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Fleshy Structures Associated with Ovule Protection and Seed Dispersal in Gymnosperms: A Systematic and Evolutionary Overview

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ABSTRACT

Fleshy structures associated with the ovule/seed arose independently several times during gymnosperm evolution. Fleshy structures are linked to ovule/seed protection and dispersal, and are present in all the four lineages of extant gymnosperms. The ontogenetic origin of the fleshy structures could be different, and spans from the ovule funiculus in the *Taxus baccata* aril, the ovule integument in *Ginkgo biloba*, to modified bracts as in case of *Ephedra* species. This variability in ontogeny is reflected in the morphology and characteristics that these tissues display among the different species. This review aims to provide a complete overview of these ovule/seed-associated fleshy structures in living gymnosperms, reporting detailed descriptions for every genus. The evolution of these independently evolved structures is still unclear, and different hypotheses have been presented—protection for the seeds, protection to desiccation—each plausible but no one able to account for all their independent origins. Our purpose is to offer an extensive discussion on these fleshy structures, under different points of view (morphology, evolution, gene involvement), to stimulate further studies on their origin and evolution on both ecological and molecular levels.

I. Introduction

Gymnosperms represent a large group of seed plants that comprise four of the five major extant lineages of the spermatophytes. These lineages are cycads, *Ginkgo*, conifers and Gnetales, known as "gymnosperms" in reference to their naked seeds, because the term gymnosperm itself derives from the ancient Greek and it literally means "naked seed." This classic interpretation derives from the observation that in most of gymnosperms at the time of pollination ovules are directly exposed at the pollen arrival. This contrasts with angiosperm, in which the ovules are additionally enclosed inside a carpel (see Tomlinson and Takaso, 2002 for a detailed discussion). In the last decades, many phylogenetic studies have contributed the understanding of the phylogenetic status of extant gymnosperms (Bowe *et al.*, 2000; Rai *et al.*, 2008; Leslie *et al.*, 2012; Lu *et al.*, 2014; Ran *et al.*, 2018). It is widely accepted that gymnosperms evolved from progymnosperms even though it is still not clear whether the gymnosperms all evolved from a common ancestor or can be traced to more than a single ancestor (Taylor *et al.*, 2009). One hypothesis suggests that the Aneurophytales were the ancestral progymnosperm group from which all gymnosperms evolved (Rothwell, 1982; Taylor *et al.*, 2009). An alternative hypothesis is that gymnosperms are polyphyletic, with the seed ferns evolving from an aneurophyte ancestor, whereas Cordaites and conifers had their origin within the Archaeopteridales (Beck and Wight, 1988; Taylor *et al.*, 2009).

The phylogenetic relationships among the five major groups of seed plants (gymnosperms and angiosperms) are not cleared up due to the lack of molecular analysis on crucial taxa (Ran *et al.* 2018; Rudall, 2021) and to the fact that extant seed plants are unrepresentative of the existed diversity because they have experienced widespread extinction (Hilton and Bateman, 2006). Although extant taxa are monophyletic, their relationships with extinct fossil groups remain elusive (Christenhusz *et al.*, 2011), because, as suggested by Hilton and Bateman (2006), extant taxa are nested within extinct taxa assigned to the pteridosperms. These authors deeply discussed the phylogenetic status of pteridosperms in relation with extant

taxa of seed plants (Hilton and Bateman, 2006). More recent molecular analyses support cycads and *Ginkgo* forming a monophyletic group, Gnetales as sister to Pinaceae, and Sciadopityaceae as sister to Cupressaceae + Taxaceae + Cephalotaxaceae, with the Taxaceae as a monophyletic group sister to Cephalotaxaceae (Ran *et al.*, 2018).

In line with these considerations, the study of gymnosperm evolution has been always considered of key importance in understanding the origin and the evolution of seed plants.

Many gymnosperms protect the ovule/seed with devoted accessory structures, often made of leathery or lignified tissues (i.e., cones in conifers). Interestingly, other species present fleshy tissues associated to the ovule and/or to the seed (Judd *et al.*, 2016; Contreras *et al.*, 2017). In fact, all four living clades of gymnosperms include representative taxa that independently evolved fleshy and edible seed-associated fruit-like structures, which originate from diverse anatomical structures (Herrera, 1989; Lovisetto *et al.*, 2012).

The evolutionary mechanisms that originated and shaped the fleshy structures present nowadays in the various lineages of extant gymnosperms still constitute a very intriguing and open topic of discussion (Herrera, 1989; Fountain *et al.*, 1989; Mack, 2000, Tiffney, 2004; Contreras *et al.*, 2017; Leslie *et al.*, 2017). Complex interactions between ecological factors, selective tradeoffs and genetic and developmental constraints determined the functional morphologies and composition of gymnosperm diaspores (Contreras *et al.*, 2017). We discuss in this review the available evolutionary hypotheses that subtended these processes.

The development of fleshy structures from different tissues surrounding or associated to the seed suggests a process of convergent evolution. The analysis of the distribution of dispersal syndromes among gymnosperms made by Givnish (1980) evidenced that there is a correlation between dioecy and animal-dispersed seeds. He developed a model in which he supported the idea that individuals allocating more resources to the production of fleshy structures greatly increase their reproductive success by attracting frugivorous to disperse seeds. However, the assumptions used by Givnish to develop his model have been questioned by Donoghue (1989). He suggested that dioecious species with fleshy structures might be the result of higher diversification in just a few clades with this characters, as also observed in flowering plant (Donoghue, 1989). Leslie *et al.* (2013) revaluated these

hypotheses, and concluded that the distribution of these reproductive characters cannot be explained by either of the previous hypotheses, but confirmed that the combination of dry-monoecy and fleshy-dioecy are found most frequently in extant species, at least for conifers.

This review aims to shed light on the diversity of these fruit-like structures overviewing them in the diverse taxa of extant gymnosperms, and considering the several hypotheses nowadays available regarding the origin of these structures. This would help plant scientists to investigate their origin and evolution, looking also at the molecular pathways controlling their development, stimulating the debate around this under-treated topic and inspiring new research questions.

II. Overview of the fleshy structures in the four extant gymnosperm lineages

A. Cycadales

Cycads species belong to the taxon Cycadopsida and date from the late Paleozoic Era (about 350–280 million years ago) (Norstog, 1987; Brenner *et al.*, 2003; Terry *et al.*, 2009; Rudall, 2021). The order Cycadales comprises ten genera (*Bowenia, Ceratozamia, Cycas, Dioon, Encephalartos, Lepidozamia, Macrozamia, Microcycas, Stangeria, Zamia*) grouped into three families (Cycadaceae, Stangeriaceae, and Zamiaceae) (Caputo *et al.*, 1991). About 330 species of cycads are currently known (Condamine *et al.*, 2015), all of them are dioecious, with the characteristic palm-like appearance (Brenner *et al.*, 2003). Three genera are endemic to Australia, two to Africa, and four to the Americas, while the species belonging to the genus *Cycas* live in Australia, in Pacific islands, in southern Asia, and in Madagascar, making this genus the most widespread (Norstog, 1987). Male and female cones bear numerous helically attached sporophylls, and they can range from one to multiple cones per plant depending on the species (Meeuse, 1963; Tang, 1987a), except for the genus *Cycas* in which the ovulate structures are not grouped to form a cone (Meeuse, 1963). The highly visible cones are produced near the stem apex (Newell, 1983), generally positioned above the leaf crown, in order to be easily accessible to animals (Mustoe, 2007). The large and heavy seeds (Figure 1a) are densely clustered in female cones and are produced in a high number, in synchronized mast-seeding events (Mustoe, 2007; Hall and Walter, 2013). Ovules have a single integument, in which three differentiated layers are distinguishable: the soft inner
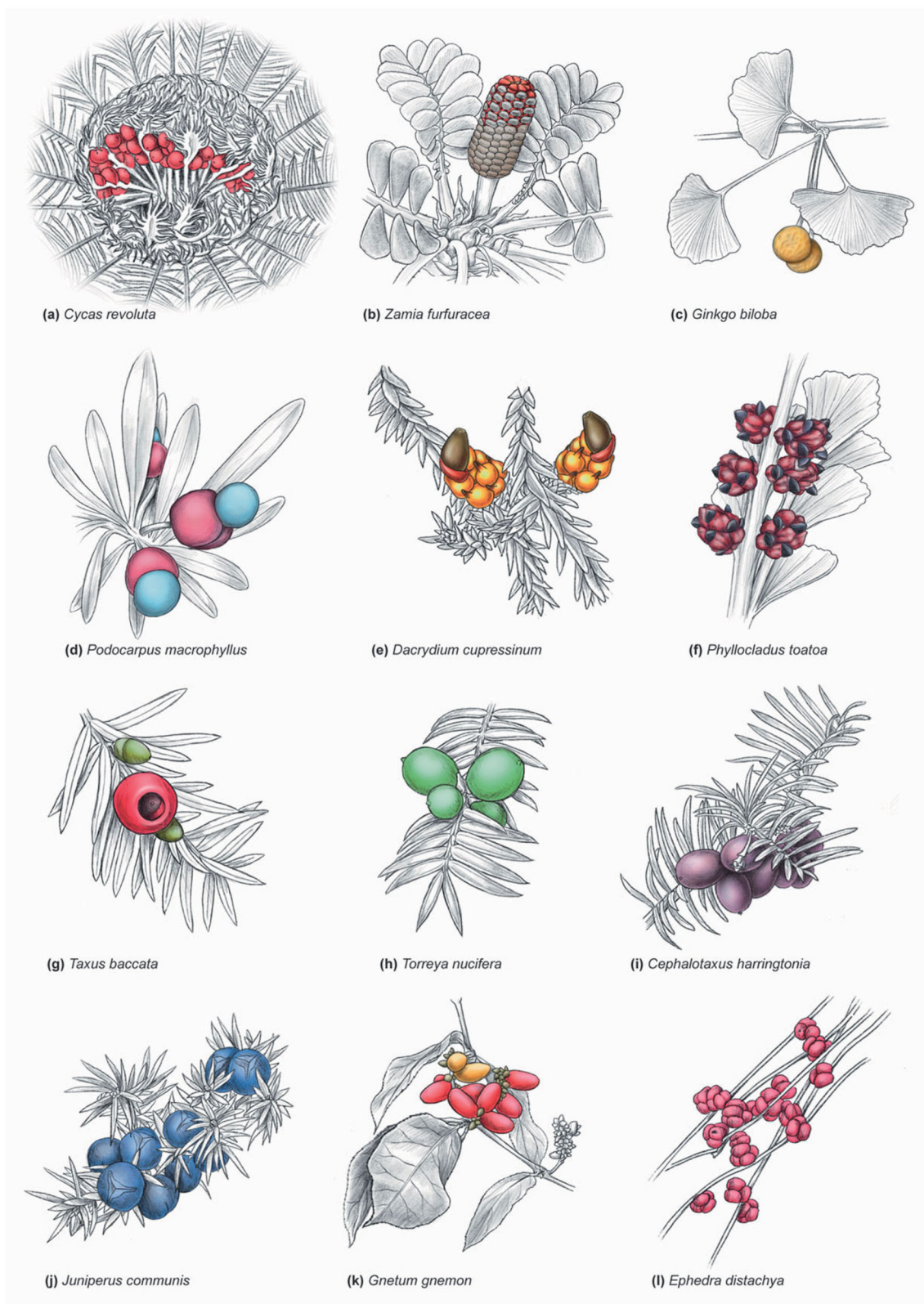
Figure 1. Illustration of some seed-associated fleshy structures found in selected gymnosperm species. Colors point out the different morphologies of the fleshy reproductive structures: (a–c) fleshy sarcotesta; (d,e) fleshy epimatium on a fleshy receptacle; (f) aril on a fleshy receptacle; (g) aril; (h) aril overgrowing from the base of the seed as a supra-integument; (i) best interpreted as an aril; (j) fusion of bract-scale complexes; (k) fleshy sarcotesta; (l) fleshy bracts.

one named endotesta that directly surrounds a massive endosperm filled with starch, the hard and stony middle layer (sclerotesta) and the outermost brightly colored, fleshy, and edible layer (sarcotesta) (Bauman and Yokohama, 1976; Mustoe, 2007; Hall and Walter, 2013). All these features suggest that cycad seeds were adapted for dispersal by large animal vectors, likely extinct megafauna (Mustoe, 2007; Hall and Walter, 2013). Indeed, the brightly-colored sarcotesta is typically red, orange or yellow, which are attractive colors especially for reptiles, which possess good color vision. Nowadays modern cycads are dispersed by birds (which have a cone visual system sensitive to red and yellow colors), bats and large mammals (Bauman and Yokohama, 1976; Mustoe, 2007). However, this process could be not very efficient, especially in regards to birds, because their small body sizes make them unfit for cycad seeds dispersal (Mustoe, 2007). Cycads seeds if ingested are toxic to vertebrates, as they contain cycasin and macrozamin, which are compounds that cause potentially fatal damage to the liver and to the nervous system, through a DNA methylation action (Whiting, 1963; Schneider et al., 2002; Hall and Walter, 2013). Only the sarcotesta is free from these toxic compounds, and instead, is rich in sugars, making this outer layer the unique edible part of the seed (Mustoe, 2007). For these reasons only animals able to ingest the whole seeds are involved in the seed dispersal. The fleshy sarcotesta is digested internally, whereas the seed kernel remains intact thanks to the resistant and protecting sclerotesta, avoiding thus the release of toxic compounds and ensuring the safe passage of the embryo through the host digestive tract (Mustoe, 2007; Hall and Walter, 2013). This enhances seeds dispersal, avoiding serious seeds predation (Burbidge and Whelan, 1982; Hall and Walter, 2013). Moreover, the removal of the fleshy sarcotesta by animals facilitates the germination of the seeds, since the sarcotesta tissue contains chemicals that inhibit germination (Newell, 1983; Mustoe, 2007).

All cycads species belonging to the Zamiaceae family are pollinated by insects, or insects are somehow involved in the process of pollination. For the species Zamia furfuracea and Zamia pumila there is some reliable evidence that the pollination is mediated by beetles (Newell, 1983). On the contrary, species belonging to the Cycadaceae family are wind-pollinated, even if for Cycas revoluta there is some evidence of ambophily (Norstog et al., 1986; Tang, 1987b; Kono and Tobe, 2007; Terry et al., 2009).

Another peculiar characteristic of cycads is the fertilization event: the flagellate spermatozoids are released by the pollen tube and swim through the archegonial chamber fluid, to reach the egg cell within the archegonium. A feature is shown only by cycads and Ginkgo biloba within seed plants (Norstog et al., 2004; Takaso et al., 2013).

The genus Cycas (Cycadaceae family) is considered basal among cycads (Brenner et al., 2003; Terry et al., 2009). Mature seeds of various species such as Cycas revoluta, C. media, C. normanbyana, C. taiwaniana, and C. wadei, have a seed coat composed by three layers: the sarcotesta, the sclerotesta, and a thin membranous jacket that encloses the female gametophyte tissues. These distinct layers derive from the differentiation of a single ovule integument (unitegmic ovules). Seeds of C. rumphii, C. circinalis, and C. thouarsii, have an additional thick layer of spongy tissue between the sclerotesta and the membranous jacket that allows seeds to float, thus seed are likely dispersed by seawater currents (Dehgan and Yuen, 1983). The inner fleshy layer is present also in the C. revoluta group, but instead of originating the spongy tissue as in the C. circinalis complex, it degenerates upon maturity (Dehgan and Yuen, 1983). The sarcotesta contains starch and is eaten by rodents, bears, bats, or other mammals, which therefore disperse seeds locally (Bauman and Yokohama, 1976; Dehgan and Yuen, 1983).

Within the Zamiaceae family, Zamia, Macrozamia and Encephalartos genera are the richest in species. Zamia species are widespread in North, South and Central America. Mature female cones (Figure 1b) drop the bright red seeds next to the parent plant (Negrón-Ortiz and Brecknon, 1989). Macrozamia spp. are endemic to Australia (Forster, 2004), seeds include the kernel (mostly female gametophyte), which contains starch, but especially the toxic poisonous glycosides "macrozamin" confined within the stony layer. The fleshy sarcotesta can be red (e.g., Macrozamia riedlei), bright orange (e.g., M. communis) or dark orange (e.g., M. lucida) (Burbidge and Whelan, 1982; Ballardie and Whelan, 1986). Several vertebrates are implicated in Macrozamia spp. seed dispersal, including emus, several species of birds, gray kangaroos, quokkas, brush wallabies and quolls, and others animals, but especially the possums (Trichosurus vulpecula), which is the main dispersal agent for M. riedlei and M. communis (Burbidge and Whelan, 1982; Ballardie and Whelan, 1986; Snow and Walter, 2007). Encephalartos African species can have solitary or multiple female cones, and seeds are dispersed by

birds, rodents and baboons (Donaldson, 2008). The genus *Dioon* also belongs to the Zamiaceae family, and seeds are characterized by a fleshy and yellow outer sarcotesta (Mora *et al.*, 2013).

B. Ginkgoales

In *Ginkgo biloba*, the fleshy structure associated with the seed is the integument of the ovule/seed that during the developmental process becomes fleshy.

Ginkgo biloba is the only surviving species in the clade Ginkgophytes, with no living relatives and its morphology has been basically unchanged for at least 200 million years (Jin *et al.*, 2012; Zhao *et al.*, 2019). The study of fossils has demonstrated that Ginkgoaceae originated in the Permian, and reached its maximum diffusion in the Jurassic, around 100 million years ago, when *Ginkgo* species were spread in both hemispheres. Later, probably due the change of climate conditions, *Ginkgo* species was restricted geographically and survived as a relict in China. From there, humans have played an important role to the spread of *Ginkgo* trees around the world, as demonstrated by genetic analysis of several trees worldwide (Hori and Hori, 1997; Zhao *et al.*, 2019).

Ginkgo biloba is a large tree, dioecious and mostly wind-pollinated. *Ginkgo biloba* is famous for its fan-shaped leaves, with dichotomous venation, that turn yellow before abscission (Judd *et al.*, 2016). Male and female individuals occur at roughly a 1:1 ratio; both plants show a long juvenile period of approximately 20 years (Del Tredici, 2007), along it the plants are morphologically indistinguishable (Hori and Hori, 1997). Vegetative and reproductive organs are produced at the end of short shoots (brachyblasts): male and female reproductive organs are located in the axils of bud scales and leaves (Del Tredici, 2007; Douglas *et al.*, 2007; Jin *et al.*, 2012). According to environmental and climatic conditions, in the Northern Hemisphere wind pollination occurs from mid-March to May (Del Tredici, 1989). Female reproductive organs consist of a stalk bearing sessile ovules on the top. A collar is located at the base of each ovule (Douglas *et al.*, 2007). At the time of buds opening, depending on latitude and climate conditions, 1–2 mm long ovules are exposed. At this stage ovules are yellow. Together with leaves expansion and elongation, ovular stalks extend rapidly pushing the ovules outward from the open bud. The resulting structure shows a spiral arrangement of ovules and leaves at the end of the short shoot (Jin *et al.*, 2012). Usually, a single stalk bears two ovules directly attached through the collar. However, *Ginkgo* plants may present up to three types of teratologies: (i) foliar, bearing ovules on or near leaf laminar structures (O-ha-tsuki, Fischer *et al.*, 2010); (ii) axial, where a general stalk differentiated into two separated ovule stalks; (iii) and two or more ovules fused together. It is still not clear whether these different morphologies reflect an altered developmental expression pattern or may be interpreted as ancestral traits (Douglas *et al.*, 2007; Jin *et al.*, 2012). At this time of development, ovules appear partially erect on the opposite sides of the ovulate stalk. The collar is irregularly lobed, and in some cases may present, between the collar and the ovule itself, an additional lobed structure named flap, which development and function are unknown (for a detailed dissertation on the ovule morphology see Douglas *et al.*, 2007). The ovule has a single integument, slightly bifid and inserted at the base of the nucellus. The process of ovule development takes a nearly a year and a half. Ovules produce the pollination drop and are receptive for the pollination in early spring, while the fertilization occurs only at the end of the summer. In this time frame, pollinated ovules undergo dramatic modification. The single integument, characteristic of gymnosperm ovules, differentiates into three distinct morphological layers: an external fleshy sarcotesta, a middle lignified sclerotesta and an inner papery endotesta. The fleshy character in *G. biloba* is thus derived from a modification of the ovule integument that after fertilization will form the structure surrounding the seed. At complete maturation, the fleshy layer of the seeds is yellow, soft and juicy (Figure 1c). The sarcotesta in fact contains fatty acids that undergo oxidation during the ripening process, producing a foul odor. After about one month from fertilization ovules drop from the tree (Singh *et al.*, 2008). Mature seeds have the characteristic bad smell, resembling rotting organic material (meat). Few data are available about how *Ginkgo* seeds are dispersed in its natural environment. Fossils from the Mesozoic era do not clarify if dinosaurs were efficient *Ginkgo* seeds dispersers (Singh *et al.*, 2008). However, it is known that Ginkgoales species coexisted with dinosaurs until their extinction, and from fossil evidence it is also known that some of them could be herbivorous, and by grazing or browsing plants they could have been disperse also *Ginkgo* seeds (Tiffney, 2004). Instead, the role of some Rodentia and

Carnivora species as dispersers has been demonstrated: after feeding with the odorous and nutrient-rich seeds they finally defecate the intact nuts presumably dispersing the plant embryos (Singh et al., 2008).

C. Coniferales

Conifers are the largest group of extant gymnosperms by number of species and have a great ecological and economical importance. This group comprises about 630 living species and its status as a monophyletic clade has been challenged in the last decade (Hilton and Bateman, 2006). Rothwell et al. (2005) states that cordaiteans and vojnovskyeans are the extinct sister groups more closely related to Voltziales taxa. The latter represents a paraphyletic group of conifers from which modern families has been postulated to have evolved (Rothwell et al., 2005; Contreras et al., 2017). Besides, molecular phylogenetic studies have placed Gnetales within conifers either as sister to Pinaceae or to all other conifer families (conifer II group) (Zhong et al., 2011; Xi et al., 2013; Lu et al., 2014; Ran et al., 2018). The first hypothesis (gnepine hypothesis) has received greater support during the years, but the question is still open.

Conifers are perennial woody trees or shrubs with well-developed wood and often needle-like or scale-like leaves (Judd et al., 2016). In conifers, both monoecious and dioecious species can be observed. Interestingly, dioecy is a characteristic often correlated with the production of fleshy cones or seeds (Givnish, 1980; Leslie et al., 2013) and it is exclusive to species belonging to the conifer II group.

These plants are named "conifers" because they carry cones (or strobili) which are complex specialized reproductive structures that protect ovules/seeds, facilitating pollination first and later seed dispersal. Conifer seed cones are compound structures derived from modified branch systems consisting of a central axis that bears modified leaves called bracts, each of which subtends a seed-bearing structure called an ovuliferous scale (Judd et al., 2016; Contreras et al., 2017). The bracts are interpreted as modified penultimate leaves, and the ovuliferous scale as reduced and flattened axillary shoot structures. This view is supported by fossil records. In particular, also in cordaitaleans – generally regarded as the extinct sister lineage of conifers (Contreras et al., 2017) – the seed cones consisted of an axis-bearing bracts (Florin, 1954 and reference therein). However, in these ancient structures, the axils of each bract presented a branch shoot system, consisting of an axis bearing sterile leaves and one to many ovules (each usually subtended by a "fertile" leaf). The transition from a structure with lateral branches bearing a number of seeds to the modern highly modified cone scale can be seen in the fossils of Cordaitales and extinct gymnosperms (Simpson, 2019). The different components of the ovuliferous cone vary extensively among conifer families, therefore few explanations about this variation are needed. For instance, bracts can be either large and conspicuous (as in Abies bracteata, in which the bract tips are exceptionally long; or Pseudotsuga menziesii, where the bracts are very large and trilobite at the apex) or very small (as in Pseudolarix amabilis). Also, the morphology of the cone scale presents a high level of variation. In Pinaceae, the scale enlarges during cone development to become the dominant component of the mature reproductive structure. Cupressaceae reproductive cones do not present morphologically distinguishable ovuliferous scales, and ovules develop from the cone axis, usually in the axil of a bract or terminally at the apex of the cone (Takaso and Tomlinson, 1989; Tomlinson et al. 1993; Tomlinson and Takaso, 2002; Schulz et al., 2003; Contreras et al., 2017). According to Florin, the absence of an ovuliferous scale can be explained as a complete fusion of the ovuliferous scale with the subtending sterile bracts (Florin, 1951, 1954).

In Araucariaceae each bract is fused with the proximal ovuliferous scale pair, while Podocarpaceae species show a wide range of cone modifications, but are most notable for presenting fleshy cone parts (epimatium and/or receptacle). Taxaceae species present highly reduced cones (small bracts and no recognizable ovuliferous scale) bearing each only a single ovule. At maturity, the seed is enclosed/enveloped by an aril, which originates from the funiculus (Contreras et al., 2017). Members of conifer group may show the two major dispersal syndromes present in gymnosperms: one involves wooden cones enclosing (usually) winged seeds that are wind- or gravity-dispersed; the other involves showy fleshy fruit-like structures that are animal-dispersed (Givnish, 1980). As demonstrated by Leslie et al. (2013) through parsimony reconstruction analyses, dry cones are plesiomorphic in conifers while the shifts to fleshy cones and the shifts back to dry cones occurred respectively between three and five times, and between one and three times within the conifer groups. All the extant genera that present fleshy structures adapted for animal mediated seed dispersal syndrome belong to families of group II of conifers. Works by Givnish (1980),

Table 1. Morphotypes of conifer dispersal units (modified from Contreras et al., 2017).

Morphotype	Genus	Family
Aggregate fructoid	*Microcachrys*	Podocarpaceae
	Saxegothaea	Podocarpaceae
Adorned fructoid	*Dacrycarpus*	Podocarpaceae
	Podocarpus	Podocarpaceae
	Acmopyle	Podocarpaceae
	Dacrydium	Podocarpaceae
	Falcatifolium	Podocarpaceae
	Phyllocladus	Podocarpaceae
	Halocarpus	Podocarpaceae
	Manoao	Podocarpaceae
Baccate fructoid	*Juniperus*	Cupressaceae
	Parasitaxus	Podocarpaceae
	Pseudotaxus	Taxaceae
	Taxus	Taxaceae
Drupe-like fructoid	*Afrocarpus*	Podocarpaceae
	Pectinopitys	Podocarpaceae
	Prumnopitys	Podocarpaceae
	Amentotaxus	Taxaceae
	Torreya	Taxaceae
	Cephalotaxus	Taxaceae

Herrera (1989), Leslie et al. (2013) and Contreras et al. (2017) confirm this interesting observation. In particular, the three families in which such type of features can be found are Podocarpaceae, Cupressaceae and Taxaceae

The great variation among the ovuliferous cone morphology in conifers can be related to the different strategies that these plants evolved to disperse their seeds, which involved one or a combination of the various components of the cone (Leslie et al., 2017). With the attempt to conduct a comprehensive analysis of conifer diaspores in relation to the different dispersal strategies, Contreras et al. (2017) distinguished, within a phylogenetic contest, nine distinct functional morphotypes of conifer diaspores. These functional structures may consist of a single seed or the seed(s) plus additional tissues which can be: (1) extra tissue derived from the adaxial surface of the ovuliferous scale, (2) entire ovuliferous scale or epimatium, (3) bract(s), (4) aril, and (5) cone axis. Four of the nine identified morphotypes are zoochorous diaspores which, based on their morphology, have been named: (1) the *aggregate fructoid* that resemble a multiple fruit and involve all the seed cone, bracts and scales together; (2) the *adorned fructoid* which display attractive accessory structures positioned below or at the base of the seed which do not fully enclose the seed (Figure 1d–f); (3) the *baccate fructoid*, formed by a fleshy colored seed cone (Figure 1g,j); and (4) the *drupe-like fructoid*, which resemble a drupe (Figure 1h,i). Both *aggregate fructoid* and *adorned fructoid* are exclusive of Podocarpaceae, with the adorned one as the plesiomorphic condition of the family (Table 1, modified from Contreras et al., 2017).

Passerine birds and rodent are the vertebrate groups that nowadays mostly interact with conifer seeds. Nevertheless, the evolution of these fleshy accessory structures to the seed predates the emergence of modern dispersal agents, as the latter radiated quite recently relative to the age of conifers themselves (Leslie et al., 2017).

1. Podocarpaceae

Podocarpaceae group are dioecious (rarely monoecious) slightly resinous trees and shrubs. They are comprised of 20 genera and 170 species (or more) and are mainly distributed in the tropical and subtropical regions of the Southern Hemisphere (Page, 1990; Farjon, 2010; Judd et al., 2016; Page, 2019).

The female cones are terminal or borne in the axil of each fertile bract, cone-like or highly reduced (Page, 1990; Farjon, 2010). In most cases during ontogenesis the number of seeds in each seed cone is reduced to one, which becomes variously enclosed or subtended with soft, often colorful tissues (Page, 1990; Farjon, 2010). In general, when the first ovule has been fertilized the other ovules are aborted and the bracts swell and fuse together to form a juicy and colorful receptacle to attract birds. The initial cup-like ovuliferous scale grows around the seed to form the epimatium, which seated on the receptacle, may swell to a succulent pulp acquiring the same attractive function (Farjon, 2010). The pseudo-fruit is generally eaten by birds (Page, 1990), thus the seed dispersed. Of the 20 genera in the Podocarpaceae only two genera do not have fleshy structures: *Pherosphaera* and the monospecific genus *Lagarostrobos* (*Lagarostrobos franklinii*).

Contreras et al. (2017) classify the fleshy structures of the monospecific genera *Microcachrys* (*Microcachrys tetragona*) and *Saxegothaea* (*Saxegothaea conspicua*) as aggregate fructoids, because in these species the seed cone comprises an axis of helically or spirally arranged bracts which become fleshy and slightly swollen at maturity, resembling a compound fruit. Each fertile scale bears an ovule. According to Farjon (2010), *Microcachrys*' agents of dispersion are still unclear, and the same can be said for *Saxegothaea*. In fact, there seems to be no clear consensus on the subject: the majority of studies (Stiles, 1908; Kelch, 1997; Contreras et al., 2017) tend to relate the structure of the seed cone of *Saxegothaea conspicua* to that of *Microcachrys tetragona*, as both appear to consist of an axis of spirally arranged bracts which become fleshy at maturity; however, Farjon (2010) suggested that *Saxegothaea*

cones could become leathery and dry at the end of maturation. In addition, *Saxegothaea* is a monoecious evergreen tree in contrast to *Microcachrys*, which is a dioecious creeping shrub.

Dacrycarpus, *Podocarpus*, *Acmopyle*, *Dacrydium*, *Falcatifolium*, *Phyllocladus* present an adorned fructoid, in which the seed is fully (first three genera) or partially (latter three genera) enclosed by the epimatium that seats on a fleshy receptacle (Figure 1d,e). However, *Phyllocladus* is an exception, as in this genus the epimatium is actually absent and replaced by an aril (Figure 1f). *Halocarpus* and *Manoao* also present adorned fructoids in whitch the fleshy portion is constituted by the epimatium. The genus *Lepidothamnus*, which consists of three species of shrubs, has a diaspora quite similar to that of *Dacrycarpus*, *Podocarpus* and *Acmopyle* (Contreras et al., 2017). This may be a case of convergent evolution, as *Lepidothamnus* is more closely related to *Phyllocladus* (according to the phylogeny by Biffin et al., 2011). The fleshy receptacle (or podocarpium) originates from sterile bracts which metamorphose in the mature post-pollination cones (Page, 1990; Mill et al., 2001). This fleshy structure has been shown to prolong seed viability through increased desiccation resistance (Fountain et al., 1989).

In the genera *Afrocarpus*, *Pectinopitys*, *Prumnopitys* and *Nageia*, the epimatium swells and becomes succulent, taking over the attractive function of the receptacle. In these genera, seeds are subtended by small scales and not by a receptacle (as in *Afrocarpus*, *Pectinopitys* and *Prumnopitys*), or by bracts either drying up or fusing and enlarging at their bases, forming a weakly developed, fleshy receptacle being usually only slightly thicker than the peduncle (as in *Nageia*), and are entirely enclosed by a fleshy epimatium usually yellow, red or black at maturity (Farjon, 2010). The monospecific genus *Sundacarpus* (*Sundacarpus amarus*) seems to have a fleshy but firm epimatium, but little is known about how the seed is dispersed (Farjon, 2010).

The five species in the genus *Retrophyllum* have a drupe-like fructoid: a large seed with a hard coat covered by a fleshy deep red, violet or purplish epimatium. *Retrophyllum minus*, the only true rheophyte among gymnosperms, has buoyant seeds that are normally transported by running water. The fleshy epimatium can be consumed by birds and fish, but also often rots away, even on the plant (Farjon, 2010). Therefore in this genus both hydrochory and zoochory can be observed. *Parasitaxus usta*, the only known parasitic gymnosperm, possesses a baccate

fructoid, where mature seeds are completely surrounded by a globose, hard, glaucous white epimatium, thus resembling a berry (Contreras et al., 2017; Farjon, 2010).

Contrary to the previous interpretations that stated that the epimatium is an entirely novel structure (Tomlinson, 1992), newer interpretations suggest that the epimatium can be considered as a fleshy seed scale (Tomlinson and Takaso, 2002; Contreras et al., 2017). Consistently, the epimatium in podocarps and ovuliferous scales in other conifers can be interpreted as homologous structures (Tomlinson, 1992; Mill et al., 2001).

2. Taxaceae

Taxaceae are small-to-moderately sized trees or shrubs, usually not resinous or only slightly resinous. Generally, are dioecious (rarely monoecious) with ovuliferous cones extremely reduced, and solitary ovules. Indeed, unlike other conifers the Taxaceae megastrobilus is not a cone, but consists of a single terminal ovule on an axillary shoot (axis of the strobilus) (Dupler, 1920; Cope, 1998). The fleshy part that surrounds the seed is commonly interpreted as an aril, an *ex-novo* developed structure, devoid of vascularization, which originates from the funiculus at the base of the seed and that at maturity changes color from green to red (Figure 1g) (Semikhov et al., 2001; Lu et al., 2008). Up to the time of pollination the ovule integument will originate the micropyle, and after the fertilization it becomes stony, forming the hard outer layer (Dupler, 1920; Judd et al., 2016), associated and partially enclosed by the fleshy cup-shaped aril (Judd et al., 2016).

Taxaceae consist of six extant genera, including two formerly segregated as Cephalotaxaceae (*Amentotaxus*, *Cephalotaxus*) and four "core" Taxaceae: *Austrotaxus*, *Pseudotaxus*, *Taxus* and *Torreya* (Ghimire and Heo, 2014; Ghimire et al., 2014). In *Austrotaxus*, *Taxus*, *Torreya*, *Amentotaxus* and *Pseudotaxus* the mature seed is surrounded by the above-mentioned fleshy aril (Figure 1h), a fruit-like structure which arises late in development, as a supra-integument arising as an overgrowth from the base of the seed (Semikhov et al., 2001; Contreras et al., 2017). The seed of *Taxus* is toxic due to the presence of cyanogenic glycosides in the seed coat; however, the fleshy aril is edible and seeds pass through the bird gut undigested (Farjon, 2010). The fleshy structure of *Cephalotaxus* has been interpreted by some authors as a sarcotesta, since it begins to develop as a ring primordium almost entirely fused with the ovule integument (Page, 1990;

Contreras *et al.*, 2017). However, considering that the mature structure is not differentiated into three layers (endotesta, sclerotesta and sarcotesta, as seen in *Ginkgo* and cycads), other authors view the fleshy outer layer of the *Cephalotaxus* seed as best interpreted as an aril (Figure 1i) (Dörken *et al.*, 2019).

Recently, Dörken *et al.* (2019), going deeper into the evolutionary origin of the Taxaceae aril, subdivided genera in two groups with respect to the structure and ontogeny of the fleshy aril. The first group, which comprises *Austrotaxus*, *Pseudotaxus* and *Taxus*, evidenced a "free cup-like" structure of the aril, whereas in the other three genera (*Amentotaxus*, *Cephalotaxus* and *Torreya*) the aril is strongly fused to the ovule. Also, the aril growth pattern and some histological characteristics showed differences between the two groups. As regards the initial stages of fleshy structure development, the authors identified three genera in which the aril is initiated by two laterals primordia (Dupler, 1920) before later forming a ring primordium (*Pseudotaxus*, *Taxus* and *Torreya*), while such distinct initial lobes were not visible in *Cephalotaxus* and *Amentotaxus* (Dörken *et al.*, 2019).

3. Cupressaceae

Cupressaceae is a relatively wide family of mostly evergreen, monoecious or dioecious conifers. The wood and foliage of these species are often aromatic, wood can be also highly valuable from an economic point of view. *Juniperus* is the only dioecious genus of the family. Moreover, it is the only genus that evolved fleshy cones, which are consumed by birds and small mammals (Judd *et al.*, 2016). Juniper seed cone consists of a fusion of fertile and sterile bract-scale complexes, which confer a globose shape to the dispersal unit, that therefore resembles a berry (Figure 1j) that fully encloses one or more seeds (Thomas *et al.*, 2007; Contreras *et al.*, 2017). At maturity, the reduced cone appears fleshy and colored. In many species, like *Juniperus communis* or *J. sabina*, the berry-like structure is bluish in color, sometimes covered in a white thin film of wax. In other species, like *J. oxycedrus*, the structure tends to be a reddish brown.

D. Gnetales

The Gnetales clade comprises Gnetaceae, Welwitschiaceae, and Ephedraceae families, each of which has a single genus within it (monotypic families). Corresponding genus are *Gnetum*, *Welwitschia*, and *Ephedra*. *Welwitschia mirabilis* is the only species in its genus and it is endemic to the Namib desert;

Ephedra comprises almost 50 species inhabiting arid and semi-arid regions of the world; *Gnetum* genus comprises almost 40 species distributed among moist tropical regions of the world, but with a concentration of species native of the Indonesian archipelago (Price, 1996; Ickert-Bond and Rydin, 2011; Hou *et al.*, 2015). All Gnetales species are functionally dioecious, even if in all three genera there are species that have functionally unisexual complexes (male) that seem to be morphologically bisexual as they have sterile or abortive ovules (Endress, 1996; Haycraft and Carmichael, 2001; Hou *et al.*, 2020). Their reproductive organ complexes do not show great differences among genera; they are compound strobili, resembling cone-like "inflorescences" borne directly on stem ends. Cones are inserted in a branched system, and sterile bracts surround both male and female structures (Doyle, 1996; Endress, 1996; Frohlich, 1999). Ovules, together with their integuments, are surrounded by one or two additional envelopes that are structures unique to Gnetales. These additional envelopes differ from the two integuments of angiosperms for having different origin and developmental pattern, being probably derived from bracts (Rydin *et al.*, 2010). The thin ovule integument extends beyond the seed envelope(s), forming the micropyle from which the pollination drop will be secreted to allow pollen capture (Ickert-Bond and Rydin, 2011; Jörgensen and Rydin, 2015; Hou *et al.*, 2019). Unlike to what happens in other gymnosperms, in *Gnetum* and *Ephedra* two simultaneous fertilization events occur. In angiosperms the process of double fertilization leads to the formation of one zygote and a secondary endosperm, while in these Gnetales species two sperms fuse with two nuclei in the ovule, producing two embryos of which only one will survive to reach maturity (Carmichael and Friedman, 1995; Carmichael and Friedman, 1996; Scutt *et al.*, 2018).

Among Gnetales only the Gnetaceae and the Ephedraceae comprise species that produce seed-associated fleshy structures. *Gnetum* species are shrubs, or climbers, except for two arborescent Indo-Pacific species (*G. gnemon* and *G. costatum*), resembling therefore dicotyledonous angiosperm species (Price, 1996; Biswas and Johri, 1997; Doyle, 1998). Reproductive structures are compound structures occurring in whorled arrangements on spikes-like cones and female or male units are interspersed with hair-like extensions (Maheshwari and Vasil, 1961; Endress, 1996; Hou *et al.*, 2015; Jörgensen and Rydin, 2015). Female strobili are composed by a central axis bearing a pair of opposite bracts at the base, followed by distally

oriented multiple collars (five to eight), each bearing five to seven ovules. Collars, connecting whorls of ovules at the central axis, can be distinctly separated, or densely placed. Each female unit (the ovule and its envelopes), inserted in a compound strobilus, produces a single large seed. Usually, the upper few collars have no ovules (Carmichael and Friedman, 1996; Endress, 1996; Price, 1996; Biswas and Johri, 1997; Manner and Elevitch, 2006; Jörgensen and Rydin, 2015; Hou et al., 2015). Each ovule is surrounded by two envelopes, which arise acropetally on ovular primordium, enclosing the nucellus and the integument, and forming the seed coat. These two envelopes differentiate into an outer thick, fleshy and edible layer (sarcotesta), and in a middle stony layer (sclerotesta) (Rodin and Kapil, 1969; Endress, 1996; Biswas and Johri, 1997; Rudall, 2021). They resemble more closely the integumental layers of some pteridosperms, especially the Trigonocarpales, than the two integuments typical of angiosperms (Rodin and Kapil, 1969; Endress, 1996; Biswas and Johri, 1997). At maturity the ovule integument is completely free from the above-described envelopes, except for its basal attachment, and it extends beyond them forming the micropylar tube. Envelopes are rich in vascular tissues that extend toward the apical region, and the sclerotesta is particularly rich in sclereids which cause the hardness of this layer. At maturity the sarcotesta becomes fleshy and bright in color (Figure 1k), becoming attractive to birds and mammals that eat this fruit-like structure, ingest the seed, and disperse it (Rodin and Kapil, 1969; Takaso and Bouman, 1986; Endress, 1996; Price, 1996; Biswas and Johri, 1997). It appears that most species with corky fruit-like structures disperse their seeds by flotation (hydrochory). There is evidence to suggest that fish-mediated dispersal occasionally occurs in Gnetum venosum (Price, 1996). The dispersal unit is a drupe-like structure, whose fleshy outer envelope derives from cupule-like bracts (Hrabovský et al., 2017). The two envelopes probably represent fused bract-like telome (Rodin and Kapil, 1969; Takaso and Bouman, 1986; Endress, 1996). Seed coat layers can be free or nearly free, representing the ancestral condition (e.g., G. gnemon and G. leyboldii). The two outer layers can be partially fused, remaining free in the apical portion (e.g., G. ula), or can be completely fused (e.g., G. montanum and G. neglectum), representing the highest degree of specialization (Rodin and Kapil, 1969).

Ephedra species are perennial (Ickert-Bond and Renner, 2016) shrubs, or vines, with ephemeral non-photosynthetic leaves and green branches (Hollander and Van der Wall, 2009). As in the other Gnetales genera both the female and the male strobili are compound complexes (Endress, 1996; Rydin et al., 2010) that grow from the leaf axils (Endress, 1996). Ephedra species are dioecious, and there is only one extant species that bear sterile ovules on male cones: E. foeminea (Jörgensen and Rydin, 2015). Male and female cones have decussate pairs of bracts, which bear reproductive complexes (Endress, 1996; Rydin et al., 2010). In particular, female cones appear in form of axillary buds and are composed by four to seven pairs of opposite and decussate (or ternate) bracts, fused at the base. Of this cup-shaped structure only the distal pair of bracts encloses one to three seeds, each surrounded by a seed envelope. Most commonly, fertile bracts are the uppermost two (e.g., E. distachya, E. sinica) but, even if rarer, there are species that bear three female units on a single female cone (e.g., E. intermedia has tri-ovulate cones). In both cases, fertile bracts subtend female units while the lower bracts are sterile. Ovules within the fertile bracts are surrounded by one additional envelope in which the outer epidermis, the mesophyll, and the inner epidermis are distinguishable. In general, the seed envelope is partially sclerenchymatous (Endress, 1996; Yang, 2004; Rydin et al., 2010; Ickert-Bond and Rydin, 2011; Ickert-Bond and Renner, 2016). The integument is thin and protrudes beyond the nucellus and beyond the outer envelope to form the outward orientated micropylar tube, while the outer envelope is thicker, completely free from the integument, and originates from the lateral abaxial side of the ovulate unit. The outer envelope is probably derived from modified and specialized foliar organs (Endress, 1996; Yang, 2004; Rydin et al., 2010). Primordia of female reproductive units are oriented obliquely with respect to the axis, indeed in the apical part, between the two ovules primordia, a furrow is formed and ovules primordia are outward oriented (Takaso, 1985; Yang, 2004). The dispersal units of Ephedra spp. are, therefore, compound pseudo-fruits derived from the entire fleshy cone composed by whorls of membranous to fleshy bracts, which surround one to three seeds, which at maturity become ivory white, bright red (Figure 1l) or orange in color (Price, 1996; Endress, 1996; Yang, 2004; Ickert-Bond and Renner, 2016; Hrabovský et al., 2017). Thirty-eight Ephedra species are known to have fleshy and brightly colored female cones that are dispersed by frugivorous birds eating the succulent, fruit-like bracts and dispersing the seeds through feces (Freitag and Maier-Stolte, 1989; Rodriguez-Pérez et al., 2005; Hollander and Van der Wall, 2009;

Table 2. Summary of the fleshy parts that constitute the dispersal units across gymnosperm genera.

Higher classification	Genus	Fleshy part	References
Cycadaceae	*Cycas*	Sarcotesta	Givnish, 1980; Mustoe, 2007 and references therein
	Lepidozamia	Sarcotesta	
	Encephalartos	Sarcotesta	
	Macrozamia	Sarcotesta	
	Dioon	Sarcotesta	
	Bowenia	Sarcotesta	
	Ceratozamia	Sarcotesta	
	Stangeria	Sarcotesta	
	Zamia	Sarcotesta	
	Microcycas	Sarcotesta	
Ginkgoaceae	*Ginkgo*	Sarcotesta	Hori and Hori, 1997
Cupressaceae	*Juniperus*	Bract-scale complexes	Hall, 1952; Givnish, 1980
Taxaceae	*Cephalotaxus*	Aril/Sarcotesta	Florin, 1954; Price, 1990; Dörken et al., 2019
	Torreya	Aril	
	Amentotaxus	Aril	
	Taxus	Aril	
	Pseudotaxus	Aril	
	Austrotaxus	Aril	
Podocarpaceae	*Saxegothaea*	Bracts (possibly dry at maturity)	Page, 1990; Farjon, 2010; Contreras et al., 2017
	Prumnopitys	Epimatium	
	Pectinopitys	Epimatium	
	Sundacarpus	Epimatium	
	Parasitaxus	Epimatium	
	Halocarpus	Epimatium	
	Lepidothamnus	Bracts + Receptacle	
	Phyllocladus	Aril + Receptacle	
	Microcachrys	Bracts	
	Nageia	Epimatium + weakly developed receptacle	
	Retrophyllum	Epimatium	
	Afrocarpus	Epimatium	
	Podocarpus	Receptacle + leathery Epimatium	
	Acmopyle	Receptacle + Epimatium	
	Dacrycarpus	Receptacle + Epimatium	
	Falcatifolium	Receptacle + reduced Epimatium	
	Dacrydium	Receptacle + reduced Epimatium	
	Manoao	Epimatium	
Ephedraceae	*Ephedra*	Fertile bracts	Florin, 1951; Price, 1996; Dörken et al., 2019
Gnetaceae	*Gnetum*	Sarcotesta	Rodin and Kapil, 1969

The table shows genera including species with fleshy fruit-like structures dispersed by animals.

Hollander *et al.*, 2010; Ickert-Bond and Renner, 2016). Indeed, seeds that traverse bird digestive tracts have an increased germination rate (Traveset, 1998; Hollander and Van der Wall, 2009). Few *Ephedra* species have winged wind-dispersed dry fruit-like structures, or wingless dry fruit-like structures (Price, 1996; Hollander *et al.*, 2010). In most cases, pollination of *Ephedra* female cones occurs by wind, even if there are exceptions, for example *Ephedra foeminea* and *E. aphylla* are entomophilous species (Bino *et al.*, 1984; Bolinder *et al.*, 2016). The process of double fertilization gives rise to two diploid nuclei: a zygote nucleus, derived from the fertilization of the egg nucleus by a sperm nucleus, and a second supernumerary zygote nucleus, formed from the fusion of the ventral canal nucleus with a second sperm nucleus (Friedman, 1990, 1991). Nevertheless, only one embryo typically survives to reach maturity (Friedman and Carmichael, 1996). Seeds are fully mature about two months after pollination (Cutler, 1939; Hollander and Van der Wall, 2009).

III. Evolutionary perspectives on the fleshiness of gymnosperm seed-associated structures

Given that plants are sessile organisms, and that biological mechanisms that prevent inbreeding by favoring dispersion are costly and essential to preserve genetic diversity, it is clear how pollination mechanisms and seed dispersal strategies are vital for the plant's fitness and are expected to be under strong selective pressures. The fleshiness character present in many gymnosperm diaspores that we widely described in this review is one of the functional traits that surely enhanced reproductive success in many seed plants.

Beside providing a detailed description of seed-associated fleshy structure morphology and ontogenetic origins in different genera of extant gymnosperms, this review shows how evolution produced, numerous times independently, functionally similar structures. A summary of these fleshy structures is presented in Table 2.

These structures develop via complex regulative networks involving many actors (genes, transcription

factors, hormones) and several levels of regulation (hormonal, environmental conditions, epigenetics mechanisms) (Lovisetto *et al.*, 2012; Brenner *et al.*, 2005). It is also plausible that developmental constraints played a role in shaping such structures (Rudall *et al.*, 2011), and it could be worth to further study this topic.

The molecular mechanisms that control and lead the development of seed-associated fruit-like organs in gymnosperms are only described for few species, and the knowledge regarding these developmental processes are still limited to few categories of master regulators, such for instance MADS-box genes. Shindo *et al.* (1999), Becker *et al.* (2003), Hou *et al.* (2020) and Deng *et al.* (2020) studied the expression domains of several MADS-box genes in *Gnetum parvifolium*, *G.gnemon*, and *G. luofuense* respectively, whereas Gramzow *et al.* (2014) provided an overview of MADS-box genes in conifers. Lovisetto *et al.* (2012) demonstrated that MADS-box genes are crucially involved also in the differentiation of the integument in *G. biloba* and of the aril in *Taxus baccata*. Moreover, Yamada *et al.* (2008) and Shigyo and Ito (2004) studied the expression pattern of the orthologues of the Arabidopsis *AINTEGUMENTA* (*ANT*) gene in *Gnetum* (*GpANTL1*) and in *Pinus* (*PtANT*) ovules. Gymnosperm seed-linked fleshy structures may display morphologic characteristics similar to those found on true botanical fruits, such as attractive colors, a pleasing taste, and a desirable texture. Indeed, as in real botanical fruits, gymnosperm fleshy structures undergo a process of ripening that confers softness, sweetness, colorfulness and attractiveness (Lovisetto *et al.*, 2012). The process of maturation and ripening encompasses a complex array of changes in the dedicated tissues involving key regulatory genes (Lovisetto *et al.*, 2012). For instance, the maturation and/or ripening of *Ginkgo* sarcotesta and of *Taxus* aril involves the plant hormone ethylene, and the softening process entails cell wall modifications (Lovisetto *et al.*, 2012), as occurs during the ripening of fleshy fruits such as tomato fruits (reviewed by Seymour *et al.*, 2013). These aspects are mainly due to a fine regulation of several genetic and hormonal pathways, and are well described for angiosperm model plants and for those plants with economic value, but they remain poorly investigated in gymnosperms.

Over the years, the origin and evolution of fleshy structures has been discussed by various authors, and different hypotheses have been put forward. Mack (2000) suggested a scenario in which fleshy pulp evolved from ancestral structures that in the primitive seed plants served as a defence against herbivores. Fleshy fruit-like structures and endozoochory appeared in that order. First, vulnerable naked seeds evolved fleshy pulp to defend themselves. Then, some herbivores overcame these defenses, but the consumption of the fleshy structure did not kill the enclosed seed (proto-frugivores stage). These first "proto-frugivores" seed dispersers would have dispersed seeds of pulp-defended plants better than abiotic dispersers, triggering a coevolutionary process. In this framework, the function of fleshy structures as promoters of endozoochory would be considered an exaptation (Mack, 2000). Even though Mack focused on angiosperm fruits, seeing how gymnosperm fleshy structures are functionally equivalent to them (Herrera, 1989), his hypothesis could be applied to gymnosperms as well. Mack's explanation could account for many seed plant species, but it is challenged by those genera in which the fleshy structures do not completely enclose the seed, such as *Dacrydium* and *Phyllocladus* (Contreras *et al.*, 2017). In these groups the defensive function of the fleshy tissues was either lost during the course of their evolution or was never present in the first place. The genera *Dacrydium, Falcatifolium, Phyllocladus, Halocarpus, Manoao, Taxus* and *Pseudotaxus*, as described in this review, all possess unenclosed seeds (Farjon, 2010), and it is possible that in these groups seed dispersal constituted the original function that drove the evolution of their fleshy structures.

The existence of nontoxic fleshy structures in toxic plants is another challenging matter. Ehrlén and Eriksson (1993), starting from the hypothesis made by Herrera (1982), proposed an evolutionary pathway able to explain such phenomenon. Herrera in 1982 proposed, looking at angiosperms, that toxic fruit pulp may be a suboptimal adaptation for avoiding seed destruction by nondispersing frugivores. Ehrlén and Eriksson (1993) however, testing the predictions made by Herrera, concluded that this might not be the case, and proposed a nonadaptive view of the phenomenon, in which toxic pulp is a by-product of plants evolving anti-herbivory mechanisms. This parsimonious interpretation views toxins occurring in the pulp as of general anti-herbivore origin, and predicts it would be adaptive to exclude them from the pulp of ripe fruits if they are too costly to produce, if they serve no other functions (such as being cues for potential dispersers, but see Cipollini and Levey, 1997) or if they steeply reduce dispersal rate (Ehrlén and Eriksson, 1993). In the genera *Taxus* and *Cycas,* the aril and the sarcotesta respectively are the only nontoxic edible parts of the plant for those who disperse

their seeds. It is possible that the process described by Ehrlén and Eriksson (1993) was the one undertaken by their fleshy structures, but this claim needs to be proven by further studies. Showing that the unripe aril in *Taxus,* and the undifferentiated integument in *Cycas* present some degree of toxicity would be a first step in that direction. For now, to our knowledge, a scenario in which the aril and the integument originally evolved to enhance seed dispersal cannot be ruled out.

Fleshy structures have also been shown to serve other functions. When studying the dispersal unit of *Dacrycarpus dacrydioides* (Podocarpaceae), Fountain *et al.* (1989) demonstrated that the fleshy receptacle of the seed cone protects seeds from desiccation-associated damage, prolonging seed viability. Although the study does not draw any evolutionary hypotheses from its results, one could speculate that certain gymnosperm fleshy structures might have evolved as a way to prolong seed viability by shielding it from dehydration, and that in a later stage the succulent tissues became attractive to animals which started to disperse them.

Realistically, the fleshy-structure trait has evolved independently many times via numerous different ontogenetic pathways, and no single evolutionary explanation is reasonably sufficient to account for all these different origins. Leslie *et al.* (2013) showed how fleshy cones have arisen independently between three and five times in conifers alone. Although recent studies bring a lot of data and underlay trends between fleshy structures and other traits in conifers (Leslie *et al.,* 2013; Contreras *et al.,* 2017; Leslie *et al.,* 2017), the ecological conditions that select for cone type and the ultimate causes of the phenomenon are still poorly understood. Species with animal-dispersed syndromes appear to possess larger seeds than unspecialized, wind-dispersed ones. In conifers, seeds enclosed by anatomically distinct types of fleshy structures appeared to vary significantly in size, with seeds enclosed by fleshy tissue derived from less than three cone scales being the largest ones (Leslie *et al.*, 2017). In turn, seed size is linked to many variables such as latitude, climate, genome size, forest structure and reproductive system (Beaulieu *et al.,* 2007; Díaz et al., 2016; Leslie *et al.,* 2017; Rueda *et al.,* 2017). For gymnosperms other than conifers less information is available, but what is known seems to confirm similar trends to those observed in conifers (Endress, 1996; Haycraft and Carmichael, 2001; Del Tredici, 2007; Mustoe, 2007; Snow and Walter, 2007; Hall and Walter, 2013). Furthermore, developmental constraints and selective tradeoffs should also be taken into account. Most dispersal structures have not evolved *de-novo*, but probably arose as modifications of already existing structures that served other purposes in the reproductive cycle of the plant. These functions may be context-dependent and may hinder the acquisition of other functions (Ridley, 1930; Contreras *et al.,* 2017). All this outlines a complex network of relationships, mutable in different ecosystems at different times, and to disentangle this web of causes and effects is a delicate and complex process, but once done it may shed light on the selective pressures that drive the appearance (and disappearance) of fleshy fruit-like structures in gymnosperms.

One final point needs to be addressed: across the four extant lineages of gymnosperms, fleshy structures and zoochory originated at different times. Leslie *et al.* (2017) predicted that the Podocarpaceae and Taxaceae lineages evolved animal-dispersed seeds during the Mesozoic (from late Triassic to mid-Jurassic), while the trait originated within Cupressaceae not earlier than 50 Mya, during the Cenozoic. Ginkgoales radiated significantly at the beginning of the Triassic (Zhou and Wu, 2006), and by that time fleshy structures might already have been present in the group. Various authors have speculated that *Ginkgo* seeds may have been dispersed by animals such as early birds and non-avian dinosaurs during the Mesozoic (Van der Pijl, 1982; Tiffney, 1984; Del Tredici, 1989; Zhou *et al.*, 2002). Cycadales may have already possessed fleshy tissues enclosing their seeds by the end of the Paleozoic (Tiffney, 2004), and it is generally agreed that during the Mesozoic cycads seeds were dispersed by different groups of animals (Mustoe, 2007; Butler *et al.,* 2009). The three extant families of Gnetales likely diverged during the Mesozoic (Won and Renner, 2006), but there is little evidence to support any hypothesis on when seed-associated fleshy structures might have arisen in the group.

This sketched chronology serves to highlight once more how different evolutionary explanations are needed to account for the origins of gymnosperm fleshy structures. One must inquire whether the ecosystem dynamic of different periods and places is homologous, examining climate, forest structure, presence of herbivores and major dispersers (Tiffney, 2004). Only under similar condition may fleshy structures of different lineages have independently evolved under similar selective pressures.

IV. Conclusions

In conclusion, besides being a systematic overview on the fleshy structures associated with ovule/seed in

living gymnosperms, this review presents and discusses the main hypotheses that have been elaborated in the years on the evolution of these fleshy structures. This work considers also the molecular pathways that subtend their development, introducing the involvement of some regulatory genes in these processes. All these information might give a global vision on a crucial aspect in gymnosperm reproduction and Spermatophyte evolution.

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