

Pollen development and anther morphology in 14 species of Rhamnaceae

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Abstract Microsporogenesis, microgametogenesis, and anther structure of 14 species belonging to Rhamneae (Rhamnoids clade), Paliureae, Pomaderreae, Colletieae, and Gouanieae (Ziziphoids clade) of the Rhamnaceae family were analyzed using bright-field, scanning, and transmission electron microscopy. The aim is to look for morphological characters that have systematic value on a suprageneric level within the family. The character states of the number of nuclei in tapetal cells, the endothelial thickening pattern, and the number of endothelial layers are consistent on tribal level. Tribes were previously delineated based on molecular phylogenetic evidence. On the other hand, pollen morphology does not seem to have systematic value at tribal level in Rhamnaceae. The presence or absence of orbicules and their morphology vary within tribes. It seems that for Rhamnaceae, the anatomy of the reproductive sporophytic structures could have more systematic value than the gametophytic structures. However, more studies are needed to confirm this.

Keywords Microsporangium · Orbicules · Pollen · Rhamnaceae · Systematics

Introduction

Rhamnaceae comprises about 900 species in 55 genera, and it has a cosmopolitan distribution, although it mostly occurs in the tropics and in warm temperate regions (Medan and Schirarend 2004; Perveen and Qaiser 2005). The historical classification of Rhamnaceae was largely based on fruit characters, and five tribes were recognized: Colletieae, Gouanieae, Rhamneae, Ventilagineae, and Zizipheae (Suessenguth 1953). The first phylogenetic analysis of Rhamnaceae using *rbcL* and *trnL-F* plastid DNA sequences demonstrated that Rhamnaceae is a monophyletic family closely related to Dirachmaceae and Barbeyaceae (Richardson et al. 2000a). However, it also showed that not all five original tribes are supported, for example, the tribes Rhamneae and Zizipheae as circumscribed by Suessenguth (1953) are unnatural. Three strongly supported clades were identified (Ziziphoids, Rhamnoids and Ampeloziziphoids), but morphological characters could not be found to underpin a formal taxonomic description of these three clades as subfamilies. In light of these findings, the tribal classification was revised and 11 new tribes were delineated (Richardson et al. 2000b). However, to date, it remains difficult to find morphological characters that have systematic value on a suprageneric level within the family.

One of the morphological characters that is more studied in the Rhamnaceae family is the pollen exine (Papagiannes 1974; Schirarend and Köhler 1993; Punt and Hoen 1995; Perveen and Qaiser 2005). Schirarend and Köhler (1993) described 12 types of pollen based mainly on tectum architecture. In spite of all these reports on pollen morphology, descriptions comparing general aspects of the microsporogenesis and microgametogenesis are rare (Gotelli et al. 2012).

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The systematic value of orbicules was studied in a few angiosperm taxa: Chloranthaceae, now included in Lamiaceae (Raj and El-Ghazaly 1987); *Euphorbia* L. (El-Ghazaly 1989; El-Ghazaly and Chaudhary 1993); the family Rubiaceae (Huysmans et al. 1997; D'Hondt et al. 2004; Verstraete et al. 2011); the order Gentianales (Vinckier et al. 2000; Vinckier and Smets 2002a, b, c, 2003); the genera *Dioscorea* L. (Schols et al. 2001) and *Oxalis* L. (Rosenfeldt

and Galati 2008); Annonaceae (Huysmans et al. 2010), and Poaceae (Lovisolo and Galati 2012). A general overview of presence/absence of orbicules in the angiosperms and a phylogenetic perspective on their form and function is discussed by Verstraete et al. (2014). Information on orbicules in Rhamnaceae is however rather scarce, and the presence of orbicules was only demonstrated for *Colletia paradoxa* and *Discaria americana* (Gotelli et al. 2012).

Table 1 Materials studied and Summary of the most important anther anatomy features, pollen morphology, and orbicule type for each species

Clade	Tribe	Species	Material provenance	Tapetum # nuclei	Endothecium		Pollen grain type	Orbicules aspect
					# layers	Thickenings shape		
Rhamnoids	Rhamneae	<i>Scutia buxifolia</i>	Lucien Hauman Botanical Garden	1	2–3	U-shape	Rhamnus	–
		<i>Condalia buxifolia</i>	Lucien Hauman Botanical Garden	1	2–3	U-shape	Rhamnus	Cup
Ziziphoids	Paliureae	<i>Hovenia dulcis</i>	Lucien Hauman Botanical Garden	1	1	U-shape with basal anastomosis tympanate baseplate	Hovenia	Hemispherical to plaque
		<i>Ziziphus mucronata</i>	Lucien Hauman Botanical Garden	2	1	U-shape with basal anastomosis	Lasiodiscus	–
		<i>Z. mistol</i>	Lucien Hauman Botanical Garden	2	1	U-shape with basal anastomosis	Lasiodiscus	Spherical to subspherical
		<i>Z. jujuba</i>	Lucien Hauman Botanical Garden	2	1	U-shape with basal anastomosis	Lasiodiscus	–
	Pomadereae	<i>Cryptandra tomentosa</i>	FAA (Coll. J. Kellermann)	1	1	U-shape	Lasiodiscus	Hemispherical
		<i>Siegfriedia darwinioides</i>	FAA (coll. N.G. Walsh)	1	1	U-shape	Lasiodiscus	Hemispherical
		<i>Stenanthemum humile</i>	FAA (Coll. J. Kellermann)	1	1	U-shape	Pomaderris	Hemispherical
	Colletieae	<i>Colletia paradoxa</i>	Lucien Hauman Botanical Garden	2	2–3	U-shape with basal anastomosis	Colletia (Gotelli et al. 2012)	Spherical to subspherical (Gotelli et al. 2012)
		<i>C. spinosissima</i>	Lucien Hauman Botanical Garden	2	2–3	U-shape with basal anastomosis	Colletia	Spherical to subspherical
		<i>Retanilla patagonica</i>	FAA (Rada Tilly, leg. M.E. Arce and D. Medan)	2	2–3	U-shape	Hovenia	Hemispherical
Gouanieae	<i>Gouania ulmifolia</i>	<i>Kentrothamus weddellianus</i>	FAA (Jujuy: Cuesta de Toquero, Medan and Aagesen 1995)	2	2–3	U-shape	Hovenia	Hemispherical to plaque
			Lucien Hauman Botanical Garden	1	2–3	U-shape tympanate baseplate	Sageretia	–

Although the general anatomy of the anther, particularly the thickening patterns of the endothecium, appears to reflect phylogenetic constraints, the anatomy of the anther was not studied in detail for any species of Rhamnaceae.

The aim of this paper is to study pollen grain and microsporangium development, anther morphology, and orbicules of 14 species of the tribes Pomaderreae, Colletieae, Paliureae, and Gouanieae (from the Ziziphoids clade), and Rhamneae (from the Rhamnoids clade) in order to broaden the current embryological knowledge of the Rhamnaceae. We also discuss the systematic value of these morphological and anatomical features.

Materials and methods

Samples of *Colletia paradoxa* (Spreng.) Escal., *C. spinosissima* J.F.Gmel. (Colletieae), *Ziziphus mucronata* Willd., *Z. mistol* Griseb., and *Z. jujuba* Mill. (Paliureae), *Gouania ulmifolia* Triana & Planch. (Gouanieae), and *Scutia buxifolia* Hutch. & M.B.Moss, *Condalia buxifolia* Reissek, and *Hovenia dulcis* Thunb. (Rhamneae) were collected from individuals cultivated at the Lucien Hauman Botanical Garden of the Facultad de Agronomía, Universidad de Buenos Aires. Reference specimens were deposited in the Herbarium Gaspar Suarez (BAA). Flowers of *Retanilla patagonica* (Speg.) Tortosa, and *Kentrothamnus weddellianus* (Miers) M.C.Johnst. (Colletieae), *Stenanthemum humile* Benth., *Siegfriedia darwinioides* C.A.Gardner, and *Cryptandra tomentosa* Lindl. (Pomaderreae) were obtained from previous collections fixed in FAA (formalin-alcohol-acetic acid) (Table 1).

Anthers were prefixed overnight in 2.5 % glutaraldehyde in phosphate buffer (pH 7.2) and then postfixed in OsO₄ at 2 °C in the same buffer for 3 h. Following dehydration in acetone series, the material was embedded in Spurr's resin. Sections of 1 µm were cut and stained with toluidine blue following Zarlavsky's technique (2014) and mounted in synthetic resin. Photographs were taken with a digital camera mounted on a Motic light microscope. Ultrathin sections (75 to 90 nm) of *Gouania ulmifolia* were made on a Reichert ultramicrotome, stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963), and observed and photographed with a JEOL-JEM 1200 EX II TEM at 85.0 kV.

For scanning electron microscopy studies, the material previously fixed in FAA was dehydrated in an ethanol series (70, 80, 90, 100 %), critical point dried with liquid CO₂, and sputter-coated with gold-palladium for 3 min (Zarlavsky 2014). Scanning micrographs were taken with a Philips XL 30 microscope.

In order to observe endothecium thickenings, mature anthers previously fixed in FAA were clarified according to Zarlavsky's technique (2014).

In order to analyze and discuss the evolution of the character states, these were manually plotted on the single optimal SW tree from the combined *rcbL/trnL-F* analysis published by Richardson et al. (2000a). The three taxa (*Stenanthemum*, *Kentrothamnus*, *Retanilla*) that were not represented in this tree were added, some double genera were reduced to one, and the specific epithet of species belonging to the same genera was added. The outgroup was simplified to one line stating "outgroup", and bootstrap values were eliminated.

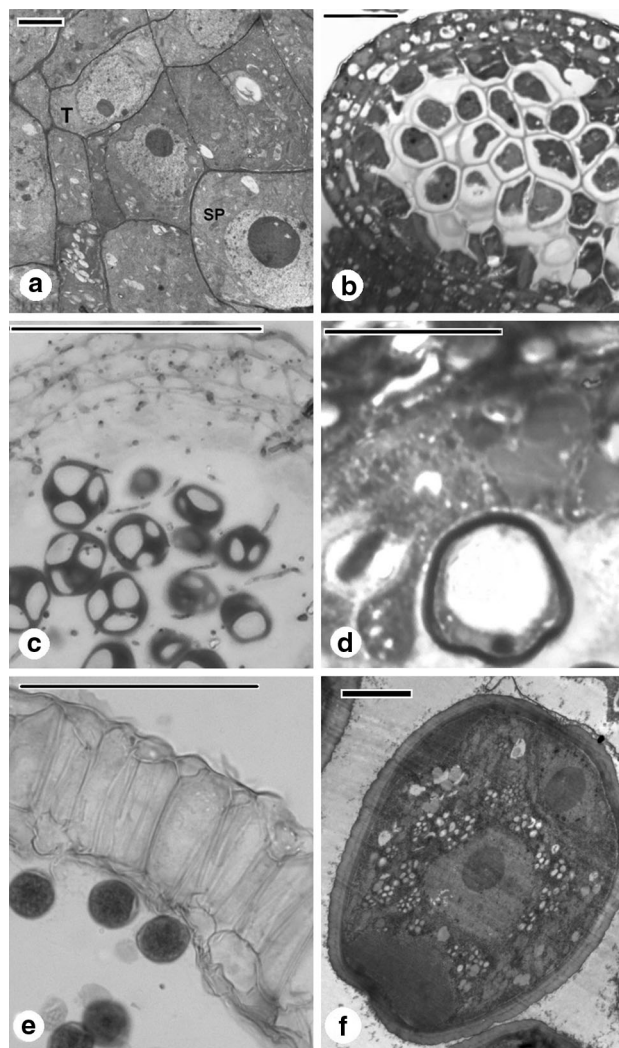


Fig. 1 Microsporogenesis and microgametogenesis. **a, f** Transmission electron microscopy, **a** *Gouania ulmifolia*, **f** *Colletia paradoxa*. **b–e** Bright-field microscopy, *Kentrothamnus weddellianus*. **a** Sporogenous tissue (sp) and tapetum (t). **b** Microspore mother cell stage. **c** Microspore tetrad stage. **d** Free microspore stage. **e** Pollen grain stage. **f** Detail of mature pollen grain. Scale bar **a, f** 1 µm; **b, d, e** 25 µm; **c** 50 µm

Results

Microsporogenesis and microgametogenesis

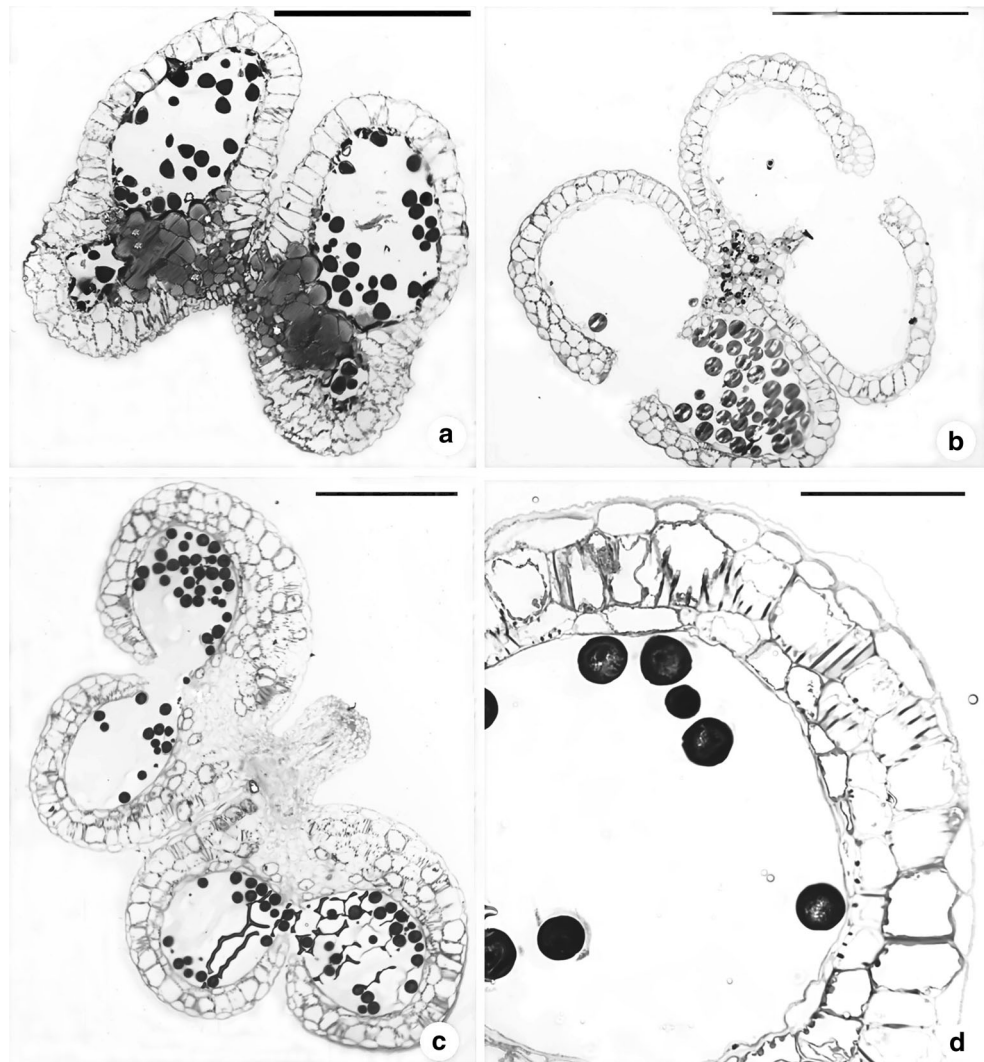
The young anther wall consists of: epidermis (ep), endothecium (en), two or three middle layers (ml), and a secretory tapetum (t). One middle layer has a common origin with the endothecium and the other with the tapetum. Tapetal cells are binucleate and sometimes divide periclinally to form another layer of cells toward the locule in *Colletia spinosissima*, *C. paradoxa*, *Retanilla patagonica*, *Kentrothamnus weddellianus*, *Ziziphus jujuba*, *Z. mistol*, and *Z. mucronata* and uninucleate in *Scutia buxifolia*, *Condalia buxifolia*, *Hovenia dulcis*, *Gouania ulmifolia*, *Stenanthemum humile*, *Siegfriedia darwinioides*, and *Cryptandra tomentosa* (Fig. 1a).

Microspore mother cells show a conspicuous nucleus. Many dictyosomes, small vacuoles, and mitochondria are observed in the cytoplasm (Fig. 1a). A thick callosic wall forms between

the plasmalemma and the primary wall (Fig. 1b). Microspore mother cells undergo simultaneous meiosis, forming tetrads with a tetrahedral arrangement (Fig. 1c). After the dissolution of the callose wall, the sporopollenin wall continues to form. Microspores have large vacuole that limits the cytoplasm to a parietal position (Fig. 1d). At this stage, the middle layers are almost completely degraded and the tapetal cells seem to remain conspicuous (Fig. 1d).

At the pollen grain stage, endothecium thickenings can be observed (Fig. 1e). In young pollen grains, the generative cell formed by a mitotic division of the microspore occupies a parietal position. The nucleus occupies most of the cytoplasm, and mitochondria are observed. A thin wall transparent to electrons delimits this cell, and a few connections with the vegetative one can be observed. The vegetative cell has a dense cytoplasm filled with amyloplasts and rough endoplasmic reticulum (Fig. 1f). The pollen grains are shed at the bicellular stage in all species studied.

Fig. 2 Mature anther structure, bright-field microscopy. **a** *Hovenia dulcis*. **b** *Ziziphus mucronata*. **c–d** *Colletia spinosissima*. Scale bar **a–c** 250 μ m; **b** 50 μ m



Mature anther structure

Scutia buxifolia, *Condalia buxifolia*, *Hovenia dulcis*, *Retanilla patagonica*, *Kentrothamnus weddellianus* *Cryptandra tomentosa*, *Stenanthemum humile*, and *Siegfriedia darwinioides* have connective parenchymatous tissue that is well developed and has a large amount of tannin cells (Fig. 2a). In contrast, this parenchymatous tissue has only some cells with low tannin content in *Colletia spinosissima*, *C. paradoxa*, and *Gouania ulmifolia*. All species of *Ziziphus* have a reduced connective parenchymatous tissue with some tannin cells (Fig. 2b).

In both species of *Colletia*, the epidermis is conspicuous in the mature anther (Fig. 2c, d), while in the remaining species it is reduced (Fig. 2a, b). The endothecium is composed by only one cell layer in *Hovenia dulcis*, all species of *Ziziphus*, *Cryptandra tomentosa*, *Stenanthemum humile*, and *Siegfriedia darwinioides* (Fig. 2a). In *Scutia buxifolia*, *Condalia buxifolia*, *Kentrothamnus weddellianus*, *Colletia spinosissima*, and *C. paradoxa*, the endothecium is 2–3 cell layers thick (Fig. 2c, d), while *Gouania ulmifolia* and *Retanilla patagonica* have an endothecium with 2 or 3 cell layers only in the contact zone with the connective tissue.

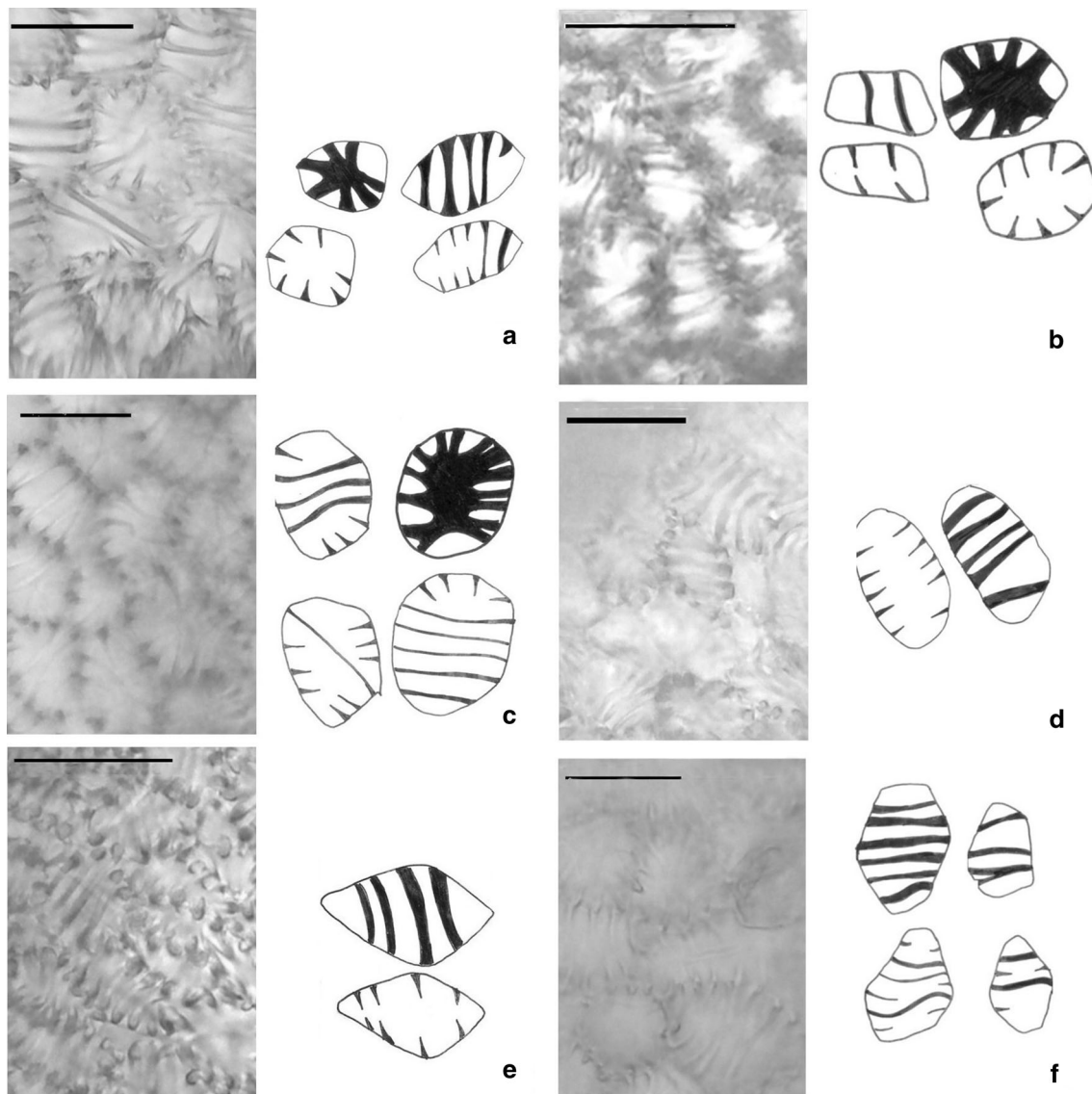


Fig. 3 Endothecium thickenings, bright-field microscopy. **a** *Hovenia dulcis*, u-shape with basal anastomosis and tympanate baseplate.; **b** *Gouania ulmifolia* u-shape with basal anastomosis and tympanate baseplate.; **c** *Ziziphus jujuba* u-shape patterns with basal anastomosis.;

d *Cryptandra tomentosa* u-shape pattern.; **e** *Retanilla patagonica* u-shape pattern.; **f** *Colletia paradoxa* u-shape patterns with basal anastomosis. Scale bar 250 μ m

Tapetal cells are persistent in *Hovenia dulcis* and *Gouania ulmifolia*, and a tapetal membrane is present in *Colletia spinosissima* and *C. paradoxa*. Anther width ranges from 300 μm in *Gouania ulmifolia* to 1000 μm in *Colletia spinosissima*.

Types of endothecium thickenings

A u-shape pattern of endothecium thickening is observed in *Scutia buxifolia*, *Condalia buxifolia*, *Cryptandra tomentosa*, *Siegfriedia darwinioides*, *Stenanthemum humile*,

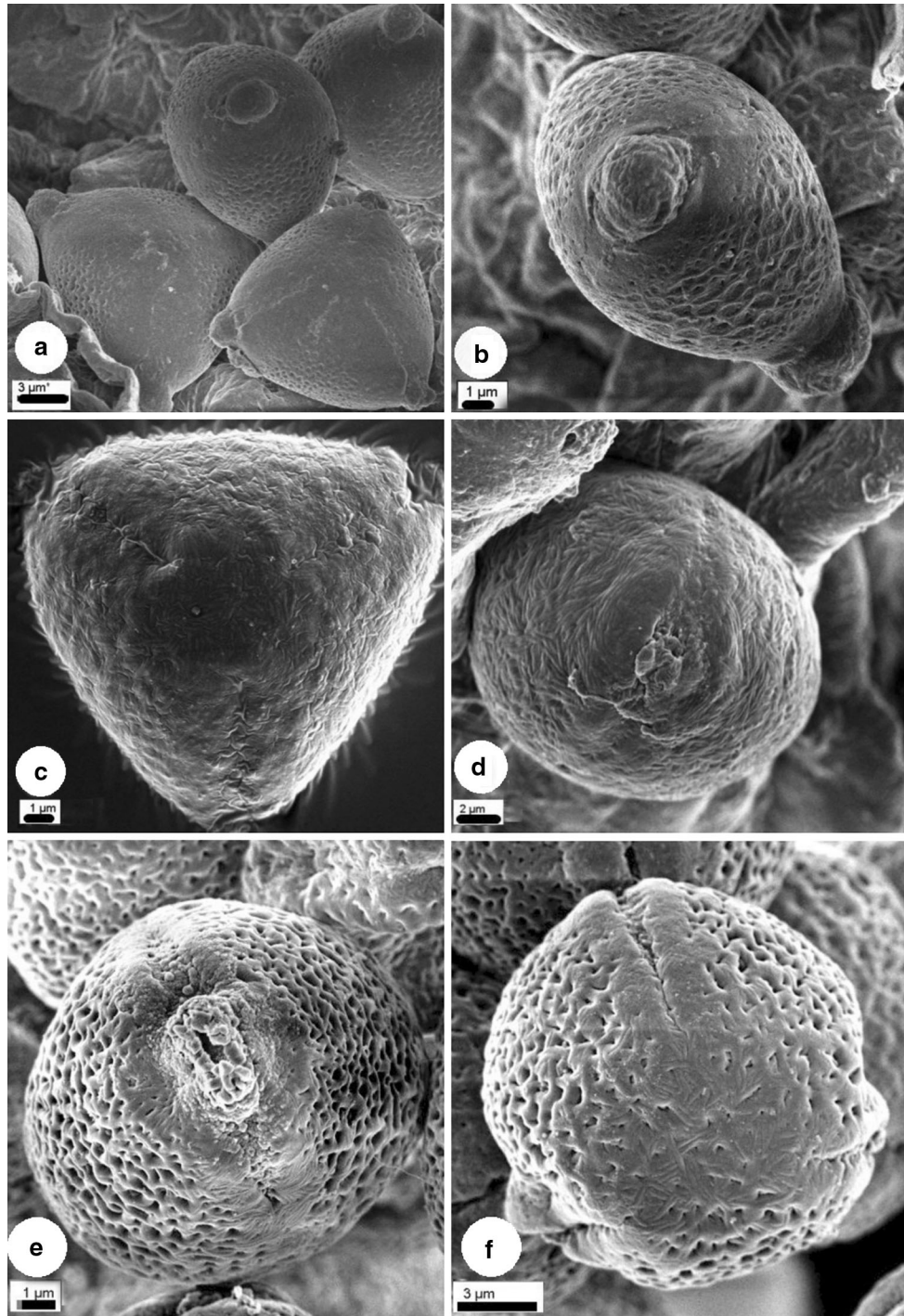


Fig. 4 Pollen grain morphology, scanning electron microscopy. **a** *Scutia buxifolia*.; **b** *Condalia buxifolia*.; **c, d** *Hovenia dulcis*.; **e, f** *Ziziphus mistol*

Retanilla patagonica, and *Kentrothamnus weddellianus*. U-shape patterns with basal anastomosis are present in *Ziziphus mucronata*, *Z. mistol*, *Z. jujuba*, *Colletia paradoxa* and *C. spinosissima*. In *Hovenia dulcis* and *Gouania ulmifolia*, thickenings show a tympanate baseplate (Fig. 3a–f; Table 1).

Pollen grain morphology (Table 1)

Scutia buxifolia

Pollen suboblate, anguloaperturate, three-colporate, triangular shape in polar view, convex mesocolpia. The pore has conspicuous intine protrusion with hemispheric aspect and irregular surface. Exine reticulate-rugulate, smooth in the pole and surrounding the colpi (*Rhamnus*-type) (Fig. 4a).

Condalia buxifolia

Pollen suboblate, three-colporate, triangular shape in polar view, plane mesocolpia with protruding apertures. The pore has conspicuous intine protrusion with hemispheric aspect and irregular surface. Exine reticulate-rugulate and smooth in the pole and surrounding the colpi (*Rhamnus*-type) (Fig. 4b).

Hovenia dulcis

Pollen oblate-spheroidal, three-colporate, triangular shape in polar view, slightly convex mesocolpia. The operculum is small with irregular surface. Exine striate-rugulate (*Hovenia*-type) (Fig. 4c, d).

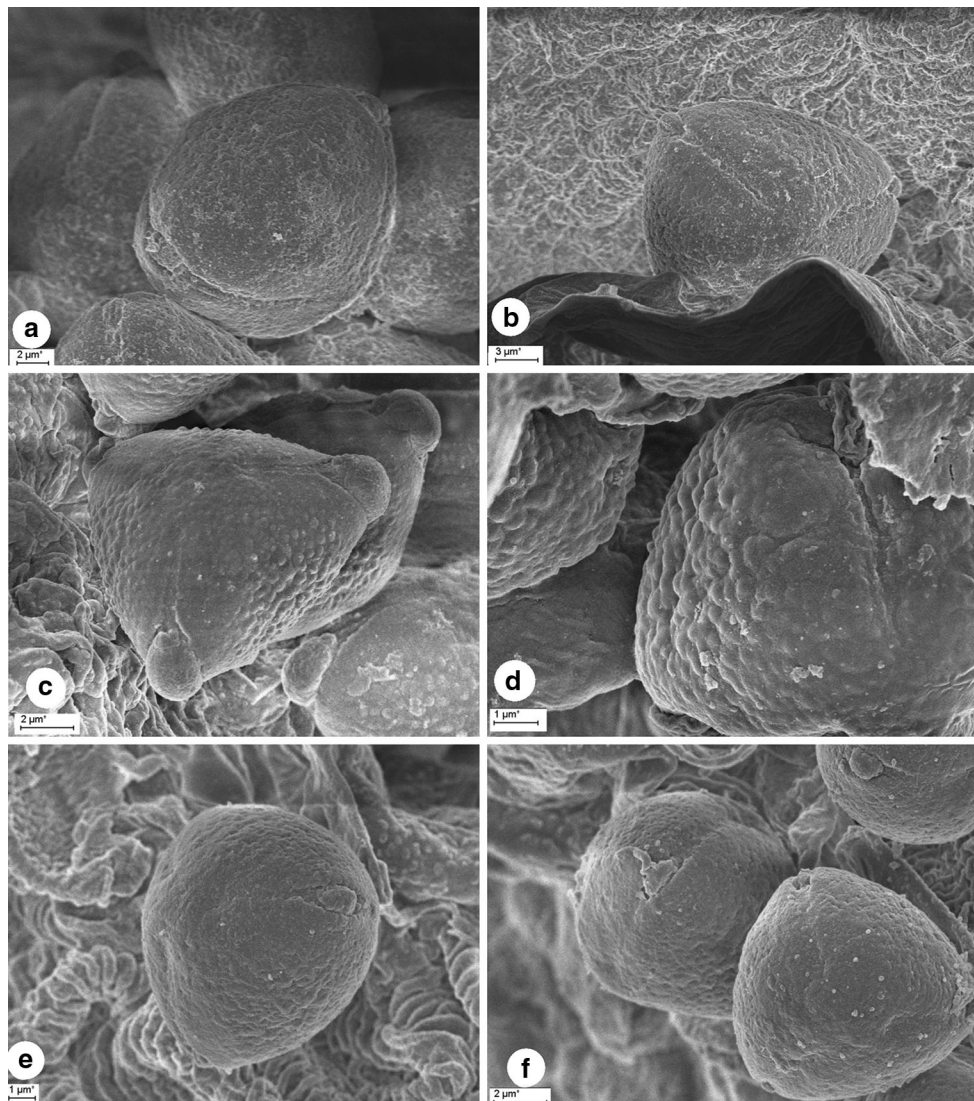


Fig. 5 Pollen grain morphology, scanning electron microscopy. **a, b** *Siegfriedia darwinioides*. **c, d** *Stenanthemum humile*. **e, f** *Cryptandra tomentosa*

Ziziphus mistol

Pollen suboblate, three-colporate, subtriangular shape in polar view, convex mesocolpia. Exine rugulate diminishing toward the poles and apertures (*Lasiodiscus*-type) (Fig. 4e, f).

Ziziphus mucronata

Pollen suboblate, three-colporate, subcircular shape in polar view. The operculum is small with irregular surface. Exine rugulate, smooth in the poles and surrounding the apertures (*Lasiodiscus*-type).

Ziziphus jujuba

Pollen suboblate, three-colporate, subcircular shape in polar view. Colpi narrow. The operculum is small with irregular surface. Exine rugulate-perforate diminishing toward the poles and apertures (*Lasiodiscus*-type).

Siegfriedia darwinioides

Pollen oblate-spheroidal, three-colporate, subtriangular shape in polar view, convex mesocolpia. Endoapertures alongate, colpi wide. Exine lightly rugulate (*Lasiodiscus*-type) (Fig. 5a, b).

Stenanthemum humile

Pollen suboblate, three-colporate, triangular in polar view. The operculum is conspicuous and has a smooth surface. Exine verrucate (*Pomaderris*-type) (Fig. 5c, d).

Cryptandra tomentosa

Pollen oblate-spheroidal, three-colporate, subtriangular shape in polar view, convex mesocolpia. The operculum is small with irregular surface. Exine lightly rugulate (*Lasiodiscus*-type) (Fig. 5e, f).

Kentrothamnus weddellianus

Pollen oblate-spheroidal, three-colporate operculate, triangular shape in polar view. The operculum is conspicuous with hemispheric aspect. Exine striate-rugulate (*Hovenia*-type) (Fig. 6a, b).

Retanilla patagonica

Pollen prolate spheroidal, three-colporate operculate, subtriangular in polar view, plane mesocolpia. Colpi narrow. Exine striate-rugulate (*Hovenia*-type) (Fig. 6c).

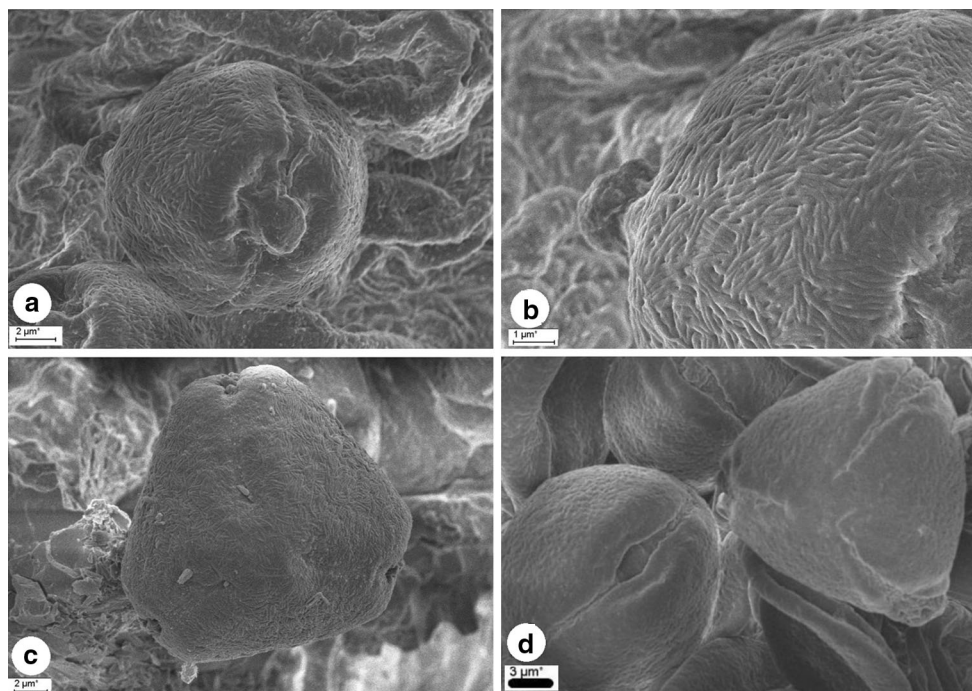


Fig. 6 Pollen grain morphology, scanning electron microscopy. **a–b** *Kentrothamnus weddellianus*. **c** *Retanilla patagonica*. **d** *Gouania ulmifolia*

Gouania ulmifolia

Pollen oblate-spheroidal, three-colporate, subtriangular shape in polar view, convex mesocolpia. Operculum reduced to absent. Exine fossulate-perforate, smooth in the poles and surrounding the apertures. (*Sageretia*-type) (Fig. 6d).

Orbicules (Table 1)

Orbicules are present in *Condalia buxifolia*, *Hovenia dulcis*, *Ziziphus mistol*, *Cryptandra tomentosa*, *Siegfriedia*

darwinioides, *Stenanthemum humile*, *Retanilla patagonica*, *Kentrothamnus weddellianus*, *Colletia paradoxa*, and *C. spinosissima*. No orbicules were observed in *Scutia buxifolia*, *Ziziphus mucronata*, *Z. jujuba*, and *Gouania ulmifolia* (Table 1; Fig. 7a).

The morphology of the orbicules was described following Galati's (2003) classification. In *Condalia buxifolia*, the orbicules have a cup aspect (Fig. 7b), while in *Hovenia dulcis* and *Kentrothamnus weddellianus*, they have a hemispherical to plaque aspect (Fig. 7c). Orbicules of *Ziziphus mistol* and both species of *Colletia* are spherical

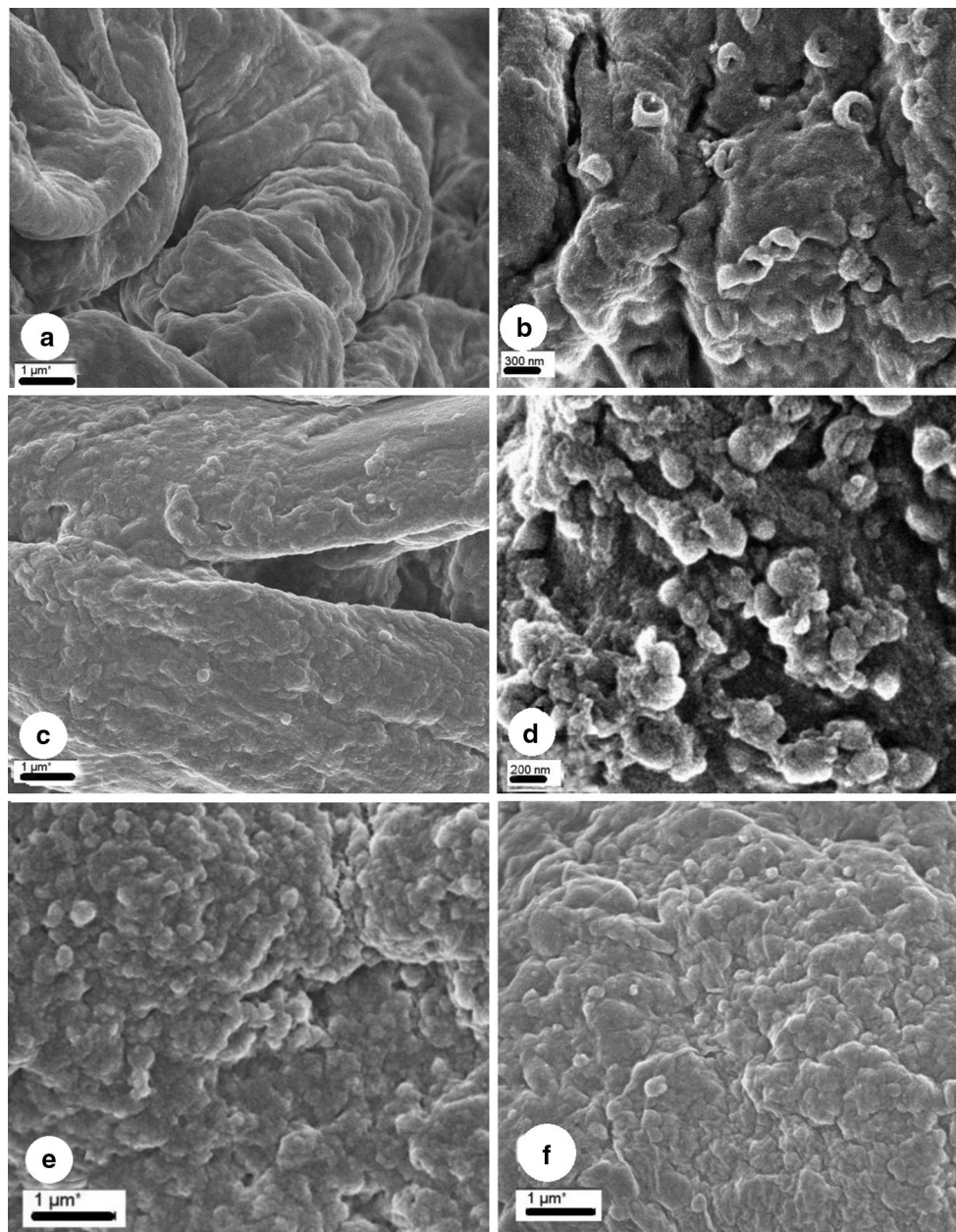


Fig. 7 Orbicules, scanning electron microscopy. **a** *Scutia buxifolia*. **b** *Condalia buxifolia*. **c** *Hovenia dulcis*. **d** *Ziziphus mistol*. **e** *Retanilla patagonica*. **f** *Cryptandra tomentosa*

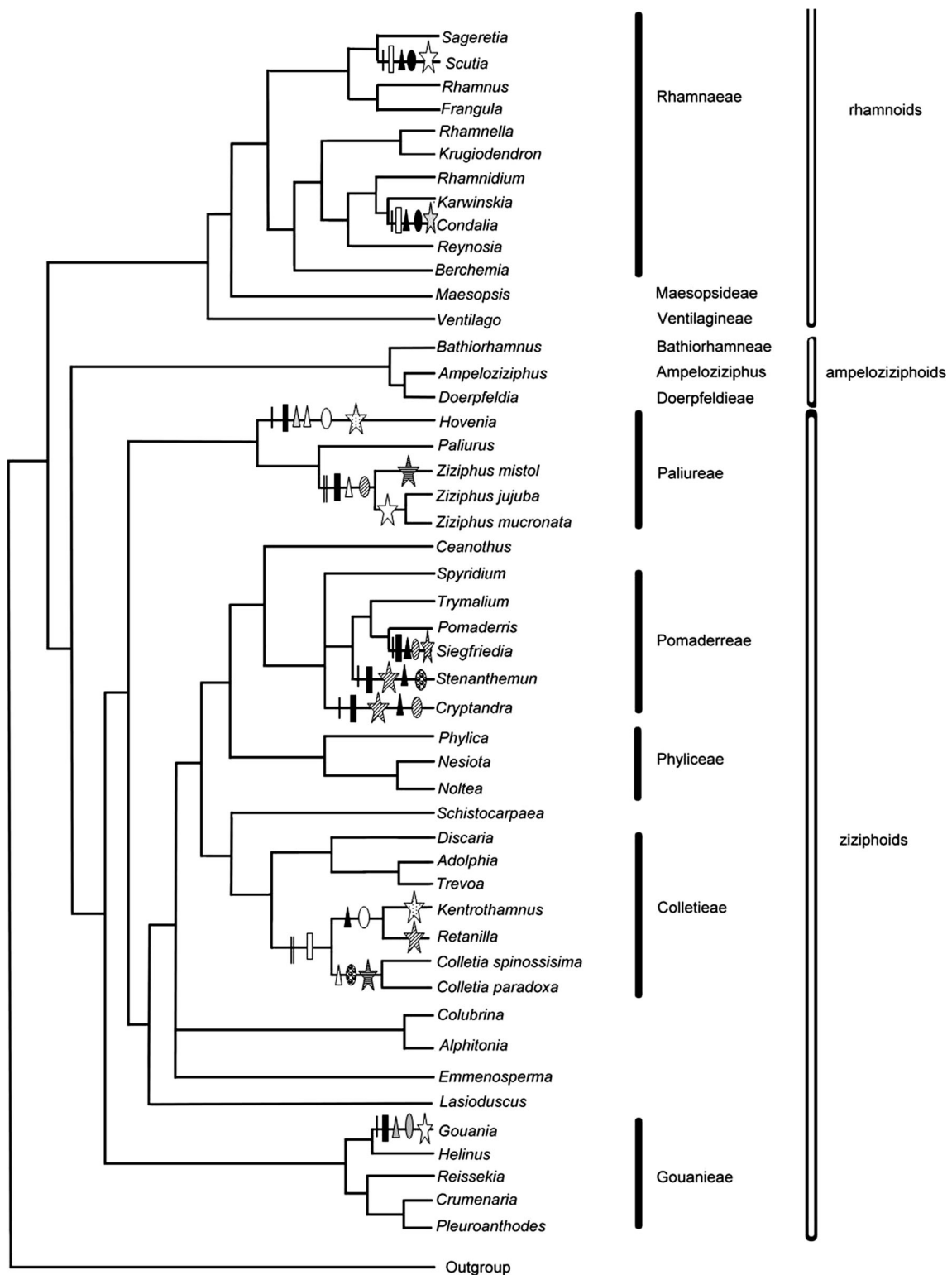


Fig. 8 Characters hand mapped in the modified single optimal SW tree from the combined *rbcL/trnL-F* from Richardson et al. (2000). Vertical bar Tapetal cells with one nucleus. Parallel to Tapetal cells with two nuclei. Black bar One endothecium layer; White bar Two to three endothecium layers. Black up pointing pointer U-shape endothecium thickening; white up pointing pointer basal anastomosis; shaded up pointing pointer tympanate baseplate. Pollen grain type:

stripped oval *Lasiodiscus*; *Pomaderris*; *Colletia*; white oval *Hovenia*; shaded oval *Sageretia*; black oval *Rhamnus*. Orbicules: black star presence; white star absence. Orbicule morphology: shaded star cup; dotted star hemispherical to plaque; stripped star spherical to subspherical; diagonally dotted star hemispherical

to subspherical (Fig. 7d). In *Stenanthemum humile*, *Cryptandra tomentosa*, *Retanilla patagonica*, and *Siegfriedia darwinioides*, the orbicules are hemispherical (Fig. 7e, f).

Discussion

This study provides detailed information about the anatomy of the anther and pollen morphology of 14 species of Rhamnaceae and a cladistic analysis using these characters (Fig. 8).

Differences found in mature anther structure seem to support tribal classification. Anther wall development is of the basic type (Davis 1966). This feature was described previously for *Colletia paradoxa* and *Discaria americana* (Gotelli et al. 2012). Tapetal cells of the species we studied from the Colletiae and Paliurae tribes, with the exception of *Hovenia dulcis*, are binucleate, while the ones of the Gouaniae and Rhamneae tribes are uninucleate. Our hypothesis is that the uninucleate tapetum is the plesiomorphic state of this character in the entire family, and the apomorphic state is the binucleate tapetum that appears in the tribe Colletiae and in the genus *Ziziphus*. However, in order to confirm this, more species of different genera of Rhamnaceae should be studied.

Another notable character is the endothecial thickening type. According to Manning (1996) thickening patterns reflect phylogenetic constraints and provide useful information since they are a stable character. In Rhamnaceae, a partial correlation between tribe and thickening type and a complete correlation between the species of a particular genus and the same state of this character are observed. Also, all genera of a particular tribe present the same number of endothecial layers, presenting a complete correlation.

Verstraete et al. (2014) reviewed the distribution of orbicules in the angiosperms and the only report of their presence in Rhamnales is in *Colletia paradoxa* and *Discaria americana*. In this paper, we found orbicules in *Condalia buxifolia*, *Ziziphus mistol*, *Hovenia dulcis*, *Cryptandra tomentosa*, *Siegfriedia darwinioides*, *Stenanthemum humile*, *Colletia spinosissima*, *Retanilla patagonica*, and *Kentrothamnus weddellianus*. In *Hovenia dulcis*, orbicules are scarce and were previously described by Gotelli et al. (2015) as orbicule-like structures. The systematic value of the orbicules was studied in a few angiosperm taxa (Raj and El-Ghazaly 1987; El-Ghazaly 1989; El-Ghazaly and Chaudhary 1993; Huysmans et al. 1997; Vinckier et al. 2000; Schols et al., 2001; Vinckier and Smets 2002a, b, c, 2003; D'Hondt et al.

2004; Rosenfeldt and Galati 2008; Huysmans et al. 2010; Verstraete et al. 2011; Lovisollo and Galati 2012). However, in this study, we did not find a clear correlation between tribes and orbicule morphology. What is more, we found both, presence and absence of orbicules, in a single genus (*Ziziphus*). Although there are no reports on presence and absence of orbicules within the same genus, it is noteworthy that this character is not usually studied in all the species of a genus. In Pomaderreae, all species have the same orbicule morphology (viz. hemispherical). There seems to be pattern of absence at the base of the family and presence in the more derived clades. However, we do not have enough information to support this hypothesis.

Pollen grain morphology is mostly in accordance with the general morphological pattern of the family Rhamnaceae (Johri et al. 1992; Schirarend and Köhler 1993; Punt and Hoen 1995; Perveen and Qaiser 2005). Schirarend and Köhler (1993) described 12 pollen types based on the tectum architecture for Rhamnaceae. The exine type of *Siegfriedia darwinioides* was described as fossulate—perforate (Schirarend and Köhler 1993), but we consider it as rugulate. The pollen morphology of *Ziziphus mistol*, *Cryptandra tomentosa*, *Stenanthemum humile*, *Retanilla patagonica*, and *Kentrothamnus weddellianus* was described for the first time in this paper and the observations support the classification of Schirarend and Köhler (1993). Previous reports show ample evidence of the systematic value of the pollen morphology (Erdtman 1986). In this study, we can observe some correlation, though not complete, between pollen morphology and the tribal classification made by Richardson et al. (2000a, b). However, according to Schirarend and Köhler (1993), the same pollen types appear in different tribes and each tribe presents several pollen types as well. Therefore, we cannot claim pollen morphology has systematic value at tribal level in Rhamnaceae.

It seems that for Rhamnaceae, morphological characters that have systematic value on a suprageneric level within the family are related to the anatomy of the reproductive sporophytic structures, while the gametophytic structures seem to be more variable. However, more studies are needed to confirm this.

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Compliance with ethical standards

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

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