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RESEARCH ARTICLE

Evolution of pollen grain morphology in *Amorimia* and allies evidences the importance of palynological apomorphies and homoplasies in Malpighiaceae systematics

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Abstract

Background and aims – Pollen grain morphology is an important morphological character for aiding the systematics of flowering plants. For Malpighiaceae, only a single unpublished palynological study has comprehensively sampled ca 60 of this family's 75 currently accepted genera. To test the systematic relevance of pollen morphology in *Amorimia* and allies, we characterised the pollen morphology of these lineages. We scored, coded, and mapped 12 characters onto the most recent molecular phylogeny of *Amorimia* and allies.
Material and methods – We sampled 13 species of *Amorimia* as ingroup and two species of *Mascagnia* and *Ectopopterys soejartoi* as outgroup. Pollen grains were acetolised, characterised, and measured using light microscopy and scanning electron microscopy. Pollen quantitative measurements were submitted to a PCA multivariate analysis. Additionally, quantitative and qualitative characters were scored and coded into 12 characters and mapped onto the molecular phylogeny of *Amorimia* and allies.
Key results – *Amorimia* and allies are stenopalynous due to all species showing the same pollen type, with some subtle differences between the pollen grains, such as details of ornamentation, shape, size, and thickness of the pollen exine. However, the patterns of pollen grain evolution showed that few qualitative and apomorphic characters are informative for intrageneric distinction (i.e. type and number of apertures), and almost all quantitative and homoplastic characters analysed were informative at infrageneric levels within Malpighiaceae.
Conclusion – Our results demonstrate that even though the pollen morphology characters of *Amorimia* and allies show subtle variation, both qualitative and quantitative apomorphic and/or homoplastic characters are highly informative for intra- and infrageneric levels in Malpighiaceae when analysed in a phylogenetic context.

Keywords

Ectopopterys, Malpighiales, *Mascagnia*, light microscopy, taxonomy, scanning electron microscopy

INTRODUCTION

Pollen grains (i.e. male gametophytes) are one of the key innovations that allowed seed plants to successfully colonise terrestrial habitats (Wallace et al. 2011). These male gametophytes comprise an inner triploid reproductive cell and an outer protective wall (i.e. the exine) made mainly of sporopollenin, which is

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incredibly resistant to degradation (Wallace et al. 2011; Williams et al. 2014). The pollen wall and other pollen morphological traits show different layers, structures, and ornamentations used over the past two centuries to improve the taxonomic classification of different ranks of flowering plants (Melhem 1978; Bahadur et al. 2022). The discovery of pollen grains was made by Marcello Malpighi in 1670, but studies detailing their morphology in several groups of plants have only arisen about two centuries later (i.e. 19th and 20th centuries) due to innovations in light microscopy (Melhem 1978; Melhem et al. 2003). Since then, pollen morphology has been widely used to aid plant taxonomic studies for the past two centuries (Lindley 1830).

Nonetheless, its central role in plant systematics was only established three decades ago by the first molecular phylogenetic studies of flowering plants. These studies found that the traditional division of angiosperms in dicots/monocots was artificial, with only monocots representing a natural group (Chase et al. 1993). The dicots represented, in fact, several early diverging or derived lineages in flowering plants, with its largest clade, the eudicots (i.e. the new dicots), being solely differentiated by their tricolpate pollen grains from the remaining angiosperms (i.e. basal angiosperms and monocots) showing monosulcate pollen grains (APG 1998, 2016). Since then, several studies have been published to explore the morphological characterisation and phylogenetic relevance of pollen in several major lineages of angiosperms (i.e. basal angiosperms – Lu et al. 2015; monocots – Furness and Rudall 2001; Lu et al. 2015; and eudicots – Yu et al. 2018), orders (e.g. Myrtales – Kriebel et al. 2017), and families (e.g. Amaranthaceae – Müller and Borsch 2005; Annonaceae – Doyle and Thomas 2012; Euphorbiaceae – Cardinal-McTeague and Gillespie 2016; Loranthaceae – Grímsson et al. 2019; Myrtaceae – Thornhill and Crisp 2012; Rubiaceae – Dessein et al. 2005; Zingiberaceae – Zou et al. 2022).

Malpighiaceae is a medium-sized family of flowering plants comprising 75 genera and ca 1,400 species, mostly endemic to the Neotropics (Almeida and van den Berg 2021; POWO 2023). In Brazil, 56 genera and 592 species of this family are recorded (Flora e Funga do Brasil 2023). Its species are characterised by a conspicuous floral conservatism represented by calyx oil glands, unguiculate petals, and Malpighiaceous pollen type (Anderson 1979, 1981). Due to molecular phylogenetic studies, this family has undergone unprecedented changes in its traditional classification in the past few years (Cameron et al. 2001; Davis et al. 2001; Davis and Anderson 2010). The recognition of new lineages brought to light deep taxonomic problems regarding the monophyly of subfamilies, tribes, and genera (Cameron et al. 2001; Davis et al. 2001; Davis and Anderson 2010; Almeida et al. 2017a; Almeida and van den Berg 2021). Since then, different authors have gradually proposed new genera and combinations (Anderson 2006; Davis and Anderson 2010) to accommodate these newly identified lineages.

Some studies describe the palynology of Malpighiaceae, but most of these only present details of a few genera or isolated species. In Erdtman (1952), Lobreau (1967), Anderson (1982), Lobreau-Callen (1983), Makino (1986), Makino-Watanabe et al. (1993a, 1993b, 1998), Gonçalves-Esteves et al. (2007), Sebastiani et al. (2014), Belonsi and Gasparino (2015), and Chaisongkram et al. (2022), we can observe the characterisation of pollen diversity in the family, and sometimes, its use in the taxonomy of some groups. As occurs in most eudicot families, it is common to observe stenopalynous genera in eurypalynous families (Harley 1991; Luz et al. 2013; Teixeira et al. 2013; Gasparino et al. 2021). For Malpighiaceae, Belonsi and Gasparino (2015) highlighted the taxonomic importance of pollen characters, such as polarity, exine ornamentation, and the type of apertures, in distinguishing genera (especially stenopalynous ones). In some cases, such as in *Banisteriopsis*, the amb, details of the apertures (presence of aspides), and thickness of the exine can help distinguish species (Belonsi and Gasparino 2015).

Amorimia W.R.Anderson is one of the several new lineages identified on those previous molecular phylogenies (Anderson 2006; Davis and Anderson 2010), representing one of the eight genera segregated from the polyphyletic *Mascagnia* (Bertero ex DC.) Bertero, but remaining closely related to this genus. *Amorimia* was described by Anderson (2006) to accommodate ten species of lianas and shrubs mostly confined to Seasonally Dry Tropical Forests of South America. It is currently distinguished from other Malpighiaceae by the presence of glands on the abaxial side of inflorescence bracts, petals pubescent on both sides, and straight styles (Almeida et al. 2016; Almeida 2018). The monophyly of *Amorimia* was corroborated by Almeida et al. (2017a) and Almeida (2018), with two subgenera being proposed for their currently 15 accepted species. The same authors recovered several macro- and micromorphological synapomorphies supporting the recognition of both subgenera, including pollen amb (Almeida et al. 2017a).

In this study, we describe in detail the pollen morphology of 13 (out of 15) accepted species of *Amorimia* and allies (*Ectopopterys* W.R.Anderson and *Mascagnia*) and use the phylogenetic framework presented by Almeida (2018) as the basis for further understanding the patterns of pollen morphology evolution in the genus. We scored and coded 12 micromorphological characters to test for secondary homologies.

MATERIAL AND METHODS

Sampling

A total of 13 species of *Amorimia* were sampled (out of 15), comprising both subgenera currently recognised by Almeida et al. (2017a) (Supplementary material 1). Only two species of *Amorimia* could not be sampled (*A.*

andersonii R.F.Almeida and *A. tumida* R.F.Almeida & A.C.Marques) due to the lack of flower buds or flowering specimens. *Ectopopterys soejartoi* W.R.Anderson and *Mascagnia cordifolia* (A.Juss.) Griseb. were sampled as outgroup (Supplementary material 1).

Pollen analysis

For light microscopy (LM), the pollen grains were treated with the acetolysis method (Erdtman 1960), with modifications cited by Melhem et al. (2003). The obtained microscope slides were incorporated into the pollen slide collection from the Plant Morphology and Palynology Lab, São Paulo State University, Campus Jaboticabal. Pollen micrographs were taken using an optic microscope Leica IM50 coupled with a video camera and computer, and digitally treated and edited using Photoshop. For scanning electron microscopy (SEM), the material was prepared following Melhem et al. (2003) for non-acetolised pollen grains. The pollen electron micrographs were generated using a JEOL JSM5410 scanning electron microscope.

Pollen descriptions and measurements

Pollen descriptions follow the terminology by Barth and Melhem (1988), Punt et al. (2007), and Bellonzi et al. (2020) for LM. The pollen grains of the analysed *Amorimia* species using SEM are described following Halbritter et al. (2018). Shape and size terminology follows Erdtman (1952), and exine thickness follows the classification (i.e. very thin, thin, or thick) proposed by Faegri and Iversen (1950). Diameter measurements (DI and DII) were taken within seven days of acetolysis to avoid pollen grain alterations related to the method (Melhem and Matos 1972). All measurements were randomly taken for 25 pollen grains. In contrast, other pollen characters (i.e. aperture, total exine, sexine, nexine, and tectum) were randomly measured for only ten pollen grains (Melhem and Matos 1972; Salgado-Laboriau 1973). The measurements referring to the tectum are included in the value of the sexine, as this structure is part of the sexine.

Statistical analyses

We calculated the arithmetic mean (x), average standard deviation (sx), sample standard deviation (s), coefficient of variability (CV), and 95% confidence interval following Zar (1996) and Vieira (2011). Only the arithmetic average (x) was calculated for measurements of n = 10. Diameter I and II measurements were used to compare diameter values of the analysed pollen grains for *Amorimia* and outgroups using MINITAB v.14 (Zar 1996; Vieira 2011). One principal component analysis (PCA) was performed using FITOPAC 1 (Shepherd 1996) and PC-ORD 5 (McCune and Mefford 2011) to verify the influence of pollen grain quantitative data on species ordination and grouping. The analysis used nine metric variables: diameter I (DIAI), diameter II (DIAII), ectoaperture length (ECLEN), ectoaperture width (ECWID), exine (EXIN), sexine (SEXI), nexine (NEXI), tectum (TECT), and shape (SHAP) (Supplementary material 2).

Character mapping analyses

The consensus phylogenetic tree by Almeida (2018) was pruned with Mesquite v.2.73 (Maddison and Maddison 2006) to show only the outgroup sampled in our study and used for the character mapping of the pollen morphology. Character coding followed the recommendations of Sereno (2007) for morphological analyses. Primary homology hypotheses (De Pinna 1991) were proposed for pollen shape, size, ornamentation, exine structure, and apertures. A total of 12 pollen characters were scored for *Amorimia* and outgroups (Supplementary material 3). In addition to *Mascagnia cordifolia*, we sampled *M. sepium* from the palynological literature (Makino 1986) for the character mapping analyses. All characters were optimised on the concatenated tree using the Maximum Likelihood function (mk1 model) using Mesquite v.2.73 (Maddison and Maddison 2006) and visualised with Winclada (beta) v.0.9 (Nixon 1999).

RESULTS

Qualitative data – general description

Pollen grains of all studied species of *Amorimia*, *Ectopopterys*, and *Mascagnia* are monads (Figs 1–4), polar in *Ectopopterys* or apolar, medium (Figs 1–2, 4–5; Supplementary material 4) to large (Figs 3–5), with circular amb, oblate-spheroidal to prolate-spheroidal (Figs 1–4). Apertures zonocolporate, 3-colporate, with long and narrow ectoaperture and circular endoaperture in *Ectopopterys* (Fig. 3F–G) or pantoporate 6-porate in *Amorimia*, and 8-porate with colpoids (Figs 1–4) in *Mascagnia* (Fig. 3H–I). Exine tectate, sexine rugulate (Fig. 2C–D), with or without areolae (Fig. 1L) or psilate (Fig. 1B) regions near the pores or distributed over the pollen grain surface (Fig. 1G–H). The exine thickness varies from very thin (*M. cordifolia*) to thin or thick (Supplementary material 5) according to the averages of the diameters 1 and 2, with sexine thicker than the nexine (Fig. 3F; Supplementary material 5).

Amorimia (Figs 1–2, 3A–E, 4)

Pollen grains are monads, apolar, medium or large (*Amorimia velutina* and *A. kariniana*), oblate-spheroidal or prolate-spheroidal, 6-porate, pantoporate, with colpoids (sometimes not evident in *A. amazonica*) (Supplementary material 4). Exine tectate, sexine rugulate, with psilate regions (only in *A. candidae*, *A. velutina*, *A. amazonica*, and *A. septentrionalis*; Figs 1A–C, 2C–F, 3C–E, 4A–D, N–O, Q–R) or with areolate regions. The exine thickness

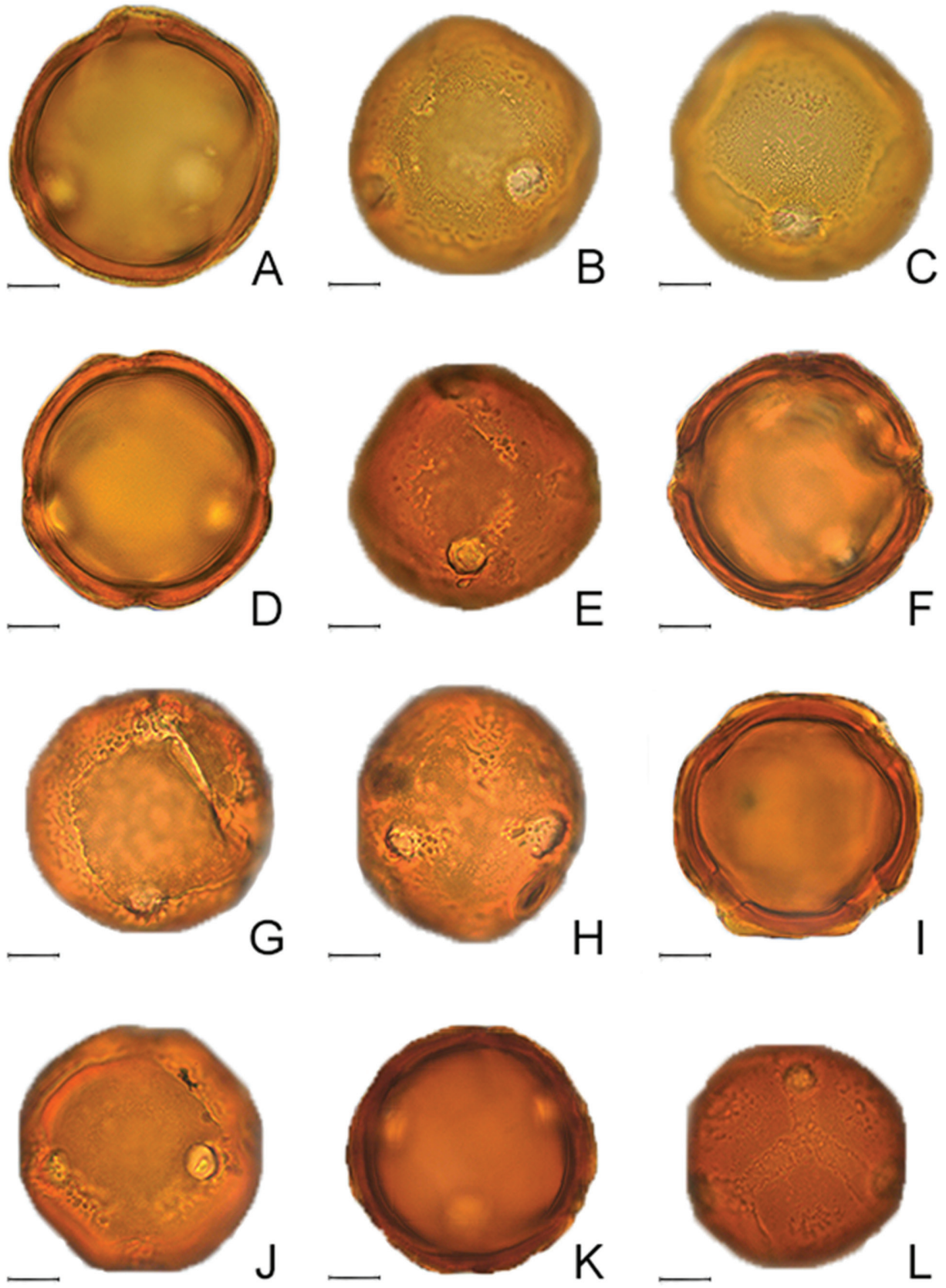

Figure 1. Photomicrographs of *Amorimia* species from light microscopy. **A**–**B**. *Amorimia candidae*. **C**–**E**. *Amorimia coriacea*. **F**–**H**. *Amorimia exotropica*. **I**–**J**. *Amorimia maritima*. **K**–**L**. *Amorimia pellegrinii*. **A**, **D**, **F**, **I**, **K**. Exine. **B**–**C**, **E**, **G**–**H**, **J**, **L**. Ornamentation and apertures. Scale bars: 10 µm.

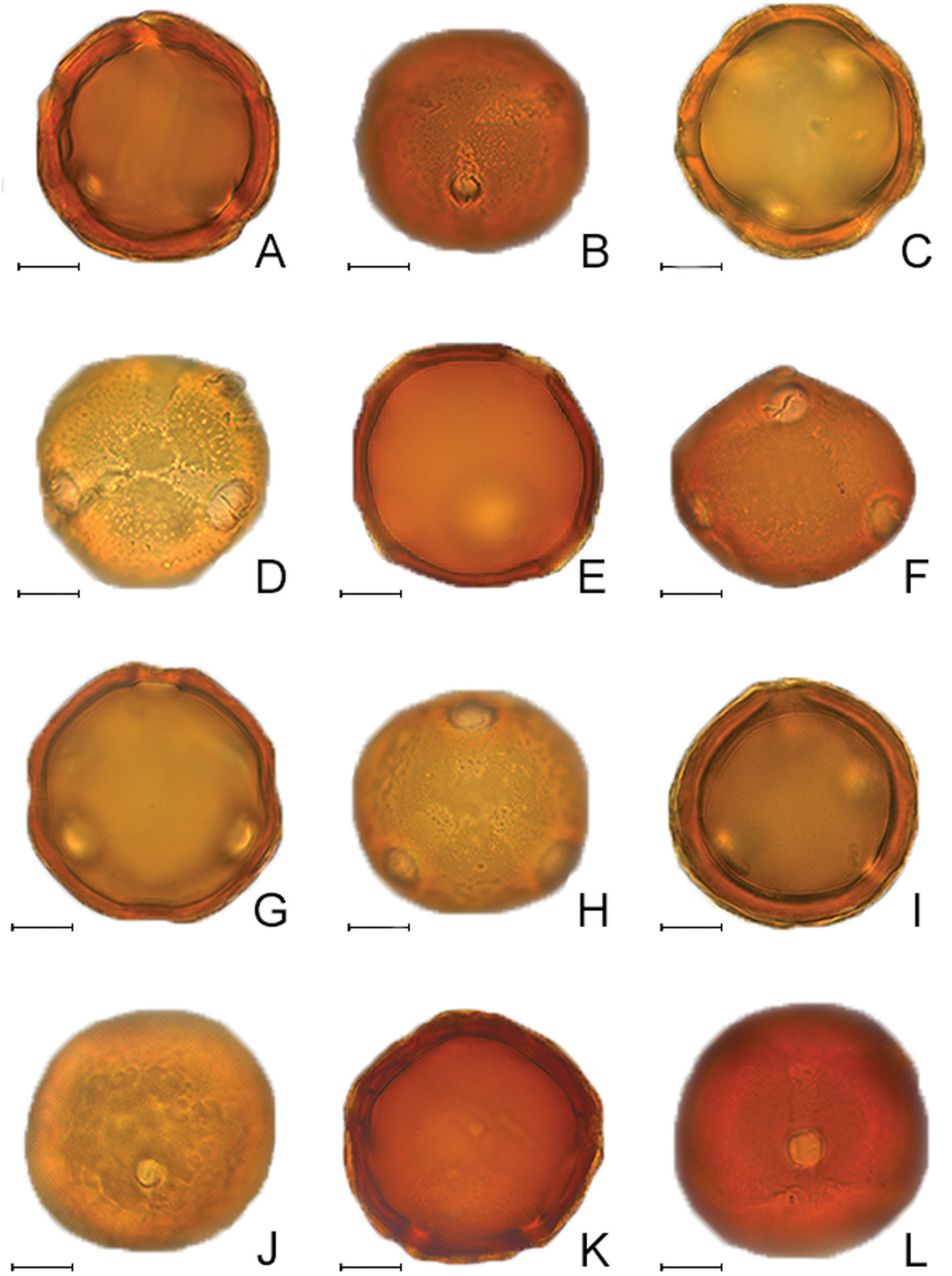

Figure 2. Photomicrographs of *Amorimia* species from light microscopy. **A**–**B**. *Amorimia rigida*. **C**–**D**. *Amorimia velutina*. **E**–**F**. *Amorimia amazonica*. **G**–**H**. *Amorimia camporum*. **I**–**J**. *Amorimia concinna*. **K**–**L**. *Amorimia kariniana*. **A**, **C**, **E**, **G**, **I**, **K**. Exine. **B**, **D**, **F**, **H**, **J**, **L**. Ornamentation and apertures. Scale bars: 10 μm.

varies from thin to thick (*A. maritima*, *A. rigida*, *A. velutina*, *A. camporum*, and *A. concinna*) and sexine is thicker than nexine (Supplementary material 5). In SEM, the pollen grains are clypeate (except for *A. amazonica*; Fig. 4A–B) and the exine fossulate (*A. amazonica*, *A. pubiflora*, *A. velutina*; Fig. 4A–B, N–P) or psilate-perforate (other species; Fig. 4C–G, I–M, Q–T).

Ectopopterys (Fig. 3F–G)

Pollen grains are monads, polar, medium, circular amb, prolate-spheroidal, 3-colporate, zonocolporate, with long and narrow ectoaperture, without margo, and circular endoaperture. Exine tectate, sexine rugulate with areolate regions. The exine thickness is thin, sexine thicker than nexine (Supplementary material 5).

Mascagnia (Fig. 3H–I)

Pollen grains are monads, apolar, medium, prolate-spheroidal (Supplementary material 4), 8-porate, pantoporate, with not evident colpoids. Exine tectate, sexine rugulate with psilate regions. The exine thickness is very thin, sexine thicker than nexine (Supplementary material 5).

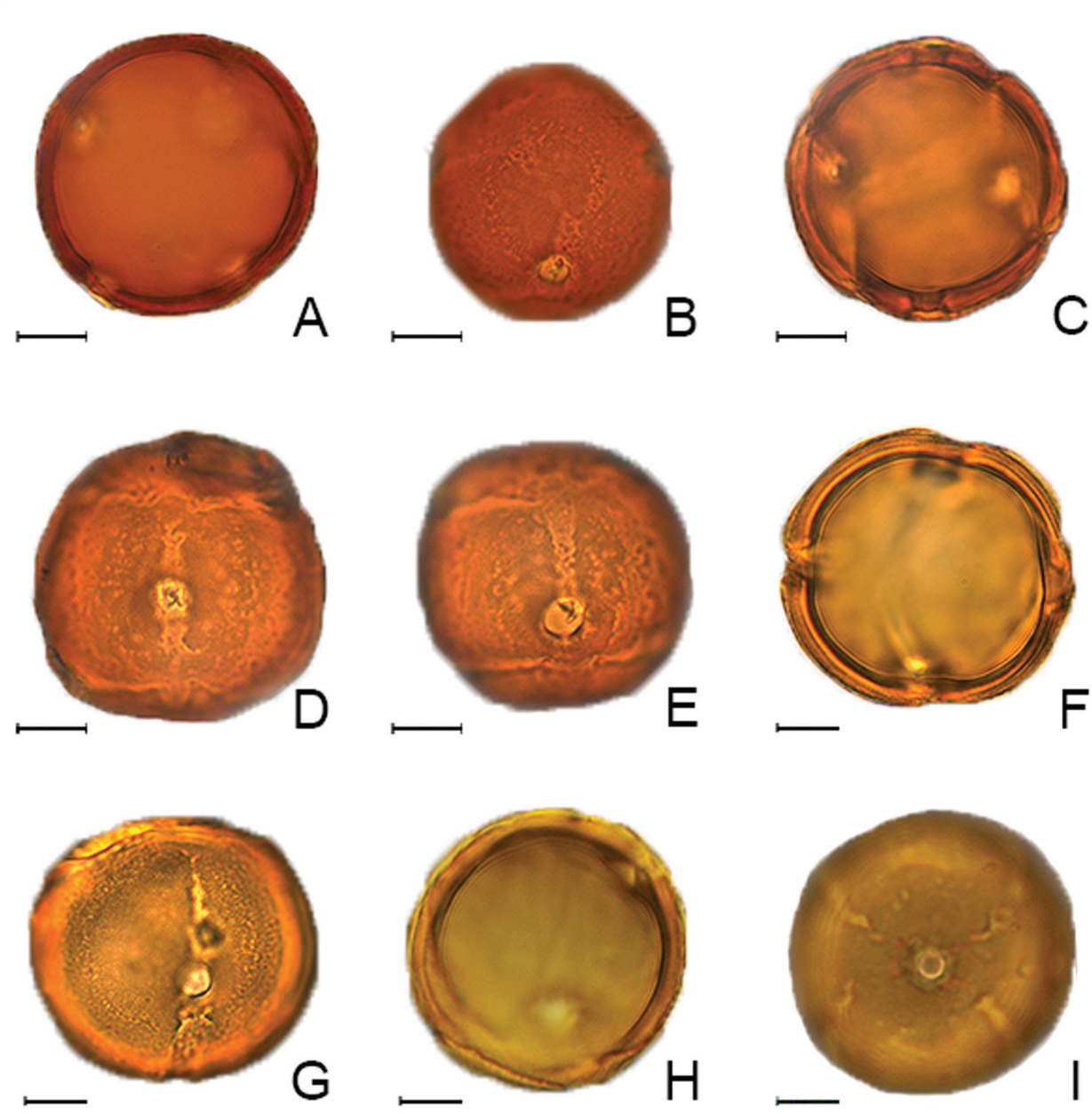

Figure 3. Photomicrographs of *Amorimia* species and outgroups from light microscopy. **A**–**B**. *Amorimia pubiflora*. **C**–**E**. *Amorimia septentrionalis*. **F**–**G**. *Ectopopterys soejartoi*. **H**–**I**. *Mascagnia cordifolia*. **A**, **C**, **F**, **H**. Exine. **B**, **D**–**E**, **G**. Ornamentation and apertures. **I**. Aperture. Scale bars: 10 μm.

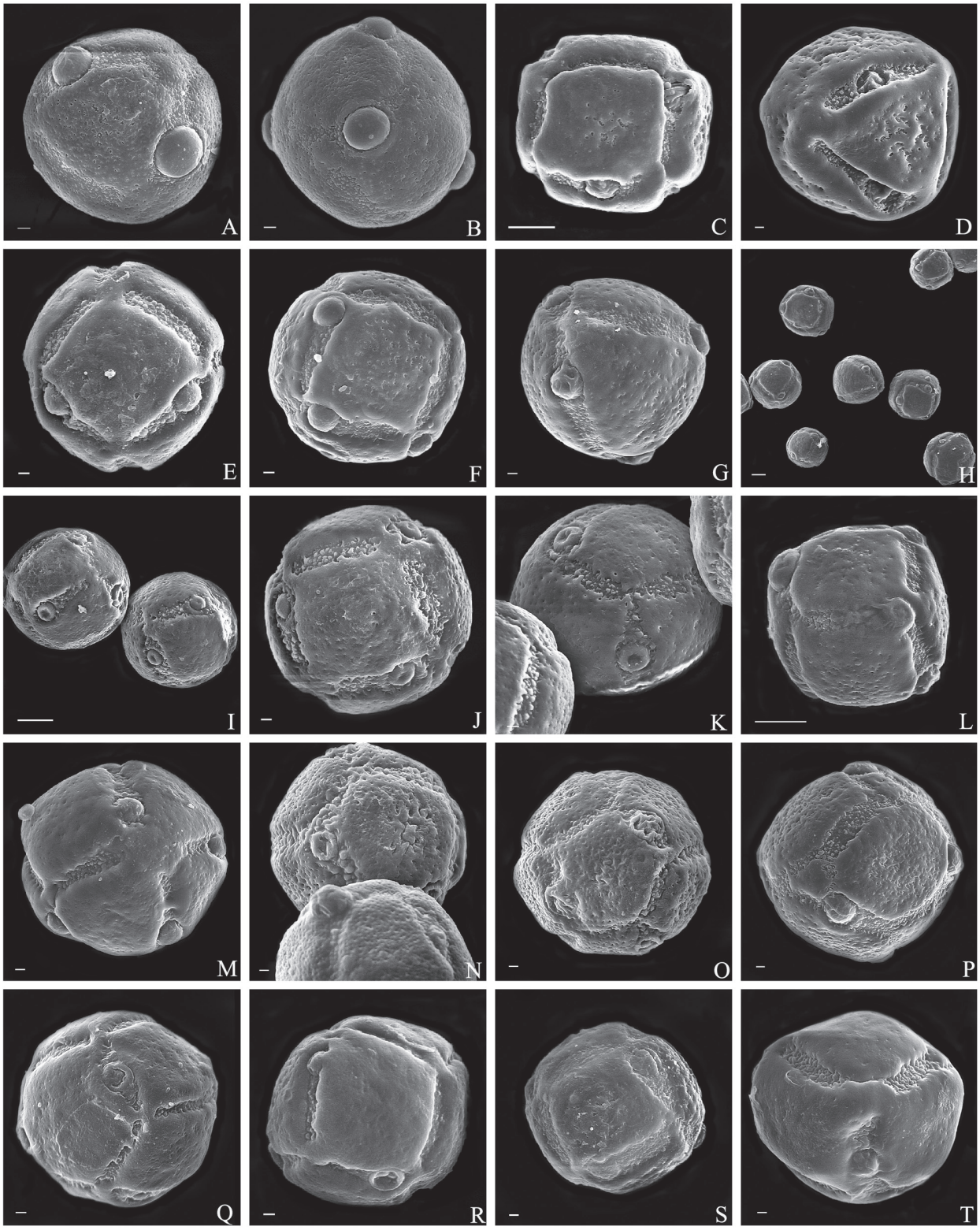

Figure 4. Scanning electron micrographs of *Amorimia* species. **A**–**B**. *Amorimia amazonica*. **C**–**D**. *Amorimia candidae*. **E**. *Amorimia coriacea*. **F**–**H**. *Amorimia exotropica*. **I**–**K**. *Amorimia maritima*. **L**–**M**. *Amorimia rigida*. **N**–**O**. *Amorimia velutina*. **P**. *Amorimia pubiflora*. **Q**–**R**. *Amorimia septentrionalis*. **S**–**T**. *Amorimia camporum*. **A**, **C**, **L**, **N**, **R**, **S**. Ornamentation. **B**, **D**, **M**, **O**, **Q**, **T**. Apertures. **H**–**I**. General view. **E**–**G**, **J**–**K**, **P**. Ornamentation and apertures. Scale bars: A–B, D–G, J–K, M–T = 2 µm; C, H–I, L = 10 µm.

Identification key for the studied species of *Amorimia*, *Ectopopterys*, and *Mascagnia* (based on light microscopy)

1.	Pollen grains colporate	***Ectopopterys soejartoi***
–	Pollen grains porate	**2**
2.	Pollen grains 8-porate	***Mascagnia cordifolia***
–	Pollen grains 6-porate	**3**
3.	Pollen grains large	**4**
–	Pollen grains medium	**5**
4.	Exine thin	***Amorimia kariniana***
–	Exine thick	***Amorimia velutina***
5.	Exine thick	**6**
–	Exine thin	**9**
6.	Pollen grain diameter < 40 µm	**7**
–	Pollen grain diameter > 40 µm	**8**
7.	Pore size < 5 µm	***Amorimia concinna***
–	Pore size > 5 µm	***Amorimia camporum***
8.	Pollen grain diameter on average 43–44 µm	***Amorimia rigida***
–	Pollen grain diameter on average 45–46 µm	***Amorimia maritima***
9.	Exine rugulate with psilate regions	**10**
–	Exine rugulate with areolate regions	**12**
10.	Oblate-spheroidal pollen grains	***Amorimia candidae***
–	Prolate-spheroidal pollen grains	**11**
11.	Exine thickness < 3.4 µm	***Amorimia amazonica***
–	Exine thickness > 3.4 µm	***Amorimia septentrionalis***
12.	Exine thickness < 3.8 µm	**13**
–	Exine thickness > 3.8 µm	**14**
13.	Pore size < 6.4 µm	***Amorimia pubiflora***
–	Pore size > 6.4 µm	***Amorimia coriacea***
14.	Pollen grain diameter on average < 46.5 µm	***Amorimia exotropica***
–	Pollen grain diameter on average > 46.5 µm	***Amorimia pellegrinii***

Quantitative data

The quantitative data analyses used pollen grain diameters and their respective averages and confidence intervals (Supplementary materials 4–5). We observed three distinct groups when analysing the metric values of the diameters (Fig. 5): 1. Smallest diameter species (*A. amazonica*, *A. camporum*, and *A. concinna*), 2. Intermediate diameter species (*A. coriacea*, *A. exotropica*, *A. maritima*, *A. rigida*, *A. pubiflora*, *A. septentrionalis*, and *M. cordifolia*), and 3. Largest diameter species (*A. candidae*, *A. kariniana*, *A. pellegrinii*, *A. velutina*, and *E. soejartoi*).

The PCA summarised 78.82% of the total variability of the data, in which axis 1 was more informative to the PCA since it summarised 43.47% of the variability (Fig. 6). The analysed *Mascagnia* and *Ectopopterys* were recovered far from those of *Amorimia* (Fig. 6). The species of *A.* subg. *Uncinae* showed lower values for all metric variables analysed (negative side of axis 1), except for *A. camporum* and *A. kariniana*, which were positioned with the species of *A.* subg. *Amorimia.* For axis 1, the most significant variable was ECLEN (Supplementary material 2), which distinguished *Ectopopterys* from other species by the colporate ectoaperture. Axis 2 summarised 31.34 % of the variability in our data; the most significant variables for this axis were SEXI and ECWID (Supplementary material 2). Note that the species of *Mascagnia* and *Ectopopterys* had lower values for these variables and were close to *A. amazonica*. In general, the species of *A.* subg. *Uncinae* are positioned on the positive side of axis 2, except for *A. camporum*, which appears alongside the species of *A.* subg. *Amorimia* with higher values for the variables that stand out in the ordination of axis 2.

Character mapping

All lineages from the molecular phylogeny were recovered with at least one or more homoplasies/apomorphies, except for both *Amorimia* subgenera (*A.* subg. *Amorimia* and *A.* subg. *Uncinae*). *Ectopopterys soejartoi* was recovered supported by three homoplasies regarding exine thickness (3.00–3.99 µm), tectum thickness (1.00–1.50 µm), and aperture width (4.00–4.99 µm), and a single synapomorphy regarding the exine ornamentation (rugulate with areolate regions). The *Amorimia* + *Mascagnia* clade was recovered supported by a single homoplasy regarding the aperture length (6.00–6.99 µm) and two synapomorphies regarding the apertures type (porate) and aperture number (= 6). *Mascagnia* was recovered supported by one homoplasy regarding sexine thickness (1.00–1.99 µm) and two synapomorphies

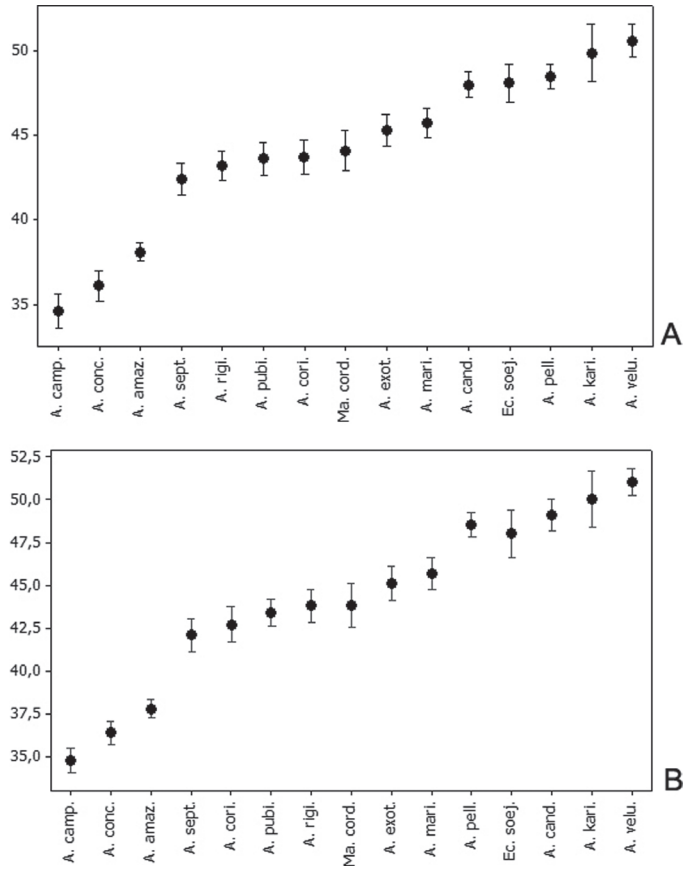

Figure 5. Diameter averages of the pollen grains of *Amorimia* and outgroups. **A**. Diameter I. **B**. Diameter II. Circles show the arithmetic average of the diameter values of pollen grains and their variation limits represented by the confidence interval. A. camp. = *Amorimia camporum*; A. conc. = *Amorimia concinna*; A. amaz. = *Amorimia amazonica*; A. sept. = *Amorimia septentrionalis*; A. cori. = *Amorimia coriacea*; A. pubi. = *Amorimia pubiflora*; A. rigi. = *Amorimia rigida*; Ma. cord. = *Mascagnia cordifolia*; A. exot. = *Amorimia exotropica*; A. mari. = *Amorimia maritima*; A. pell. = *Amorimia pellegrinii*; Ec. soej. = *Ectopopterys soejartoi*. A. cand. = *Amorimia candidae*; A. kari. = *Amorimia kariniana*; A. velu. = *Amorimia velutina*.

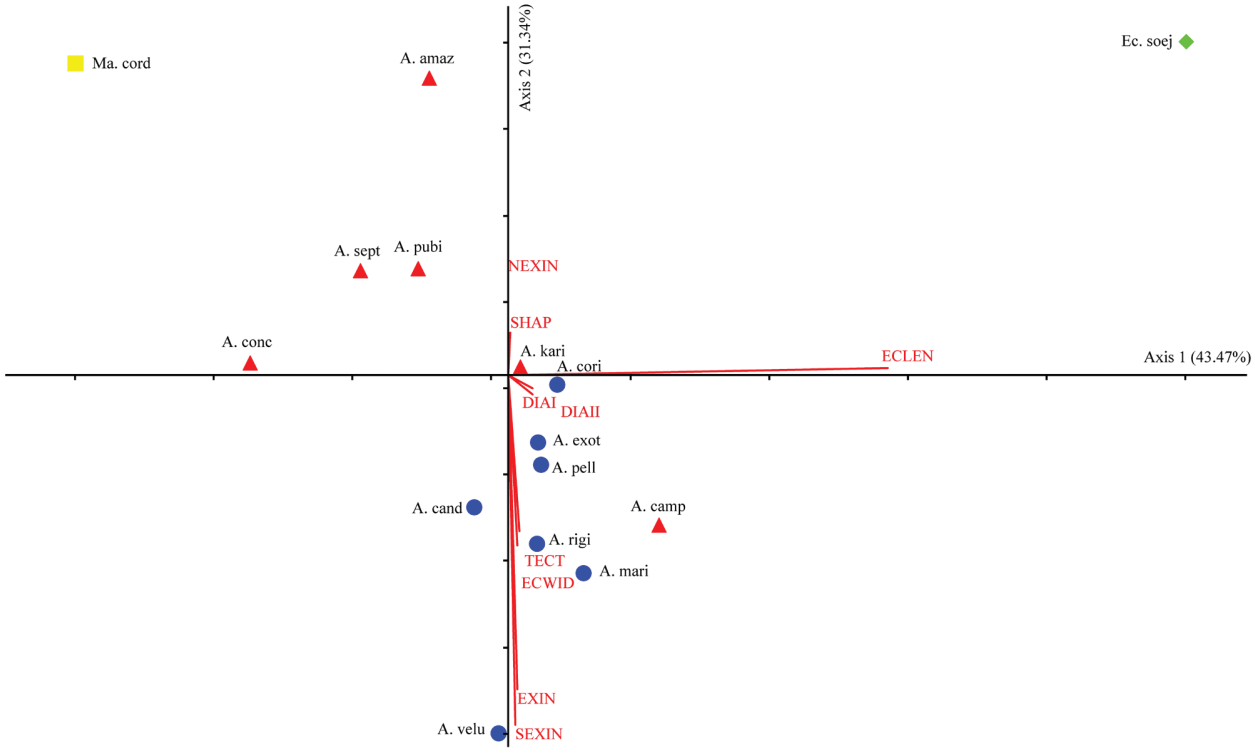

Figure 6. PCA ordination of the species of *Amorimia* subg. *Amorimia* (blue circles), *Amorimia* subg. *Uncinae* (red triangles), *Ectopopterys* (green diamond), and *Mascagnia* (yellow square). A. cand = *Amorimia candidae*; A. cori = *Amorimia coriacea*; A. exot = *Amorimia exotropica*; A. mari = *Amorimia maritima*; A. pell = *Amorimia pellegrinii*; A. rigi = *Amorimia rigida*; A. velu = *Amorimia velutina*; A. amaz = *Amorimia amazonica*; A. camp = *Amorimia camporum*; A. conc = *Amorimia concinna*; A. kari = *Amorimia kariniana*; A. pubi = *Amorimia pubiflora*; A. sept = *Amorimia septentrionalis*. Ec. soej = *Ectopopterys soejartoi*; Ma. cord = *Mascagnia cordifolia*.

Table 1. List of homoplasies and apomorphies (including synapomorphies and autapomorphies) recovered for all lineages in this study.

Lineages	Homoplasies	Apomorphies
Ectopopterys soejartoi	exine thickness 3.00–3.99 µm; tectum thickness 1.00–1.50 µm; aperture width 4.00–4.99 µm	aperture type colporate; sexine rugulate with areolate regions
Mascagnia + *Amorimia* clade	aperture length 6.00–6.99 µm	aperture type porate; aperture number 6
Mascagnia	sexine thickness 1.00–1.99 µm	aperture number 8; exine thickness very thin
Mascagnia cordifolia	–	aperture length 3.00–3.99 µm
Mascagnia sepium	aperture size 5.00–5.99 µm width	exine thickness 5.00–5.99 µm; nexine thickness 3.00–3.99 µm
Amorimia	nexine thickness 0.01–0.99 µm	exine thickness 4.00–4.99 µm; aperture width 6.00–6.99 µm; sexine psilate-perforate
Amorimia subg. ***Amorimia***		
Amorimia exotropica	sexine thickness 3.00–3.99 µm; aperture width 7.00–7.99 µm	–
A. velutina + *A. coriacea* + *A. maritima* + *A. candidae* + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade	pollen grain shape oblate-spheroidal; nexine thickness 1.00–1.99 µm; aperture length 5.00–5.99 µm	–
Amorimia velutina	pollen grain size large; tectum thickness 1.51–1.99 µm; ornamentation type rugulate with psilate regions; exine thick	exine thickness 6.00–6.99 µm; sexine thickness 4.00–4.99 µm
A. coriacea + *A. maritima* + *A. candidae* + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade	–	aperture length 7.00–7.99 µm
Amorimia coriacea	pollen grain shape prolate-spheroidal; exine thickness 3.00–3.99 µm; nexine thickness 0.01–0.99 µm	–
A. maritima + *A. candidae* + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade	tectum thickness 0.51–0.99 µm; aperture width 7.00–7.99 µm	–
Amorimia maritima	sexine thickness 3.00–3.99 µm; exine thick	–
A. candidae + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade	aperture length 6.00–6.99 µm	–
Amorimia candidae	exine thickness 3.00–3.99 µm; nexine thickness 0.01–0.99 µm; tectum thickness 1.51–1.99 µm; ornamentation type rugulate with psilate regions	–
A. pellegrinii + *A. andersonii* + *A. rigida* clade	aperture length 6.00–6.99 µm	–
Amorimia pellegrinii	pollen grain shape prolate-spheroidal	–
A. andersonii + *A. rigida* clade	sexine thickness 3.00–3.99 µm; tectum thickness 0.51–0.99 µm; exine thick	–
Amorimia subg. ***Uncinae***		
Amorimia tumida	–	–
A. pubiflora + *A. septentrionalis* clade	exine thickness 3.00–3.99 µm; aperture length 5.00–5.99 µm	–
Amorimia pubiflora	ornamentation type fossulate	–
Amorimia septentrionalis	nexine thickness 1.00–1.99 µm; ornamentation type rugulate with psilate regions; aperture width 5.00–5.99 µm	–
A. camporum + *A. kariniana* + *A. amazonica* + *A. concinna* clade	sexine thickness 3.00–3.99 µm; tectum thickness 1.00–1.50 µm	–
Amorimia camporum	pollen grain shape oblate-spheroidal; tectum thickness 0.51–0.99 µm; exine thick	aperture length 8.00–8.99 µm; aperture width 9.00–9.99 µm

Table 1 (continued). List of homoplasies and apomorphies (including synapomorphies and autapomorphies) recovered for all lineages in this study.

Lineages	Homoplasies	Apomorphies
Amorimia kariniana	pollen grain size large; exine thickness 3.00–3.99 μm; sexine thickness 2.00–2.99 μm	–
A. amazonica + *A. concinna* clade	–	ornamentation type rugulate
Amorimia amazonica	exine thickness 2.00–2.99 μm; sexine thickness 1.00–1.99 μm; nexine thickness 1.00–1.99 μm	tectum thickness 0.01–0.50 μm
Amorimia concinna	pollen grain shape oblate-spheroidal; aperture length 4.00–4.99 μm; aperture width 4.00–4.99 μm; exine thick	–

regarding the number of apertures (= 8) and exine thickness being very thin. *Mascagnia cordifolia* was recovered supported by a single synapomorphy regarding the aperture length (3.00–3.99 μm), while *M. sepium* was recovered supported by a single homoplasy regarding aperture width (5.00–5.99 μm) and two synapomorphies regarding exine thickness (5.00–5.99 μm) and nexine thickness (3.00–3.99 μm) (Fig. 7, Table 1, Supplementary material 3).

Amorimia was recovered supported by a single homoplasy regarding nexine thickness (0.01–0.99 μm) and three synapomorphies regarding exine thickness (4.00–4.99 μm), aperture width (6.00–6.99 μm), and exine ornamentation (psilate-perforate). Both subgenera of *Amorimia* were not recovered, supported by any homoplasy or synapomorphy. Within *A.* subg. *Amorimia*, *A. exotropica* was recovered supported by two homoplasies regarding sexine thickness (3.00–3.99 μm) and aperture width (7.00–7.99 μm). The *A. velutina* + *A. coriacea* + *A. maritima* + *A. candidae* + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade was recovered supported by three homoplasies regarding pollen grains shape (oblate-spheroidal), nexine thickness (1.00–1.99 μm), and aperture length (5.00–5.99 μm). *Amorimia velutina* was recovered supported by four homoplasies regarding pollen grain size (large), tectum thickness (1.51–1.99 μm), exine thickness (thick) and ornamentation type (rugulate with psilate regions), and two autapomorphies regarding exine thickness (6.00–6.99 μm) and sexine thickness (4.00–4.99 μm). The *A. coriacea* + *A. maritima* + *A. candidae* + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade was supported by a single synapomorphy regarding aperture length (7.00–7.99 μm). *Amorimia coriacea* was recovered supported by three homoplasies regarding pollen grain shape (prolate-spheroidal), exine thickness (3.00–3.99 μm), and nexine thickness (0.01–0.99 μm). The *A. maritima* + *A. candidae* + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade was supported by two homoplasies regarding tectum thickness (0.51–0.99 μm) and aperture width (7.00–7.99 μm). *Amorimia maritima* was recovered supported by two homoplasies regarding sexine thickness (3.00–3.99 μm) and exine thickness (thick). The *A. candidae* + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade was supported by a single homoplasy regarding aperture length (6.00–6.99 μm). *Amorimia candidae* was recovered supported by four homoplasies regarding exine thickness (3.00–3.99 μm), nexine thickness (0.01–0.99 μm), tectum thickness (1.51–1.99 μm), and ornamentation type (rugulate with psilate regions). The *A. pellegrinii* + *A. andersonii* + *A. rigida* clade was recovered supported by a single homoplasy regarding aperture length (6.00–6.99 μm). *Amorimia pellegrinii* was recovered supported by a single homoplasy regarding pollen grain shape (prolate-spheroidal). The *A. andersonii* + *A. rigida* clade was recovered supported by three homoplasies regarding sexine thickness (3.00–3.99 μm), tectum thickness (0.51–0.99 μm), and exine thickness (thick) (Fig. 7, Table 1, Supplementary material 3).

Finally, within the *Amorimia* subg. *Uncinae*, *A. tumida* was not recovered as supported by any homoplasy or autapomorphy. The *A. pubiflora* + *A. septentrionalis* clade was recovered supported by two homoplasies regarding exine thickness (3.00–3.99 μm) and aperture length (5.00–5.99 μm). *Amorimia pubiflora* was recovered supported by a single homoplasy regarding exine ornamentation type (fossulate). *Amorimia septentrionalis* was recovered supported by three homoplasies regarding nexine thickness (1.00–1.99 μm), ornamentation type (rugulate with psilate regions), and aperture width (5.00–5.99 μm). The *A. camporum* + *A. kariniana* + *A. amazonica* + *A. concinna* clade was recovered supported by two homoplasies regarding sexine thickness (3.00–3.99 μm) and tectum thickness (1.00–1.50 μm). *Amorimia camporum* was recovered supported by three homoplasies regarding pollen grain shape (oblate-spheroidal), tectum thickness (0.51–0.99 μm), and exine thickness (thick), and two autapomorphies regarding aperture length (8.00–8.99 μm) and aperture width (9.00–9.99 μm). *Amorimia kariniana* was recovered supported by three homoplasies regarding pollen grain size (large), exine thickness (3.00–3.99 μm), and sexine thickness (2.00–2.99 μm). The *A. amazonica* + *A. concinna* clade was recovered as supported by a single synapomorphy regarding exine ornamentation (rugulate). *Amorimia amazonica* was recovered supported by three homoplasies regarding exine thickness (2.00–2.99 μm), sexine thickness

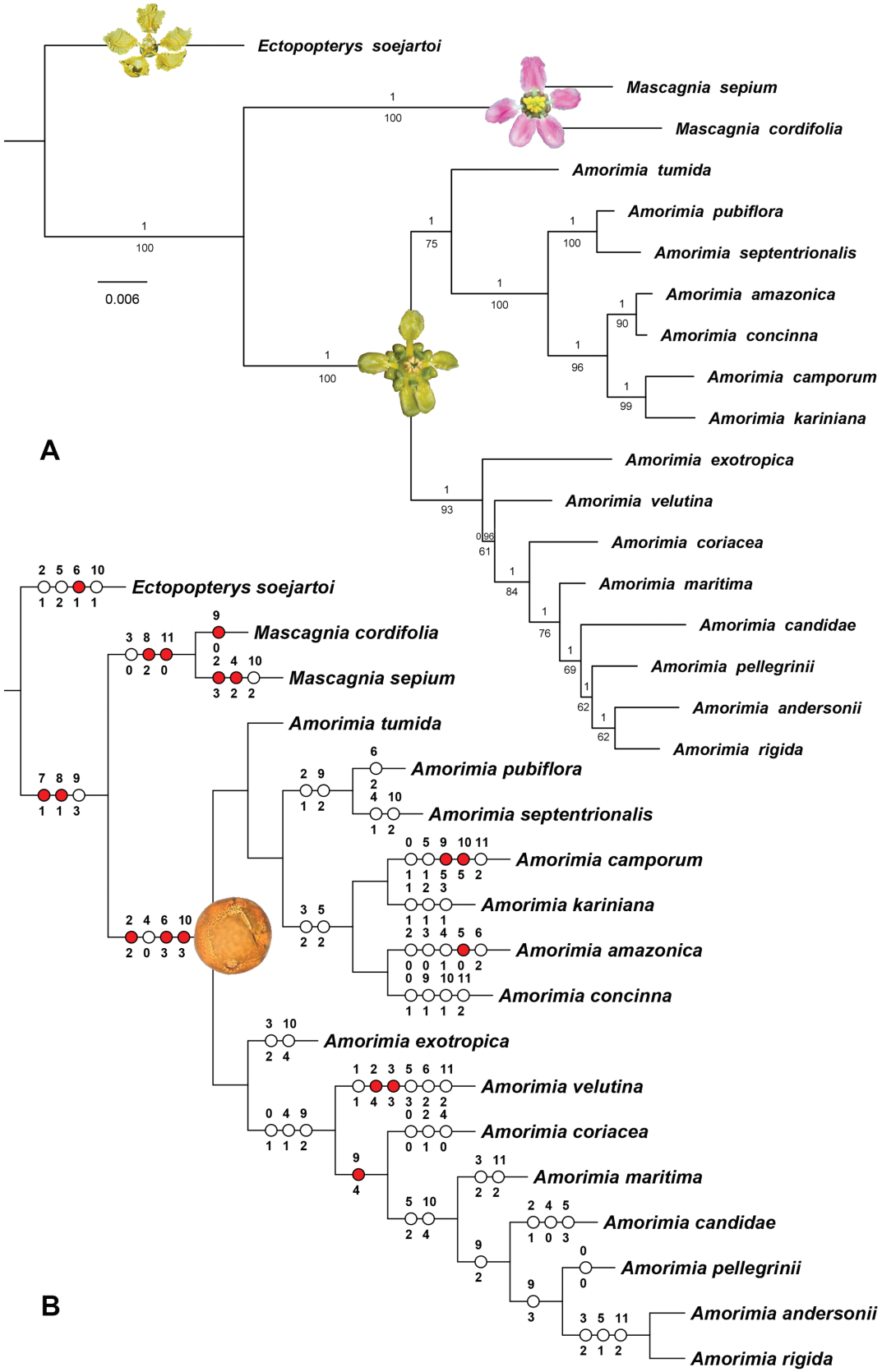

Figure 7. Molecular phylogeny and pollen character mapping of *Amorimia* and allies (Malpighiaceae) pruned from Almeida (2018). **A**. Phylogenetic tree – numbers above and below branches represent posterior probability and bootstrap values, respectively. **B**. Character mapping tree – red circles represent apomorphies (synapomorphies and autapomorphies); white circles represent homoplasies; numbers above circles represent the number of the pollen character; numbers below circles represent the number of the pollen character state reconstructed.

(1.00–1.99 μm), nexine thickness (1.00–1.99 μm), and exine ornamentation type (fossulate), besides a single autapomorphy regarding tectum thickness (0.01–0.50 μm). Finally, *Amorimia concinna* was recovered supported by four homoplasies regarding pollen grain shape (oblate-spheroidal), aperture length (4.00–4.99 μm), aperture width (4.00–4.99 μm), and exine thickness (thick) (Fig. 7, Table 1, Supplementary material 3).

DISCUSSION

Palynology of *Amorimia* and allies

The genus *Amorimia* was recently segregated from *Mascagnia*, and, unfortunately, no palynological evidence was included in its original description (Anderson 2006). Lowrie (1982) performed a comprehensive study of the pollen morphology of 60 out of the 75 currently accepted genera of Malpighiaceae, describing three main morphological types and a few subtypes for this family. Radially symmetric pollen grains divided into 1. 3-colporate, 2. parasyntricolporate, 3. syntricolporate, 4. 4-colporate, or 5. polycolporate genera. Globally symmetric pollen grains lacking ectoapertures divided into 1. Aspidopteroid, 2. Bunchosioid, and 3. Ryssopteroid types, and globally symmetric pollen grains with ectoapertures divided into 1. Banisterioid, 2. Clonodioid, 3. Mascagnioid, and 4. Tetrapteroid types (Lowrie 1982). This author described the pollen grains of some species of *Mascagnia*, now treated in *Amorimia*, as of the Mascagnioid subtype, characterised as pollen grains with branched exine ornamentation with fused rugae and more than six pores randomly dispersed by the intersection of two rugae. Our results demonstrated clypeate pollen grains with fossulate or psilate-perforate exine for all species of *Amorimia*, different from those presented by Lowrie (1982). *Amorimia* pollen grains are similar to the pollen grains of the genus *Mascagnia* but differ concerning the number of apertures, corroborating the taxonomic changes proposed by Anderson (2006). In general, our data agree with the denomination of Mascagnioid pollen grains, as previously done by Lowrie (1982), for *Amorimia* species, since the pollen grains present patterns of aperture and ornamentation somewhat similar to those observed by Lowrie (1982), varying however in the number of apertures and ornamentation type, which allows to use them as diagnostic traits to easily distinguish *Amorimia* from *Mascagnia*.

Makino (1986) and Belonsi and Gasparino (2015) also found 8-porate pollen grains in *Mascagnia cordifolia* and *M. sepium*. Still, they observed nexine thicker than sexine, different from the data found in this study (i.e. sexine thicker than nexine). Therefore, the number and type of apertures corroborate the pollen pattern found for the species of *Mascagnia,* even with slight differences observed in the present study. The pollen grains of *Ectopopterys soejartoi* were briefly described by Anderson (1980) as 3-colporate, with the present study also corroborating the description presented by this author. Pollen grain type and number of apertures are also useful to differentiate *Amorimia* species from their allied genera (*Ectopopterys* and *Mascagnia*).

Another factor to be highlighted is the corroboration obtained from the ancestral pollen character reconstructions that placed colporate pollen grains (as in *Ectopopterys soejartoi*) as the probable ancestral state and porate pollen grains (*Amorimia* and *Mascagnia*) as a derived character in the Malpighioid clade. We corroborated that the pollen morphology of *Amorimia* regarding qualitative characters is constant for the species analysed, but showed that quantitative characters are very informative for their taxonomy. According to traditional palynological classification, *Amorimia* can be considered stenopalynous (i.e. with minor discrete morphological variations). As abovementioned, the type and number of apertures allow the distinction of *Amorimia* from the closely related *Mascagnia* and *Ectopopterys*. In the PCA analysis, the metric variables of the pollen grains confirm the qualitative data and help to distinguish the analysed genera since the differences in the measurements of the ectoapertures and the exine layers allowed *Mascagnia* and *Ectopopterys* to be separated from *Amorimia*.

In LM, the pollen grains of the species analysed here present sexine rugulate with areolate or psilate areas, which was also verified in previous studies for Malpighiaceae (Makino 1986; Makino-Watanabe et al. 1993a, 1993b, 1998; Belonsi and Gasparino 2015). For the description of pollen ornamentation in SEM, clypeate pollen grains were observed (as described by Halbritter et al. 2018), and the sexine is fossulate or psilate-perforate, details not previously described for species of *Amorimia*.

Evolution of pollen grains in *Amorimia* and allies

Cameron et al. (2001) tested the phylogenetic relevance of Lowrie's (1982) three main micromorphological pollen groups in Malpighiaceae. These authors found that radially symmetrical pollen grains are probably plesiomorphic in Malpighiaceae, occurring in the Byrsonimoid, Acridocarpoid, Mcvaughioid, and Ptilochaetoid clades. Since this author did not sample any outgroups outside Malpighiaceae in his study, it is impossible to confidently state the plesiomorphic nature of this pollen grain morphology in Malpighiaceae. Nonetheless, Perveen and Quaiser (1995) show that the pollen grains of two species of *Bergia* L. (Elatinaceae) are indeed radially symmetrical, corroborating this character state as probably plesiomorphic for the Elatinaceae + Malpighiaceae clade. The same pattern of pollen morphology was also reported for two species of *Elatine* L. (Ramayya and Rajagopal 1971), increasing the chances of radially symmetrical pollen grains being, indeed, a synapomorphy for the clade formed by both families. Additional studies for the remaining 54 accepted species in Elatinaceae are needed for an in-depth assessment of this hypothesis.

In contrast, globally symmetrical pollen grains with ectoapertures were recovered as a synapomorphy for the Bunchosioid + Hiraeoid + Tetrapteroid + Malpighioid + Stigmaphylloid clade by Cameron et al. (2001). Globally symmetrical pollen grains without ectoapertures were recovered as homoplastic, having independently arisen at least seven times mostly in Old World genera of Malpighiaceae (except for the New World *Barnebya* W.R.Anderson & B.Gates, *Bunchosia* Rich. ex Kunth, and *Heladena* A.Juss.; Cameron et al. 2001). *Amorimia* and allies have constantly been placed by several molecular phylogenetic studies (Cameron et al. 2001; Davis et al. 2001, 2014; Davis and Anderson 2010; Almeida et al. 2017a; Almeida 2018) as early diverging lineages in the Malpighioid clade, showing globally symmetrical pollen grains with ectoapertures, as described by Lowrie (1982). The remaining lineages of the Malpighioid clade are endemic to the Old World (i.e. Africa + Asia) and show globally symmetrical pollen grains without ectoapertures as an adaptation to the generalist pollination syndrome arisen in Old World lineages due to the lack of oil collecting bees in this region of the planet (Lobreau 1968; Lowrie 1982; Cameron et al. 2001; Davis et al. 2014).

Our results recovered two evolutionary patterns regarding homoplastic and apomorphic pollen micromorphological characters. Regarding synapomorphies or autapomorphies (i.e. apomorphies), few qualitative characters, such as the type of pollen aperture (colporate or porate) and the number of apertures (3, 6, or 8), were very informative in distinguishing lineages at the generic rank. A few quantitative characters, such as exine thickness and aperture width, were informative at the generic level for the species sampled in this study. On the other hand, a few quantitative characters such as exine, nexine, sexine, and tectum thickness, and aperture length and width were informative to distinguish lineages at an infrageneric rank. Only the ornamentation type was informative to distinguish species at an infrageneric level. Regarding homoplasies, all quantitative and almost all qualitative micromorphological pollen characters analysed were informative both at the generic and infrageneric levels in *Amorimia* and allies. It is also worth mentioning that most of the homoplastic characters recovered as informative in our results were related to quantitative pollen characters, which are frequently underexplored in evolutionary studies of pollen grains.

Finally, Almeida et al. (2017a) proposed two subgenera within *Amorimia*: *A.* subg. *Amorimia*, or Atlantic clade, and *A.* subg. *Uncinae*, or Amazonian clade. As the clade names suggest, these species are already geographically separated. Molecular parsimony and bootstrap analyses showed that this separation is supported, and some morphological characters also support both subgenera. The authors suggest a differentiation of both subgenera based on pollen characters such as pollen amb and size (Almeida et al. 2017b). Nonetheless, the data from the present study do not corroborate Almeida et al. (2017b), since their species sampling for *Amorimia* was incomplete. In fact, when this classification was proposed, *Amorimia tumida* was still unknown to science and was not included in the molecular phylogeny published by these authors (Almeida et al. 2017b). This species is sister to the remaining species of the *A.* subg. *Uncinae* and was described only based on molecular data, and vegetative and fruit morphology, with its flowers still being unknown to science (Almeida et al. 2017b). Consequently, it was impossible to analyse this species' pollen morphology in the present study. Since *A. tumida* is a crucial species placed at the base of the *A.* subg. *Uncinae* clade, this taxon's missing data directly impacts the phylogenetic reconstruction of the analysed pollen micromorphological characters. Thus, only future studies focusing on the pollen morphology of *A. tumida* will be able to shed light on the relevance of pollen morphology to corroborate the classification system of *Amorimia*.

CONCLUSIONS

According to palynological standards of pollen morphology variation, *Amorimia* can be categorised as stenopalynous since all species show the same pollen type, with some subtle differences between the pollen grains, such as ornamentation, shape, size, and thickness of the exine. The micromorphological patterns of pollen grain evolution found by Lowrie (1982) showed several qualitative and only a few quantitative pollen characters informative at suprageneric levels (i.e. phylogenetic clades). On the other hand, the patterns of pollen grain evolution demonstrated by our results showed few qualitative characters informative at intergeneric levels. Still, almost all quantitative characters analysed were informative at infrageneric levels. The quantitative and qualitative analyses do not corroborate the currently recognised subgenera of *Amorimia* due to not sampling *A. tumida*, a critical species in the phylogenetic backbone known only by fruiting specimens. Sampling *A. tumida* should be a future priority to shed light on the evolutionary patterns of pollen micromorphology in *Amorimia*.

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REFERENCES

Almeida RF (2018) Taxonomic revision of *Amorimia* W.R.Anderson (Malpighiaceae). Hoehnea 45(2): 238–306. https://doi.org/10.1590/2236-8906-47/2017

Almeida RF, van den Berg C (2021) Molecular phylogeny and character mapping support generic adjustments in the Tetrapteroid clade (Malpighiaceae). Nordic Journal of Botany 39(1): e02876. https://doi.org/10.1111/njb.02876

Almeida RF, van den Berg C, Amorim AMA (2016) Untangling the *Amorimia rigida* complex, a puzzling group of lianescent Malpighiaceae from Eastern Brazil. Phytotaxa 284: 1–23. https://doi.org/10.11646/phytotaxa.284.1.1

Almeida RF, Amorim AMA, Corrêa AMS, van den Berg C (2017a) A new infrageneric classification for *Amorimia* (Malpighiaceae) based on morphological, phytochemical, and molecular evidence. Phytotaxa 313: 231–248. https://doi.org/10.11646/phytotaxa.313.3.1

Almeida RF, Mello ACMP, Oliveira DMT, Amorim AMA (2017b) Leaf anatomy and micromorphology uncover a new species of *Amorimia* (Malpighiaceae) from Southeastern Brazil. Phytotaxa 305: 179–190. https://doi.org/10.11646/phytotaxa.305.3.5

Anderson WR (1979) Floral Conservatism in Neotropical Malpighiaceae. Biotropica 11: 219–223. https://doi.org/10.2307/2388042

Anderson WR (1980) *Ectopopterys*, a new genus of Malpighiaceae from Colombia and Peru. Contributions from the University of Michigan Herbarium 14: 11–15. https://www.biodiversitylibrary.org/page/12698759

Anderson WR (1981) Malpighiaceae. Botany of Guayana Highland – Part IX. Memoirs of The New York Botanical Garden 32: 21–305.

Anderson C (1982) A monograph of the genus *Peixotoa* (Malpighiaceae). Contribution from the University of Michigan Herbarium 15: 1–92.

Anderson WR (2006) Eight segregates from the Neotropical genus *Mascagnia* (Malpighiaceae). Novon 16: 168–204. https://doi.org/10.3417/1055-3177(2006)16[168:ESFTNG]2.0.CO;2

APG (1998) An ordinal classification for the families of flowering plants. Annals of the Missouri Botanical Garden 18: 531–553. https://doi.org/10.2307/2992015

APG IV (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society 181: 1–20. https://doi.org/10.1111/boj.12385

Bahadur S, Taj S, Long W, Ahamad M (2022) Pollen morphology and its implication in the taxonomy of some selected tribes of the Asteraceae of Hainan Island South China. The Botanical Review 88: 271–298. https://doi.org/10.1007/s12229-022-09277-3

Barth OM, Melhem TS (1988) Glossário Ilustrado de Palinologia. Editora da Universidade Estadual De Campinas, Campinas, 1–67.

Belonsi TK, Gasparino EC (2015) Pollen morphology of Malpighiaceae from Brazilian forest fragments. Brazilian Journal of Botany 38: 379–393. https://doi.org/10.1007/s40415-015-0134-1

Bellonzi TK, Dutra FV, Souza CN, Rezende AA, Gasparino EC (2020) Pollen types of Sapindaceae from Brazilian forest fragments: apertural variation. Acta Botanica Brasilica 34: 327–341. https://doi.org/10.1590/0102-33062020abb0022

Cameron KM, Chase MW, Anderson WR, Hills HG (2001) Molecular systematics of Malpighiaceae: evidence from plastid *rbcL* and *matK* sequences. American Journal of Botany 88: 1847–1862. https://doi.org/10.2307/3558361

Cardinal-McTeague WM, Gillespie LJ (2016) Molecular phylogeny and pollen evolution of Euphorbiaceae tribe Plukenetieae. Systematic Botany 41(2): 329–347. https://doi.org/10.1600/036364416X691759

Chaisongkram W, Tubtamat M, Kulpholmuang W, Chantaranothai P (2022) Palynological studies and lectotypification of Malpighiaceae in Thailand. Tropical Natural History 22(1): 108–118.

Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price R A, Hills HG, Qiu Y, Kron KA (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. Annals of the Missouri Botanical Garden 80(3): 528–580. https://doi.org/10.2307/2399846

Davis CC, Anderson WR (2010) A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. American Journal of Botany 97: 2031–2048. https://doi.org/10.3732/ajb.1000146

Davis CC, Anderson WR, Donoghue MJ (2001) Phylogeny of Malpighiaceae: evidence from chloroplast *ndhF* and *trnL-F* nucleotide sequences. American Journal of Botany 88: 1830–1846. https://doi.org/10.2307/3558360

Davis CC, Schaefer H, Xi Z, Baum DA, Donoghue MJ, Harmon LJ (2014) Long-term morphological stasis maintained by a plant-pollinator mutualism. Proceedings of the National Academy of Sciences 111: 5914–5919. https://doi.org/10.1073/pnas.1403157111

De Pinna MCC (1991) Concepts and tests of homology in the cladistic paradigm. Cladistics 7: 367–394. https://doi.org/10.1111/j.1096-0031.1991.tb00045.x

Dessein S, Ochoterena H, De Block P, Lens F, Robbrecht E, Schols P, Smets E, Vinckier S, Huysmans S (2005) Palynological characters and their phylogenetic signal in Rubiaceae. Botanical Review 71: 354–414. https://doi.org/10.1663/0006-8101(2005)071[0354:PCATPS]2.0.CO;2

Doyle JA, Thomas AL (2012) Evolution and phylogenetic significance of pollen in Annonaceae. Botanical Journal of the Linnean Society 169(1): 190–221. https://doi.org/10.1111/j.1095-8339.2012.01241.x

Erdtman G (1952) Pollen Morphology and Plant Taxonomy: Angiosperms. Alquimist and Wiksell, Stockholm, 1–539.

Erdtman G (1960) The acetolysis method - a revised description. Svensk Botanisk Tidskrift 54: 561–564.

Faegri G, Iversen J (1950) Textbook of modern pollen analysis. Second edition. Scandinavian University Books, Copenhagen, Denmark, 1–168.

Flora e Funga do Brasil (2023) Malpighiaceae. Jardim Botânico do Rio de Janeiro. https://floradobrasil.jbrj.gov.br/FB155 [accessed 27.06.2023]

Furness CA, Rudall PJ (2001) Pollen and anther characters in monocot systematics. Grana 40(1–2): 17–25. https://doi.org/10.1080/00173130152591840

Gasparino EC, Souza CN, Dutra FV, Cruz-Barros MAV, Chautems A (2021) Pollen morphology of Ligeriinae Hanst. (Gesneriaceae): diagnostic features and their systematic importance. Review of Paleobotany and Palynology 285: 104363. https://doi.org/10.1016/j.revpalbo.2020.104363

Gonçalves-Esteves V, Soares Júnior EF, Mendonça CBF (2007) Palinologia de espécies de Malpighiaceae Juss. ocorrentes nas restingas do Estado do Rio de Janeiro. Hoehnea 34: 519–529. https://doi.org/10.1590/S2236-89062007000400007

Grímsson F, van Valkenburg JLCH, Wieringa JJ, Xafis A, Jacobs BF, Zetter R (2019) Pollen morphology of the African *Sclerosperma* (Arecaceae). Grana 58(2): 99–113. https://doi.org/10.1080/00173134.2018.1519033

Halbritter H, Ulrich S, Grímsson F, Weber M, Zetter R, Hesse M, Buchner R, Svojtka M, Frosch-Radivo A (2018) Illustrated Pollen Terminology. Second edition. Springer, Vienna, 1–483. https://doi.org/10.1007/978-3-319-71365-6

Harley MM (1991) The pollen morphology of Sapotaceae. Kew Bulletin 46(3): 379–491. https://doi.org/10.2307/4110538

Kriebel R, Khabbazian M, Sytsma KJ (2017) A continuous morphological approach to study the evolution of pollen in a phylogenetic context: an example with the order Myrtales. PLoS ONE 12(12): e0187228. https://doi.org/10.1371/journal.pone.0187228

Lindley J (1830) The Genera and Species of Orchidaceous Plants. Ridgways, London, 1–553. https://doi.org/10.5962/bhl.title.499

Lobreau D (1967) Contribution à l'étude du pollen des Malpighiaceae d'Afrique. Pollen Spores 9: 241–277.

Lobreau D (1968) Le pollen des Malpighiacees d'Afrique et de Madagascar. Bulletin de l'Institut français d'Afrique noire Série A 30: 59–83.

Lobreau-Callen D (1983) Analyse de la répartition géographique des Malpighiaceae d'après les caractères du pollen et de la pollinisation. Bothalia 14 (3/4): 871–881.

Lowrie SR (1982) The palynology of the Malpighiaceae and its contribution to family systematics. PhD Thesis, University of Michigan, USA.

Lu L, Wortley AH, Li DZ, Wang H, Blackmore S (2015) Evolution of angiosperm pollen. 2. The basal angiosperms. Annals of the Missouri Botanical Garden 100(3): 227–269. https://doi.org/10.3417/2012048

Luz CFP, Maki ES, Korák-Terra I, Vidal-Torrado P, Filho CVM (2013) Pollen grain morphology of Fabaceae in the Special Protection Area (SPA) Pau-de-Fruta, Diamantina, Minas Gerais, Brazil. Anais da Academia Brasileira de Ciências 85(4): 1329–1344. https://doi.org/10.1590/0001-3765201380511

Maddison WP, Maddison DR (2006) Mesquite: a modular system for evolutionary analysis. Version 3.61. https://www.mesquiteproject.org/ [accessed 17.10.2023]

McCune B, Mefford MJ (2011) PC-ORD: multivariate analysis of ecological data. MjM Software Design, Oregon. https://www.wildblueberrymedia.net/pcord [accessed 10.10.2023]

Makino WH (1986) Flora polínica da Reserva do Parque Estadual das Fontes do Ipiranga – Família 125-Malpighiaceae. Hoehnea 13: 21–30.

Makino-Watanabe H, Melhem TS, Barth MO (1993a) Morfologia dos grãos de pólen de espécies de *Banisteriopsis* C.B.Robinson ex Small (Malpighiaceae). Revista Brasileira de Botânica 16: 47–67.

Makino-Watanabe H, Melhem TS, Barth MO (1993b) Morfologia dos grãos de pólen de espécies brasileiras de *Janusia* A.Juss. e *Schwannia* Endl. (Malpighiaceae). Hoehnea 20: 79–86.

Makino-Watanabe H, Melhem TS, Barth MO (1998) Morfologia polínica de *Camarea* St.-Hil. (Malpighiaceae). Revista Brasileira de Botânica 21: 1–6.

Melhem TS (1978) Palinologia suas aplicações e perspectivas no Brasil. Coleção Museu Paulista, Série ensaios (2): 325–368.

Melhem TS, Matos MER (1972) Variabilidade de forma dos grãos de pólen de *Eriope crassipes* Benth – Labiate. Hoehnea 2: 1–10.

Melhem TS, Cruz-Barros MAV, Corrêa MAS, Makino-Watanabe H, Silvestre-Capelato MSF, Gonçalves-Esteves VL (2003) Variabilidade polínica em plantas de Campos do Jordão (São Paulo, Brasil). Boletim do Instituto de Botânica de São Paulo 16: 1–104.

Müller K, Borsch T (2005) Phylogenetics of Amaranthaceae based on *matK/trnK* sequence data: evidence from parsimony, likelihood, and Bayesian analyses. Annals of the Missouri Botanical Garden 92(1): 66–102. http://www.jstor.org/stable/3298649

Nixon KC (1999) Winclada (beta). Version 0.9. Published by the author, Ithaca, NY. https://www.cladistics.com [accessed 07.02.2023]

Perveen A, Quaiser M (1995) Pollen flora of Pakistan - III. Elatinaceae. Pakistan Journal of Botany 27(1): 87–92.

POWO (2023) Plants of the World Online. https://powo.science.kew.org/ [accessed 10.10.2023]

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. Review of Paleobotany and Palynology 143: 1–81. https://doi.org/10.1016/j.revpalbo.2006.06.008

Ramayya N, Rajagopal T (1971) Systematics, distribution and anatomy of the two Indian species of the genus *Elatine* L. Bulletin of the Botanical Survey of India 13(3–4): 328–337.

Salgado-Laboriau ML (1973) Contribuição a palinologia dos Cerrados. Academia Brasileira De Ciências, Rio De Janeiro.

Sebastiani R, Cruz-Barros MAV, Corrêa MAS (2014) Palynological study of *Janusia* A. Juss. and related genera (Malpighiaceae). Brazilian Journal of Botany 37(4): 543–559. https://doi.org/10.1007/s40415-014-0082-1

Sereno PC (2007) Logical basis for morphological characters in phylogenetics. Cladistics 23: 565–587. https://doi.org/10.1111/j.1096-0031.2007.00161.x

Shepherd GJ (1996) Fitopac 1: Manual do Usuário. Departamento de Botânica, Universidade Estadual de Campinas, Campinas, 1–96.

Teixeira MDR, Amorim AM, Santos FAR (2013) Pollen morphology of Menispermaceae in the state of Bahia, Brazil. Acta Botanica Brasilica 27(2): 436–444. https://doi.org/10.1590/S0102-33062013000200016

Thornhill AH, Crisp MD (2012) Phylogenetic assessment of pollen characters in Myrtaceae. Australian Systematic Botany 25: 171–187. https://doi.org/10.1071/SB11019

Vieira S (2011) Introdução à Bioestatística. Elsevier, Rio de Janeiro, 1–345.

Wallace S, Fleming A, Wellman CH, Beerling DJ (2011) Evolutionary development of the plant spore and pollen wall. AoB PLANTS 2011: plr027. https://doi.org/10.1093/aobpla/plr027

Williams JH, Taylor ML, O'Meara BC (2014) Repeated evolution of tricellular (and bicellular) pollen. American Journal of Botany 101(4): 559–571. https://doi.org/10.3732/ajb.1300423

Yu Y, Wortley AH, Lu L, Li DZ, Wang H, Blackmore S (2018) Evolution of angiosperm pollen. 5. Early diverging Superasteridae (Berberidopsidales, Caryophyllales, Cornales, Ericales, and Santalales) plus Dilleniales. Annals of the Missouri Botanical Garden 103(1): 106–161. https://doi.org/10.3417/2017017

Zar JH (1996) Biostatistical Analysis. Second edition. Prentice Hall, Englewood Cliffs, New Jersey, 1–662.

Zou P, Newman M, Liao JP (2022) Systematics of Zingiberaceae. Grana 61(6): 448–470. https://doi.org/10.1080/00173134.2022.2127328

SUPPLEMENTARY MATERIALS

Supplementary material 1
List of herbarium specimens sampled in this study for 13 species of *Amorimia*, *Ectopopterys soejartoi*, and *Mascagnia cordifolia*.
https://doi.org/10.5091/plecevo.102524.suppl1

Supplementary material 2
Pearson and Kendall correlation coefficients among all metric variables of pollen grains and two initial PCA ordination axes for the studied species.
https://doi.org/10.5091/plecevo.102524.suppl2

Supplementary material 3
List of morphological characters of pollen grains and their character states for the sampled *Amorimia* species and outgroup.
https://doi.org/10.5091/plecevo.102524.suppl3

Supplementary material 4
Quantitative data of pollen grains of *Amorimia* and outgroups. x = arithmetic mean, sx = sample standard deviation, s = standard deviation of the sample, IC = 95% confidence interval, CV = variation coefficient. * n = 25.
https://doi.org/10.5091/plecevo.102524.suppl4

Supplementary material 5
Arithmetic mean (µm) of apertures (pores, endoapertures*, and colpi) and exine measurements of pollen grains of the studied species of *Amorimia*, *Ectopopterys*, and *Mascagnia*. n = 10.
https://doi.org/10.5091/plecevo.102524.suppl5