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Evolution of pollen grain morphology in *Amorimia* W.R.Anderson and allies (Malpighiaceae)

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Abstract

Background and aims: The genus *Amorimia* W.R. Anderson (Malpighiacerae), was recently segregated from species previously treated as the genus *Mascagnia* Bertero and then subdivided into two subgenera *Amorimia* subgenus *Amorimia* and *Amorimia* subgenus *Uncinae*, based on morphological and molecular data. Pollen grains have been used to improve the taxonomic classification of many ranks (i.e., species, genus, family) over the past two centuries. The pollen grains of the species of *Amorimia* were not previously analyzed. Therefore, we describe in detail the pollen morphology of 13 (out of 15) species of *Amorimia*, 1 species of *Mascagnia* Bertero and 1 species of *Ectopopterys* W.R Anderson and reconstruct the phylogeny framework to understand the patterns of micromorphological pollen evolution in the genus.
Material and Methods: The pollen grains were acetolysed, measured and photographed under light microscope. The quantitative data were submited to a multivartiate analysis and were also made a character-mapping to identify which pollen characters are important in distinguishing species.
Key Results: The pollen grains of the analyzed species are monads, apolar, medium to large, with circular amb, oblate-spheroidal to prolate-spheroidal, presenting apertures that may be 3-colporate, with long and narrow colpi and circular endoaperture (*Ectopopterys*), 6-porate (*Amorimia*) or 8-porate (*Mascagnia*). Exine presenting a rugulate ornamentation which may or may not have areolate or psilate areas close to the pore or distributed by the surface of the pollen grain.
Conclusion: According to the results of this study, *Amorimia* is a stenopolinic genus and the characteristics of the pollen grains are important to segregate the genus. Nonetheless, these characteristics do not corroborate with the subdivision of this genus.

Keywords

Ectopopterys, Malpighiales, *Mascagnia*, light microscopy, systematics, taxonomy

INTRODUCTION

Pollen grains (i.e., male gametophytes) are one of the key innovations that allowed seed plants to successfully colonise terrestrial habitats due to a durable wall capable of withstanding the harsh desiccating and UV-B-rich environment encountered on land (Wallace et al. 2011). These male gametophytes are composed by an inner triploid reproductive cell and an outer protective wall (i.e., the exine, sometimes called the pollen coat) largely made of sporopollenin, which is incredibly resistant to degradation (Wallace et al. 2011; Williams et al. 2014). The pollen wall shows different layers, structures and ornamentations that have been used over the past two centuries to improve the taxonomic classification of different ranks (i.e., species, genus, family) of flowering plants (Melhem 1978). 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 latter (i.e., 19th and 20th centuries) as a result of innovations on light microscopy (Melhem 1978, 2003). Since then, pollen morphology has been widely used to aid plant taxonomic studies for the past two centuries (Lindley 1830).

Nonetheless, its major role for plant systematics was only established three decades ago by the first molecular phylogenetic studies of flowering plants. These studies evidenced 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 derivate lineages in flowering plants, with its largest clade, the Eudicots (i.e., the new dicots), being solely differentiated by their tricolpate pollen from the remaining angiosperms (i.e., Basal Angiosperms and Monocots) showing monosulcate pollen grains (APG 1998). Since then, several studies have been published to explore the

morphological characterisation and phylogenetic relevance of pollen in several major lineages (i.e. Basal Angiosperms - Lu et al. 2015; Monocots – Furness and Rudall 2001; Luo et al. 2015; and Eudicots - Yu et al. 2018), orders (e.g., Myrtales – Kriebel et al. 2017), and families (e.g. Amaranthaceae - Muller and Borsch 2005; Annonaceae - Doyle and Thomas 2012; Euphorbiaceae - Cardinal-McTeague and Gillespie 2016; Loranthaceae - Grimsson et al. 2018; Myrtaceae - Thornhill and Crisp 2012; Zingiberaceae – Zou et al. 2022) of angiosperms.

Malpighiaceae is a medium-sized family of flowering plants comprising 77 genera and ca. 1,400 species mostly endemic to the Neotropics (Almeida and van den Berg 2020). Its species are characterised by a conspicuous floral conservatism represented by calyx oil glands, unguiculate petals, and Malpighiaceous pollen type (Anderson 1981). In the past few years, this family has gone through unprecedented changes in its traditional classification due to molecular phylogenetic studies (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. 2017; Almeida and van den Berg 2020). Since then, different authors have gradually proposed new genera and combinations (Anderson 2006; Davis and Anderson 2006; Anderson 2011) to accommodate these newly identified lineages.

Amorimia W.R.Anderson is one of the several new lineages identified on those previous molecular phylogenies (Anderson 2006; Davis & Anderson 2010), representing one of the eight genera segregated from the polyphyletic *Mascagnia* (Bertero ex DC) Bertero, but still 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 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). The monophyly of *Amorimia* was corroborated by Almeida et al. (2017, 2018), with two subgenera being proposed for their currently 15 accepted species. In this same study, the authors recovered several macro and micromorphological synapomorphies supporting the recognition of both subgenera, of of them represented by pollen shape.

In this study, we describe in detail the pollen morphology of 13 (out of 15) accepted species of *Amorimia* and reconstruct the phylogenetic framework presented by Almeida et al. (2017, 2018) as the basis to further understanding the patterns of micromorphological pollen evolution in the genus. More specifically, we scored and coded 12 micromorphological characters in order to test for secondary homologies.

MATERIAL AND METHODS

Sampling

A total of 13 species of *Amorimia* were sampled (out of 15 species) comprising both subgenera currently recognised by Almeida et al. (2017): *A.* subg. *Amorimia* - *A. candidae* R.F.Almeida, *A. coriacea* (Griseb.) R.F.Almeida, *A. exotropica* (Griseb.) W.R.Anderson, *A. maritima* (A.Juss.) W.R.Anderson, *A. pellegrinii* R.F.Almeida, *A. rigida* (A.Juss.) W.R. Anderson, and *A. velutina* W.R.Anderson; *A.* subg. *Uncinae* R.F.Almeida - *A. amazonica* (Nied.) W.R.Anderson, *A. camporum* W.R.Anderson, *A. concinna* (C.V.Morton) W.R.Anderson, *A. kariniana* W.R.Anderson, *A. pubiflora* (A.Juss.) W.R.Anderson, and *A. septentrionalis* W.R. Anderson. 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 limitations

regarding the lack of flower buds or flowering specimens. Additionally, a single species of the genera *Ectopopterys* W.R.Anderson (*E. soejartoi* W.R.Anderson) and *Mascagnia* (Bertero ex DC) Bertero [*M. cordifolia* (A.Juss.) Griseb.] were sampled as out groups. Information of all sampled specimens is found on Table 1.

Laboratory analyses

Mature floral buds of all sampled species were dissected under a stereomicroscope for anther removal and storage in labelled test tubes for a modified version (Melhem et al. 2003) of the classical acetolysis by Erdtman (1960). The acetolysis method destroys the protoplasm turning the pollen grains empty and translucid enabling the visualisation of the exine micromorphology. The subsequent microscope slides generated were incorporated to the pollen slide collection from the Plant Morphology and Palynology Lab, São Paulo State University, Campus Jaboticabal. Pollen photographs were documented using an optic microscope Leica IM50 coupled with a video camera and computer and digitally treated and edited using the Photoshop software.

Pollen descriptions and measurements

Pollen descriptions followed the terminology proposed by Bellonzi et al. (2020), Barth and Melhem (1988), and Punt et al. (2007). Shape and size terminology followed Erdtman (1952), and exine thickness followed Faegri and Iversen (1950). Diameter measurements (d1 and d1) 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 (n=25), while other pollen characters (i.e., aperture, total exine, sexine, nexine, and tectum) were randomly measured for only 10 pollen grains (n=10, Melhem and Matos 1972; Salgado-Laboriau 1973).

Statistical analyses

We calculated the arithmetic average (x), average standard deviation (sx), sample standard deviation (s), coefficient of variability (CV), and 95% confidence interval following Vieira (2011) and Zar (1996). Only the arithmetic average (x) was calculated for measurements of n=10. Measurements of diameter I and II were used to compare diameter values of the analysed pollen grains for *Amorimia* and outgroups on the MINITAB software (Vieira 2011, Zar 1996). Two principal component analyses (PCA) were performed using the FITOPAC (Shepherd 1996) and PC-ORD (McCune and Mefford 1999) software to verify the influence of pollen grains quantitative data on species ordination and grouping. The first analysis used 14 metric variables: diameter I (DIAI), diameter II (DIAII), pore length (PCOMP), pore width (PLAR), colpus length (CCOMP), colpus width (CLAR), endoaperture length (ECOMP), endoaperture width (ELAR), exine (EXIN), sexine (SEXIN), nexine (NEXIN), tectum (TETO), shape (FORMA), and exine thickness (ESPEXI). The second analysis used only metric variables present in all pollen grains: diameter I (DIAI), diameter II (DIAII), pore length (PCOMP), pore width (PLAR), exine (EXIN), sexine (SEXIN), nexine (NEXIN), tectum (TETO), shape (FORMA), and exine thickness (ESPEXI), excluding colpi values present only in *E. soejartoi*. For this species, only the endoaperture values were used to compare with the pores of the remaining analysed species.

Character mapping analyses

The consensus phylogenetic tree presented by Almeida et al. (2018) was pruned to show only the outgroups sampled in our study and used for character-mapping of pollen

micromorphology. 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 micromorphological characters were scored for *Amorimia* and outgroups (Table 2). In addition to M. cordifolia, we sampled Mascagnia sepium for the character-mapping analyses based on Makino (1986). All characters were optimized on the concatenated tree using the Maximum Likelihood function (mk1 model) using Mesquite 2.73 (Maddison and Maddison 2006) and visualised on Winclada (Nixon 1999).

RESULTS

Qualitative data

Pollen grains of all studied species of *Amorimia*, *Ectopopterys*, and *Mascagnia* are monads, apolar (Figs. 1-3), medium to large (Fig. 4a-b), with circular amb (Figs. 1-3), and shapes varying from oblate-spheroidal to prolate-spheroidal (Figs. 1-3). They can also be 3-colporate with long and narrow colpi and circular endoaperture in *Ectopopterys* (Fig. 3d-e), 6-porate in *Amorimia* (Figs. 1-2) or 8-porate in *Mascagnia* (Fig. 3f-g). The exine ornamentation is rugulate (Fig. 2c-d), with or without areolate (Fig. 1l) or psilate (Fig. 1b) regions near the pores or distributed over the pollen grain surface (Fig. 1g-h). The exine thickness can also vary from very thin, thin or thick (Table 4) and the sexine is always thicker than the nexine (Fig. 3f) in all analysed species (Table 1).

Identification key for the studies species of *Amorimia, Ectopopterys*, and *Mascagnia*

1. Pollen grains colporate…..... *Ectopopterys soejartoi*
1’ Pollen grains porate…..... 2

2. Pollen grains 8-porate…..... *Mascagnia cordifolia*
2’ Pollen grains 6-porate..... 3

3. Pollen grains large..... 4
3’ Pollen grains medium..... 5

4. Exine thin..... *Amorimia kariniana*
4’ Exine thick..... *Amorimia velutina*

5. Exine thick..... 6
5’ Exine thin..... 9

6. Prolate-spheroidal, rugulate..... *Amorimia amazonica*
6’ Oblate-spheroidal, rugulate with areolate regions…..... 7

7. Pollen grain diameter average 34-35 µm…..…… *Amorimia camporum*
7’ Pollen grain diameter average 43-46 µm……..…… 8

8. Pollen grain diameter average 43-44 µm……..…….. *Amorimia rigida*
8’ Pollen grain diameter average 45-46 µm……..…….. *Amorimia maritima*

9. Exine rugulate with areolate regions..... 10
9’ Exine rugulate with psilate regions…..... 12

10. Pollen grain diameter average 43-44 µm……... *Amorimia pubiflora*
10’ Pollen grain diameter average 45-49 µm……..…… 11

11. Pollen grain diameter average 45-46 µm……...…. *Amorimia exotropica*
11’ Pollen grain diameter average 48-49 µm…..……..... *Amorimia pellegrinii*

12. Prolate-espheroidal..... *Amorimia septentrionalis*
12’ Oblate-espheroidal..... *Amorimia candidae*

Quantitative data

Pollen grain diameters and their respective averages and confidence intervals were used for the quantitative data analyses. We observed three distinctive groups when analysing the metric values of the diameters (Fig. 34a-b): 1. lowest 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 first PCA (Fig. 5) summarised 95.77% of the total variability of the data, in which the axis 1 was more informative to the PCA since it summarised 91.21% of the variability. The analysed species of *Mascagnia* and *Ectopopterys* were recovered quite distant from those of *Amorimia* (Fig. 5). Species of *A.* subg. *Uncinae* were placed both in the positive (*A. amazonica*, and *A. concinna*, *A. kariniana*, *A. pubiflora*, and *A. septentrionalis*) and negative (*A. camporum*) sides of the axis (Fig. 5). The most significant variables supporting this separation were colpi length (CCOMP) and colpi length and width (PCOMP and PLAR, respectively) (Table 5). The axis 2 summarised only 4.56% of the variability in our data, with all species of *Amorimia* placed in the positive side, while the species of *Ectopopterys* and *Mascagnia* were placed in the negative side due to three variables (SEXIN, EXIN, and PLAR; Fig. 5).

The second PCA (Fig. 6) summarised 85.15% of the variability of the data in its two initial axes. The axis 1 summarised 57.00% of the variability of the data, with pore length and width (PCOMP and PLAR, respectively) and sexine (SEXIN) being the most informative metric variables for this grouping (Table 6). The positive side of this axis only included *A. velutina* due to the largest exine values which were one of the most important variables to this analysis, and a group including *A. candidae*, *A. maritima*, *A. pellegrinii*, and *A. rigida*. Another group evidenced in this analysis only included *M. cordifolia*, which shows the lowest values for pore length and width and sexine in this study. And *E. soejartoi* and *A. concinna* which shows similar values of pore length and width. In the negative side of the axis the highest values of pore length and width are observed with a group comprising *A. kariniana*, *A. coriacea* and *A. exotropica*, a group only comprising *A. amazonica*, another only comprising *A. camporum*, and a fourth group comprising *A. pubiflora* and *A. septentrionalis*. The axis 2 summarised 28.15% of the variability of the data, with tectum (TETO), exine (EXIN), and sexine (SEXIN) being the most important metric variables. In the positive side of the axis, species with the highest tectum length were observed and species with the lowest values were observed in the negative side of the axis. *A. amazonica* showed the lowest values for tectum and exine among all studied species (Table 6), alongside *E. soejartoi* and *M. cordifolia*. When taking into account both axes, a group comprising species of *A.* subg. *Amorimia* is observed in the positive side of the axes, except for *A. camporum*.

Character-mapping

All lineages from the molecular phylogeny were recovered with at least a single or more homoplasies/apomorphies, except for both *Amorimia* subgenera (*A.* subg. *Amorimia* and *A.* subg. *Uncinae*). For additional information see Table 7.

DISCUSSION

The genus *Amorimia* was recently segregated from *Mascagnia* (Bertero ex DC) Bertero and, unfortunately, no palynological evidence was included in its original description. Lowrie (1982) described the pollen grains of some species of *Mascagnia*, now treated i*n Amorimia*, generically as Mascagnioid pollen types, defined by him as pollen grains with exine ornamentation branched with fused rugae and more than six pores randomly dispersed by the intersection of two rugae. In the present study, pollen grains with rugulate ornamentation were found, most of them with psilate or areolate regions on the surface, usually close to its six pores. These data probably confirm the type of ornamentation observed by Lowrie (1982) for the Mascagnioid pollen type, but this similarity is only reflected on the type of ornamentation. *Amorimia* pollen grains are similar to the pollen grains of the genus *Mascagnia*, but differ in relation to the number of apertures, corroborating the taxonomic changes proposed by Anderson (2006). In general, our data disagree with the denomination of Mascagnioid pollen grains, as previously made by Lowrie (1982), for species of *Amorimia* since even though these pollen grains show similar ornamentation patterns observed by Lowrie (1982), the difference in the number of openings allows the use of pollen micromorphology as a diagnostic character for *Amorimia*.

Belonsi and Gasparino (2015) and Makino (1986) also found 8-porate pollen grains in *Mascagnia cordifolia* and *M. sepium*, but observed thicker nexine than sexine, different from the data found in this study (i.e. sexine thicker than nexine). Therefore, the number and type of apertures confirm 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. Another character that allows us to use the type and number of openings of the pollen grains to differentiate *Amorimia* species from those present in the close genera (*Ectopopterys* and *Mascagnia*). Another factor to be highlighted is the confirmation obtained by reconstructing the ancestral pollen characters in the analysed species of the positioning of colporate pollen grains (as in *Ectopopterys soejartoi*) as an ancestral character and porate pollen grains (*Amorimia* and *Mascagnia*) as a derived character. The present study can confirm that the pollen morphology of *Amorimia* is constant qualitative characters for the species analysed, but with quantitative characters being very informative for their taxonomy. Therefore, the genus can be regarded as stenopollinic (i.e. with small discrete morphological variations). And as previously commented, the type and number of openings allows the distinction of *Amorimia* from the closely related *Mascagnia* and *Ectopopterys*.

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 molecularly supported, and some morphological features also show this separation. The authors suggest a differentiation of both subgenera based on pollen characters such as pollen outline and size (Almeida et al. 2017a). Nonetheless, the data from the present study do not corroborate Almeida et al. 2017b. In fact, when this classification was proposed, *Amorimia tumida* R.F.Almeida & A.C.Marques 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, vegetative and fruit morphology, with its flowers being still unknown to science (Almeida et al. 2017b; Almeida et al. 2020). Consequently, it was not possible to analyse the pollen morphology of this species in the present study. Since *A. tumida* is a key species placed at the base of the A. subg. Uncinae clade, the missing data represented by this taxon direct impact 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 into the relevance of pollen morphology to corroborate the classification system of *Amorimia*.

On the other hand, the data referring to the thickness of the exine and shape of the pollen grains allow to distinguish several groups of species within the analysed species, evidencing the great relevance of quantitative pollen data to the taxonomy of *Amorimia*. Species with thick exine differ from those with thin exine in the pollen grains, as well as species with prolate-spheroidal pollen grains stand out from the others with oblate-spheroidal shape.

CONCLUSIONS

According to the results found in the present study, it can be considered that the genus Amorimia is stenopollinic, since the species have the same pollen type, with some subtle differences between the pollen grains, such as ornamentation, shape, size and thickness of the pollen exine. The quantitative and qualitative analyses carried out do not corroborate the morphological and molecular differentiation, which divides the genus into two subgenera: Amorimia subgenus Amorimia and Amorimia subgenus Uncinae. Confirming then that the pollen morphology is a character that defines the species of the genus. The pattern of ornamentation of Amorimia pollen grains confirms the main type of ornamentation (rugulate, with some variations) observed for pollen grains of species of the family.

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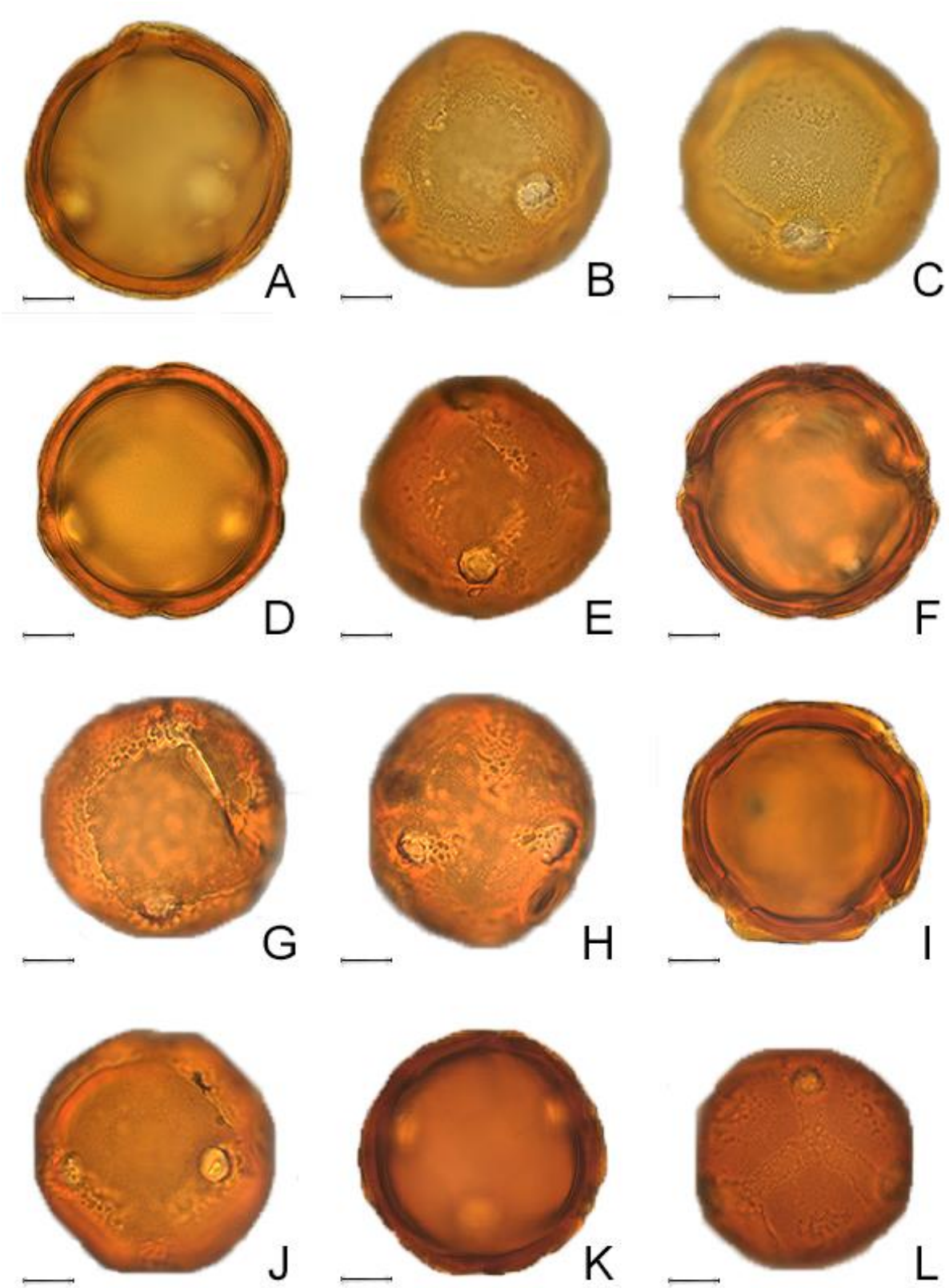

Figure 1. Photomicrographs of species of *Amorimia* in optical microscopy: *Amorimia* candidae - **A.** Exine. **B.** Ornamentation and apertures. *Amorimia coriacea* - **C.** Exine. **D-E.** Ornamentation and aperture. *Amorimia exotropica* - **F.** Exine. **G-H.** Ornamentation and apertures. *Amorimia* maritima - **I.** Exine. **J.** Ornamentation and apertures. *Amorimia pellegrinii* - **K.** Exine. **L.** Ornamentation and apertures. Scales: 10 μm.

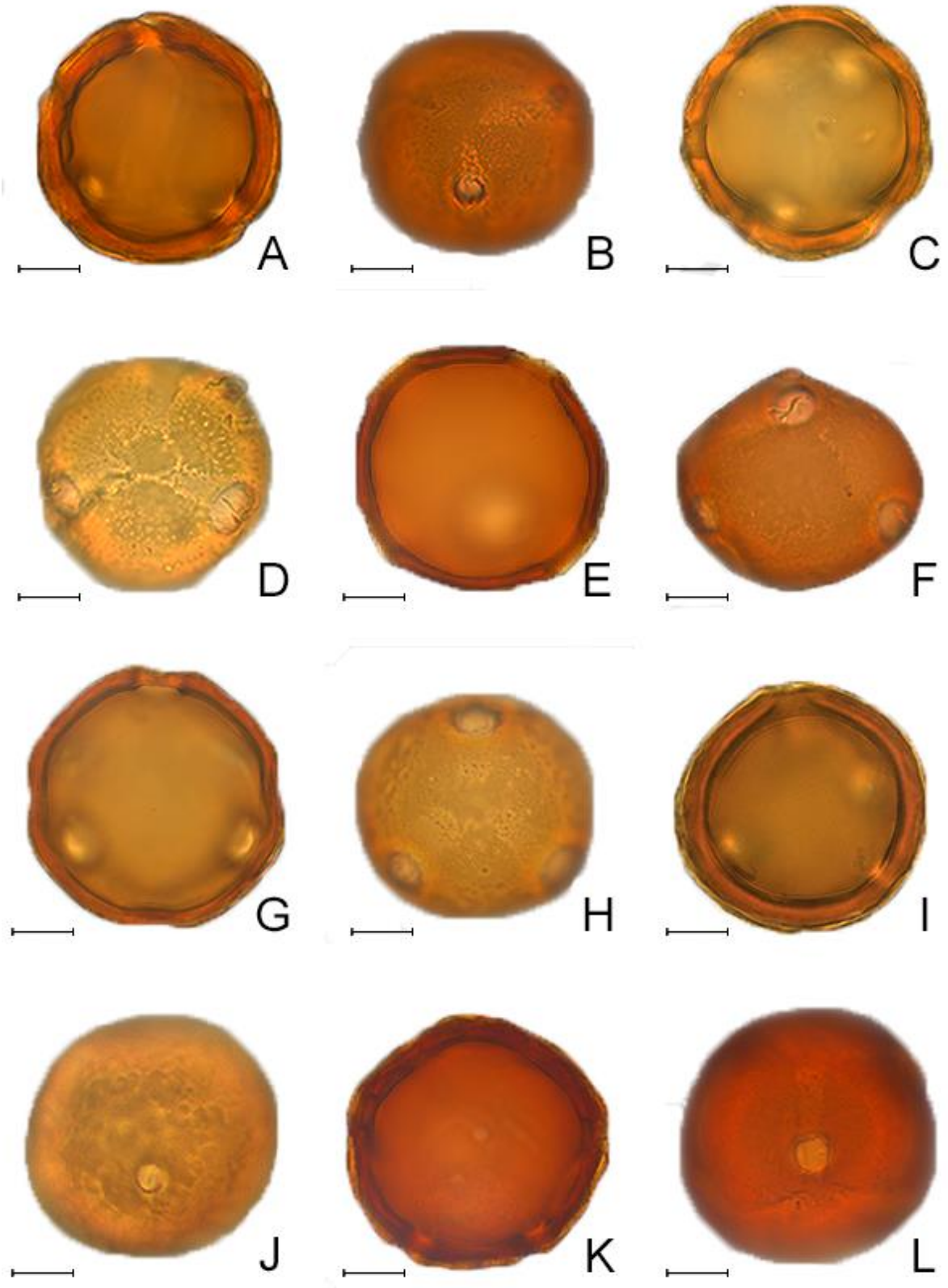

Figure 2. Photomicrographs of species of *Amorimia* in optical microscopy: *Amorimia rigida* - **A.** Exine. **B.** Ornamentation and aperture. *Amorimia velutina* - **C.** Exine. **D.** Ornamentation and apertures. *Amorimia amazonica* - **E.** Exine. **F.** Ornamentation and apertures. *Amorimia camporum* - **G.** Exine. **H.** Ornamentation and apertures. *Amorimia concinna* - **I.** Exine. **J.** Ornamentation and apertures. *Amorimia kariniana* - **K.** Exine. **L.** Ornamentation and apertures. Scales: 10 µm.

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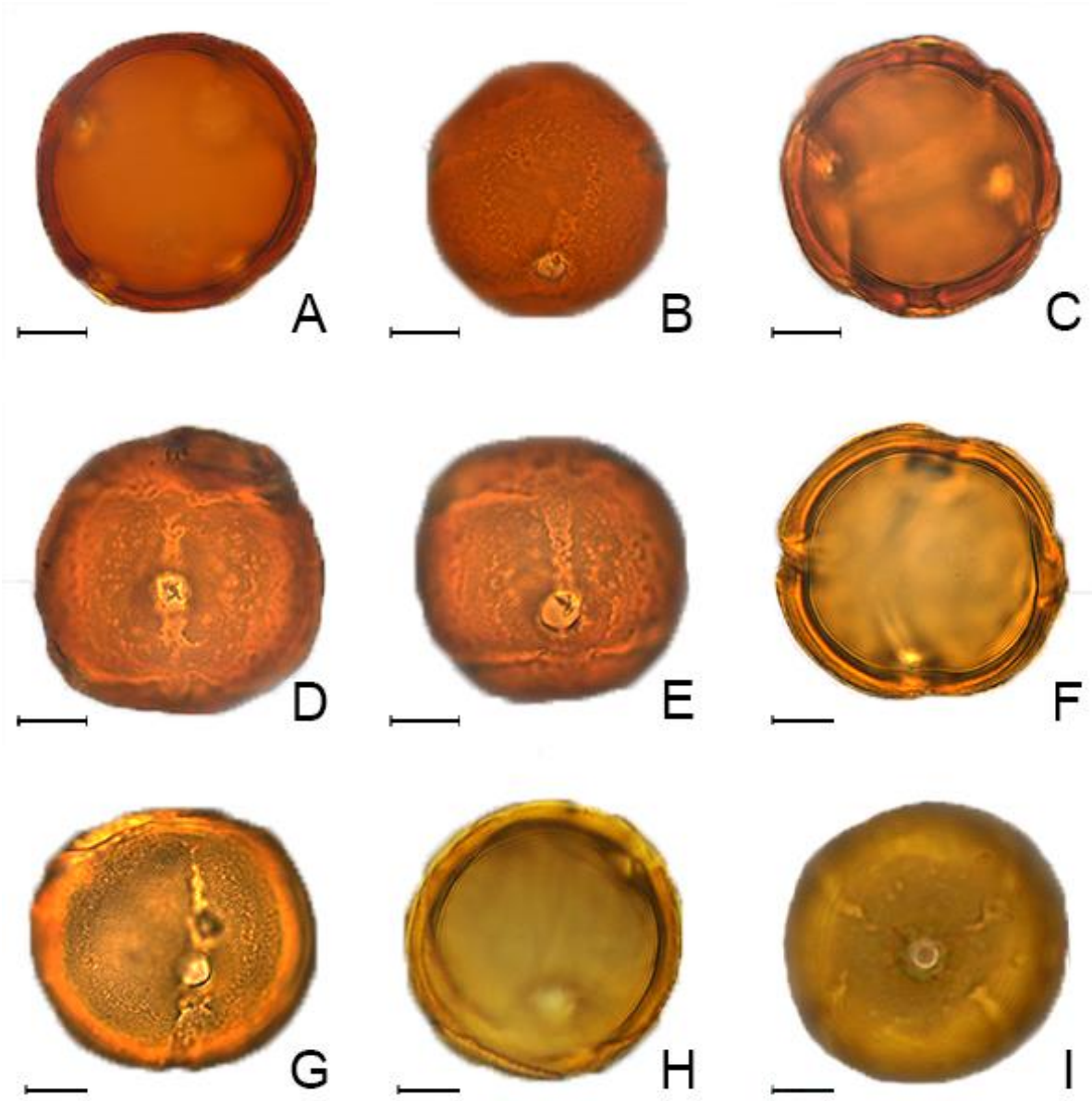

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549 **Figure 3.** Photomicrographs of species of *Amorimia* and outgroups in optical microscopy:
550 *Amorimia pubiflora* - **A.** Exine. **B.** Ornamentation and apertures. *Amorimia septentrionalis* -
551 **C.** Exine. **D-E.** Ornamentation and apertures. *Ectopopterys soejartoi* - **F.** Exine. **G.**
552 Ornamentation and aperture. *Mascagnia cordifolia* - **H.** Exine. **I.** Aperture. Scales: 10 µm.

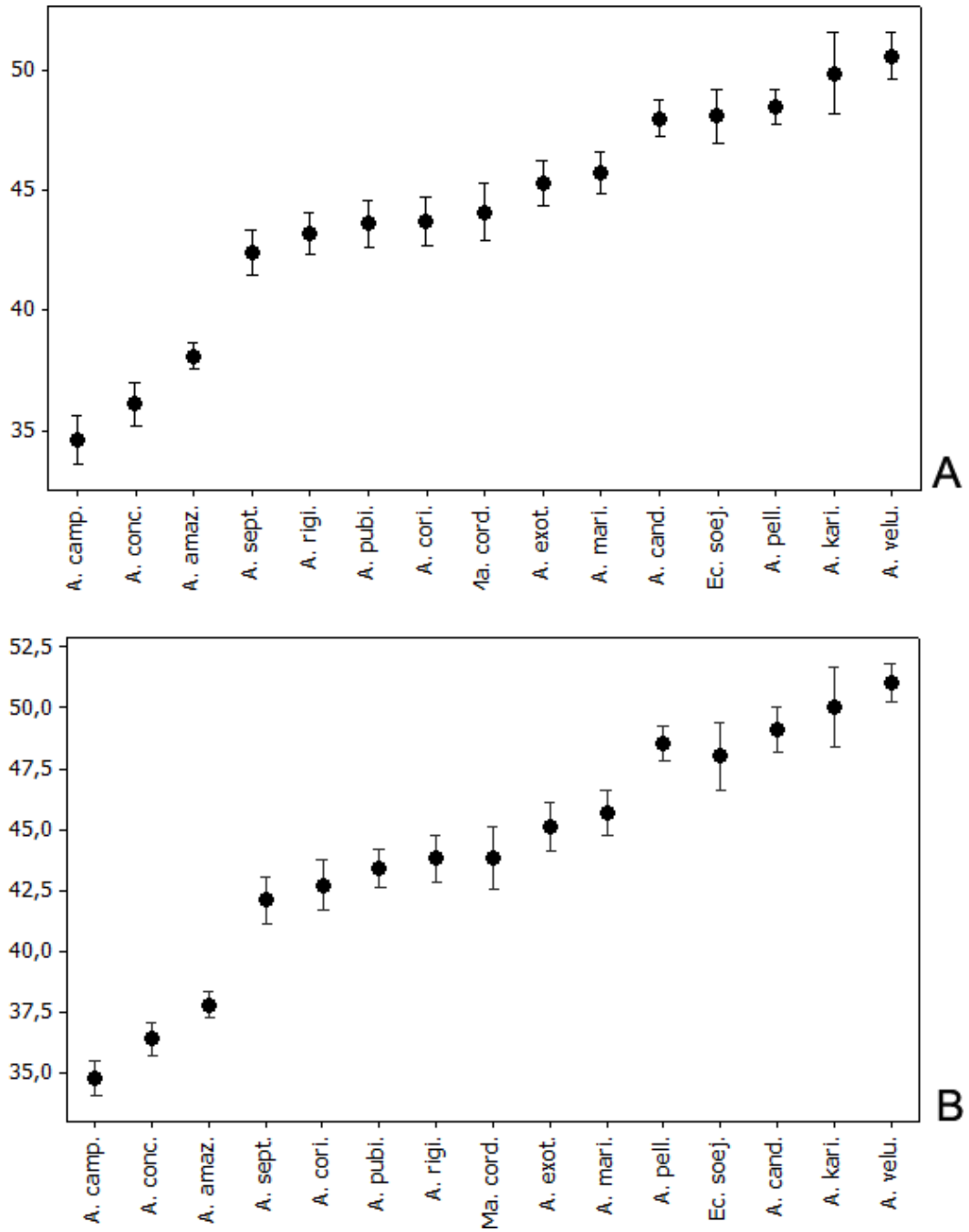

Figure 4. Graphics from the diameter average of the pollen grains of *Amorimia* and outgroups. **A.** Diameter I. **B.** Diameter II. Circles show the arithmetic average of the diameter values of pollen grains and their variation limits represented by the confidence interval. A. camp = *Amorimia camporum*; 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*.

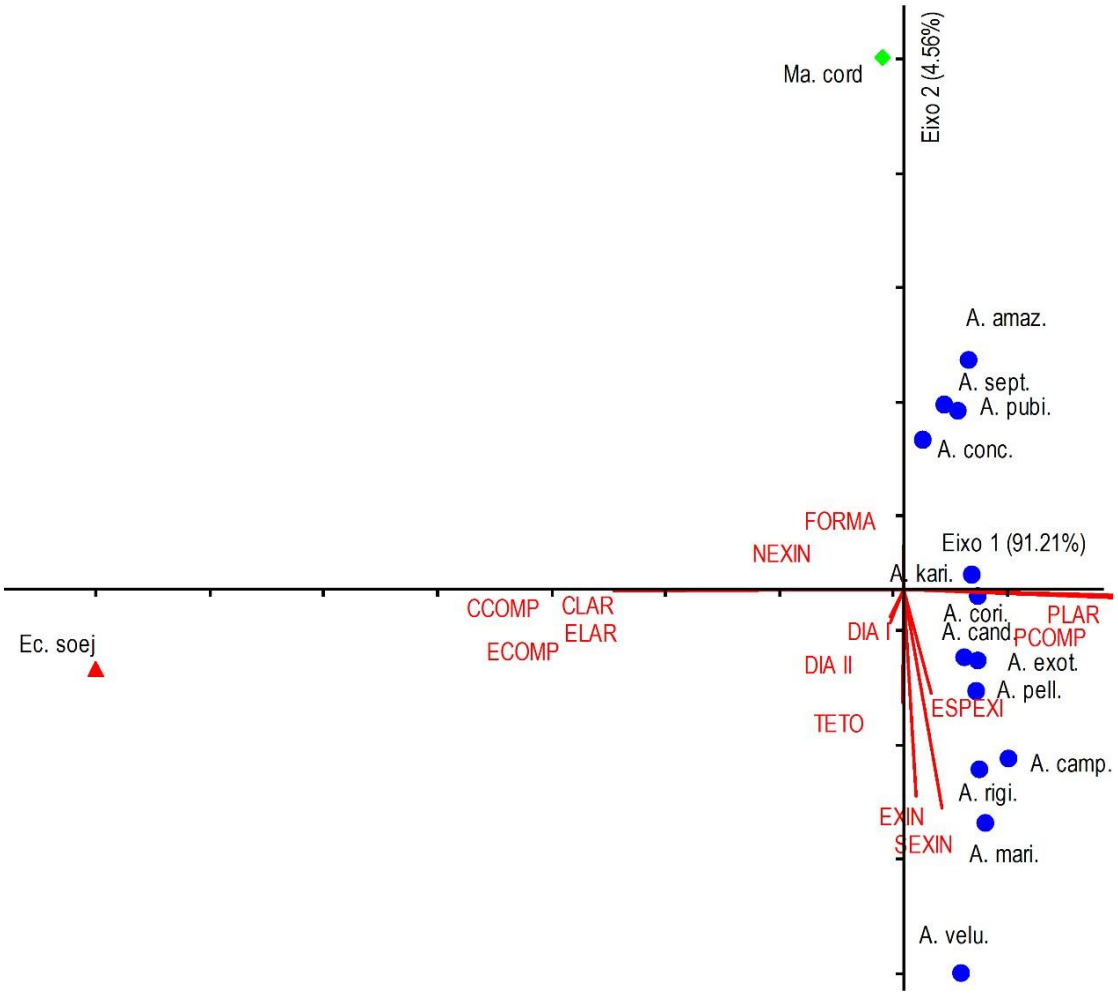

Figure 5. PCA ordination of the species of *Amorimia*, *Ectopopterys* and *Mascagnia* regarding all metric variables of pollen grains. A cand, = *Amorimia candidae*; A. cori. = *Amorimia coriacea*; A. exot. = *Amorimia exotropica*; A. mari. = *Amorimia maritima*; A. pell. = *Amorimia pellegrinii*; A. 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*.

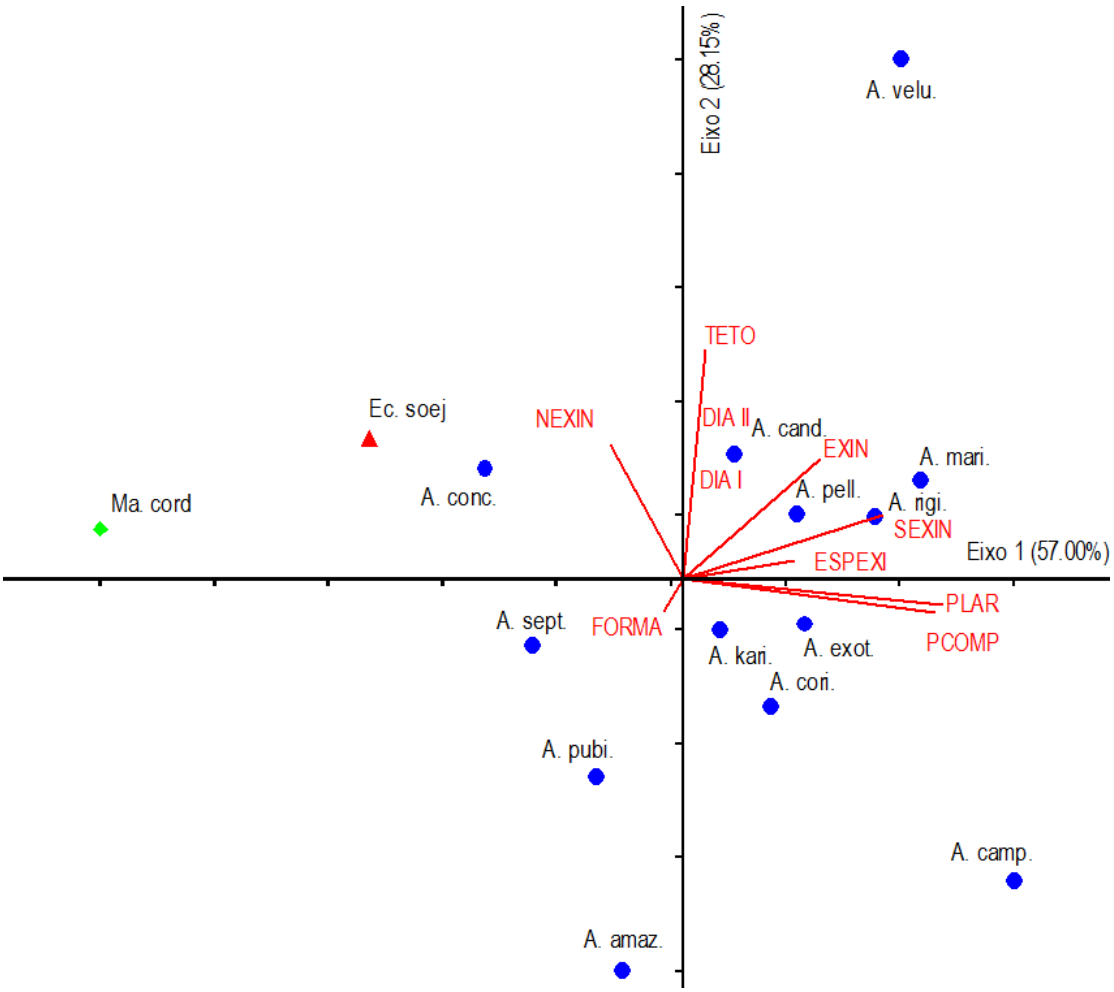

Figure 6. PCA ordination of the species of *Amorimia*, *Ectopopterys* and *Mascagnia* regarding metric variables from all pollen grains. A cand, = *Amorimia candidae*; A. cori. = *Amorimia coriacea*; A. exot. = *Amorimia exotropica*; A. mari. = *Amorimia maritima*; A. pell. = *Amorimia pellegrinii*; 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*.

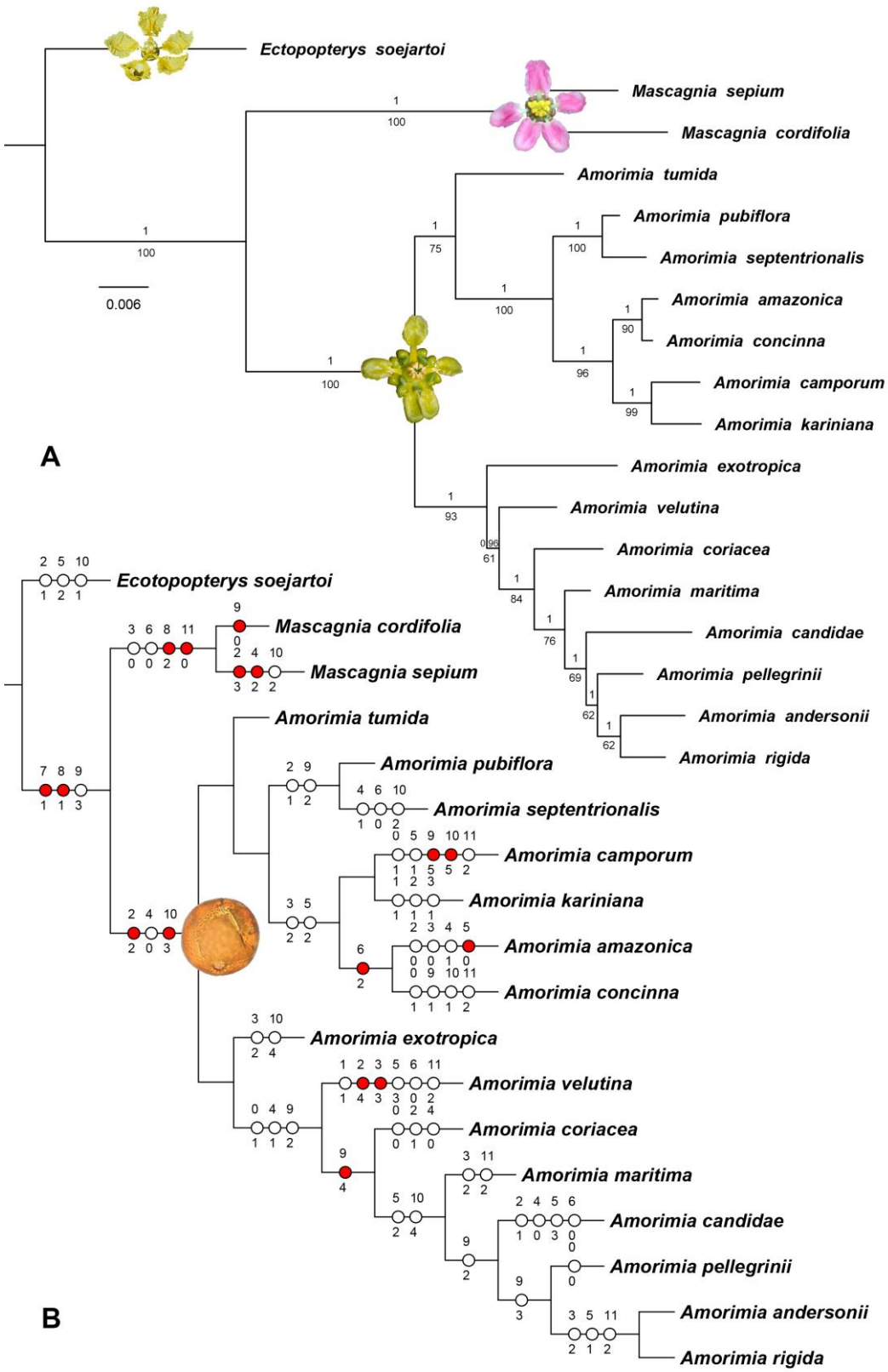

Figure 7. Molecular phylogeny and pollen character-mapping of *Amorimia* and allies (Malpighiaceae). **A.** Phylogenetic tree - numbers above and below branches represent posterior probability and bootstrap values, respectively. **B.** Character mapping tree - red circles represent apomorphies (synapomorphies 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.

Table 1. List of herbarium specimens sampled in this study for 13 species of *Amorimia*, *Ectopopterys soejartoi* and *Mascagnia cordifolia*.

Species	Subgenera	Sampled specimens (acronyms following Thiers cont. updated)
Ectopopterys soejartoi W.R.Anderson	Outgroup	Peru. Alto Amazonas. Loreto. Rio Maranon, 21 October 1972, *Wurdack 2356* (US).
Mascagnia cordifolia (A.Juss.) Griseb.	Outgroup	Brazil. Mato Grosso do Sul. Corguinho, Fazenda Colorado, 19 October 2012, *Francener et al. 1172* (CGMS).
Amorimia candidae R.F.Almeida	*A.* subg. *Amorimia*	Brazil. Bahia. Santa Terezinha, Serra da Jibóia, S12°47'46" W39°31'37", 303 m, 9 October 2010, *Melo 8557* (HUEFS). Brazil. Bahia. Itaberaba, pastagem, S12°28' W40°18', 15 October 2002, *Moura 3* (HUEFS).
Amorimia coriacea (Griseb.) R.F.Almeida	*A.* subg. *Amorimia*	Brazil. Rio de Janeiro. Niterói, Itaipuaçu, Pico Alto Moirão, 17 June 1985, *Andreata 708* (HUEFS). Brazil. Rio de Janeiro. Peró, Praia das Conchas, 14 January 2016, *Almeida & Pellegrini 615* (HUEFS).
Amorimia exotropica (Griseb.) W.R.Anderson	*A.* subg. *Amorimia*	Brazil. Paraná. *Dusén 14093* (MBM). Brazil. Rio Grande do Sul. Nova Petrópolis, Bairro Joaneta, 4 January 1990, *Schlindwein 534* (MPUC).
Amorimia maritima (A.Juss.) W.R.Anderson	*A.* subg. *Amorimia*	Brazil. Bahia. Ilhéus, ramal que separa a Fazenda Alegrias do Campus da Universidade Estadual de Santa Cruz, 7 June 1995, *Mattos-Silva 3136* (CEPEC). Brazil. Bahia. Barro Preto, Serra da Pedra Lascada, 26 April 2004, *Amorim 4102* (CEPEC)
Amorimia pellegrinii R.F.Almeida	*A.* subg. *Amorimia*	Brazil. Bahia. Tanquinho, estrada para Exu, S12°42' W39°43', 2 June 2005, *Carvalho 111, 114* (HUEFS) Brazil. Bahia. Ipirá, S11°22' W38°41', 14 October 2002, *Moura s.n.* (HUEFS)
Amorimia rigida (A.Juss.) W.R.Anderson	*A.* subg. *Amorimia*	Brazil. Minas Gerais. São Miguel do Jequitinhonha, 26 June 2013, *Almeida 557* (HUEFS). Brazil. Minas Gerais. São Miguel do Jequitinhonha, Reserva Biológica da Mata Escura, 29 June 2013, *Almeida 561* (HUEFS)
Amorimia velutina W.R.Anderson	*A.* subg. *Amorimia*	Brazil. Minas Gerais. Itaobim, entre Itaobim e Jequitinhonha. Km 4, 9 March 1977, *Shepherd et al. 4409* (UEC). Brazil. Bahia. Caetité, Fazenda Baixa Grande, caminho para Pajeú do Vento,

		S14°04’03” W42°38’12”, 820 m, 9 February 1997, *Stannard 5312* (HUEFS)
Amorimia amazonica (Nied.) W.R.Anderson	*A.* subg. *Uncinae*	Peru. Amazonas, Bosque Seco, 6 November 1999, *Rojas 753* (US).
Amorimia camporum W.R.Anderson	*A.* subg. *Uncinae*	Peru. Cajamarca. San Ignacio, District Huarango, 26 April 1996, *Campos & Díaz 2658* (US). Peru. San Martín. Juan Jui, Alto Rio Huallaga, February 1936, *Klug 4259* (US).
Amorimia concinna (C.V.Morton) W.R.Anderson	*A.* subg. *Uncinae*	Colombia. Magdalena. Magdalena, Valledupar, 12 January 1944, *Haught 3927* (US).
Amorimia kariniana W.R.Anderson	*A.* subg. *Uncinae*	Ecuador. Guayas. Pedro Carbo, 8 July 1940, *Haught 3070* (US).
Amorimia pubiflora (A.Juss.) W.R.Anderson	*A.* subg. *Uncinae*	Brazil. São Paulo. Castilho, 20 August 1972, *Melichenko s/n* (IAC).
Amorimia septentrionalis W.R.Anderson	*A.* subg. *Uncinae*	Brazil. Ceará. Itaitinga, Sererau, 10 April 2003, *dos Santos s.n.* (HUEFS). Brazil. Ceará. sin. loc., 22 July 1958, Döbereiner 538 (RFA).

Table 2. List of morphological characters of pollen grains and their character states for the species of *Amorimia* and outgroups sampled.

Character 0	Pollen grain, shape: (0) prolate-spheroidal, (1) oblate-spheroidal
Character 1	Pollen grain, size: (0) medium, (1) large
Character 2	Pollen grain, exine, thickness: (0) 2.00-2.99, (1) 3.00-3.99, (2) 4.00-4.99, (3) 5.00-5.99, (4) 6.00-6.99
Character 3	Pollen grain, sexine, thickness: (0) 1.00-1.99, (1) 2.00-2.99, (2) 3.00-3.99, (3) 4.00-4.99
Character 4	Pollen grain, nexine, thickness: (0) 0.01-0.99, (1) 1.00-1.99, (2) 3.00-3.99
Character 5	Pollen grain, tectum, thickness: (0) 0.01-0.50, (1) 0.51-0.99, (2) 1.00-1.50, (3) 1.51-1.99
Character 6	Pollen grain, ornamentation, type: (0) rugulate with psilate regions, (1) rugulate with areolate regions, (2) rugulate
Character 7	Pollen grain, aperture, type: (0) colporate, (1) porate
Character 8	Pollen grain, aperture, number: (0) 3, (1) 6, (2) 8
Character 9	Pollen grain, aperture, size, long: (0) 3.00-3.99, (1) 4.00-4.99, (2) 5.00-5.99, (3) 6.00-6.99, (4) 7.00-7.99, (5) 8.00-8.99
Character 10	Pollen grain, aperture, size, width: (0) 3.00-3.99, (1) 4.00-4.99, (2) 5.00-5.99, (3) 6.00-6.99, (4) 7.00-7.99, (5) 9.00-9.99
Character 11	Pollen grain, exine, thickness: (0) very thin, (1) thin, (2) thick

Table 3. Quantitative data of pollen grains of *Amorimia* and outgroups. x = average, sx = standard deviation of the average, s = standard deviation of the sample, IC = 95% confidence interval, CV = variation coefficient. * *n*=25.

Subgenus	Species	(Xmin – Xmax) X ±sx	s	IC	CV%
		Diameter I (μm)			
Amorimia	*Amorimia candidae*	(45.00-50.00) 48.00 ± 0.35	1.77	(47.27-48.73)	3.68
	Amorimia coriacea	(40.00-50.00) 43.70 ± 0.48	2.41	(42.71-44.69)	5.51
	Amorimia exotropica	(42.50-50.00) 45.30 ± 0.44	2.20	(44.39-46.21)	4.86
	Amorimia maritima	(42.50-50.00) 45.70 ± 0.42	2.11	(44.83-46.57)	4.61
	Amorimia pellegrinii	(45.00-52.50) 48.50 ± 0.35	1.77	(47.77-49.23)	3.64
	Amorimia rigida	(40.00-47.50) 43.20 ± 0.42	2.11	(42.33-44.07)	4.88
	Amorimia velutina	(45.00-55.00) 50.60 ± 0.46	2.13	(49.64-51.56)	4.57
Uncinae	*Amorimia amazonica*	(35.00-40.00) 38.10 ± 0.29	1.31	(37.56-28.64)	3.43
	Amorimia camporum	(30.00-37.50) 34.60 ± 0.49	2.47	(33.58-35.62)	7.13
	Amorimia concinna	(32.50-40.00) 36.10 ± 0.43	2.17	(35.20-37.00)	6.02
	Amorimia kariniana	(42.50-55.00) 49.88 ± 0.68	3.42	(48.47-51.29)	6.86
	Amorimia pubiflora	(40.00-50.00) 43.60 ± 0.48	2.40	(42.61-44.59)	5.51
	Amorimia septentrionalis	(37.50-47.50) 42.40 ± 0.47	2.34	(41.44-43.36)	5.51
-	*Mascagnia cordifolia*	(37.50-50.00) 44.10 ± 0.58	2.88	(42.91-45.29)	6.52
-	*Ectopopterys soejartoi*	(42.50-52.50) 48.10 ± 0.55	2.73	(46.97-49.23)	5.67
		Diameter II (μm)			
Amorimia	*Amorimia candidae*	(45.00-52.50) 49.10 ± 0.45	2.27	(48.16-50.04)	4.62
	Amorimia coriacea	(40.00-47.50) 42.70 ± 0.50	2.49	(41.67-43.73)	5.84
	Amorimia exotropica	(40.00-50.00) 45.10 ± 0.49	2.45	(44.09-46.11)	5.42
	Amorimia maritima	(42.50-50.00) 45.70 ± 0.45	2.23	(44.78-46.62)	4.87
	Amorimia pellegrinii	(42.50-50.00) 47.70 ± 0.45	2.27	(46.76-48.64)	4.77
	Amorimia rigida	(40.00-50.00) 43.80 ± 0.46	2.30	(42.85-44.75)	5.24
	Amorimia velutina	(47.50-55.00) 51.00 ± 0.38	1.91	(50.21-51.79)	3.74
Uncinae	*Amorimia amazonica*	(35.00-40.00) 37.80 ± 0.26	1.31	(37.26-38.34)	3.48
	Amorimia camporum	(32.50-37.50) 34.80 ± 0.35	1.76	(34.08-35.52)	5.05
	Amorimia concinna	(32.50-40.00) 36.40 ± 0.33	1.63	(35.73-37.07)	4.47
	Amorimia kariniana	(45.00-55.00) 50.00 ± 0.66	3.31	(48.63-51.37)	6.61
	Amorimia pubiflora	(40.00-47.50) 43.40 ± 0.38	1.89	(42.62-44.18)	4.36
	Amorimia septentrionalis	(37.50-45.00) 42.10 ± 0.47	2.36	(41.13-43.07)	5.60
-	*Mascagnia cordifolia*	(37.50-50.00) 43.80 ± 0.61	3.07	(42.53-45.07)	7.01
-	*Ectopopterys soejartoi*	(42.50-55.00) 48.00 ± 0.68	3.39	(46.60-49.40)	7.05

Table 4. Arithmetic average, in μm, of apertures (pores, endoapertures* and **colpi**) and exine measurements of pollen grains of the studied species of *Amorimia*, *Ectopopterys* and *Mascagnia*, *n*=10.

Subgenus	Species	Lenght	Width	**Lenght**	**Width**	Exine	Sexine	Nexine	Tectum
Amorimia	*Amorimia candidae*	5.96	7.36	-	-	3.71	2.96	0.95	1.51
	Amorimia coriacea	7.39	6.96	-	-	3.78	2.85	0.93	0.86
	Amorimia exotropica	6.98	7.00	-	-	4.16	3.17	0.99	0.85
	Amorimia maritima	7.27	7.60	-	-	4.74	3.73	1.01	1.20
	Amorimia pellegrinii	6.86	7.15	-	-	4.00	2.94	1.06	1.29
	Amorimia rigida	6.80	7.23	-	-	4.87	3.78	1.09	0.95
	Amorimia velutina	5.85	6.09	-	-	6.08	4.95	1.13	1.81
Uncinae	*Amorimia amazonica*	6.44	6.68	-	-	2.87	1.53	1.34	0.39
	Amorimia camporum	8.86	9.1	-	-	4.21	3.63	0.58	0.78
	Amorimia concinna	4.27	4.32	-	-	4.08	3.09	0.99	1.15
	Amorimia kariniana	6.78	6.90	-	-	3.49	2.53	0.96	1.02
	Amorimia pubiflora	5.91	6.14	-	-	3.27	2.37	0.90	0.66
	Amorimia septentrionalis	5.30	5.39	-	-	3.69	2.46	1.22	0.73
-	*Ectopopterys soejartoi*	4.28*	4.38*	**24.06**	**3.74**	3.48	2.16	1.34	1.10
-	*Mascagnia cordifolia*	3.18	3.12	-	-	2.96	1.76	1.20	0.82

Table 5. Pearson and Kendall correlation coefficients among all metric variables of pollen grains and two initial PCA ordination axes for the studied species.

Variables		Principal Components	
		Axis 1	Axis 2
DIAI	Diameter I	-0.0185	-0.1156
DIAII	Diameter II	-0.0182	-0.1281
PCOM	Pore length	0.3955	-0.3077
PLAR	Pore width	0.4013	-0.3542
CCOM	Colpus length	-0.6180	-0.2720
CLAR	Colpus width	-0.2984	-0.1313
ECOM	Endoaperture length	-0.3191	-0.1404
ELAR	Endoaperture width	-0.3227	-0.1420
EXIN	Exine	0.0244	0.4342
SEXI	Sexine	0.0532	-0.5671
NEXI	Nexine	-0.0374	0.0210
TETO	Tectum	-0.0097	-0.3273
FORMA	Pollen grain shape	0.0000	0.0077
ESPEXI	Exine thickness	0.0039	-0.0330

Table 6. Pearson and Kendall correlation coefficients among metric variables of all pollen grains and two initial PCA ordination axes for the studied species.

Variables		Principal Components	
		Axis 1	Axis 2
DIAI	Diameter I	0.0041	0.2734
DIAII	Diameter II	0.0106	0.2901
PCOM	Pore length	0.5593	-0.2908
PLAR	Pore width	0.5977	-0.2711
EXIN	Exine	0.2964	0.4004
SEXI	Sexine	0.4558	0.3740
NEXI	Nexine	-0.1378	0.2645
TETO	Tectum	0.1204	0.5590
FORMA	Pollen grain shape	-0.0044	-0.0078
ESPEXI	Exine thickness	0.0285	0.0167

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Table 7. 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 size 4.00-4.99 µm width	-
Mascagnia + *Amorimia* clade	aperture size 6.00-6.99 µm long	aperture type porate; aperture number 6
Mascagnia	sexine thickness 1.00-1.99 µm; ornamentation type rugulate with psilate regions	aperture number 8; exine thickness very thin
Mascagnia cordifolia	-	aperture size 3.00-3.99 µm long
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 size 6.00-6.99 µm width
Amorimia subg. ***Amorimia***	-	-
Amorimia exotropica	sexine thickness 3.00-3.99 µm; aperture size 7.00-7.99 µm width	-
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 size 5.00-5.99 µm long	-
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 size 7.00-7.99 µm long
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* +	tectum thickness 0.51-0.99 µm; aperture size 7.00-7.99 µm	-

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A. andersonii + *A. rigida* clade	width	
Amorimia maritima	sexine thickness 3.00-3.99 µm; exine thick	-
A. candidae + *A. pellegrinii* + *A. andersonii* + *A. rigida* clade	aperture size 6.00-6.99 µm long	-
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 size 6.00-6.99 µm long	-
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***	-	-
A. pubiflora + *A. septentrionalis* clade	exine thickness 3.00-3.99 µm; aperture size 5.00-5.99 µm long	-
Amorimia septentrionalis	nexine thickness 1.00-1.99 µm; ornamentation type rugulate with psilate regions; aperture size 5.00-5.99 µm width	-
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 size 8.00-8.99 µm long; aperture size 9.00-9.99 µm width
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 size 4.00-4.99 µm long; aperture size 4.00-4.99 µm width; exine thick	-