



# Orbicules in south American Balanophoraceae: Analysis of its possible role and its taxonomic value in the family<sup>☆</sup>

Virginia Cristina Gómez Villafañe<sup>a</sup>, Héctor Arnaldo Sato<sup>a,\*</sup>, Diego Demarco<sup>b</sup>, Ana María Gonzalez<sup>c</sup>

<sup>a</sup> Centro de Estudios e Investigaciones Botánicas (CEIBo), Alberdi 47, Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, Jujuy, Argentina

<sup>b</sup> Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brasil

<sup>c</sup> Instituto de Botánica del Nordeste (IBONE), UNNE-CONICET, Facultad de Ciencias Agrarias, Sargento Cabral 2131, Corrientes, Argentina.

## ARTICLE INFO

Edited by: Louis Ronse De Craene

### Keywords:

Anther  
Holoparasite  
*Lophophytum*  
*Langsdorffia*  
*Ombrophytum*  
*Helosis*  
Pollen

## ABSTRACT

Balanophoraceae are root parasites, lacking both chlorophyll and typical cormophytic organization. There have been few studies of pollen dispersal mechanisms in Balanophoraceae, and no studies on structures accompanying pollen, such as orbicules. Anthers were analyzed in five South American taxa of Balanophoraceae (*Langsdorffia hypogaea*, *Lophophytum pyramidale*, *L. mirabile* subsp. *bolivianum*, *Ombrophytum subterraneum* and *Helosis cayenensis*), in order to: (1) determine the presence of orbicules, characterize them and relate their presence with the tapetum type, characteristics of the anther wall and dehiscence, and the pollination mode; (2) and analyze its possible taxonomic value in relation to the phylogeny of the family Balanophoraceae. In three genera (*Lophophytum*, *Langsdorffia* and *Ombrophytum*), the anthers were found to be ditheous, tetrasporangiate with longitudinal dehiscence. In these taxa the endothecium is present and is responsible for dehiscing the anthers. In *Helosis* the pollen sacs are welded in a synandrium with apical grooves and lacking an endothecium. In all species, a secretory tapetum is responsible for nourishing the pollen grains during their formation. The presence of orbicules is variable among the species studied: it is found in *Lophophytum* and *Langsdorffia*, and is absent in *Ombrophytum* and *Helosis*. In *Lophophytum* the orbicules appear as small and spheroidal corpuscles with smooth surfaces. In *Langsdorffia hypogaea* the orbicules are conspicuous spherical, ellipsoid to ovoid forms. This study shows that there is no relationship between layers present in mature anthers, dehiscence type, pollination mode and orbicules presence. We report for the first time the presence of orbicules in the holoparasitic family Balanophoraceae belonging to the order Santalales, in which there is scarce information available regarding the presence of orbicules. Given the intergeneric variations and the consistency at generic level observed in the taxa here analyzed, orbicules may have predictive value and potential for systematically oriented research questions.

## 1. Introduction

Orbicules or Ubsch bodies are corpuscles smaller than 1  $\mu\text{m}$ , but can reach up to 20  $\mu\text{m}$ , composed of sporopollenin and located in the tapetal anthers membrane (Erdtman, 1960; Pacini et al., 1985; Huysmans et al., 1998; Galati et al., 2010; Ruggiero and Bedini, 2020; Shamrov et al., 2021). They are formed simultaneously with the pollen grains, and exhibit similar reaction to dyes, electron density, autofluorescence and resistance to the acetolysis as the exine (Heslop-Harrison, 1962; Christensen et al., 1972). In most plants, the orbicules were associated with

the presence of a secretory tapetum (Huysmans et al., 1998). However, over time, they were also found in anthers with plasmodial and invasive non-syncytal tapetum (Gotelli et al., 2023).

Various hypotheses exist regarding the potential functions of these corpuscles ranging from dispersing pollen grains to playing a role in pollination (Huysmans et al., 1998; Vaknin et al., 2000; Vinckier and Smets, 2001), but no consensus has yet been reached. Some authors consider them to be no more than a by-product of tapetal metabolism, however in wheat they have been shown to carry a sporophytic protein (RAFTIN) that is targeted to the microspore exine and is essential for

<sup>☆</sup> This article is part of a special issue entitled: "Clonal plants" published at the journal *Flora*.

\* Corresponding author.

E-mail addresses: [vir.gomezvillafane@fca.unju.edu.ar](mailto:vir.gomezvillafane@fca.unju.edu.ar) (V.C. Gómez Villafañe), [hector.a.sato@gmail.com](mailto:hector.a.sato@gmail.com) (H.A. Sato), [diegodemarco@usp.br](mailto:diegodemarco@usp.br) (D. Demarco), [anagonzalez.ibone@gmail.com](mailto:anagonzalez.ibone@gmail.com) (A.M. Gonzalez).

<https://doi.org/10.1016/j.flora.2024.152642>

Received 15 July 2024; Received in revised form 9 November 2024; Accepted 11 November 2024

Available online 12 November 2024

0367-2530/© 2024 Elsevier GmbH. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

pollen development (Wang et al., 2003). It has even been proposed that their study has practical implications related to wellness and health, since the orbicules may play a possible role as a vector of pollen allergens. Vinckier et al. (2005) report the presence of allergens in the orbicules, and if these microscopic particles are dispersed into the atmosphere and are inhaled by individuals, they may act as very effective vectors of allergens.

The presence of orbicules is confirmed in 1150 taxa. On the contrary, orbicules have been reported to be absent in 619 taxa (Moon, 2018). Generally, orbicule development has been reported from the ANA grade (the earliest diverged group) the most primitive taxa, even within pteridophytes, gymnosperms and angiosperms (Verstraete et al., 2014). Therefore, orbicules can be considered as a primitive character whose presence has been found even in fossil records of flowering plants (Taylor, 1976; Zetter et al., 2002; Balthazar et al., 2005). As well as pollen morphological traits, orbicules may provide important clues to identify plant species and infer their evolutionary history (Moon, 2018).

In angiosperms, they are common in monocots but are absent in late-branching clades such as the majority of Asteraceae and the majority of Lamiaceae, revealing an evolutionary trend from presence to absence along the phylogenetic tree. Orbicules are a plesiomorphic trait; nevertheless, their occurrence represents a consistent characteristic within specific taxa at the family and genus levels. Therefore, orbicule distribution might be a useful diagnostic character in systematics (Banerjee, 1967; Heslop-Harrison and Dickinson, 1969; Christensen et al., 1972; Raj and El-Ghazaly, 1987; El-Ghazaly, 1989; El-Ghazaly and Chaudhary, 1993; Vinckier et al., 2000; Schols et al., 2001; Vinckier and Smets, 2002a–c, 2003; Galati, 2003; Rosenfeldt and Galati, 2008; Lovisollo and Galati, 2012; Verstraete et al., 2014; Moon, 2018; Ruggiero and Bedini, 2020).

In parasitic plant groups, studies on the presence of orbicules on anthers are limited. In hemiparasitic plants, Ruggiero and Bedini (2020) investigated orbicules in *Krameria lanceolata* Torr. (Krameriaceae) and *Osyris alba* L. (Santalaceae) but reported that the internal surface of the anthers was smooth and lacked orbicules; however, among the Loranthaceae they evidenced the presence of orbicules of a size below a micrometer in *Loranthus europaeus* Jacq., *Alepis flavida* (Hook.f.) Tiegh. and *Tripodanthus flagellaris* (Cham. & Schltdl.) Tiegh. In holoparasitic plants, very prominent orbicules have been observed in *Cuscuta europaea* L. (Govil and Lavania, 1980) and *Cuscuta obtusiflora* H. B. and K. (Rodríguez-Pontes, 2009).

The Balanophoraceae L. C. Richard et A. Richard, are holoparasitic root species, living underground, devoid of chlorophyll and lacking the typical structures of cormophytic organization. They present inflorescences totally or partially above-ground, or even completely underground, of fungoid aspect, with a large number of tiny unisexual flowers (Kuijt, 1969; Hansen, 1980a; Mauseth et al., 1992; Hsiao et al., 1993, 1994; Gedalovich-Shedletzky, 1990; Gonzalez and Mauseth, 2010; Sato and Gonzalez, 2016).

Balanophoraceae is a family of mostly tropical or subtropical distribution, presenting 13 genera, 7 of which are in America, with 18 represented species (Hansen, 1980b; Nickrent, 1997; Nickrent and Musselman, 2004; Su et al., 2015). The presence of orbicules has not been previously reported among the species of this family.

*Langsdorffia hypogaea* Mart. (subfamily Balanophoroideae) is often collected in various habitats including neotropical savanna and tropical rainforest, from Mexico to Southeast Brazil and Paraguay (Hansen, 1980a; Pott and Pott, 1994; Fox and Zardini, 1998; da Silva Freitas et al., 2017; Santos et al., 2017; Thorogood and Santos, 2020). It is a dioecious species with reddish inflorescences (Cardoso et al., 2011). No anatomical studies have been carried out on this taxon, and the pollen grains have been described as isopolar, tetraporate and suboblate (Hansen, 1980a). Investigations on its floral biology report nectar secretion by extrafloral structures, as well as a high diversity of floral visitors, and the presence of a bird species visiting inflorescences has even been recorded (Santos et al., 2017). It is worth noting that Coleoptera of the family

Nitidulidae were the most abundant pollinators (da Silva Freitas et al., 2017).

*Lophophytum pyramidale* (Leandro) L.J.T. Cardoso & J.M.A. Braga grows in mixed tropical forest in NE Argentina and SE Brazil, and *L. mirabile* subsp. *bolivianum* (Wedd.) B. Hansen is found in evergreen forests in Argentina, Bolivia, Brazil and Paraguay (subfamily Lophophytoideae) (Burkart, 1949; Hansen, 1980a, 1980b, 1987; Xifreda, 1999; Zuloaga et al., 2008; Novara, 2009; Sato, 2015). Both species are monoecious and have inflorescences composed of unisexual flowers. Embryological studies on these species described the embryo-sac as of the *Adoxa* type and tends to take a “J” shape (Sato and Gonzalez, 2016, 2017, 2022). The anatomy and development of the staminate flowers of *L. pyramidale* and *L. mirabile* subsp. *bolivianum* were investigated by Sato and Gonzalez (2013, 2022), who reported that microsporogenesis and microgametogenesis develop normally, but the presence of orbicules was not recorded in these investigations.

*Ombrophytum subterraneum* (Aspl.) B. Hansen (subfamily Lophophytoideae) inhabits Andine highlands in dry bushland (Puna) and evergreen and deciduous mountain forests in Argentina, Chile, Bolivia and the Galapagos Islands (Kuijt, 1969; Hansen, 1980a). It is a monoecious species, but there are collections of individuals with pistillate flowers only (Mauseth et al., 1992). No reproductive anatomy or embryology studies have been carried out on this taxon. Only the pollen grains have been described as predominantly tricolporate, spheroid and having an almost smooth exine (Hansen, 1980a). The economic importance of this species was reported by Sato et al. (2024), who describe the food and medicinal potential of these plants consumed by rural populations.

*Helosis cayennensis* (Sw.) Spreng. var. *cayennensis* (subfamily Helosidoideae) grows in tropical rain forests and montane forests in damp places in Central America as far as NE Argentina (Hansen, 1980a; Fontana and Popoff, 2006). The protogynous inflorescence is spadix-like, with tiny unisexual flowers. The complete development of unisexual flowers, including normal sporogenesis, gametogenesis and its particular type of embryo-sac, named *Helosis*-type has been described in this species, but there is no mention of the presence of orbicules in the welded pollen sacs in a synandrium (Gonzalez et al., 2013, 2019).

In this paper the anthers of five taxa from the family Balanophoraceae are analyzed: *Langsdorffia hypogaea* (subfamily Balanophoroideae), *Ombrophytum subterraneum*, *Lophophytum pyramidale*, *L. mirabile* subsp. *bolivianum* (subfamily Lophophytoideae), and *Helosis cayennensis* var. *cayennensis* (subfamily Helosidoideae) in order to: (1) determine the presence of orbicules, characterize them and relate their presence with the tapetum type, characteristics of the anther wall and dehiscence, and the pollination mode; (2) and analyze its possible taxonomic value in relation to the phylogeny of the family Balanophoraceae.

## 2. Materials and methods

### 2.1. Plant materials

Morphological observations of orbicules were based on plants specimens collected and deposited in the Instituto de Botánica del Nordeste Herbarium (CTES), Universidade Federal de Minas Gerais Herbarium (BHCB) and Universidad Nacional de Jujuy Herbarium (JUA), voucher information is listed in Table 1. One inflorescence with mature pollen grains was taken per specimen and then ten staminate flowers were excised from each for observation and analysis by light and scanning electron microscopy.

### 2.2. Light microscopy (LM)

Anthers were fixed in FAA (formaldehyde, 70% ethyl alcohol, acetic acid 5:90:5). The material was dehydrated in series of histological dehydrating (Gonzalez and Cristóbal, 1997), paraffin embedded, transversally cut with rotary microtome Microm HM350 into 10 µm

**Table 1**  
Summary of the characteristics of staminate flowers, androecium, anther wall, orbicules, pollen grains and pollination mode in the five Balanophoraceae species analyzed, based on the observations of the present work and also on previous research.

Species	Staminate flowers, androecium, anthers	Dehiscence	Endothecium	Tapetum	Orbicules			Pollen	Pollination mode	Voucher information
					Shape	Size [ $\mu\text{m}$ ], min-max (mean, standard deviation)	Characteristics			
<i>Lophophytum mirabile</i>	1 piece of perianth, 2 free stamens, anther ditheous and tetrasporangiate (Sato and Gonzalez, 2013)	longitudinal	1-3 layers, well-developed lignified thickenings	secretory	spheroidal	small, 0.27 - 0.41 (X: 0.34, SD: 0.047)	without outstanding surface characteristics, with a low electron density central core	spheroidal, tricolporate, sincolpate, with a thin exine without conspicuous sculptures	cantharophily syndrome (Ferrer et al., 2011; Sato, 2015)	ARGENTINA Prov. Jujuy: National Park Calilegua, Sato 430: 23°56'57.3"S/ 64°55'46"W; Sato 432, 434, 436: 23°45'30"S/ 64°51'5"W (CTES) Prov. Salta: Caraparí, Sato 202: 20°20'47.4"S/ 63°48'11.1"W (CTES)
<i>L. pyramidale</i>	2 pieces of perianth 2 free stamens, anther ditheous and tetrasporangiate (Sato and Gonzalez, 2013)	longitudinal	1-3 layers, well-developed lignified thickenings	secretory	spheroidal	small, 0.27 - 0.41 (X: 0.34, SD.: 0.047)	without outstanding surface characteristics, with a low electron density central core	spheroidal, tricolporate, sincolpate, with a thin exine without conspicuous sculptures	cantharophily syndrome (Ferrer et al., 2011; Sato, 2015)	ARGENTINA Prov. Misiones: San Ignacio and Salto Tabay; Sato 114: 27°16'27.6"S/ 55°31'34.0"W, Sato 421, 422, 423: 26°59'49.9"S 55°10'48.6"W (CTES)
<i>Langsdorffia hypogaea</i>	3 pieces of perianth, 3 stamens fused into a synandrium, anther ditheous and tetrasporangiate	extrorse and longitudinal	1 layer, underdeveloped lignified thickenings	secretory	spheroidal, ellipsoidal to ovoid	large: 4.81 - 6.42 (X: 5.86, SD: 0.48) wide: 3.99 - 4.91 (X: 4.39, SD: 0.26)	it was not possible to determine whether they are solid type or with central core	isopolar, tetraporate, equinulate	cantharophily syndrome (da Silva Freitas et al., 2017)	BRAZIL. Minas Gerais: São Joao do Rio Preto, Mota 2862: 20°25'59.34"S/ 43°30'23.26"W, (BHCB)
<i>Ombrophytum subterraneum</i>	lack perianth parts, 2 free stamens, anther ditheous and tetrasporangiate	longitudinal	1-3 layers, well-developed lignified thickenings	secretory	absent	absent	absent	spheroidal, tricolporate with an almost smooth exine	cantharophily syndrome	ARGENTINA Prov. Jujuy: Rodeo, Yavi department, Gómez Villafañe & Sato 4, 9, 17, 18, 30: 22°17'47.60"S/ 65°55'9.73"W (JUA)
<i>Helosis cayennensis</i>	3 tepals, 3 stamens fused into a synandrium, with 9 pollen sacs (Gonzalez et al., 2013)	3 apical longitudinal grooves	absent, dehiscence due to epidermal and parietal layers rupture	secretory	absent	absent	absent	spheroidal, tricolpate, granular exine (Gonzalez et al., 2013)	unknown	ARGENTINA Prov. Corrientes: Apipé Grande Island, Ituzaingó department, Gonzalez & Popoff 239, Gonzalez & Sato 470: 27°26'49.5"S/ 56°56'50.5"W (CTES)

thick sections. It was coloured using safranin - fast-green (Johansen, 1940) and with safranin-astra blue combination (Luque et al., 1996). It also included material in synthetic resin (Historesin Leica) prior dehydration in increasing ethanol series (O'Brien et al., 1981). Cuts of 7 µm thick were made and stained with toluidine blue (O'Brien et al., 1964). Histochemical tests were performed in these cuts with Lugol for detecting starch, and Sudan III for lipid substances (Ruzin, 1999). The observations were made with an LEICA DMLB2 light microscope equipped with a polarizing filter and recorded with a LEICA ICC50HD digital camera.

### 2.3. Scanning electron microscopy (SEM)

The material fixed in FAA was dehydrated in ascending acetone series, dried at critical point with CO<sub>2</sub> (Denton Vacuum LLC, DCP-1) and metalized with palladium gold (Denton Vacuum, Desk II). A MEB Jeol LV5800 (Electron Microscopy Service of the Universidad Nacional del Nordeste, Corrientes, Argentina) and a Jeol LV6480 (Electron Microscopy Service of the Universidad Nacional de Salta, Salta, Argentina) were used for observations.

### 2.4. Transmission electron microscopy (TEM)

Observations using this equipment could only be made on *Lophophytum* species. Three anthers of each specimen of *Lophophytum* in different stages of development were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and post-fixed in OsO<sub>4</sub> at 2°C in the same buffer. In the fixation protocol, the pH value was adjusted to a value of 7.2 after several trials to observe these species. Dehydration was carried out in acetone and embedded in Spurr synthetic resin. With ultramicrotome Sorvall MT1 ultrathin sections (750 nm to 900 nm) were made, which were contrasted with uranyl acetate and lead citrate (O'Brien & McCully 1981) and observed with TEM Zeiss EM 109T equipped with digital camera Gatan ES1000W from the Laboratorio Nacional de Investigación y Servicios de Microscopía Electrónica de la Universidad de Buenos Aires (Argentina).

### 2.5. Orbicules analysis

The orbicules measurements were performed using free image analysis software ImageJ® (Rasband, 2023; Gonzalez, 2018). The mean (X) and standard deviation (SD) were calculated based on the measurement of 100 orbicules taken at random from one anther of at least five different flowers of each species. Measurements were taken from SEM and TEM images for *Lophophytum* and LM and SEM for *Langsdorffia*.

## 3. Results

Table 1 summarizes the characteristics of staminate flowers, androecium, anther wall, orbicules and pollen grains in the five Balanophoraceae species analyzed, compiling information from the present work and also from previous research.

The inflorescence of *Lophophytum mirabile* and *L. pyramidale* have a primary rachis bearing secondary rachises densely covered with unisexual flowers (Fig. 1A-B). The pistillate flowers are located in the proximal third and the staminate flowers in the distal part of the primary rachis. Staminate flowers of both species are reduced to two stamens and pieces of perianth of fleshy nature, one piece in *L. mirabile* and two in *L. pyramidale*, they do not cover the anthers, leaving these exposed throughout development (Fig. 2A-B). The anthers are dithecous, tetrasporangiate (Fig. 2C) and their dehiscence is longitudinal. In the transversal section of a young anther, a tapetum of secretory type overlaying the pollen sacs can be seen (Fig. 2D-E). This tapetum is composed of a

single layer of uninucleate cells with a dense cytoplasm. Mature anther wall is formed by epidermis and one to three layers of endothecium with well-developed lignified thickenings (Fig. 2C, F).

The orbicules are attached to the inner walls of the tapetum and are visible from the free microspore stage. They remain evident even in dehiscent anthers with mature pollen grains (Fig. 2F). TEM observations of anthers in microspore-free stages revealed spheroid corpuscles of different electron density in the cytoplasm of the tapetal cells, corresponding to pro-orbicules (Fig. 2G). The already formed orbicules are first associated with invaginations of the plasmatic membrane of the tapetal cells and then they can be observed along the walls of the tapetal cells (Fig. 2H). With LM, small orbicules can be observed and have identical coloration to the microspore wall (Fig. 2E). At the pollen grain stage, the orbicules of both *Lophophytum* taxa are of spheroidal type, small in size ranging from 0.27 µm to 0.41 µm (X: 0.34 µm, SD: 0.047), without prominent surface features with a central core of low electron density (Fig. 2H-I).

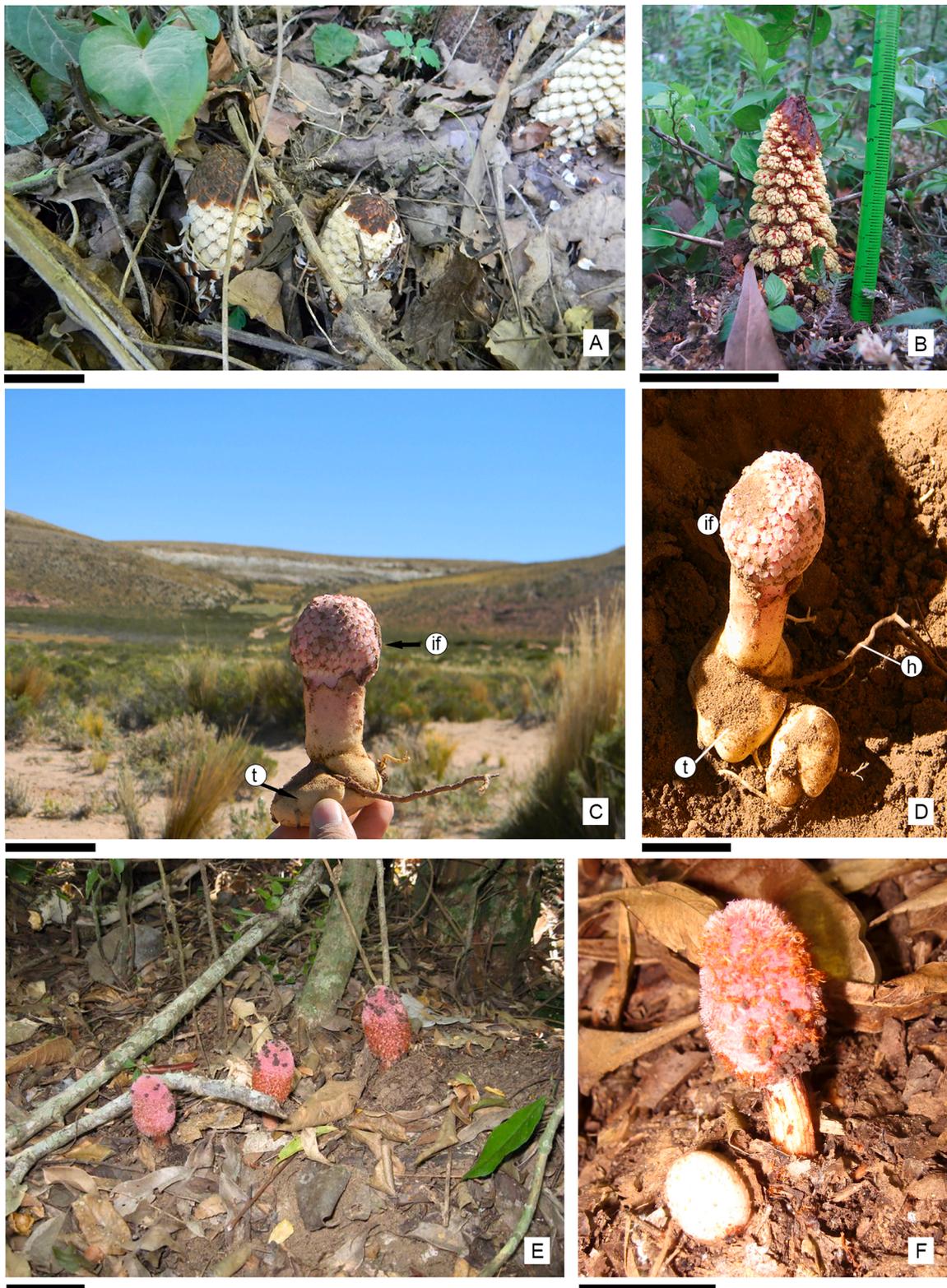
Pollen grains are spheroidal, tricolporate, sincolpate, with a thin exine without conspicuous sculptures (Fig. 2E-F). Pollen grains are released from the anther at the bicellular stage, with orbicules still present above the remnants of the tapetal membrane, where the cells have already collapsed.

In *Langsdorffia hypogaea* the inflorescences are unisexual, staminate flowers are monochlamydeous, generally trimerous (less common dimerous and tetramerous), formed only by perianth and stamens (Fig. 3A, 4A). The androecium is formed for 3 stamens (less often 1-2) fused into a synandrium (Fig. 3A-B, E). Anthers are bithecal and tetrasporangiate, with extrorse longitudinal dehiscence (Fig. 3E). When pollen sacs have free microspores, the anther wall consists of epidermis, a layer of underdeveloped endothecium without thickenings and a secretory-like tapetum (Fig. 3D). At the time of dehiscence, the tepals covering synandrium open and recurve, the walls of anthers break longitudinally because of the wall weakness, not specifically by the action of poorly developed endothecium, both in cell size measured in radial development as in the presence of lignified thickenings (Fig. 3C).

The orbicules are observed both at the free microspore stage (Fig. 3D), as in dehiscent anthers with mature pollen grains, where remnants remain adhered to the inner walls of the pollen sacs (Fig. 3C). Conspicuous orbicules are observed on the inner wall of the pollen sacs and also scattered between the pollen grains (Fig. 3C-D). They have spherical, ellipsoidal to ovoid shapes (Fig. 4C-D). Its size is 4.81 µm to 6.42 µm in length (X: 5.86 µm, SD: 0.48) and 3.99 µm to 4.91 µm in width (X: 4.39 µm, SD: 0.26). They have the same reaction to the dyes as the exine (Fig. 3D); they have negative reactions to the starch and lipid substances. Given the inability to obtain adequate samples for processing with TEM, it was not possible to determine whether they are solid type or with central core. The pollen grains are dispersed in a bicellular state, they are isopolar, tetraporate, equinulate, and they measure from 15 µm to 22 µm (Fig. 4B).

In *Ombrophytum subterraneum* the inflorescences are formed by a primary subterranean rachis carrying numerous secondary rachises on which the unisexual flowers are arranged (Fig. 1C-D). The primary rachis have pistillate flowers in the proximal part and staminate flowers in the distal third. Inflorescences with only pistillate flowers have also been found. The staminate flowers lack perianth parts and each flower is composed of two stamens, with longitudinal dehiscence (Fig. 5A). The anthers are dithecous and tetrasporangiate. The wall of the mature anther consists of epidermis, one to three layers of endothecium with conspicuous lignified thickening and secretory tapetum type (Fig. 5B-C). The anthers do not have orbicules, in any of the stages analyzed (Fig. 5B-D). The pollen grains are spheroidal, tricolporate and have an almost smooth exine (Fig. 5C-D).

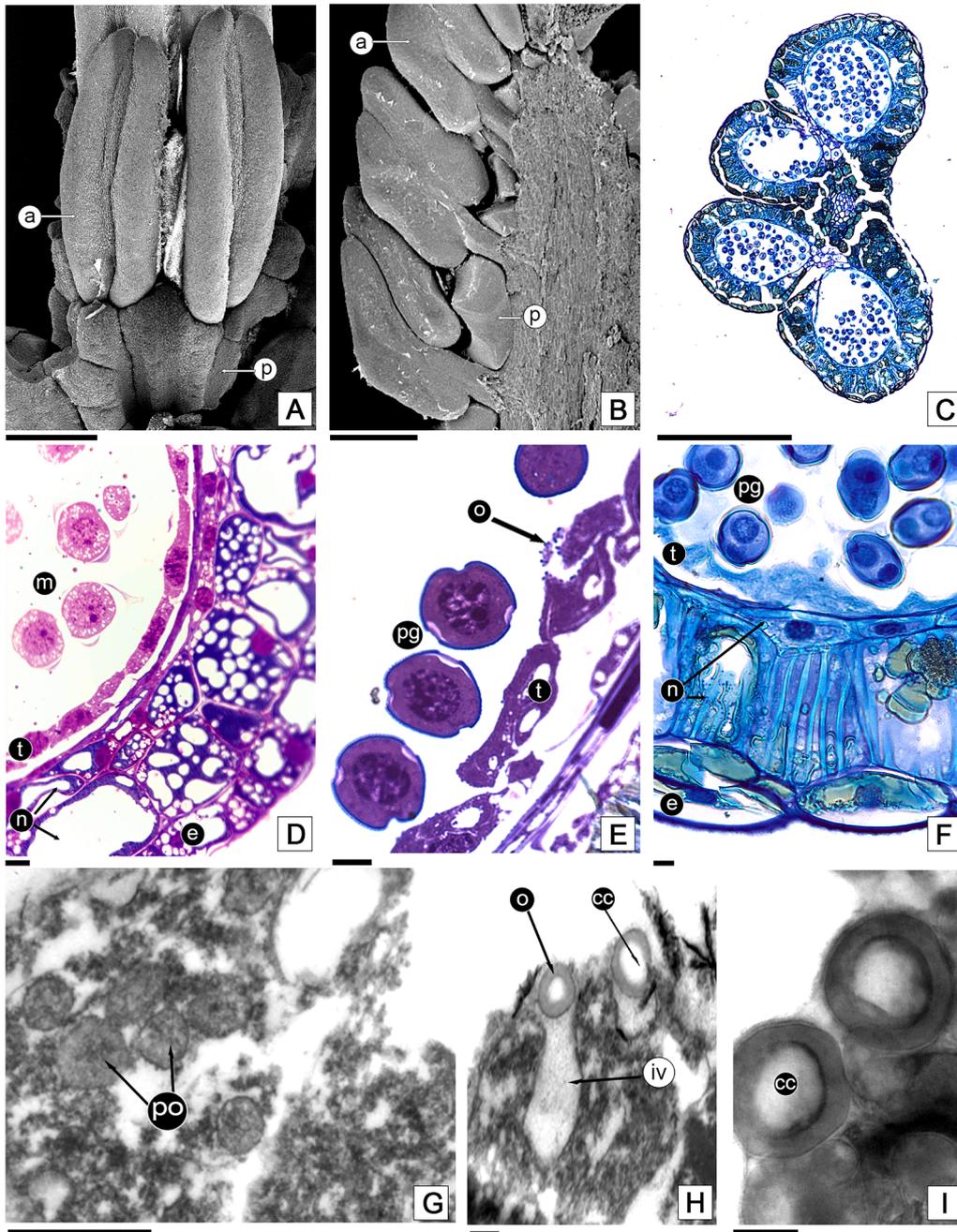
In *Helosis cayennensis* the inflorescence is covered with bracts



**Fig. 1.** Habitat of the different species. *L. mirabile* (A), *L. pyramidale* (B), *O. subterraneum* (C-D), *H. cayennensis* (E-F). (A-B) inflorescences at the stage of drooping scales emerging from the soil surface. (C) Plant recently collected in a locality of the Puna. (D) Recently excavated individual, showing tuber (t), inflorescence (if) and host root (h). (E) three inflorescence emerging from the ground litter. (F) Detail of an inflorescence with drooping scales exposing its flowers. Scales bars = (A, E) 10 cm; (B-D, F) 5 cm.

(Fig. 1E-F). The unisexual flowers are embedded in a dense mass of phyllaries. The pistillate flower is reduced to a pistil. Staminate flowers have a perigonium formed by three tepals, androecium with filaments

and three anthers with three thecae each welded in synandrium (Fig. 5E). The anther wall possesses epidermis, parietal layers, and a secretory tapetum, lacks endothecium. No orbicules were detected



**Fig. 2.** Staminate flower, anther wall and Orbicules of *Lophophytum*. *L. mirabile* (A, H), *L. pyramidale* (B-G, I). SEM (A-B), LM (C-F), TEM (G-I). (A-B) Staminate flowers showing: one perianth piece (p) and anthers (a). (C) Cross section of indehiscent anther. (D) Detail of the anther wall layers in microspores mother cell stage, epidermis (e), middle layers (n), tapetum (t), microspores mother cell (m). (E) Anther cross section in the unicellular pollen stage showing: tapetum (t) with orbicules (o) in its internal tangential wall (arrow). (F) cross section of mature anther in bicellular pollen stage, anther wall showing: epidermis (e), 2-layered endothecium with characteristic lignified thickenings (n), secretory tapetum degraded (t), pollen grains (pg). (G) Detail of cell tapetum to its internal tangential wall showing the pro-orbicules (po). (H) Orbicules (o) and its central core (cc) on the inner wall of the tapetum, emerging from invaginations of the cell membrane of the tapetum (iv). (I) Orbicules showing central core of low electron density (cc). Scales bars = (A-B) 1 mm; (C) 0.5 mm; (D-F) 10  $\mu$ m; (G) 1  $\mu$ m; (H-I) 0.25  $\mu$ m.

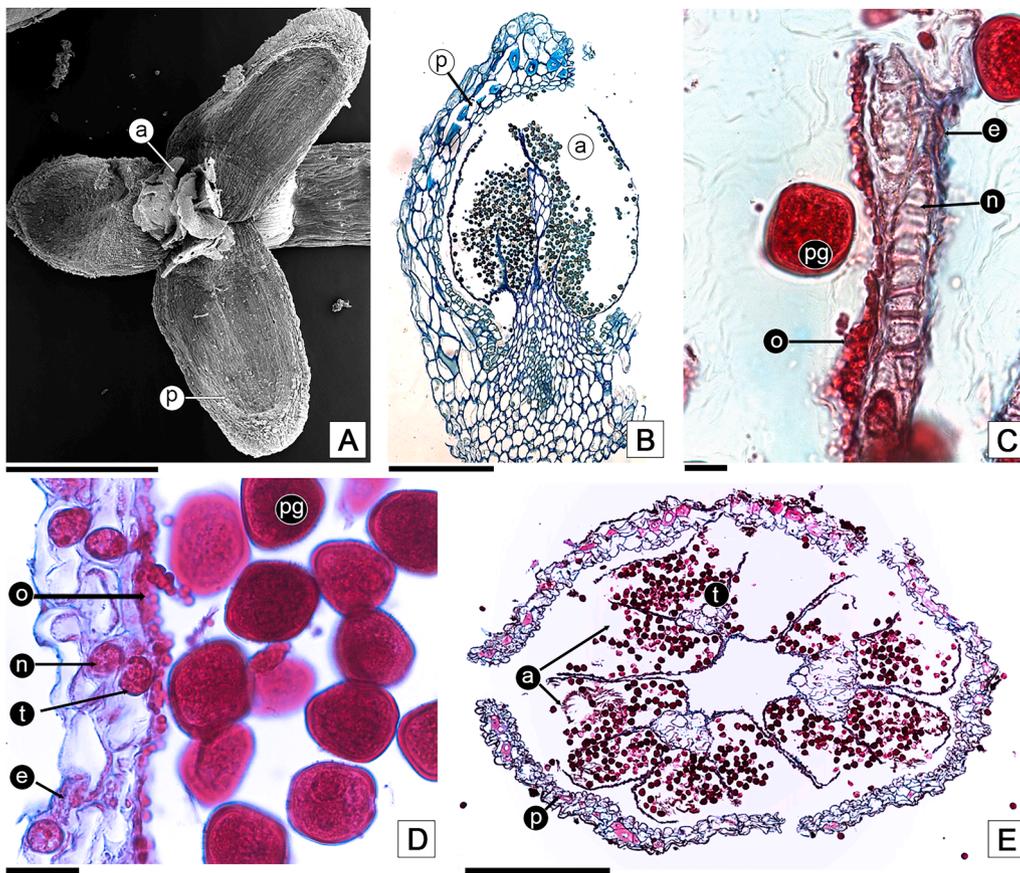
(Fig. 5F-G). Dehiscence occurs through three apical longitudinal grooves that converge at the apex and then open toward the base of the synandrium, and is caused by lysis of the epidermis and parietal layers. Pollen grains are spheroidal, tricolpate, the exine is granular type (Fig. 5G).

#### 4. Discussion

Orbicules presence have been observed in the most primitive taxa, the ANITA group (the earliest diverged grade) and even within

pteridophytes, gymnosperms and angiosperms (Moon, 2018). Thus orbicules have been suggested to have a positive correlation with the primitive, secretory tapetum type (Huysmans et al., 1998; Verstraete et al., 2014). Nevertheless, it has been confirmed the presence of orbicules in species with plasmodial or non-invasive syncytial tapetum type and also the absence of orbicules in species with secretory tapetum type (Strittmatter et al., 2000; Galati et al., 2007; Moon, 2018; Gotelli et al., 2023).

The association between the presence of orbicules and the secretory



**Fig. 3.** Staminate flower, anthers and pollen grain from *Langsdorffia hypogaea*. SEM (A), LM (B-E). (A) Flower showing three tepals (p) and anthers (a). (B) Longitudinal section of an anther showing: one tepal (p) and anthers synandrium with microspores (a). (C) Cross section of mature dehiscent anther showing: epidermis (e), endothecium with lignified thickening (n), degraded tapetum with abundant orbicules (o) and pollen grains (pg). (D) Cross section of young anther showing the wall layers: epidermis (e), endothecium (n), tapetum (t) with orbicules (o) and pollen grains (pg). (E) Cross section showing full staminate flower, three tepals (p), synandrium of three anthers (a) and connective tissue (t). Scales bars = (A) 5 mm; (B, E) 200  $\mu$ m; (C) 10  $\mu$ m; (D) 20  $\mu$ m.

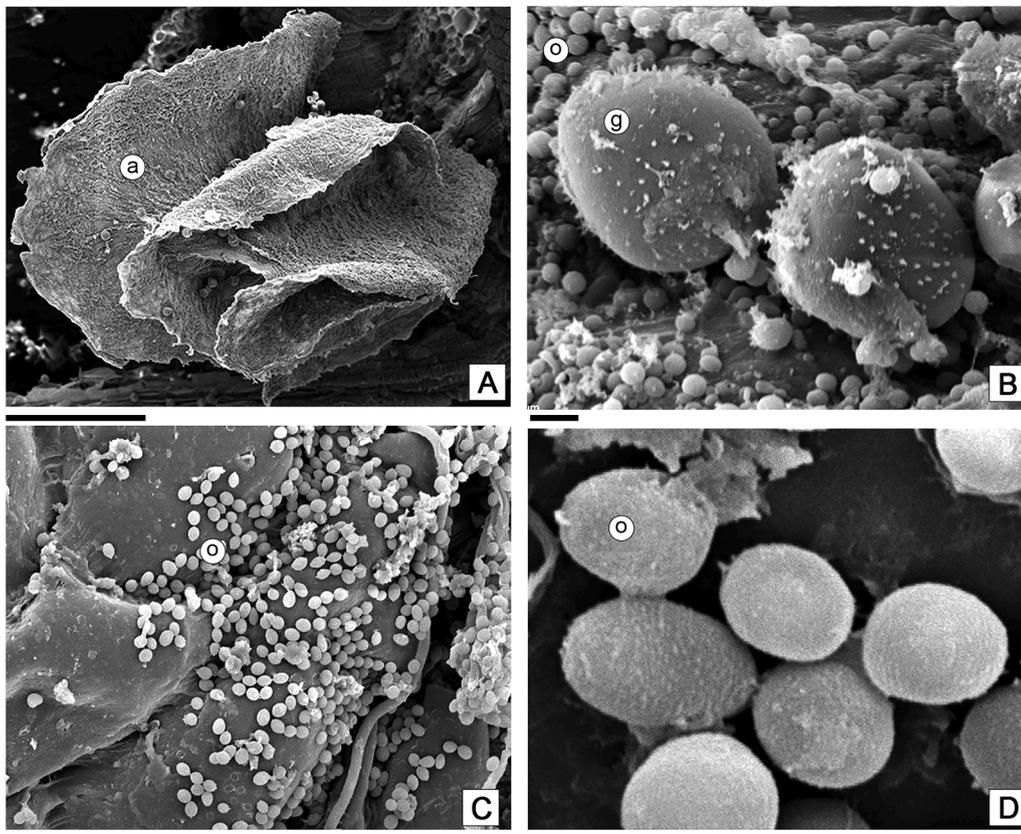
tapetum described by Pacini et al. (1985) was observed in three of the five taxa studied in this work, since all of them have a secretory tapetum but *Langsdorffia hypogaea* and both *Lophophytum* species possess orbicules and *O. subterraneum* y *Helosis cayennensis* lack orbicules.

The description of the presence of orbicules in *L. pyramidale*, *L. mirabile* and *Langsdorffia hypogaea* represent the first record for the Balanophoraceae family, they are absent in *O. subterraneum* and *H. cayennensis*. As these are a very specialized group among angiosperms (currently located in the Santalales order), it highlights the fact that orbicules are not limited to certain taxonomic groups in particular. However, for several angiosperm orders, no data on orbicules are available. What has been described for Balanophoraceae reinforces the fact that further study is necessary to investigate the occurrence of orbicules and their characters.

In regard to the function of orbicules, numerous initial hypotheses were proposed (Huysmans, 1998). Two primary theories were put forth: one suggesting that orbicules are a mere byproduct of tapetal metabolism, and another that they serve as a transport system for sporopollenin between the tapetum and microspores (Maheshwari, 1950; Heslop-Harrison and Dickinson, 1969; Christensen et al., 1972; Verstraete et al., 2014). The *Lophophytum* and *Ombrophytum* species exhibit a remarkable endothecial development, with up to three distinct layers and a precisely defined stomium region. This intricate structure enables anther dehiscence, as evidenced in previous research (Sato and Gonzalez, 2013; this study). It should be noted, however, that in *Lophophytum* the orbicules are clearly small, while in *Ombrophytum* they are not present. In *Langsdorffia*, however, the endothecium has a low participation in the process of opening anthers; it is limited to a single and

underdeveloped layer. Orbicules in *Langsdorffia* are large and are observed in high numbers during pollen grain development. They are even present on the tapetal membrane after pollen release. In *Helosis*, as in *Langsdorffia*, the anthers are welded in a synandrium, but the dehiscence is produced by rupture of the apical portion of the anther (Gonzalez et al., 2013). However, the endothecium and the orbicules are absent in *Helosis*, so that the function of pollen dispersal cannot be attributed to the orbicules.

Galati et al. (2010) studied the relationship between the orbicule morphology and the pollination system. This type of orbicules would suggest melittophily for the *Lophophytum* species. However, in this genus there exists strong evidence that supports cantharophilous syndrome (Ferrer et al., 2011; Sato, 2015). On the other hand, there have also been meliponines collecting and transporting pollen from *Lophophytum* flowers, but without the possibility of acting as effective pollinators (Sato, 2015; Sato and Gonzalez, 2022). Nevertheless, it is important to emphasize that in the studies of reproductive anatomy carried out in both species of *Lophophytum* and in *Helosis*, the existence of apomixis was proposed, justified by the formation of endosperm and embryo in the absence of pollen tube formation and double fertilization (Sato and Gonzalez, 2016, 2017, Gonzalez et al., 2019). For the type of *Langsdorffia* orbicules, correspondence to any of the types of pollination proposed by Galati et al. (2010) was not found, but a Nitidulidae (Coleoptera) is known to be responsible for pollination, also presenting cantharophily syndrome (da Silva Freitas et al., 2017). In *O. subterraneum* the presence of Coleoptera as floral visitors has been observed (pers. obs.), so as well as the other species mentioned above, it could present cantharophily. Nevertheless some authors have raised the



**Fig. 4.** Staminate flowers and orbicules in *Langsdorffia hypogaea*. SEM (A-D). (A) Detail of open anthers (a). (B) Two pollen grains (g) surrounded by orbicules (o) on the inner wall of the anther. (C) Orbicules (o) on the inner wall of the anther. (D) Magnification of orbicules (o) in (C). Scales bars = (A) 1 mm; (B-D) 5  $\mu$ m.

possibility that it reproduces asexually as *Balanophora* (Kuijt, 1969; Murata, 1990) due to its totally subterranean life.

The orbicules of *L. pyramidale* and *L. mirabile* are spheroidal, small (average 0.68  $\mu$ m), with a smooth surface and a central core; whereas the orbicules in *Langsdorffia hypogaea* are ellipsoid to ovoid and large (average 5.8  $\mu$ m x 4.4  $\mu$ m), according to the classifications of Galati (2003) and Ruggiero and Bedini (2020).

It is notable that the two species of *Lophophytum* under consideration have identical orbicules, while those of *Langsdorffia hypogaea* are substantially different. Furthermore, the anthers of *O. subterraneum* and *H. cayennensis* have no orbicules. This is consistent with findings in other angiosperms, where numerous studies have been conducted analyzing the taxonomic value of orbicules in different groups (Banerjee, 1967; Heslop-Harrison and Dickinson, 1969; Christensen et al., 1972; Raj and El-Ghazaly, 1987; El-Ghazaly, 1989; El-Ghazaly and Chaudhary, 1993; Vinckier et al., 2000; Schols et al., 2001; Vinckier and Smets, 2002a–c, 2003; Galati, 2003; Rosenfeldt and Galati, 2008; Lovisolo and Galati, 2012). It has been generally observed that all major (informal) groups of angiosperms, such as magnoliids, monocots, basal eudicots, rosids and asterids, show a mixed picture with both positive and negative observations. However, the magnitude of these variations decreased considerably at the family and genus level, in which orbicule distribution data are surprisingly constant (Verstraete et al., 2014; Moon, 2018). Thus orbicules may have predictive value and potential for systematically oriented research questions, given the intergeneric variations and the consistency at generic level observed in the taxa here analyzed, in agreement with what was previously observed by Verstraete et al. (2014) and Moon (2018). In this regard, it is of great interest to consider the case of the family Rosaceae, that was reported as a consistent occurrence group of orbicules (Verstraete et al., 2014), but recent studies showed the distribution patterns are varying according to the taxa with consistent trend at generic level (Song et al., 2016, 2017a,

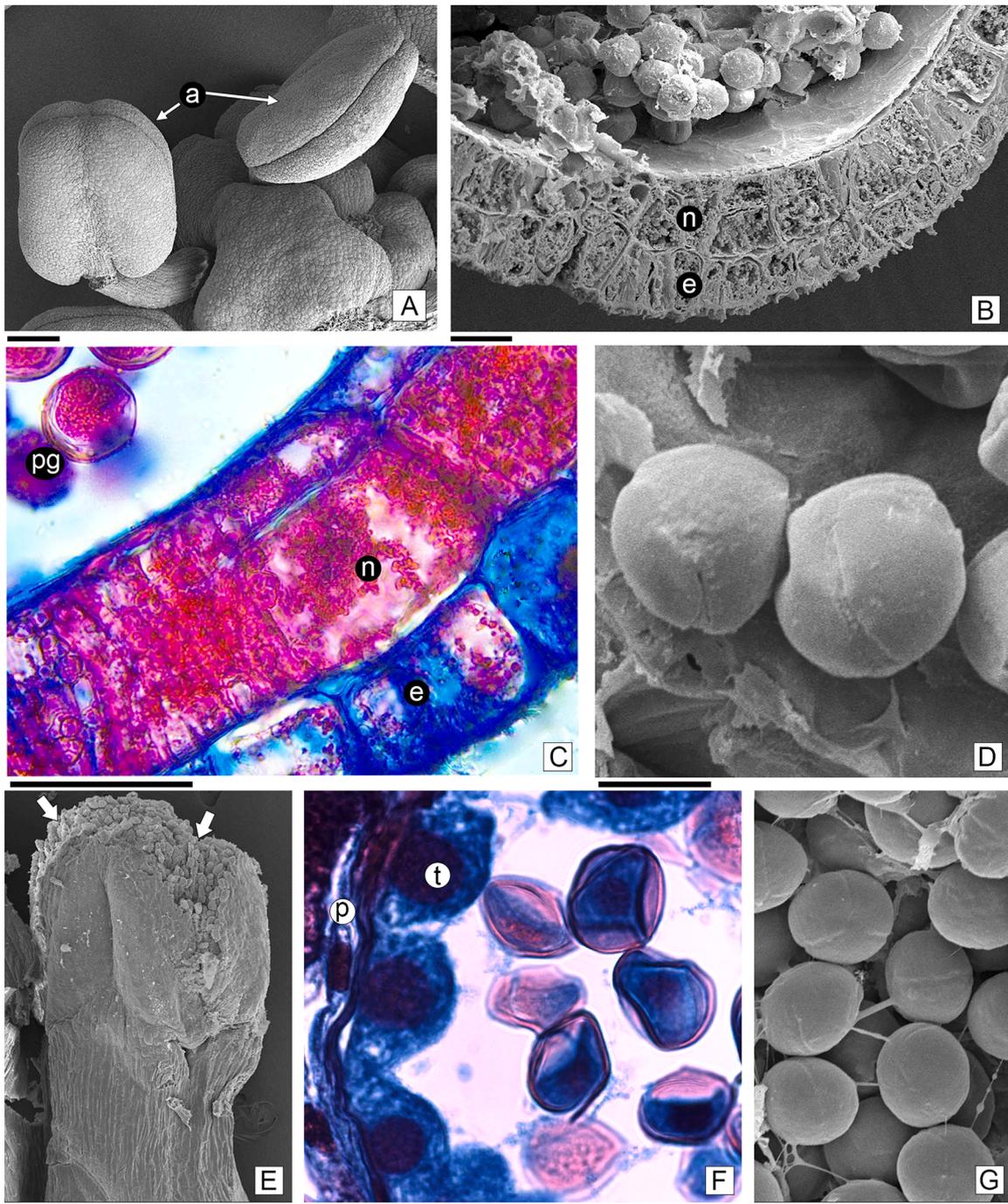
2017b), as well as we report in Balanophoraceae.

If we relate the phylogeny of the family Balanophoraceae (Sanchez-Puerta et al., 2023) with the evolutionary trend towards a reduction in size of orbicules along a phylogenetic tree that might have led to the absence of orbicules in most late-branching clades, reported by Verstraete et al. (2014) and Ruggiero and Bedini (2020), we can speculate that: (1) in the monophyletic group, recognized as the subfamily Balanophoroideae, *Langsdorffia* maintained the character of the presence of orbicules, which are of large size; (2) on the other hand, in the monophyletic group represented by *Lophophytum* and *Ombrophytum* (named subfamily Lophophytoideae), orbicules were only preserved in *Lophophytum*, which are of small size, and in *Ombrophytum* they seem to have been lost since they are absent; (3) in the subfamily Helosidoideae, sister clade of subfamily Lophophytoideae, *Helosis* had not maintained the presence of orbicules either.

The evolutionary trend mentioned above has been observed in families in which the presence of orbicules has been thoroughly studied, such as Annonaceae (Huysmans et al., 2010) and Rubiaceae (Verstraete et al., 2011). Therefore, it would be necessary to extend the present study, incorporating more species of Balanophoraceae to achieve a better understanding of the possible evolution of this character in this group of holoparasitic plants.

## 5. Conclusions

The form and function of the orbicules cannot be analyzed separately from the tapetum, because it is the tissue in which they originate. Thus, the positive correlation between orbicules and secretory tapetum type has been widely documented since the discovery of these tiny particles. However, since two of the five species analyzed with a secretory tapetum lacked orbicules, we consider that the presence of a secretory tapetum type does not ensure the presence of orbicules.



**Fig. 5.** Staminate flower and anther wall of *Ombrophytum subterraneum* (A-D) and *Helosis cayennensis* (E-G). LM (C, F), SEM (A-B, D-E, G). (A) Secondary axis bearing staminate flowers showing anthers (a). (B) Cross section of mature anther showing the wall layers, epidermis (e), endothecium (n). (C) Cross section of mature anther in bicellular pollen stage, anther wall showing: epidermis (e), 2-layered endothecium with characteristic lignified thickenings (n), pollen grains (pg). (D) Pollen grains on the inner anther wall in the surface view. (E) Synandrium with apical dehiscence (arrows). (F) Cross section of anther in microspore stage showing parietal layer (p), and secretory tapetum (t). (G) pollen grains. Scales bars = (A) 1 mm; (B-C) 50  $\mu$ m; (D, F-G) 20  $\mu$ m; (E) 0.5 mm.

Based on the literature cited (orbicules as potential participants in anther opening) and according to what we have observed in the Balanophoraceae studied, we consider that there is no association between the type of anther wall structure, orbicules and the mechanism of anther dehiscence.

Pollination modes associated with a type of orbicules do not seem to correspond to the studied species, although it is necessary to strengthen pollination studies in this group of plants.

According to what we observed in this work in the species of the family Balanophoraceae studied, the two species of *Lophophytum* have

the same type of orbicules, however two genera that belong to the same subfamily and have similar life forms as *Lophophytum* and *Ombrophytum* differ in the presence of orbicules, and another more distant genus as *Langsdorffia* has orbicules with very different characteristics. *Helosis cayennensis* (subfamily Helosiodoideae) lacks orbicules, although it has an androecium with stamens welded onto a synandrium, similar to *Langsdorffia*. Given this variability among genera, a possible relationship with the taxonomy of the family could be suggested, however studies in more species are required.

Finally, we report for the first time the presence of orbicules in the

holoparasitic family Balanophoraceae belonging to the order Santalales, in which there is scarce information available regarding the presence of orbicules. Thus, it is necessary to study the presence and type of orbicules in a greater number of species of Balanophoraceae to achieve a better understanding of its function and its diagnostic value in taxonomy.

### CRedit authorship contribution statement

**Virginia Cristina Gómez Villafañe:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Héctor Arnaldo Sato:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Diego Demarco:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Ana María Gonzalez:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization.

### Declaration of competing interest

Orbicules in south American Balanophoraceae: analysis of its possible role and its taxonomic value in the family.

Virginia Cristina Gómez Villafañe, Héctor Arnaldo Sato, Diego Demarco and Ana María Gonzalez.

The authors have no conflicts of interest to declare that are relevant to the content of this article.

### Acknowledgements

This work was supported by Secretaría de Ciencia y Técnica y Estudios Regionales de la Universidad Nacional de Jujuy.

### Data availability

Data will be made available on request.

### References

- Balthazar, M.V., Pedersen, K.R., Friis, E.M., 2005. *Teixeria lusitanica*, a new fossil flower from the early cretaceous of Portugal with affinities to Ranunculales. *Plant Syst. Evol.* 255, 55–75. <https://doi.org/10.1007/s00606-005-0347-z>.
- Banerjee, U., 1967. Ultrastructure of the tapetal membranes in grasses. *Grana* 7, 2–3. <https://doi.org/10.1080/00173136709430043>.
- Burkart, A., 1949. La Balanoforácea *Lophophytum leandrii* en Misiones y apuntes sobre una especie vecina en Salta. *Darwiniana* 9, 169–172.
- Cardoso, L.J.T., Alves, R.J.V., Braga, J.M.A., 2011. A new species and a key for *Langsdorffia* (Balanophoraceae). *Syst. Bot.* 36 (2), 424–427. <https://doi.org/10.1600/036364411X569606>.
- Christensen, J.E., Horner, H.T., Lersten, N.R., 1972. Pollen wall and tapetal orbicular wall development in *Sorghum bicolor* (Gramineae). *Am. J. Bot.* 59, 43–58. <https://doi.org/10.1002/j.1537-2197.1972.tb10061.x>.
- da Silva Freitas, L., Moreira, L.M., De Avila Jr, R.S., Felestrino, É.B., Demarco, D., de Sousa, H.C., Ribeiro, S.P., 2017. Reproductive phenology and floral visitors of a *Langsdorffia hypogaea* (Balanophoraceae) population in Brazil. *Flora* 233, 51–57. <https://doi.org/10.1016/j.flora.2017.02.023>.
- El-Ghazaly, G., 1989. Pollen and orbicule morphology of some *Eurphorbia* species. *Grana* 28, 243–259. <https://doi.org/10.1080/00173138909427439>.
- El-Ghazaly, G.A., Chaudhary, R., 1993. Pollen morphology of some species of the genus *Euphorbia* L. *Rev. Palaeobot. Palynol.* 78, 293–319. [https://doi.org/10.1016/0034-6667\(93\)90069-7](https://doi.org/10.1016/0034-6667(93)90069-7).
- Erdtman, G., 1960. The acetolysis method. A revised description. *Svensk Botanisk Tidskrift* 54, 561–564.
- Ferrer, M.S., Marvaldi, A.E., Sato, H.A., Gonzalez, A.M., 2011. Biological notes on two species of *Oxycorynus* (Coleoptera: *Belidae*) associated with parasitic plants of the genus *Lophophytum* (Balanophoraceae), and new distribution records in Argentina. *Revista de la Sociedad Entomológica Argentina* 70 (3–4), 351–355.
- Fontana, J.L., Popoff, O.F., 2006. *Helosis* (Balanophoraceae) en Argentina. *Bol. Soc. Argent. Bot.* 41 (1–2), 85–90.
- Fox, C., Zardini, E., 1998. *Langsdorffia* Mart. (Balanophoraceae), un nuevo género para Paraguay. *Candollea* 53, 118–119.
- Galati, B.G., 2003. Ubisch bodies in Angiosperms. In: Pandey, K, Dhakal, MR (Eds.), *Advances in plant reproductive biology II*, eds. Narendra Publishing House, Delhi, India, pp. 1–20.
- Galati, B.G., Gotelli, M.M., Rosenfeldt, S., Torretta, J.P., Zarlavsky, G., 2010. Orbicules in relation to the pollination modes. In: Kaiser, BJ (Ed.), *Pollen: Structure, Types and Effects*. Nova Science Publishers, Huntington, New York, USA.
- Galati, B.G., Monacci, F., Gotelli, M.M., Rosenfeldt, S., 2007. Pollen, tapetum and orbicule development in *Modiolastrum malvifolium* (Malvaceae). *Ann. Bot.* 99, 755–763. <https://doi.org/10.1093/aob/mcm011>.
- Gedalovich-Shedletzky, E., Kuijt, J., 1990. An ultrastructural study of the tuber stands of *Balanophora*: Balanophoraceae. *Canad. J. Botany* 68, 1271–1279. <https://doi.org/10.1139/b90-162>.
- Gonzalez, A.M., 2018. ImageJ: una herramienta indispensable para medir el mundo biológico. *Folium* 1, 1–17.
- Gonzalez, A.M., Cristóbal, C.L., 1997. Anatomía y ontogenia de semillas de *Helicteres lhotzkyana* (Sterculiaceae). *Bonplandia* 9 (3–4), 287–294. <https://doi.org/10.30972/bon.93-41497>.
- Gonzalez, A.M., Mauseth, J.D., 2010. Morphogenesis is highly aberrant in the vegetative body of the holoparasite *Lophophytum leandrii* (Balanophoraceae): All typical vegetative organs are absent and many tissues are highly modified. *Int. J. Plant Sci.* 171, 499–508. <https://doi.org/10.1086/651947>.
- Gonzalez, A.M., Popoff, O.F., Salgado Laurenti, C.R., 2013. Structure of staminate flowers, microsporogenesis, and microgametogenesis in *Helosis cayennensis* var. *cayennensis* (Balanophoraceae). *Anales del Jardín Botánico de Madrid* 70 (2), 113–121. <https://doi.org/10.3989/ajbm.2362>.
- Gonzalez, A.M., Sato, H.A., Marazzi, B., 2019. Embryology in *Helosis cayennensis* (Balanophoraceae): structure of female flowers, fruit, endosperm and embryo. *Plants* 8 (3), 74. <https://doi.org/10.3390/plants8030074>.
- Gotelli, M., Lattar, E., Zini, L.M., Rosenfeldt, S., Galati, B., 2023. Review on tapetal ultrastructure in angiosperms. *Planta* 257 (6), 100. <https://doi.org/10.1007/s00425-023-04138-8>.
- Govil, C.M., Lavania, S., 1980. Floral anatomy and embryology of some species of *Cuscuta* L. *Proc. Plant Sci.* 89, 219–228. <https://doi.org/10.1007/BF03046208>.
- Hansen, B., 1980a. Balanophoraceae. *Flora Neotropica* 23, 1–80.
- Hansen, B., 1980b. Pollen dimorphism in *Lophophytum mirabile* Schott & Endl. (Balanophoraceae). *Grana* 19, 189–191. <https://doi.org/10.1080/00173138009425003>.
- Hansen, B., 1987. Balanophoraceae. *Flora del Paraguay* 7–11.
- Heslop-Harrison, J., 1962. Origin of exine. *Nature (Lond.)* 195, 1069–1071. <https://doi.org/10.1038/1951069a0>.
- Heslop-Harrison, J., Dickinson, H.G., 1969. Time relationships of sporopollenin synthesis associated with tapetum and microspores in *Lilium*. *Planta* 84, 199–214. <https://doi.org/10.1007/BF00388106>.
- Hsiao, S.C., Mauseth, J.D., Gomez, L.D., 1993. Growth and anatomy of the vegetative body of the parasitic angiosperm *Helosis cayennensis* (Balanophoraceae). *Bullet. Torrey Botanical Club* 120, 295–309. <https://doi.org/10.2307/2996994>.
- Hsiao, S.C., Mauseth, J.D., Gomez, L.D., 1994. Growth and anatomy of the vegetative body of the parasitic Angiosperm *Langsdorffia hypogaea* (Balanophoraceae). *Bullet. Torrey Botanical Club* 121, 24–39. <https://doi.org/10.2307/2996881>.
- Huysmans, S., El-Ghazaly, G., Smets, E., 1998. Orbicules in angiosperms: morphology, function, distribution, and relation with tapetum types. *Botanical Review* 64, 240–272. <https://doi.org/10.1007/BF02856566>.
- Huysmans, S., Verstraete, B., Smets, E., Chatrou, L.W., 2010. Distribution of orbicules in Annonaceae mirrors evolutionary trend in angiosperms. *Plant Ecol. Evol.* 143, 199–211. <https://doi.org/10.5091/plecevo.2010.438>.
- Johansen, D.A., 1940. *Plant microtechnique*. McGraw-Hill Book, New York, p. 511.
- Kuijt, J., 1969. *The biology of parasitic flowering plants*. University of California Press, Berkeley, USA, p. 248.
- Lovisolo, M.R., Galati, B.G., 2012. Diversidad de orbículas en Poaceae. *Boletín de la Sociedad Argentina de Botánica* 47, 87–96.
- Luque, R.H., Souza, C., Kraus, J.E., 1996. Métodos de coloração do Roeser (1972) Modificado – E Kropp (1972), visado a substituição do azul de astra por azul de alciano 8GS on 8GX. *Acta Botanica Brasílica* 10, 199–212. <https://doi.org/10.1590/S0102-33061996000200001>.
- Maheshwari, P., 1950. *An introduction to the embryology of the angiosperms*. New York, USA. 453p. <https://doi.org/10.5962/bhl.title.5681>.
- Mauseth, J.D., Hsiao, S.C., Montenegro, G., 1992. Vegetative body of the parasitic angiosperm *Ombrophytum subterraneum* (Balanophoraceae). *Bullet. Torrey Botanical Club* 119, 407–417. <https://doi.org/10.2307/2996729>.
- Moon, H.K., 2018. The phylogenetic potential of orbicules in angiosperms. *Korean J. Plant Taxon.* 48 (1), 9–23. <https://doi.org/10.11110/kjpt.2018.48.1.9>.
- Murata, J., 1990. Agamic species of *Balanophora* in Japan. *Mem. Nation. Sci. Mus. (Tokyo)* 23, 43–50.
- Nickrent, D.L., 1997. *The parasitic plant connection, Balanophoraceae* Páginas. Department of Plant Biology, Southern Illinois University, Carbondale. <http://www.parasiticplants.siu.edu>. accessed July 14 2024.
- Nickrent, D.L., Musselman, L.J., 2004. Introduction to parasitic flowering plants. *The Plant Health Instructor* 13, 300–315. <https://doi.org/10.1094/PHI-I-2004-0330-01>.
- Novara, L.J., 2009. *Balanophoraceae*. In: LJ Novara (ed.), *Flora del valle de Lerma* 8: 1–7. Salta, Argentina.
- O'Brien, T.P., Feder, N., McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59 (2), 368–373. <https://doi.org/10.1007/BF01248568>.
- O'Brien, T.P., McCully, M.E., 1981. *The study of plant structure. Principles and selected methods*, Melbourne, Australia, p. 357.
- Pacini, E., Franchi, G., Hesse, M., 1985. The tapetum: its form, function and possible phylogeny in Embriophyta. *Plant Syst. Evol.* 149, 155–185. <https://doi.org/10.1007/BF00983304>.
- Pott, A., Pott, V.J., 1994. *Plantas do Pantanal*. Brasília, Embrapa, p. 320.

- Raj, B., El-Ghazaly, G., 1987. Morphology and taxonomic application of orbicules (Ubusch bodies) in *Chloranthaceae*. *Pollen et Spores* 29, 151–166.
- Rasband, W.S., 2023. ImageJ, 1997–2023. US National Institutes of Health, Bethesda, Maryland, USA. <https://imagej.nih.gov/ij>. accessed May.
- Rodríguez-Pontes, M., 2009. Seed formation and pollination system in *Cuscuta obtusiflora*: first record of preanthesis cleistogamy in parasitic plants and some functional inferences. *Flora-Morphol. Distrib. Function. Ecol. Plants* 204 (3), 228–237. <https://doi.org/10.1016/j.flora.2008.02.005>.
- Rosenfeldt, S., Galati, B.G., 2008. Orbicules diversity in *Oxalis* species from the province of Buenos Aires (Argentina). *Biocell* 32, 41–47. <https://doi.org/10.32604/biocell.2008.32.041>.
- Ruggiero, F., Bedini, G., 2020. Phylogenetic and morphologic survey of orbicules in angiosperms. *Taxon*. 69 (3), 543–566. <https://doi.org/10.1002/tax.12281>.
- Ruzin, S.E., 1999. Plant microtechnique and microscopy. New York. USA.
- Sanchez-Puerta, M.V., Ceriotti, L.F., Gatica-Soria, L.M., Roulet, M.E., Garcia, L.E., Sato, H.A., 2023. Invited Review Beyond parasitic convergence: unravelling the evolution of the organellar genomes in holoparasites. *Ann. Bot.* 132 (5), 909–928. <https://doi.org/10.1093/aob/mcad108>.
- Santos, J.C., Nascimento, A.R.T., Marzinek, J., Leiner, N., Oliveira, P.E., 2017. Distribution, host plants and floral biology of the root holoparasite *Langsdorffia hypogaea* in the Brazilian savanna. *Flora* 226, 65–71. <https://doi.org/10.1016/j.flora.2016.11.008>.
- Sato, H.A., 2015. Anatomía reproductiva de las especies de *Lophophytum* Schott & Endl. (Balanophoraceae) de la Argentina y revisión taxonómica del género en América. Tesis doctoral Facultad de Ciencias Agrarias. Universidad Nacional del Nordeste, Argentina.
- Sato, H.A., Gómez Villafañe, V.C., Bonillo, G.A., Gonzalez, A.M., 2024. Redefining Parasitic Plants: An Analysis of Economically Important Balanophoraceae Species in Argentina. *IntechOpen*. <https://doi.org/10.5772/intechopen.114247>.
- Sato, H.A., Gonzalez, A.M., 2013. Anatomía y desarrollo de la flor estaminada, microsporogénesis y microgametogénesis en especies de *Lophophytum* (Balanophoraceae) en la Argentina. *Boletín de la Sociedad Argentina de Botánica* 48 (1-2), 59–72.
- Sato, H.A., Gonzalez, A.M., 2016. Floral development and anatomy of pistillate flowers of *Lophophytum* (Balanophoraceae), with special reference to the embryo sac inversion. *Flora* 219, 35–47. <https://doi.org/10.1016/j.flora.2016.01.002>.
- Sato, H.A., Gonzalez, A.M., 2017. Embryogenesis, endospermogenesis and fruit development in *Lophophytum* (Balanophoraceae). *Flora* 233, 79–89. <https://doi.org/10.1016/j.flora.2017.05.001>.
- Sato, H.A., Gonzalez, A.M., 2022. Anatomy, embryology and life cycle of *Lophophytum*, a root-holoparasitic plant. *Parasitic Plants*. *IntechOpen*. <https://doi.org/10.5772/intechopen.99981>.
- Schols, P., Furness, C.A., Wilkin, P., Huysmans, S., Smets, E., 2001. Morphology of pollen and orbicules in some *Discorea* species and its systematic implications. *Botanical Journal of the Linnean Society* 136, 295–311. <https://doi.org/10.1111/j.1095-8339.2001.tb00574.x>.
- Shamrov, I.I., Anisimova, G.M., Babro, A.A., 2021. Tapetum types and forms in angiosperms. In: *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences*, 75, pp. 167–179. <https://doi.org/10.2478/prolas-2021-0026>.
- Song, J.-H., Moon, H.-K., Hong, S.-P., 2016. Pollen morphology of the tribe Sorbarieae (Rosaceae). *Plant Syst. Evol.* 302, 853–869. <https://doi.org/10.1007/s00606-016-1303-9>.
- Song, J.-H., Moon, H.-K., Oak, M.-K., Hong, S.-P., 2017a. Phylogenetic evaluation of pollen and orbicule morphology in Rosaceae tribe Neillieae (subfamily Amygdaloideae). *Botanical J. Linnean Soc.* 183, 439–453. <https://doi.org/10.1093/botlinnean/bow019>.
- Song, J.-H., Oak, M.-K., Roh, H.-S., Hong, S.-P., 2017b. Morphology of pollen and orbicules in the tribe Spiraeae (Rosaceae) and its systematic implications. *Grana* 56, 351–367. <https://doi.org/10.1080/00173134.2016.1274334>.
- Strittmatter, L.L., Galati, B.G., Monacci, F., 2000. Übusch bodies in the peripetal membrane of *Abutilon pictum* Gill (Malvaceae). *Beiträge zur Biologie der Pflanzen* 71, 1–10.
- Su, H., Hu, J., Anderson, F.E., Der, J.P., Nickrent, D.L., 2015. Phylogenetic relationships of Santalales with insights into the origins of holoparasitic Balanophoraceae. *Taxon*. 64, 491–506. <https://doi.org/10.12705/643.2>.
- Taylor, T.N., 1976. The ultrastructure of Schopfipollenites: orbicules and tapetal membranes. *Am. J. Bot.* 63 (6), 857–862. <https://doi.org/10.1002/j.1537-2197.1976.tb11877.x>.
- Thorogood, C., Santos, J.C., 2020. *Langsdorffia*: Creatures from the deep? *Plants. People Planet*. 2 (3), 181–185. <https://doi.org/10.1002/ppp3.10102>.
- Vaknin, Y., Gan-Mor, S., Bechar, A., Ronen, B., Eiskowitch, D., 2000. The role of electrostatic forces in pollination. *Plant Syst. Evol.* 222 (1), 133–142. <https://doi.org/10.1007/BF00984099>.
- Verstraete, B., Groeninckx, L., Smets, E., Huysmans, S., 2011. Phylogenetic signal of orbicules at family level: Rubiaceae as case study. *Taxon*. 60, 742–757. <https://doi.org/10.1002/tax.603010>.
- Verstraete, B., Moon, H.K., Smets, E., Huysmans, S., 2014. Orbicules in flowering plants: a phylogenetic perspective on their form and function. *Botanical Review* 80 (2), 107–134. <https://doi.org/10.1007/s12229-014-9135-1>.
- Vinckier, S., Cadot, P., Smets, E., 2005. The manifold characters of orbicules: structural diversity, systematic significance, and vectors for allergens. *Grana* 44 (4), 300–307. <https://doi.org/10.1080/00173130510031654>.
- Vinckier, S., Huysmans, S., Smets, E., 2000. Morphology and ultrastructure of orbicules in the subfamily *Ixoroideae* (Rubiaceae). *Rev. Palaeobot. Palynol.* 108, 151–174. [https://doi.org/10.1016/S0034-6667\(99\)00036-6](https://doi.org/10.1016/S0034-6667(99)00036-6).
- Vinckier, S., Smets, E., 2001. The potential role of orbicules as a vector of allergens. *Allergy* 56, 1129–1136. <https://doi.org/10.1034/j.1398-9995.2001.00172.x>.
- Vinckier, S., Smets, E., 2002a. Morphology, ultrastructure and typology of orbicules in family Loganiaceae s.l. and related genera, in relation to systematics. *Rev. Palaeobot. Palynol.* 119, 161–189. [https://doi.org/10.1016/S0034-6667\(01\)00137-3](https://doi.org/10.1016/S0034-6667(01)00137-3).
- Vinckier, S., Smets, E., 2002b. Morphological and ultrastructural diversity of orbicules in relation to evolutionary tendencies in Apocynaceae s.l. *Ann. Bot.* 90, 647–662. <https://doi.org/10.1093/aob/mcf243>.
- Vinckier, S., Smets, E., 2002c. Systematic importance in orbicule diversity in Gentianales. *Grana* 4, 158–182. <https://doi.org/10.1080/001731302321042623>.
- Vinckier, S., Smets, E., 2003. Morphological and ultrastructural diversity of orbicules in Gentianaceae. *Ann. Bot.* 92, 657–672. <https://doi.org/10.1093/aob/mcg187>.
- Wang, A., Xia, Q., Xie, W., Datla, R., Selvaraj, G., 2003. The classical Ubusch bodies carry a sporophytically produced structural protein (RAFTIN) that is essential for pollen development. *Proc. Natl. Acad. Sci.* 100, 14487–14492. <https://doi.org/10.1073/pnas.2231254100>.
- Xifreda, C.C., 1999. Balanophoraceae. In: F. Zuloaga & O. Morrone (eds.), *Catálogo de las plantas vasculares de la Rep. Argentina II. Monographs in Systematic Botany from the Missouri Botanical Garden* 74: 353–354.
- Zetter, R., Weber, M., Hesse, M., Pinggen, M., 2002. Pollen, pollenkitt, and orbicules in *Craigia brononii* flower buds (Tilioidae, Malvaceae) from the Miocene of Hambach, Germany. *Int. J. Plant Sci.* 163 (6), 1067–1071. <https://doi.org/10.1086/342520>.
- Zuloaga, F.O., Morrone, O.N., Belgrano, M.J., Marticorena, C., Marchesi, E., 2008. *Catálogo de las plantas vasculares del Cono Sur. Monographs in Syst. Botany Missouri Botanical Garden* 107, 1–3348.