



Pliocene *Lythrum* (loosestrife, Lythraceae) pollen from Portugal and the Neogene establishment of European lineages

Manuel Vieira ^a, Reinhard Zetter ^b, Mario Coiro ^c, Friðgeir Grímsson ^{d,*}

^a GEOBIOTEC, Department of Earth Sciences, NOVA School of Science and Technology, Campus de Caparica, P-2829 516 Caparica, Portugal

^b University of Vienna, Department of Paleontology, Althanstraße 14, 1090 Vienna, Austria

^c Ronin Institute for Independent Scholarship, Montclair, NJ, USA

^d University of Vienna, Department of Botany and Biodiversity Research, Division of Structural and Functional Botany, Rennweg 14, 1030 Vienna, Austria

ARTICLE INFO

Article history:

Received 8 July 2021

Received in revised form 17 October 2021

Accepted 19 October 2021

Available online 23 October 2021

Keywords:

Dated phylogeny

Paleovegetation

Phytogeography

Piacenzian

Speciation

Wetland

ABSTRACT

The fossil record of *Lythrum* is scarce and every new find brings an extra piece of the puzzle to the obscure phyto-geographic history of this genus. *Lythrum* pollen is unique and has the potential to be recognized in palynological assemblages. Therefore, the few pre-Holocene fossil records are all pollen described from North America, Russia, and Europe. The European records are both most numerous and geologically younger than those from other parts of the Northern Hemisphere. Earliest European *Lythrum* pollen records are of late Miocene age and parallel to starting crown group radiation in the Eurasian clade of *Lythrum*. European Miocene to Pliocene *Lythrum* pollen morphology is comparable to that of extant species. Interestingly, the late Miocene expansion of *Lythrum* into Europe coincides with the decline of *Decodon*, and *Lythrum* appears to replace *Decodon* in late Neogene fossil palynological assemblages.

© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The genus *Lythrum* L. comprises approximately 37 species of herbaceous plants, ranging from single small (10–30 cm) to relatively tall (up to ca 1.5 m) plants forming large colonies (Koehne, 1903; Webb, 1967; Graham et al., 1987; Morris, 2007; POWO, 2019). Most of the species are found in wetland areas, marshlands and swamps, in water or close to the shoreline of lakes/ponds. *Lythrum* produces small seeds and all species are insect pollinated (Koehne, 1903; Graham et al., 1987; Morris, 2007; POWO, 2019). The fossil record of *Lythrum* is sparse and there are no reliable macro- or mesofossil records (vegetative parts, leaves, fruits, seeds) from sediments older than the Holocene (Graham, 2013). The pollen record of *Lythrum* has been summarized by Graham and Graham (1971), Muller (1981), Grímsson et al. (2011), and Graham (2013). Its earliest known pollen record, *Lythrum elkensis* Grímsson, R.Zetter et C.C. Hofm., is from the early Campanian (Late Cretaceous) of Wyoming, northwestern USA (Grímsson et al., 2011). Younger records include late Miocene pollen from southeastern Austria (*Lythrum wilhelmii* Grímsson, R. Zetter et C. C. Hofmann; Grímsson et al., 2011), as well as pollen from the late Miocene to Pliocene of Spain (Van Campo, 1989; Peñalba, 1985), the Pliocene of Germany (Menke, 1976), the Pleistocene of Greece (Wijmstra, 1969), and the

Pleistocene to Holocene of France (Bakels, 1995; Andrieu et al., 1997) (Fig. 1A).

Here we add new Pliocene records of *Lythrum* pollen from the Atlantic coast of Portugal. The pollen is described and compared to previously reported European records as well as pollen of extant species in this part of the globe. Based on the fossil record and a newly built dated phylogenetic framework, the timing for dispersal of *Lythrum* into Europe as well as the evolution and possible origin of European lineages are discussed. The new *Lythrum* pollen from Portugal is also evaluated in relation to: 1) previous palynological records from the same basin, 2) its potential paleohabitat, 3) and role in the paleovegetation.

2. Materials and methods

2.1. Sample preparation and study technique

Standard laboratory processing for palynomorphs (e.g. Fægri and Iversen, 1989) was applied to the samples; HCl (55%) and HF (37%) were used for acid maceration, followed by 125 µm and 10 µm sieving to remove coarse and fine fractions respectively. Due to the high quantities of amorphous organic matter and charcoal fragments, the samples were boiled in a sodium perborate solution for about 5 min, and then washed and centrifuged three times. No oxidizing reagents or alkalis that can be harmful to pollen grains were used. The final residues were diluted in glycerin and mounted on slides without cover slips for

* Corresponding author.

E-mail address: fridgeir.grimsson@univie.ac.at (F. Grímsson).

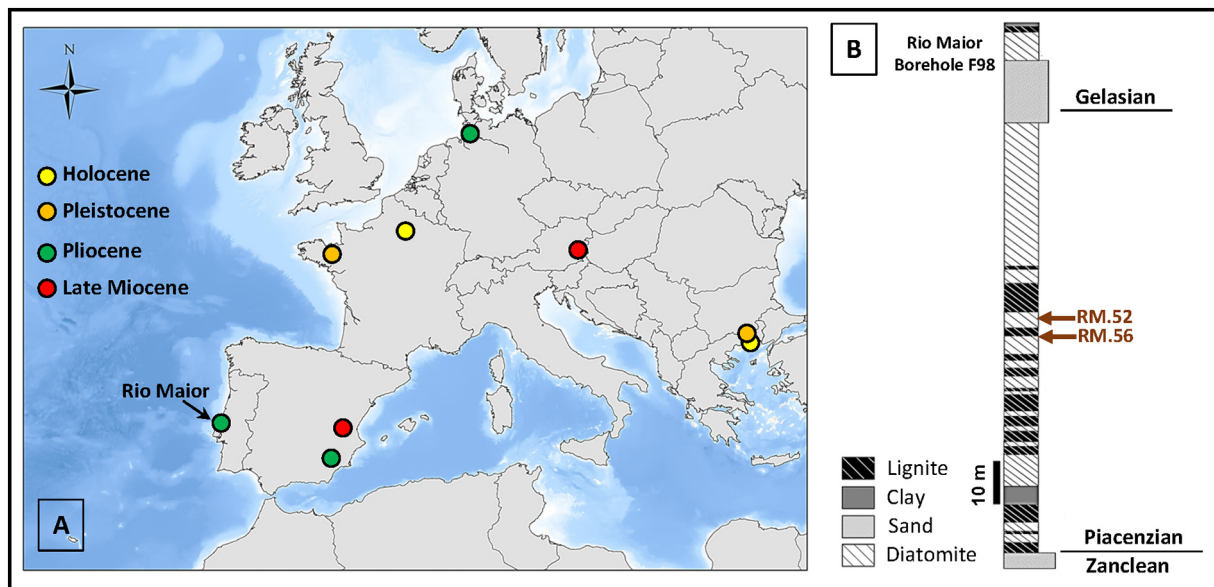


Fig. 1. A. European fossil *Lythrum* pollen records. B. Lithology of the F98 borehole and stratigraphic position of samples with fossil *Lythrum* pollen.

selection and extraction of individual pollen grains. The individual grains were investigated using the single-grain method that combines light (LM) and scanning electron microscopy (SEM) (Zetter, 1989; Halbritter et al., 2018). They were extracted using a micromanipulator (preparation needle with an eyebrow hair fixed to it) and transferred into a clean drop of glycerin on a separate slide for photography with LM. Using the micromanipulator, the same grains were then transferred onto a SEM specimen stub. To dissolve the remaining glycerin, the pollen grains were washed with small drops of absolute ethanol. The SEM specimen stubs containing the pollen were then sputter coated with gold and the pollen was observed and photographed with SEM. Terminology for pollen description follows Punt et al. (2007; LM) and Halbritter et al. (2018; SEM).

2.2. Phylogenetic analysis

For the dated phylogeny we used GenBank to assemble a matrix including 14 species of *Lythrum* and three outgroups covering three loci: the chloroplast *rbcl* and *matK* genes and the nuclear 18S–5.8S–26S ribosomal DNA locus (Table 1). Sequences were aligned using the MAFFT online service (Katoh et al., 2019) using the default settings. A dated analysis using the fossilized birth–death prior was run using MrBayes

ver. 3.2.7 (Ronquist et al., 2012) as implemented on the CIPRES Science Gateway (Miller et al., 2010). The prior on the age of the tree was set as a uniform distribution between the oldest age for the oldest fossil of *Lythrum* (84 Ma) and the age of the oldest eudicot fossil (125 Ma) (Coiro et al., 2019). A separate constraint was put on the crown group of *Lythrum*, constraining its origin between the oldest age of the oldest fossil (83 Ma) and the age of the oldest Cenozoic fossil (9.4 Ma). A GTR plus Gamma model was employed on three separate partitions (one per loci), with the substitution rates and the Gamma shape parameters unlinked over the partitions. A single Independent Gamma Rate clock model was applied to all partitions. The sampling probability parameter of the fossilized birth–death prior was set to 0.06, the proportion of extant species of Lythraceae sampled in our matrix, and the sampling strategy was set to ‘diversity’. Two independent Metropolis-coupled Monte Carlo runs (mcmc; with one cold chain and three heated chains) were run for 10,000,000 generations, sampling every 5000 generations. Convergence of the mcmc chains was checked using Tracer (Rambaut et al., 2018), using an Effective Sample Size of > 200. A consensus tree was then generated using the ‘allcompat’ option in MrBayes.

2.3. Provenance of studied fossil material

Along the Atlantic coast of Portugal, three main Cenozoic basins, the Tagus, Mondego, and Alvalade, preserve Pliocene sediments. The here described fossil pollen comes from the Rio Maior sub-basin, an intrabasin at the northwestern margin of the Tagus Basin in central western Portugal. Due to its geographic position, the Rio Maior sub-basin has attracted a lot of scientific attention and the deposits have been subject to palaeobotanical studies over the last c. 40 years (e.g., Diniz, 1984a, 1984b; Vieira, 2009; Vieira et al., 2011, 2018; Vieira and Zetter, 2020). The Pliocene deposits within the sub-basin cover an area that is 7.5 km long and 2 km wide and overly an unconformity with Jurassic, Cretaceous, Paleogene and Miocene strata. From the bottom towards the top, the Pliocene strata of the Rio Maior sub-basin comprise fine caulinitic sandstones, lignites, and diatomites, and the uppermost layers are clays and sands (Fig. 1B) (Zbyszewski, 1967). The most complete sedimentary succession is located near the eastern border of the basin and constitutes a small anticline with a NW–SE orientation that is 3.5 km long and 1 km wide, with a maximum sediment thickness of about 120 m. The Borehole F98, sampled for this study, was

Table 1
GenBank accessions used in the phylogenetic analysis for this study.

Species	rbcl	matK	Ribosomal RNA
<i>Decodon verticillatus</i>	AY905406.1	GU228434.1	AY905421.1
<i>Heimia salicifolia</i>	AY905410.1	GU228438.1	
<i>Lawsonia inermis</i>	AY496863.1	GU228442.1	KF850586.1
<i>Lythrum alatum</i>	KJ773667.1	MK520296.1	MG236674.1
<i>Lythrum borysthenicum</i>	HM850152.1	HM850985.1	
<i>Lythrum californicum</i>	MF963134.1	MF963454.1	MF964073.1
<i>Lythrum curtissii</i>	KX397855.1	KY607284.1	
<i>Lythrum flagellare</i>	KJ773668.1	EU002183.1	
<i>Lythrum hyssopifolia</i>	HM850153.1	KF224979.1	MG975399.1
<i>Lythrum junceum</i>	HM850154.1	HM850987.1	MG975401.1
<i>Lythrum lineare</i>	KJ773669.1		AY910748.1
<i>Lythrum ovalifolium</i>		GU228443.1	
<i>Lythrum portula</i>	HM850155.1	JN895968.1	MG237615.1
<i>Lythrum salicaria</i>	KM360870.1	MH659998.1	MG975396.1
<i>Lythrum thymifolia</i>			MG975400.1
<i>Lythrum tribracteatum</i>			MG975398.1
<i>Lythrum virgatum</i>		GU228444.1	MG975397.1

located in the central part of the anticline to capture the most complete sequence (see Vieira et al., 2018).

The fossil *Lythrum* pollen grains reported were discovered in samples RM.52 and RM.56, in association with lignite bearing units of the sedimentary strata (Fig. 1B). The age of these deposits was refined by Vieira (2009) and Vieira et al. (2018) following regional sedimentological and lithostratigraphical studies (e.g., Cachão, 1990; Cunha et al., 1993; Silva, 2001; Diniz, 2003; Ramos and Cunha, 2004; Vieira et al., 2006). A marine transgression took place along the Atlantic coast of Portugal during the late Zanclean–early Piacenzian (dated around 3.6–3.5 Ma) (Cunha et al., 1993; Ramos and Cunha, 2004). The shallow marine deposits outcropping at Vale do Freixo (locality situated north of Rio Maior sub-basin within the Tagus Basin) document this transgression. The study of calcareous nannofossils (Cachão, 1990), isotopic analyses from pectinid shells (Silva, 2001), and stratigraphic correlation using dinoflagellate cysts (Vieira et al., 2006) allowed for a confident dating of this transgression. The basal sands in the Rio Maior sub-basin show the same marine influence as in Vale do Freixo, and the overlying lacustrine diatomite/lignite sequence corresponds mainly to Piacenzian (late Pliocene) sedimentation (Vieira et al., 2018). The uppermost sediments of the Rio Maior sub-basin, outcropping in the Abum area, have been dated by magnetostratigraphy to the Gauss–Matuyama reversal at 2.58 Ma (Diniz and Mörner, 1995). This suggests an early Gelasian (earliest Pleistocene) age for the final infilling of the Rio Maior sub-basin (Diniz, 2003). The pollen bearing sediments containing the *Lythrum* pollen (Fig. 1B) can therefore be considered to be of late Pliocene (Piacenzian) age (3.6–2.58 Ma; following ICS, 2020).

3. Results

3.1. Systematic description

Order: MYRTALES Juss. Ex Bercht. & J.Presl

Family: LYTHRACEAE J.St.-Hil.

Genus: **Lythrum** L.

“Generic pollen diagnoses:” The description of the fossil pollen follows the framework provided by Graham et al. (1987) and Grímsson et al. (2011). The pollen of extant *Lythrum* species (excl. *Peplis* type taxa) feature a combination of three colpi and three pseudocolpi, along with parallel striae running along the polar axis. The colpi are longer and wider than the pseudocolpi. The endopori are always present within the margin of the colpi and are equipped with an annulus. The SEM sculpture of the colpus membrane and pseudocolpi is usually microverrucate, microechinate, or microrugulate, and with widely spaced sculpture elements. The combination of these features is not found in any other Lythraceae.

Species: *Lythrum portugallense* sp. nov. (Plate I; Table 2).

“Diagnosis:” Tricolporate pollen with three alternating pseudocolpi; colpi longer than pseudocolpi. Endoporus large and circular with an annulus surrounding the porus. Pollen wall tectate; sculpture psilate to striate in LM, striate in SEM, striae are parallel and running along the polar axis, some striae are interwoven and/or curve towards colpus/pseudocolpus margin (SEM); microverrucate to microrugulate sculpture elements widely spaced in both colpi and pseudocolpi.

“Holotype:” UMP 2009-F98-RM52-01 (Plate I, 1–3).

“Paratypes:” UMP 2009-F98-RM52-02 (Plate I, 4–6), UMP 2009-F98-RM52-03 (Plate I, 7–9), UMP 2009-F98-RM56-01 (Plate I, 10–12).

“Type locality:” Rio Maior, Lower Tagus Basin, Portugal (lat. 39°20' N, long. 8°56' W).

“Age:” Piacenzian (3.6–2.58 Ma; following ICS, 2020).

“Etymology:” The species is named after Portugal, the country where the sediments containing the *Lythrum* pollen are found. The Latin name for Portugal is Portugallia and the ending “ense” refers to “from”, meaning “*Lythrum* from Portugal”, with a “-e” at the end because it’s an adjective of the i-stem class.

“Description:” Pollen, monad, P/E ratio oblate to isodiametric, shape spheroid-hexagonal prism, outline hexagonal in polar view, elliptic to circular in equatorial view, 21–25 µm wide in LM, 21–25 µm in SEM, polar axis 21–24 µm in LM, 21–23 µm in SEM; tricolporate, with three alternating pseudocolpi (heteroaperturate); colpi longer than pseudocolpi (LM, SEM), colpi 4.5–6.5 µm wide (SEM); endopori large and circular with an annulus surrounding the porus (LM, SEM); pseudocolpi 2.2–4.8 µm wide (SEM); exine 2.5–3.5 µm thick, nexine as thick or slightly thicker than sexine (LM); pollen wall tectate; sculpture psilate to striate in LM, striate in SEM, striae (0.3–0.5 µm wide) are parallel and running along the polar axis, some striae are interwoven and/or curve towards colpus/pseudocolpus margin (SEM); colpi and pseudocolpi with widely spaced microverrucate to microrugulate sculpture elements (SEM).

“Comparison:” The fossil *Lythrum portugallense* sp. nov. pollen from the Rio Maior sub-basin can be compared in size, outline, shape, aperture arrangement, wall thickness, and sculpture with pollen of both extant North American [for examples see *Lythrum alatum* (Plate II, 1–3) and *Lythrum curtissi* (Plate II, 4–6)] and European *Lythrum* species [for examples see *Lythrum hyssopifolia* (Plate II, 7–9) and *Lythrum salicaria* (Plate II, 10–12)]. Size-wise the Rio Maior pollen is most similar to that of *L. hyssopifolia*, the width of the colpi and pseudocolpi are also comparable, while the exine is much thicker in the fossil pollen than in pollen of *L. hyssopifolia* (Table 2). Its exine thickness is the same as in pollen of *L. salicaria* and *L. virgatum*. Based on the combined morphological features observed with LM and SEM the Rio Maior pollen resembles most pollen of *L. hyssopifolia* and *L. salicaria* (Table 2). Fossil *Lythrum* pollen grains have previously been described/recorded from the late Miocene to Holocene of Europe (Table 3), but based mostly on LM studies. The only comparable Neogene fossil is *L. wilhelmii* from the Late Miocene of Austria, also studied with combined LM and SEM (see fig. 1G–L in Grímsson et al., 2011). The Rio Maior pollen matches the Austrian fossils fairly well in size, shape outline, and sculpture. The pollen wall (exine) is considerably thicker in the fossil grains from Rio Maior, and sculpture elements (microverrucae and microrugulae) in the aperture and pseudoaperture regions of *L. wilhelmii* are often densely spaced, a feature not observed in the fossils from Portugal. Based on these differences, we refrain from assigning the Portuguese pollen to *L. wilhelmii* and describe it as a new species.

“Remarks:” This is the first *Lythrum* pollen record of Piacenzian age documented for south Europe using the single-grain method (combined LM and SEM; Zetter, 1989).

3.2. Phylogenetic analysis

Our dated phylogenetic analysis results in a well-resolved tree with eight clades with a posterior probability (pp) of more than 0.9; two clades receiving between 0.6 and 0.7 pp., and only four clades receiving less than 0.5 pp. (Fig. 2). The analysis retrieves two separate American and Eurasian clades of *Lythrum* with strong support (1 and 0.96 pp. respectively), though the relationships between the American species are unresolved. The modern genus *Lythrum* is inferred to have radiated between the latest Cretaceous and the mid-Oligocene (*Lythrum* crown age of 73.9–29.4 Ma; Fig. 2). The radiation of the American clade is inferred to have started between the mid-Oligocene and the latest Miocene (27.7–4.8 Ma; Fig. 2), while the Eurasian clade is inferred to have radiated earlier, between the late Paleocene and the earliest Miocene (58.6–21.4 Ma; Fig. 2).

4. Discussion

4.1. The Pliocene environment and vegetation units at Rio Maior: Does *Lythrum* fit the picture?

During the Pliocene, Mediterranean-type taxa expanded across the eastern part of the Iberian Peninsula (Barrón et al., 2010). In coeval

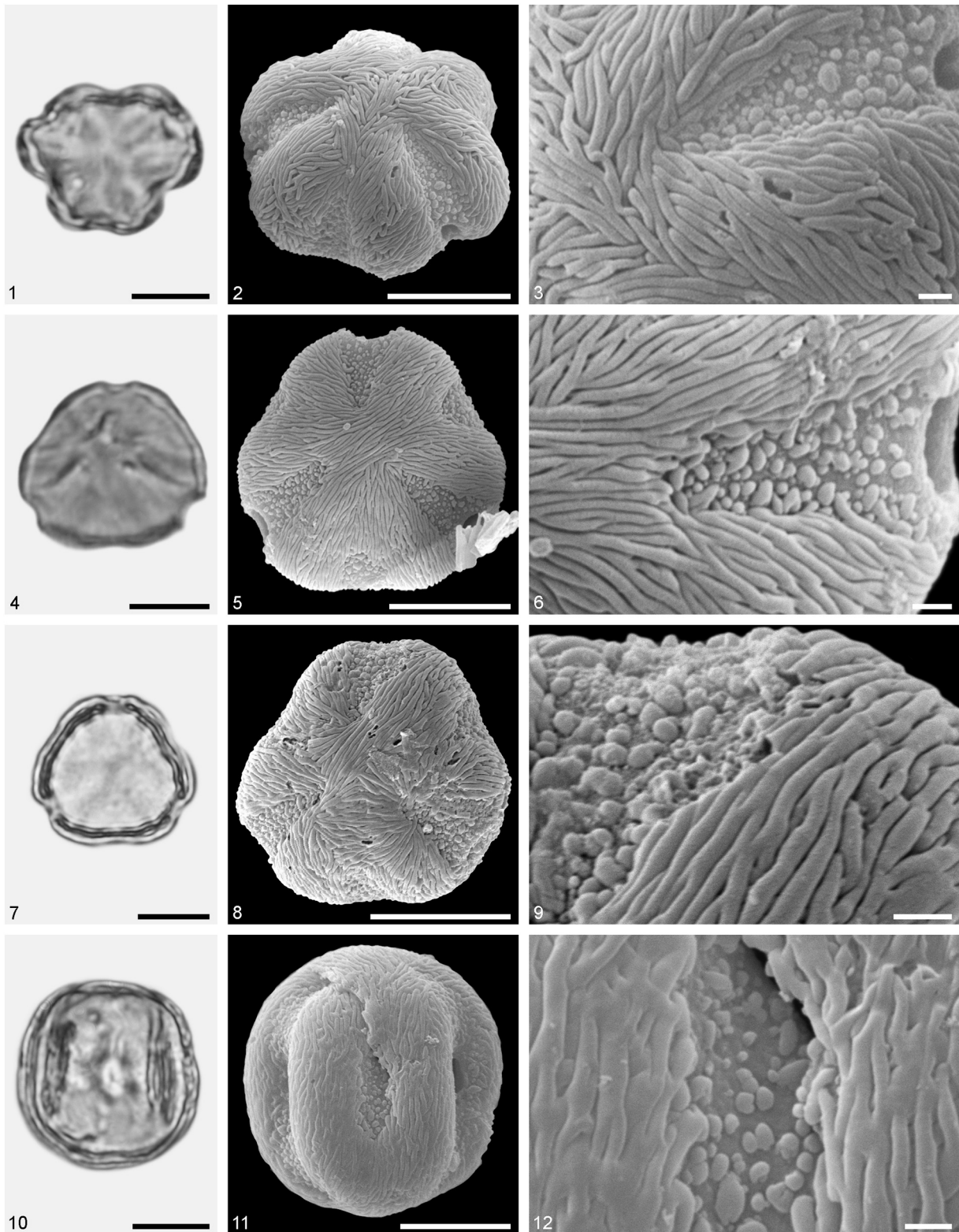


Plate I. Fossil *Lythrum portugallense* sp. nov. pollen from Rio Maior (1–9 from Sample RM 52; 10–12 from sample RM 56). (1, 4, 7, 10) Light micrographs, polar and equatorial views. (2, 3, 5, 6, 8, 9, 11, 12) Scanning electron micrographs of the same pollen grains showing the sculpture. (1–3) Holotype. (4–12) Paratypes. Scale bar = 10 μ m in 1, 2, 4, 5, 7, 8, 10, and 11; 1 μ m in 3, 6, 9 and 12.

western Portugal, a mixed broadleaved evergreen vegetation has been recorded dominated by Engelhardioideae, Ericaceae, *Myrica/Morella*, and *Quercus*. Furthermore, the progressive extirpation of warmth loving taxa, such as *Arecaceae*, *Castanea/Castanopsis*, Engelhardioideae, *Eurya*,

Nyssa, Rutaceae, Sapotaceae, *Symplocos*, and *Tetraclinis*, towards the late Piacenzian is evident (Vieira, 2009; Vieira et al., 2011; Martinetto and Vieira, 2020). Nonetheless, the Atlantic coast of the Iberian Peninsula appears to have maintained a warm and humid climate throughout

Table 2
Pollen morphology of fossil and extant *Lythrum* taxa.

	<i>L. elkensis</i> Late Cretaceous Wyoming, USA	<i>L. wilhelmii</i> Late Miocene Rechnitz, Austria	<i>L. portugallense</i> sp. nov. Late Pliocene Portugal	<i>L. hyssopifolia</i> Extant Eurasia	<i>L. salicaria</i> Extant Eurasia	<i>L. virgatum</i> Extant Eurasia
P/E ratio	Prolate to subprolate	Oblate to isodiamteric	Oblate to isodiamteric	Isodiamteric to subprolate	Isodiamteric to subprolate	Isodiamteric to subprolate
Outline eq.v.	Elliptic	Elliptic to circular	Elliptic to circular	Circular to elliptic	Circular to elliptic	Circular to elliptic
Outline p.v.	Triangular to hexagonal	Hexagonal to circular	Hexagonal to circular	Circular to hexagonal	Circular to hexagonal	Circular to hexagonal
Shape	Spheroid-hexagonal prism	Spheroid-hexagonal prism	Spheroid-hexagonal prism	Spheroid-hexagonal prism	Spheroid-hexagonal prism	Spheroid-hexagonal prism
Size in LM (E/P; μm)	18–20 / 21–23	22–25 / 21–23	21–25 / 21–24	21–26 / 20–25	21–35 / 20–35	20–39 / 20–38
Size in SEM (E/P; μm)	14–16 / 18–20	21–25 / 21–22	21–25 / 21–23	23–25 / 22–24	18–28 / 19–31	19–20 / 18–19
Aperture	Tricolporate	Tricolporate	Tricolporate	Tricolporate	Tricolporate	Tricolporate
Colpi width (μm)	1.5–2.1	4.1–6.2	4.5–6.5	5.8–6.9	5.2–8.5	5.5–6.5
Colpi vs. pseudocolpi	Equal in length	Colpi longer	Colpi longer	Colpi longer	Colpi longer	Colpi longer
Width of pseudocolpi in SEM (μm)	5.0–6.0	2.1–5.1	2.2–4.8	2.4–3.4	1.0–3.0	1.9–2.2
Exine thickness (μm)	2.0–3.5	1.3–1.7	2.5–3.5	1.5–2.2	2.5–3.5	2.5–3.5
Exine	Nexine thinner than sexine	Nexine as thick or slightly thicker than sexine	Nexine as thick or slightly thicker than sexine	Nexine thinner or as thick as sexine	Nexine thinner or as thick as sexine	Nexine thinner than sexine
Sculpture in SEM	Striate, striae parallel and running along the polar axis, sometimes irregularly arranged around pori	Striate, striae parallel and running along the polar axis	Striate, striae parallel and running along the polar axis, striae exhibiting some braiding	Striate, striae parallel and running along the polar axis	Striate, striae parallel and running along the polar axis	Striate, striae parallel and running along the polar axis
Width of striae in SEM	0.2–0.4	0.4–0.6	0.3–0.5	0.3–0.5	0.3–0.6	0.2–0.4
Colpus membrane	Microrugulate to microverrucate, elements densely packed	Microverrucate to microrugulate, elements densely to widely spaced	Microverrucate to microrugulate, elements widely spaced	Microverrucate to microrugulate to microechinate, elements widely spaced	Microverrucate to microrugulate, elements widely spaced	Microverrucate to microrugulate, widely spaced
Pseudocolpi	Rugulate to microechinate, elements densely packed	Microrugulate to microechinate	Microverrucate to microrugulate, widely spaced	Microverrucate to microechinate, elements widely spaced	Microverrucate to microrugulate, elements widely spaced	Microverrucate to microrugulate, widely spaced

Note: Both the fossil pollen grains and the pollen of extant European taxa listed here was studied using combined LM and SEM. E = equatorial diameter; P = polar axis; eq.v. = equatorial view; p.v. = polar view; LM = light microscope; SEM = scanning electron microscope. Descriptions and measurements based on this study and Graham et al. (1987); Booi et al. (2003); Grímsson et al. (2011); Halbritter (2016), and Halbritter et al. (2021).

the Piacenzian. The relatively high precipitation reconstructed from the palynological record of the Rio Maior sub-basin differs substantially from other Piacenzian reconstructions on the Mediterranean side of the Iberian Peninsula (Vieira et al., 2018). According to Vieira et al. (2018), the pollen assemblages recorded from the Rio Maior sub-basin (core F98) reflect different wetland habitats and surrounding vegetation units. Within swamps and riparian areas surrounding lakes, trees such as *Alnus*, *Craigia*, Engelhardioideae, *Liquidambar*, *Nyssa*, Sapotaceae, *Taxodium*, and *Zanthoxylum* were prominent, in analogy to records from Pliocene central Mediterranean sites (e.g. Bertini, 2010). In well drained areas, the forest was dominated by *Acer*, *Carya*, *Juglans*, Myricaceae, *Quercus*, *Trigonobalanopsis*, and *Ulmus* with abundant Ericaceae. Additional components included shrubs and lianas, such as *Hedera*, *Lonicera*, Rosaceae, and Vitaceae. Pollen of *Buxus*, *Cathaya*, *Keteleeria*, *Picea*, *Pinus*, *Sequoia*, and *Tsuga* were associated with vegetation surrounding the lowland wetland, such as highland and/or mountain forest elements. There was also a high diversity of herbaceous plants, including Apiaceae, Asteraceae, Fabaceae, Campanulaceae, Euphorbiaceae, and Poaceae (e.g. Vieira, 2009). Based on previously identified pollen and spores, as well as the palaeoenvironment and vegetation units that have been suggested so far (e.g. Vieira, 2009; Vieira et al., 2011; Martinetto and Vieira, 2020; Vieira and Zetter, 2020), we conclude that the *Lythrum portugallense* sp. nov. pollen originates from plants that were part of riparian vegetation surrounding paleolakes within the Rio Maior sub-basin. It is likely that

this vegetation unit was shared with other wetland herbaceous plants, such as Amaranthaceae, Osmundaceae, Pteridaceae, *Ranunculus*, *Typha*, and growing in close proximity to fully aquatic plants like *Myriophyllum*, *Nuphar*, and *Utricularia*.

The samples investigated for this study belong to Pollen Zone RM5 as circumscribed by Vieira et al. (2018). The palynoflora of this Pollen Zone contains a number of taxa with potential modern analogues typical of warm temperate climates (sensu Kottke et al., 2006). Based on the climate requirements of potential modern analogues, with mean annual temperatures reconstructed between 18.2–22.0 °C, and the coldest month mean temperature > 6 °C, the RM5 Pollen Zone is believed to represent the warmest interval of the entire Rio Maior record (Vieira et al., 2018).

4.2. Preservation potential of *Lythrum* and implications of the find

The macro- and mesofossil records from Rio Maior have so far not yielded any vegetative parts of *Lythrum*, such as leaves, fruits or seeds (Vieira, 2009). The absence of other *Lythrum* organs in the fossil record of Rio Maior is not surprising. Extant *Lythrum* plants are herbaceous and do not shed their leaves throughout their life cycle. The leaves remain attached to the stem and decompose along with the rest of the plant. Also, they are usually more membranous than those of woody plants, increasing the probability to be destroyed during transport and

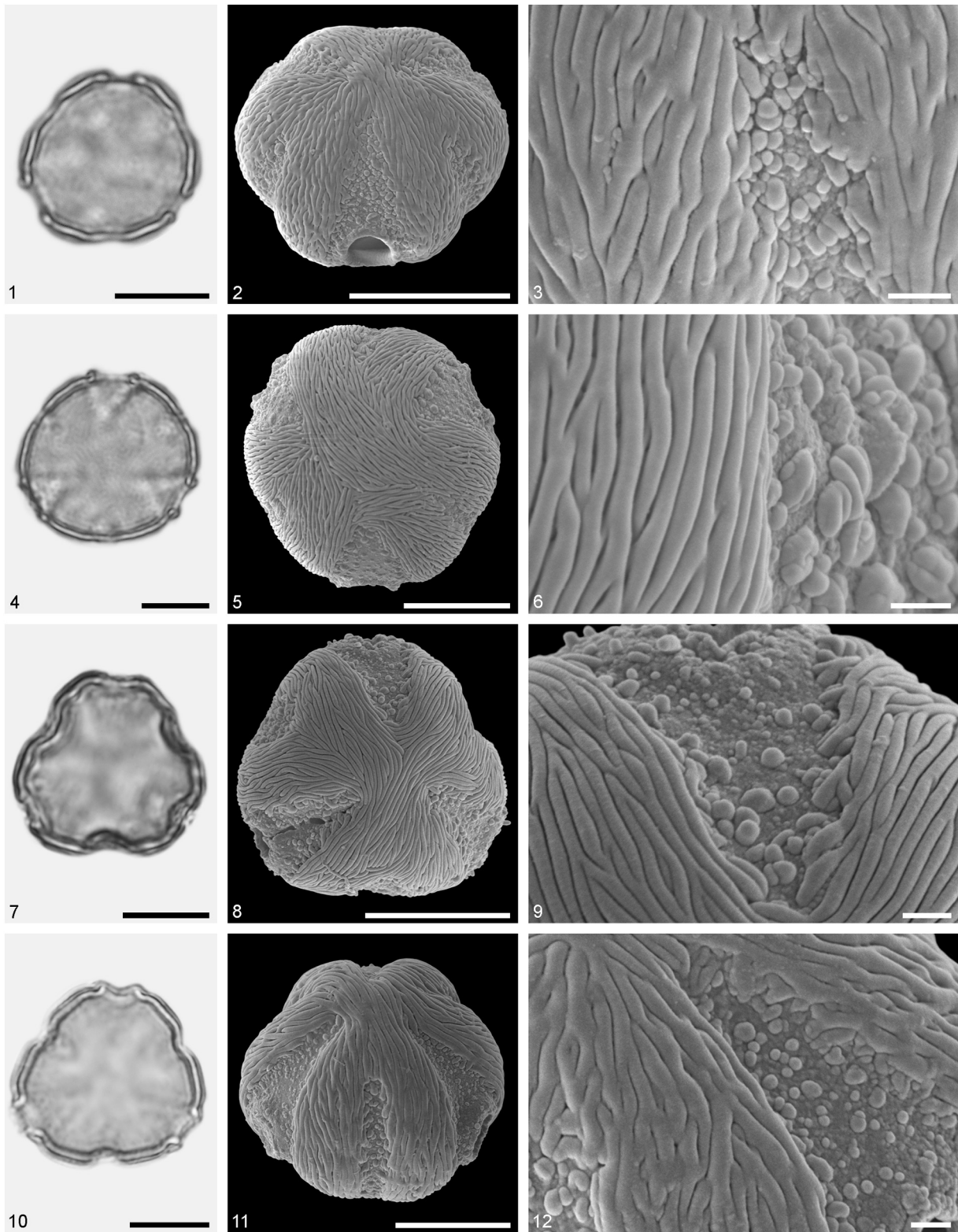


Plate II. Extant *Lythrum* pollen. (1–3) *Lythrum alatum*, (4–6) *Lythrum curtissi*, (7–9) *Lythrum hyssopifolia*, (10–12) *Lythrum salicaria*. (1, 4, 7, 10) Light micrographs in polar view. (2, 3, 5, 6, 8, 9, 11, 12) Scanning electron micrographs in polar view and close-ups of sculpture showing irregular striae and colpus membrane.

decreasing their preservation potential. The seeds are relatively small, usually less than 1 mm (or less than 0.5 mm) and easily overlooked (e.g., Koehne, 1903; Ferguson, 1985; Grímsson et al., 2011). Most *Lythrum* species grow in wetland areas, in water or close to the shoreline of lakes, in marshlands, and in swamps (Koehne, 1903; Graham et al., 1987; Morris, 2007). All *Lythrum* species are insect pollinated. Plants

with this pollination strategy produce less pollen when compared with wind pollinated plants, making their occurrence in palynological records sparse (Fægri and Iversen, 1989). Interestingly, *Lythrum portugallense* sp. nov. pollen is relatively frequent in some samples from Rio Maior (in the studied samples 4/5 *Lythrum* grains have been counted in the general scanning), suggesting that the parent plants

Table 3
European fossil *Lythrum* pollen records.

Taxon (reference)	Organ (study type)	Locality	Age
<i>Lythrum</i> sp. (Bakels, 1995)	Pollen (LM, no micrograph)	Maizy-Cuiry and Bazoches, Soissonais, France	Holocene (Bakels, 1995), <0.0117 Ma (ICS, 2020)
<i>Lythrum</i> sp. (Wijmstra, 1969)	Pollen (LM, no micrograph)	Tenaghi Philippon, Macedonia, Greece	Late Pleistocene to Holocene (Wijmstra, 1969), <0.129 Ma (ICS, 2020)
<i>Lythrum</i> sp. (Andrieu et al., 1997)	Pollen (LM, no micrograph)	Dingé, Ille-et-Vilaine, France	Middle Pleistocene, Chibanian (Andrieu et al., 1997), 0.774–0.129 Ma (ICS, 2020)
<i>Lythrum portugallense</i> sp. nov. (this study)	Pollen (LM + SEM)	Rio Maior, Portugal	Late Pliocene, Piacenzian (Diniz and Mörner, 1995; Diniz, 2003; Vieira et al., 2018), 3.6–2.58 (ICS, 2020)
<i>Lythrum</i> sp. (Menke, 1976)	Pollen (LM)	Oldenswort, Schleswig-Holstein, Germany	Pliocene (Menke, 1976), 5.33–2.58 Ma (ICS, 2020)
<i>Lythrum</i> sp. (Peñalba, 1985)	Pollen (LM)	Casa del Pino, Spain	Pliocene, Zanclean (Barrón et al., 2010), 5.33–3.6 Ma (ICS, 2020)
<i>Lythrum</i> sp. (Van Campo, 1976, 1989); Lythraceae (Casas-Gallego et al., 2015)	Pollen (LM)	Venta del Moro, Spain	Late Miocene, middle Messinian, c. 6.23 Ma (Gibert et al., 2013)
<i>Lythrum wilhelmii</i> (Grímsson et al., 2011)	Pollen (LM + SEM)	Rechnitz, Badersdorf, Austria	Late Miocene, middle Tortonian, c. 9.5 Ma (Rögl and Daxner-Höck, 1996; Hofmann and Zetter, 2005)

Note: Only a handful of records are supported by LM and/or SEM micrograph documentation.

were living at the fringes of the paleolake as part of the riparian vegetation. The number and preservation grade of the pollen suggest short transportation. Possible pollen sources would be 1) flowers that fell directly into the reservoir, 2) dried pollen not extracted by insects that fell from the anthers directly into the water, and/or 3) pollen flushed into the lake following intense rain.

4.3. Dispersal of *Lythrum* into Europe and the origin of modern lineages

Paleobotanical evidence from North America shows that the lineage leading to *Lythrum* (incl. *Peplis* type pollen) was already established on that continent in the early Campanian, 82–81 Ma. Slightly younger records from the late Campanian/early Maastrichtian, 72–68 Ma, are also

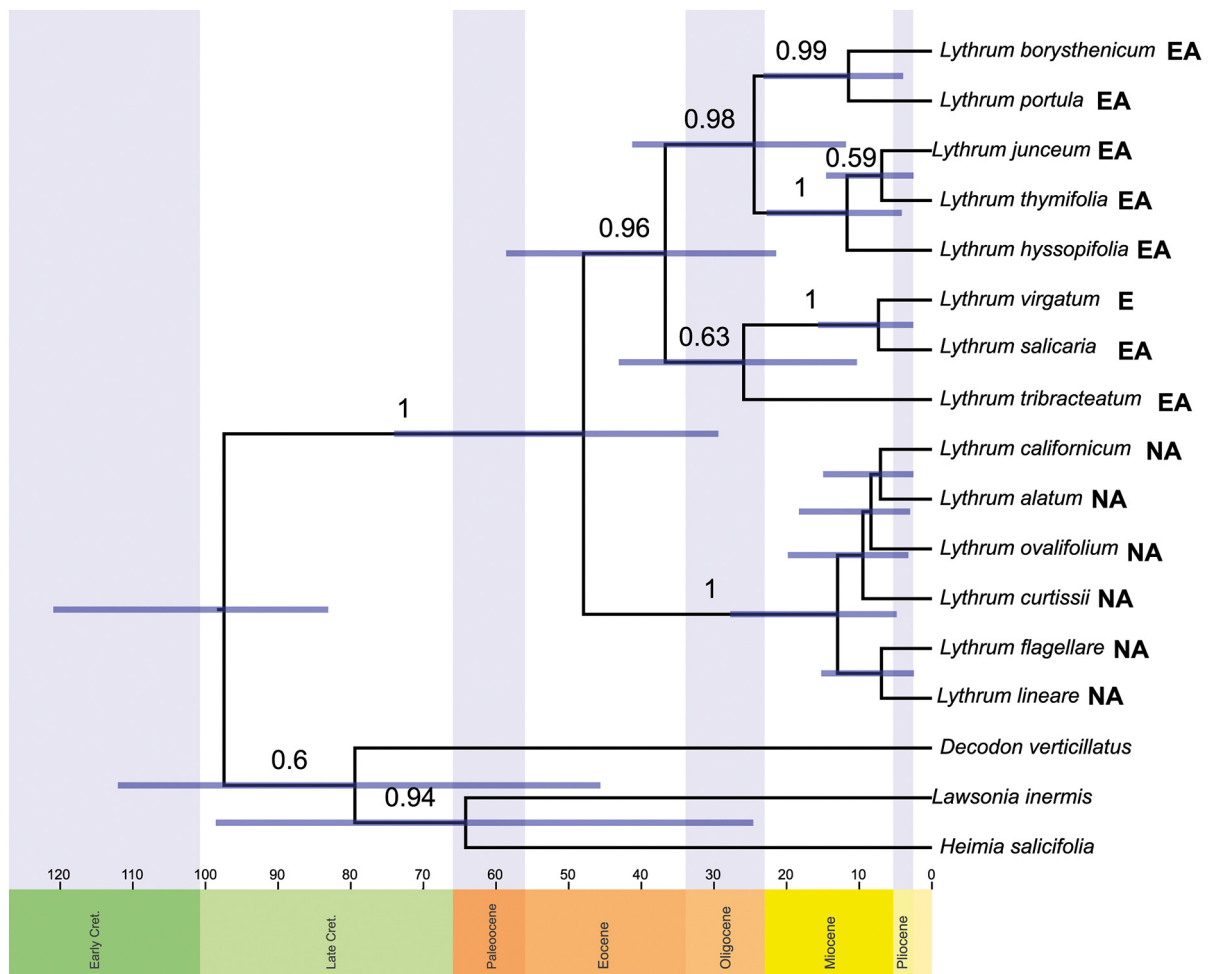


Fig. 2. Dated phylogeny of *Lythrum*, with node support (posterior probability) shown above the branches. Blue bars indicate 95% Highest posterior density intervals. Geographical distribution of the species in either Europe (E), Eurasia (EA), or North America (NA) is indicated after the name.

known from Russia (Grímsson et al., 2011). These earliest records are followed by a large gap in the fossil record lasting until the Neogene when *Lythrum* pollen starts to occur in European floras (Fig. 1; Table 3). Based on published European palynofloras (e.g., Muller, 1981; Mai, 1995; Stuchlik et al., 2014) and our cumulative experience with Cenozoic European angiosperm pollen record over the past 40 years, we confidently conclude that the absence of *Lythrum* in Pre-Neogene European palynofloras is not an artifact and that this genus did not reach Europe prior to the late Miocene. The fossil record shows that Lythraceae already had a North American-Russian distribution in the Late Cretaceous, suggesting a Beringian dispersal that most likely continued into the early Cenozoic. The dated phylogeny (Fig. 2) and current distribution of extant species indicates that the North American clade split from the Eurasian clade during the Paleogene, with no additional dispersal events occurring between the two continents during younger times. How and when *Lythrum* dispersed into Europe can be traced based on the overall fossil record and the dated phylogeny presented herein. Dispersal from the west via the North Atlantic Land Bridge (NALB) can be excluded. The key areas for Paleogene and Neogene plant dispersal across the NALB are Greenland, Iceland, and the Faroe Island. The Paleocene to Eocene palynofloras of Greenland and the Faroes (e.g., Grímsson et al., 2016, 2021, and references therein) and the Neogene of Iceland (e.g., Denk et al., 2011, 2013, and references therein) have not revealed any *Lythrum* pollen. Noteworthy is that pollen of *Decodon*, also an insect pollinated Lythraceae that thrives (thrived) in comparable wetland habitats as *Lythrum*, has been identified from the Paleocene of the Faroe Islands (Grímsson et al., 2014) and the Miocene of Iceland (Grímsson et al., 2012). Even though the earliest fossil *Lythrum* pollen pinpoints a minimum age of 82–81 Ma for the genus, the main divergence in the Eurasian clade apparently did not occur until the Eocene-Miocene (20–60 million years later). The fossil record shows that *Lythrum* first appeared in Europe in the late Miocene parallel to starting crown group radiation in the Eurasian clade (Fig. 2), probably originating from an older Asian stock. The morphology (LM and SEM) of late Miocene to Pliocene *Lythrum* pollen from Europe clearly shows that the pollen is \pm identical to that of extant species (Table 2). The expansion of *Lythrum* into Europe coincides with the decline of *Decodon* from its early to middle Miocene peak distribution (Grímsson et al., 2012). *Lythrum* appears to replace *Decodon* in fossil palynological assemblages, finally culminating in their complete conquest of European wetland environments during the Pleistocene–Holocene and contemporaneous extinction of *Decodon* in this part of the globe.

5. Conclusions

This contribution proves that the single-grain method is vital for the study of microfloras, allowing to find and identify rare elements such as *Lythrum*. This record is relevant to resolving the phytogeographic history of this genus and it also shows the importance of the Rio Maior flora as a source of information for west European Pliocene floras. The pollen morphology of Miocene to Pliocene *Lythrum* is comparable to that of extant species. The earliest European *Lythrum* pollen records are of late Miocene age and parallel to starting crown group radiation in the Eurasian clade and coincide with the decline of *Decodon* in late Neogene fossil palynological assemblages.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Guido W. Grimm, Orleans, France for commenting an earlier version of this manuscript, and two anonymous reviewers for their suggestions to improve this manuscript.

References

- Andrieu, V., Field, M.H., Povel, P., Guiot, J., Guenet, P., de Beaulieu, J.-L., Reille, M., Morzadec-Kerfourn, M.-T., 1997. Middle Pleistocene temperate deposits at Dingé, Ille-et-Vilaine, northwest France: pollen, plant and insect macrofossil analysis. *J. Quat. Sci.* 12, 309–332.
- Bakels, C., 1995. Late Glacial and Holocene pollen records from the Aisne and Vesle valleys, Northern France: the pollen diagrams Maizy-Cuiry and Bazoches. *Med. Rijks Geol. Dienst.* 52, 223–234.
- Barrón, E., Rivas-Carballo, R., Postigo-Mijarra, J.M., Alcalde-Olivares, C., Vieira, M., Castro, L., Pais, J., Valle-Hernández, M., 2010. The Cenozoic vegetation of the Iberian Peninsula: a synthesis. *Rev. Palaeobot. Palynol.* 162, 382–402.
- Bertini, A., 2010. Pliocene to Pleistocene palynoflora and vegetation in Italy: state of the art. *Quat. Int.* 225, 5–24.
- Booi, M., Punt, W., Hoen, P.P., 2003. Lythraceae. The northwest European pollen flora 68. *Rev. Palaeobot. Palynol.* 123, 163–180.
- Cachão, M., 1990. Posicionamento biostratigráfico da jazida pliocénica de Carnide (Pombal). *Gaia* 2, 11–16.
- Casas-Gallego, M., Lassaletta, L., Barrón, E., Bruch, A.A., Montoya, P., 2015. Vegetation and climate in the eastern Iberian Peninsula during the pre-evaporitic Messinian (late Miocene). *Palynological data from the Upper Turolian of Vente del Moro (Spain)*. *Rev. Palaeobot. Palynol.* 215, 85–99.
- Coiro, M., Doyle, J.A., Hilton, J., 2019. How deep is the conflict between molecular and fossil evidence on the age of angiosperms? *New Phytol.* 223, 83–99.
- Cunha, P.M., Barbosa, B.P., Reis, R., 1993. Synthesis of the Piacenzian onshore record, between the Aveiro and Setúbal parallels (Western Portuguese margin). *Ciê. Terra* 12, 35–43.
- Denk, T., Grímsson, F., Zetter, R., Simonarson, L.A., 2011. Late Cainozoic Floras of Iceland: 15 Million Years of Vegetation and Climate History in the Northern North Atlantic. Springer, Dordrecht.
- Denk, T., Grimm, G.W., Grímsson, F., Zetter, R., 2013. Evidence from “Köppen signatures” of fossil plant assemblages for effective heat transport of Gulf Stream to subarctic North Atlantic during Miocene cooling. *Biogeosciences* 10, 7927–7942.
- Diniz, F., 1984a. Etude palynologique du bassin pliocène de Rio Maior. *Paléobiol. Cont.* 14, 259–267.
- Diniz, F., 1984b. Apports de la palynologie à la connaissance du pliocène portugais. Rio Maior: un bassin de référence pour l'histoire de la flore, de la végétation et du climat de la façade atlantique de l'Europe méridionale. Ph.D. Thesis University of Montpellier, Montpellier, France.
- Diniz, F., 2003. Os depósitos detritico-diatomíticos de Abum (Rio Maior). *Novos aspectos paleoflorísticos e implicações paleoclimáticas*. *Ciê. Terra* V, 7 CD Rom A49–A52.
- Diniz, F., Mörmner, N.-A., 1995. The Reuverian / Pretiglian transition in Rio Maior, Portugal. XIV International Congress INQUA, Schriften der Alfred-Wegener-Stiftung 2/95, p. 64.
- Fægri, K., Iversen, J., 1989. In: Fægri, K., Kaland, P.E., Krzywinski, K. (Eds.), *Textbook of Pollen Analysis*, 4th ed Blackburn Press, Caldwell, New Jersey, USA revised by.
- Ferguson, D.K., 1985. The origin of leaf-assemblages - new light on an old problem. *Rev. Palaeobot. Palynol.* 46, 117–188.
- Gibert, L., Scott, G.R., Montoya, P., Ruiz-Sánchez, F., Morales, J., Luque, L., Abella, J., Leria, M., 2013. Evidence for an African-Iberian mammal dispersal during the pre-evaporitic Messinian. *Geology* 41, 691–694.
- Graham, S.A., 2013. Fossil records in the Lythraceae. *Bot. Rev.* 79, 48–145.
- Graham, A., Graham, S.A., 1971. The geologic history of the Lythraceae. *Brittonia* 23, 335–346.
- Graham, A., Nowicke, J.W., Skvarla, J.J., Graham Patel, S.A.V., Lee, S., 1987. Palynology and systematics of the Lythraceae. II. Genera *Haitia* through *Peplis*. *Am. J. Bot.* 74, 829–850.
- Grímsson, F., Zetter, R., Hofmann, C.-C., 2011. *Lythrum* and *Peplis* from the Late Cretaceous and Cenozoic of North America and Eurasia: new evidence suggesting early diversification within the Lythraceae. *Am. J. Bot.* 98, 1801–1815.
- Grímsson, F., Ferguson, D.K., Zetter, R., 2012. Morphological trends in the fossil pollen of *Decodon* and the paleobiogeographic history of the genus. *Int. J. Plant Sci.* 173, 297–317.
- Grímsson, F., Højgaard, B., Hofmann, C.-C., Pott, C., Grimm, G.W., Friðgeirsson, G.R., Denk, T., Zetter, R., 2014. Revisiting the late Paleocene Mykines macrofossil site, Faeroe Islands. 9th European Palaeobotany-Palynology Conference. Abstract Book, pp. 85–86.
- Grímsson, F., Pedersen, G.K., Grimm, W., Zetter, R., 2016. A revised stratigraphy for the Palaeocene Agatdalen flora (Nuussuaq Peninsula, western Greenland): correlating fossiliferous outcrops, macrofossils, and palynological samples from phosphoritic nodules. *Acta Palaeobot.* 56, 307–327.
- Grímsson, F., Denk, T., Zetter, R., 2021. Cenozoic vegetation and phytogeography of the sub-arctic North Atlantic. In: Panagiotakopulu, E., Sadler, J.P. (Eds.), *Biogeography in the Sub-Arctic: The Past and Future of the North Atlantic Biotas*. John Wiley & Sons Ltd, pp. 29–49.
- Halbritter, H., 2016. *Lythrum hyssopifolia*. PalDat - A Palynological Database . https://www.paldat.org/pub/Lythrum_hyssopifolia/302888 accessed 2021-06-28.
- Halbritter, H., Ulrich, S., Grímsson, F., Weber, M., Zetter, R., Hesse, M., Buchner, R., Svojtka, M., Frosch-Radivo, A., 2018. *Illustrated Pollen Terminology*. Springer, Cham, Switzerland.
- Halbritter, H., Weber, M., Heigl, H., Auer, W., 2021. *Lythrum salicaria*. PalDat - A Palynological Database . https://www.paldat.org/pub/Lythrum_salicaria/306279 accessed 2021-06-28.
- Hofmann, C.-C., Zetter, R., 2005. Reconstruction of different wetland plant habitats of the Pannonic Basin system (Neogene, Eastern Austria). *Palaios* 20, 266–279.
- ICS, 2020. International Chronostratigraphic Chart v2020/03. <http://www.stratigraphy.org/ICSChart/ChronostratChart2020-03.pdf>.

- Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* 20, 1160–1166. <https://doi.org/10.1093/bib/bbx108>.
- Koehne, E., 1903. Lythraceae. In: Engler, A. (Ed.), *Das Pflanzenreich IV*. 216. Heft 17. W. Engelmann, Germany.
- Kottek, M., Grieser, J., Beck, D., Rudolf, B., Rubel, F., 2006. World map of Köppen-Geiger climate classification updated. *Meteorol. Z.* 15, 259–263. <https://doi.org/10.1127/09412948/2006/0130>.
- Mai, D.H., 1995. *Tertiäre Vegetationsgeschichte Europas*. Jena, Gustav Fischer.
- Martinetto, E., Vieira, M., 2020. New Pliocene records of plant fossil-taxa from NW Portugal and their relevance for the assessment of diversity loss patterns in the late Cenozoic of Europe. *Rev. Palaeobot. Palynol.* 281, 104286. <https://doi.org/10.1016/j.revpalbo.2020.104286>.
- Menke, B., 1976. Pliozäne und ältestquartäre Sporen- und Pollenflora von Schleswig-Holstein. *Geol. Jahrb. A* 32, 3–197.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA, 14 November 2010, pp. 1–8 <https://doi.org/10.1109/GCE.2010.5676129>.
- Morris, J.A., 2007. *A molecular phylogeny of the Lythraceae and inference of the evolution of heterostyly*. Ph.D. Thesis Kent State University, Kent, Ohio, USA.
- Muller, J., 1981. Fossil pollen records of extant angiosperms. *Bot. Rev.* 47, 1–142.
- Peñalba, C., 1985. *Estudio esporopolínico del Neógeno occidental de la Cuenca del Guadalquivir*. MS thesis University of Salamanca, Spain.
- POWO, 2019. *Plants of the World Online*. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet <http://www.plantsoftheworldonline.org/> accessed 2021-03-21.
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A., 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.* 143, 1–81.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* 67, 901–904.
- Ramos, A., Cunha, P.P., 2004. Facies associations and palaeogeography of the zancleanpiacenzian marine incursion in the Mondego cape-Nazaré area (onshore of central Portugal). Abstracts of the 23rd International Meeting of IAS, 227, Coimbra, Portugal.
- Rögl, F., Daxner-Höck, G., 1996. Late Miocene Paratethys correlations. In: Bernor, R.L., Fahlbusch, V., Mittmann, H.-W. (Eds.), *The Evolution of Western Eurasian Neogene Mammal Faunas*. Columbia University Press, New York, USA.
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542. <https://doi.org/10.1093/sysbio/sys029>.
- Silva, C.M., 2001. *Gastropodes pliocénicos Marinhas de Portugal*. Sistemática, paleobiologia, paleoecologia e paleobiogeografia. Ph.D. thesis Faculdade de Ciências, Universidade Lisboa.
- Stuchlik, L., Ziemińska-Tworzydło, M., Kohlman-Adamska, A., Grabowska, I., Słodkowska, B., Worobiec, E., Durska, E., 2014. *Atlas of pollen and spores of the Polish Neogene*. Volume 4. Angiosperms. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków 2.
- Van Campo, E., 1976. *La flore sporopollenique du gisement Miocène terminal de Venta del Moro (Espagne)*. Ph.D. thesis University of Science and Techniques of Languedoc, Montpellier II, Montpellier, France.
- Van Campo, E., 1989. *Flore pollinique du Miocène supérieur de Venta del Moro (Espagne)*. *Acta Palynol.* 1, 9–32.
- Vieira, M., 2009. *Palinologia do Pliocénico da Orla Ocidental Norte e Centro de Portugal: Contributo para a compreensão da cronostatigrafia e da evolução paleoambiental*. Ph.D. thesis University of Minho, Braga, Portugal.
- Vieira, M., Zetter, R., 2020. New pollen record of megathermal and mesothermal elements in the late Pliocene from West Portugal revealed by combined light microscope and scanning electron microscope studies. *Grana* 59, 114–126. <https://doi.org/10.1080/00173134.2019.1690572>.
- Vieira, M., Sousa, L., Pais, J., Pereira, D., 2006. Palynological study of Vale do Freixo Pliocene (Pombal, Mondego Basin, Portugal). 7th European Palaeobotany-Palynology Conference, Prague, Czech Republic. Abstract book, p. 151.
- Vieira, M., Poças, E., Pais, J., Pereira, D., 2011. Pliocene flora from S. Pedro da Torre deposits (Minho, NW Portugal). *Geodiversitas* 33, 71–85.
- Vieira, M., Pound, M., Pereira, D.L., 2018. The late Pliocene palaeoenvironments and palaeoclimates of the western Iberian Atlantic margin from the Rio Maior flora. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 495, 245–258. <https://doi.org/10.1016/j.palaeo.2018.01.018>.
- Webb, D.A., 1967. Generic limits in European Lythraceae. *Fed. Repert.* 74, 10–13.
- Wijmstra, T.A., 1969. Palynology of the first 30 meters of a 120 m deep section in northern Greece. *Acta Bot. Neerl.* 18, 511–527.
- Zbyszewski, G., 1967. Estudo geológico da bacia dos lignitos de Rio Maior. *Estud. Not. Trabal. Serv. Foment. Mineir.* 17, 5–105.
- Zetter, R., 1989. Methodik und Bedeutung einer routinemäßig kombinierten lichtmikroskopischen und rasterelektronenmikroskopischen Untersuchung fossiler Mikroflora. *Cour. Forschungsinst. Senck.* 109, 41–50.