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Diversity, biogeography, evolutionary relationships, and conservation of Eastern Mediterranean freshwater mussels (Bivalvia: Unionidae)

Manuel Lopes-Lima^{a,b,c,*}, Mustafa Emre Gürlek^d, Ümit Kebapçı^e, Hülya Şereflişan^f,
Telat Yanık^g, Alireza Mirzajani^h, Eike Neubert^{i,j}, Vincent Prié^{a,k}, Amilcar Teixeira^l,
André Gomes-dos-Santos^{b,m}, David Barros-García^b, Ivan N. Bolotov^{n,o,p},
Alexander V. Kondakov^{n,o,p}, Ilya V. Vikhrev^{n,o,p}, Alena A. Tomilovaⁿ, Tahir Özcan^f,
Ayhan Altun^f, Duarte V. Gonçalves^b, Arthur E. Bogan^q, Elsa Froufe^b

^a CIBIO/InBIO – Research Center in Biodiversity and Genetic Resources, University of Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

^b CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, P 4450-208 Matosinhos, Portugal

^c SSC/IUCN – Mollusc Specialist Group, Species Survival Commission, International Union for Conservation of Nature, c/o The David Attenborough Building, Pembroke Street, CB2 3QZ Cambridge, United Kingdom

^d Burdur Vocational School of Food Agriculture and Livestock, Mehmet Akif Ersoy University, 15100 Burdur, Turkey

^e Biology Department of Art and Science Faculty, Mehmet Akif Ersoy University, Burdur, Turkey

^f Marine Sciences and Technology Faculty, Iskenderun Technical University, 31200 Iskenderun, Hatay, Turkey

^g Ataturk University, Faculty of Fishery, 25240 Erzurum, Turkey

^h Inland Waters Aquaculture Research Center, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), P.O. Box 66, Bandar-e Anzali, Iran

ⁱ Natural History Museum Bern, Bernstr. 15, CH-3005 Bern, Switzerland

^j Institute of Ecology and Evolution, University of Bern, CH-3012 Bern, Switzerland

^k Institute of Systematics, Evolution, Biodiversity (ISYEB), National Museum of Natural History (MNHN), CNRS, SU, EPHE, UA CP 51, 57 rue Cuvier, 75005 Paris, France

^l Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^m Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 1021/1055 Porto, Portugal

ⁿ N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Northern Dvina Emb. 23, 163000 Arkhangelsk, Russia

^o Northern Arctic Federal University, Northern Dvina Emb. 17, 163017 Arkhangelsk, Russia

^p Saint-Petersburg State University, Universitetskaya Emb. 7/9, 199034 Saint Petersburg, Russia

^q North Carolina Museum of Natural Sciences, 11 West Jones St, Raleigh, NC 27601, United States

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ABSTRACT

Located at the junction between Europe, Africa, and Asia, with distinct evolutionary origins and varied ecological and geographical settings, together with a marked history of changes in orogeny and configuration of the main river basins, turned the Eastern Mediterranean into a region of high diversity and endemism of freshwater taxa.

Freshwater mussels (Bivalvia, Unionidae) from the Western Palearctic have been widely studied in their European range, but little attention has been dedicated to these taxa in the Eastern Mediterranean region and their diversity and phylogeography are still poorly understood.

The present study aims to resolve the diversity, biogeography, and evolutionary relationships of the Eastern Mediterranean freshwater mussels. To that end, we performed multiple field surveys, phylogenetic analyses, and a thorough taxonomic reevaluation. We reassessed the systematics of all Unionidae species in the region, including newly collected specimens across Turkey, Israel, and Iran, combining COI + 16S + 28S and COI phylogenies with molecular species delineation methods. Phylogeographical patterns were characterized based on published molecular data, newly sequenced specimens, and species distribution data, as well as ancestral range estimations.

We reveal that Unionidae species richness in the Eastern Mediterranean is over 70% higher than previously assumed, counting 19 species within two subfamilies, the Unioninae (14) and Gonideinae (5). We propose two new species, *Anodonta seddoni* sp. nov. and *Leguminaia anatolica* sp. nov. Six additional taxa, *Unio delicatus* stat.

* Corresponding author.

E-mail address: manuelmplopeslima@gmail.com (M. Lopes-Lima).

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rev., *Unio eucirrus* stat. rev., *Unio hueti* stat. rev., *Unio sesirmensis* stat. rev., *Unio terminalis* stat. rev. removed from the synonymy of *Unio tigridis*, as well as *Unio damascensis* stat. rev. removed from the synonymy of *Unio crassus*, are re-described. The nominal taxa *Unio rothi* var. *komarowi* O. Boettger, 1880 and *Unio armeniacus* Kobelt, 1911 are proposed as new synonyms of *Unio bruguierianus*, and *Anodonta cyrea* Drouët, 1881 and *Anodonta cilicica* Kobelt & Rolle, 1895 as new synonyms of *Anodonta anatina*. Also, the presence of *Unio tumidus* in the Maritza River is confirmed. The phylogeographic patterns described here are interpreted concerning major past geological events.

Conservation needs and implications are presented, together with populations and species conservation priorities.

1. Introduction

Freshwater bivalves within the order Unionida also known as freshwater mussels are recognized for their important ecosystem functions and services (Vaughn, 2018); peculiar life-cycle, with a unique parasitic stage on fish (Modesto et al., 2018); uncommon doubly, maternal and paternal, mitochondrial inheritance (Guerra et al., 2019); and globally poor conservation status (Lopes-Lima et al., 2014, 2018a). They are also extraordinarily useful to understand past geological and hydrological events due to their stable biogeography associated with low dispersal and restriction to freshwater habitats (Zieritz et al., 2020).

Accordingly, describing their diversity, distribution, and evolution is extremely important, which is reflected in the growing research attention, especially in the Western Palearctic region (e.g. Froufe et al., 2016a, 2016b; Araujo et al., 2017, 2018; Tomilova et al., 2020a). However, across this region, the available knowledge is still unbalanced, with species diversity and distribution being considerably well-known in northern, western, and central Europe, while many gaps persist in their southeastern range, especially in the Balkans, Turkey, and the Middle East (Lopes-Lima et al., 2017a). Cryptic diversity has been identified in Italy, the Balkans, and Turkey (Lopes-Lima et al., 2017a), which led to the re-description and recognition of additional freshwater mussel species in Italy (Froufe et al., 2017; Riccardi et al., 2020) and the Balkans (Froufe et al., 2016a, 2017; Araujo et al., 2017, 2018).

In the Eastern Mediterranean and adjacent countries (here including Turkey, Syria, Armenia, Azerbaijan, Iran, Iraq, Georgia, and the Levant countries Israel, Jordan, and Lebanon), this conspicuous group of bivalves has been extensively described since the 19th century. By that time, notorious and prolific French malacologists such as Henri Drouët, Jules René Bourguignat, and his disciple Arnould Locard, benefiting from the many shell specimens collected across this region by several missionaries and amateur naturalists, e.g. Louis Lortet and Huet du Pavillon, described more than 50 freshwater mussel species (see Sup. Appendix I). This proliferation of species descriptions continued until the beginning of the 20th century, with the descriptive works by Isaac Lea in the United States of America and later by Wilhelm Kobelt in Germany that contributed to a substantial increase in the freshwater mussel species names (to more than 100) described for this area (Lea, 1863, 1865, 1870; Kobelt and Rolle, 1895; Kobelt, 1911, 1912, 1913, 1915). All of these descriptions were mainly based on shell characters, especially shape, colour, and sculpture. Soon, several malacologists began to recognize that using shell characters alone was misleading to discriminate species of freshwater mussels, due to their high shell plasticity in response to environmental variations (e.g. Ortman, 1912; Prashad, 1931). By the middle of the 20th century, a comprehensive review of the world's freshwater mussels, lumped and synonymised all the described species from this region into only 11 species with 5 additional subspecies (Haas, 1969). However, this review was again mainly based on shell characters. Very few subsequent works were dedicated to freshwater mussels from this area, but by the 1980s, German scientists connected with the Senckenberg Natural History Museum in Frankfurt, made several expeditions across Turkey, Syria, and the Middle East collecting many specimens of freshwater molluscs. From these expeditions, several works were published on the species

identification, distribution, and biogeography of freshwater mussels (e.g. Schütt, 1983; Kinzelbach, 1987, 1989). However, they relied on shell characters for species identification and inferred all demographic and biogeographic patterns using the distribution of those morpho-species.

Currently, the modern taxonomy of freshwater mussels does not rely on conchological features alone but rather on an integrative approach including other characters such as molecular and anatomical data (e.g. Prié and Puillandre, 2014; Lopes-Lima et al., 2017b, 2018b). Unfortunately, until now, there have been very few surveys and published molecular studies on freshwater mussels from the Eastern Mediterranean countries and the Middle East. Nevertheless, the most recent freshwater mussels inventories from the study area recognize the existence of species belonging to the genera *Anodonta* Lamarck, 1799, *Unio* Philipsson in Retzius, 1788, *Potomida* Swainson, 1840, *Leguminaria* Conrad, 1865, and *Pseudodontopsis* Kobelt, 1913 (Falkner, 1994; Graf and Cummings, 2021) and some recent research (see details below) started to unveil the species diversity and identity in the Eastern Mediterranean, although in a very restricted number of species and populations.

Phylogenetic studies on the genus *Potomida* recognized a single species from this region, but the number of populations used was limited, and previously described regional subspecies had not been sampled (Froufe et al., 2016a; Araujo et al., 2017). Another phylogenetic study covering the genus *Unio* Philipsson in Retzius, 1788 also included specimens from Turkey and Israel, but only a few sequences from three populations were analysed (Araujo et al., 2018). Furthermore, studies on the taxonomic validity and phylogeny of two previously described species from the Orontes (Asi) basin with uncertain status, i.e. *Anodonta pseudodontopsis* Locard, 1883 and *Pseudunio homsensis* (Lea, 1865), have been recently published but using only specimens from a very restricted area, i.e. the Lower Orontes catchment (Vikhrev et al., 2018; Tomilova et al., 2020b).

Due to its geographical location, at the crossroads between three continents, the region presently occupied by the Eastern Mediterranean countries, established strong paleobiogeographic associations with the Balkans, Central Europe, East Asia, and Africa, between the late Oligocene and early Pleistocene, 24–2 Ma (Steininger and Rögl, 1984). During the Early Miocene, this region remained largely isolated between the Neotethys Sea (the origin of the Mediterranean), and its northern branch, i.e. the Paratethys (that gave birth to the Black and Caspian Sea basins) (Steininger and Rögl, 1984). The collision of the Arabian and Eurasian plates during the Middle Miocene promoted the gradual uplifting of this region (Şengör et al., 1988), leading to the closure of the Neotethyan Ocean (Jaffey and Robertson, 2005; Bialik et al., 2019). It also caused the gradual transformation of the Paratethys into a series of smaller brackish/freshwater lakes in Europe (Magyar et al., 1999; Krstić et al., 2012), contemporary with, a series of Neogene Central Anatolian interconnected lacustrine systems (Meijers et al., 2018, 2020; Lazarev, 2020), which functioned as Paratethyan refugia and/or corridors for fresh/brackish water biota (Wesselingh et al., 2008; Kebapçı et al., 2012; Rausch et al., 2020). During the Pliocene and Pleistocene, the substantial tectonic uplift and faulting that occurred in parallel over the past 5–6 million years (Ma) have shaped the complex drainage systems in Anatolia, creating many of the major hydrographic basins (Göktaşan et al.,

1997; Bozkurt, 2001). However, sporadic stream capture mechanisms functioned as a bridge for freshwater life forms enabling more recent faunal exchanges among different bioregions (Por and Dimentman, 1985). Due to dramatic changes in the connectivity between the Paratethys and the Mediterranean, a succession of freshwater lakes, brackish, and marine environments severely affected the dispersal of the aquatic fauna between southeastern Europe and western Anatolia since the Miocene (Krijgsman et al., 2020). However, this connectivity was largely unaffected by events in the Mediterranean Sea, like the Zanclean flood, indicating that the aquatic systems at the northwestern corner of Anatolia remained essentially fresh or brackish water even in the earliest Pliocene.

Being located at the intersection of bordering areas, with distinct evolutionary origins and varied ecological and geographical environments, this region provided a high potential for faunal interchanges, especially over the last 24 million years. The evolution of the hydrographic networks that changed considerably from the Miocene until the present day, turned the Eastern Mediterranean into a region of high diversity and endemism of freshwater taxa such as fish and gastropods (Heller, 2007; Bektas et al., 2019, 2020). However, the phylogeography of the freshwater mussels from this area is poorly understood, hampering the evaluation of biogeographic hypotheses.

The present study aims to unveil the systematics, taxonomy, phylogeny, and phylogeography of freshwater mussels from Eastern Mediterranean and adjacent countries through extensive surveys by i) evaluating and identifying species boundaries and distributions; ii) estimating phylogenies, with newly collected specimens; iii) revising species taxonomy and systematics; and iv) discussing the conservation implications of our results.

2. Materials and methods

2.1. Taxon sampling

In a series of surveys from 2013 to 2020, a total of 338 Unionidae specimens were sampled from 87 sites in Turkey, Iran, and Israel for DNA extraction, barcoding, and subsequent phylogenetic and phylogeographic interpretations (Fig. 1: TOP-LEFT; Sup. Table 1). Available samples from 26 other taxa representatives of the main groups within the Unionidae and one from the Margaritiferidae were also selected for extraction, to be included as an outgroup in the following COI + 16S + 28S phylogenies (Sup. Table 1).

2.2. DNA extraction, amplification, and sequencing

A small foot tissue sample was collected (following Naimo et al., 1998), placed in 96% ethanol and mussels were then returned to their original habitats. Selected voucher specimens from each population and species were collected and deposited in the Gürlek collection in Mehmet Akif Ersoy University, Turkey; the Kebapçı collection in the Art and Science Faculty, Mehmet Akif Ersoy University, Burdur, Turkey, the North Carolina Museum of Natural Sciences Museums, Raleigh, United States of America and the Russian Museum of Biodiversity Hotspots, N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia (Sup. Table 1). Genomic DNA was extracted from the tissue samples, using a standard high-salt protocol (Sambrook et al., 1989) or the Jetquick tissue DNA Spin Kit (Genomed) following the manufacturer's protocol. For each population selected for barcoding, one to six (mean = 4.0) specimens per species were sequenced for cytochrome c oxidase subunit 1 (COI) and

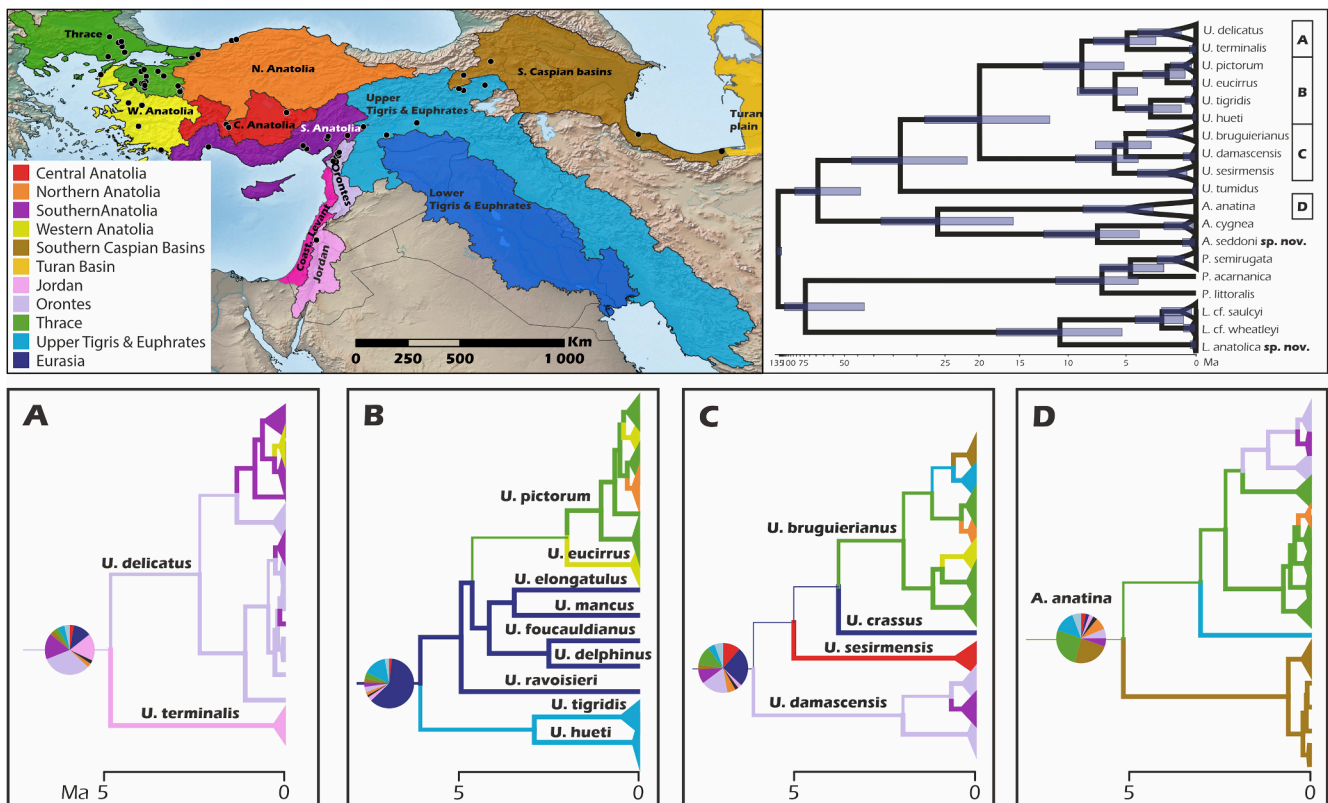


Fig. 1. Ancestral range reconstruction of freshwater mussel species occurring in the study region. Geographic entities correspond to the freshwater ecoregions by Abell et al. (2008). TOP-LEFT: Map of sampling sites (black spots) and freshwater ecoregions. TOP-RIGHT: BEAST fossil-calibrated tree (time axis, in Ma, is on a logarithmic scale for ease of representation). BOTTOM: details of selected sections of the tree. Branch thickness is relative to the support for the most likely range and goes from 1 at the tips to around 0.1–0.2 at each group's root. Branch colours correspond to regions in the map. Pie-charts represent support for each potential ancestral area. A complete tree can be found in the Supplementary Materials section.

three for the large mitochondrial ribosomal subunit rRNA 16S and the nuclear ribosomal rRNA subunit 28S (Sup. Table 1). PCR conditions for all markers COI (LCO22me2 + HCO700dy2; Walker et al., 2006, 2007), 16S (16SL + 16SH; Palumbi et al., 1991) and 28S (28S-RD1.3f + 28S-RD4b; Whiting, 2002) were described in Froufe et al. (2016a). Annealing temperatures of 48 °C were used for 16S and 28S; and 50 °C for COI. Amplified DNA templates were purified and sequenced using the same primers.

2.3. Phylogenetic analyses and species delineation

Three sequence alignments, COI, 16S, and 28S, were constructed using all newly sequenced specimens with GUIDANCE2 (Sela et al., 2015), following Fonseca et al. (2016). These alignments were then concatenated into a COI + 16S + 28S dataset and analysed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods, in IQ-TREE v 2.1.2 (Minh et al., 2020) and MrBayes 3.2.7a (Ronquist et al., 2012), respectively. For the BI analyses, the best-fit models of nucleotide substitution and partition schemes were selected using PartitionFinder 2 (Lanfear et al., 2016) under the Bayesian Information Criterion. BI analyses were initiated with program-generated trees and four Markov chains with default incremental heating. Two independent runs of 20×10^6 generations were sampled at intervals of 1,000 generations, producing a total of 20,000 trees. Burn-in was determined upon the convergence of loglikelihood and parameter values using Tracer 1.7.1 (Rambaut et al., 2018). For the ML phylogenetic analyses, the best-fit models of nucleotide substitution and partition schemes were selected using ModelFinder (Kalyaanamoorthy et al., 2017) inside IQ-TREE. Maximum Likelihood analyses were then conducted with initial tree searches, followed by 10 independent runs and 10,000 ultrafast bootstraps replicates.

A time-calibrated phylogeny of the COI + 16S + 28S dataset was then reconstructed in BEAST v. 1.10.4 (Drummond and Rambaut, 2007). We used one partition per gene with unlinked GTR + G substitution models, unlinked uncorrelated relaxed clock models, and a birth–death speciation tree prior, to accommodate both the deep and the intra-specific phylogenetic depths. Time calibration was based on mitochondrial substitution rates estimated from Zieritz et al. (2020) and the expected time-depth of our analysis. We used an average of 0.5%/Ma for both mitochondrial markers and an SD of 0.1% and 0.2% for 16S and COI, respectively. 28S ran with a uniform prior of 0–0.2%/Ma, estimated from preliminary runs. Tree root height was also set at 152 Ma (SD 10 Ma) based on Zieritz et al. (2020). Three independent runs of 10^7 generations were checked for convergence, adequate burn-in, and ESS values >200, on Tracer v1.7. These were then merged with Log-Combiner and the maximum clade credibility tree was identified and annotated by Tree Annotator (both in BEAST package). Calculations were performed in CIPRES Science Gateway (Miller et al., 2010).

To reconstruct the ancestral biological exchanges among local freshwater ecoregions, we performed a Bayesian phylogeographic inference in discrete space using the Bayesian Stochastic Search Variable Selection (BSSVS; Lemey et al., 2009) in BEAST. Given the complexity of the geologic history of the region since the Miocene, we decided to use geographic delimitations more representative of Pleistocene and late Pliocene conformation. The freshwater ecoregions defined by Abell et al. (2008) were used to classify samples into nine regions. We used the same dataset, models, and prior settings as in the dating analysis. BSSVS parameters were symmetric substitution model, strict location-trait clock, and an exponential distribution for the location rates prior (mean = 1, offset = 0).

Five additional COI + 16S + 28S datasets were constructed, three for each of the genera *Anodonta*, *Potomida*, and *Leguminaia* and two for each of the main groups within the genus *Unio*, i.e. the *crassus*- and *pictorum*-group. In each dataset, all specimens previously published (for the three genes) for each genus or *Unio* group were included for comparison (Sup. Table 1). The five datasets were then analysed using ML and BI

methodologies using the same parameters used for the whole species (COI + 16S + 28S) phylogenies described above.

For species delineation, six COI datasets were assembled, three for the genera *Anodonta*, *Potomida*, and *Leguminaia*, and three for the *crassus*-, *pictorum*-, and *tumidus*-groups of the genus *Unio*, with all newly sequenced and published sequences (Sup. Tables 2–7). Three distinct methods were then applied to each COI dataset, to determine the number of Molecular Operational Taxonomic Units (MOTUs). The BIN system implemented in BOLD (Ratnasingham and Hebert, 2013), the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012), and haplotype network reconstructions (95% connection limit) in TCS 1.21 (Clement et al., 2000). For the BINs system, each dataset was analysed with the Cluster Sequences tool implemented in BOLD 4 (<http://v4.boldsystems.org>) (Ratnasingham and Hebert, 2013). The ABGD was analysed for each dataset using its online version (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>) with the default settings and the Kimura-2-parameter (K2P) distance matrix (Puillandre et al., 2012). The TCS parsimony method was accomplished by reconstructing haplotype networks on each dataset with a 95% statistical parsimony connection limit and considering each separate network as a MOTU. Each of the COI datasets was then reduced to its haplotypes. Except for the *Unio tumidus* dataset that presented a very low genetic diversity, the remaining COI datasets were then analysed for phylogeny using ML and BI methods with the same parameters as described above. Interspecific and intraspecific COI sequence divergences (uncorrected *p*-distance) were estimated using MEGA X (Kumar et al., 2018).

2.4. Taxonomy, systematics, and distribution

A taxonomic reevaluation of all nominal taxa in the study area was accomplished by comparing morphological characters with the existing type specimens for each nominal taxon (when available) and the available molecular data. Type materials analysed were included in Supplementary Appendix I. When types were not found or available for inspection, due to the COVID19 pandemic restrictions, figured specimens or high-quality photographs were examined instead. The distribution of each species in the study area was inferred using the current data and previous reference works (Bourguignat, 1852, 1856, 1857, 1880, 1881; Küster, 1861, 1862; Lea, 1863, 1865, 1870; Tristram, 1865; Boettger, 1880; Martens, 1880; Drouët, 1881; Locard, 1883; Kobelt and Rolle, 1895; Simpson, 1900, 1914; Sturany, 1902; Kobelt, 1911, 1912, 1913, 1915; Ovtshinnikov, 1935; Pallary, 1939; Haas, 1969; Modell, 1951; Schütt, 1983; Kinzelbach, 1987, 1989; Falkner, 1994; Graf and Cummings, 2021) and the IUCN < <http://www.iucnredlist.org> > and GBIF < <http://www.gbif.org> > databases which include a large number of museum records. Distribution data were then integrated and represented as coloured potential distribution maps using level 6 HydroBASINS (Lehner et al., 2013) shapefile, where small subbasins were grouped with their main drainage or with other small drainages (especially along the coast). Vector and raster map data from Earth topography layers made by Natural Earth < <http://naturalearthdata.com> > were also included for illustrative reasons.

3. Results and discussion

3.1. Phylogenies, species delineation, and distributions

The composition, size, and parameters of all 13 datasets (7 COI + 16S + 28S and 6 COI individual datasets) together with the partition schemes and nucleotide substitution models for all analyses are presented in Table 1. No indels or stop codons were found in any of the COI datasets.

Molecular species delineation for the COI individual datasets revealed the existence of 17 MOTUs (Tables 2 and 3) that are here recognized as species.

We present and discuss here the revised systematics of Eastern

Table 1

Number of sequences (N), haplotypes (Haps), and sizes of all datasets used, as well as substitution models for each partition for all phylogenetic analyses. *not analysed for phylogeny.

Dataset	N (new)	Haps	Size (nt)	Models									
				COI codon 1		COI codon 2		COI codon 3		16S		28S	
				BI	ML	BI	ML	BI	ML	BI	ML	BI	ML
COI + 16S + 28S													
All species	283	–	1871	GTR +	TN + F +	F81	F81 + F	GTR	TIM3 + F	GTR +	TIM2 + F	GTR +	TIM2 + F
	(230)			I + G	I + G4	+ I	+ I	+ G	+ G4	I + G	+ I + G4	I + G	+ I + G4
All species (BEAST)	283 (230)	–	1871	GTR +	–	GTR	–	GTR	–	GTR +	–	GTR +	–
				G		+ G		+ G		G		G	
Unio													
<i>pictorum</i> - group	84(72)	–	1911	HKY +	TIM2 + F	F81	F81 + F	HKY	TPM3u +	HKY +	TIM2 + F	HKY +	TPM2 + F
				I	+ I			F	I	I	+ I	I + G	+ I + G4
<i>crassus</i> - group	80(60)	–	1948	HKY +	TN + F +	F81	F81 + F	HKY	HKY + F	HKY +	TN + F + I	HKY +	TN + F + I
				I	I			+ I	I	I		I	
<i>Anodonta</i>	133 (42)	–	1861	HKY +	TPM2 +	F81	TN + F	HKY	TIM3 + F	HKY +	TPM2 + F	HKY +	TN + F + I
				I	F + I		+ I	I	I	I	+ I	I + G	
<i>Leguminaia</i>	15(14)	–	1936	GTR +	TIM2e + I	F81	TN + F	HKY	TPM3 + F	GTR +	TIM2e + I	HKY +	TN + F + I
				I			+ I	+ G	I	I		I + G	
<i>Potomida</i>	51(6)	–	1948	K2P + I	TNe + I	F81	F81 + F	GTR	TIM3 + F	K2P + I	TNe + I	F81	F81 + F
								+ I	+ G4				
COI													
Unio													
<i>pictorum</i> - group	803 (130)	154	620	SYM +	TN + F +	F81	F81 + F	GTR	TPM3 + F	–	–	–	–
				G	G4			+ G	+ G4				
<i>crassus</i> - group	260 (104)	68	657	K2P + I	TN + F +	F81	F81 + F	HKY	HKY + F	–	–	–	–
					I			+ G	+ G4				
<i>tumidus</i> - group*	72 (5)	13	660	–	–	–	–	–	–	–	–	–	–
<i>Anodonta</i>	499 (63)	128	620	GTR +	TN + F +	F81	F81 + F	GTR	TIM3 + F	–	–	–	–
				I	I			+ G	+ G4				
<i>Leguminaia</i>	24(22)	10	633	GTR +	HKY + F	F81	HKY +	GTR	HKY + F	–	–	–	–
				G	+ I		F + I	+ G	+ I				
<i>Potomida</i>	59(14)	29	660	HKY +	TPM3 +	F81	TPM3 +	HKY	TPM3 + F	–	–	–	–
				G	F + I		F + I	+ G	+ I				

Mediterranean Unionidae, based on the first comprehensive phylogeny of this fauna and comparative morphological analyses. This includes the description of two new species, i.e. *Anodonta seddoni* sp. nov. and *Leguminaia anatolica* sp. nov., and several species re-descriptions (see Taxonomic account section and Sup. Appendix I). A full taxonomic revision of all the Unionidae species described from this region is also presented in Sup. Appendix I.

The combined (COI + 16S + 28S) dataset phylogeny confirms the division of the species present in the area into two subfamilies, the Unioninae Rafinesque, 1820 and the Gonideinae Ortmann, 1916 (Fig. 1: TOP-RIGHT; Sup. Figs. 1 and 2). Based on our results and on morphological characteristics of two species for which no molecular data were available, we recognize here 19 species in total as valid for the study area (see below). The Unioninae is represented by 14 spp. from the genera *Unio* and *Anodonta* while the Gonideinae is represented by 5 spp. belonging to the genera *Leguminaia*, *Potomida*, and *Pseudodontopsis* (Tables 2 and 3).

Unioninae Rafinesque, 1820

Unio Philipsson in Retzius, 1788

As in most other Unionida groups, shell plasticity due to environmental factors is extremely high in the *Unio* genus. Until the 20th century, morphologically based species descriptions led to over-description (Graf, 2010). Therefore, in his comprehensive revision of the Unionida, Haas (1969) integrated all available names within the *Unio* genus into 12 fundamental species further divided into 49 subspecies. This work, although still based on morphology established the basis for the classification of the order until now. By the end of the 20th century and beginning of the current century, with the appearance of molecular tools and the development of statistical models to infer phylogenetic relationships and delineate species, it became obvious that morphological features were not enough to characterize the diversity and evolutionary relationships within this genus (Lopes-Lima et al., 2017b). Many recent

molecular works have been published since the beginning of the 2000s, with revaluations of the *Unio* species diversity, mainly in North Africa and Europe (Araujo et al., 2005, 2009, 2018; Khalloufi et al., 2011; Prié and Puillandre, 2014; Froufe et al., 2016b). Less is known about the *Unio* diversity in the Eastern Mediterranean countries, where until now almost no molecular data were available. In his review, Haas (1969) recognized five *Unio* species from the study area (Table 2). Since then, there was not a consensual view on the number of *Unio* species and their distributions in the region (Table 2), with every study presenting distinct interpretations (e.g., Modell, 1951; Schütt, 1983; Kinzelbach 1987, 1989). A recent molecular study revisited the phylogeny, systematics, and species within *Unio* (Araujo et al., 2018), however only three populations from the study region were included, two in Turkey and one in Israel, and therefore, as these authors recognized, their interpretation was limited.

Based on the currently accepted phylogeny and the molecular data available, the *Unio* genus is now divided into four main species groups, i. e., the *pictorum*-, the *crassus*-, the *tumidus*- and the *gibbus*-groups (Lopes-Lima et al., 2017a; Araujo et al., 2018).

In the COI + 16S + 28S phylogeny of the present study, the *Unio* species from the study region, are divided into three clades corresponding to three of the previously known *Unio* groups, i.e. the *pictorum*, *crassus*, and *tumidus* groups (Fig. 1: TOP-RIGHT; Sup. Figs. 1 and 2).

Pictorum-group

There has been considerable confusion about the identity and distribution of the several *Unio* species within this group originally described by Bourguignat in the middle of the 19th century, especially concerning *Unio terminalis* Bourguignat, 1852, *U. tigris* Bourguignat, 1852 and *U. eucirrus* Bourguignat, 1857 (Falkner, 1994, Araujo et al., 2018). In his comprehensive review, Haas (1969) considered only three *Unio* species in the *pictorum*-group for the Eastern Mediterranean countries: two subspecies of *U. pictorum* in northwestern Turkey, *Unio*

Table 2

Historical classification systems of the Unionidae from the study area. * now reassigned to the family Margaritiferidae.

This study	Haas 1969	Kinzelbach 1987/Kinzelbach 1989	Falkner 1994	Graf & Cummings 2021
Anodonta Lamarck, 1799				
<i>A. cygnea</i> (Linnaeus, 1758)	<i>A. cygnea</i>	<i>A. cygnea</i>	<i>A. (Anodonta) cygnea gravida</i>	<i>A. cygnea</i>
<i>A. anatina</i> (Linnaeus, 1758)	<i>A. (Gabillotia) pseudodopsis</i>	<i>A. anatina</i>	<i>A. (Anodonta) anatina ssp.</i>	<i>A. anatina</i>
<i>A. seddoni</i> sp. nov.	<i>A. (Anodonta) vescoiana</i>	<i>A. pseudodopsis</i>	<i>A. (Anodonta) anatina cilicica</i>	<i>A. cyrea</i>
<i>A. vescoiana</i> Bourguignat, 1856		<i>A. (Sinanodonta) vescoiana</i>	<i>A. (?Euphrata) cyrea</i>	<i>A. vescoiana</i>
			<i>A. (Euphrata) vescoiana</i>	
			<i>A. (Gabillotia) pseudodopsis</i>	
Unio Philipsson in Retzius, 1788				
(pictorum-group)				
<i>U. pictorum</i> (Linnaeus, 1758)	<i>U. pictorum ascanius</i>	<i>U. mancus</i>	<i>U. (Unio) pictorum gaudioni</i>	<i>U. pictorum</i>
<i>U. delicatus</i> Lea, 1863	<i>U. pictorum gaudioni</i>	<i>U. tigridis tigridis</i>	<i>U. (Unio) mancus eucirrus</i>	<i>U. tigridis</i>
<i>U. eucirrus</i> Bourguignat, 1857	<i>U. elongatulus eucirrus</i>	<i>U. terminalis terminalis</i>	<i>U. (Unio) mancus kruperi</i>	
<i>U. hueti</i> Bourguignat, 1855	<i>U. tigridis tigridis</i>	<i>U. terminalis delicatus</i>	<i>U. (Unio) terminalis</i>	
<i>U. terminalis</i> Bourguignat, 1852	<i>U. tigridis terminalis</i>		<i>U. (Unio) tigridis</i>	
<i>U. tigridis</i> Bourguignat, 1852				
(crassus-group)				
<i>U. bruguierianus</i> Bourguignat, 1853	<i>U. crassus bruguierianus</i>	<i>U. crassus bruguierianus</i>	<i>U. (Crassunio) crassus bruguierianus</i>	<i>Unio bruguierianus</i>
<i>U. damascensis</i> Lea, 1863	<i>U. crassus gontieri</i>	<i>U. crassus damascensis</i>	<i>U. (Crassunio) crassus damascensis</i>	
<i>U. sesirmensis</i> Kobelt, 1913		<i>U. crassus gontieri</i>	<i>U. (Crassunio) crassus gontieri</i>	
(tumidus-group)				
<i>U. tumidus</i> Philipsson in Retzius, 1788	<i>U. tumidus</i>			<i>U. tumidus</i>
Leguminaria Conrad, 1865				
<i>L. cf. wheatleyi</i> (Lea, 1862)	<i>L. wheatleyi</i>	<i>L. wheatleyi</i>	<i>L. wheatleyi</i>	<i>L. wheatleyi</i>
<i>L. cf. saulcyi</i> (Bourguignat, 1852)	<i>L. saulcyi</i>	<i>L. saulcyi</i>	<i>L. saulcyi</i>	<i>L. saulcyi</i>
<i>L. anatolica</i> sp. nov.				
Potomida Swainson, 1840				
<i>P. semirugata</i> (Lamarck, 1819)	<i>P. littoralis semirugata</i>	<i>P. littoralis semirugata</i>	<i>P. littoralis semirugata</i>	<i>P. semirugata</i>
	<i>P. littoralis delesserti</i>	<i>P. littoralis delesserti</i>	<i>P. littoralis delesserti</i>	
	<i>P. littoralis komarowi</i>	<i>P. littoralis tracheae</i>	<i>P. littoralis tracheae</i>	
	<i>P. littoralis homsensis</i> *		<i>P. littoralis komarowi</i>	
Pseudodontopsis Kobelt, 1913				
<i>P. euphratica</i> Bourguignat, 1852	<i>P. euphraticus</i> Bourguignat, 1852		<i>P. euphratica</i>	<i>P. euphratica</i>

elongatulus eucirrus in the coastal streams from southern Turkey to northern Israel, and two subspecies of *U. tigridis*, i.e. *U. tigridis tigridis* in the Tigris-Euphrates (Tr. Dicle-Fırat) and Orontes basins and *U. tigridis terminalis* in the River Jordan but also present in southern Turkey.

Unio eucirrus was originally described by Bourguignat (1857) and although the described specimen was said to have two probable origins, one in the vicinity of Beirut in Lebanon and the other in a stream running to the Dardanelles strait (Tr. Çanakale Boğazı) in Anatolia, Bourguignat clearly states that he believes the true origin is the Dardanelles given its shell shape (Bourguignat, 1857). However, this last thought had been overlooked by later authors who considered Beirut as the true type locality. Therefore, most of the subsequent works considered *Unio eucirrus* a species occurring in the coastal streams in the Levant. However, these populations have been confused with coastal stream forms of *U. delicatus* Lea, 1863 (see below). Although specimens from the Jordan Basin were generally considered by most authors as *U. terminalis* and those from the Tigris and Euphrates as *U. tigridis*, many of the specimens collected in the River Orontes basin overlapped in shape with both forms leading to a “puzzling form chaos” (Falkner, 1994; Araujo et al., 2018). This problem was mitigated by Haas (1969) who included both forms under *U. tigridis* and then followed by most subsequent authors (e.g. Araujo et al., 2018; Graf and Cummings, 2021).

In the present study, our *pictorum*-group ML and BI COI + 16S + 28S phylogenies exhibited similar topologies, retrieving six well-supported clades of specimens from the study area (Fig. 2). Both COI ML and BI phylogenies for the same group presented similar topologies, retrieving 15 clades that were defined as MOTUs by all species delineation methods (Sup. Fig. 3; Sup. Table 2). Uncorrected *p*-distances among the delineated MOTUs ranged from 2.3% (between *U. pictorum* and *U. eucirrus*) to 6.9% (between *Unio ravoisieri* and *U. terminalis*) (Sup. Table 8).

The six MOTUs occurring in the study area are here recognized as valid species, i.e. *Unio pictorum*, *U. eucirrus*, *U. delicatus*, *U. terminalis*, *U. tigridis*, and *Unio hueti* Bourguignat, 1855.

Unio pictorum occurs in European Turkey and northwest Anatolia in the large Maritsa (Gr. Evros, Tr. Meriç), Sakarya, Susurluk, Gediz, and Karamenderes river basins and coastal streams in-between, although its distribution might extend east by the Black Sea coast given that no surveys were made in the area (Figs. 2 and 3). The species exhibits an interesting genetic structure with haplotypes from Anatolia being related to others across Europe, while those from the Maritsa basin being exclusive and divergent, suggesting a long period of isolation from the remaining Anatolian and Danubian basins (Fig. 3). Previously published sequences on Greek populations from the River Axios and Lake Trichonida were here recognized as MOTUs but were not examined in detail because they are outside the study area and therefore considered within *U. pictorum* (Fig. 3 and Sup. Fig. 3).

Unio eucirrus is endemic to Turkey and occurs only in the Karamenderes River in sympatry with *U. pictorum*, and *U. bruguierianus* from the *crassus*-group (Figs. 2 and 3). *Unio eucirrus* is sister to the *U. pictorum* clade (Figs. 1 and 2) being only 2.3% divergent (Sup. Table 8), nevertheless, we recognize here the species validity based solely on the joint molecular species delineation results, given the similarity of shell shape. However, future molecular studies with additional markers and specimens and/or a genomic approach should be used to test the validity of our decision.

Unio delicatus occurs from the Çine River in southwest Anatolia east to the large river basins of Seyhan, Ceyhan, and Orontes, and the coastal basins of Syria, and presumably Lebanon in the Levant (Fig. 4). The species, as recognized here, includes all nominal taxa previously synonymised under *U. eucirrus*, *U. terminalis*, and *U. tigridis* from these basins and streams (see taxonomic account and Sup. Appendix I). It was placed under *U. tigridis* in the only molecular study using Turkish specimens (Araujo et al., 2018), but no “true” *U. tigridis* specimens from the Tigris River basin were included in that study, therefore, misleading their results. *Unio delicatus* has a high genetic diversity with 14 unique haplotypes but not a marked geographic genetic structure (Fig. 4).

Unio terminalis clusters with *U. delicatus* but presents a 3.1%

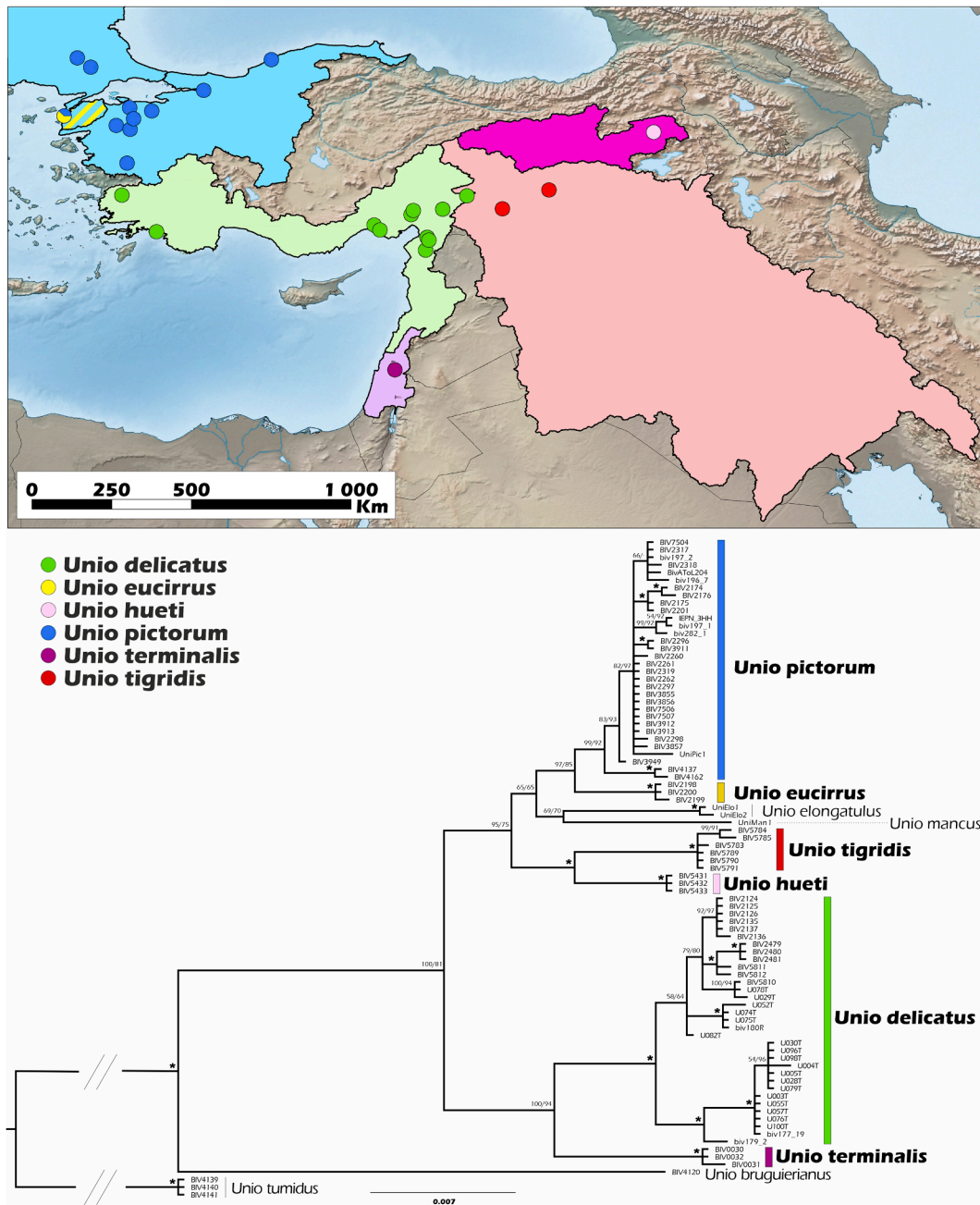


Fig. 2. TOP: Distribution map of all *Unio* (*pictorum*-group) species depicted across the main river basins in shaded colours and coloured dots representing sampled populations. BOTTOM: Bayesian consensus tree for the *Unio* (*pictorum*-group) species inferred from the combined COI + 16S + 28S gene fragments. The values by the nodes indicate Bayesian posterior probability percentage/Maximum Likelihood ultrafast bootstrap values. Values over 95% are represented by an asterisk. Taxa and sequence reference from the study area are represented in bold.

uncorrected *p*-distance divergence in COI (Sup. Table 8). It occurs only in the Jordan River basin, including Lake Kinneret, also known as the Sea of Galilee or Lake Tiberias (Figs. 2 and 4), which is probably its last refuge, given the disappearance of Lake Hula in Israel in the 1950s and the poor conservation status of the Jordan River network (Karmon, 1960; Barinova et al., 2010).

The last two species here recognized from the *pictorum*-group, are *U. tigridis* and *U. hueti*. *Unio tigridis* is widely distributed in the Tigris and Euphrates basins and is genetically distinct from both *U. delicatus* and *U. terminalis* (Figs. 1 and 2, Sup. Fig. 3). *Unio tigridis* is more closely related to a MOTU from the River Murat in the upper Euphrates basin here recognized as *U. hueti* (Sup. Fig. 3; Sup Table 8). While *U. tigridis* specimens exhibit the traditional wedge-shaped shell, those from the

Murat River are elliptical and quite distinct (Fig. 5). Searches and observations of *Unio* nominal taxa and type specimens from the study area led us to assign the name *Unio hueti* Bourguignat, 1855 described from the upper Euphrates to the species collected from Murat River. Although we could not find the type specimen of *U. hueti* described by Bourguignat in Paris and Geneva museums, where most of this author’s collection is deposited, the shell shape of the figured specimen from the original description publication is almost an exact match to our collected specimens (Fig. 5). Additionally, the type locality (the upper Euphrates, near Erzurum), concurs with the place where our specimens were collected. *Unio hueti* should be restricted to the rivers Murat and Karasu on the upper reaches of the Euphrates (Fig. 2) although more surveys are needed in Eastern Turkey to better characterize its distribution. Not

Unio pictorum & Unio eucirrus

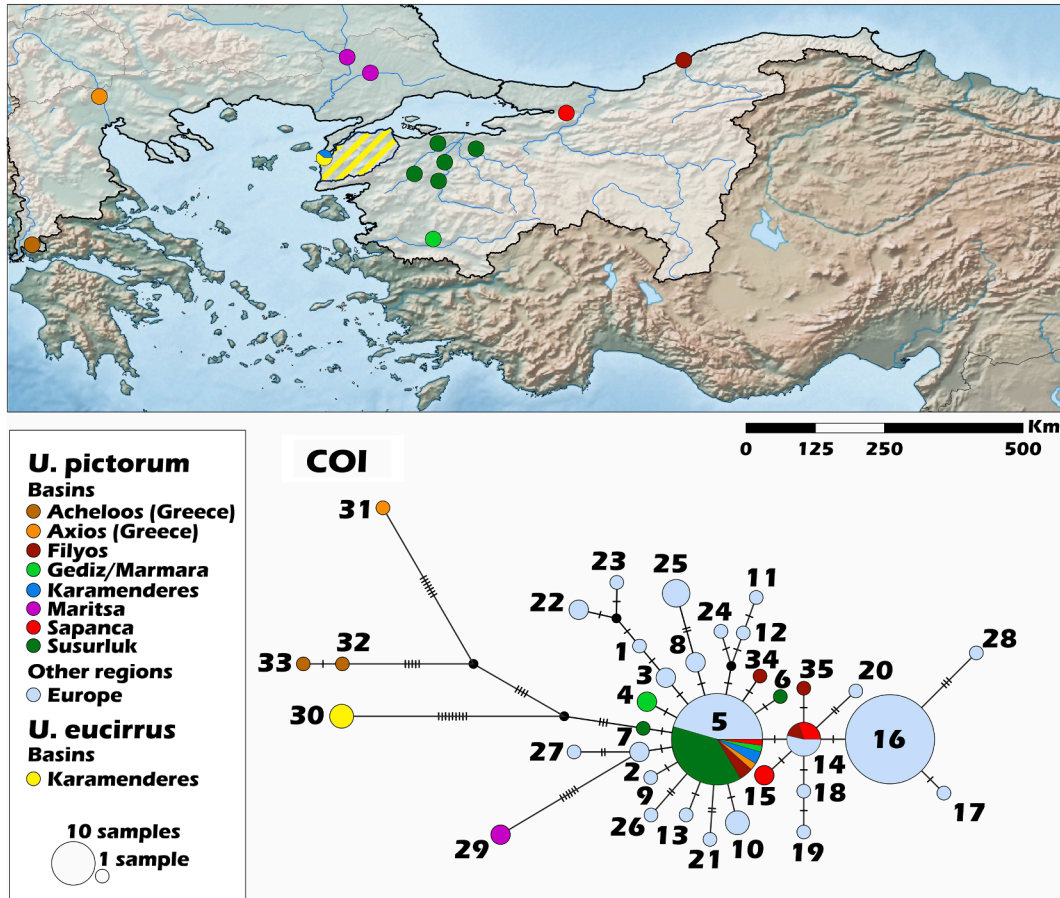


Fig. 3. TOP: Distribution map of *Unio pictorum* and *Unio eucirrus* in the study area, depicting their potential distribution across the main river basins in white (*U. pictorum*) and yellow stripes (*U. eucirrus*) and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *U. pictorum* + *U. eucirrus* sequences (Supp. Table 2). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enough specimens were collected and sequenced from both *U. tigridis* and *U. hueti* to elaborate on their genetic geographic structures.

Crassus-group

Unio species from the *crassus*-group are generally stricter in terms of habitat requirement than those from the *pictorum* and *tumidus* groups, most occurring in lotic habitats of rivers and streams and fewer in lakes and reservoirs (Lopes-Lima et al., 2017a). The shells of these species are generally shorter and taller than those from the *pictorum* and *tumidus* groups, although intermediate forms can be found among the three groups. The umbo sculpture on *crassus*-group species is generally wrinkled but it varies among species, populations, and even with age, with some specimens presenting double-looped and even nodulous sculptures (Klishko et al., 2017). This led to greater confusion for species identification among the authors that used mostly this character to distinguish *Unio* species from the study region (e.g. Modell, 1945, 1951; Kinzelbach et al., 1987, 1989). Although a multitude of nominal taxa was described for the *crassus*-group of *Unio* species in this area, since Haas (1969) and until the present study, only a single species was consistently recognized in the area, i.e. *Unio bruguierianus* Bourguignat, 1853 (Araujo et al., 2018; Graf and Cummings, 2021).

The ML and BI *crassus*-group COI + 16S + 28S phylogenies of the current study exhibited similar topologies, retrieving three well-supported clades from specimens in the study area (Fig. 6). Both COI ML and BI phylogenies for the *crassus*-group presented similar topologies, retrieving 2 clades that were defined as MOTUs by all species

delineation methods, one including specimens collected in the Tarsus, Ceyhan and Orontes basins (here recognized as *Unio damascensis* Lea, 1863) and the other specimens from the Lakes Beyşehir and Tuz endorheic basins (here recognized as *Unio sesirmensis* Kobelt, 1913) (Sup. Fig. 4; Sup. Table 3). Although only identified as a MOTU by two of the species delineation methods used, we also recognize *U. bruguierianus* as a valid species, for specimens across North Anatolia and eastern Greece, given its monophyly in the three gene phylogenies (Fig. 6 and Sup. Fig. 4), its genetic divergence from the other species ($\geq 2.8\%$) and the results from a previously published study (Araujo et al., 2018). Uncorrected *p*-distances among the delineated MOTUs ranged from 2.7% (between *Unio crassus* Philipsson in Retzius, 1788 and *Unio cf. courtilieri* Hattemann, 1859) to 9.8% (between *U. sesirmensis* and *Unio tumidiformis*) (Sup. Table 8).

The species from the *crassus*-group here recognized, i.e. *U. bruguierianus*, *U. sesirmensis*, and *U. damascensis*, are distributed in exclusive allopatric regions in the study area (Fig. 6).

Unio bruguierianus occurs from the Thessaly and Macedonian regions in Greece to Thrace and north Anatolia, east to the upper Tigris and Euphrates basins in Turkey, Syria, and Iraq, and the Arax (Tr. Aras) River basin in Turkey, Armenia, Azerbaijan, and Iran (Araujo et al., 2018; Fig. 7). It reveals a marked geographic genetic structure, with divergent lineages in most independent river basins. Exceptions are the Lissos River basin in Greece, and the Gediz and Karamenderes River basins in Turkey, whose individuals share the same haplotypes (Fig. 7:

Unio delicatus

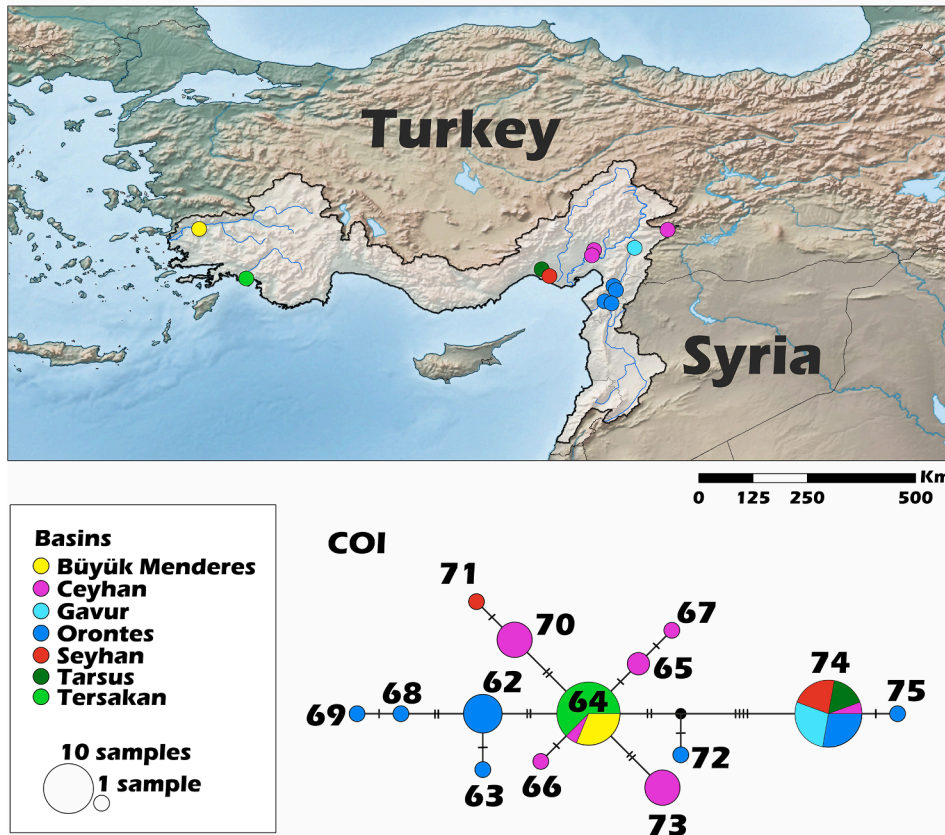


Fig. 4. TOP: Distribution map of *Unio delicatus* in the study area, depicting its potential distribution across the main river basins in white and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *U. delicatus* sequences (Sup. Table 2). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin.

H3), therefore suggesting a recent connection between these basins by the Dardanelles strait (Fig. 7).

Unio sesirmensis occurs in streams of the Beyşehir and Tuz endorheic basins and likely in other freshwater habitats in the Central Anatolian Plateau (Figs. 6 and 8). This species was described based on specimens from the Bourguignat collection, collected in Lake Suğla, which is connected to Lake Beyşehir (Kobelt, 1913). This species was described as having a short, ventrally rounded shell shape and not elongated as most of the species in the *pictorum*-group (Kobelt, 1913). Nevertheless, Kobelt wrote that the species belongs to the *Unio pictorum*-group but without presenting a rationale. The most plausible explanation for this placement is the presence of nodules or tubercles in the umbo sculpture as indicated in the original description (Kobelt, 1913). This type of sculpture features is typical of *U. pictorum* although it may also be found in other *Unio* species, including some from the *crassus*-group (Klishko et al., 2017). A thorough inspection of the specimens collected for this study from Lakes Beyşehir and Tuz basins showed a remarkably similar shell shape with the *U. sesirmensis* figured specimen (Fig. 5) with most of them revealing the presence of well-expressed nodules in the umbo. Therefore, based on the molecular data, shell morphology, and the taxonomic investigation, we place *Unio sesirmensis* within the *crassus*-group recognizing it as the valid name for the *Unio* species occurring in the Central Anatolian Plateau. *Unio sesirmensis* reveals some geographic genetic structure, with unique haplotypes in the populations for each lake basin (i.e. H13, H14, and H15 from Lake Beyşehir and H16 from Lake Tuz, Fig. 8), but the area should be surveyed more intensely to understand this pattern in detail (Fig. 8).

Unio damascensis Lea, 1863 occurs in southwestern Anatolia, in the Tarsus, Ceyhan, and possibly Seyhan basins. It also occurs in the Orontes basin in Turkey and Syria. From the nominal species previously described from the Southern Anatolian and Levante rivers running to the Mediterranean, only three of them, i.e. *Unio damascensis*, *U. orontesensis*

Lea, 1863, and *U. syriacus* Lea, 1863 were synonymized by Haas (1969) under *Unio bruguierianus* and, therefore, placed clearly in the *crassus*-group. The three nominal species are similar in shape among themselves and with the specimens collected from the Tarsus, Ceyhan, and Orontes basins (Sup. Appendix I). Therefore, we consider them all to belong to the same species. The three nominal taxa were described in the same publication (Lea, 1863), and using the first reviser principle (Article 24.2 of the International Code of Zoological Nomenclature), Falkner (1994) gave priority to *U. damascensis*. Sequences from *U. damascensis* cluster in a well-supported clade, although the phylogenetic relationships of this clade could not be well established (Figs. 1 and 6). The species has a marked geographic genetic structure with individuals from each main river basin having unique haplotypes (i.e. H24, H67, and H68 for Ceyhan, H66 for Tarsus, H25, H26, H61 and H62 for Orontes, and H18 for Gavur, Fig. 9). The haplotypes from the specimens collected in a channel near Türkoğlu (i.e. H18, Fig. 9) are more divergent with two species delineation methods considering them as a separate MOTU (Fig. 9 and Sup. Fig. 4). This channel belongs to an irrigation network that now connects the Ceyhan and the Orontes basins but was once part of the isolated endorheic basin of Lake Gavur, drained in the 1960s (Yarar and Magnin, 1997) for agriculture purposes. These divergent haplotypes might be explained by a long isolation period of this extinct lake from the coastal basins.

Tumidus-group

The *tumidus*-group of *Unio* species contains a single species, i.e. *Unio tumidus* Philipsson, 1788 (Lopes-Lima et al., 2017a; Araujo et al., 2018). The species has a wide distribution in Europe, mainly in higher latitudes, not occurring in the southern European Peninsulas of Iberia, Italy, and most of the Balkans south of the Danube Basin. The species had been previously detected in the River Tundza (Tr. Tunca), a tributary of the River Maritsa in Bulgaria and uncertainly in the western (Greek) bank of the River Maritsa (Reischütz et al., 2008; Georgiev, 2012). We have only



Fig. 5. Shells of Eastern Mediterranean freshwater mussels within the Unioniinae subfamily. *Anodonta anatina* (Lake Uluabat – BIV2279); *Anodonta cygnea* (Lake Taşkısıği – BIV2352); *Anodonta seddoni* sp. nov. (Kocaçay, Beyşehir – Holotype: NCSM 107600), *Anodonta vescoiana* (Konya, Turkey probable erroneous locality – not sequenced Holotype: MHNG-Moll 101552); *Unio pictorum* (River Susurluk – BIV3911); *Unio eucirrus* (River Karamenderes – BIV2198); *Unio delicatus* (unnamed stream by Lake Gölbaşı – U51T); *Unio terminalis* (Lake Kinneret, Jordan, Israel – NCSM 27719.1); *Unio hueti* (River Murat, Euphrates-Tigris, Turkey – BIV5432); *Unio tigridis* (Karaardıç stream, Euphrates-Tigris, Turkey – BIV5788); *Unio damascensis* (unnamed stream by Lake Gölbaşı – LG9); *Unio bruguierianus* (inlet of Lake Sapanca – BIV2324); *Unio sesirmensis* (Melendiz stream, Tuz, Turkey – BIV4081); *Unio tumidus* (Söğütlük, Maritsa, Turkey – BIV4139). Specimen codes refer to Sup. Fig. 1.

detected one population of *Unio tumidus* in the study area from the River Maritsa (Figs. 1 and 10). The species global genetic structure was never studied in detail but from the scarce previously available molecular data and the newly sequenced individuals it seems to have some genetic structure, with all the River Maritsa COI haplotypes being unique (H6 and H7, Fig. 10; Sup. Table 4).

Anodonta Lamarck, 1799

Until the middle of the 20th century, the taxonomy of the genus *Anodonta* suffered from similar over description problems already described above for *Unio*. This confusion led to the synonymization of all nominal taxa in Europe under *Anodonta cygnea* (Linnaeus, 1758) by Haas (1969). Since then, two other species have been studied in detail and separated from *A. cygnea*, i.e. *Anodonta anatina* (Linnaeus, 1758) and *Anodonta exulcerata* Porro, 1838 (Froufe et al., 2014, 2017; Riccardi

et al., 2020; Tomilova et al., 2020a). More than 10 nominal *Anodonta* taxa had also been described for the study area before the beginning of the 20th century. Except for *Anodonta vescoiana* Bourguignat, 1856 and *Anodonta pseudodopsis* Locard, 1883, all of them were later synonymised with *A. cygnea* (Haas, 1969). A study published in the late 1980s revised the *Anodonta* diversity in the study area recognizing four species, i.e. *A. cygnea* for the River Maritsa basin, *Anodonta palustris* Ferussac, 1822, distributed across western Turkey, *A. pseudodopsis* in the River Orontes basin, and *A. vescoiana* in the River Tigris and Euphrates Basin (Kinzelbach, 1989). That study further divided *Anodonta palustris* into three subspecies, i.e. *Anodonta palustris gravida* Drouët, 1879 for the Western Anatolia, *Anodonta palustris cilicica* Rolle & Kobelt, 1895 for the populations in the Çukurova region, and a third unnamed subspecies *Anodonta palustris* ssp. for the Central Anatolia Plateau populations (Kinzelbach, 1989). *Anodonta pseudodopsis* was recently considered a junior synonym of *A. anatina*, based on morphological and molecular data (Tomilova et al., 2020b). As for *A. vescoiana*, the type specimen was described by Bourguignat as having been collected near Konya in central Anatolia. However, this has been considered by many authors as a misplacement, given that the species was never recorded anywhere near this area (Pallary, 1939; Schutt, 1983). We concur with these authors and for this reason, we consider that this species only occurs in the middle and lower sections of the Tigris and Euphrates. We had not surveyed this region, and therefore, no access to materials of *A. vescoiana* for genetic analyses. Nevertheless, given its distinctive shell morphology, we recognize it as a valid species (Table 3).

In the present study, the ML and BI *Anodonta* COI + 16S + 28S phylogenies exhibited similar topologies, retrieving three well-supported clades from specimens in the study area (Fig. 11). Both COI ML and BI phylogenies for the *Anodonta* presented similar topologies, retrieving four clades that were defined as MOTUs by all species delimitation methods (Sup. Fig. 5; Sup. Table 5). Uncorrected *p*-distances among the delineated MOTUs ranged from 7.7% (between *A. cygnea* and *A. exulcerata*) to 14.5% (between *A. anatina* and *A. exulcerata*) (Sup. Table 8).

The three MOTUs occurring in the area are here recognized as valid species, i.e., *Anodonta anatina*, *A. cygnea*, and the newly described *A. seddoni* sp. nov. (see formal description in the taxonomic account section). While *Anodonta anatina* has a wide distribution from European Turkey in the west to the Arax basin in eastern Anatolia and the southern Caspian Iranian wetlands in Iran, *A. cygnea* is restricted to Northwest Turkey and *A. seddoni* sp. nov. to the central Anatolian Plateau (Fig. 11).

Anodonta anatina is divided into five haplogroups with allopatric distributions (Fig. 12). One in the River Maritsa basin, one in the northwest Anatolian basins of Sakarya, Susurluk, and Karamenderes, one in Southern Anatolia until the River Orontes basin, and two more divergent haplogroups, one in the upper Euphrates and the other in the Arax and southern Caspian basins (Fig. 12). All previously available nominal *Anodonta* taxa available for the study area, including those from the south and eastern Caspian basins until now considered as *Anodonta cyrea* Drouët, 1881, are here synonymised under *A. anatina* based on the shell morphology and the observed type specimens (Sup. Appendix 1).

Anodonta cygnea is restricted to the Rivers Maritsa and Sakarya basins in Turkey, although its distribution might extend east by the Black Sea coast given that no surveys were made in the area (Fig. 13). Contrary to the distribution of the species across Europe, it showed some geographic structure, with all sequenced haplotypes being unique to the study region (Fig. 13).

Anodonta seddoni sp. nov. is here formally described (see taxonomic account) for specimens collected in Kocaçay Deresi one of the inlets of Lake Beyşehir (Figs. 5 and 13). The species is related to the Pan-European *A. cygnea*, and *A. exulcerata* present in Italy and coastal Adriatic countries (Figs. 11 and 13; Froufe et al., 2017). The species distribution should extend to most freshwater habitats on the Central Anatolian Plateau because *Anodonta* specimens previously collected from Lake Beyşehir and the Ereğli Marshes (Kinzelbach, 1989) should

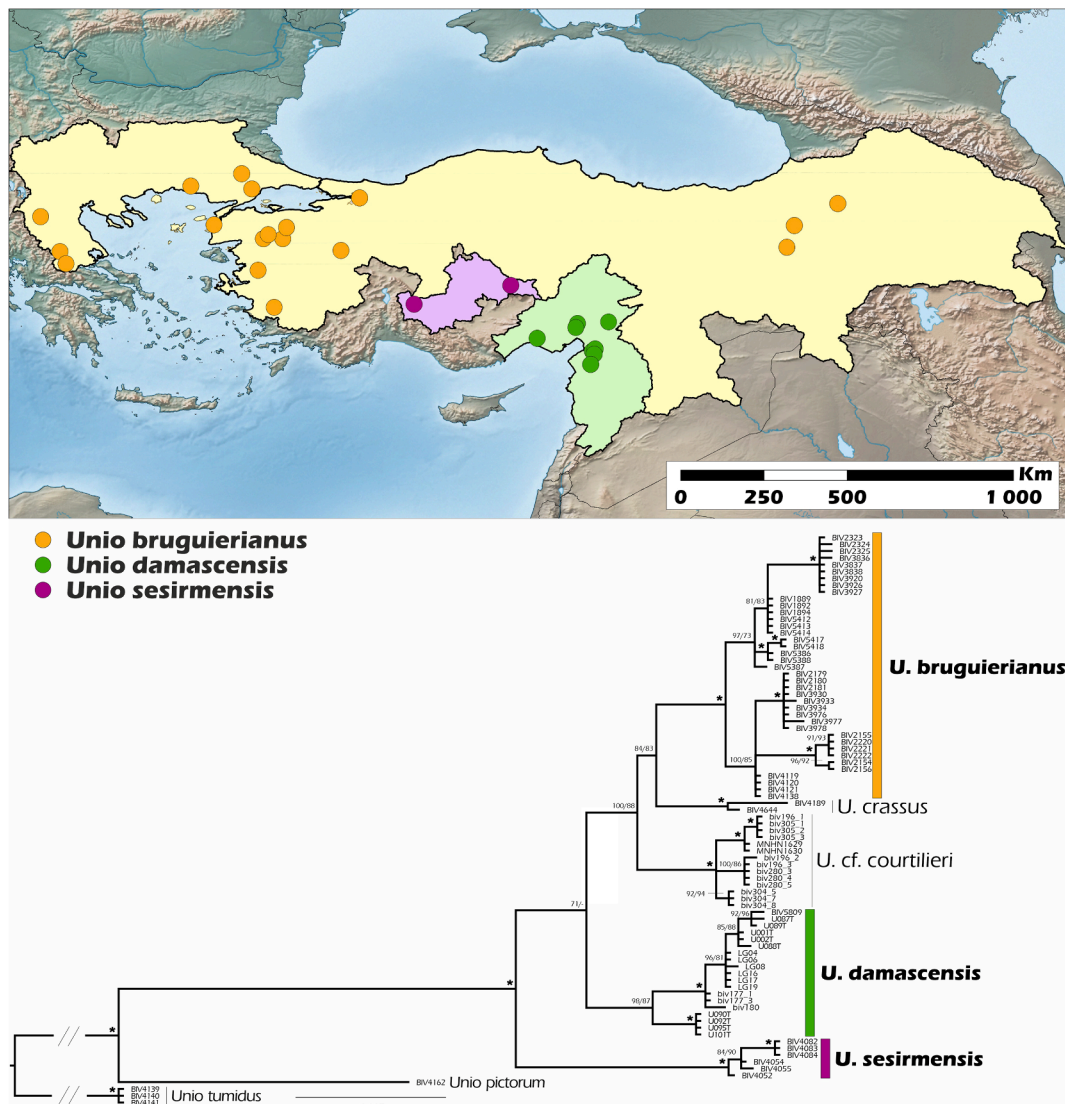


Fig. 6. TOP: Distribution map of all *Unio* (*crassus*-group) species depicted across the main river basins in shaded colours and coloured dots representing sampled populations. BOTTOM: Bayesian consensus tree for the *Unio* (*crassus*-group) species inferred from the combined COI + 16S + 28S gene fragments. The values by the nodes indicate Bayesian posterior probability percentage/Maximum Likelihood ultrafast bootstrap values. Values over 95% are represented by an asterisk. Taxa and sequence reference from the study area are represented in bold.

belong to the same species. Kinzelbach (1989) considered this species as an unnamed subspecies of *Anodonta palustris* (originally described for freshwater habitats of France and England) which is a junior synonym of *A. anatina*. This author already recognized that this form had a concentric umbo sculpture more similar to *Anodonta cygnea* and unlike the remaining forms of *A. palustris*. However, he refrained to describe it formally and kept it as *Anodonta palustris* ssp. (Kinzelbach, 1989). Given that no additional name was found for *Anodonta* populations in this region, the species is here newly described (see taxonomic account).

Gonideinae Ortman, 1916

Leguminaia Conrad, 1865

Leguminaia, although being restricted to the study area, had also been over-described (Sup. Appendix I). Haas (1969) recognized this and lumped all available nominal species into two main forms that he recognized as species, i.e. *Leguminaia wheatleyi* Lea, 1862 and *L. saulcyi* (Bourguignat, 1852). However, the only difference found by Haas (1969) was that the shell shape in *Leguminaia wheatleyi* was elongated while it was shorter and almost rhombic in *L. saulcyi*. Furthermore, *L. wheatleyi* was described as occurring mainly in the Tigris and Euphrates basin, while *L. saulcyi* occurred in the coastal basins of Israel,

Lebanon, and Syria. More recently, a single *Leguminaia* specimen collected from the upper Euphrates near Erzurum showed considerable differences in shell shape from the two previously known forms and thought to belong to a distinct new species from the upper Euphrates reaches (Falkner, 1994).

In the present study, both ML and BI *Leguminaia* COI + 16S + 28S phylogenies exhibited similar topologies, retrieving two well-supported clades from specimens in the study area (Fig. 14). Within one of these clades, three divergent specimens from the Karasu river, tributary of the Orontes made an additional well-supported subclade (Fig. 14).

Both COI ML and BI phylogenies for the *Leguminaia* presented similar topologies, retrieving three MOTUs by all species delineation methods (Sup. Fig. 6; Sup. Table 6), one for the specimens collected in River Murat, Euphrates Basin (here recognized as *Leguminaia anatolica* sp. nov.), a second including all specimens of the River Orontes and Lake Gölbaşı (here recognized as *L. cf. wheatleyi*), and a third for three specimens collected in the River Karasu, Orontes basin (with unclear taxonomic status and here referred to as *L. cf. saulcyi*) (Sup. Fig. 6). Uncorrected *p*-distances among the delineated MOTUs ranged from 2.8% (between *L. cf. wheatleyi* and *L. cf. saulcyi*) to 9.0% (between *L. cf.*

Unio bruguierianus

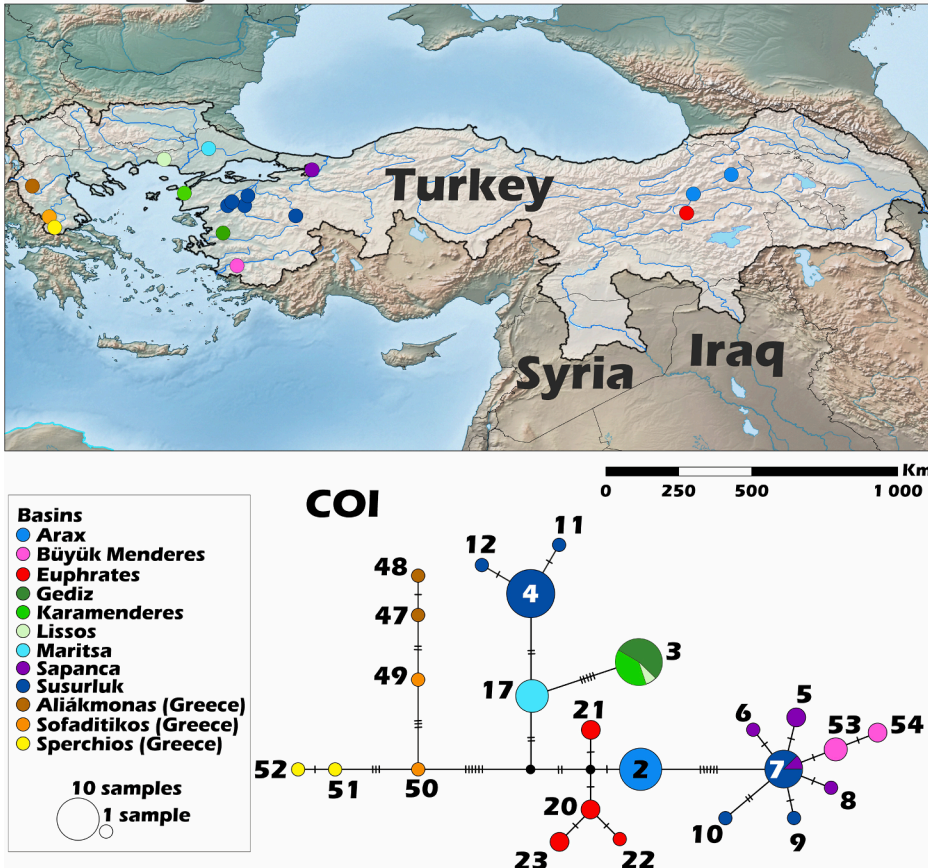


Fig. 7. TOP: Distribution map of *Unio bruguierianus* in the study area, depicting its potential distribution across the main river basins in white and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *U. bruguierianus* sequences (Sup. Table 3). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin.

Unio sesirmensis

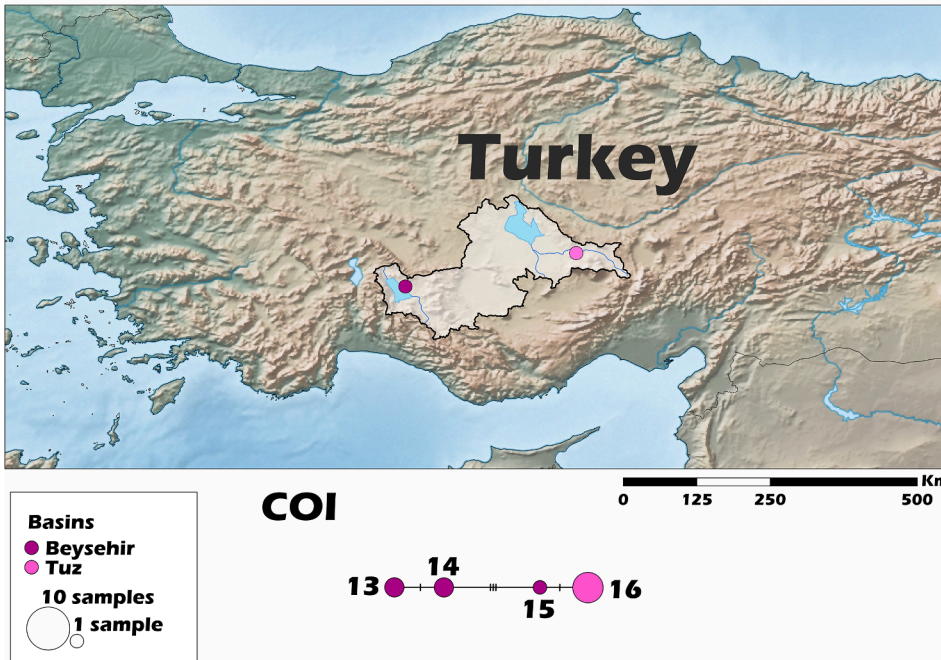


Fig. 8. TOP: Distribution map of *Unio sesirmensis* in the study area, depicting its potential distribution across the main river basins in white and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *U. sesirmensis* sequences (Sup. Table 3). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin.

saulcyi and *Leguminaia anatolica* sp. nov.) (Sup. Table 8).

The results from our molecular analyses did not show any concordance between shell shape and species discrimination methods between

both lineages. i.e. *Leguminaia* cf. *wheatleyi* and *L. cf. saulcyi*, found in the River Orontes basin, and sympatry in the River Karasu (Figs. 14 and 15). Also, both the COI + 16S + 28S and the COI phylogenies (Fig. 14 and

Unio damascensis

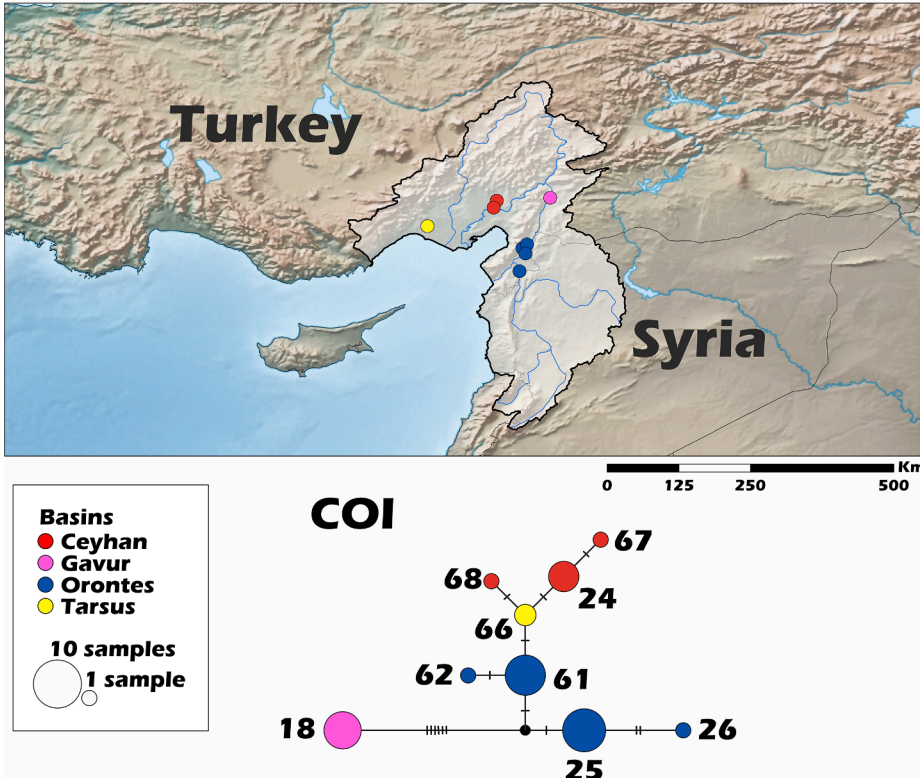


Fig. 9. TOP: Distribution map of *Unio damascensis* in the study area, depicting its potential distribution across the main river basins in white and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *U. damascensis* sequences (Sup. Table 3). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin.

Unio tumidus

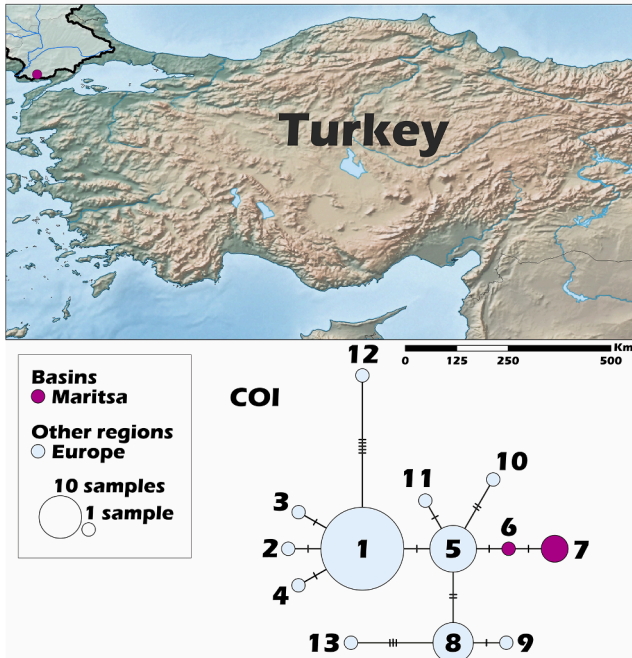


Fig. 10. TOP: Distribution map of *Unio tumidus* in the study area, depicting its potential distribution across the main river basins in white and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *U. tumidus* sequences (Sup. Table 4). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin.

Sup. Fig. 6) were not well resolved in separating these lineages and therefore a more detailed work with more markers and samples is needed to verify the taxonomic status of both taxa.

Given the lack of reliable morphological characters to identify the two lineages collected from the Orontes basin and the polyphyly of *Leguminaia cf. wheatleyi*, it is difficult to establish which one corresponds to *Leguminaia cf. wheatleyi* and *L. cf. saulcyi*. We attributed *Leguminaia cf. wheatleyi* to the taxon present in the three surveyed populations in the Rivers Orontes and the Karasu (Fig. 14) because it is represented by more central haplotypes in the COI + 16S + 28S phylogenies and COI haplotype network (H2, H5, H6, H7, H9, and H10, Fig. 14), which probably represents the species present in the middle and lower sections of the Tigris and Euphrates, considered as the origin of the genus (Falkner, 1994). We then attribute *Leguminaia cf. saulcyi* to the sequenced specimens collected only in the River Karasu, Orontes basin in sympatry with *L. cf. wheatleyi*. Nevertheless, these assumptions are still fragile, and it is crucial to obtain specimens and molecular data from populations in the middle reaches of the Tigris and the Nahr Al-Kebir, closer to the type localities to test them.

Given that the previous species determinations are flawed, the distribution here proposed and based on previous records should be considered with caution. *Leguminaia cf. saulcyi* was originally distributed in the Orontes basin and other coastal basins of Syria, Lebanon, and Israel (Haas, 1969). Given that most of these coastal rivers are now dried or highly impacted by pollution, the species should have been extirpated in most of them, possibly only remaining in the Orontes and Nahr Al-Kebir basins (Fig. 14). *Leguminaia cf. wheatleyi* was mainly distributed in the middle reaches of the Tigris and Euphrates border but with sporadic records across the whole basin (Fig. 14).

The specimens sequenced from the River Murat in the upper Euphrates were collected far away from the type localities of previous nominal taxa. Therefore, we decided to describe them as belonging to a novel distinct species based on shell morphological and molecular differences with the other two taxa (see Taxonomic account section; Sup.

Table 3

List of species within the study region, closest related taxa, and country-level presence for Armenia (AR), Azerbaijan (AZ), Israel (IL), Iran (IR), Iraq (IQ), Jordan (JO), Lebanon (LB), Syria (SY), and Turkey (TR). Native (NAT); Endemic (END); *No molecular data available.

Taxon	Mean COI <i>p</i> -distance % (nearest neighbour)	Countries							
		AR	AZ	IL	IR	IQ	JO	LB	SY
UNIONINAE									
Unionini Rafinesque, 1820									
<i>Unio</i> (<i>pictorum</i> -group)									
<i>Unio pictorum</i> (Linnaeus, 1758)	2.3 (<i>Unio eucirrus</i>)								NAT
<i>Unio delicatus</i> Lea, 1863	3.1 (<i>Unio terminalis</i>)							NAT	NAT
<i>Unio eucirrus</i> Bourguignat, 1857	2.3 (<i>Unio pictorum</i>)								END
<i>Unio hueti</i> Bourguignat, 1855	3.0 (<i>Unio tigridis</i>)								END
<i>Unio terminalis</i> Bourguignat, 1852	3.1 (<i>Unio delicatus</i>)			NAT			NAT		NAT
<i>Unio tigridis</i> Bourguignat, 1852	3.0 (<i>Unio hueti</i>)				NAT	NAT			NAT
<i>Unio</i> (<i>crassus</i> -group)									
<i>Unio bruguierianus</i> Bourguignat, 1853	2.8 (<i>Unio cf. courtilieri</i>)	NAT	NAT		NAT	NAT			NAT
<i>Unio damascensis</i> Lea, 1863	3.2 (<i>Unio cf. courtilieri</i>)							NAT	NAT
<i>Unio sesirmensis</i> Kobelt, 1913	5.1 (<i>Unio cf. desectus</i>)								END
<i>Unio</i> (<i>tumidus</i> -group)									
<i>Unio tumidus</i> Phillipson in Retzius 1788	10.4 (<i>Unio foucauldianus</i>)								NAT
Anodontini Rafinesque, 1820									
<i>Anodonta cygnea</i> (Linnaeus, 1758)	7.7 (<i>Anodonta exulcerata</i>)								NAT
<i>Anodonta anatina</i> (Linnaeus, 1758)	13.5 (<i>Anodonta cygnea</i>)	NAT	NAT		NAT				NAT
<i>Anodonta seddoni</i> sp. nov.	8.3 (<i>Anodonta cygnea</i>)								END
<i>Anodonta vescoiana</i> Bourguignat, 1856 *	–					NAT			
GONIDEINAE									
Gonideini Ortmann, 1916									
<i>Leguminaia cf. wheatleyi</i> Lea, 1862	2.8 (<i>Leguminaia cf. saulcyi</i>)				NAT	NAT			NAT
<i>Leguminaia cf. saulcyi</i> (Bourguignat, 1852)	2.8 (<i>Leguminaia cf. wheatleyi</i>)						NAT	NAT	NAT
<i>Leguminaia anatolica</i> sp. nov.	8.2 (<i>Leguminaia cf. wheatleyi</i>)								END
Lamprotulini Modell, 1942									
<i>Potomida semirugata</i> (Lamarck, 1819)	3.0 (<i>Potomida acarnanica</i>)			NAT			NAT	NAT	NAT
Incertae sedis									
<i>Pseudodontopsis euphratica</i> (Bourguignat, 1852) *	–				NAT	NAT			

Table 8). Falkner (1994) already mentioned that a distinct species occurred in the isolated upper Euphrates basins. Although the specimen he analysed came from the River Karasu (Euphrates basin) and ours from the River Murat, these basins are adjacent, connecting at their mouths and sharing the same ecological conditions. We consider that these specimens from both rivers should belong to the same species, i.e. the newly described *Leguminaia anatolica* sp. nov.. This species should occupy the Rivers Murat and Karasu basins in the upper Euphrates (Figs. 14 and 15), although systematic surveys in both basins, coupled with molecular work, are needed to confirm this distribution.

Potomida Swainson, 1840

The genus *Potomida* has been previously studied in the area, with a single species being recognized across the whole study area, i.e. *P. semirugata* (Lamarck, 1819) (Froufe et al., 2016a). We found and sequenced two additional populations to those used in the previous study, one in a coastal spring system in Antalya and another from the River Karasu, a tributary of the Orontes.

Both COI ML and BI phylogenies for the *Potomida* presented similar topologies, retrieving a single MOTU by all species delineation methods (Sup. Fig. 7; Sup. Table 7).

Potomida semirugata was originally distributed in Southern Anatolia and the coastal rivers of Syria, Lebanon, and Israel (Fig. 16). It was also present in the endorheic basins of the River Jordan in Israel and the River Kuweik in Syria. However, there are no recent records of the species from the Jordan, and the River Kuweik was almost completely dried in the 1960s. Given that the Jordan basin represents a distinct ecoregion for fish and *Unio* species, it might also mean that the *Potomida* present here belongs to a distinct species. However, given the lack of molecular data and shell morphological similarity, we kept the Jordan populations within *Potomida semirugata*. Additional nominal taxa of the Arax River Basin, which flows in Northeast Anatolia to the Caspian Sea, were previously placed under *Potomida*, i.e. *Unio rothi* var. *komarowi* O. Boettger, 1880 and *Unio armeniacus* Kobelt, 1911 (Haas, 1969; Graf and Cummings, 2021). However, in the current study, many samples were

collected from the type locality of *U. r.* var. *komarowi* in the Kars River by the city of Kars and in the Arax main channel, and no *Potomida* specimens were found. Kobelt (1911) in the *U. armeniacus* original description, considered the species to belong to the *U. gregorii* (= *Unio crassus*) group and not to the *Potomida littoralis* group of species. Later, Modell (1951) also considered that these *Potomida* taxa from the Arax were peculiar forms of *Unio bruguierianus*. Our examination of the original description and type specimens supports this placement. Specimens of *Unio bruguierianus* abundantly found in Kars and Arax rivers have a more rounded shell shape and are more similar to *Potomida*. Therefore, we have considered both names *Unio rothi* var. *komarowi* and *Unio armeniacus* as junior synonyms of *Unio bruguierianus* and excluded the Arax basin from the distribution of the *Potomida* genus (Sup. Appendix I).

Pseudodontopsis Kobelt, 1913

This genus is only represented by a single species *Pseudodontopsis euphratica* (Bourguignat, 1852) (Fig. 15) occurring in the middle and lower sections of the Tigris and Euphrates basin. Unfortunately, we were not able to survey this area and thus no available molecular data is available. Based on a dichotomous key with simple morphological characters it has been placed within Gonideinae (Pfeiffer et al., 2019).

3.2. Biogeography

An exhaustive biogeographic reconstruction of the region would require data from more species in bordering regions since those could influence possible colonisation paths or the interpretation of putative geological drivers. However, we were able to postulate broad patterns by using previously published phylogeographical and geological data, together with the time-calibrated phylogeny of local diversity and a phylogenetic prediction of biological exchanges among regions in the study area.

Most of the Unionidae genera recorded in the region appear to be ancient (Late Cretaceous to Paleogene) derivatives of the East Asian

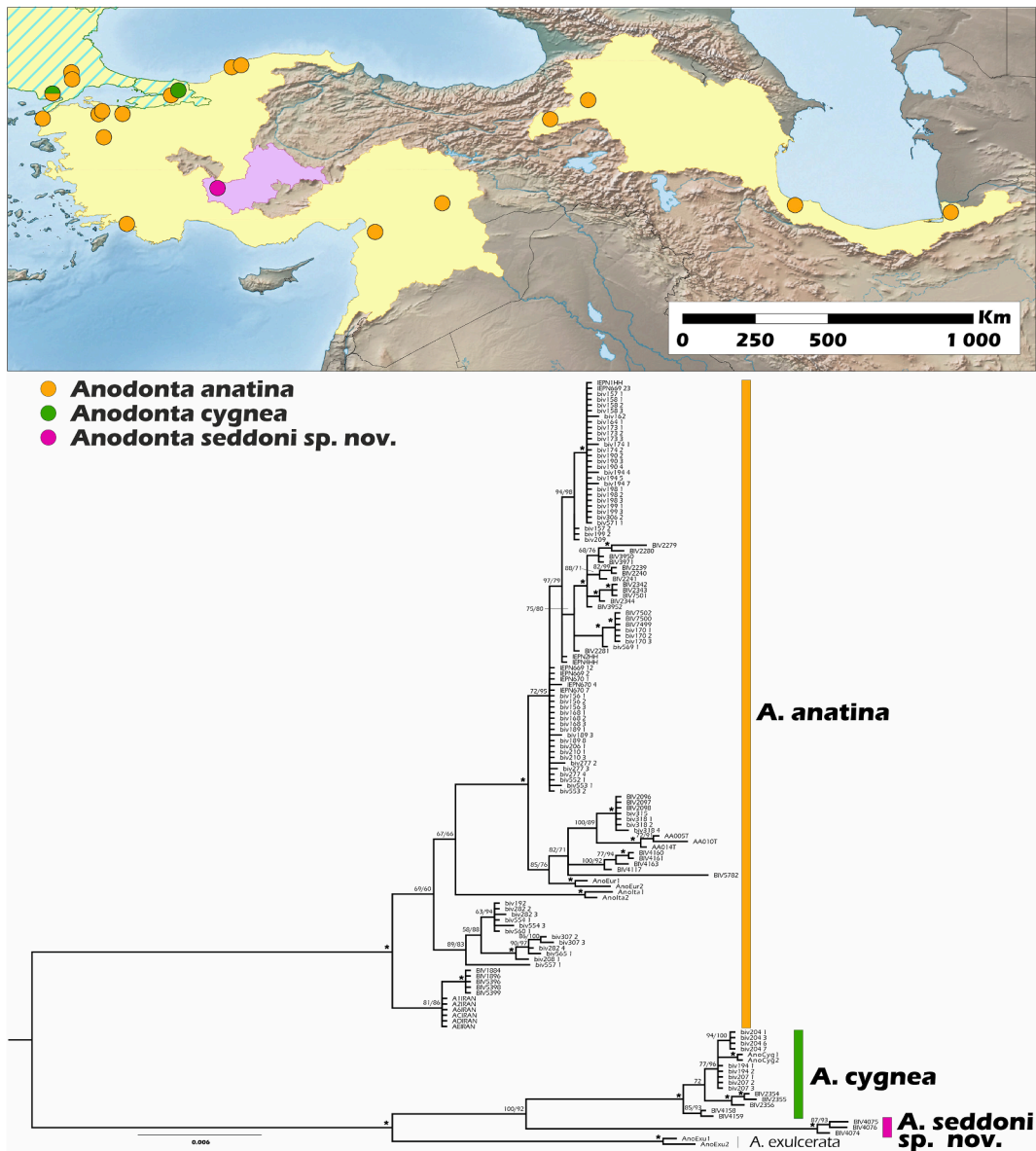


Fig. 11. TOP: Distribution map of all *Anodonta* species depicted across the main river basins in shaded colours and coloured dots representing sampled populations. BOTTOM: Bayesian consensus tree for the *Anodonta* species inferred from the combined COI + 16S + 28S gene fragments. The values by the nodes indicate Bayesian posterior probability percentage/Maximum Likelihood ultrafast bootstrap values. Values over 95% are represented by an asterisk. Taxa and sequence reference from the study area are represented in bold.

fauna (Bolotov et al., 2017; Froufe et al., 2020). In particular, *Potomida* is a sister group to the *Lamprotula* Simpson, 1900 + *Pronodularia* Starobogatov, 1970 clade (Froufe et al., 2020). In its turn, a group, which contains *Leguminaia*, *Microcondylaea* Vest, 1866 (Southern Europe), and *Gonidea* Conrad, 1857 (western North America), shares a common ancestor with the East Asian genus *Sinosolenia* Bolotov et al., 2021 (Lopes-Lima et al., 2017; Froufe et al., 2020). The genus *Unio* is most closely related to a clade containing several East Asian genera such as *Nodularia* Conrad, 1853, *Cuneopsis* Simpson, 1900, and *Schistodesmus* Simpson, 1900 (Lopes-Lima et al., 2020). Finally, *Anodonta* is closely related to a group of North American genera but the entire clade derived from common ancestors with the tribes Lancelariini and Cristariini, both of which are clades endemic to East Asia (Lopes-Lima et al., 2020). Thus, it suggests that there were at least four independent colonization events of freshwater mussels from East Asia towards Europe, the Middle East, and the Mediterranean (the ancestors of: i) *Unio*, ii) *Anodonta*, iii) *Leguminaia* + *Microcondylaea* + *Pseudodontopsis*, and iv) *Potomida*), most

likely through coastal basins of the proto-Paratethys Sea, existing in Central Asia during the Late Cretaceous and Paleogene epochs (Kaya et al., 2019, 2020).

Based on the genera present in the study region, the radiations of the Unioninae *Anodonta* and *Unio* appear to have a European origin with several waves of range expansion into Anatolia south to the Middle East since the early Miocene (Modell, 1951; Falkner, 1994). Species within the *pictorum*-group of *Unio* seem to have further expanded their distribution to the Horn of Africa via the coastal route or by the Nile, and then to Southern Africa (Lopes-Lima et al., 2017b).

The appearance of the Central Anatolian lacustrine system, the separation of the proto-basins, and the link between west Anatolia and eastern Greece likely affected the distribution of the distinct Unionidae species and lineages that are highly concordant with the ecoregion division previously determined for fish (Abell et al., 2008) (Fig. 1). The more widespread *Anodonta* and *Unio* species follow this pattern, with mutually exclusive species or lineages in each of the ecoregions, likely

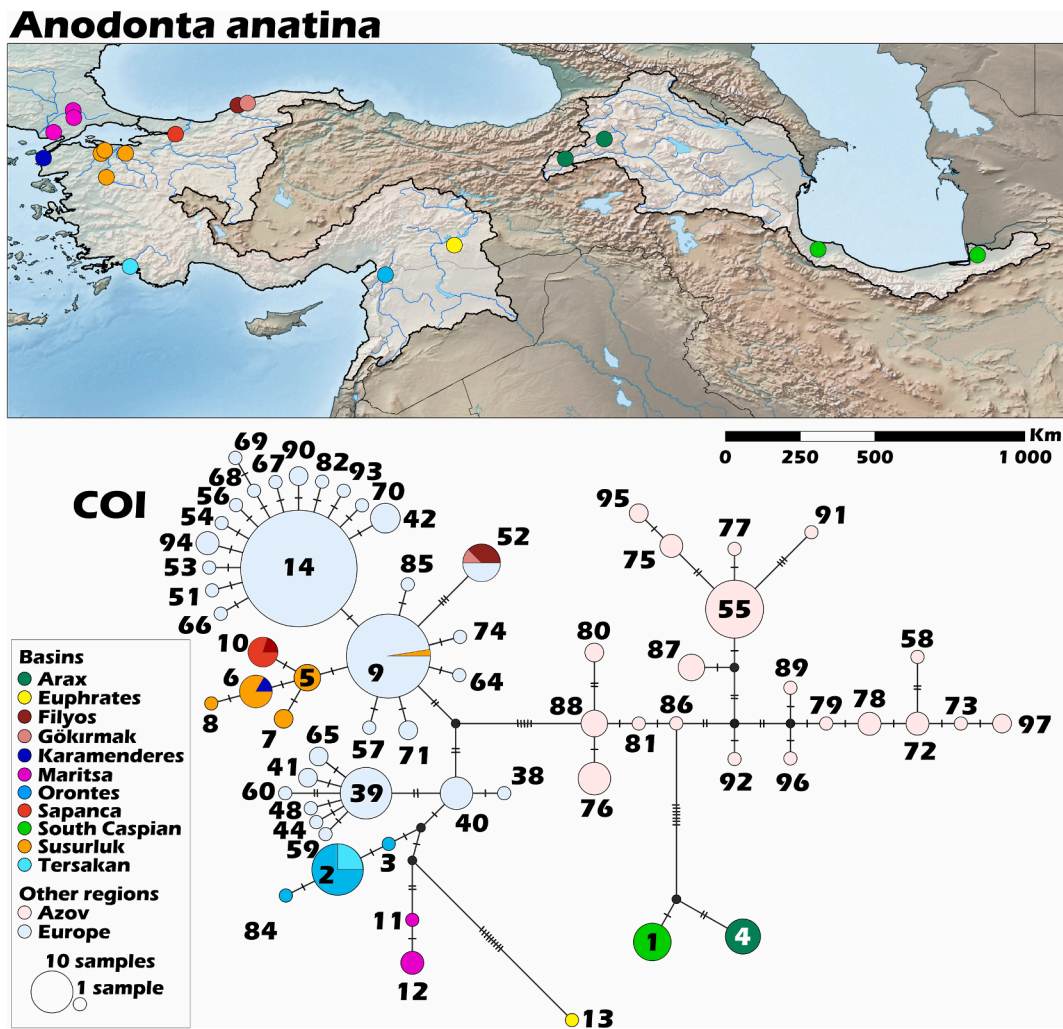


Fig. 12. TOP: Distribution map of *Anodonta anatina* in the study area, depicting its potential distribution across the main river basins in white and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *A. anatina* sequences (Sup. Table 5). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin.

split earlier by the isolation of the central Anatolian lakes and the following geological uplift and separation of the main proto-basins in the early Pliocene. This can be seen in the early-branching patterns of the endemic *Anodonta seddoni* sp. nov. and *Unio sesirmensis*, suggesting they are relicts of the Neogene Central Anatolian lakes (Meijers et al., 2020) (Figs. 1, 6, and 11) but also in the more recent speciation of other *Unio* and the divergent lineages of *U. bruguierianus*, *U. pictorum*, and *A. anatina* endemic to the study area (Figs. 2–13; Table 3).

The colonization of Western Anatolia by *Unio pictorum sensu stricto* possibly occurred in two main waves of range expansion from central Europe (Figs. 1 and 3). The first was in the Pleistocene, possibly through the series of freshwater lakes that connected Western Anatolia and Greece (Krijgsman et al., 2020) and then a post-glacial second wave via the Black Sea coastal route (Bektas et al., 2020). While the ancient distribution expansion can be seen in the divergent haplotypes of the Greek populations and the Rivers Maritsa and the closely related species *Unio eucirrus* from River Karamenderes in Turkey, the recent invasion is evident in the related or shared haplotypes of the remaining populations in Anatolia with those from Central Europe (Fig. 3). Sister taxa *Unio terminalis* and *U. delicatus* (Fig. 2) should represent an early (mid-Miocene) divergent wave of the *pictorum*-group (Fig. 1). The formation of the River Jordan basin possibly isolated populations of the common ancestor of both species that then diverged into *U. terminalis* (Fig. 1A).

The Jordan River basin, formed during the Pliocene, has a complex history involving marine transgressions and Late Pleistocene lacustrine phases (Por and Dimentman, 1985; Vai, 2016). A later (Pliocene) range expansion gave rise to *Unio huerti* and *U. tigridis* from the Upper and Lower Euphrates basin (Figs. 1 and 2).

Unio bruguierianus probably benefited from very recent connections of the upper reaches of the Tigris and Euphrates with the Arax and Black Sea coastal basins, leading to several successful transitions among ecoregions (Fig. 1C), having a higher number of haplotypes than most species in the region (Fig. 7). This species' distribution possibly expanded in the Pleistocene from the Caucasus area through Black Sea drainages south into the Euphrates-Tigris upper reaches colonizing Sakarya and Susurluk Basins, and southwest to the Büyük Menderes Basin. Another range expansion wave colonized the Maritsa and other Thracian basins and then crossed back to Anatolia by the Dardanelles strait. This can be seen in the common single haplotype (Fig. 7: H3) from Lissos, Gediz, and Karamenderes and its closer relationship to most of the Susurluk (Fig. 7: H4, H11, and H12) and the single Maritsa haplotypes (Fig. 7: H17). The remaining *crassus*-group species *U. damascensis* probably derives from an ancient Euphrates-Orontes connection while the relict *Unio sesirmensis* was probably originated earlier during the formation of the Central Anatolian Basin in the Miocene (Figs. 1 and 6).

The phylogeographic patterns of *Anodonta anatina* are more difficult

Anodonta cygnea & Anodonta seddoni sp. nov.

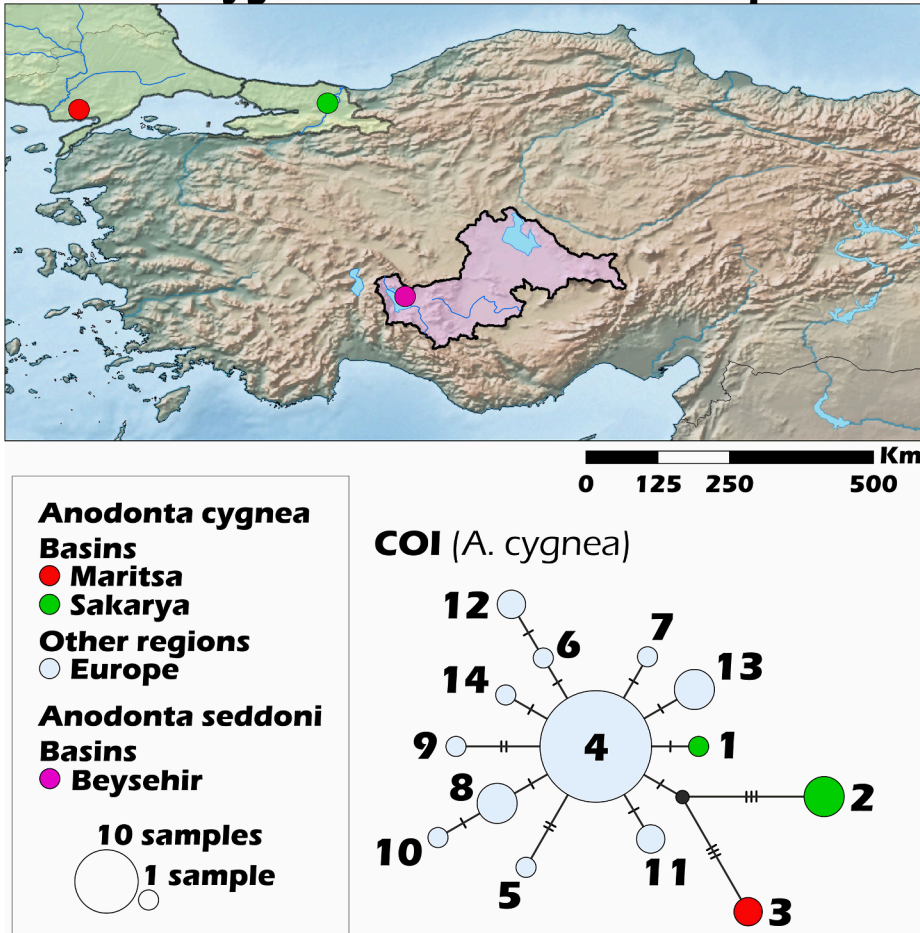


Fig. 13. TOP: Distribution map of *Anodonta cygnea* and *Anodonta seddoni* in the study area, depicting their potential distribution across the main river basins shaded in green (*A. cygnea*) and pink (*A. seddoni*) and outlined in black. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *A. cygnea* (Sup. Table 5). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to interpret. While the South Anatolian, Orontes, and upper Euphrates populations are related to those in the Maritsa basin and some Central European populations (Fig. 12), a more recent connection of the northwest Anatolian populations with Central Europe is likely, given their shared and related COI haplotypes (Fig. 12). The populations from the Arax and southern Caspian wetlands are interrelated and form a COI haplotype cluster. These last populations should have a distinct origin probably somewhere around the Caspian basin, given their lower divergence with populations from this region (Fig. 12).

The Gonideinae genera *Leguminaia* and *Pseudodontopsis* are restricted to the study area, while *Potomida* occurs in the Western Mediterranean and mainland Greece as well (Figs. 14–16; Froufe et al., 2016a).

The Tigris-Euphrates system holds the highest endemism and species richness of freshwater mussels in the entire region. Originating in the mid-Miocene and initially flowing west into the Neotethys (now Mediterranean) Sea, the ancient Proto-Euphrates shifted east towards the Mesopotamian Basin. This system was then formed by the Late Miocene (Demir et al., 2007; Stow et al., 2020). The freshwater mussel faunal interchanges of the Euphrates with the Orontes, can be explained by the formation of a patchwork of connections 6,000 years ago (Por and Dimentman, 1985), but this event still needs further research (Bektas et al., 2020). The River Orontes, having the highest density of fish endemism in the Mediterranean, also had past faunal connections with rivers Seyhan, Ceyhan, and Jordan (Por and Dimentman, 1985), explaining the distribution of *Unio delicatus*, *U. damascensis*, *Potomida semirugata*, and the southern Anatolian *Anodonta anatina* lineage.

The genus *Leguminaia* is thought to have originated in the Tigris-Euphrates basin expanding its range northwest to the Orontes during the Pliocene probably by a temporary connection with the Kuweik and

the Euphrates via Karasu, and south to the Levant coastal basins and the Jordan River (Kinzelbach, 1987).

The fossil record exhibits *Potomida*-like fossils widely distributed in lakes of eastern Europe during the late Tertiary, eventually becoming extinct at the end of this period (Haas, 1969). The Eastern-Western Mediterranean disjunct distribution pattern of *Potomida* may be explained by a dispersal event during the brief freshwater phase of the Mediterranean Salinity Crisis, known as ‘Lago Mare’ (Froufe et al., 2016a; Stoica et al., 2016; Araujo et al., 2017). The dispersal of the Eastern Mediterranean *Potomida semirugata* west to Muğla Province (Figs. 14–16), and south to the River Jordan Basin, probably indicate Plio-Pleistocene range expansions, reflecting the patterns of *Unio delicatus* and *U. terminalis*, respectively. *Potomida semirugata* has been also recorded from the endorheic Kuweik River (Kinzelbach, 1987) which provides further evidence for the existence of recent connections between the Orontes and the Kuweik basins.

Besides the ancient Unionidae Asian range expansions at the end of the Cretaceous/beginning of the Paleogene, other links of the fauna of the region with some Asian taxa are doubtful. The monotypic genus *Pseudodontopsis* and *Anodonta vescoiana* are both endemic to the Lower Tigris-Euphrates basin and no molecular data is available for these species. While the former seems to have an ancient origin similar to the other gonidein genera *Potomida* and *Leguminaia*, the latter is placed by several authors under the East Asian genus *Sinanodonta* Modell, 1945 due to umbo inflation, large size, and general shell outline (e.g. Modell, 1945, 1951; Kinzelbach, 1987). However, this is highly unlikely since all *Sinanodonta* species are native to Asia, east of the River Mekong, therefore with a huge gap between both this region and Mesopotamia (Lopes-Lima et al., 2020).

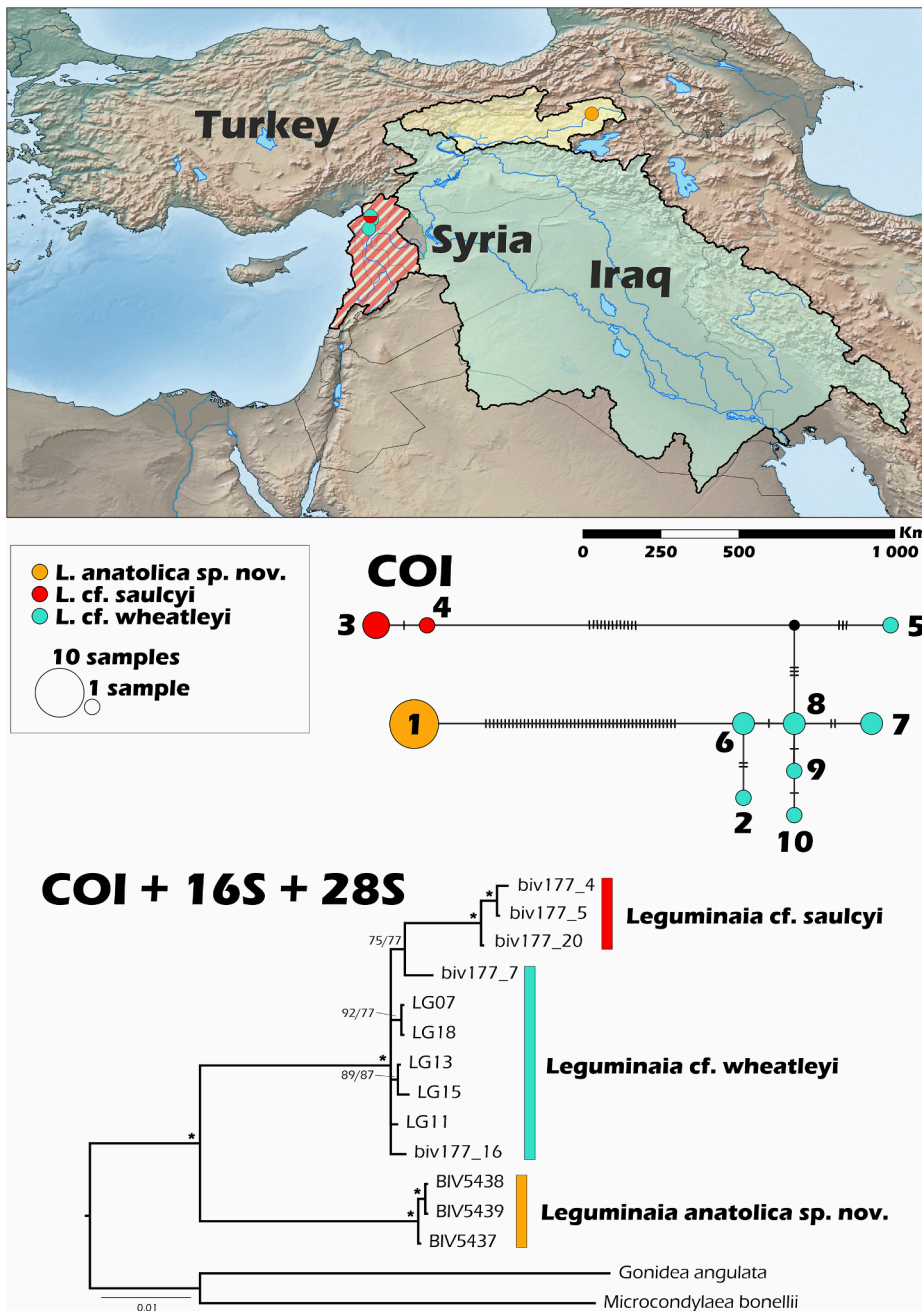


Fig. 14. TOP: Distribution map of *Leguminaia* species in the study area, depicting their potential distribution across the main river basins shaded in different colours and outlined in black. Coloured dots represent sequenced populations. CENTRE: COI Haplotype (TCS) network showing the relationships of all new and previously published *Leguminaia* sequences (Sup. Table 6). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution. Colours represent the proportion of individuals from each basin. BOTTOM: Bayesian consensus tree for the *Leguminaia* species inferred from the combined COI + 16S + 28S gene fragments. The values by the nodes indicate Bayesian posterior probability percentage/Maximum Likelihood ultrafast bootstrap values. Values over 95% are represented by an asterisk. Taxa and sequence reference from the study area are represented in bold.

3.3. Conservation implications

The present results have extensive conservation implications regarding the freshwater mussels of the Eastern Mediterranean and adjacent countries. The freshwater habitats in this region are highly threatened by water shortage in the semi-arid and arid regions in the south. In the Levant coastal basins, freshwater mussels have been mostly extirpated due to the high levels of pollution and lack of water (Mienis, 2002; Lopes-Lima and Seddon, 2014). Also, in the Tigris and Euphrates basins, the many dams planned, under construction, or already constructed, and the increased water extraction for agriculture and urban purposes is posing an increasing pressure on freshwater habitats and taxa (Munro and Tournon, 1997). Drainage projects, such as the Ereğli, Amik, and the Hula wetlands for agriculture, have been implemented since the 1950s leading to the extirpation of large freshwater mussel populations. In Western Anatolia, the increasing human density and

touristic development are leading to higher pollution levels and the increased transformation of coastal rivers into channels and ditches. Therefore, there is an urgent need to make action plans for the conservation of freshwater species in this region and freshwater mussels in particular. Species conservation status assessments are urgently needed to identify the species here recognized, which should be prioritized for conservation and protection. Extensive surveys on *Anodonta seddoni* sp. nov., *U. hueti*, and *Leguminaia anatolica* sp. nov. are required to accurately evaluate their distribution ranges and subsequently their conservation status. Restricted range species like *Anodonta seddoni* sp. nov., *Unio eucirrus*, *Unio sesirmensis*, and *Unio terminalis* require urgent protection measures. Information provided here on the genetic structure of each species is also important to help in future prioritization of populations for conservation and management actions, such as translocations, propagation, and assisted migration.

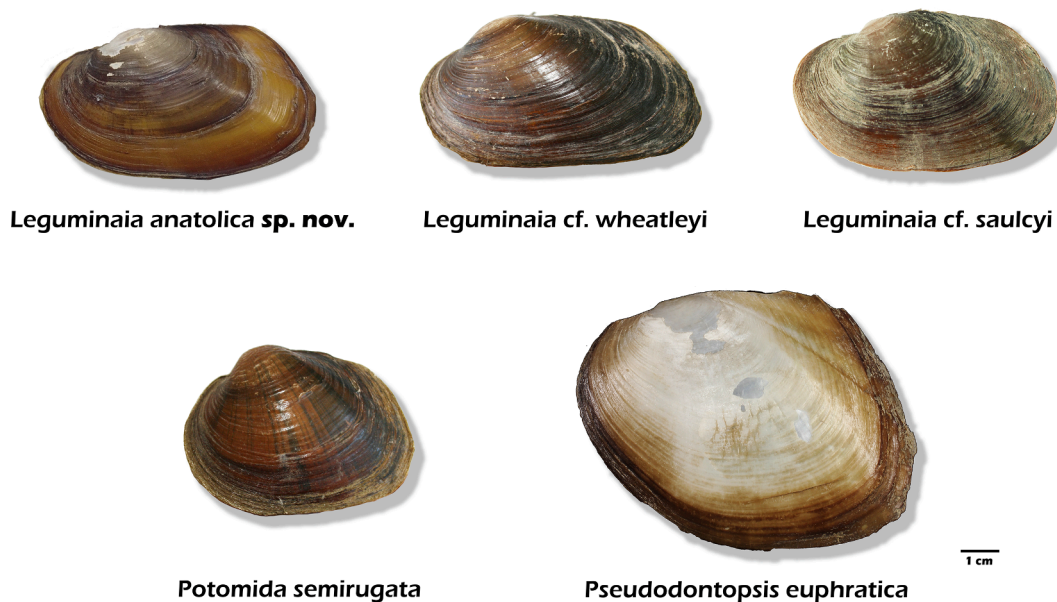


Fig. 15. Shells of Eastern Mediterranean freshwater mussels within the Gonideinae subfamily. *Leguminaia* cf. *wheatleyi* (unnamed stream by Lake Gölbaşı, Turkey – LG007); *Leguminaia* cf. *saulcyi* (River Karasu, Orontes, Turkey – BIV177_17); *Leguminaia anatolica* **sp. nov.** (River Murat, Turkey – Holotype: NCSM 107598); *Potomida semirugata* (unnamed stream by Lake Gölbaşı, Turkey – PL626); *Pseudodontopsis euphratica* (River Tigris, Baghdad, Iraq - not sequenced Holotype: MNHN_IM_2000-1691). Specimen codes refer to [Sup. Fig. 1](#).

4. Taxonomic account

Family Unionidae Rafinesque, 1820

Subfamily Unioninae Rafinesque, 1820

Tribe Unionini Rafinesque, 1820

Genus *Unio* Philipsson in Retzius, 1788

Type species: *Mya pictorum* Linnaeus, 1758 (orig. desig.) = *Unio pictorum* (Linnaeus, 1758)

Comments: This genus includes nine species in the Eastern Mediterranean region: *Unio pictorum* (Linnaeus, 1758), *U. delicatus* Lea, 1863, *U. eucirrus* Bourguignat, 1857, *U. hueti* Bourguignat, 1855, *U. terminalis* Bourguignat, 1852, *U. tigridis* Bourguignat, 1852, *U. bruguierianus* Bourguignat, 1853, *U. damascensis* Lea, 1863, and *U. sesirmensis* Kobelt, 1913.

Tribe Anodontini Rafinesque, 1820

Genus *Anodonta* Lamarck, 1799

Type species: *Mytilus cygneus* Linnaeus, 1758 (orig. desig.) = *Anodonta cygnea* (Linnaeus, 1758)

Comments: This genus includes four species in the Eastern Mediterranean region: *Anodonta cygnea* (Linnaeus, 1758), *Anodonta anatina* (Linnaeus, 1758), *Anodonta seddoni* **sp. nov.**, and *Anodonta vescoiana* Bourguignat, 1856.

***Anodonta seddoni* Gürlek, Kebapçı & Lopes-Lima sp. nov.**

Type material: Holotype NCSM 107600 deposited in the North Carolina State Museum of Natural Sciences, Raleigh, United States of America: Collected at Kocaçay Deresi, Konya, Turkey, 37.710335 N, 31.736377 E, 1135 m a.s.l., 10.vi.2016, M.E. Gürlek leg. Shell length 75.7 mm, height 48.4 mm, width 25.4 mm. Paratype (1 specimen) NCSM 107597 deposited in the North Carolina State Museum of Natural Sciences, Raleigh, United States of America, same collection data as the holotype.

Etymology: This species is dedicated to our esteemed colleague Dr. Mary Seddon for her comprehensive efforts on the global conservation of molluscs.

Diagnosis: The new species is similar to *A. anatina* in shell outline, but differs by presenting concentric rings in the umbo instead of the wavy rugae characteristic of *A. anatina*. It differs from other similar species recognized in the genus, i.e. *A. anatina*, *A. cygnea*, and

A. exulcerata by multiple fixed nucleotide substitutions in the COI and 16S markers.

Description: Shell ovate in outline and thin across the whole shell. Anterior margin rounded, posterior margin narrowly rounded, ventral margin convex. Pseudocardinal and lateral teeth absent. Umbo sculpture consisting of simple concentric rings, umbo cavity deep, narrow. Shell length 75.7–77.1 mm, height 48.4–49.5 mm, width 25.4–28.5 mm. Periostracum colour varies from horn-coloured to brownish yellow, nacre white, and translucent. Mantle is white to yellowish. Foot dull-orange and yellowish. Visceral mass yellowish, outer gills brownish and dark.

Habitat and ecology: Inhabits mud and clay substrata rich in vegetation.

Distribution: Occurs in Lake Beyşehir and surrounding streams, possibly occurring in other freshwater systems in the Central Anatolian Plateau.

Subfamily Gonideinae Ortmann, 1916

Tribe Gonideini Ortmann, 1916

Genus *Leguminaia* Conrad, 1865

Type species: *Monocondyloea mardinensis* Lea, 1865 (orig. desig.) = *Leguminaia wheatleyi* (Lea, 1862)

Comments: This genus includes three species in the Eastern Mediterranean region: *Leguminaia wheatleyi* (Lea, 1862), *Leguminaia saulcyi* (Bourguignat, 1852), and *Leguminaia anatolica* **sp. nov.**

***Leguminaia anatolica* Gürlek, Kebapçı and Lopes-Lima sp. nov.**

Type material: Holotype NCSM 107598 deposited in the North Carolina State Museum of Natural Sciences, Raleigh, United States of America, collected at Murat river, Tutak-Ağrı, Turkey, 39.536614 N, 42.808281 E, 1570 m a.s.l., 5.v.2018, M.E. Gürlek leg. Shell length 75.3 mm, height 42.4 mm, width 21.7 mm. Paratype NCSM 107599 (n = 3), deposited in the North Carolina State Museum of Natural Sciences, Raleigh, United States of America, same collection data as the holotype. **Additional material examined:** Eight specimens: GCMAE M1 (n = 8), same data as the holotype deposited in the Gürlek collection (Mehmet Akif Ersoy University, Turkey)

Etymology: This species is named after Anatolia, the large peninsula in western Asia, making up the majority of modern-day Turkey's territory on this continent.

Potomida semirugata

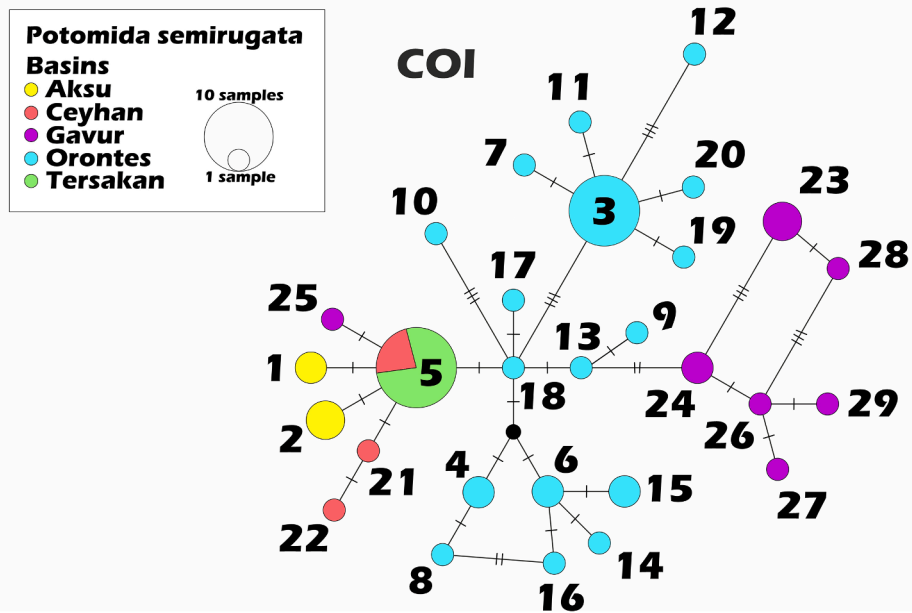
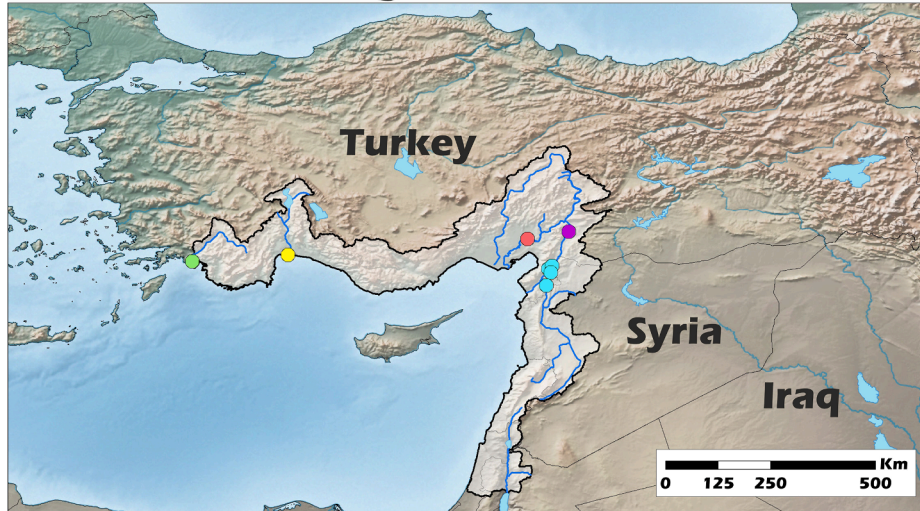


Fig. 16. TOP: Distribution map of *Potomida semirugata* in the study area, depicting its potential distribution across the main river basins shaded in different colours. Coloured dots represent sequenced populations. BOTTOM: COI Haplotype (TCS) network showing the relationships of all new and previously published *Potomida semirugata* (Sup. Table 7). Circle size is proportional to the observed haplotype frequencies each dash indicates a nucleotide substitution.

Diagnosis: The new species *Leguminaia anatolica* sp. nov can be distinguished from *Leguminaia saulcyi* by being more elongated in shell shape and a straight posterior dorsal margin generally parallel to the ventral margin. *Leguminaia anatolica* sp. nov has more curved convex ventral margins that are typically straight or slightly convex in *Leguminaia wheatleyi*. The posterior ridge is also more clearly marked in *L. anatolica* sp. nov. than in the two other *Leguminaia* species.

Description: Shell semi-elliptical, anterior end rounded, posterior dorsal margin straight and rounded at the posterior end. Ventral margin curved. Umbo sculpture not very evident due to umbo erosion. Periostracum colour varies from yellow, red to dark brown. Nacre white with yellow umbo cavity. Shell length 58.5–91.4 mm, height 55.2–37.8 mm, width 29.3–21.1 mm. Mantle, foot, and visceral mass are creamy-white, outer gills dark.

Habitat and ecology: Inhabits mud, clay substrata, occasionally with stone cover.

Distribution: Occurs in the River Murat, possibly occurring in the associated streams of this and other tributaries of the Upper Euphrates

basin.

Tribe Lamprotulini Modell, 1942

Genus *Potomida* Swainson, 1840

Type species: *Mysca corrugata* Swainson, 1840 (orig. desig.) = *Potomida littoralis* (Cuvier, 1798)

Comments: This genus includes a single species in the Eastern Mediterranean region, i.e. *Potomida semirugata* (Lamarck, 1819).

Incertae sedis within Gonideinae

Genus *Pseudodontopsis* Kobelt, 1913

Type species: *Unio euphraticus* Bourguignat, 1852 (orig. desig.) = *Pseudodontopsis euphratica*

Comments: This monotypic genus includes only the type species *Pseudodontopsis euphratica* (Bourguignat, 1852) endemic to the Eastern Mediterranean region (Fig. 15).

5. Conclusions

The present study represents a significant advance in our

understanding of the diversity and evolutionary relationships of the Eastern Mediterranean Unionidae and adjacent regions. We reveal a much higher diversity than expected for the region. Based on these results, four major biogeographic regions can be delimited, i.e. the Tigris and Euphrates, Southern, Central, and Northern Anatolia. This division and its subdivisions are coherent with what has been previously described for fish, which are the main (obligatory) hosts and dispersal vectors. The ancestral lineages of this fauna colonized the Middle East and the Mediterranean from East Asia during the Late Cretaceous – Paleogene. While the southern regions of Anatolia and the Middle East host regional endemic radiations of *Leguminaia*, *Microcondylaea*, *Potomida*, and *Pseudodontopsis*, waves of *Unio* and *Anodonta* repeatedly expand towards Northern Europe and Northern Africa. Central Anatolia is a place of great endemism in terrestrial and freshwater ecosystems and suggests even earlier colonizations of *Unio* and *Anodonta*. Finally, Northern Anatolia is more closely linked with Europe supported by the multiple more recent secondary European range expansions of several taxa. This knowledge is incredibly important for future conservation planning in terms of delineating taxa and areas of conservation importance. The conservation status of the species here defined should be assessed independently, especially for species here identified with restricted ranges. Areas of high endemism such as the upper Euphrates and Tigris and the central Anatolian Lake systems are also worth special attention, given their high level of threat.

The present study also provides an important baseline for future research to improve our knowledge about the bio- and phylogeographic patterns of the Unionidae from the Eastern Mediterranean. Further studies should aim to integrate wider surveys on the surrounding regions of the Balkan, East Anatolian, Syrian, Iraqi, and Iranian basins with more robust genomic approaches.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2021.107261>.

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