon state, which shifted directly to three other states: blade-like hypophyll, phaneromer, and coleoptile. In the cyperid clade, which comprises Juncaceae, Thurniaceae, and Cyperaceae, the first two families have a phaneromer, whereas in Cyperaceae, the cotyledon develops into a coleoptile (Tillich 2007). The phaneromer corresponds to the proximal part of the cotyledonary hyperphyll, which develops into a tubular structure that raises the seed well above the soil surface. In contrast, the coleoptile, which is also a tubular structure, is produced by the meristematic activity of the marginal tissue of the cotyledonary sheath and is usually short (Tillich 2007).

In this context, the present study analyzes the development of the seed coat and seedlings of *J. tenuis* with a morphological, micromorphological, and anatomical approach, aiming to provide new information about these processes in Juncaceae. The following questions were addressed: What is the origin of the mucilaginous layer of the seed coat of *J. tenuis*? How is the anatomical structure of the seed coat and its relation to dispersal? Does *J. tenuis* share the same morphological pattern of seedling development as other previously studied species of Juncaceae? Finally, how can the early seedling development be characterized from an anatomical perspective?

Materials and methods

Botanical material

Mature seeds of *J. tenuis* were collected from the mature fruits of plants growing in open areas, roadsides, and human-impacted areas in the Lagoa Pequena, Florianópolis, Santa Catarina, Brazil (SisGen register number ADE51D9). The seeds were stored in closed paper bags and kept at room temperature. Flowers and fruits at different developmental stages were also collected, fixed in FAA 50 (37% formaldehyde, glacial acetic acid, 50% ethanol, 1:1:18 v/v) (Johansen 1940) for 24 h, and stored in 70% ethanol for the study of seed coat development. Vouchers were identified and deposited in the *Herbarium Rioclarense* (HRCB 77282) of the Instituto de Biociências at Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil.

Germination and seedling development

For the analysis of seedling development, 100 mature seeds were placed to germinate in four Petri plates (25 seeds per plate). The Petri plates were lined with filter paper moistened with distilled water and stored in an air-conditioned room at a constant temperature ($25 \pm 2^\circ\text{C}$) and fluorescent light ($\approx 40 \mu\text{mol.m}^{-2}\text{s}^{-1}$ PAR).

The seeds were observed once every 24 h for 60 days, with germination identified as the rupture of the seed coat followed by the emergence of the phaneromer. The results were recorded by images obtained with a Canon Power Shot S80 digital camera coupled to a Leica EZ4 stereomicroscope. Seedlings at different developmental stages were fixed in FAA 50 for 24 h and then transferred to 70% ethanol for the anatomical study. The terminology adopted follows Tillich (2007), where definitions and examples for the use of the terms are given.

Light microscopy (LM)

Flowers, fruits, and seedlings at different developmental stages and fixed in FAA 50 were dehydrated in an n-butyl alcohol series. These materials were then embedded in 2-hydroxyethyl methacrylate (Leica Histo-resin Embedding Kit) (Gerrits and Smid 1983) and sectioned (5–7- μ m thick) on a rotary microtome (Leica, RM 2245). Anatomical sections were stained with periodic acid-Schiff (PAS) reagent and 0.05% toluidine blue in sodium borate (O'Brien et al. 1964; Feder and O'Brien 1968), and then mounted on permanent slides with Entellan (Merck). Selected sections were registered under an image-capturing device (Leica, DFC 450) attached to a microscope (Leica, DM 4000B). Image processing was performed with the LAS digital imaging system (Leica Application Suite V4.0.0).

Scanning electron microscopy (SEM)

Seeds and seedlings at different developmental stages previously fixed in FAA 50 were dehydrated in an ethanol series (30%, 50%, 70%, 90%, and 100%) for 15 min in each solution, and then critical point dried (Balzers CPD 050). The dried samples were fixed in stubs and coated with gold by a Bal-Tec SCD 050 sputtering system. Observations were carried out under a Jeol JSM IT 300 microscope, and the images were obtained using the microscope image-capturing system.

Results

Seed coat development

Juncus tenuis has a tricarpeal gynoecium with anatropous, bitegmic ovules, and parietal placentation. The outer and inner integuments have two layers of rectangular cells with a conspicuous nucleus and dense cytoplasm (Fig. 1a–c). Both integuments delimit the micropyle (Fig. 1b). In addition, a cuticle is observed between the outer and inner integuments and between the inner integument and the nucellus (Fig. 1a–c).

The integuments undergo modifications during endosperm and embryo development (Fig. 1d–g). After fertilization, the cells of the inner layer of the inner integument increase in size and accumulate phenolic compounds (Fig. 1c–e), giving rise to the tanniferous endotegmen (Fig. 1d–g). The cells of the outer layer of the inner integument do not undergo evident changes until the stage when the nucellus is absorbed by the developing endosperm (Fig. 1b–e). From that stage onward, these cells begin to disintegrate (Fig. 1f) and are completely absent in the mature seed (Fig. 1g). The cuticle between the inner and

outer integuments becomes thicker and more sinuous, with changes in its chemical composition evidenced by changes in its color (Fig. 1f, g). The cells of the inner layer of the outer integument elongate, accommodating to the sinuosity of the cuticle (Fig. 1f, g). The cells of the outer layer of the outer integument, which form the exotesta, elongate tangentially (Fig. 1d–f). In the mature seed, they become mucilaginous by accumulating polysaccharides as indicated by the PAS-positive reaction (Fig. 1g).

In the mature seeds the seed coat comprises a tanniferous endotegmen, endotesta, and a mucilaginous exotesta (Fig. 1g). Numerous ellipsoid to ovoid seeds are formed per capsule, all yellow-orange and with a reticulated surface when dry (Fig. 2a, b). The mucilaginous exotesta can be observed under SEM (Fig. 3b); since the walls of its cells are thin, they usually break, leaving only parts of the anticlinal walls (Fig. 3a).

Germination and morphological aspects of seedling development

The germination percentage of *J. tenuis* was 28%. Table 1 shows the time for the appearance of the seedling structures.

Germination begins between 16–20 days after imbibition with the rupture of the seed coat tissues at the micropylar end, followed by the emergence of the phaneromer (Figs. 2a, b, 3a). The phaneromer curves toward the substrate and develops into a long, cylindrical structure that raises the seed (Figs. 2c, 3b). At one end of the phaneromer, where the extremely reduced hypocotyl-radicular axis is found, dense and long rhizoids are distinguishable two to four days after the phaneromer appears (Figs. 2d, e, 3c, d). Subsequently, the primary root begins to develop between the rhizoids (Figs. 2e, 3d, e). The primary root grows fast and becomes well-developed with many root hairs after few days (Figs. 2f, 3f). The formation of shoot-born roots starts two to six days after the emergence of the primary root (Figs. 2f, 3f). Six to ten days after the appearance of the first shoot-born root (35–38 days after germination), the first plumular leaf appears as a lanceolate eophyll (Figs. 2g, 3g). The first eophyll is fully expanded approximately 40 days after germination, by which time more shoot-born roots are formed (Fig. 2g).

Anatomical aspects of seedling development

After seed imbibition and before germination, mucilage secretion is observed at the micropylar end of the seed (Fig. 4a). In this region, the seed coat disrupts with the protrusion of the phaneromer (Fig. 4b, c). The cells of the phaneromer divide rapidly, forming a solid and cylindrical structure, which then elongates (Fig. 4d, e). The cells of the outermost layer at the end of the axis differentiate into the

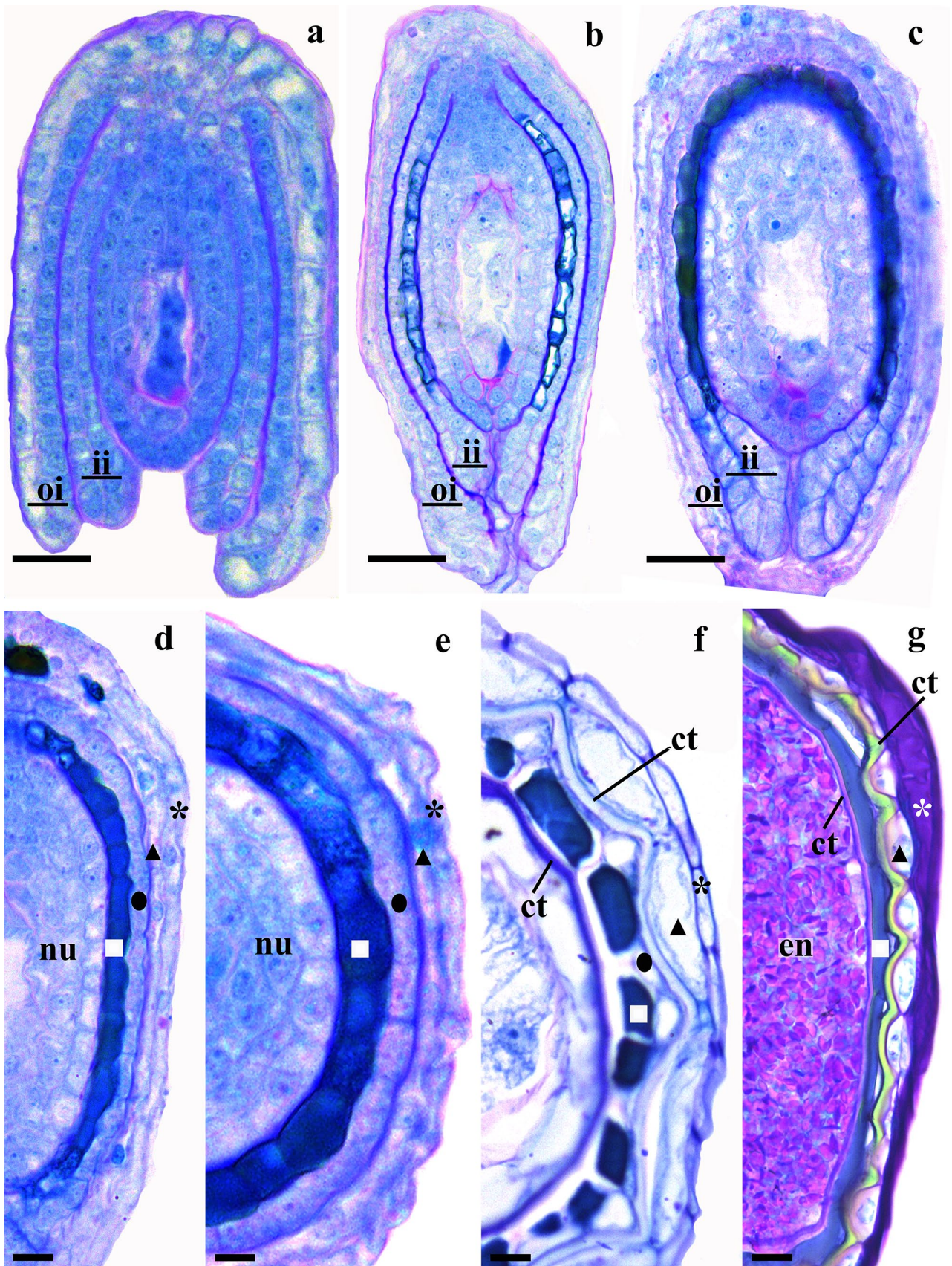


Fig. 1 Seed coat development in *Juncus tenuis*. **a–c** Longitudinal sections of ovules in successive developmental stages, before **a** and after fertilization **b**, **c**. **d**, **e** Details of the ovule integuments after fertilization, in longitudinal and cross sections, respectively. **f** Cross section of the seed coat of the immature seed. **g** Longitudinal section of the seed coat of the mature seed. *ct* cuticle, *en* endosperm, *ii* inner integument, *nu* nucellus, *ot* outer integument. Symbols: square endotegmen, circle exotegmen, triangle endotesta, asterisk exotesta. Scale bars 50 μm **a–c**, 25 μm **d–g**

rhizoids (Fig. 4e). Concomitantly, the root apical meristem is formed, and both the root and stem poles become evident, positioned on the same orientation of the phaneromer (Fig. 4e, f). After the development of the primary root, the shoot apical meristem becomes organized (Fig. 4g). The rhizoids remain in the collar region, which corresponds to the transition between the hypocotyl and the primary root. The shoot-born roots, which have an endogenous origin, are formed from the division of hypocotyl cells (Fig. 4h, i). The cells of the shoot apical meristem divide, elongating the epicotyl and giving rise to the first plumular leaf primordium, both of which protected by the cotyledonary sheath (Fig. 4j).

Discussion

Seed coat development and ecological considerations

The seed coat in *J. tenuis* is formed by three layers: the endotegmen, endotesta, and exotesta. The endotegmen is tanniferous, similarly to the seeds of representatives of other Poales families like the Xyridaceae (Oriani and Scatena 2014, 2017; Nardi et al. 2015); Eriocaulaceae (Scatena and Bouman 2001; Coan et al. 2010); Mayacaceae (Venturelli and Bouman 1986); Restionaceae (Rudall and Linder 1988); Centrolepidaceae (Hamann 1975); Rapateaceae (Venturelli and Bouman 1988); Poaceae (Nakamura et al. 2009); Bromeliaceae (Magalhães and Mariath 2012; Prado et al. 2014); as well as Thurniaceae, including *Thurnia* Hook.f. species (Thimm 1985) and *Pronium serratum* Baill. (formerly included in Juncaceae) (Munro and Linder 1997). In Cyperaceae, which forms the cyperid clade along with Juncaceae and Thurniaceae, the phenolic compounds are accumulated in the exotesta cells, as described for *Rhynchospora* Wild. species (Coan et al. 2008). Therefore, the tanniferous endotegmen, although common in Poales, is not a feature shared by all cyperids.

It is believed that the presence of phenolic compounds can inhibit germination (Mayer and Poljakoff-Mayber 1989), thus playing a regulatory role in the germination process. In addition, the phenolic compounds may act as defense against herbivores and play antibacterial and antifungal activities (Levin 1976).

The number of layers that constitute the seed coat is a taxonomic trait that differentiates Poales families and even genera, as reported for Xyridaceae (Oriani and Scatena 2014, 2017; Nardi et al. 2015). As shown in the present study for *J. tenuis*, the seed coat is composed of endotegmen, endotesta, and exotesta, with the exotegmen being absorbed during seed development. In the seeds of *J. prismatocarpus* and *J. effusus*, Zaman (1950) observed the absorption of the outer layer of both outer and inner integuments, thus the seeds do not have the exotegmen and exotesta. In species of Cyperaceae, the exotesta is present and forms the seed coat along with the endotegmen, but neither of these layers is mucilaginous (Makde and Bhuskute 1987; Coan et al. 2008).

The number of layers in the outer integument is variable for Cyperaceae, as shown in species of *Hypolytrum* Pers. –with three cell layers– and *Rhynchospora* –with two cell layers (Coan et al. 2008); the last character state is the most common for this family (Makde and Bhuskute 1987; Zhang 1999). However, this variation in the number of layers of the outer integument is not significant; during seed development, some layers disintegrate, and in the mature seed, the testa is composed only of the exotesta, with one layer of thin-walled cells (Coan et al. 2008). In *Thurnia* (Thurniaceae), the testa is formed by multiple layers, with lignified cells, whereas the endotegmen and exotegmen cells are thin-walled and filled with tannins, making the limits of the cells almost imperceptible (Thimm 1985). The seed coat composed of exotesta, exotegmen, and endotegmen is therefore shared by Juncaceae and Thurniaceae, whereas in Cyperaceae, the seed coat comprises only the exotesta and endotegmen. The reduction in the number of cell layers of the seed coat in Cyperaceae is probably related to the presence of indehiscent fruits (nutlets).

The anatomical characteristics of the seed coat in cyperids may also be related to environmental factors, since these species occur in different environments. The species of *Thurnia*, for instance, are usually found in wet areas and creek banks, suggesting that the presence of the testa with multiple layers of lignified cells is an adaptation to prevent the seed from rotting in the water, allowing it to remain viable for a longer period. On the other hand, most Cyperaceae species are found in open and dry areas, and their seeds, having few layers in the seed coat, are protected by the pericarp since their fruits are indehiscent. Studies of the anatomy of the Cyperaceae fruit wall show that it is generally composed of lignified cells in the mesocarp (Shah 1968; Ragonese et al. 1984; Browning and Gordon-Gray 1993, 1996; Coan et al. 2008). Thus, the mechanical protection of the seed is transferred to the pericarp, while the testa, whose cells contain phenolic compounds (Coan et al. 2008), provides chemical protection against predators and pathogens. For *J. tenuis* and other Juncaceae species occurring in dry and open areas with high light exposure, a mucilaginous testa may help protect

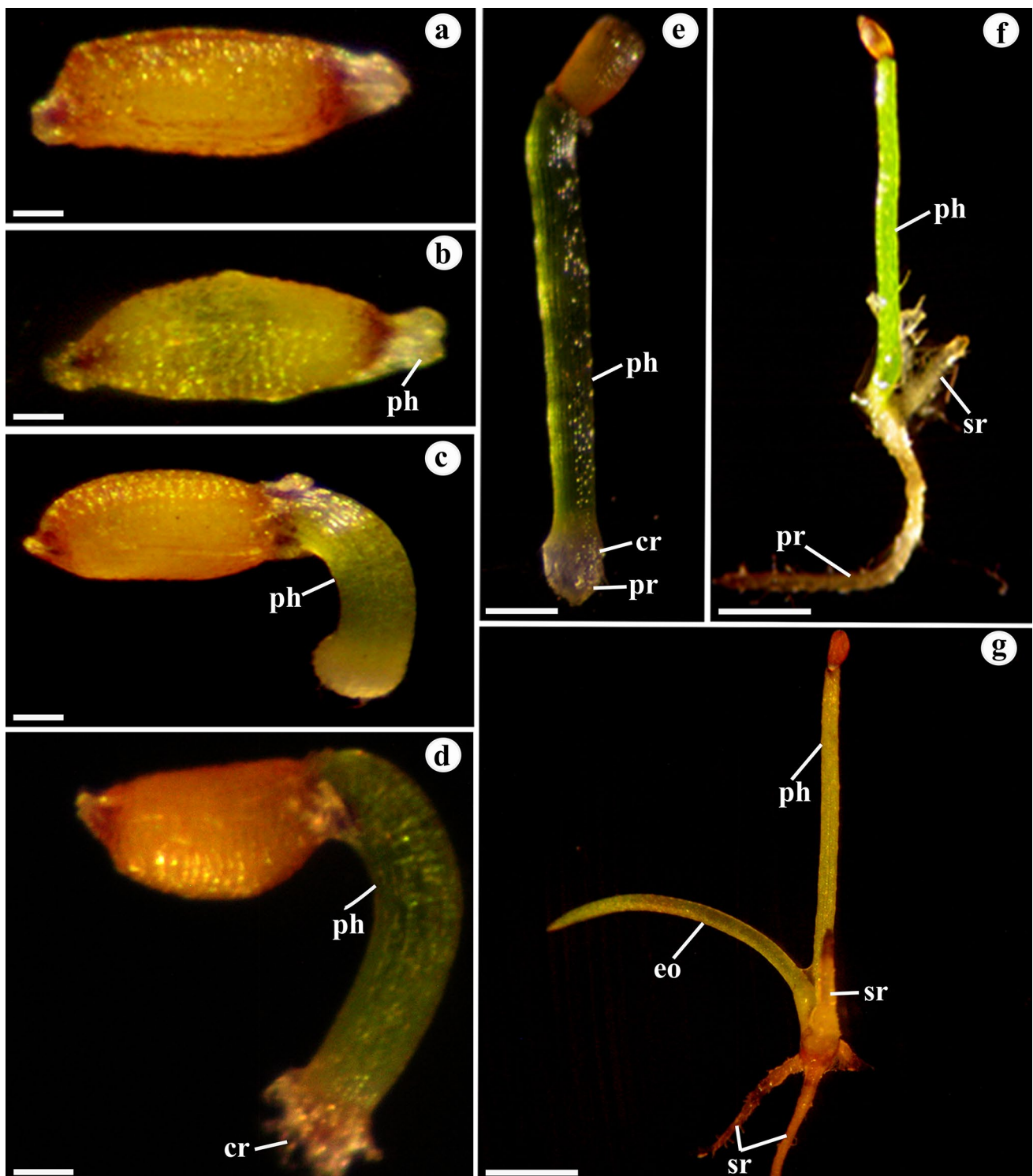


Fig. 2 Morphological features of seedling development in *Juncus tenuis*. **a, b** Rupture of the seed coat and phaneromer emergence. **c** Phaneromer development. **d** Differentiation of the rhizoids. **e** Phaneromer elongation and beginning of primary root development. **f** Primary

root elongation and shoot-born root in early development. **g** Seedling with the first developing eophyll and several shoot-born roots. *eo* eophyll, *ph* phaneromer, *cr* collar rhizoids, *pr* primary root, *sr* shoot-born root. *Scale bars* 1.0 mm **a–c**, 1.2 mm **d**, 1.5 mm **e–g**

Fig. 3 Micromorphological features of seedling development in *Juncus tenuis*. **a** Beginning of seed germination. **b** Development of the phaneromer and beginning of the rhizoids differentiation; note the seed's mucilaginous testa. **c** Seedling showing an elongated phaneromer and long, dense collar rhizoids. **d** Detail of the collar rhizoids and beginning of primary root development between the rhizoids. **e** Primary root development. **f** Developed primary root and beginning of shoot-born root development. **g** Seedling with the first developing eophyll. *eo* eophyll, *ph* phaneromer, *cr* collar rhizoids, *pr* primary root, *sr* shoot-born root. Scale bars 100 μm **a**, **b**, 200 μm **c**, **f**, 50 μm **d**, 20 μm **e**, 500 μm **g**

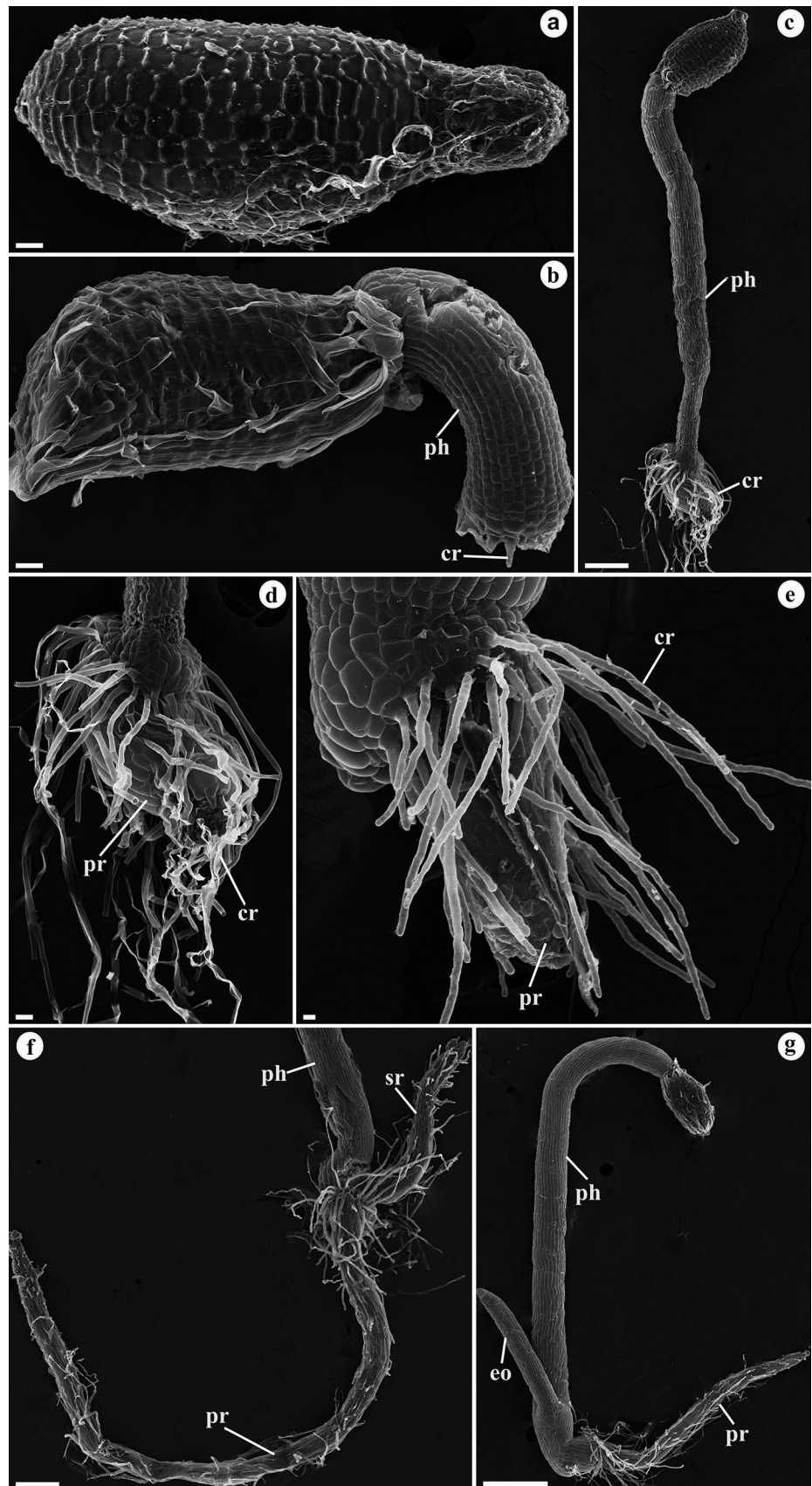


Table 1 Time for the emergence of different seedling structures in *Juncus tenuis* after the beginning of germination experiment

Structures	Days
Phaneromer	16–20
Collar rhizoids	18–24
Primary root	23–26
First shoot-born root	25–32
First eophyll	35–38

the seed against desiccation, since their fruits are dehiscent (loculicidal capsules), and there is no layer with lignified cells composing the seed coat. We believe that the cuticle layer between the tegmen and the testa, which thickens during seed maturation, provides mechanical protection.

Because the mucilaginous layer of the seeds of *Juncus tenuis* corresponds to the exotesta, it may be termed sarcotesta (Corner 1976). Among the Poales, seeds with a mucilaginous testa have also been reported for Bromelioideae species (Bromeliaceae) (Silva et al. 2020). In these species, as is in *J. tenuis*, the number of layers in the outer integument does not increase during seed development, but its cells do increase in size by accumulating hydrophilic substances, resulting in a mucilaginous testa. The mucilaginous nature of the seed testa in *J. tenuis* was observed soon after imbibition, when the mucilage increased the seed's adherence to the substrate. The occurrence of a sticky substance adhering the seeds to the substrate was also observed by Kraus et al. (1996) in germination experiments of *Paepalanthus* Mart. species (Eriocaulaceae) and, according to the authors, this feature facilitates the imbibition.

Studies on the functions of the seed coat mucilage of a number of angiosperms suggest that it has multiple ecological roles. These include facilitation of seed hydration, mediation of germination under waterlogged conditions, regulation of seed dormancy by reduction of oxygen diffusion, and promotion of seed dispersal by attachment to animals (Western 2012; Yang et al. 2012). The precise role of mucilages appears to be dependent on species and their environmental context (Western 2012). Since representatives of *J. tenuis* generally occur in open environments under high light exposure and also in disturbed areas, it is believed that the mucilaginous exotesta regulates germination by keeping a sufficient concentration of water around the seed, as well as protecting against desiccation. Furthermore, as already demonstrated for other species of angiosperms, the presence of a mucilaginous seed coat facilitates germination in soils with low water potential (Young et al. 1970; Sun et al. 2012), such as the sandy soils of grassland vegetation, where many species of Juncaceae grow.

In addition to regulating germination and protecting the seed against desiccation, we believe that the mucilaginous seed coat may also affect seed dispersal, since the fruiting and seed dispersal of *J. tenuis* occur in the rainy season

(Salisbury 1974; Balslev and Stefano 2015). Thus, when the seeds come into contact with water, they swell up and form large viscous masses, causing the capsules to open, a mechanism previously described by Balslev (1996, 1998) for other species of *Juncus*. Furthermore, depending on the hydration level, the mucilage can become extremely sticky, working as an adhesive and increasing seed adherence (Kreitschitz et al. 2015). This mechanism also facilitates dispersal by fixing the seeds to dispersal agents and then to the substrate, increasing the probability of germination.

We believe that there is also a relationship between seed size and the need for light as an ecological strategy for germination since small seeds tend to sink less, remaining in the surface layers of the soil, which may facilitate the emergence of the small seedlings. The mucilaginous seed coat increases seed adherence to surface soil particles and is also important for early seedling development.

In a manner similar to *Alyssum minus* (L.) Rothm. (Brassicaceae) (Sun et al. 2012), we believe that the mucilage of the seeds of *J. tenuis* plays an important role in seed dispersal, adhesion of seeds to the substrate, and hydration, as well as functioning as a water reservoir for germination, especially in conditions of water stress. This feature probably explains the wide distribution of *J. tenuis* through different habitats, including disturbed environments such as roadsides, trails, and even sidewalks in urban areas in the Americas, Japan, New Zealand, and Europe (Balslev and Stefano 2015). According to Salisbury (1974), humans can also affect the dispersion of *J. tenuis* by taking the seeds adhered to the footwear or tires, which is facilitated by the presence of the mucilaginous seed coat, meaning that human activity and urban mobility can help expand the species' distribution. Also according to Salisbury (1974), the minute size of the seeds, combined with their shape prevent them from injury by the moving footwear or rotating tires to which they adhere, while such movements may disrupt the seed-aggregates resulting from the mucilaginous seed coat. The relationship between the presence of seeds with mucilage and the ability to colonize disturbed areas has already been demonstrated in other herbaceous species of Brassicaceae, Euphorbiaceae, Lamiaceae, Onagraceae, and Plantaginaceae, in which mucilage has been identified as an ecological advantage since such seeds do not require soil to germinate (Young and Evans 1973), including increasing their invasive potential (Bangle et al. 2008).

Germination and seedling development in an ecological and evolutionary context

Juncus tenuis seeds are small, with many of their features visible only under a stereomicroscope. Seed mass affects several aspects of plant ecology (Moles et al. 2005). Small-seeded species can produce more seeds for a given amount

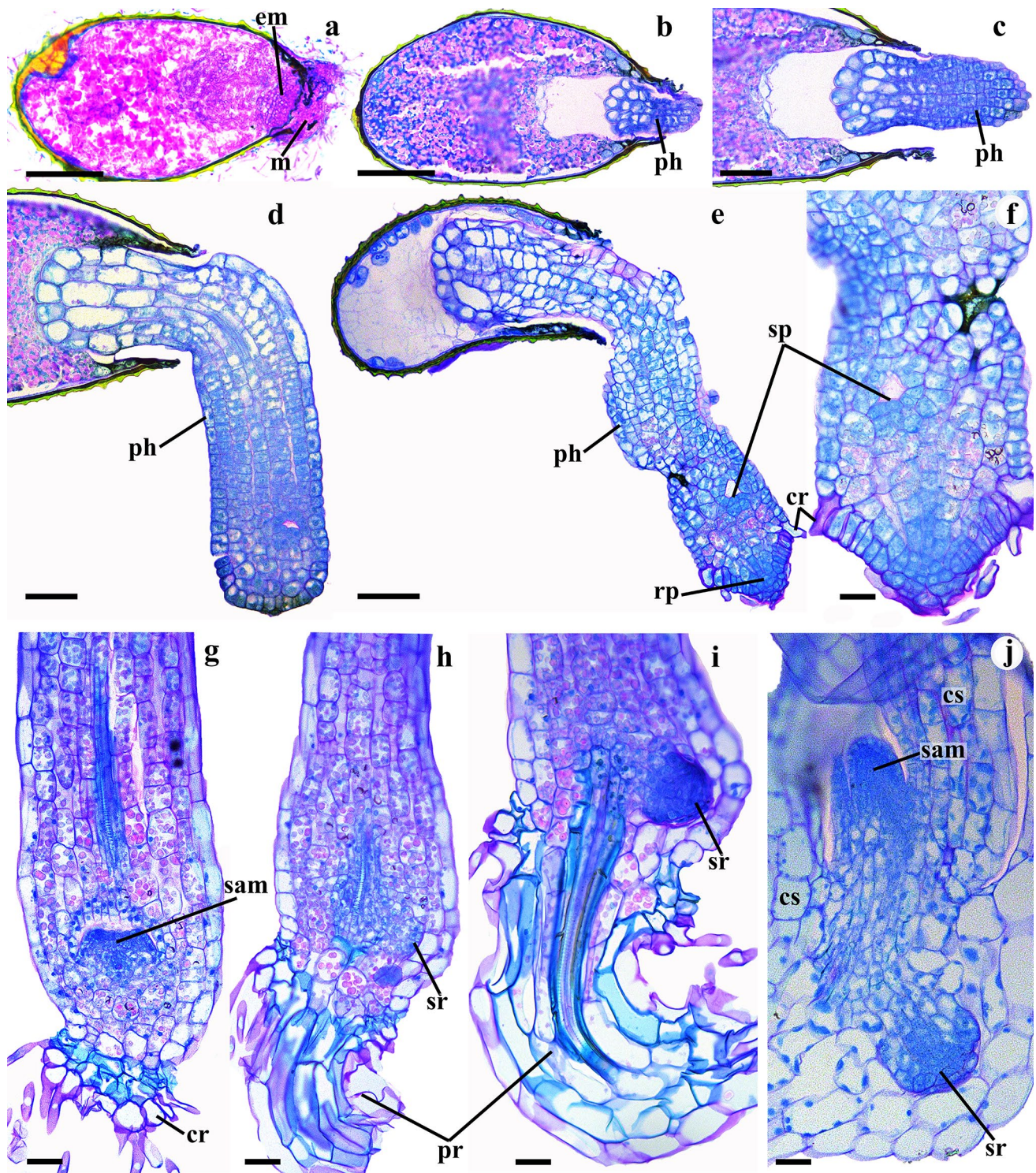


Fig. 4 Anatomical features of seedling development in *Juncus tenuis*. **a** Imbibed seed showing the mucilaginous testa and mucilage secretion in the micropylar region. **b, c** Phaneromer emergence. **d** Phaneromer development. **e** Collar rhizoids formation, and shoot and root poles differentiation. **f** Detail of the hypocotyl-radicle axis, with an organized root apical meristem. **g** Organization of the shoot apical meristem. **h, i** Primary root development and initiation of shoot-born

root formation from hypocotyl cells. **j** Detail of the shoot apical meristem, with leaf primordia protected by the cotyledonary sheath. *cs* cotyledonary sheath, *em* embryo, *m* mucilage, *ph* phaneromer, *pr* primary root, *cr* collar rhizoids, *rp* root pole, *sam* shoot apical meristem, *sp* shoot pole, *sr* shoot-born root. Scale bars 100 μ m **a, b**, 50 μ m **c, d**, 60 μ m **e**, 25 μ m **f–j**

of energy than those with large seeds (Leishman et al. 2000; Moles et al. 2005, 2007).

Species with small seeds are often associated with open (Leishman and Westoby, 1994; Seiwa and Kikuzawa, 1996) or disturbed environments (Fenner 1995), as observed for *J. tenuis*. Although this species has a widespread distribution and grow in different conditions, its germination percentage is low. We believe that the seeds require special conditions to germinate, such as specific light, humidity, and temperature, as reported for other monocot species including members of Xyridaceae and Eriocaulaceae (Garcia and Oliveira 2007) as well as Velloziaceae (Mota and Garcia 2012), which also grow in open environments with high light exposure. The lack of specific conditions may thus explain the low germination percentage observed for *J. tenuis* in our experiment and in the experiments by Salisbury (1974) with seeds of *J. tenuis* from Hampshire (in which 39% of 6800 seeds germinated) and from Sussex (in which 32% of 700 seeds germinated). Since this species is perennial and have rhizomes densely branched (Kirschner et al. 2002c), the vegetative propagation may be one of its strategies to maintain the natural populations.

As described for *J. tenuis*, seedling development in Juncaceae is marked by the rupture of the seed coat at the micropylar region followed by the development of the phaneromer. The cells of the proximal end of the cotyledonary hyperphyll divide, forming an elongated tubular structure (phaneromer), which pushes the embryonic axis out of the seed. The shoot and root apical meristems are only evident after the development of the phaneromer. This late development of both meristems is not a common feature in angiosperms and is described here for Juncaceae for the first time.

After the development of the phaneromer and before the emergence of the primary root, dense and long rhizoids appear at the collar region for fixation of the seedling to the substrate and absorption of water and nutrients for seedling development, while the primary root is still undeveloped. These rhizoids differ from the root hairs, as the former differentiate from the epidermis of the collar region (Tillich 2007), whereas the latter differentiate from the protoderm, originating from the root apical meristem. The collar rhizoids, the first structure to come into contact with the substrate, is considered a derived character that increases the likelihood of seedling establishment (Tillich, 2000). Unlike some Poales, in which the primary root does not develop or aborts early in development (Kraus et al. 1996; Tillich 2007; Corredor et al. 2015; Mascarenhas and Scatena, 2021), the primary root and shoot-born roots of Juncaceae develop a few days after the formation of the collar rhizoids and replace them in the function of fixation and absorption.

With respect to the cotyledon, Tillich (2007) identifies two regions: the hypophyll, corresponding to its basal and bifacial portion, and the hyperphyll, which is the distal and

unifacial portion. The hyperphyll can be entirely haustorial, retained in the seed, or elongated, forming an apocole or a phaneromer. The apocole is short and restricted to hypogeal palm seedlings of which the plumule is buried into the soil during germination (Tillich 2007). By comparison, the phaneromer is more elongated and raises the seed well above the soil surface, as occurs in Juncaceae and other monocot families (Tillich 2007).

In *J. tenuis*, only the most distal portion of the hyperphyll is retained inside the seed during seedling development, with a haustorial function, while the proximal portion of the hyperphyll elongates, forming the phaneromer. The phaneromer is a specialized structure, which, in addition to the considerable elongation between the seed and the cotyledon sheath, is the first assimilated organ in the seedling (Tillich 2007). The presence of the phaneromer can be considered an ecological adaptation favoring germination, since this structure raises the seed well above the ground surface, facilitating light exposure and, consequently, promoting seedling development.

In Poales, the phaneromer occurs in Juncaceae and Thurniaceae (cyperid clade), in Typhaceae (an early diverging family), and in the families of the restiid clade (Restionaceae, Centrolepidaceae, and Anarthriaceae) (Tillich 2007). According to Tillich (2007), the phaneromer has derived from the compact cotyledon, which is the ancestral condition for the order (Tillich 2007). Thus, considering the occurrence of the phaneromer in distinct clades of Poales, it probably had several independent origins during the evolution of the order. Because the phylogenetic studies based on morphological and molecular data show Thurniaceae as a sister group of the clade formed by Juncaceae and Cyperaceae (e.g., Bremer 2002; Linder and Rudall 2005; Chase et al. 2006; Givnish et al. 2010, 2018; Bouchenak-Khelladi et al. 2014), the phaneromeric cotyledon is most likely the ancestral character state in the cyperid clade.

Conclusions

In *J. tenuis*, the seed coat is formed by a tanniferous endotegmen, endotesta, and a mucilaginous exotesta. We suggest that the mucilaginous exotesta plays multiple roles, facilitating seed hydration and promoting seed dispersal. The tanniferous endotegmen probably serves as chemical protection for the seed, while mechanical protection is provided by the thick cuticle between the tegmen and the testa.

Our results show that *J. tenuis* shares the same pattern of seedling development and the same type of cotyledon as the other species of the family. Germination is marked by the rupture of the seed coat at the micropylar region and

the protrusion of the phaneromer. The shoot and root poles become evident later, only after the elongation of the phaneromer. This study provides new anatomical data on the seed coat and seedling development in Juncaceae, contributing to understand the ecology and evolution of the family.

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Declarations

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

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