

Research Article

Cenozoic *Ampelopsis* and *Nekemias* leaves (Vitaceae, Ampelopsidae) from Eurasia: Paleobiogeographic and paleoclimatic implicationsAixa Tosal^{1,2,3*} , Alba Vicente^{1,2,4,5}, and Thomas Denk⁶¹Departament de Dinàmica de la Terra i de l'Oceà (DDTO), Facultat de Ciències de la Terra, Universitat de Barcelona (UB), C/de Martí Franqués s/n, Barcelona 08028, Spain²Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), C/de Martí Franqués s/n, Barcelona 08028, Spain³Centre de Recherche sur la Paléobiodiversité et les Paléoenvironnements (CR2P-UMR 7207), Centre national de la recherche scientifique (CNRS), Muséum National d'Histoire Naturelle (MNHN), Sorbonne Université (UPMC), 57 rue Cuvier, Cedex 05, Paris 75231, France⁴Instituto Politécnico Nacional (IPN), Av. Instituto Politécnico Nacional s/n, Colonia Playa Palo de Santa Rita, La Paz 23096, Mexico⁵Institut Català de Paleontologia Miquel Crusafont, ICTA-ICP Building, Universitat Autònoma de Barcelona (UAB), c/de les Columnes s/n, Cerdanyola del Vallès 08193, Spain⁶Department of Palaeobiology, Swedish Museum of Natural History, Box 50007, Stockholm 10405, Sweden

*Author for correspondence. E-mail: atosal@ub.edu

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Abstract We describe a new species of Ampelopsidae (Vitaceae), *Nekemias mucronata* sp. nov., from the Rupelian of Cervera (Spain) and revise another fossil species, *Ampelopsis hibschi*, originally described from Germany. Comparison with extant Ampelopsidae suggests that the North American species *Nekemias arborea* is most similar to *Nekemias mucronata* while the East Mediterranean *Ampelopsis orientalis* is the closest living relative of *A. hibschi*. Our review of fossil data indicates that, during the Eocene, four species of Ampelopsidae occurred in Eurasia, that is, *N. mucronata* in the Czech Republic, *A. hibschi* in Kazakhstan, and two fossil species in the Far East (*Ampelopsis cercidifolia* and *Ampelopsis protoheterophylla*). In the Oligocene, a new species, *Ampelopsis schischkinii*, appeared in Kazakhstan; meanwhile, *N. mucronata* spread eastwards and southwards, and *A. hibschi* mainly grew in Central Europe. In the late Oligocene, *N. mucronata* became a relict in the Iberian Peninsula and *Nekemias* might have persisted in Western Eurasia until the latest Miocene ("*Ampelopsis*" *abkhasica*). The last occurrence of *A. hibschi* was in the Middle Miocene in Bulgaria, probably a refuge of humid temperate taxa, along with *Ampelopsis* aff. *cordata*. Carpological remains suggest that this lineage persisted in Europe at least until the Pleistocene. Our data confirm previous notions of the North Atlantic and Bering land bridges being important dispersal routes for Ampelopsidae. However, such dispersion probably occurred during the Paleogene rather than the Neogene, as previously suggested. A single species of Ampelopsidae, *A. orientalis*, has survived in Western Eurasia, which appears to have been linked to a biome shift.

Key words: Cenozoic, leaf fossils, paleobiogeography, paleoclimate, Vitaceae, Western Eurasia.

1 Introduction

Vitaceae Jussieu (the grape family) is a plant family with about 15 genera and 950 species with leaves ranging from simple to compound (Chen & Manchester, 2007; Jones et al., 2013). It is mainly distributed in Southern Asia, Africa, Australia, and the Pacific islands, with only a few species reported in temperate regions (Süssenguth, 1953; Chen et al., 2007; Wen, 2007). Recent phylogenetic studies of Vitaceae have provided a framework for its infrafamilial relationships and the biogeographic diversification

of the family (e.g., Rossetto et al., 2001, 2002, 2007; Ingrouille et al., 2002; Soejima & Wen, 2006; Wen et al., 2007, 2013, 2018; Chen, 2009; Lu et al., 2018). The phylogenetic data supports five major clades within Vitaceae referred to as tribes: (i) Ampelopsidae, (ii) Parthenocisseae, (iii) Viteae, (iv) Cisseae, and (v) Cayratieae (Wen et al., 2018).

The Ampelopsidae is a relatively small tribe within Vitaceae and comprises 47 species distributed in Asia, Europe, America, Africa, and Australia (Soejima & Wen, 2006; Wen et al., 2007, 2013, 2018; Ren et al., 2011; Nie et al., 2012; Liu et al., 2013).

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The origin of the tribe is still under debate. Based on a time-calibrated phylogeny by Nie et al. (2012), the Ampelopsideae presumably appeared during the late Paleocene–Early Miocene (61.0–23.4 Ma) in North America (Manchester, 1994; Manchester & McIntosh, 2007; Nie et al., 2012). An alternative hypothesis was proposed based on late Cretaceous fossil seeds found in India with anatomical features characteristic of different extant Vitaceae tribes (Manchester et al., 2013). These similarities suggested that these seeds could be ancestral to the modern Ampelopsideae (Manchester et al., 2013). Based on this, it was suggested that the tribe Ampelopsideae originated during the Cretaceous in India (Southern Hemisphere) rather than during the Paleocene–Early Miocene in North America (Northern Hemisphere), as was previously claimed.

Prior to the valid publication of Wen et al. (2018), the tribe Ampelopsideae was informally referred to as the Ampelopsis clade and was divided into three groups (North I, North II, and South) based on phylogenies from chloroplast and nuclear markers (Nie et al., 2012). *Ampelopsis* and *Nekemias*, two important genera in this clade, belong to the North I and North II groups, which include the modern Northern Hemispheric taxa of the Ampelopsideae.

Nuclear data provided strong support for a paraphyletic *Ampelopsis*; therefore, the traditional *Ampelopsis* was split into two genera, *Ampelopsis* s.s. A. Rich. ex Michx. and *Nekemias* Raf. (Wen et al., 2014). These genera can be distinguished by their leaf architecture (Wen et al., 2018; Gray, 2019). *Ampelopsis* leaves are simple, trifoliate, or palmately compound, while those in *Nekemias* are pinnately compound (Wen et al., 2014). One exception to this rule is the western Asian species *Ampelopsis orientalis* (Lam.) Planch., which has bipinnate leaves with leaflets usually arranged in groups of three but belongs to *Ampelopsis* s.s. based on molecular data (Hearn et al., 2018). Molecular phylogenetic studies further suggest that the compound leaves and the venation pattern of *Nekemias* and *Ampelopsis* are the most ancestral character states in Vitaceae (Wen et al., 2018).

In Europe, the occurrence of *Ampelopsis* s.s. dates back to the Paleocene/Eocene based on unambiguous records of reproductive structures (Mai, 1995; Collinson et al., 2012). The oldest leaf fossils with clear affinity to the Ampelopsideae date back to the Oligocene (Bůžek et al., 1981). These authors described several fossil leaflets with intact cuticle from the Czech Republic (České Středohoří) and Germany (Haselbach, Schichten, and Seifhennersdorf) and assigned them to *Ampelopsis hirschi* Bůžek, Kvaček et Walther. In some of the leaf-bearing strata, seeds, and pollen grains of *Ampelopsis* were also found, confirming the presence of this genus in Central Europe during the late Paleogene. Bůžek et al. (1981) suggested that *A. hirschi* dispersed southwards and westwards along with other Arctotertiary floral elements during the Oligocene in response to the cooling event that characterized the Eocene–Oligocene Transition (EOT). Thereafter, *A. hirschi* remained in this paleogeographic area together with *Ampelopsis* aff. *cordata* Bozakov being an indicator of the Western and Central European bioprovince until the Middle Miocene, when its area became restricted to refugia in the Balkans (e.g., Satovcha, Bulgaria; Bozakov, 2000). Carpological studies indicate that *Ampelopsis* persisted in Central Europe until the Pleistocene (Dunarobba, Central

Italy, Pini et al., 2014; Cheb Basin, Czech Republic; Teodoridis et al., 2017). Unlike for *Ampelopsis*, there is no fossil record of *Nekemias* to date.

In Asia, the fossil record of Ampelopsideae is sparser than in Europe. The oldest leaf record dates back to the Middle Eocene (Kodrul, 1999). This author described a new species from the Russian Far East (*Ampelopsis protoheterophylla* Kodrul) with leaf morphological affinities with *Ampelopsis glandulosa* var. *heterophylla* (Thunb.) Momi. Based on the fossil assemblage, this fossil species was part of a warm-temperate, mesophilic, and deciduous flora (Akhmetiev, 2007). The *Ampelopsis* lineage persisted in the North Pacific region until the Neogene: *Ampelopsis cercidifolia* Budants (Budantsev, 1997) in late Eocene, *Ampelopsis angulata* Pavlyut (Pavlyutkin et al., 2014) in Oligocene, *Ampelopsis amgensis* Klimova (Krassilov, 1984) in Miocene, and *Ampelopsis populifolia* Hu et Chaney (Hu & Chaney, 1940) in Late Miocene deposits. Notably, all these fossil species have been considered closely similar to the extant species *A. glandulosa* (Wall.) Momi., which has a modern range that overlaps with the ranges of the fossil species.

In the present study, we describe a new Western Eurasian leaf fossil species of Ampelopsideae, which we assigned to the genus *Nekemias* based on a detailed analysis of the leaf architecture. Second, the original material of *A. hirschi* from the Czech Republic and Germany was reassessed and other Eurasian records of *Ampelopsis* were reviewed. Third, the relationships were established between the fossil species studied and modern members of Ampelopsideae. Fourth, the paleogeographic distribution of the fossil leaf species across the Cenozoic in Eurasia was analyzed and compared to the modern ranges of genera in the Ampelopsideae. Finally, the particular climate types that characterized the fossil species' ranges were assessed.

2 Material and Methods

The study material comes from the Cervera paleobotanical site situated in the eastern Ebro Basin. This basin is triangular and corresponds to the foreland basin of the Pyrenees (Fig. 1A). The Ebro Basin was formed during the Paleogene due to the collision between the Iberian and the Eurasian plates (Puigdefàbregas et al., 1992), resulting in the uplift of the Pyrenees and the Catalan Coastal Chain. However, the marine environment remained in the eastern Ebro Basin until the late Eocene, when the Ebro Basin became endorheic (Costa et al., 2010). Five fluvio-lacustrine systems were formed from the late Eocene (Priabonian) to the late Miocene (Anadón et al., 1989). The Cervera plant fossil site belongs to the third lacustrine system (La Segarra lacustrine system), which is Rupelian in age, based on charophyte biostratigraphy (Sanjuan et al., 2012, 2014). In parallel, magnetostratigraphic studies allowed a more precise dating for the plant bed studied here, with a Rupelian age (early Oligocene) of 32.4–32 Ma, corresponding to chron C12 (Barberà et al., 2001).

The La Segarra lacustrine system is mainly constituted by the Montmaneu Formation composed of marls, marlstones, and limestones interpreted as a permanent lake (Colldeforns et al., 1994). Based on these authors, this formation locally

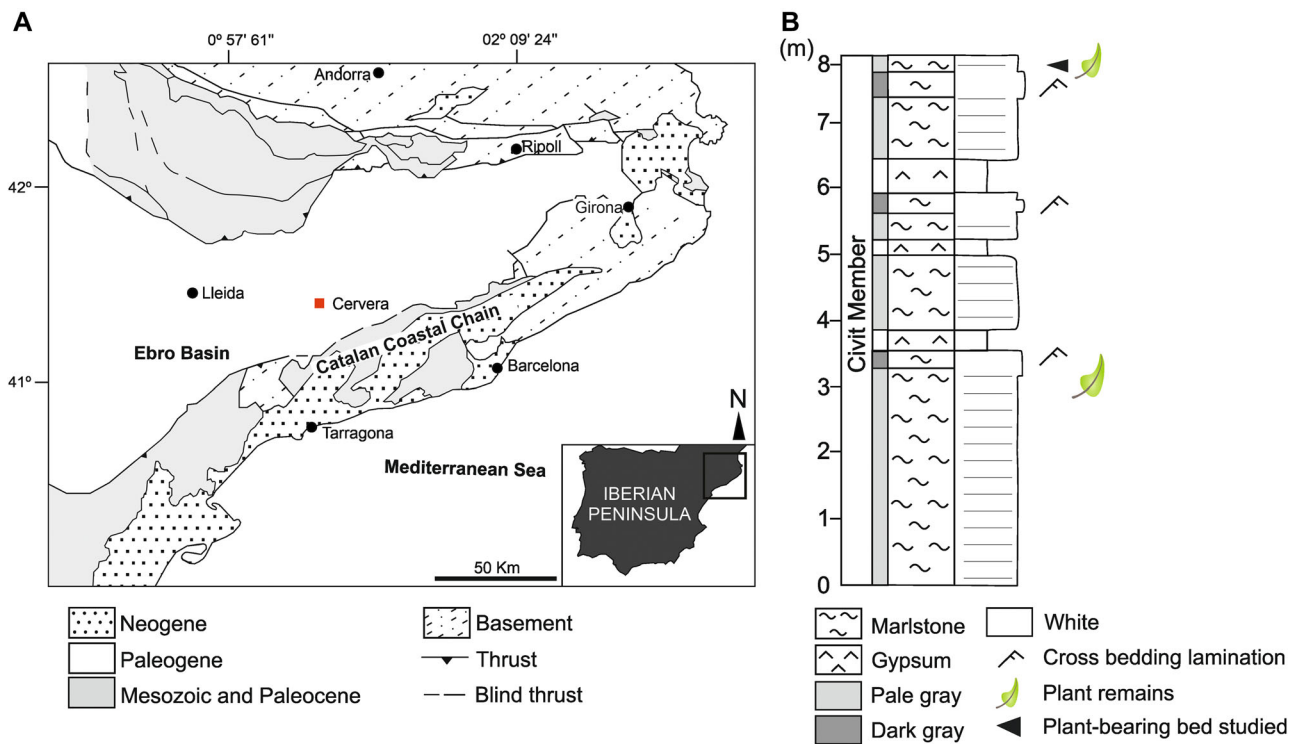


Fig. 1. Geological setting of the area studied. **A**, Geological map of the Ebro Basin with the location of the study area (red square), modified from Vergés et al. (1998). **B**, Stratigraphic log of the Carulla quarry with the location of the plant bed studied. Modified from Tosal & Martín-Closas (2016).

passes to intercalations of pale and dark gray marlstones corresponding to the 14-m-thick Civit Member, which contains the fossil leaflets studied here (Fig. 1B). The paleoflora from the Civit Member was buried in the distal areas of a permanent lake, characterized by a poorly oxygenated lake bottom, which favored the preservation of the plant remains (Tosal & Martín-Closas, 2016).

A total of 83 isolated leaflets preserved as adpressions without cuticular details preserved were recovered from Cervera (NE Spain) and are kept in the Museu de Ciències Naturals de Barcelona under catalog numbers MGB 33090; MGB 85001a,b; MGB 85002; MGB 85006; MGB 85007; MGB 85008a,b; MGB 85010; MGB 85012; MGB 94155–MGB 94227.

The specimens were photographed using a Nikon 5300 camera with a 105 mm Macro lens. Up to 30 pictures were taken of each specimen and later compiled using the Helicon Focus 5.3 software (www.heliconsoft.com). The processed photographs were used to describe the leaf features presented here. Descriptions of leaf morphology mainly followed the terminology proposed by Ellis et al. (2009). The leaf measurements were taken using the ImageJ free software (https://imagej.net/Welcome).

The taxonomic attribution of the adpressions studied here was based on the resemblance with living species of *Ampelopsis* s.l., that is, *Ampelopsis* s.s. and *Nekemias*. The Oligocene *Nekemias* specimens studied herein were compared with other Oligocene specimens assigned to *Ampelopsis*, including the type material of *A. hirschi* stored at the Geological Survey of the Czech Republic (Prague, Czech Republic), *Ampelopsis* aff. *cordata* stored at the Institute of

Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences (Sofia, Bulgaria). In addition, the fossil specimens from Cervera were compared with living *Ampelopsis* and *Nekemias* with compound leaves stored at the herbarium of the Muséum National d'Histoire Naturelle (Paris, France) and the online herbarium of the University of South Florida in order to propose the nearest living relative (NLR) of the fossil species.

Paleoenvironmental information of the fossil *Ampelopsis* and *Nekemias* from the late Paleogene and Neogene of Western Eurasia was compiled from previous published data. To compare precipitation figures derived from the Climate Leaf Analysis Multivariate Program (CLAMP) and Coexistence Approach methods, the equation of Tanrattana et al. (2020) was used and the mean annual precipitation (MAP) was obtained from CLAMP parameters.

3 Results

Systematic paleobotany

Family Vitaceae Jussieu

Tribe Ampelopsideae J. Wen & Nie

Genus *Nekemias* Rafinesque

Nekemias mucronata sp. nov. Tosal, Vicente & Denk (Figs. 2, 3)

Other records:

1971 *Rhus pyrrhae* Unger—Fernández-Marrón, 95, pl. 7, fig. 2

1992 *Rhus pyrrhae* Unger—Sanz de Siria Catalan, 335, pl. 6, fig. 14

2004 *Ampelopsis* sp.—Kvaček & Walther, 20, pl. 3, fig. 3

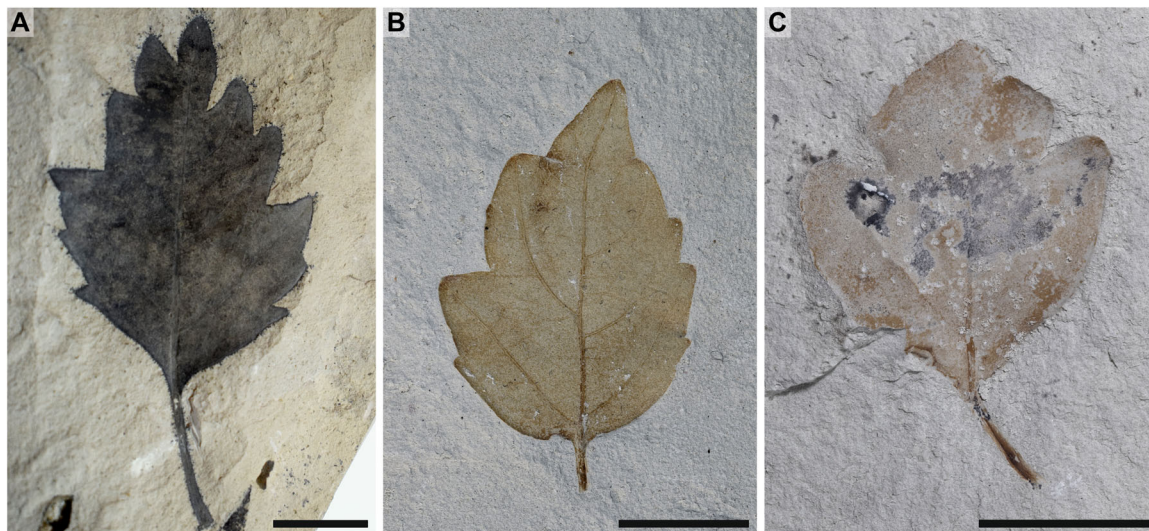


Fig. 2. Apical leaflets of *Nekemias mucronata* sp. nov. **A**, Complete leaflet corresponding to the holotype MGB 33090. **B**, Paratype, MGB 85001b showing rounded base and a mucro at the tooth tip. **C**, Paratype, MGB 85002 displaying craspedodromous secondary venation pattern. Scale: 1 cm.

2011 cf. *Ampelopsis* sp.—Kvaček & Teodoridis, 94, pl. 5, fig. 12
(?) 2012 *Ampelopsis hibschii* Bůžek, Kvaček et Walther—
Averyanova, pl. 10, fig. 8; pl. 75, fig. 8

2014 aff. *A. hibschii*—Velitzelos et al., 66, pl. VI, fig. 4

2019a No *Rhus* “Group 2”—Tosal et al., 72, pl. V, figs. 1–6

2021 *Ampelopsis iljinskiae* Averyanova nom. inval.—
Averyanova et al., pl. I, fig. 7

2021 *Liriodendron* sp. Moreno-Domínguez et al., pl. 4,
fig. 12

Material Seventy-three specimens from the La Segarra lacustrine system, Cervera (Catalonia, Spain).

Holotype Specimen MGB 33090 from the Museu de Ciències Naturals de Barcelona, collected by M. Madern (Fig. 2A).

Paratypes Specimens MGB 85001b and MGB 85002 stored at the Museu de Ciències Naturals de Barcelona corresponding to apical leaflets (Figs. 2B, 2C) and specimens MGB 85010 and MGB 94211; corresponding to lateral leaflets (Fig. 3).

Type stratum Marlstones of Montmaneu Formation (Civit Member) from the Carulla quarry section near Cervera (Tosal & Martín-Closas, 2016).

Chronostratigraphic range From late Eocene to late Oligocene.

Paleogeographic distribution Late Eocene in Kučlín (Czech Republic); early Oligocene in Bechlejovice (Czech Republic) and Cervera (Spain); late Oligocene in La Val (Spain); and Oligocene in Laginia (Greece) and Kusto (Kazakhstan).

Derivation of name The epithet refers to the distinctive mucro at the tooth tip of the leaflets.

Diagnosis Terminal leaflet symmetric or slightly asymmetric with a broad lanceolate shape and toothed margin. Tooth apex mucronate. Base straight or rounded with a petiolule. Apex straight. Primary vein pinnate. Secondary vein pattern craspedodromous. Intercostal tertiary veins sinuous. Exterior tertiary veins looped.

Lateral leaflet bilobate or trilobate, petiolulate. Lamina shape broad ovate. Base cordate or rounded. Apex straight.

Margin toothed. Teeth broad and blunt with mucronate apex. Primary venation pattern pinnate. Secondary veins craspedodromous without branching. Two basal secondary veins run into the tooth apex of the lateral lobes or the basal-most tooth. Intercostal tertiary veins sinuous. Exterior tertiary course ending in tooth tip.

Description The number of leaflets per leaf remains unknown because the leaflets studied are found isolated.

Terminal leaflets The terminal leaflet is symmetric (Fig. 2A) or slightly asymmetric with an ovate or broad lanceolate (rhomboid) shape and toothed margin (Figs. 2B, 2C). The lamina is 3.6–4.8 cm long and 1.8–3.6 cm wide. The number of teeth varies from 5 to 11 and teeth show a mucro at the apex (Fig. 2B). The mucro is usually bent into the sediment and is not very visible. The lamina base is straight or rounded with a petiolule up to 1.2 cm long. The apex is straight. Tooth shape is straight (53%), convex (34%), and rarely flexuous (13%) in the proximal flank while the distal flank is concave (60%) or straight (40%). The primary venation is pinnate. The secondary vein pattern is craspedodromous and the attachment to the midvein is excurrent (Fig. 2C). Secondary veins rise from the midvein at angles of 40–60° and reach the tip of the tooth. Intercostal tertiary veins are sinuous. Occasionally, a tertiary vein departs from a secondary vein and ends in the sinus of the tooth. A pair of tertiary loops rise from the primary vein at approximately 2–4 mm below the first pair of secondary veins, at angles of 40–70° to the midvein.

Lateral leaflets Lateral leaflets are petiolulate and display symmetric (Figs. 3A, 3B) or asymmetric blades. The lamina is 3.5 cm long and 2.9 cm wide. The asymmetric leaflets show basal extension asymmetry or medial asymmetry. The base is cordate and occasionally shows a short (5 mm) petiole (Fig. 3B). The apex is bluntly acute. The blade is bilobate or trilobate and the margin is toothed. The total number of teeth ranges from 5 to 11 (mean 7). Symmetric specimens display the same number of teeth on both sides of the lamina while the asymmetric ones show unequal distribution of the

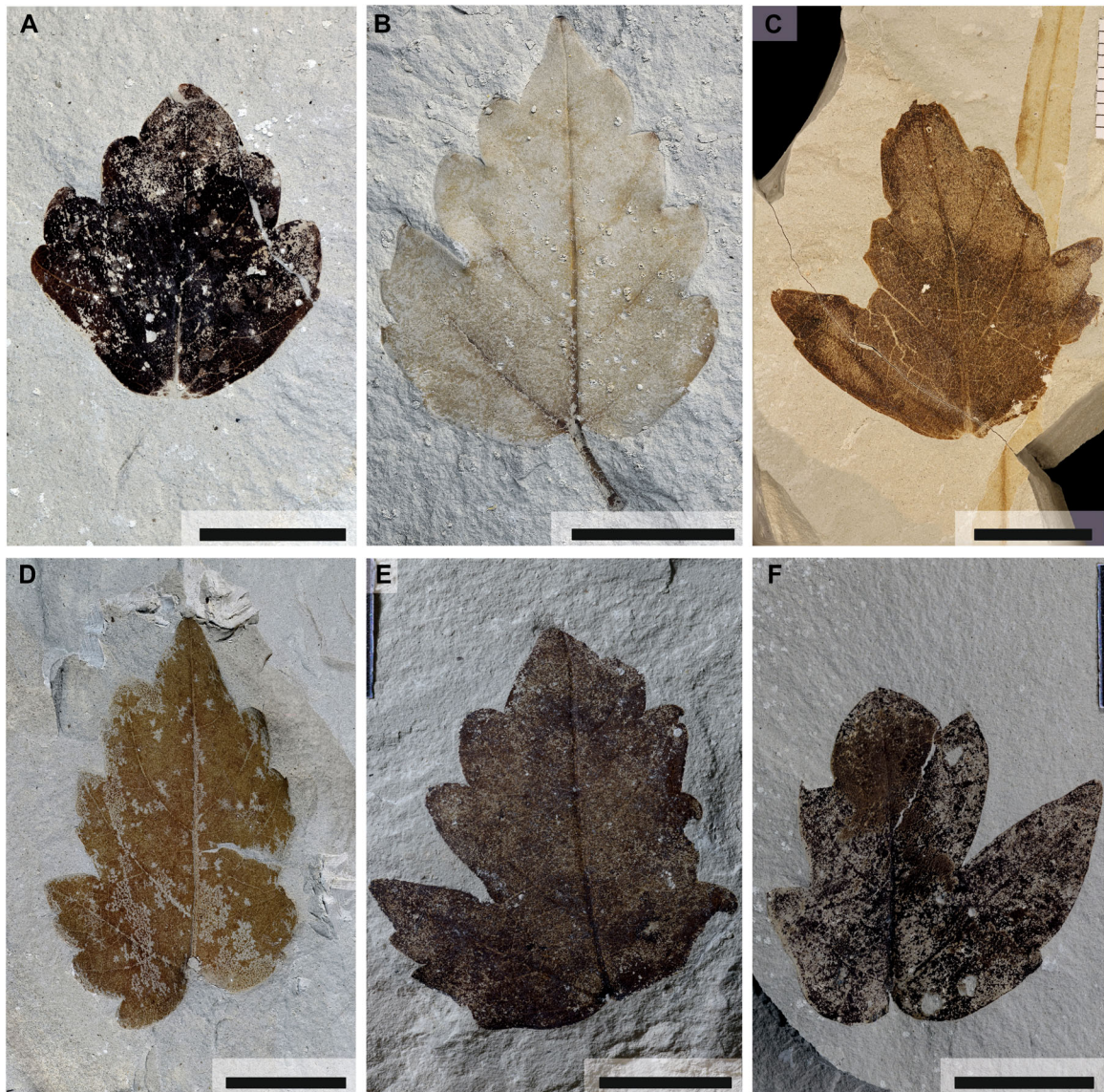


Fig. 3. Lateral leaflets of *Nekemias mucronata* sp. nov. stored at the Museu de Ciències Naturals de Barcelona. **A**, Paratype with a complete symmetric leaflet showing the first pair of secondary veins rising from the base MGB 94211. **B**, Complete leaflet showing the petiole still attached and the mucro at the tooth tip, MGB 85007. **C**, Paratype MGB 85010 displaying well-preserved tertiary venation framework. (**D–F**) Evolution of lobe development. **D**, little developed MGB 85008b; **E**, MGB 94212 with a lobe more developed than the other side; and **F**, MGB 94210 with a lobe significantly more developed than the other side. Scale: 1 cm.

teeth on both sides of the blade. The asymmetric specimens display 3–7 teeth (mean 5) on the side of the lamina where the lobe is well-developed, while 1–5 teeth (mean 4) occur on the other side (Figs. 3D–3F). The tooth shape is also variable. Both flanks may be convex (58%), straight (41%), and rarely flexuous (1%). Tooth spacing is irregular and the sinus is angular. The teeth are mucronate (Fig. 3B). The pattern of the primary venation is pinnate. The secondary venation framework is craspedodromous without branching and emerging from the midvein at angles of 30–60°. Two basal secondary veins feeding the lobes rise from the midvein at 50–70°. The junction of the secondary veins with the primary vein is excurrent. The tertiary framework decreases

exmedially (Fig. 3C). The exterior tertiary veins depart from the basal secondary veins at angles of 30–35° and run into the tooth apex.

Remarks The leaflets from the Oligocene in Cervera studied here were initially attributed to *Rhus* L. by Depape & Brice (1965) and Sanz de Siria Catalan (1992). The revision of *Rhus* specimens from this locality allowed some specimens that do not belong to *Rhus* to be segregated and they were provisionally related to *Acer* L. due to their trilobate shape (Tosal et al., 2019a). The reinvestigation of these specimens reveals significant differences with *Acer*. For instance, the tooth architecture with the marked mucro as found in *Nekemias mucronata* sp. nov. distinguishes it from *Acer*.

Furthermore, the results presented here agree with the interpretation of Tosal et al. (2019a), which pointed to a lignified tooth apex. These authors interpreted this feature as a gland; however, re-examination of the leaflets does not show evidence of secretory structures. In addition, the symmetric blade of the apical leaflet was interpreted as a simple leaf (Tosal et al., 2019a) but here, it is reinterpreted as corresponding to the apical leaflets of a compound leaf.

Comparison with extant Ampelopsidae species The most noticeable feature of *Nekemias mucronata* sp. nov. is the bilobate lamina shape and the serrate margin. These features are characteristic of the Vitaceae and more specifically of *Vitis* L., *Ampelopsis* and *Nekemias* in the tribe Ampelopsidae (Bůžek et al., 1981; Bharathan et al., 2002; Wen et al., 2018). However, *Vitis* species display a basally concentric tertiary framework resembling a spider web-like ribbing, while the fossil species described here display a sinuous tertiary venation pattern, as found in many *Ampelopsis* and *Nekemias* species. The fossil

leaflets from Cervera are found isolated, challenging their attribution to one of these genera. However, based on the high level of leaf morphological similarity with a particular modern species, *N. mucronata* sp. nov. was assigned to *Nekemias* (Fig. 4; Table 1).

Six modern *Ampelopsis* species (*A. aconitifolia* Bunge, *A. cordata* Michx., *A. delavayana* Planch. ex Franch., *A. hederaceae* DC., *A. japonica* (Thunb.) Makino, and *A. orientalis* Planch.) and two *Nekemias* species (*N. arborea* (L.) J. Wen & Boggan, *N. cantoniensis* (Hook. & Arn.) J. Wen & Z.L.Nie, kept in the herbaria of the Muséum National d'Histoire Naturelle in Paris (France) and the University of South Florida (USA), were used for comparison with the fossil leaflets of *N. mucronata* sp. nov. (Table 1). Five leaf features were analyzed: (i) leaf or leaflet shape; (ii) number of lobes; (iii) apex shape; (iv) base shape; and (v) secondary and tertiary venation pattern. The primary vein pattern was excluded since it is pinnate in both genera.

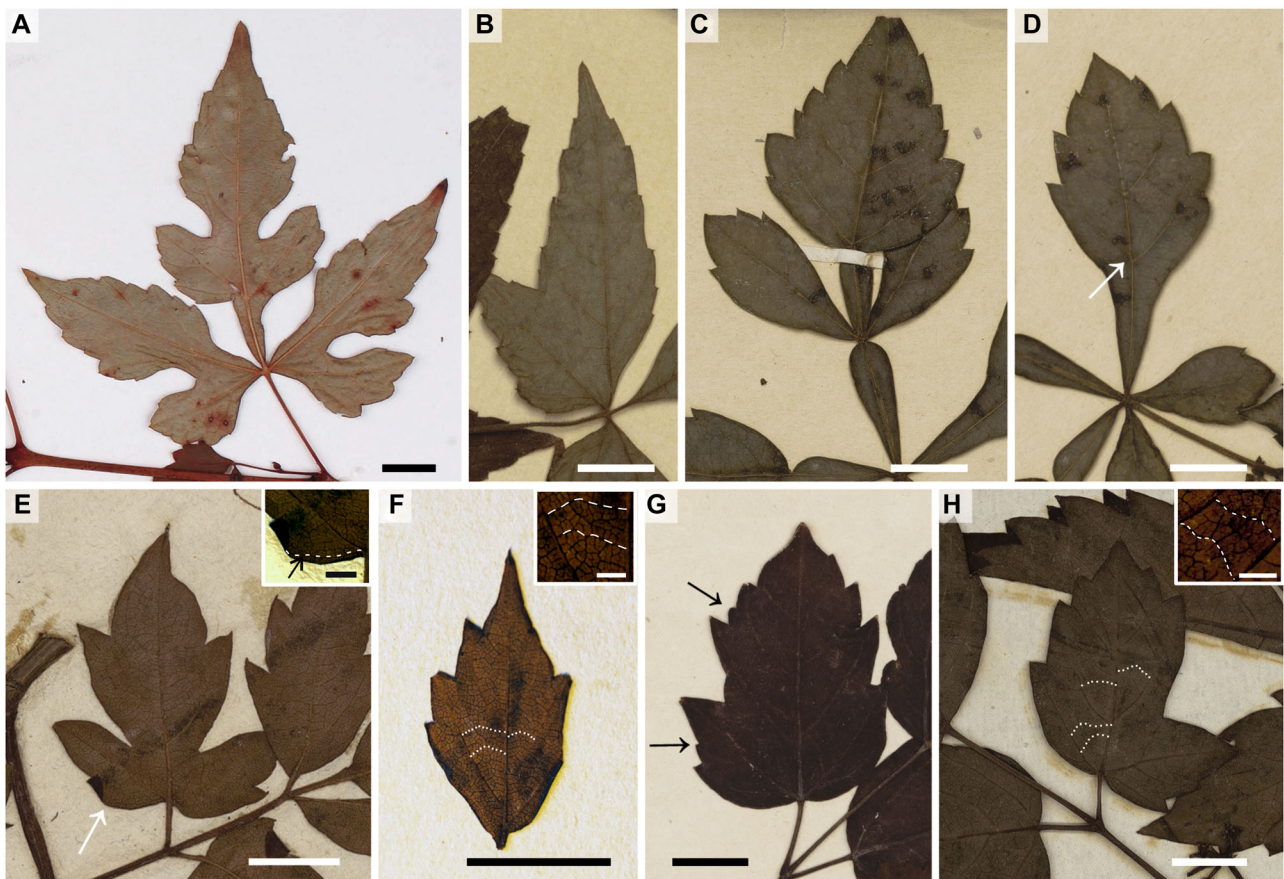


Fig. 4. Foliar features of extant *Ampelopsis* and *Nekemias* species housed in the Paris herbarium (B-H) and the University of South Florida (A). **A**, Leaflets of *Ampelopsis aconitifolia* with a marked constriction in the central part of the blade (USF-77473). **B**, Lateral leaflets of *Ampelopsis delavayana* with one basal vein (MNHN-P-03177374). **C**, Leaflet of *Ampelopsis japonica* with a winged petiolule (MNHN-P-03170159). **D**, Leaflet of *Ampelopsis japonica* showing the first pairs of secondary veins rising at a certain distance from the base (MNHN-P-03170159). **E**, Leaflet of *Nekemias arborea* showing well-developed lobes and smooth sinus. Inset: detail of the exterior tertiary veins looped (MNHN-P-03176863). **F**, *Nekemias arborea* leaflet showing sinuous tertiary framework (dashed line). Inset: detail of the sinuous tertiary veins (MNHN-P-03176862). **G**, Leaflet of *Ampelopsis orientalis* with two orders of teeth (MNHN-P-03176902). **H**, *Ampelopsis orientalis* leaflet showing the two basal secondary veins and the sinuous tertiary venation pattern (dashed lines). Inset: detail of the tertiary venation pattern (MNHN-P-03176897). Scale: 1 cm. Inset scale (E; F; H): 1 mm.

Table 1 Leaf characters of fossil species of Ampelopsidae from Western Eurasia and extant species of this tribe with lobed and serrate margin leaves

	Ampelopsis s.l. species	Blade shape	Length (cm)	Width (cm)	Symmetry of the leaflet	N° of lobes	Tooth orders	Base shape	Petiole	Secondary venation	Tertiary venation	Exterior tertiary veins	Herbarium number
Fossil	<i>Nekemias mucronata</i> sp. nov.	Lanceolate (rhombic) or ovate	3.6–4.8	1.8–3.6	Symmetric or asymmetric	0–3	1	Straight, cordate, or rounded	Presence	Craspedodromous. Lateral leaflets: 2 basal veins	Sinuuous	Reaching at the tooth tip	–
	<i>Ampelopsis hibschii</i> Bůžek, Kvaček et Wälder	Lanceolate (elliptic) or ovate	4.2–6.2	3.3–6	Symmetric or slightly asymmetric	2–3	1–2	Cordate	Presence	Craspedodromous. Lateral leaflets: 2–4 basal veins	Sinuuous	Reaching at the tooth tip	–
	<i>Ampelopsis</i> aff. <i>cordata</i> Borkov	Ovate	2.5–8.3	1.2–12.2	Asymmetric	0	1	Cordate	Presence	Craspedodromous. 2 basal veins	Sinuuous	Reaching at the tooth tip	–
Extant	<i>Ampelopsis schischkini</i>	Ovate	3–7.2	2.7–8.1	Symmetric or slightly asymmetric	0	1	Truncate rarely slightly cordate	Presence	Craspedodromous	–	Reaching at the tooth tip	–
	<i>Iljinskaja aconitifolia</i> Bunge	Lanceolate	3.7–5.5	2.3–2.6	Symmetric or asymmetric	3–8	1	Decurrent	Absence	Craspedodromous. No basal veins	Sinuuous	Forming loops	USF-77473
	<i>Nekemias arborea</i> (L.) J. Wen & Boggan	Lanceolate (rhombic) or ovate	1.8–3.25	1.2–2.2	Symmetric or asymmetric	0–3	1	Straight, cordate, or rounded	Presence	Craspedodromous. Lateral leaflets: 2 basal veins	Sinuuous	Reaching at the tooth tip	MNH-N-P-03176858; MNHN-P-03176862
	<i>Ampelopsis cantoniensis</i> (Hook. & Arn.) Planch.	Lanceolate	4.7–6.1	2.5–3.3	Symmetric	0	1	Straight	Presence or absence	Brochidodromous. 2 basal veins	Regular reticulate	Forming loops	MNH-N-P-03176863
	<i>Ampelopsis cordata</i> Michx.	Ovate	4.6–6.8	4.0–5.7	Symmetric	0–3	1	Cordate	Presence	Craspedodromous. 2 basal veins	Sinuuous	Reaching at the tooth tip	MNH-N-P-00536067
	<i>Ampelopsis delavayana</i> Franch.	Lanceolate	4.3–5.6	2.0–2.9	Symmetric or asymmetric	0–1	1	Straight	Presence (apical leaflet), absence (lateral leaflets)	Craspedodromous. Without basal veins	Sinuuous	Reaching at the tooth tip	MNH-N-P-03177374
	<i>Ampelopsis hederaceae</i> DC., (= <i>Parthenocissus quinquefolia</i>)	Lanceolate	5.7–9.6	1.6–4.0	Symmetric	0	1	Straight	Absence	Craspedodromous. Without basal veins	Regular reticulate	Forming loops	MNH-N-P-02788303; MNHN-P-04021722
	<i>Ampelopsis japonica</i> (Thunb.) Makino	Lanceolate or ovate	2.0–4.6	0.9–2.1	Symmetric or asymmetric	3–5	1	Decurrent or straight	Absence	Craspedodromous. Without basal veins	Sinuuous	Reaching at the tooth tip	MNH-N-P-02769416; MNHN-P-03170159
	<i>Ampelopsis orientalis</i> Planch.	Lanceolate or ovate	3.0–4.4	1.7–3.5	Symmetric or asymmetric	3–5	1–2	Straight or cordate	Presence	Craspedodromous. Lateral leaflets: 2 basal veins	Sinuuous	Reaching at the tooth tip	MNH-N-P-03176897; MNHN-P-03176902

The taxonomy of currently accepted extant species can be obtained from World Flora Online (<https://wfpantlist.org/plant-list>, access date: July 18, 2023).

Nekemias mucronata sp. nov. is symmetric or asymmetric with rhombic or ovate blade shape and petiolulate. This contrasts with *A. aconitifolia*, which displays a marked constriction in the central part of the blade, and the leaflets are sessile (Fig. 4A). It differs also from *A. delavayana* DC. (= *Parthenocissus quinquefolia*) because the lateral leaflets of this species display only one basal secondary vein rather than two as in *N. mucronata* sp. nov. (Fig. 4B). Another *Ampelopsis* species compared with the fossil *N. mucronata* sp. nov. is *A. japonica*. The latter species displays a winged petiolule in mature leaflets and sessile leaflets at the early stage of development (Figs. 4C and 4D respectively), while *N. mucronata* sp. nov. has a nonwinged petiolule. In addition, the first pairs of secondary veins of *A. japonica* rise at a certain distance from the base (Fig. 4D) contrasting with the basal secondary veins of *N. mucronata* sp. nov.

Leaflets of *A. orientalis* are nonwinged petiolulate, as is *N. mucronata* sp. nov. However, the serrate margin of the modern species displays two orders of teeth (Fig. 4G), while *N. mucronata* sp. nov. only has one order of teeth. The lateral lobes of *N. arborea* are as prominent as those observed in *N. mucronata* sp. nov. (Fig. 4E) and the sinus is smooth, as observed in *N. mucronata* sp. nov. (Fig. 4F). In addition, the venation pattern of *N. arborea* is similar to the fossil species *N. mucronata* sp. nov. showing close leaflet size, a pinnate

primary vein, craspedodromous secondary veins with two basal veins in the lateral leaflets, and sinuous tertiary framework (Fig. 4F; Table 1). Therefore, the extant species showing the greatest similarity with *N. mucronata* sp. nov. is *N. arborea*.

Nekemias arborea occurs in eastern North America (Fig. 5) but is sister to a number of East Asian species (Wen et al., 2007, 2014; Nie et al., 2012; Hearn et al., 2018). Molecular phylogenetic studies (Nie et al., 2012; Hearn et al., 2018) suggested that *N. arborea* diverged from its Eastern Asian sisters between ca. 40 and 15 Ma (95% highest posterior density), corresponding to the late Eocene to Late Miocene. This is consistent with the age of the fossils reported here but requires additional migration either across Eurasia or the North Atlantic Land Bridge.

Genus *Ampelopsis* Michx.

Ampelopsis hirschi Bůžek, Kvaček et Walther emended (Fig. 6).

1981 *Ampelopsis hirschi* Bůžek, Kvaček et Walther—Bůžek et al., 127, pl. 1–6 figs. 1–7

Other records:

(?) 1876 *Populus mutabilis* Heer—Engelhardt, 1876, 381, pl. 19, fig. 14, non figs. 13, 15

1885 *Populus mutabilis* Heer—Engelhardt, 29, pl. 13, fig. 9, non fig. 21

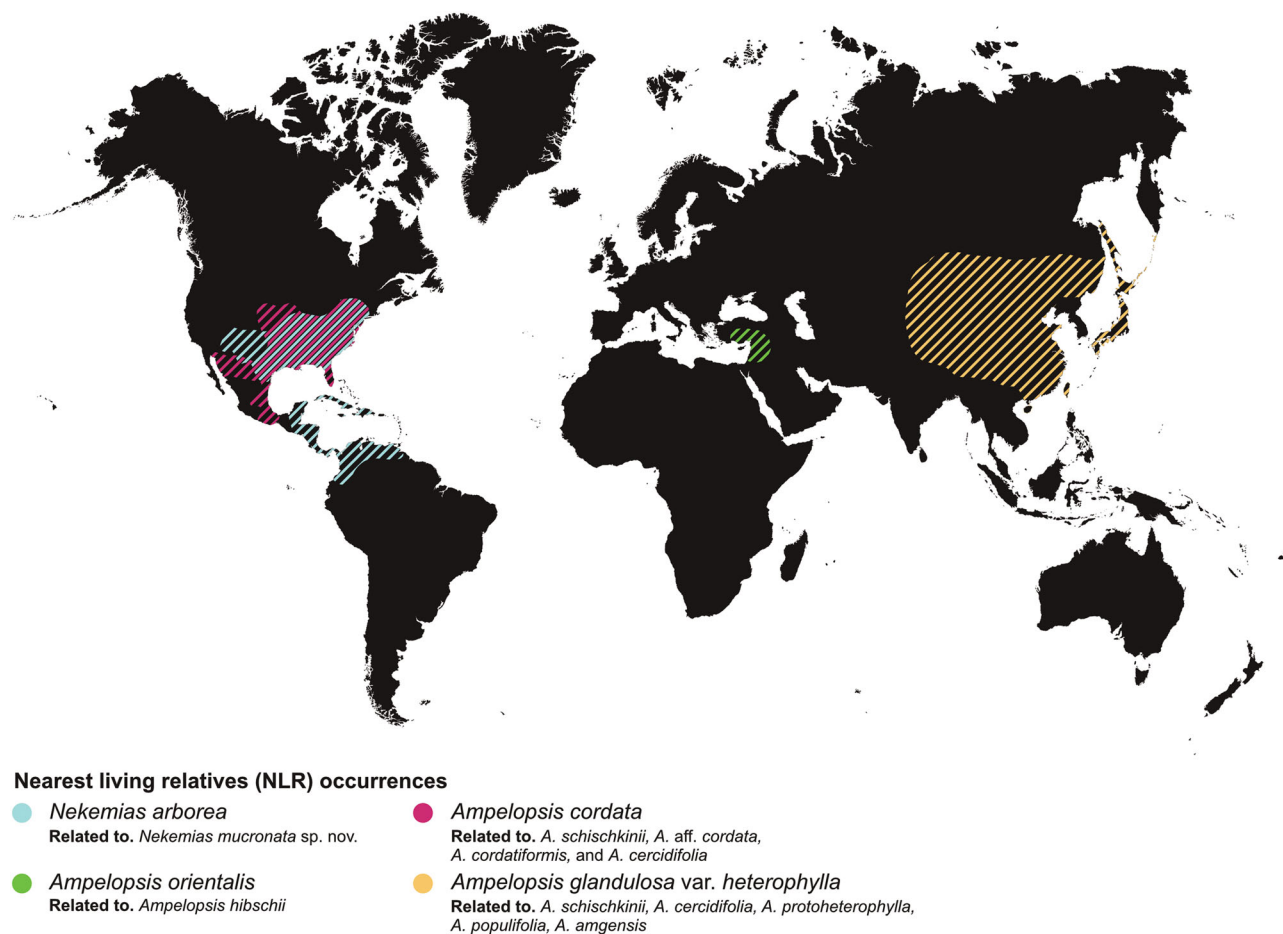


Fig. 5. Present-day world map with the occurrences of the nearest living relative (NLR) of the fossil species of the tribe Ampelopsideae (data from Table S2).

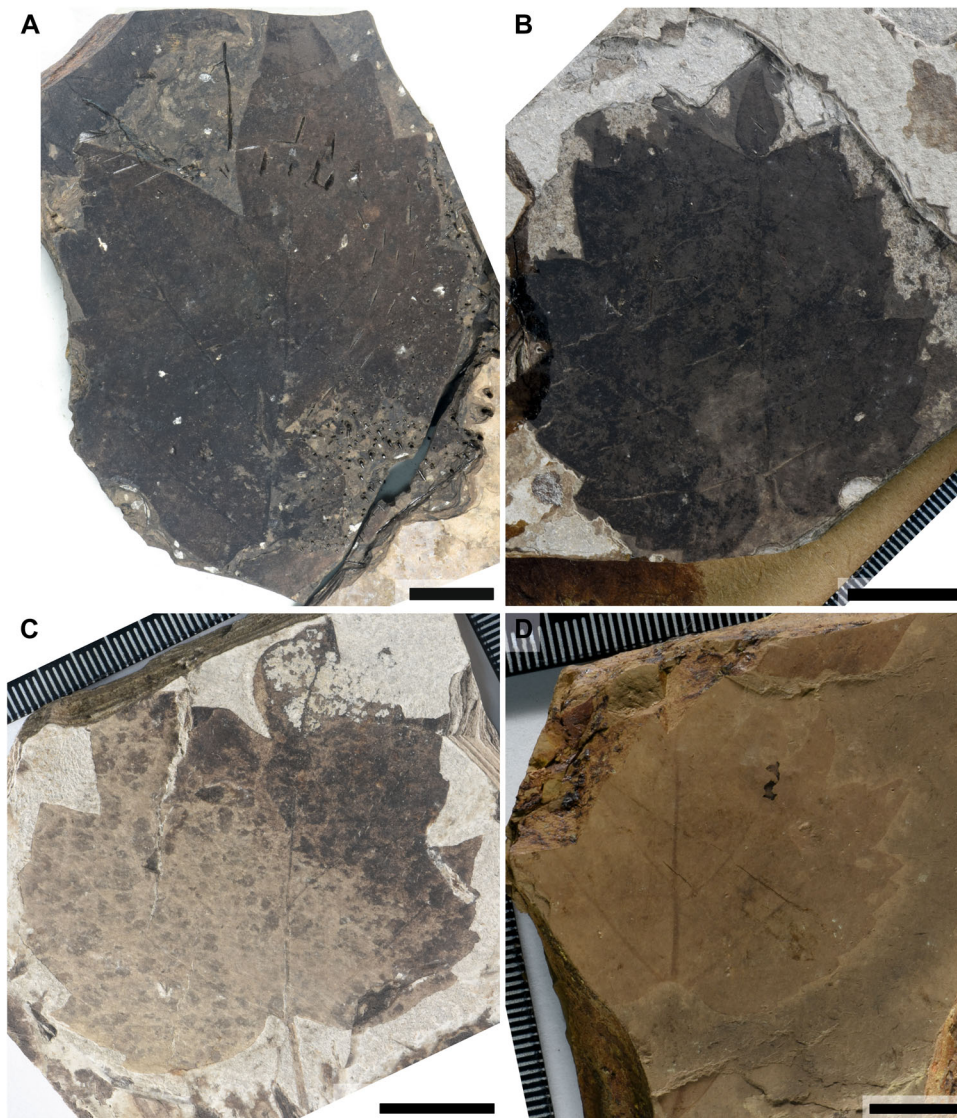


Fig. 6. *Ampelopsis hibschii* from České středohoří housed at the Czech Geological Survey. **A**, Holotype (CB 818). **B**, Specimen with one tooth at the apex showing a pronounced narrowing of the sinuses (1/4 B8 10-Be1006). **C**, Specimen showing cordate base with a reflex shape and petiolate (1/4 B8 12-Be1005). **D**, Specimen with two teeth orders (1/4 B8 13b). Scale: 1 cm.

1966 cf. Vitaceae (*Cissus*?)—Bůžek, Holý & Kvaček, 249–250

1978 cf. *Ampelopsis* sp.—Bůžek, Kvaček & Walther, 353

1978 *Ampelopsis* sp.—Mai & Walther, 126, pl. 7, figs. 1–3; pl. 46, figs. 1–6

1994 *Ampelopsis hibschii* Bůžek, Kvaček et Walther—Kvaček & Walther, 1994, 60, 7, figs. 7–8

2000 *Ampelopsis* aff. *orientalis*—Božukov, 18, pl. 2, fig. 1; 19, pl. 3, fig. 5

2000 *Ampelopsis hibschii* Bůžek, Kvaček et Walther—Božukov, 18, pl. 2, fig. 2, 19, pl. 3, fig. 3

2004 *Ampelopsis* sp.—Kvaček & Walther, 20, pl. 3, figs. 1–2, text-fig. 11.9

2007 *Ampelopsis hibschii* Bůžek, Kvaček et Walther—Kvaček & Teodoridis, 389; fig. 3P

2012 *Ampelopsis* sp. cf. *heterophylla* (Thunb.) Siebold et Zucc.—Averyanova, 99; pl. 8, fig. 11; pl. 61, figs. 2, 3

2012 *Ampelopsis* sp. cf. *heterophylla*—Averyanova, 221; pl. 61, fig. 1

2014 aff. *Ampelopsis hibschii* Bůžek, Kvaček et Walther—Velitzelos et al., 66, pl. VI, figs. 1–3

2014 *Ampelopsis dubia* (C.O. Weber) Winterscheid et Kvaček—Winterscheid & Kvaček, pl. 2, fig. 9–10, pl. 10, figs. 7–8

Chronostratigraphic range From late Eocene to Middle Miocene.

Paleogeographic distribution Late Eocene in Kiin-Kerish (Kazakhstan); Oligocene in Fylakton (Greece); early Oligocene in Dečín, Bohemia, Bechlejovice, and Útesky (Czech Republic), and Haselbach and Saxony (Germany); late Oligocene in Orsberg, Rhineland-Palatinate (Germany); and Middle Miocene in Rhodopes (Bulgaria).

Original material Fourteen isolated and fragmented leaflets from Dečín (the České Středohoří Mountains, Czech Republic). The holotype with the number CB 818 is referred

to as KU 38 (collection ÚÚG) in Bůžek et al. (1981). The specimens are quite fragmentary making it difficult to determine their position on the leaf.

Original diagnosis proposed by Bůžek et al. (1981). Blätter oval, breit-oval bis herzförmig mit einer keilförmigen, truncaten bis schief-herzförmigen Spreitenbasis. Einfach, unvollständig gelappt oder geteilt. Lamina meist asymmetrisch, oft in einseitig oder zweiseitig abgesetzten ganzrandigen, apikalen Lappen bzw. Blatteil und 2–4 Seitenlappen gegliedert. Bei einfachen Blättern nur eine mehr oder weniger ausgezogene Spitze und 2–4 laterale Hauptzähne vorhanden. Blattzähne mehr oder weniger deutlich mucronat. Nervatur craspedodrom, in Apikallappen bzw. apikalem Blatteil und in den Hauptzähnen camptodrom. Basales Sekundärnervenpaar entspringt mit dem Mittelnerv vom Blattstiel außerhalb der Blattlamina, Verzweigung fußförmig.

Emended diagnosis Leaflet symmetric or slightly asymmetric, petiolate, lamina ovate, or broad lanceolate in shape, simple or incompletely palmately lobed with serrate margin. Cordate or straight base and acute apex with one or two apical teeth. Conspicuously constricted apical lamina segment, recalling a lobe (or a single or a pair of teeth) distinct from the rest of the lamina. Primary venation pinnate and secondary framework craspedodromous. Two to four secondary veins depart from the base, attachment to midvein excurrent. Intercostal tertiary vein fabric sinuous. Exterior tertiary veins reach the tooth apex.

Description of the type material of *A. hibschi* The leaflet is symmetric or slightly asymmetric and lanceolate (elliptic) or ovate shape, with a length of 4.2–6.2 cm and a width of 3.3–6 cm. The blade is palmately lobed with two or three lobes. The apex is straight with two apical teeth and rarely only one tooth (Figs. 6A, 6B). The base is cordate with a reflex base shape and petiolate (Fig. 6C). The margin is serrate with up to 10 teeth distributed irregularly across the blade, ranked in two orders and devoid of mucro (Fig. 6D). In general, the distal flank is straight or convex, while the

proximal flank may be concave, convex, flexuous, or straight. The tooth located at the apex of the leaflet is convex and frequently the sinus of the teeth almost reaches the midvein (the apical lamina thus appearing strongly constricted), which is a characteristic feature of the species. The sinus between the teeth is angular. The primary vein is pinnate. The secondary venation is composed of four to six craspedodromous pairs of veins rising from the midvein at angles of 30–60°, having a straight way ending at the tooth apex. Two or rarely four basal secondary veins rising at angles of 70–90° to the primary vein ends at the apex of the lobule. The junction of the secondary veins with the primary vein is excurrent. The tertiary framework is sinuous. The exterior tertiary veins rise from the basal secondary veins at angles of 30–35° and reach the tooth apex.

Remarks *Ampelopsis hibschi* (Fig. 7A), *N. mucronata* sp. nov. (Fig. 7B), *A. aff. cordata* (Fig. 7C) from the Middle Miocene in Satovcha Graben, Bulgaria, and *Ampelopsis schischkinii* Iljinskaja (1963; Fig. 7D) from the early Oligocene in Kusto, Kazakhstan, have in common the toothed margin and the two basal secondary veins. However, *A. aff. cordata* and *A. schischkinii*, show a strong resemblance to each other, are unlobed, and their secondary veins are dichotomized close to the margin (Figs. 7C, 7D), while *A. hibschi* and *N. mucronata* sp. nov. display a trilobate blade and the secondary veins run directly into the tooth tip (Figs. 7A, 7B). Despite the similarities between *A. hibschi* and *N. mucronata* sp. nov., the angle between the lateral lobes and the central lobe is closer in *N. mucronata* sp. nov. (Fig. 7B) and the basal veins rise at a smaller angle than in *A. hibschi* (Fig. 7A). Although most of the type population of *A. hibschi* is represented by fragmentary leaflets, two orders of teeth without mucro at the tooth tip are recognized. This contrasts with only one tooth order in *N. mucronata* sp. nov., displaying a mucro at the tooth apex. In addition, the tooth shape of *A. hibschi* is sharper than *N. mucronata* sp. nov. Another leaf physiognomic difference between these two Eurasian fossil

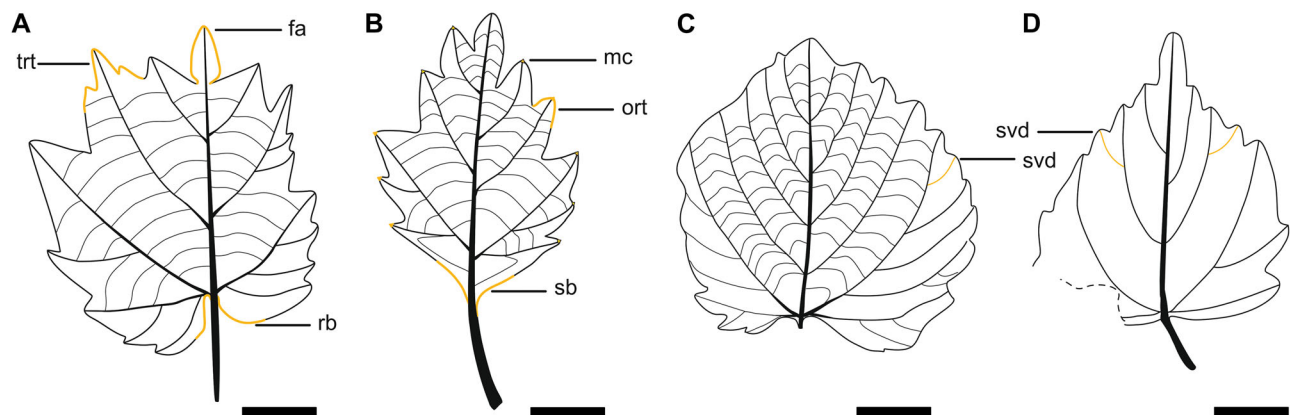


Fig. 7. Line drawing of Cenozoic apical leaflet or leaf of Western Eurasian and Central Asian species of Ampelopsidae. In yellow, are the most characteristic features of the species. **A**, *Ampelopsis hibschi* from České středohoti (Czech Republic) with reflexed base (1/4 B8 10-Be1006). **B**, *Nekemias mucronata* sp. nov. with straight base and mucronate tooth (MGB 33090). **C**, *Ampelopsis* aff. *cordata* from Satovcha Graben (Bulgaria) with symmetric and unlobed blade (Car-2249). **D**, *Ampelopsis schischkinii* from Kiin-Kerish (Kazakhstan) with secondary veins dichotomized close to the margin (72 collection 449). fa, flame-shaped apex; mc, Mucro; ort, One order of teeth; rb, Reflexed base; sb, Straight base; svd, Secondary vein dichotomized close to the margin; trt, two orders of teeth. Scale: 1 cm.

species is the apex shape. In most of the specimens of *A. hibschi*, the apex is composed of two teeth and the sinus of the tooth is sharp (Fig. 7B). These features contrast with *N. mucronata* sp. nov., in which the apex is composed of only one tooth with a poorly pronounced sinus.

Comparison with extant Ampelopsidae species Cuticle analyses carried out by Bůžek et al. (1981) showed that the epidermal structure of *A. hibschi* resembles some living *Ampelopsis*, that is, *A. bodinieri* (H.Lév. & Vaniot) Rehder, *A. aconitifolia*, *A. delavayana*, and *Ampelopsis brevipedunculata* (Maxim.) Trautv., as well as *Nekemias cantoniensis*, but differs from *Nekemias arborea*, which has a characteristic striation on the upper epidermis. In addition, Bůžek et al. (1981) interpreted the apically constricted lamina in *A. hibschi* as potentially analogous with the apical leaflets in *A. japonica* and *A. aconitifolia*.

We compared fossil leaflets of *A. hibschi* with the same extant *Ampelopsis* and *Nekemias* species as *N. mucronata* sp. nov. (Table 1). As in *N. mucronata* sp. nov., *A. hibschi* is symmetric or asymmetric with broad lanceolate or ovate blade shape with nonwinged petiolule, but it displays two orders of teeth. The petiolulate leaflets together with the serrate margin with two orders of teeth in *A. hibschi* contrast with the modern leaflets of *A. japonica*, which are sessile with one order of teeth (Fig. 4D). Furthermore, the lateral leaflets of *A. japonica* do not display secondary basal veins (Fig. 4D) as found in *A. hibschi*. Unlike *A. japonica*, modern leaflets of *A. orientalis* show two orders of teeth (Fig. 4G).

Ampelopsis orientalis shows physiognomic features similar to *A. hibschi*, namely symmetric or asymmetric blade; blade size, bi-trilobate shape with serrate margin; cordate or straight base, two orders of teeth (Fig. 4G; Table 1) and a venation pattern, including a first pair of secondary basal veins in the lateral leaflets and sinuous tertiary venation pattern (Fig. 4H). Therefore, *A. orientalis* may be the nearest living relative (NLR) of *A. hibschi*. *A. orientalis* is native to Lebanon–Syria and southern Turkey (Fig. 5) and is part of a clade including several East Asian species of *Ampelopsis* s.s. (Nie et al., 2012; Hearn et al., 2018). The latter include *A. aconitifolia* and *A. japonica* suggesting that Bůžek et al.'s (1981) interpretation of a possible analogy in the leaf morphologies of these modern species and the fossil species *A. hibschi* may be correct.

4 Discussion

4.1 Links between the European and Asian floras during the Paleogene

The revision of the leaf record of Ampelopsidae in Eurasia indicates that three fossil species of *Ampelopsis* (*Ampelopsis hibschi*, *Ampelopsis cercidifolia*, and *Ampelopsis protoheterophylla*) and one of *Nekemias* (*N. mucronata* sp. nov.) occurred in Central Europe and Asia during the middle and late Eocene (Fig. 8; Table 2). The presence of *A. protoheterophylla* was restricted to the middle Eocene deposits of Sakhalin (Kodrul, 1999), while *A. cercidifolia* is found in the late Eocene in Kamchatka (Budantsev, 1997), both localities in the Far East (Russia), and East Asia. In contrast, *A. hibschi* has a longer paleobiogeographic history. This species first occurred in the

late Eocene deposits of Kiin-Kerish in Kazakhstan, Central Asia (cf. Kvaček & Teodoridis, 2011; Averyanova, 2012). Later, it was found in Europe, in the Rupelian (early Oligocene) strata of the Czech Republic (cf. Kvaček & Teodoridis, 2011) and in Germany (Mai & Walther, 1978; Walther & Kvaček, 2007; Kvaček et al., 2015).

Moreover, the earliest record of *N. mucronata* is from the late Eocene in the Czech Republic, which later expanded both toward the south (Spain and Greece) and east (Kazakhstan) during the Oligocene (Figs. 8, 9; Tables 2, S1). This expansion was accompanied by biome shifts.

The extent and nature of different terrestrial biomes and vegetation types in Western Eurasia and Central Asia during the late Eocene and early Oligocene have been addressed in various studies (e.g., Mai & Walther, 1984; Zhilin, 1989; Popov et al., 2001, 2002; Willis & McElwain, 2014; Kvaček, 2010; Scotese, 2013a, 2013b; Scotese et al., 2014). During the late Eocene, a Subtropical Summerwet forest biome (Paratropical forest) was widespread across these regions, which was more seasonal in Southwestern Europe and parts of southern Central Europe and to the west and east of the Turgay Sea (Popov et al., 2001; Scotese, 2013a). Establishing a detailed phytogeographic zonation is hampered by the difficulties of assigning fossil plant taxa to genera and families (Popov et al., 2001). For example, the *Kazakhstanian Province* defined by oak-laurel “hard-leaved” forests might be very similar to some West European floras of the *North European Province* defined by evergreen and summer green coniferous-broad leaved, mainly oak laurel forests (Popov et al., 2001, p. S39). Different taxonomic concepts may give the false impression of a distinct vegetation zonation. Makulbekov (1972) considered several modern and a few extinct members of Fagaceae as typical of the late Eocene vegetation of the *Kazakhstanian Province* but some of these taxa may be conspecific or very similar to roughly coeval taxa from West Europe (see, e.g., Mai & Walther, 1984; Kvaček & Walther, 2010).

A comparison of the late Eocene and early Oligocene leaf assemblages of the Kiin-Kerish region (Kazakhstan; Averyanova et al., 2021) with coeval localities in Europe indicates several cases of shared taxa between the two regions. Evergreen fagaceous foliage composed of *Castanopsis furcinervis* (Rossm.) Kräusel et Weyl., *Dryophyllum corticellense* (Watelet) Saporta et Marion, *Dryophyllum dewalquei* Saporta et Marion, and *Dryophyllum kryshtofovichii* Iljinsk., among others (Iljinskaja, 1957; Makulbekov, 1972), correspond to the European Paleogene fossil species *Eotrigonobalanus furcinervis* (Rossm.) Walther et Kvaček, *Castanophyllum lonchitiforme* Kvaček et Walther, and *C. venosum* (Rossm.) Knobloch et Kvaček, respectively (e.g., Knobloch et al., 1996; Kvaček & Walther, 2010). Overall, these taxa reflect the presence of laurel forests (sensu Körner, 2013) or laurel-oak forests across a wide range of Western Eurasia and into Central Asia at this time. This forest type hosted several other plant genera and species shared between the regions (e.g., *Comptonia comptoniifolia* (Brongn.) Doweld, *Ampelopsis*, *Myrica longifolia* Unger, or *Zelkova* Spach). In addition, a number of woody angiosperms had earlier occurrences in Central Asia than in Europe (e.g., *Nelumbo protospeciosa* Saporta, *Chaneya ninae* (Iljinsk. & Akhmetiev) Averyanova, *Cercidiphyllum crenatum* (Unger)

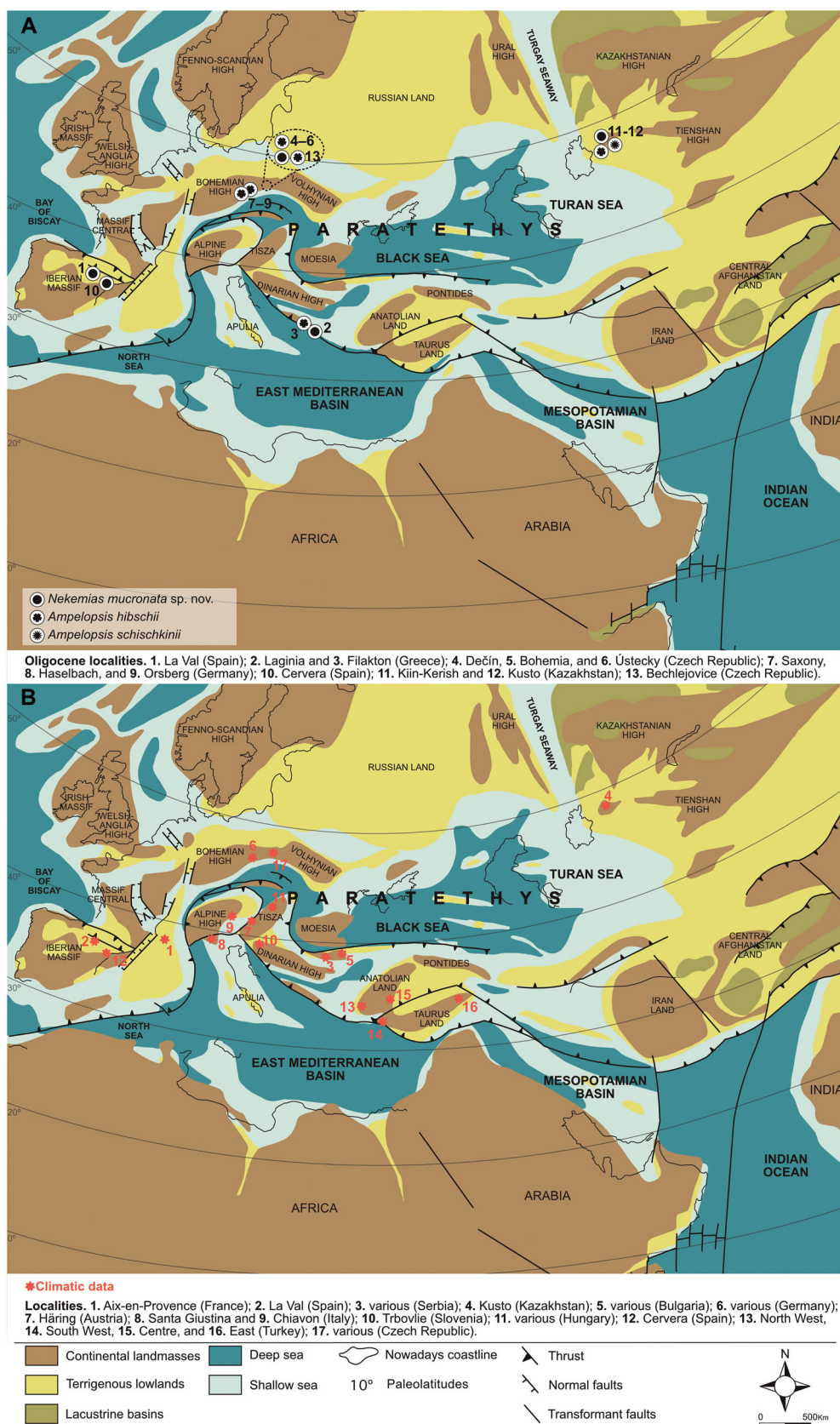


Fig. 8. Late Eocene paleogeographic map of Western Eurasia. Modified from Popov et al. (2004). **A**, Distribution of *Ampelopsis* and *Nekemias* species. **B**, Localities with paleoclimatic data (Table S1).

Table 2 Occurrence of *Ampelopsis* and *Nekemias* fossil leaves species from Eurasia

Species revisited	Age	Locality			References
		Basin	Village/province	Country	
<i>Ampelopsis cordatifformis</i>	Pliocene	-	Willershausen	Germany	Knobloch (1998)
<i>Ampelopsis amgensis</i>	Miocene	-	Far East	Russia	Krassilov (1984)
<i>Ampelopsis abkhasica</i>	Late Miocene	Kodor river	Abkhazia	Georgia	Kolakowsky (1960)
<i>Ampelopsis hibschii</i>	Middle Miocene	Satovcha Graben	Rhodopes	Bulgaria	Bozukov (2000)
<i>Ampelopsis aff. cordata</i>		Satovcha Graben	Rhodopes	Bulgaria	Bozukov (2000)
<i>Ampelopsis populifolia</i>	Early Miocene	-	Shanwang	China	Hu & Chaney (1940)
<i>Nekemias mucronata</i> sp. nov.	Oligocene	Evros Basin	Lagina	Greece	Velitzelos et al. (2014)
<i>A. hibschii</i>		Evros Basin	Filakton	Greece	Velitzelos et al. (2014)
<i>Ampelopsis schischkini</i>		Zaizan Basin	Kiin-Kerish	Kazakhstan	Ilijinskaja (1963); Averyanova (2012)
<i>A. hibschii</i>	late Oligocene	Rhine Basin	Orsberg	Germany	Winterscheid & Kvaček (2014)
<i>N. mucronata</i> sp. nov.		Ebro Basin	La Val	Spain	Moreno-Domínguez et al. (2021)
<i>N. mucronata</i> sp. nov.	early Oligocene	Ebro Basin	Cervera	Spain	Fernández-Marrón (1971); Sanz de Siria Catalan (1992); Tosal et al. (2019a)
<i>N. mucronata</i> sp. nov.		Zaizan Basin	Kusto	Kazakhstan	Averyanova (2012); Averyanova et al. (2021)
<i>N. mucronata</i> sp. nov.		České středohoří Mts.	Bechlejovice	Czech Republic	Kvaček & Walther (2004)
<i>A. hibschii</i>		České středohoří Mts.	Bechlejovice	Czech Republic	Engelhardt (1885); Bůžek et al. (1966, 1978); Kvaček & Walther (2004); Kvaček & Teodoridis (2007)
<i>A. hibschii</i>		Hrozený Hill	Ústecký	Czech Republic	Kvaček et al. (2015)
<i>A. hibschii</i>		Seiffennersdorf	Saxony	Germany	Kvaček et al. (2015)
<i>A. hibschii</i>		Weisselster Basin	Haselbach	Germany	Mai & Walther (1978); Walther & Kvaček (2007)
<i>Ampelopsis angulata</i>		-	Far East	Russia	Pavlyutkin et al. (2014)
<i>N. mucronata</i> sp. nov.	late Eocene	České středohoří Mts.	Kučlín	Czech Republic	Kvaček & Teodoridis (2011)
<i>A. hibschii</i>		Zaizan Basin	Kiin-Kerish	Kazakhstan	Averyanova (2012)
<i>Ampelopsis cercidifolia</i>		-	Far East	Russia	Budantsev (1997)
<i>Ampelopsis protoheterophylla</i>	middle Eocene	Sakhalin	Far East	Russia	Kodrul (1999)

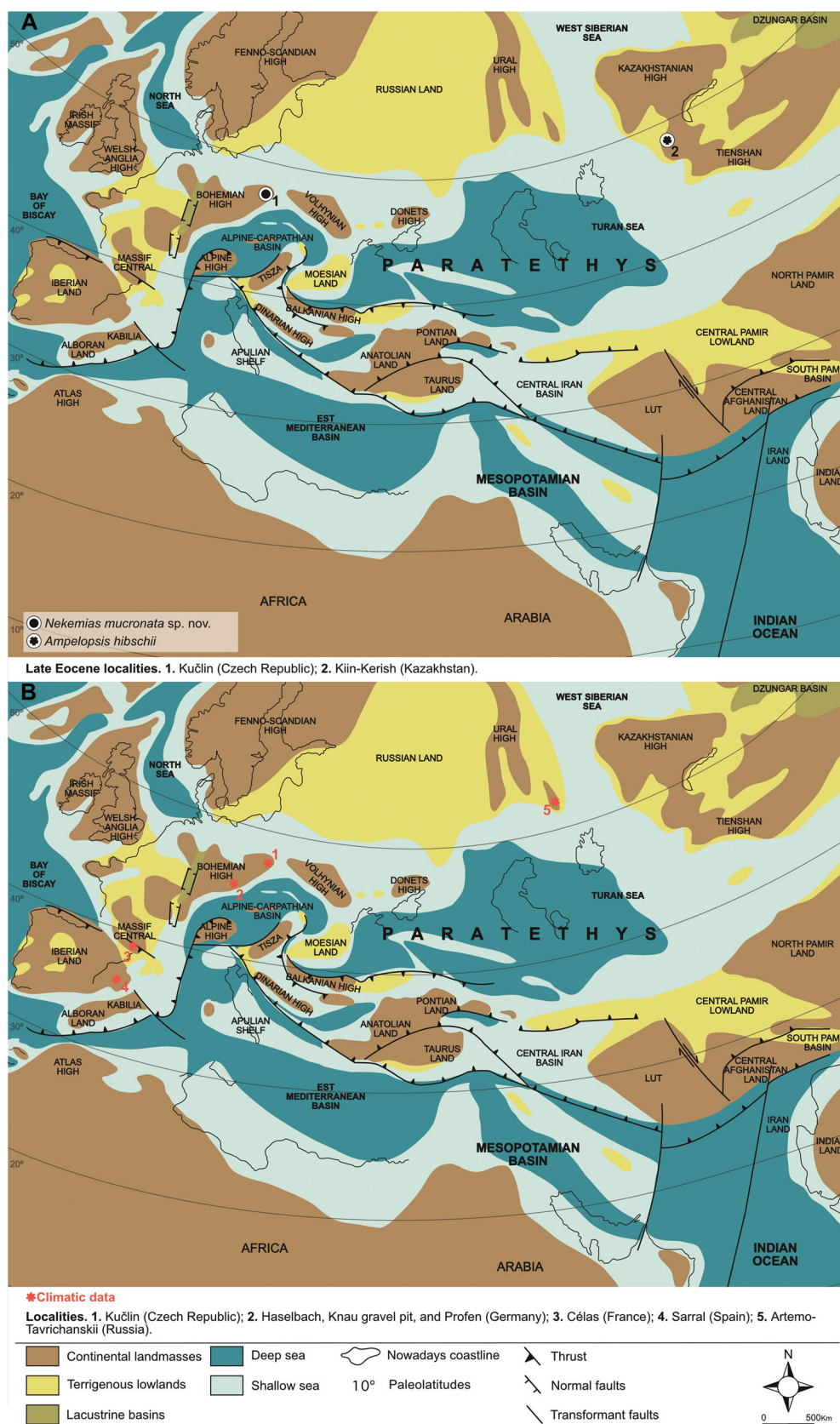


Fig. 9. Oligocene paleogeographic map of Western Eurasia. Modified from Popov et al. (2004). **A**, Distribution of *Ampelopsis* and *Nekemias* species. **B**, Localities with paleoclimatic data (Table S1).

Br., *Liquidambar europaea* Braun in Buckl., and the enigmatic Rosaceae *Phyllites kvacekii* Bůžek) and may have migrated to Western Eurasia during the Oligocene (Zhilin, 1989; Averyanova et al., 2021). This latter pattern is also seen in the *A. hibschi* investigated here.

4.2 Implications on the origin and dispersion routes of Ampelopsidae

Based on carpological remains from Upper Cretaceous deposits of India, Manchester et al. (2013) suggested a Southern Hemisphere origin for the Vitaceae (and Vitales). This out-of-India scenario could explain the relatively sudden diversification of the family in the late Paleocene and early Eocene in the Northern Hemisphere (Manchester et al., 2013; Manchester, 2020). The origin of Ampelopsidae has been broadly investigated in molecular phylogenetic studies (Wen et al., 2007; Nie et al., 2012). Nie et al. (2012) suggested a Cretaceous origin for the family Vitaceae and that the tribe Ampelopsidae (the former “Ampelopsis clade”) originated in North America between ca. 60–25 Ma (95% highest posterior density) based on molecular clock dating. These authors presented a biogeographic scenario for Ampelopsidae, which included two independent dispersals via the North Atlantic land bridges (NALBs) during the Early Miocene. The first migration via the NALB would have been the Early Miocene split between the lineage leading to the North American *Nekemias arborea* and its East Asian sister species; the second would be a late Early Miocene split between the lineage leading to the North American *Ampelopsis cordata* and the Eurasian species of *Ampelopsis*.

Our results are in general agreement with the 95% HPD node age ranges of Nie et al. (2012), but add chronostratigraphic and biogeographic information for *Ampelopsis* and *Nekemias*. For *Nekemias*, the presence of *N. mucronata* sp. nov. in early Oligocene strata of Spain and Greece (Europe) along with Kazakhstan (Central Asia) suggests that the clade had a much wider distribution during the Oligocene than today (Fig. 9). Whether the initial split between North America and Eurasia involved the NALB (as favored by Nie et al., 2012) or the Bering Land Bridge is difficult to tell based on both molecular and fossil data. The earlier occurrence of *Nekemias mucronata* in Central Europe lends credibility to an initial crossing of the NALBs as proposed for the Rosaceae *Vauquelinia* (Denk et al., 2023). In any case, unlike in the biogeographic scenario of Nie et al. (2012), the fossil record suggests that the *Nekemias* lineage was already in Eurasia during the late Eocene and persisted until the late Oligocene (Figs. 8, 9; Table S1). Although an exhaustive review of all the fossil species of Eurasia is not the aim of this study, a number of fossil leaves of the Ampelopsidae tribe formerly attributed to *Ampelopsis*, for example, “*Ampelopsis*” *abkhassica* Kolak. (late Oligocene) should be revised considering the strong resemblance to some extant *Nekemias* species, for example, *Nekemias megalophylla* (Diels et Gilg) J. Wen et Nie (Fig. 10). Such a revision will allow the paleobiogeographic evolution of this lineage to be refined.

The presence of *A. hibschi* in Europe and Central Asia during the late Eocene to Middle Miocene (e.g., Bůžek et al., 1981; Figs. 8–10), as well as *A. cercidifolia* and *A. protoheterophylla* from the late Eocene in East Asia (Budantsev, 1997 and Kodrul, 1999, respectively), and finally

Ampelopsis schischkinii and *Ampelopsis angulata* Pavlyut in the early Oligocene in Central and East Asia, respectively (Averyanova, 2012; Pavlyutkin et al., 2014; Averyanova et al., 2021), suggests that *Ampelopsis*—stem or crown group relatives—had a much wider distribution during the Paleogene than today (Fig. 10). In view of the fossil record, the intercontinental distribution of extant members of *Ampelopsis* could either be the result of NALB crossing or migration across the Bering Land Bridge or both.

4.3 Factors determining *Ampelopsis* and *Nekemias* distribution in Western Eurasia

Major changes in climate and vegetation characterize the EOT. Although these events have been studied in detail in particular plant groups, such as *Comptonia* (e.g., Teodoridis et al., 2017) and *Doliosrobis* (Kvaček, 2002), their possible impact on Vitaceae paleobiogeography remains largely unknown. Bůžek et al. (1981) undertook an exhaustive study of *A. hibschi* leaves found in Europe until the 1980s. These authors concluded that *A. hibschi* was restricted to Central Europe during the Paleogene (Kvaček, 2010). Our review of more recent records of *A. hibschi* suggests that the fossil species *A. hibschi* grew in Kazakhstan (Central Asia) in the late Eocene (Fig. 8; Table 2) and had dispersed to Central and South Europe by the Oligocene (Fig. 9; Table 2), confirming the hypothesis of Bůžek et al. (1981) of a westward area expansion. During the Middle Miocene, *A. hibschi* survived as a relict in the Balkans (Europe) and co-occurred with *A. aff. cordata* (Fig. 10; Table 2).

Nekemias mucronata sp. nov. grew in the Czech Republic in the late Eocene (Fig. 8). During the Oligocene, this species dispersed southward and thrived in Greece and Spain (Fig. 9; Table 2) and eastwards to Kazakhstan (Central Asia). In Kazakhstan, *N. mucronata* sp. nov. co-occurred with another member of Ampelopsidae, *A. schischkinii* (Fig. 9; Table 2). The last occurrences of *N. mucronata* sp. nov. are recorded in late Oligocene deposits from the Ebro Basin (NE Spain, Europe; Table 2).

The compilation of available paleoclimatic data has been studied to test whether the particular distribution of these accepted fossil Vitaceae species, that is, *A. hibschi*, *A. schischkinii*, *A. aff. cordata*, and *N. mucronata* sp. nov., was determined by climate and/or different biome types. To this end, a climate data comparison was carried out between the different localities where these fossil species occurred and with adjacent localities that lack them (Table S1), as well as with current climate data of their extant counterparts (Table S2).

4.3.1 Climatic and environmental niches in fossil and extant *Nekemias*

Available climate data for *N. mucronata* sp. nov. were revised from the late Eocene deposits of Kučlín, the Czech Republic (Teodoridis & Kvaček, 2015), and the Oligocene deposits of Cervera and La Val, Spain (Tosal et al., 2019b; Moreno-Domínguez et al., 2021 respectively), and Kusto, Kazakhstan (Averyanova et al., 2021). In the late Eocene, a warm climate without winter frosts has been inferred for Kučlín; the ratio between the precipitation during the three consecutive wettest and driest months is 4.6. This points to weak precipitation seasonality assuming that the main amount of rain was falling during the summer (see Peel et al., 2007, for threshold values of distinctly seasonal climates in summer-dry

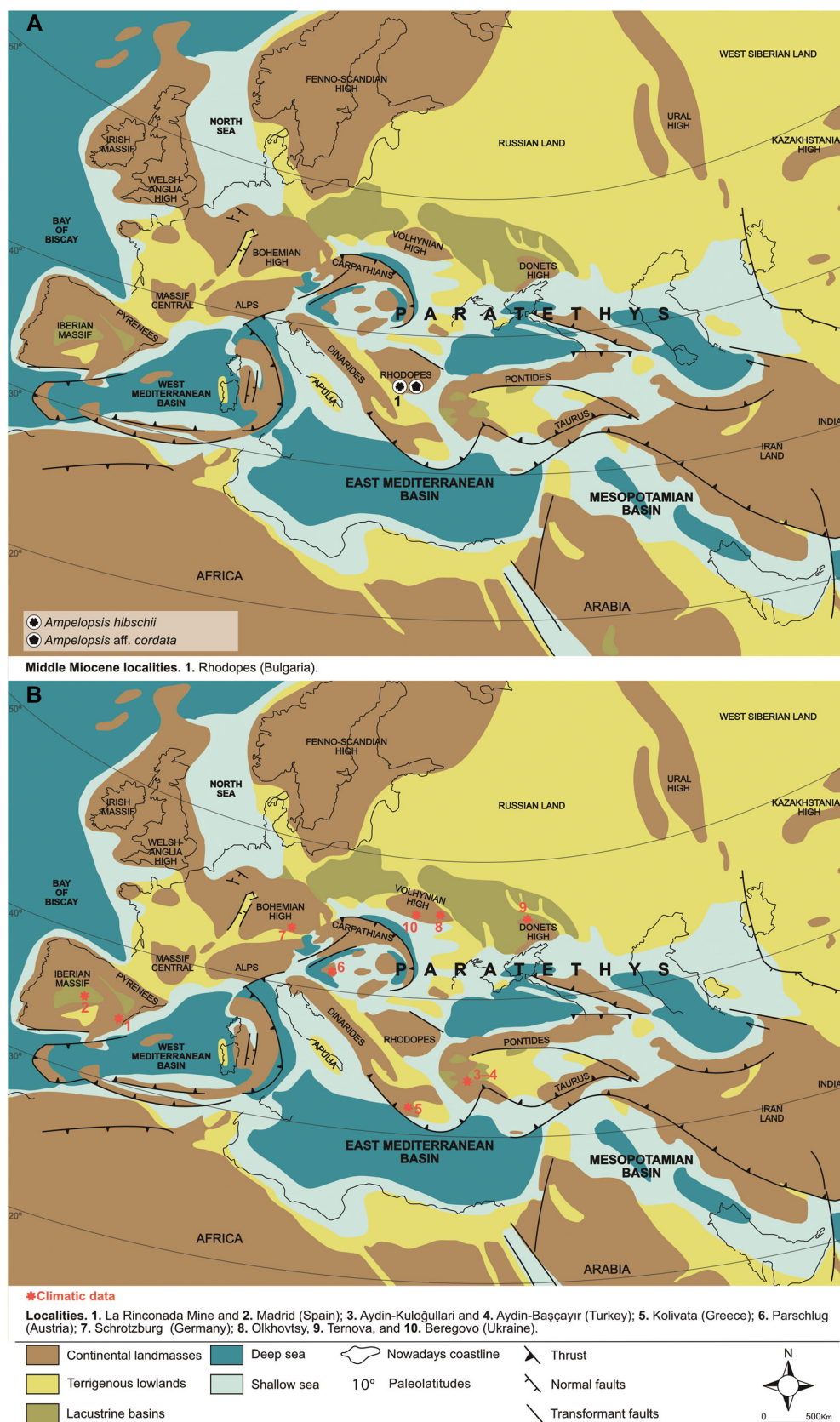


Fig. 10. Middle Miocene paleogeographic map of Western Eurasia. Modified from Popov et al. (2004). **A**, Distribution of *Ampelopsis* and *Nekemias* species. **B**, Localities with paleoclimatic data (Table S1).

versus winter-dry regions). The major vegetation type in Kučlín was broadleaved evergreen forest or its transition toward mixed mesophytic forest (Kvaček & Teodoridis, 2011). Range expansion during the early Oligocene was linked to changing climate conditions. Both in the Czech Republic and Kazakhstan, decreased precipitation seasonality has been inferred (ratio between the three wettest and driest months of 3.8; Table S1). This was accompanied by cooler temperatures. In contrast, in coeval localities in the Ebro Basin (Catalonia, Spain) where *N. mucronata* sp. nov. was recognized, the climate was much warmer but still with weak precipitation seasonality (fully humid climate assuming main rain period during the summer). To date, the youngest occurrence of *N. mucronata* sp. nov. in the late Oligocene in La Val is connected to a warm climate with significant precipitation seasonality (ratio of precipitation between the three wettest and three driest months of 7.4), supporting woodland-savannah vegeta-

tion, as previously inferred by Moreno-Domínguez et al. (2021). These gradients in climatic conditions were accompanied by distinct biome differences. Early Oligocene biomes in Kazakhstan might have included broadleaved deciduous forests (Averyanova et al., 2021), while mixed mesophytic forests and broadleaved evergreen forests prevailed in Central Europe. In South Europe, Oligocene vegetation was laurel oak forest (Greece; Velitzelos et al., 2014) and woodland and savannah (Spain; Tosal & Martín-Closas, 2016).

Most extant species of *Nekemias* grow under different temperature conditions to their fossil counterparts. For example, *Nekemias chaffanjonii* (Lév et Vaniot) J. Wen et Nie, *Nekemias gongshanensis* (Li) J. Wen et Nie or *N. megalophylla*, prefer climates with mild-warm MAT, ranging from 7.3 to 22.1°C, and freezing during the winter, with coldest mean month temperature (CMMT) ranging from −4.2 to 1.3°C (Fig. 11; Table S2). In contrast, the single North American

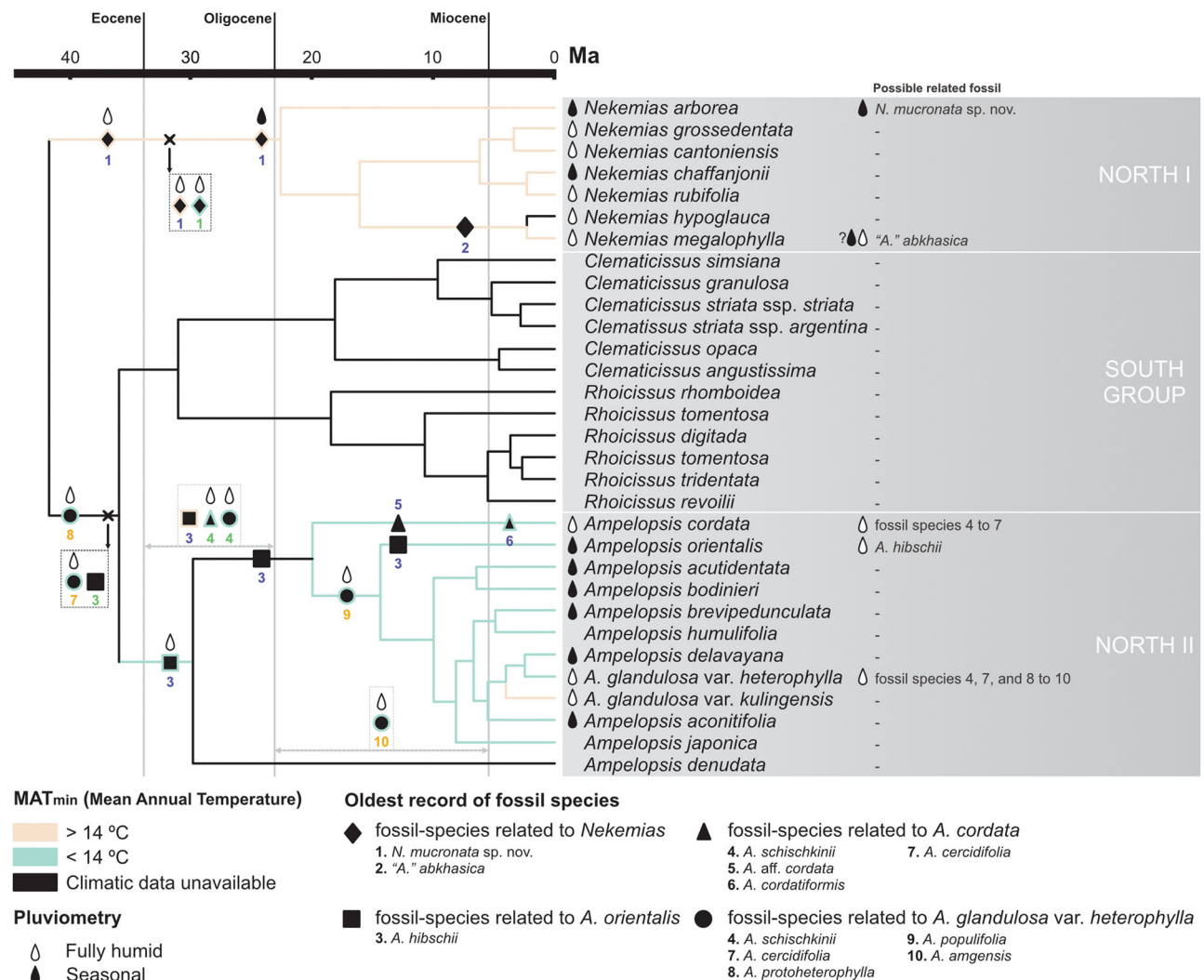


Fig. 11. A summary of phylogenetic relationships within Ampelopsidae adapted from Nie et al. (2012) and following the taxonomy of Lombardi (2015). It shows the records of the fossil leaf species from Eurasia recognized in the present study (Table 2) and the climatic requirements of the extant species.

species, *N. arborea*, sister to the remaining species in the genus (Nie et al., 2012) and considered here as the NLR of *N. mucronata* sp. nov. grows in relatively warmer temperatures from 15 to 25 °C MAT and mild or cold winters (CMMT), from 0 to 20 °C (Fig. 11; Table S2). These conditions fit well with the climatic conditions estimated for *N. mucronata* sp. nov. (Table S1).

As occurs with the temperature values, the precipitation conditions vary among extant species of *Nekemias*. For instance, one-third of the *Nekemias* species, that is, *N. arborea*, *N. chaffanjonii*, or *N. gongshanensis*, grow under seasonal or strongly seasonal precipitation conditions, while others grow under fully humid conditions, such as *Nekemias cantoniensis*, *Nekemias celebica* Suess., *Nekemias grossedentata* (Hand. Mazz.) J. Wen et Nie, *Nekemias hypoglaucula* (Hance) J. Wen et Nie, *N. megalophylla*, and *Nekemias rubifolia* (Wall.) J. Wen et Nie (Fig. 11; Table S2). The fossil evidence suggests that, initially, *N. mucronata* grew in climates with weak precipitation seasonality and later adapted to more varied climatic conditions (Fig. 11; Table S1). Hence, this fossil species appears to have been little affected by climate change. However, one possible reason for its extinction might be the increasing seasonality during the Oligocene in Spain.

4.3.2 Climatic and environmental niches in fossil and extant *Ampelopsis*

The paleoclimate data from the fossil localities with *Ampelopsis* along with climatic data from coeval plant localities from Western Eurasia (Tables S1, S2) indicates that the Western and Central Eurasian *Ampelopsis* fossil species thrived in climates from weak to moderate precipitation seasonality (ratio of precipitation between the three wettest and three driest months from 3.8 to 4.6) and in a range of climates, with CMMT close to freezing point or as high as ca. 5 °C (Fig. 11; Table S1). These values suggest that fossil species of *Ampelopsis* from this paleogeographic area grew in a wide spectrum of climates (Fig. 11).

As with the *Ampelopsis* fossil species (*A. hibschi*, *Ampelopsis amgensis*, *Ampelopsis cercidifolia*, *Ampelopsis populifolia*, *A. protoheterophylla*, and *A. schischkinii*), most of the living species, including *A. orientalis* and *Ampelopsis glandulosa*, probable NLR of the aforementioned fossil species, grow in climates with strong temperature seasonality (Fig. 11; Table S2). However, unlike these fossil species, which thrived in fully to moderately humid climates, their NLR are characterized by climates with strong precipitation seasonality (Fig. 11; Table S2). Notably, the modern NLRs are found in regions with summer-dry Mediterranean climates (*A. orientalis*) or in fully humid to winter-dry climates (*A. glandulosa*). In contrast, the modern climate of *A. cordata* matches the climate inferred for its European fossil (Neogene) counterparts, *A. aff. cordata* from Bulgaria, and *A. cordatiformis* from Germany. These species are characterized by a fully humid temperate climate (Fig. 11; Table S1).

In summary, combining the phylogenetic studies of Nie et al. (2012) with the fossil data presented here, it appears that, initially, the species of *Ampelopsis* genus would have thrived under non or weak seasonal climates, as evidenced by all fossil representatives *A. hibschi*, *A. schischkinii*, *A. aff. cordata*, *A. protoheterophylla*, *A. cercidifolia*, *A. populifolia*,

and *A. amgensis* (Fig. 11). This climatic signature is still observed in a few living species of the clade, including the NLR of *A. aff. cordata* (*A. cordata*) or *A. schischkinii* (*A. glandulosa*). In contrast, the morphological similarity of *A. hibschi* and the modern Western Eurasian *A. orientalis* points to niche evolution in the modern species in the course of the aridification of the present range of this species.

5 Conclusions

The new Ampelopsidae species, *Nekemias mucronata* sp. nov., presents a terminal leaflet with a straight base, toothed margin, and one tooth order. Teeth are mucronate. The primary vein is pinnate, the secondary veins are craspedodromous, and the tertiary framework is sinuous. The lateral leaflets differ from the apical leaflet for the asymmetric bitrilobate blade, cordate base (often asymmetric), the two basal secondary veins, and the presence of a mucro at the tooth tip. Comparison of *N. mucronata* sp. nov. with extant species of the tribe Ampelopsidae shows that *Nekemias arborea* is the most similar species and, hence, it is considered here at its NLR.

Nekemias mucronata sp. nov. resembles another fossil Vitaceae, that is, *Ampelopsis hibschi hibschi*; however, *N. mucronata* sp. nov. differs from *A. hibschi* because the latter displays a cordate leaf base with a reflex base shape, two orders of teeth, and a less conspicuous mucronate tooth apex. Additionally, the leaf apex in *A. hibschi* is characterized by a markedly constricted apical part. The comparison of *A. hibschi* with the extant species of Ampelopsidae agrees with previous studies that *A. hibschi* would be related to the modern *Ampelopsis orientalis*.

This study allows a better understanding of the paleobiogeographic distribution of Ampelopsidae. This tribe existed in East Asia since the middle Eocene with the occurrence of *Ampelopsis protoheterophylla* in the Far East (Russia, East Asia) and at least from the late Eocene in Western Eurasia (Europe and Central Asia) with the presence of *N. mucronata* sp. nov. in the Czech Republic and *A. hibschi* in Kazakhstan. By the Oligocene, *N. mucronata* sp. nov. spread southwards to Spain and Greece and eastwards to Central Asia (Kazakhstan), while *A. hibschi* thrived mostly through Central Europe but also in Greece. In addition, a new species appeared in Kazakhstan (Central Asia), that is, *Ampelopsis schischkinii*, and in the Far East (Russia, East Asia), that is, *Ampelopsis angulata*. At the end of the Oligocene, *N. mucronata* sp. nov. would have survived as a relict in the Iberian Peninsula. The distribution of *A. hibschi* is difficult to interpret since there is no record after the Oligocene until the Middle Miocene, when this species occurred in Bulgaria along with *Ampelopsis aff. cordata*. For that reason, the Balkans could have acted as a refuge for *A. hibschi*. However, the *Ampelopsis* lineage would persist in Central Europe at least until the Pliocene with the occurrence of *Ampelopsis cordatiformis* but the carpological record suggests that *Ampelopsis* lineage survived in Europe until the Pleistocene.

The distribution of Ampelopsidae leaf taxa was not determined by climate. *N. mucronata* sp. nov. thrived in climates with fully humid, weak, or marked precipitation seasonality and a relatively warm annual temperature. Its NLR, the American *N. arborea*, grows in a climate of warm and weak seasonal

precipitation, as is interpreted for the fossil species *N. mucronata* sp. nov. during the Oligocene in Spain. Maybe the increase in drought during the late Oligocene in this paleogeographic area caused the extinction of the last population of *N. mucronata* sp. nov. In contrast, *Ampelopsis* leaf fossil species occurred in fully humid or weak precipitation seasonality climates throughout the Cenozoic in Eurasia. The modern eastern Mediterranean *A. orientalis* shifted to a pronounced summer-dry Mediterranean climate after the Middle Miocene.

Finally, our data support previous notions of two possible dispersion routes of Ampelopsidae, including the North Atlantic or Bering land bridges. However, in contrast to previous time-calibrated molecular studies, the fossil record indicates that the intercontinental dispersal between North America and Eurasia occurred already during the Paleogene, rather than Neogene dispersal as previously suggested.

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Supplementary Material

The following supplementary material is available online for this article at <http://onlinelibrary.wiley.com/doi/10.1111/jse.13126/supinfo>:

Table S1. Climatic parameters of late Eocene to Middle Miocene paleobotanical sites from Western Eurasia. Localities with Ampelopsidae fossil record are marked in bold. [†]MAT, mean annual temperature; [‡]WMMT, warmest mean month temperature (°C); [§]CMMT, coldest mean month temperature (°C); [¶]GS, growing season (months); [#]MAP, mean annual precipitation (mm); [♥]GSP, growing season precipitation (mm); [♦]MMGSP, mean month growing season precipitation (mm); [‡]3Wet, three consecutive wettest months (mm); [‡]3Dry, three consecutive driest months (mm); ^{§§}RH, relative humidity (%). References: (1) Postigo-Mijarra et al. (2022); (2) Casas-Gallego et al. (2021); (3) Kayseri-Özer & Emre (2022); (4) Kayseri-Özer et al. (2014); (5) Kovar-Eder et al. (2022); (6) Uhl et al. (2006); (7) Syabryaj et al. (2007); (8) Tanrattana et al. (2020); (9) Moreno-Domínguez et al. (2021); (10) Kayseri-Özer et al. (2017); (11) Akgün et al. (2013); (12) Utescher et al. (2007); (13) Averyanova et al. (2021); (14) Bozukov et al. (2009); (15) Teodoridis & Kvaček (2015); (16) Erdei et al. (2012); (17) Roth-Nebelsick et al. (2004); (18) Mosbrugger et al. (2005); (19) Tosal et al. (2019b); (20) Kunzmann et al. (2019).

Table S2. Climatic parameters of extant *Nekemias* and *Ampelopsis* species. Data from Fang et al. (2011); GBIF; Rubel et al. (2017) (incl. a KMZ for Google Earth—high res: Global_1986-2010_KG_5m.kmz). [†]MAT, mean annual temperature (°C); [‡]WMMT, warmest mean month temperature (°C); [§]CMMT, coldest mean month temperature (°C); [¶]MAP, mean annual precipitation (mm); [#]P3 warmest, precipitation of the warmest quarter (the warmest three months); [♥]P3 coldest, precipitation of the coldest quarter. Species marked with an asterisk (*) grow under strong seasonality (either winter or summer dry). Species marked in bold, are the closest relatives of the fossil species.