A molecular phylogeny of the European nesticid spiders (Nesticidae, Araneae): Implications for their systematics and biogeography

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ABSTRACT

Nesticidae is a small family of spiders with a worldwide distribution that includes 15 genera and 272 described species. Seven genera and 56 species are known from Europe, distributed from the Iberian Peninsula to the Caucasus and the Ural Mountains. Most of these European species are cave dwellers and many of them are troglobites. In this study we present the first molecular phylogeny of the family Nesticidae in Europe with a wide geographical sampling across the continent. In our analysis the European nesticid fauna is well represented, including six genera and 40 of the 56 currently accepted species including the type species of all sampled genera. We have included in the analysis representatives of the North American and Asian fauna to test the monophyly of the European species and the phylogenetic relationships of European lineages. Phylogenetic relationships were reconstructed using maximum likelihood and Bayesian inference. As part of our Bayesian analyses, we also dated the phylogeny using two approaches, one based only on fossil calibrations and one that included an additional biogeographical constraint.

Our results show paraphyly of the European nesticids with respect to the Asian and North American taxa. We recover four main lineages within Europe. These four European lineages and all European genera have 100% bootstrap support and high posterior probability support in the BEAST2 analysis. The *Typhlonesticus* lineage is the earliest branching clade present in Europe and includes seven species, the five currently accepted species plus *T. parvus* from Bosnia and Herzegovina and *T. silvestrii* from western North America. The Eastern lineage includes the genus *Aituaria* and is the sister group of the Asian genera *Nesticella* and *Wraios*. The *Domitius* lineage is likely the sister group of the Central European lineage and spreads over the Iberian and Italian peninsulas. Finally, the Central European lineage includes three genera: *Kryptonesticus*, distributed from the karstic massifs of the Balkan Peninsula to Turkey, *Nesticus* with a single synanthropic species *N. cellulanus* and *Carpathonesticus*, exclusive to the Carpathian Mountains. With the exception of the genus *Typhlonesticus*, all European genera show an allopatric distribution (except for the two European synanthropic species). The results obtained in this study together with the revision of the original descriptions, redescriptions, and illustrations, lead us to propose 11 nomenclatural changes (new combinations) concerning the genera *Typhlonesticus*, *Nesticus* and *Carpathonesticus*.

1. Introduction

Nesticidae, also known as scaffold web spiders, is a small family of spiders distributed worldwide that live in dark and damp places, particularly in caves, where they build irregular webs in crevices along cave walls (Fig. 1). On rare occasions they are found wandering on cave walls looking for food or to mate. Outside caves, nesticids inhabit moist habitats on rock walls, rock shelters on shady slopes or in the leaf litter of wooded areas where they build their webs. The family includes 15

genera and 272 accepted species (World Spider Catalog, 2022).

Seven genera and 56 species are known from Europe (World Spider Catalog, 2022). Most of these European species are cave-dwellers and many are cave-obligate (troglobitic) species. Given their habitat preferences, most species show restricted distributions, mainly in the genera *Typhlonesticus* Kulczyński 1914, *Domitius* Ribera 2018 and *Kryptonesticus* Pavlek & Ribera 2017. Two invasive species are also present in Europe: *Eidmannella pallida* (Emerton, 1875) which is widespread in the Canary Islands and Mallorca, as well as in caves across the southern and eastern

Iberian Peninsula and Italy, and *Nesticella mogera* (Yaginuma, 1972) broadly distributed throughout the Asian continent, recorded in Azerbaijan and identified in some European countries as an introduced species (Hungary, Germany and Poland) (Kielhorn, 2009; Pfliegler, 2014; Rozwałka et al., 2013).

European Nesticids have a long taxonomic history, from the descriptions of *Nesticus cellulanus* (Clerck, 1757) and the family Nesticidae Simon, 1894 to the present day many authors have paid attention to the European representatives of this family. Most of these contributions were focused on the description of new species and provide valuable information about their geographic distribution. Biogeographic patterns and phylogenetic relationships of the family have received much less attention. Recently, several works have studied the origin, evolutionary relationships, and biogeography of European taxa (Pavlek and Ribera, 2017; Ribera, 2018; Ribera et al., 2014). These contributions focused on the genera *Typhlonesticus*, *Kryptonesticus*, and *Domitius*, respectively (for more information on taxonomy see Supplementary Materials) and included molecular phylogenies with limited taxonomic scope based on which they hypothesized that these genera have been present in Southern Europe and the Middle East since well before the Pleistocene glaciations. This hypothesis highlights the importance of Pleistocene glaciations for the evolution of Mediterranean nesticids as many species have become extinct outside caves due to changing climatic conditions (particularly decreasing in humidity) (Ribera et al., 2014), a pattern like the one observed in other Holarctic spiders such as Pimoidae (Hormiga et al., 2021; Mammola et al., 2016). However, unlike Pimoidae, nesticids have several epigean species in Europe, most of which are currently classified in the widely distributed genus *Nesticus*, suggesting potential dispersals from/to Europe and/or evolution of adaptations to drier environments.

Despite the large number of studies, a phylogenetic analysis including a broad sampling of European nesticids is still missing, which makes it impossible to test published hypotheses about their relationships based on morphological similarities and distributional patterns. In addition, recent studies have shown that some European (and perhaps also non-European) *Nesticus* species may need to be transferred to other genera (Pavlek and Ribera, 2017; Ribera et al., 2014) which would potentially have an impact on the interpretation of the biogeographical and evolutionary patterns of the family.

In this paper we use molecular data from five molecular markers to infer the phylogenetic relationships of European nesticids. In our dataset the European nesticid fauna is well represented: 40 of the 56 described species and six genera including all corresponding type species were sampled. We included a small subset of the Asian and American nesticid faunas (nine Asian and three North American species) to perform preliminary tests of the monophyly of the European lineages, although it would be necessary to include more Asian and American species to address the taxonomic status of non-European species currently placed in *Nesticus*. The genus *Daginesticus* from Georgia and the Russian Caucasus is not included in the analysis due to its recent description (Fomichev et al., 2022). Our intention is to provide a solid base for the revision of the current taxonomy of the group that allows us to delimit the genera of European nesticids and formulate hypotheses about their origin, their phylogenetic relationships, and their historical biogeography. We use fossils to date the phylogeny in order to infer the time of past diversification events and to test the hypothesis that European nesticids are old and have diversified well before the Pleistocene.

2. Material and methods

2.1. Taxon sampling and species-level identification

We sampled a large number of localities throughout Southern Europe, the Carpathian Mountains, Turkey and the Mediterranean islands in order to collect the highest possible number of European nesticid species. Samples from additional localities were provided by
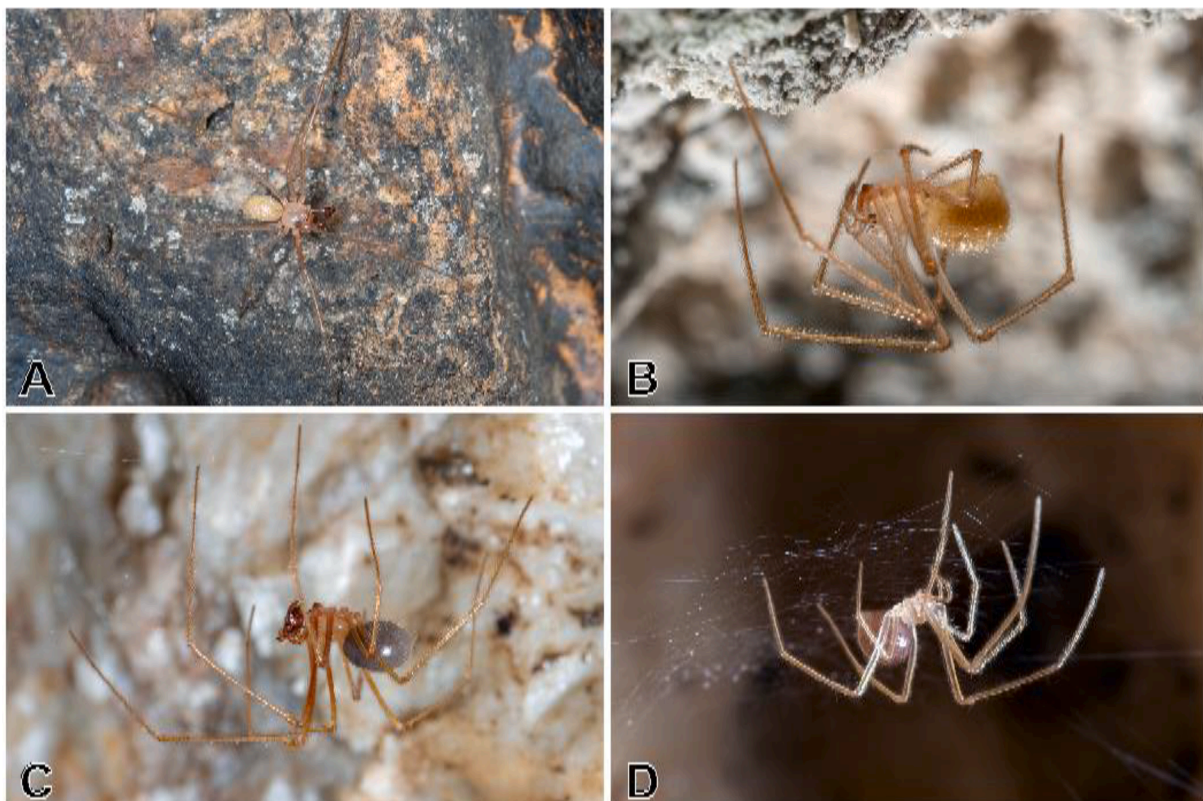

Fig. 1. Nesticids habitus and typical habitats. A = *Kryptonesticus fagei*; B = *Kryptonesticus deelemanae*; C = *Typhlonesticus absoloni*; D = *Typhlonesticus parvus*. Photographers: Martina Pavlek and Marko Lukić. Photos were processed by Tin Rožman.

colleagues (Fig. 2). Species level identifications were carried out after reviewing the original descriptions, redescriptions and illustrations by different authors that provide excellent information about the currently accepted nesticid species. Representatives of all European nesticid genera were included in the analyses including their type species. In all, 40 accepted species belonging to all European genera were obtained: *Aituaria* (3), *Carpathonesticus* (9), *Domitius* (7), *Kryptonesticus* (6), *Nesticus* (10) and *Typhlonesticus* (5). Each species is represented by a single terminal except *N. cellulanus* and *K. eremita* (Simon, 1880) for which we included two in order to check the genetic divergences between distant populations, because they are the only European synanthropic species and have a wide geographic distribution.

In order to assess the monophyly of the European nesticids we included in the analysis representatives of the North American fauna that show morphological similarities with European taxa: *Nesticus carteri* Emerton, 1875 *N. holsingeri* Gertsch, 1984 and *N. silvestrii*, Fage, 1929. In addition, several representatives of the Asian fauna were also included: *Nesticella apiculata* Liu & Li, 2013; *N. brevipes* Yaginuma, 1970; *N. falcata* Liu & Li, 2013; *N. gracilenta* Liu & Li, 2013; *Wraios longiembolus* Ballarin & Li, 2015; *Speleoticus navicellatus* (Liu & Li, 2013); *S. uenoi* (Yaginuma, 1972); *Cyclocarcina floronoides* Komatsu, 1942 and *Nesticus kyongkeomsanensis* Namkung, 2002.

Traditionally, Nesticidae were considered as a sister group to Theridiidae, however recent phylogenies place them as a sister group to other families, e.g.: Linyphiidae (Bond et al., 2014), Synotaxidae (Kallal et al., 2021) or Araneidae (Ramírez et al., 2021). Therefore, we have included a broad sample of araneoid families as outgroups. We have further expanded the outgroup taxon sampling to be able to use two fossils as calibration constraints in the molecular dating analyses: Pimoidae (Poinar and Buckley, 2012) and Nephilinae (Kuntner et al., 2019). As a result in our final dataset, representatives of the families Tetragnathidae – *Tetragnatha versicolor* Walckenaer, 1941; Araneidae – *Argiope bruennichi* (Scopoli, 1772), *Micrathena gracilis* (Walchenaer, 1805), *Trichonephila clavipes* (Linnaeus, 1767), *Trichonephila clavata* (L. Koch, 1878), *Nephilingis cruentata* (Fabricius, 1775), *Nephila pilipes* (Fabricius, 1793); Theridiidae – *Theridion nigroannulatum* Keyserling, 1884; Linyphiidae – *Linyphia triangularis* (Clerck, 1757), *Neriene radiata* (Walckenaer, 1841), *Stemonyphantes lineatus* (Linnaeus, 1758); Pimoidae – *Nanoa enana* Hormiga, Buckle & Scharff, 2005, *Pimoa trifurcata* Xu & Li, 2007 and Oxyopidae – *Peucetia viridans* (Hentz, 1832) were used as outgroups.

2.2. DNA extraction, PCR amplification and sequencing

Specimens were collected in the field, directly preserved in 96% or absolute ethanol and stored at 4 °C. Total genomic DNA was extracted from legs or thorax tissue using the QIamp® DNA Mini Kit and DNeasy Blood &Tissue Kit (QIAGEN) following the manufacturer's protocol. The approximate concentration and purity of the DNA obtained were verified using 1% agarose/TBE gel electrophoresis and a BioSpec-nano Spectrophotometer. Partial fragments of five genes, two mitochondrial – cytochrome *c* oxidase subunit I (*cox1*) and 16S rRNA (*16S*), and three nuclear – 18S rRNA (*18S*), 28S rRNA (*28S*) and Histone H3 (*H3*) were amplified and sequenced using the primers listed in the supplementary materials. PCR reactions were conducted at a final volume of 25 µL using either Taq polymerase (Promega) or Biotools Pfu DNA Polymerase (Biotools). PCR products were cycle-sequenced in both directions using the same PCR primers and the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) following (Planas et al., 2013; Planas and Ribera, 2014). The sequencing was performed on an ABI 3700 automated sequencer at the Centres Científics i Tecnològics of the University of Barcelona (CCiTUB, https://www.ccit.ub.edu) or at the Macrogen sequencing center in the Netherlands. Chromatograms were edited and assembled with Geneious v. 10.0.9 (https://geneious.com). All newly generated sequences were deposited in GenBank (see Table 1 for accession numbers).

2.3. Alignment and evolutionary model selection.

The alignment of *cox1* and *H3* was trivial, given that the amplified fragments showed no insertions/deletions and hence the sequences were adjusted manually. We translated sequences into amino acids and checked for stop-codons to avoid sequencing/editing errors.

For the ribosomal gene fragments, we performed alignments using the online version of Mafft (Katoh and Standley, 2013) using the Q-INS-i algorithm (Katoh and Toh, 2008), which considers the secondary structure of RNA, with default settings. Ribosomal gene alignments did not contain large ambiguous aligned regions in our dataset and we used the entire alignments in following analyses. The best partitioning scheme and corresponding substitution models were selected with the program PartitionFinder v.2.1.1 (Lanfear et al., 2017).

2.4. Phylogenetic analyses and molecular dating

We partitioned data by codon position (in the case of the two protein coding genes) and explored the best partitioning scheme and substitution models simultaneously using the greedy algorithm. Bayesian information criterion was used to select the best partition scheme and the corresponding best fit models. Maximum likelihood (ML) phylogenetic analyses were carried out in the computer program RAxML-NG v1.0.0 (Kozlov et al., 2019) using the partition scheme and models of molecular evolution selected by PartitionFinder. Nodal support was evaluated using 1000 nonparametric bootstrap replicates (–bs-trees 1000). In addition, joint tree inference and molecular dating using Bayesian approach was carried out in the program BEAST v2.5.1 (Bouckaert et al.,
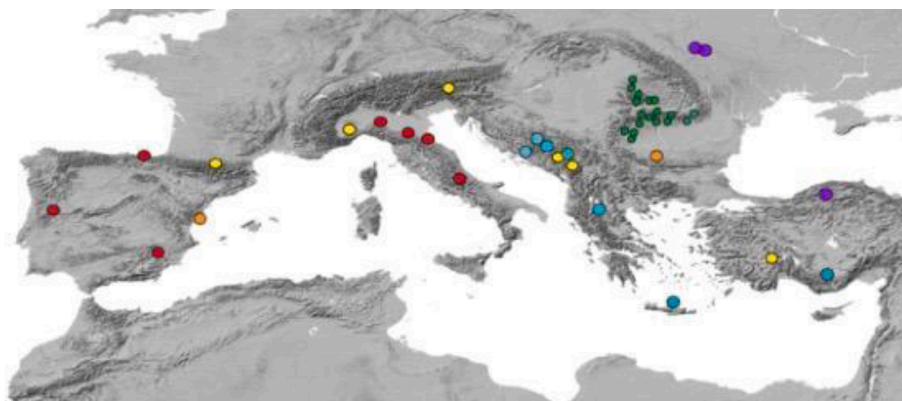

Fig. 2. Sampling localities of the European nesticids genera used in this study. Red – *Domitius*, Yellow – *Typhlonesticus*, Blue – *Kryptonesticus*, Orange – *Nesticus cellulanus*, Green – *Carpathonesticus*, Purple – *Aituaria*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
List of taxa included in this study with the corresponding accession numbers of sequences used in the molecular analyses. Location data is provided for all nesticid terminals.

Family	Species	Code	Locality	COI	16S	18S	28S	H3
Nesticidae	*Aituaria borutzkyi*	1007	Kizilema Magarasi, Zonguldak, Turkey,	OP179635	OP162536	OP162634	OP162676	OP205500
Nesticidae	*Aituaria eriashvilii*	1837	Chernivtsi City, Ukraine. in a cellar.	OP179636	OP162537	OP162635	OP162677	NO
Nesticidae	*Aituaria pontica*	1836	Chernivtsi City, Ukraine. in a cellar.	OP179634	OP162535	NO	OP162678	OP205501
Nesticidae	*Carpathonesticus balacescui*	1411	Unnamed Cave in Cheile Tatarului Gorges, Bucegi Mts., Romania	OP179644	OP162538	OP162636	OP162679	OP205517
Nesticidae	*Carpathonesticus biroi*	1048	Ursilor Cave, Chiscau, Romania	OP179637	NO	OP162637	OP162680	OP205502
Nesticidae	*Carpathonesticus carpaticus*	1659	Polatistea Valley, Parang Mountains, Romania.	OP179645	OP162539	OP162638	OP162681	OP205510
Nesticidae	*Carpathonesticus cernensis*	1424	Pestera Hotilor, Baile Herculane, Romania. 44°53′51.95″N 22°25′45.67″E	OP179649	OP162540	OP162639	OP162682	OP205511
Nesticidae	*Carpathonesticus constantinescui*	1405	Pestera Doranca, Piatra Crailui Mts, Zarnesti, Romania. 45°33′49.18″N 25°20′8.52″E	OP179650	NO	OP162640	OP162683	OP205512
Nesticidae	*Carpathonesticus diaconui*	1417	Pestera Muierii, Baia de Fier, Serbea Massif, Capatina Mts., Romania. 45° 1′31.96″N 22°39′55.14″E	OP179648	OP162541	OP162641	OP162684	OP205513
Nesticidae	*Carpathonesticus fodinarum*	1051	Pesterea Poarte lui Joarnele, Giarda, Alba, Garda, Romania.	KX632162	KX632158	OP162642	OP162685	MF693108
Nesticidae	*Carpathonesticus hungaricus*	1448	Pestera Cave. Liliecilor, Cheile Ampoitei Gorges, Romania. 46°08′21.7748″N 23°23′39.8507E	KF417412	KF417402	OP162643	OP162686	KF417419
Nesticidae	*Carpathonesticus ionescui*	1421	Pestera Tismana, Tismana. Cv. inside the Monastir. Romania, 45° 4′50.24″N 22°55′40.32″E	OP179652	OP162542	OP162644	OP162687	EU746443
Nesticidae	*Carpathonesticus lotriensis*	1454	Unnamed Cave in Lotrioara Valley, Lotrului Mountains, Sibiu, Romania. 45°34′45.8319″N 24°11′16.7493″E	KF417413	KF417399	OP162645	OP162688	KF417418
Nesticidae	*Carpathonesticus orghidani*	1418	Pestera Polovragi, Oltet Gorges, Capatina Mts., Romania. 45°11′40.88″N 23°47′27.56″E	OP179653	OP162543	OP162646	OP162689	OP205514
Nesticidae	*Carpathonesticus plesai*	1666	Huda de la Chiscau, Bihor, Romania.	OP179654	OP162544	OP162647	OP162690	OP205515
Nesticidae	*Carpathonesticus puteoum*	1401	Pestera Bolii, Cheile Banitei, Merisor, Pui, Huneadora, Romania. 45°27′16.50″N 23°18′57.58″E	OP179639	OP162545	OP162648	OP162691	OP205503
Nesticidae	*Carpathonesticus racovitzai*	1403	Pestera La Corovan, Scarissoara, Romania.	OP179640	NO	OP162649	OP162692	OP205516
Nesticidae	*Carpathonesticus simoni*	1413	Unnamed Cave in Bisbrita Gorges, Stogu-Vinturarita Mts., Romania. 45°11′42.2789″N 024°02′03.2702′′ E	KF417408	KF417398	OP162650	OP162693	KF417417
Nesticidae	*Carpathonesticus spelaus*	1663	Pestera Lucia Mare, Sohodol, Campeni, Romania.	OP179641	OP162546	OP162651	OP162694	OP205506
Nesticidae	*Carpathonesticus wiehlei*	1660	Pestera de Sub Pod, Rosia - Petrila Valley, Parang Mountains, Romania.	OP179656	OP162547	OP162652	OP162695	OP205518
Nesticidae	*Carpathonesticus* sp.	1464	Voineasa, Vâlcea, Oltenia, Romania. 45°25′52.45″N 23°50′59.08″E	OP179642	OP162548	OP162653	OP162696	OP205504
Nesticidae	*Domitius sbordonii*	2702	Tana degli orchetti, near Supino, Lazio. Italy	MF693110	MF693116	OP162654	OP162697	MF693102
Nesticidae	*Domitius menozzii*	1725	Grotta do Pertuzo do Canté, Apparizione, Genova, Italia	MF693111	MF693117	OP162655	OP162698	MF693103
Nesticidae	*Domitius luquei*	3801	Cueva de la Picona, San Pedro de Carmona, Cabuérniga, Cantabria. Spain	MF693112	EU746439	OP162656	OP162699	MF693104
Nesticidae	*Domitius lusitanicus*	1612	Algar de Marradinhas II, Concelho de Alcanena, Portugal.	MF693113	EU746429	OP162657	OP162700	MF693105
Nesticidae	*Domitius baeticus*	1670	Cueva del Castillo. Siles. Jaén. Spain	MF693114	MF693118	OP162658	OP162701	MF693106
Nesticidae	*Domitius culsu*	D555	Tana delle Fate di Coreglia Antelminelli, Coreglia Antelminelli, Toscana, Italy	MK860152	MK860134	NO	NO	MK860143
Nesticidae	*Domitius speluncarum*	D557	Tana di Magnano, Canigiano, Lucca, Toscana, Italy	MK860153	MK860135	NO	NO	MK860144
Nesticidae	*Kryptonesticus arenstorffi*	1356	Čora pećina, Crni nugli, Dragaljsko polje, Gornje Krivošije, Selakov do, Risan Distr., Montenegro 42°35′36,5′′ N 18°41′41.6′′ E	KF417407	KF417403	OP162659	OP162702	KF417422
Nesticidae	*Kryptonesticus deelemanae*	AR3936	Samogorska špilja, Biokovo Mt., Croatia 43°19′5,71′′ N, 17°7′29,9′′ E	KX632167	KX632160	NO	NO	KX611237
Nesticidae	*Kryptonesticus dimensis*	2595	Dim Cave. Taurus Mountains, Alanya District. Turkey, 36°32′3.79′′ N 32°07′0.20′′ E	KF417406	KF417401	OP162660	OP162703	KF417420
Nesticidae	*Kryptonesticus eremita*	1493	Pišurka, town of Korčula, Korčula Isl., Croatia 42°57′33.83′ N 17°7′45.26′′ E	KX632165	EU746445	OP162661	OP162704	OP205508
Nesticidae	*Kryptonesticus eremita*	1111	Shpella e Dragoit, Gjirokastër, Albania,	OP179643	NO	OP162662	OP162705	OP205507
Nesticidae	*Kryptonesticus fagei*	AR1510		KX632166	KX632159	OP162663	OP162706	KX611236

Table 1 (*continued*)

Family	Species	Code	Locality	COI	16S	18S	28S	H3
			Bjelušica, Popovo polje, Bosnia and Herzegovina 42°50′41′′ N 17°58′43′′ E					
Nesticidae	*Kryptonesticus henderickxi*	1554	Kournas Cave, Kournas, Crete	KX632164	KF417404	OP162664	OP162707	OP205509
Nesticidae	*Nesticus cellulanus*	1022	Manantiales Monte Castro, Sueces, Castelló, Spain.	KX632163	EU746444	OP162665	OP162708	KX611235
Nesticidae	*Nesticus cellulanus*	1023	Bg. West.Rhodope Mt Cave, Escopria, Mostovo, Bulgary	OP179647	OP162549	NO	NO	OP205519
Nesticidae	*Typhlonesticus absoloni*	1355	Baba Tusha Cv., Trnovo, Virpazar Distr., Montenegro, 42°17′25.1′′ N 19°02′10.8′′ E	KF417410	KF417397	OP162666	OP162709	KF417416
Nesticidae	*Typhlonesticus gocmeni*	2598	Keloğlan Cave. Dodurgalar Town, Acıpayam District. Denizli Province. Turkey, 37°23′14.74′′N 29°34′18.29′′E	KF939310	KF939307	OP162667	OP162710	KF939313
Nesticidae	*Typhlonesticus idriacus*	1677	Grotte Pre Oreak, Nimis, Friuli. Italy	KF939312	OP162550	OP162668	OP162711	OP205522
Nesticidae	*Typhlonesticus morisii*	1676	Sotterranei del Forte di Vernante, Vernante, Cuneo, Italy	KF939311	KF939308	OP162669	OP162712	OP205523
Nesticidae	*Typhlonesticus obcaecatus*	1035	Cueva del Molino de Aso, Boltaña, Prov. Huesca, Spain.	KF939309	EU746437	OP162670	OP162713	MF693109
Nesticidae	*Typhlonesticus parvus*	1832	Pavlova Pecina, Bihovo, Trvinje, Bosnia Herzegovina.	OP179638	OP162551	OP162671	OP162714	OP205505
Nesticidae	*Typhlonesticus silvestrii*	1864	Nacimiento_Fergusson Road, Monterrrey, California USA. 35°59′43.80′′ N 121°29′6.72′′W	OP179655	OP162552	OP162672	OP162715	OP205504
Nesticidae	*Cyclocarcina floronoides*	Z183	Tatoro cave, Tatoro mountain, Kanna Town, Tano District, Gunma Prefecture, Japan.	MG201049	MG200520	MG200695	MG200869	MG201226
Nesticidae	*Nesticus kyongkeomsanensis*	LEGO_12_2	Kyongkeomsangul-cave, Gwangha-ri, Jeongseon- eup, Jeongseon-gun, Gangwon-do, South Korea	JN817084	JN816451	NO	JN816884	NO
Nesticidae	*Nesticus carteri*	1862	Lick Creek, Country Park, Pike Co., Kentuky. USA. 37°23′58.56′′N 82°18′20.52′′W	OP179645	NO	OP162673	OP162716	OP205521
Nesticidae	*Nesticus holsingeri*	1861	Blount Co., Catfish Cave, Alabama, USA.	OP179651	NO	OP162674	OP162717	NO
Nesticidae	*Nesticella apiculata*	83	Xuehua Cave, Fuxi Mt., Xinzhong Town, Gongyi County, Zhengzhou City, Henan Province, China	MG200895	MG200365	MG200541	MG200718	MG201072
Nesticidae	*Nesticella huomachongensis*	HU	Yanzi Cave, Huomachong Town, Chenxi Co., Huaihua City, Hunan Province, China	KF359043	KF359043	KF359244	KF359345	KF359136
Nesticidae	*Nesticella falcata*	288	Biyun Cave, Panxian County, Guizhou Province, China	MG200948	MG200419.	MG200594	MG200838	MG201125
Nesticidae	*Nesticella gracilenta*	294	Jinqian Cave, Huachu Town, Puding County, Guizhou Province, China.	MG200968	MG200447.	MG200614.	MG200831	MG201145
Nesticidae	*Wraios longiembolus*	Z035	Xishuangbanna Nature Reserve, Guanping Township, Jinghong County, Yunnan Province, China.	MG201038	MG200509	NO	MG200862	MG201215
Nesticidae	*Speleoticus navicellatus*	Z272	Qiaoxu cave, Qiaoxu Village, Qibainong Township, Dahua County, Hechi Prefeture, Guangxi Province, China,	MG201046	MG200515	MG200692	MG200870	MG201223
Nesticidae	*Speleoticus uenoi*	Z186	Hitoana cave, Hitoana sengen Shrine, Hitoana, Fujinomiya City, Shizuoka Prefecture, Japan	MG201048.	MG200516	MG200694	MG200871	MG201225
Tetragnathidae	*Tetragnatha versicolor*			FJ525317	FJ525350	FJ525387	FJ525369	FJ525336
Linyphiidae	*Linyphia triangularis*			AY078693	AY078664	AY078668	AY078682	AY078702
Linyphiidae	*Neriene radiata*			GU338672	GU338623	GU338463.	GU338574	AY078709
Linyphiidae	*Stemoniphantes lineatus*			FJ838667	FJ838691	FJ838715	FJ838738	FJ838761
Pimoidae	*Nanoa enana*			JN010203	NO	JN010184	JN010189	NO
Pimoidae	*Pimoa trifurcata*			JN010205	JN010168	JN010186	JN010187	NO
Oxyopidae	*Peucetia viridans*			FJ607580	FJ607467	FJ607506	FJ607541	FJ607618
Araneidae	*Argiope bruenichi*			KC193952	KC849106	JN018309	JN018406	KC849021
Araneidae	*Micrathena gracilis*			KJ157252	KJ157084	FJ525396	FJ525377	FJ525343
Araneidae	*Trichonephila clavipes*			KC849081	KC849124	JN010173	EU003422	KC849040
Araneidae	*Trichonephila clavata*			KC849082	KC849125	AB917959	KC848999	KC849041
Araneidae	*Nephilingis cruentata*			KC849096	KC849137	NO	KC849011	KC849052
Araneidae	*Nephila pilipes*			KC849088	KC849130	KC848963	JN018407	KC849045
Theridiidae	*Theridion nigroannulatum*			EF050324	EF050178	EF458147	MK957477	EF050369

2014). The same partition and models were used with linked clock models for the mitochondrial partitions and all loci were linked to a single tree model. Analyses were run under an uncorrelated relaxed clock model with lognormal distribution using a birth–death tree prior (Gernhard, 2008). To calibrate the tree, we used a combination of two fossils and one biogeographical dating constraint. We used the estimated age of pimoids from the Baltic amber (Wunderlich, 2004) as a stem constraint for Pimoidae. This prior was set as a gamma distribution prior with an offset of 43 Ma and alpha 1.0478 and beta 2.0 (which results in a 97.5% quantile of probability density between 43 and 50.6 Ma). We

used the fossil *Geratonephila burmanica* Poinar and Buckley described from Burmese amber (Poinar and Buckley, 2012) to constrain the stem age of Nephilinae following Kuntner et al. (2019). We set this prior as a gamma distribution prior with an offset of 97 Ma, and alpha 1.05 and beta 2.0 (97.5% quantile of probability density between 97 and 105 Ma). Finally, we also ran a dating analysis including one additional biogeographical constraint based on the time of separation between the island of Crete and Turkey. Aegean islands were connected to continental Greece and Turkey forming a continuous landmass known as Ägäis. Its breakup 9–12 Ma resulted in the formation of the Aegean Islands (Lymberakis and Poulakakis, 2010). The age of the node corresponding to the split of the species *Kryptonesticus dimensis* and *K. henderickxi* was set to a minimum age of 12 Ma based on their present distributions - Turkey and Crete respectively. The split between Crete and Turkey has been used to date the divergence of *Sulcia* species (Leptonetidae) with analogous distribution (Ledford et al., 2021). We applied this constraint

as a uniform prior with min bound of 12 Ma and max bound of 120 Ma that corresponds to recent estimates of the minimum age of Araneoidea (Kallal et al., 2021). Molecular dating analyses in BEAST2 were run for 400 million generations (for the dating without biogeographic constraints) and 280 million (generations for the dating implementing the biogeographic constraint). For each combination of constraints two independent runs were carried out. We used the program Tracer v1.7.1 (Rambaut et al., 2018) to evaluate run statistics and convergence. Beast output files we process with the programs LogCombiner and TreeAnnotator distributed together with BEAST 2.

3. Results

3.1. Data

Specimens, localities and GenBank accession numbers of the
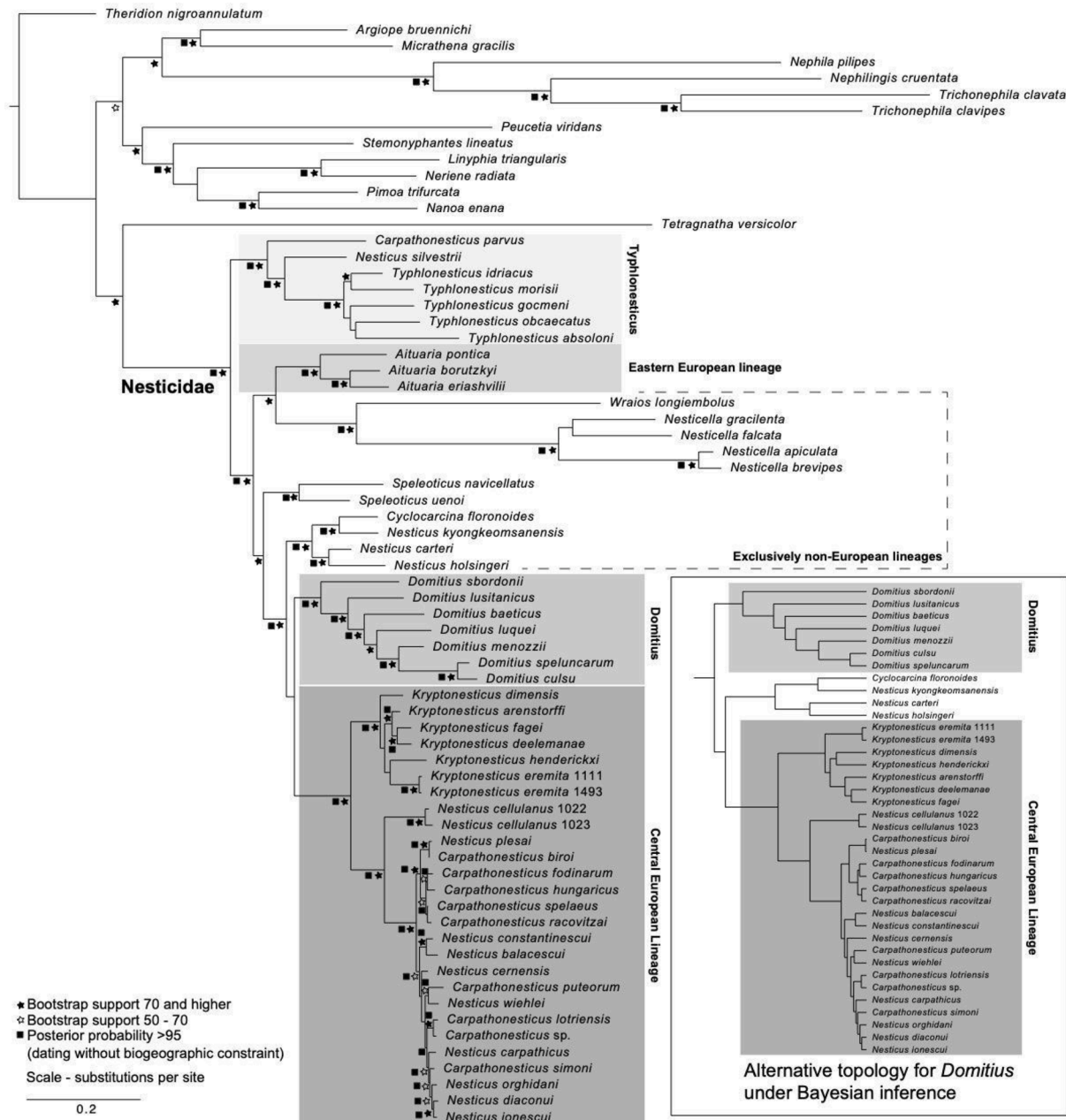

Fig. 3. Tree from the maximum likelihood analyses summarizing supports from maximum likelihood (bootstrap) and Bayesian analyses (posterior probability), and alternative topology from Bayesian analysis.

sequences used in this study are listed in Table 1. The final concatenated matrix included 69 terminals (55 nesticids and 14 outgroups) and 3430 aligned characters (cox1 = 930, 16S = 489, H3 = 317, 18S = 820 and 28S = 874). The best partitioning scheme obtained with PartitionFinder includes nine partitions: the first, the second and the third position of *cox1*, the first, the second and the third position of *H3* and *16S*; *18S* and *28S*. The following were the best fit models of molecular evolution selected by PartitionFinder under the AICc criterion: GTR + I + G for *cox1* 1st and 2nd position, *16S*, *18S* and *28S*; GTR + G for *cox1* 3rd position and *H3* 1st position; TVMEF + I for *H3* 2nd position; SYM + G for *H3* 3rd position.

3.2. Maximum likelihood analyses

Figure 3 shows the ML tree obtained with RAxML-NG analyzing the concatenated matrix under the best partition scheme and best fit models selected with PartitionFinder. We recovered four main lineages within the European nesticids which we refer to as the *Typhlonesticus* lineage, the Eastern European lineage (represented by *Aituaria* in our dataset but putatively also including *Daginesticus*), the *Domitius* lineage and the Central European lineage (*Kryptonesticus*, *Nesticus* and *Carpathonesticus*). These results show paraphyly of the European nesticids with respect to the Asian and North American fauna. The four European lineages as well as all European genera (after transfer of several misplaced species) have 100% bootstrap support and high posterior probability support in BEAST2 results. *Typhlonesticus* is the earliest branching European nesticid lineage and includes seven species (six of them European), the five currently accepted species plus *T. parvus* from Bosnia and Herzegovina and *T. silvestrii* from western North America. The Eastern European lineage groups three species included in our dataset (*Aituaria pontica*,
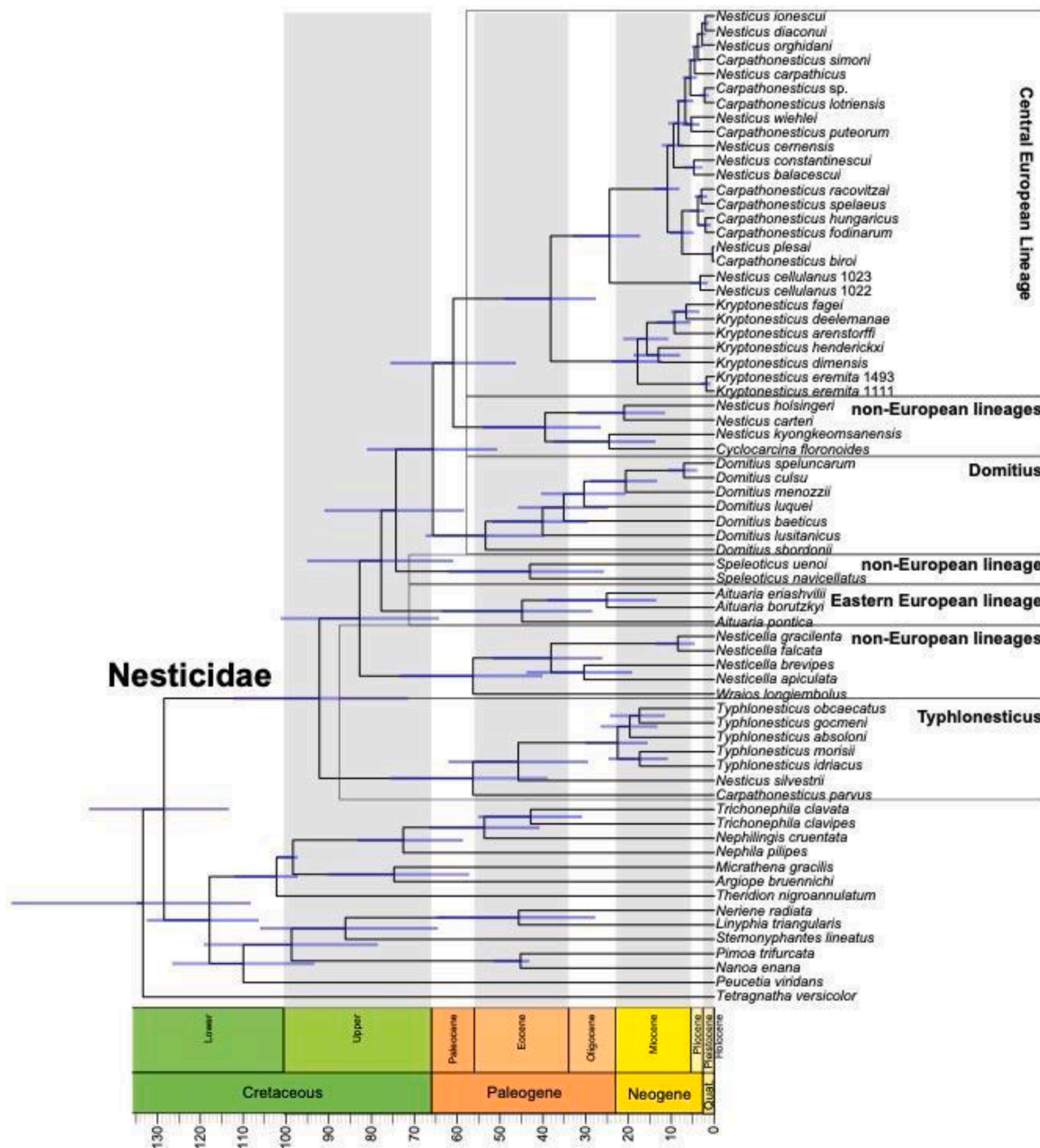

Fig. 4. Dated tree of European nesticids based on the dating strategy without biographical constraint.

A. eriashvilii, A. borutzkyi) and is the sister group of the Asian genera *Nesticella* and *Wraios*. Although the genus *Daginesticus* was not included in the analysis due to its recent description, based on its morphological features we hypothesize that it likely belongs to the Eastern European lineage as sister to *Aituaria*. The genus *Domitius* is the sister group of the Central European Lineage, although this placement received a low bootstrap support (49%). Finally, the Central European Lineage includes three genera: *Kryptonesticus*, distributed throughout the karstic reliefs of southern central Europe and Turkey, *Nesticus* with a single synanthropic species *N. cellulanus* appear as the sister group to *Carpathonesticus*, and the *Carpathonesticus*, exclusive to the Carpathian Mountains.

The ML analysis (Fig. 3) and Bayesian reconstruction in BEAST2 (Fig. 4) resulted in identical topologies except for the position of the *Domitius* lineage. The ML analysis place *Domitius* as sister group of the Central European Lineage albeit with 49% bootstrap support, while in the BEAST2 results two species from Korea and Japan (*N. kyongkeomsanensis* and *C. floronoides*) and two from the USA (*N. carteri* and *N. holsingeri*) constitute the sister group of the Central European Lineage. However, this alternative topology did not receive a high support either (posterior probability < 95).

3.3. Molecular dating

Independent runs for each set of constraints showed convergence and results were therefore combined using LogCombiner and discarding 72% of the samples as burn-in for the dating without biogeographical constraint and 71% for the dating with biogeographical constraint. For both dating schemes, the combined post burn-in samples were summarized with TreeAnnotator on the highest clade credibility tree using mean node heights. Despite the high number of generations, after discarding pre-stationary posterior samples as burn-in, some parameters such as rates of change from C to G for some of the data partitions did not reach ESS above 200 and this did not improve with increased number of generations. However, in both analyses most parameters showed high ESS (more than 200), including likelihood, posterior, prior, mrca.age, etc. Thus, parameters that we discuss here such as inferred ages and tree topology have all received high ESS. The results from the dating analyses are presented in Figs. 4 and S1. Both analyses resulted in highly congruent topologies with the only difference referring to some relationships within *Kryptonesticus* and the placement of the genus *Aituaria*, which in the results based on analysis including the biogeographical constraint was sister to *Wraios* + *Nesticella* (a topology also recovered by the ML analyses). Estimates of divergence times are highly consistent for shallower nodes, however there are some significant differences in the mean age estimates for deeper splits. For example, dating without biogeographic constraint (Fig. 4) results in a mean age estimate for the split between *Typhonesticus* and the rest of the nesticids of 91.9 Ma (71.1–111.9 Ma) while the age of the same node is estimated at about 84 Ma (58.3–107.4 Ma) when the biogeographic constraint is used (Fig. S1). This bias towards younger ages for the deeper nodes is observed across the topology of the analysis including the biogeographic constraint. However, when 95% highest probability density intervals of age estimates are considered there is a significant overlap between the two analyses.

It is worth noting that when biogeographic constraint was not used the age of the Cretan species was estimated to be about 12.9 Ma (7.8–18.6 Ma) which is consistent with the time when the island became separated from continental landmasses. Because use of biogeographical constraints in general implies strong assumptions about the effects of biographical processes on the evolution of clades and to avoid circularity when discussing biogeographical patterns (Sauquet, 2013), all further discussion is based on the analysis which did not include the biogeographic constraint.

4. Discussion

4.1. Systematics and taxonomy

Mediterranean nesticids are a textbook example of a group described long ago in which there are no recent taxonomic revisions or broad phylogenetic analyses, and where genera are not properly diagnosed. Our results show that several European lineages are supported as monophyletic, but specific composition of genera as currently described is controversial, and several species are misplaced, mainly in the genera *Nesticus* and *Carpathonesticus*. Currently, Mediterranean nesticids are grouped into seven genera: *Aituaria, Daginesticus, Carpathonesticus, Domitius, Kryptonesticus, Nesticus, Typhlonesticus*. Here we review the specific composition of each of them in light of our results.

Our findings regarding the genus *Nesticus* are perhaps the most significant. This genus was described by Thorell, 1869 and includes 125 species distributed worldwide (World Spider Catalog, 2022). In the European/Mediterranean area 11 species are present, including the type species of the genus, *N. cellulanus*, which is distributed in Europe and Turkey and is introduced to North America. Of the remaining ten species, nine are described from the Romanian Carpathians and one (*N. birsteini* Charitonov, 1947 not included in the analysis) is reported from the Caucasus (Russia and Georgia). Our phylogenetic analyses show that the nine *Nesticus* species from the Romanian Carpathians included in our analyses, belong to the genus *Carpathonesticus*. After a detailed review of the original description and illustrations provided from different authors, we can also confirm that *N. birsteini* is not congeneric with *N. cellulanus* and likely belongs to the Eastern European lineage (with the genera *Aituaria* or *Daginesticus*). Regarding the remaining *Nesticus* species included in the analysis, our results place *N. silvestrii* from western North America within the genus *Typhlonesticus* and suggest that *N. carteri* and *N. holsingeri* from USA and *N. kyongkeomsanensis* from Korea should be transferred to other genera.

It is interesting to note that *N. cellulanus*, the type species of the genus, is included within the Central European lineage together with the genera *Kryptonesticus* and *Carpathonesticus* (Figs. 3 and 4). This suggests that, as currently defined, *Nesticus* may be a European monospecific genus sister to *Carpathonesticus*. The lineage including *Nesticus* and *Carpathonestius* and its distribution in central Europe suggests that many (perhaps all) of the more than 100 *Nesticus* species currently described worldwide are likely misplaced and in need of revision to clarify their taxonomic status. Based on our phylogenetic results, we propose 10 new combinations and 1 status restored (Table 2).

The genus *Carpathonesticus* was described by Lehtinen & Saaristo (1980) and includes 17 accepted species (World Spider Catalog, 2022). We have included in the analysis nine *Carpathonesticus* representatives, most from Romania (Carpathian Mountains) and *C. parvus* from Bosnia and Herzegovina. Our analyses show that *C. parvus* belongs to the *Typhlonesticus* lineage. For the remaining eight species not included in the analysis, after a close inspection of the genitalic morphology based on illustrations provided by different authors (Evtushenko, 1993; Nae,

Table 2
List of proposed nomenclatural changes.

List of nomenclatural changes proposed in this study
Carpathonesticus balacescui (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Carpathonesticus carpaticus (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Carpathonesticus cernensis (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Carpathonesticus constantinescui (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Carpathonesticus diaconui (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Carpathonesticus ionescui (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Carpathonesticus orghidani (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Carpathonesticus plesai (Dumitrescu, 1980) comb. nov., ex *Nesticus*
Carpathonesticus wiehlei (Dumitrescu, 1979) comb. nov., ex *Nesticus*
Typhlonesticus silvestrii (Fage, 1929) comb. nov., ex *Nesticus*
Typhlonesticus parvus Kulczynski, 1914 stat. rest.

2013; Weiss, 1981; Weiss and Heimer, 1982) we conclude that five of the species reviewed (*C. avrigensis* Weiss & Heimer, 1982; *C. cibiniensis* Weis, 1981; *C. galotshkae* Evtushenko, 1993; C. orolesi Nae, 2013 and C. paraavrigensis Weiss & Heimer, 1982) all of them from the Carpathian Mountains, are correctly diagnosed. The information available about the three remaining *Carpathonesticus* species from the Georgian and Russian Caucasus (*C. zaitzevi* (Charitonov, 1939), *C. caucasicus* (Charitonov, 1947), and *C. ljovuschkini* (Pichka, 1965)) is scarce (only original descriptions) and does not allow us to assign them to a genus with confidence. We can only affirm that they belong to the Eastern European lineage (genera *Aituaria* or *Daginesticus*).

The genus *Typhlonesticus* was described by Kulczyński (1914) (type species: *T. absoloni* (Kratochvil, 1933 but see; Lehtinen and Saaristo, 1980). In the same paper *T. parvus*, a second species of this genus was also described. Subsequently Brignoli (1971) transferred *T. parvus* to the genus *Nesticus* and later, Lehtinen and Saaristo (1980) included it in the genus *Carpathonesticus*. Currently *Typhlonesticus* includes five species spread throughout the Mediterranean basin, from Turkey to the Pyrenees (Ribera et al., 2014).

Results of our molecular analysis show that *C. parvus* is included in the lineage of the genus *Typhlonesticus*, as is *N. silvestrii* from the USA, therefore, both species must be transferred to this genus. Regarding *N. silvestrii*, there are several species morphologically very similar to it, such as *N. potterius* (Chamberlin, 1933) and *N. sodanus* Gertsch, 1984 (Gertsch, 1984). Surely, when the North American species are included in the analysis, it will be possible to clearly define the genera included in this lineage. For now, and provisionally, we transfer *N. silvestrii* to the genus *Typhlonesticus*.

The genera *Aituaria* and *Daginesicus* have been recently revised (Fomichev et al., 2022). The former was described by Spassky (1932) and includes four species whose distribution covers Easter Ukraine, Crimea, Turkey, Georgia, and Iran (*A. pontica*, *A. borutzkyi*, *A. eriashvilii* and *A. iranica*) (World Spider Catalog, 2022). *Daginestius* has been recently described from the Russian Caucasus and Georgia including two species (*D. dzhamirzoevi* Fomichev, Ballarin & Marusik, 2022 and *D. mamajevae* (Marusik, 1987)). Both genera are sympatric, and their distributions spread to the southeast of the European plate in the areas where the Turgai Strait isolated Europe from Asia.

The genera *Kryptonesticus* and *Domitius* have been recently described (Pavlek and Ribera, 2017; Ribera, 2018) and include nine and eight species respectively (World Spider Catalog, 2022). *Kryptonesticus* is spread from Bulgaria and Turkey to Croatia, including Montenegro, Bosnia and Herzegovina and Crete, while *Domitius* is found in the western European peninsulas (Iberian and Italian). These genera are well established and do not require any nomenclatural changes.

Finally, we shall note that species of the genera *Typhlonesticus*, *Domitius* and *Kryptonesticus* have narrow distribution ranges and are restricted to the karstic areas of the southern European mountain ranges. In addition, almost all of them are highly troglomorphic species. We interpret that these three genera should be considered as endemic lineages that only occur over relatively small ranges and they should have special protection since they correspond to the last representatives of these old lineages.

All our analyses recover the existence of four independent lineages within the Mediterranean Nesticidae. Two of them are formed by a single genus: the *Typhlonesticus* and the *Domitius* lineages. The Eastern lineage groups two: *Aituaria* and *Daginesticus* (*Daginesticusi* is not present in our analyses, but it shares morphological similarities with *Aituaria* which suggest that it belongs to the same lineage) whereas the Central and most recent lineage contains three: *Kryptonesticus*, *Nesticus* and *Carpathonesticus*. Because in our analysis only the European Nesticid fauna is well represented, some of the relationships inferred here for the European lineages may change, particularly in the case of the genus *Nesticus*, including inferences about their biogeographic history. However, the results obtained here allow us to provide hypotheses about European nesticids origins and evolution.

4.2. Biogeography

Our molecular dating analysis estimate the origin of the group that includes both European and non-European taxa at 128 Ma (113–145.7 Ma) in the Early Cretaceous (Fig. 4). At this time the landmasses of the northern hemisphere formed the supercontinent of Laurasia (Asia, Europe, and North America) which was largely covered by a subtropical forest where nesticids were likely broadly distributed. Europe was partially isolated from Asia during this period by the Turgai Strait (120–29 Ma), an epicontinental seaway that stretched from the present-day Caspian Sea northward, following the Ural Mountains. The Turgai Sea was not continuous throughout this period, but it was persistent (Brikiatis, 2014). The estimated divergence times of the European lineages are between 91.9 (71.1–111.9) Ma and 60.7 (46.1–75.5) Ma supporting the hypothesis that European nesticids have been present in Europe well before the Pleistocene. During this time Euramerica was starting to break up although multiple land bridges across the Atlantic Ocean allowed the exchange of flora and fauna between Europe and North America. Although some discrepancies exist between different authors, the Tulean land bridge was the most important route for exchange of temperate biota (Sanmartín et al., 2001) until it broke about 50 Ma. A more northern *trans*-Atlantic connection, the De Geer land bridge, persisted until the Late Eocene (39 Ma Sanmartín et al., 2001 but see; Brikiatis, 2014). However, this route is considered less important for biotic exchange than the Tulean bridge. A third connection, the Greenland–Faroes Bridge, probably persisted until the Miocene but this connection is not considered to have been an important dispersal route (Sanmartín et al., 2001). The climate during this period was boreotropical and Euramerica was covered with subtropical forests that extended throughout its geography (Sanmartín et al., 2001), conditions favorable for nesticids across its entire territory which remained largely unchanged until the end of the Eocene (Brikiatis, 2014). Although our representation of non-European taxa is scarce, our results suggest that current distributional patterns of European and North American nesticids have likely resulted from vicariance due to the opening of the Atlantic Ocean, like other spiders found across North America and Europe (Ledford et al., 2021; Opatova et al., 2013). For example, the North American species *T. silvestrii* is nested within Mediterranean species from which it diverged c.a. 45.6 Ma (29.3–61.8 Ma). All *Typhonesticus* species are found in caves and most of the Mediterranean representatives diversified between much later in the last in the last 22.4 Ma (15.4–30 Ma) (except *T. parvus* which is older) at a time when the Aegean Sea was not yet open (Dermitzakis and Papanikolaou, 1981; Lymberakis and Poulakakis, 2010; Papadopoulou et al., 2010) making it possible to follow southern European mountain ranges from Turkey to the Balkan Peninsula.

In the late Eocene and early Oligocene, the collision between the continental plates of Africa, Asia and Europe triggered the Alpine Orogeny (the uplift of the main current mountain ranges in southern Europe, North Africa, and Asia). This collision also led to the closing of the Turgai Strait, the isolation of the remains of the Tethys Sea, and the formation of the Mediterranean Sea (Sanmartín et al., 2001). These geological events led to the establishment of a Mediterranean climate in southern Europe. The action of glaciations in central and northern Europe and the establishment of the Mediterranean climate in the south have likely altered the distribution of nesticids restricting the areas with suitable climate and isolating several species in underground environments where the necessary conditions for their survival existed. The Pleistocene glaciations have been associated with mass extinctions but also with elevated speciation rates. For instance, most divergence in terrestrial arthropods groups at the species level has traditionally been explained because of isolation in glacial refugia (Sanmartín et al., 2001). The combination of these geological processes and global climate fluctuations have had a profound effect on the distribution and diversity patterns of European nesticids due to the fragmentation and sharp decrease in suitable habitats. As a result, a large number of species have

very restricted ranges (e.g. *Typhlonesticus, Domitius, Krytonesticus*).

Several species from the Eastern and the Central European lineage still live outside caves, however most of them also have very restricted distributions and are often associated with caves although they may lack troglomorphic characters. For example, most species of *Carpathonesticus* have been collected inside caves or mines, but some of them are also found outside (e.g. *C. spelaeus* (Szombathy, 1917) and *C. racovitzai* (Dumitrescu, 1979). Two species are never found in caves, however they are always found in humid and shady places, (*C. lotriensis* Weiss, 1983 and *C. cibiniensis* Weiss, 1983). Some show preadaptative characters to the underground environment (depigmentation and slight ocular reduction) (e.g. Kratochvíl, 1978). The absence of troglomorphic species highly adapted to the subterranean environment, as occurs in the rest of the European lineages of this family (except for the genus *Nesticus* and the Eastern European lineage) may be due to the recent radiation of *Carpathonesticus* in the last 24 Ma (17–33 Ma) in combination with somewhat milder effect of the Quaternary glaciations in the Romanian Carpathians (Batchelor et al., 2019).

In Europe, species with wider distributions are only found in the Eastern European lineage and in the genus *Nesticus*. The genera *Aituaria* (four species) and *Daginisticus* (two species) constitute the easternmost lineage of the European Nesticidae, which has diverged about 77.5 Ma (60.7–94.9 Ma). Its species are widely spread at the boundaries of the European and Asian continental plates (Eastern Ukraine, Russia (Caucasus), Georgia, Turkey, and Northern Iran). The current distribution of these genera suggests that they were likely ancestrally distributed in western Asia and after the closure of the Turgai Strait (29 Mya) were able to colonize the easternmost part of Europe. In addition, they are not related to any European lineage, but rather to other Asian taxa.

Both genera show sympatric distribution (Fomichev et al., 2022) and some species could be defined as synanthropic species since they have been collected in cellars in some cities (*D. mamajevae, A. eriashvilii, A. pontica*) and/or shows a wide distribution area: *A. borutzkyi* (Crimea, Turkey, Georgia). *A. eriashvilii* (Ukraine, Georgia) (Fomichev et al., 2022; Marusik et al., 2017). We shall note that, there are four more described nesticid species from the Russian Caucasus (*N. birsteini* and *C. ljovuschkini*) and Georgia (*C. zaitzevi* and *C. caucasicus*). It is likely that these species belong to the Eastern European lineage since morphologically they do not belong to the genera *Nesticus* or *Carpathonesticus* (see taxonomy discussion).

4.3. Cave adaptation from tropical ancestral climates

Our results suggest that ancestrally European nesticids have likely been adapted to a tropical climate (warm and humid) and possibly also had affinities to cave entrances, since these had similar ecological conditions. The potential role of changes in climatic conditions and particularly decrease in humidity and increase in seasonality have long been suggested as one of the important drivers for cave adaptations (e.g. Barr and Holsinger, 1985) with examples from spiders such as the Asian nesticid genus *Nesticella* (Ballarin and Li, 2018) and Leptonetidae (Ledford et al., 2021). It is interesting to note that, of the seven genera present in Europe, only three have numerous cave-obligate (troglobitic) species (*Typhlonesticus, Kryptonesticus* and *Domitius*). The eastern lineage (genera *Aituaria* and *Daginiesticus*) does not have any troglobitic species. There are also no cave-obligate species in the genera *Nesticus* and *Carpathonesticus*.

The genera *Typhlonesticus* and *Kryptonesticus* are sympatric and their distribution covers the mountain ranges of the northern Mediterranean, from Turkey to the Pyrenees. Most likely both genera have persisted in caves refugia while their populations outside caves have gone extinct as a result of drastic changes in climatic conditions. Although both genera diverged at different times, their diversification patterns coincide with periods when climate has changed becoming hotter and drier (*Kryptonesticus* species diversified between 17.8 and 6.5 Ma, while in *Typhlonesticus*, *T. parvus* originated c.a. 56.1 Ma (38.6–75.5 Ma) and the rest of

the 5 European species diversified between 22 and 17 Ma).

Throughout the Oligocene the climate in Europe gradually changed with increasing seasonality and aridity (Bruch et al., 2011; Eronen et al., 2009). Palynological data suggest that in the Early Miocene (ca. 20–15 Ma) the Iberian Peninsula had an overall warm, subtropical climate, mostly dry but with humid conditions near the mountain ranges (Jiménez-Moreno et al., 2010). The current Mediterranean climate, with a marked seasonality of moderate winters and hot and dry summers began to settle during the middle and late Miocene (Serravalian and Tortonian, c.a.15–7 Ma) (Jiménez-Moreno et al., 2010; Suc, 1984). The climate change in the Serravalian drove the extinction of many thermophilus and high water-requirement plant families, which were progressively substituted by mesothermic plants in the Mediterranean (e.g., Quercus, Fagus, Alnus), high-elevation conifers, and xerophytes in the lowlands (Bidegaray-Batista et al., 2014). In this scenario the mountain ranges acted as climatic refuges for *Typhlonesticus* and *Kryptonesticus* until the climate changed progressively towards cooler and dryer conditions in the Quaternary, ca. 3.4 Ma (Bidegaray-Batista et al., 2014). Quaternary glacial cycles have likely caused extinctions of nestícids in the north and center of the continent, and across the high elevation regions in the southern European peninsulas, while in regions where glaciations were milder (e.g. the Carpathians and in southern Europe) extinction processes have affected mostly epigean taxa, leaving cave populations from different mountain massifs isolated. Our results show that Quaternary glacial cycles have likely had a minor impact on the speciation processes in *Typhlonesticus,* and *Kryptonesticus* as inferred time of divergence events predate the Quaternary. Therefore, most likely diversification in these genera was driven by the Miocene climatic transition and the subsequent establishment of the Mediterranean climate. Similar effects on diversification patterns have been inferred for Dysderidae spiders in Europe (Bidegaray-Batista et al., 2014) suggesting that the Miocene climatic transition has affected spider diversity in many European lineages.

Most of the species of the genus *Domitius* from western European Peninsulas are cave-obligate but the adaptation to the cave-life, although it has certain similarities to that of the *Typhlonesticus* and *Kryptonesticus* genera, shows a different pattern. The genus originated about 65.5 Ma (50.4–80.9 Ma), and most extant species diverged between 53.1 and 20.6 Ma long before the Miocene climatic transition and the establishment of the Mediterranean climate. Only the divergence between the Italian species *D. culsu* and *D. speluncarum* (7.07 Ma, 3.8–10.8 Ma) and perhaps *D. menozzii* (20.6 Ma, 13.2–28.9 Ma) could be directly attributed to the Miocene climatic transition. It is possible that in the case of the Iberian Peninsula, the Miocene climatic transition isolated the extant species in climatic refuges of its periphery and caused extinctions of most epigean species, mainly in the Iberian central massif.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2022.107685.

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