



Potamophilus acuminatus (Fabricius, 1792): distribution update in North Africa confirmed by COI barcoding sequencing (Coleoptera, Elmidae)

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Abstract

Potamophilus acuminatus (Fabricius, 1792) is here recorded for the first time from Morocco and the recent distribution in Slovakia is updated. The North African distribution was hitherto based only on two larvae from Tunisia. DNA-barcoding confirmed the identification of specimens from Morocco after comparing ten sequences of *P. acuminatus* from Germany, France and Slovakia. The COI haplotype of the Moroccan samples was quite divergent (0.033–0.035 uncorrected *p*-distance) compared with European specimens. These high *p*-distances suggest the existence of a different intraspecific lineage. A brief morphological diagnosis of adults and larvae is given.

Key words: Riffle Beetles, distribution, sampling methods, cytochrome oxidase I, Morocco, Europe

Introduction

The genus *Potamophilus* Germar (1811) represents a small genus of the family Elmidae with one Palaearctic and five oriental species (Kodada *et al.* 2022). The type species *P. acuminatus* (Fabricius, 1792) is described from the River Saale near Halle (Germany) and is widely distributed in the Palaearctic Region, where it has been reported in 28 countries (Jäch *et al.* 2016).

Adults are active fliers, and their life span lasts only several weeks. They are not strictly aquatic and often dwell on submerged wood or other riparian vegetation above or below the water line. They can be found singly or swarming in aggregations containing several to hundreds of specimens, thus resembling swarming bees (observed on a partly submerged wooden pile in the River Latorica in Slovakia, J. Kodada, pers. obs.). Some adults have also been collected on shore in the splash zone on downstream surfaces of rocks (Buczyński *et al.* 2011). Specimens can fly readily and quickly when disturbed and they feed on the leaves of aquatic mosses, such as *Fontinalis antipyretica* L. However, the larvae are obligatory xylophagous as they feed on submerged dead wood of deciduous trees in running water and their development lasts up to several years (Gerber 1993). Standard macroinvertebrate benthological sampling methods are often insufficient for collecting adults and require additional searching on partly submerged wood, rootlets, growth on macrophytes, beating and sweeping the riparian vegetation and using light traps during the several weeks of their life spans (generally in June–August for Central Europe).

Many European records are old, dating back to the first half of the previous century and the species is classified as strongly in danger of extinction in some countries (e.g., Spain: Ribera 2000; Austria: Jäch *et al.* 2005; Czechia: Boukal 2005). However, newer data for Austria, Hungary, Poland and Serbia show that the species is not as rare as formerly assumed (Kálmán *et al.* 2009; Csabai *et al.* 2010; Buczyński *et al.* 2011; Jäch *et al.* 2013; Novaković *et al.* 2016).

Few records exist from the Iberian Peninsula and these are based on only 20 specimens from 10 localities with irregularly distributed populations, which are more common in the southern half of the Peninsula (Rico 1996; Millán *et al.* 2014). From North Africa, only two larvae were collected in Joumine aval, northern Tunisia (Touaylia *et al.* 2010).

We present here the first record of *P. acuminatus* collected recently in Morocco. The fresh material also enabled obtaining DNA barcodes used to support specimen identification and comparing DNA sequences with samples from other regions.

Material and methods

Collecting was performed in several streams in the Tétouan Province of northern Morocco (2022) and the small, lowland Rivers Ipeľ (2022) and Latorica (1992, 2017) of south-western and south-eastern Slovakia, respectively. We used a D-frame hand-net, applying a multi-habitat scheme to sample major habitats proportionally according to their presence within a sampling reach. Additionally, we sampled microhabitats, such as submerged moss on stones, exposed submerged rootlets of riparian vegetation and submerged wood. We sampled by positioning the net and disturbing the substrate for a distance that equalled the square of the frame width upstream of the net. When taking samples from moss, we positioned the net immediately downstream of the substrate while the moss was gently combed by hand, enabling the specimens to float into the net. Submerged rootlets were similarly combed by hand or with a soft brush. After several replicates, we rinsed the collected material 2–3 times with stream water and sorted specimens directly in the field. The submerged wood of physically manageable size was taken ashore and inspected for the presence of larvae. The best results were obtained by waiting for the wood pieces to dry until the larvae start to move. Specimens were immediately preserved in 96% ethanol.

The material examined is deposited in the Ján Kodada Collection, Comenius University in Bratislava, Slovakia (CKB) and in the Collection of Nard Bennis (CBT) in Laboratoire Ecologie, Systématique, Conservation de la Biodiversité (LESCB), Université Abdelmalek Essaâdi, Tétouan, Morocco.

As the mature larvae were covered by dense secretions/debris, we treated some specimens with proteolytic enzymes and some with lactic acid. The treatment with lactic acid for four days gave the best results significantly and these larvae were used for photography.

We examined specimens using a Leica M205C stereomicroscope with fusion optics and diffuse lighting at magnifications up to 160×. Habitus photographs were produced with a Zeiss Axio-Zoom V–16 stereomicroscope, diffuse LED lighting and a Canon 5D Mark IV camera. The habitus photographs were created by using the image-stacking software ZereneStacker. The morphological terminology follows Kodada *et al.* (2016).

Three species from two genera of the family Elmidae were included in the DNA barcoding. The dataset comprised data obtained from *Potamophilus acuminatus*, collected during sampling in Morocco and Slovakia; their origin and GenBank accession numbers are summarised in Table 1. Sequences from French and German specimens were retrieved from the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>). In the dataset, data of *Potamophilus kelabitensis* Kodada, Boukal, Vďáčný, Goffová & Ondřejková, 2022 from Sarawak and *Elmis maugetii* Latreille, 1802 from Slovakia were included.

For DNA extraction, tissue samples containing the abdomen or one leg with the coxa and attached muscle were used. DNA was isolated with the E.Z.N.A.® Tissue DNA kit (OMEGA Bio-tek Inc., Norcross, GA, USA), following the manufacturer's protocol. The barcoding fragment of the 5' end of the mitochondrial gene coding for cytochrome *c* oxidase subunit I (COI) was amplified with the standard primers LCO1490 and HCO2198 (Folmer *et al.* 1994). PCR reactions were set according to the protocols in 'The Beetle DNA Lab' (https://zsm-entomology.de/wiki/The_Beetle_D_N_A_Lab). The quality of PCR products was checked by electrophoresis in 1% TBE agarose gel. PCR products were purified with the Exo-CIP™ Rapid PCR Cleanup Kit (New England Biolabs® Inc., Ipswich, MA, USA) according to the manufacturer's instructions. Macrogen Europe B.V. (Amsterdam, The Netherlands) sequenced purified PCR products from both directions. Sequences were trimmed and assembled into contigs in Geneious 6.1.8 (<https://www.geneious.com>).

The nucleotide sequences were aligned with the Muscle codon algorithm, considering the invertebrate mitochondrial genetic code. Phylogenetic trees were constructed in maximum likelihood (ML) and neighbour joining (NJ) methods. ML analyses were conducted in IQ-TREE ver. 1.6.10 (Nguyen *et al.* 2015) on the IQ-TREE web

server (<http://iqtree.cibiv.univie.ac.at/>) (Trifinopoulos *et al.* 2016). Prior parameters individual molecular markers were estimated with IQ-TREE and then implemented with the ‘prset’ command. The GTR+F+I+G4 model with 1,000 bootstrap repetitions was assigned. The NJ tree was constructed using the K2P model and 1,000 bootstrap repetitions in MEGA-X software (Kumar *et al.* 2018). ML and NJ trees were computed as unrooted and were rooted with the outgroup taxa in FigTree ver. 1.2.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). The maximum likelihood tree, based on amino acid sequences, was measured by the JTT matrix-based model with bootstrap support (1,000 replicates) and performed in MEGA 7 software. Pairwise uncorrected *p*-distances of nucleotide sequences were calculated in MEGA-X (Kumar *et al.* 2018). Sequences are available in the GenBank database. A haplotype network was calculated from the COI alignment using the TCS method (Clement *et al.* 2000) in PopART ver. 1.7 (Leigh & Bryant 2015). Mutations were displayed as numbers along the network edges.

TABLE 1. Origin and GenBank accession numbers of COI sequences used in analyses and haplotype names in haplotype network.

| Species | Sample | Haplotype | Locality | GenBank accession no |
|----------------------------------|------------|-----------|---|----------------------|
| <i>Potamophilus acuminatus</i> | JK1084 | H1 | Slovakia, Salka env., Ipeľ river | OP325456 |
| <i>P. acuminatus</i> | JK1085 | H1 | Slovakia, Salka env., Ipeľ river | OP325457 |
| <i>P. acuminatus</i> | JK1250 | H1 | Slovakia, Latorica river | OP325458 |
| <i>P. acuminatus</i> | JK1251 | H1 | Slovakia, Latorica river | OP325459 |
| <i>P. acuminatus</i> | JK1141 | H4 | Morocco, Rif Mountains, Oued Laou, Afertane | OP325460 |
| <i>P. acuminatus</i> | JK1249 | H4 | Morocco, Rif Mountains, Oued Laou, Afertane | OP325461 |
| <i>P. acuminatus</i> | HM422045.1 | H3 | Germany, Eglfing, near Ach river | HM422045.1 |
| <i>P. acuminatus</i> | KM448250.1 | H1 | France, Brecy-Brieres, Aisne | KM448250.1 |
| <i>P. acuminatus</i> | KU907242.1 | H2 | France, Brecy-Brieres, Aisne | KU907242.1 |
| <i>P. acuminatus</i> | KU908112.1 | H1 | France, Brecy-Brieres, Aisne | KU908112.1 |
| <i>P. acuminatus</i> | KU912942.1 | H1 | France, Brecy-Brieres, Aisne | KU912942.1 |
| <i>P. acuminatus</i> | KU913515.1 | H1 | France, Brecy-Brieres, Aisne | KU913515.1 |
| <i>Potamophilus kelabitensis</i> | JK540 | | Malaysia, Sarawak, Bario env. | MW649804 |
| <i>Elmis maugetii</i> | JK637 | | Slovakia, Záhorie env., Rudava river | MW684762 |

Results

Potamophilus acuminatus (Fabricius, 1792)

Material examined. Adults. 2 ♂♂, 1 ♀ (CKB, CBT): MOROCCO, Tétouan Province, Afertane Oued Laou 35°21'11" N, 5°11'9" W, 30 m a.s.l., 9. VI. 2022, J. Kodada leg.; 4 ♂♂, 5 ♀♀, 41 specimens (CKB): SLOVAKIA, Latorica River, ca. 48°30'19.213"N, 22°2'50.328"E, 15. 7. 1992, J. Kodada leg.

Larvae. 5 specimens (CKB): SLOVAKIA, Salka env., Ipeľ River, 47°53'06.5"N 18°45'47.9"E, 6. 05. 2022, J. Kodada & D. Selnekovič leg.; 10 specimens (CKB): SLOVAKIA, Vrbovka env., Ipeľ River 48°05'19.7"N 19°24'35.6"E, 26. 08. 2020 S. Krčmárik leg.; 15 specimens (CKB): SLOVAKIA, Latorica River, ca. 48°29'58.055"N, 22°3'10.355"E, 25. 8. 2017, J. Kodada leg.; 15 specimens (CKB): SLOVAKIA, Latorica River, ca. 48°29'58.055"N, 22°3'10.355"E, 15. 7. 2019, J. Kodada leg.

Potamophilus acuminatus is the largest European Elmid and the single representative of the subfamily Larainae LeConte, 1861.

Barcoding. Maximum likelihood and neighbour joining trees, inferred from the COI dataset (603 nucleotide positions), show identical topologies. Therefore, posterior probabilities (PP) were mapped along with bootstrap support on to the best-scoring ML tree. The specimens from Morocco grouped with specimens of *P. acuminatus* from western and central Europe with maximum statistical support (Fig. 1). Given the taxon sampling, the Moroccan

specimens were depicted as their nearest relatives with strong support (100% ML and NJ bootstrap). Sequences from four widely separated, allopatric populations of the well-supported *P. acuminatus* clade show pairwise genetic distances ranging from 0.000–0.035 (uncorrected *p*-distance, Table 2). Specimens were grouped into two main lineages with high statistical support, separated by a relatively high uncorrected *p*-distance (0.033–0.035). The first lineage includes specimens from France, Germany and Slovakia and the second one represents specimens from Morocco.

TABLE 2. Pairwise uncorrected *p*-distances of COI nucleotide sequences (codes without species name represent *P. acuminatus*—see Table 1).

| species | JK1084 | JK1085 | JK1250 | JK1251 | JK1141 | JK1249 | HM422045.1 |
|------------------------|--------|--------|--------|--------|--------|--------|------------|
| JK1084 | | | | | | | |
| JK1085 | 0.000 | | | | | | |
| JK1250 | 0.000 | 0.000 | | | | | |
| JK1251 | 0.000 | 0.000 | 0.000 | | | | |
| JK1141 | 0.033 | 0.033 | 0.033 | 0.033 | | | |
| JK1249 | 0.033 | 0.033 | 0.033 | 0.033 | 0.000 | | |
| HM422045.1 | 0.002 | 0.002 | 0.002 | 0.002 | 0.035 | 0.035 | |
| KM448250.1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.033 | 0.002 |
| KU907242.1 | 0.002 | 0.002 | 0.002 | 0.002 | 0.035 | 0.035 | 0.003 |
| KU908112.1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.033 | 0.002 |
| KU912942.1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.033 | 0.002 |
| KU913515.1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.033 | 0.002 |
| <i>P. kelabitensis</i> | 0.131 | 0.131 | 0.131 | 0.131 | 0.139 | 0.139 | 0.133 |
| JK540 | | | | | | | |
| <i>E. maugetii</i> | 0.179 | 0.179 | 0.179 | 0.179 | 0.177 | 0.177 | 0.181 |
| JK637 | | | | | | | |

TABLE 2. Continued.

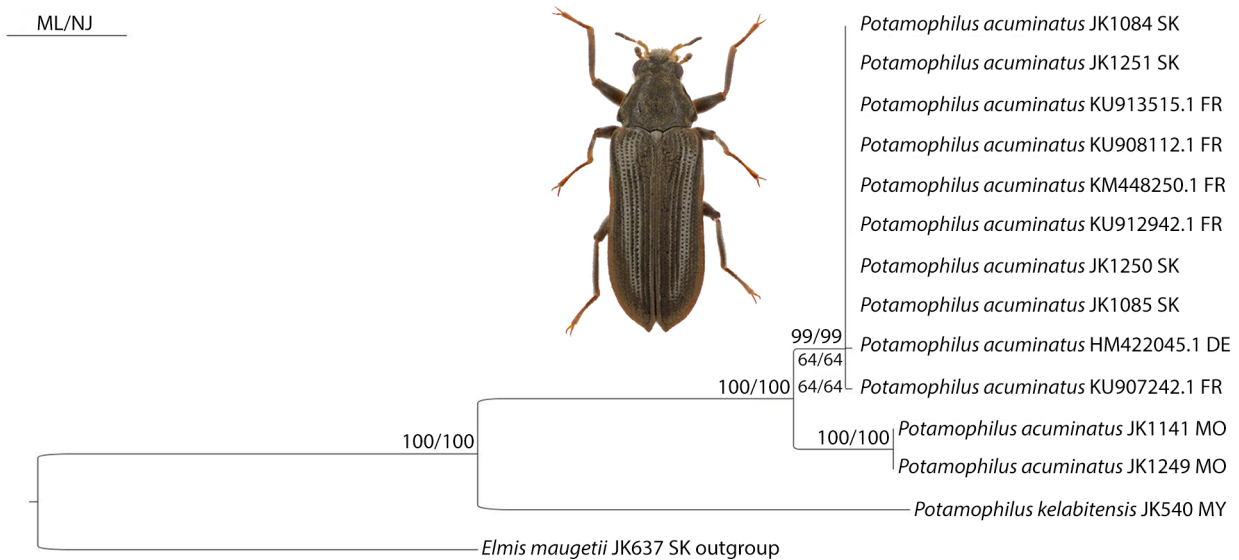
| species | KM448250.1 | KU907242.1 | KU908112.1 | KU912942.1 | KU913515.1 | JK540 | JK637 |
|------------------------|------------|------------|------------|------------|------------|-------|-------|
| JK1084 | | | | | | | |
| JK1085 | | | | | | | |
| JK1250 | | | | | | | |
| JK1251 | | | | | | | |
| JK1141 | | | | | | | |
| JK1249 | | | | | | | |
| HM422045.1 | | | | | | | |
| KM448250.1 | | | | | | | |
| KU907242.1 | 0.002 | | | | | | |
| KU908112.1 | 0.000 | 0.002 | | | | | |
| KU912942.1 | 0.000 | 0.002 | 0.000 | | | | |
| KU913515.1 | 0.000 | 0.002 | 0.000 | 0.000 | | | |
| <i>P. kelabitensis</i> | 0.131 | 0.131 | 0.131 | 0.131 | 0.131 | | |
| JK540 | | | | | | | |
| <i>E. maugetii</i> | 0.179 | 0.177 | 0.179 | 0.179 | 0.179 | 0.177 | |
| JK637 | | | | | | | |

Haplotype network analyses supported the monophyletic origin of *P. acuminatus* specimens by 71 shared nucleotide positions within the barcoding fragment. However, the haplotype from Morocco differs in 20 nucleotide

positions from the most frequent European haplotype (Fig. 2). This deep intraspecific divergence is probably congruent with the divergent geographic distribution of populations.

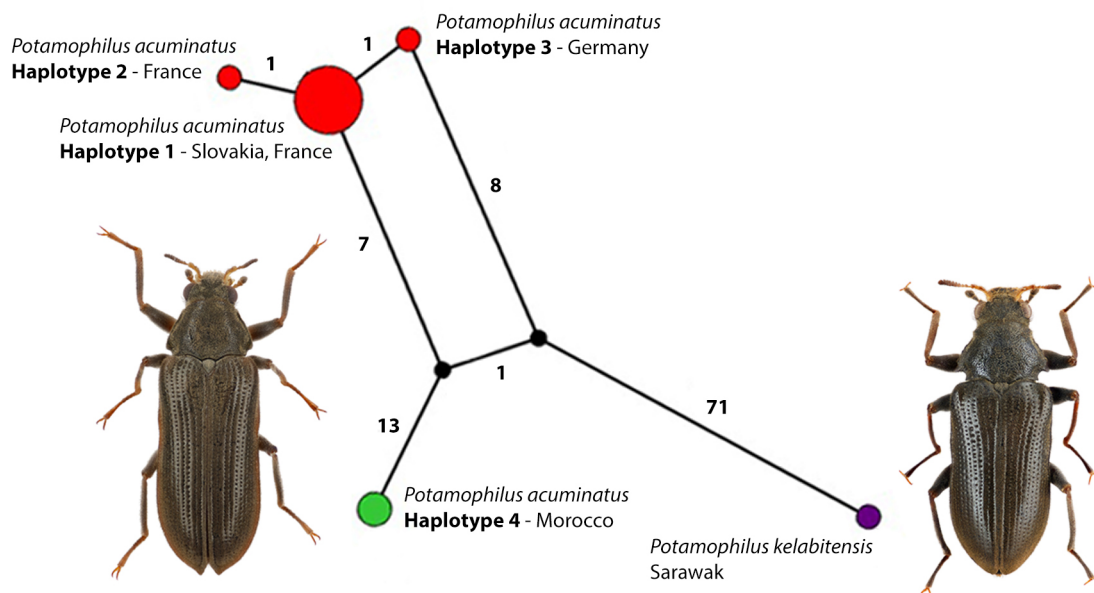
All examined adults are morphologically very similar and all barcoded specimens were grouped into a single well-supported, unstructured lineage in the tree inferred from amino acid sequences. Their very uniform genital morphology also confirms this status.

The separation of *P. kelabitensis* from *P. acuminatus* is corroborated by their deep genetic divergence in the barcoding COI gene (0.131–0.139 uncorrected *p*-distance).



1

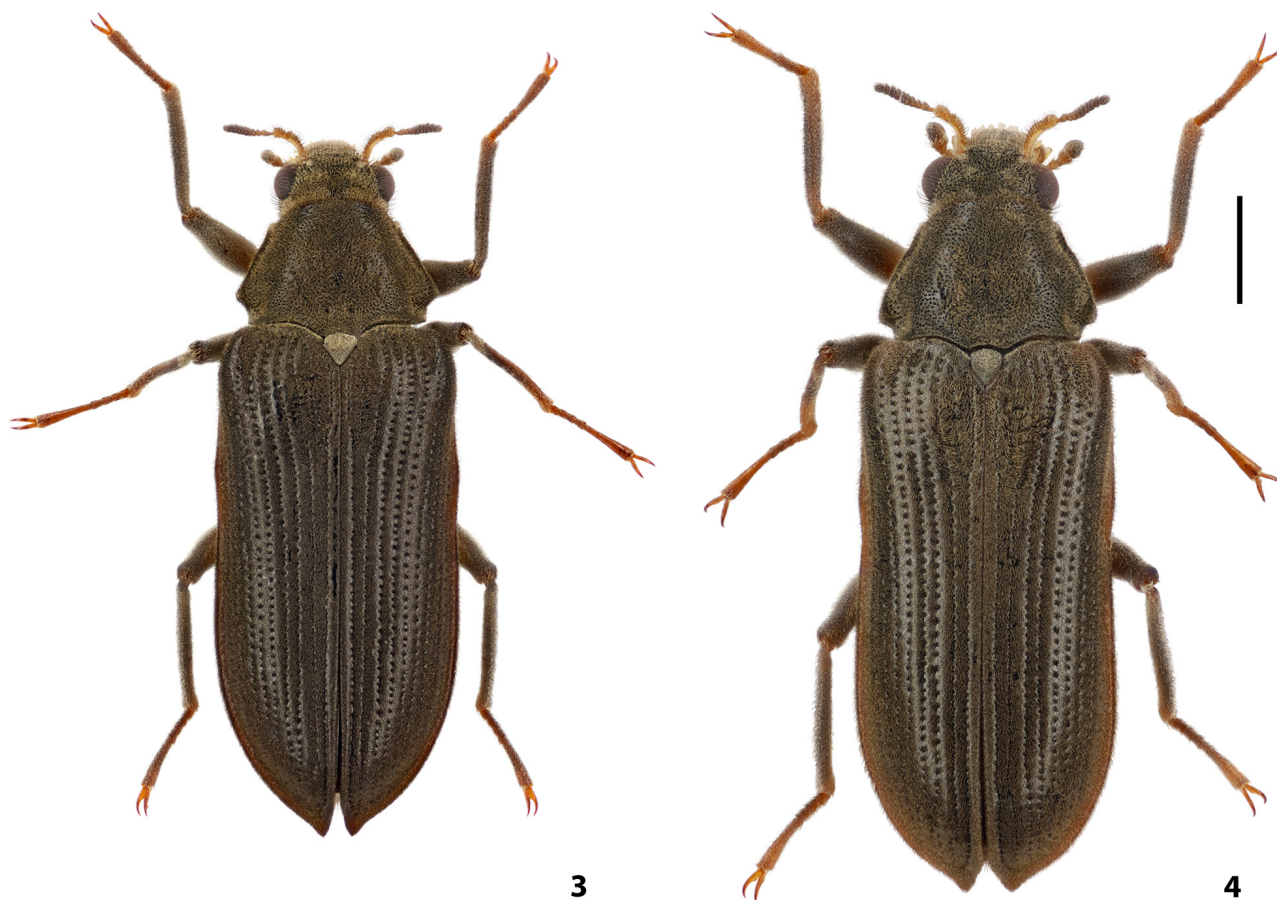
Haplotype network



2

FIGURES 1–2. 1) Phylogenetic tree based on the mitochondrial COI gene of *Potamophilus acuminatus* and *P. kelabitensis*. *Elmis maugetii* served as an outgroup taxon in analyses. Bootstrap values for ML analyses performed in IQ-Trees and distance NJ analyses performed in MEGA-X were mapped on to the best-scoring IQ-Tree; 2) TCS haplotype network of COI sequences of *Potamophilus acuminatus* and *P. kelabitensis*. Numbers along the edges represent mutational steps; small black dots represent undetected haplotypes. For specimens belonging to individual haplotype groups, see Table 1.

Diagnosis of the adult (Figs 3, 4). Body elongated, parallel-sided, length excluding head: 6.1–7.7 mm and maximal elytral width: 2.4–3.0 mm. Colour predominantly black, except reddish-brown antennal segments 1–4, distal portion of femora, trochanters, anterior face of femora, claws and abdominal apex. The surface with dense short yellowish decumbent setae and moderately long and less dense, dark suberect setae.



FIGURES 3–4. *Potamophilus acuminatus*, dorsal habitus; **3**) male from Morocco; **4**) female from Morocco. Scale bar: 1 mm.

The pronotum gradually expanded posteriad, broadest in front of the posterior margin and deeply excised in hind angles; sides smooth, not crenate, finely arched or nearly straight; the pronotal disc with broad, distinct depression in front of the scutellum. Pronotal surface with more or less elongated punctures that vary in size; longest diameter of largest punctures ca. twice the facet diameter; smallest punctures slightly smaller than facets. Punctures moderately irregularly spaced, from nearly confluent to separate by distances up to the largest puncture diameter.

Elytra long and narrow (ca. 3.6–4.0× as long as pronotum), moderately convex dorsally, with ten punctate striae and one accessory basal stria between the sutural and second stria. Sutural interval is raised in the posterior 0.8 of its length, other intervals flat to feebly convex. Strial punctures on elytra moderately coarse, sharply impressed, rounded and separated by about their diameter. Apex of each elytron produced and angulated in males while acuminate and protruding with divergent apices in females.

Meso- and metatibia are weakly sinuous; mesotibia in males usually more strongly enlarged distally with an indistinct median tubercle.

The aedeagus is trilobate and symmetrical; parameres in the apical portion are narrowed, flattened, abruptly bent ventrad and less sclerotised; apices do not reach the apex of the penis.

Diagnosis of the mature larva (Figs 7–10). Length from anterior margin of head to apex of abdomen: 12.5 mm; greatest width across metanotum: 3.0 mm. Body elongated, tapered dorsad, ventrally almost flat, dorsally convex; dorsal sagittal line present from prothorax up to sixth abdominal segment; cross-section subtriangular. Prevailing ground colour black with yellowish-brown mouth parts, antennae and claws. Head with a cluster of 5 stemmata on each side; without tooth on anterior margin between base of antenna and clypeus. Antennae short and three-segmented. Frontoclypeal suture distinct. Mandibles short and wide, very strong, with three apical teeth;

penicillus with short, dense setae. Maxilla with palpus, four-segmented; galea and lacinia separate and apex of each densely setose. Labrum with anterior margin nearly straight, with a fringe of dense short setae. Labium with palpus, short, two-segmented; postmentum undivided; gula large.

The pronotum ca. twice as wide as long, with transverse depression in the anterior third, anterolateral and posterolateral angles rounded; sides moderately arcuate, slightly converging anteriorly; surface with three pairs of smooth areas lacking granulation (signa) situated on each side of meson. Mesonotum and metanotum about 2.6× as broad as long, 0.7× as long as the pronotum and each with six pairs of signa. Margins of thoracic segments moderately explanate. Terga of all thoracic segments with two longitudinal rows of short setae situated on each meson side. Ventral side with a small subtriangular presternum. Prothorax with one anterolateral and two lateral sclerites on each side of meson and a sizable subtriangular sclerite between coxae; sclerites do not surround coxae posteriorly; lateral sclerites are well-discernible in cleared specimens. Ventral portions of both meso- and metathorax with five sclerites: one large anterior sclerite projecting widely between coxae; each lateral side with two moderately large lateral sclerites, the latter not projecting behind coxae. Legs stout, five-segmented, about 0.5× as long as thorax width, all similar in shape and length.

Abdominal terga 1–8, each with four rows of setae on each side of meson, admedian rows more distinct than sublateral ones. Sternopleural sclerites are present on segments 1–7; sclerites become progressively narrower posteriorly; ventrites 1–8 subequal in length. Terminal segment with dorsal longitudinal mesal keel, as long as combined length of the four preceding segments, gradually narrowed posteriorly; apex strongly bifurcated; operculum sub-pentagonal ca. 0.3× segment length, opercular hooks long and narrow. All spiracles, small and subequal in form and size, present anterolaterally on the mesothorax and lying more laterally on abdominal segments 1–8; all spiracles open on small tubercles.

Habitat of specimens examined (Fig. 5). **Morocco.** Three adult specimens were collected downstream of the Oued Laou River near Afertane Village. The permanent river is ca. 30 m wide, with a rocky substrate. Near the river grow native wild oleander shrubs (*Nerium oleander* L.) and bunches of *Carex* sp. Adult specimens were collected either from exposed submerged roots of *Carex* sp. or of *Nerium oleander*; although, as both plants were growing next to each other, it was impossible to identify which plant the individuals were on. Further collecting on surrounding vegetation was unsuccessful and no waterlogged wood was found in this river section. The area is subject to adverse impacts, such as domestic water pollution, grazing, extraction of water, sand and gravel, bathing and the use of detergents for washing clothes. **Slovakia.** The Latorica River flows from Ukraine to eastern Slovakia on lowlands and gives rise to the Bodrog River, itself a tributary of the Tisza River in Hungary. The river is ca. 10–15 m wide, regulated (canalised) at collecting places with sandy substrate. As riparian vegetation is dense, mainly with *Salix* sp., *Populus alba* L. and *P. nigra* L., numerous fallen branches, broken trees or exposed rootlets are in the water. Hundreds of adult specimens were observed swarming during the hot summer days in July 1992, so a sample of about 100 specimens was taken. On submerged branches, numerous large larvae were observed; *Macronychus quadrituberculatus* P.W.J. Müller, 1806, co-occurs very frequently on the same microhabitat. Adults and larvae were repeatedly observed in 2017 and 2019 and a persisting high population density of *P. acuminatus* was confirmed. The Ipeľ River is a left-hand tributary of the Danube flowing through a large alluvial floodplain in southern Slovakia. The river is 20–30 m wide and shallow, with macrophytes and gravel substrate at collecting places near the village of Salka (Fig. 6). Riparian vegetation is dense, but confined to the narrow area around the river; mainly *Salix* sp., *Populus alba* and *P. nigra* are present here. From the Ipeľ River estuary to the Danube, *P. acuminatus* has already been recorded (Brtek & Rotschein 1964). Searching on waterlogged wood in 2022 revealed eleven larvae; the population density here was relatively low and no adults were found. On the contrary, the population density of *M. quadrituberculatus* was higher. Sampling in the river sections upstream near the villages of Vrbovka, Bušince and Balog nad Ipľom showed higher population densities and even adults were captured by S. Benedikt (<https://www.biomonitoring.sk/OccurrenceData>). *Potamophilus* from Slovakia is known from a few published records only: Šamorín (Kelecsényi 1896), Bratislava (Roubal 1936, Kodada 1991), the River Danube near the estuary of the Ipeľ River (Brtek & Rotschein 1964), Rajčianka River near Čičmany (Šporka *et al.* 1998), Hronský Beňadik (Kodada 1991), Latorica River near the bridge of road Kráľovský Chlmec—Veľké Kapušany (Majzlan 1997), the Bodrog River and Ondava River near Kladzany (Jászay & Harman 2019) and Gajary (Majzlan 2014). The distribution, including unpublished data, was summarised by Kodada *et al.* (2003): the Danube River Basin, the lower Váh River Basin, Hron and Ipeľ Rivers, the Latorica River Basin and the Tisa River. Larger populations with observable adults and easily findable larvae are still preserved in the Latorica, Ondava, Bodrog and Tisa Rivers in eastern Slovakia

and the middle course of the River Ipeľ in southern Slovakia. Most lowland rivers in Slovakia are in a suboptimal condition, usually degraded on water regimes during the 20th century by numerous regulations and often affected by different kinds of pollution. Fine-grained silt, driven by run-off from arable land, resulting in the silting of interstitial space of the stream bed substrate, also has an adverse impact. Larvae are associated with submerged wood and, as this microhabitat is removed as part of the watercourse and riparian vegetation maintenance, the population is adversely affected to the point of extinction. Currently, *P. acuminatus* is classified as Critically Endangered in Slovakia (Holecová & Franc 2001).

Distribution (Fig. 11). Afghanistan, Albania, Austria, Azerbaijan, Belarus, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Israel, Italy, Lebanon, The Netherlands, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Syria, Tunisia, Turkey, Turkmenistan and Ukraine (Jäch *et al.* 2016). The species is here recorded for the first time in Morocco. This record increases Moroccan Elmidae richness to 22 species and the Rif Mountains to 15 (Bennas & Sáinz Cantero 2007; Benamar *et al.* 2022).



FIGURES 5–10. 5–6) habitat of *Potamophilus acuminatus*: 5) permanent River Oued Laou near Afertane Village, Morocco; 6) the River Ipeľ near Salka, Slovakia; 7–9) mature larvae of *Potamophilus acuminatus* from the Ipeľ River, specimen treated by lactic acid: 7) dorsal view; 8) same in lateral view; 9) same in ventral view. Scale bar: 1 mm. 10) living mature larvae from the Ipeľ River covered by secretions and debris on a wood piece, length of larvae approximately 12 mm.



FIGURE 11. The distribution map of *Potamophilus acuminatus* in North Africa and Spain. Data from Spain are adapted from Rico (1996).

Discussion

Potamophilus acuminatus is widely distributed and recorded in numerous countries (Jäch *et al.* 2016). However, the actual distribution during the global climatic change is somewhat unclear. The species monitoring is more successful for long-living larvae than adults. Larvae are mainly found on partly submerged wood, less on submerged roots (Gerend 2011; Kodada, pers. obs.) or were also reported from stones covered with *Fontinalis antipyretica* (Buczyński *et al.* 2011). Collecting adults requires beating and sweeping the riparian vegetation, examining submerged wood or rootlets, the growth of *F. antipyretica* and using light traps during the several weeks of their life.

The species inhabits streams and rivers of different sizes, from hyporhithral to hypopotamal. Medium and larger rivers, surrounded by trees providing autochthonous submerged wood, are the preferred larval habitat. The natural watercourses are probably the best, although large populations can also be found in canalised channels (e.g., the River Latorica in Slovakia). Surprisingly, the species was recorded even in a low water quality river (Bug River, Buczyński *et al.* 2011). In Europe, localities with high populations density are known from Austria, France, Hungary and Slovakia (Kovács *et al.* 1999; Kovács & Merkl 2005; Csabai *et al.* 2010; Gerend 2011; Jäch *et al.* 2013; Jászay & Harman 2019) and Hungary is considered as a European refuge of the species (Jäch *et al.* 2005).

Most lowland rivers in Europe are in a suboptimal condition, usually degraded on water regimes during the 20th century by numerous regulations and the populations are becoming extinct or fluctuate enormously in size. Determining the current distribution and population sizes requires long-term monitoring using appropriate collecting methods because data from most countries are probably deficient. In the case of Morocco, the lack of complete inventories of water beetles is due to a low number of specialists in that specific order and a lack of molecular biology laboratories as a crucial tool for taxonomic resolution (Belhaj *et al.* 2022). Considerable information is still required to provide an accurate picture of aquatic insect richness patterns in Morocco and north-western Africa.

Potamophilus has not yet been taxonomically revised and the first comprehensive diagnoses of its species were published only recently (Kodada *et al.* 2022). The examination of morphological characters did not show diagnostic features separating the specimens from Morocco from those of Central Europe. Their differences fit into local population variability. In this respect, the recorded high uncorrected *p*-distance is surprising. However, the low variability of European haplotypes can reflect the bottleneck effect more than a small sample size. Contrarily, the South American Larinae, for example, *Disersus otoganchi* Čiampor, Kodada, Božáňová & Čiamporová-Zaťovičová, 2021 differs significantly from congeners in male genitalia, while the differences in DNA sequences from the closest species are minor (Čiampor *et al.* 2021). Nevertheless, a more significant number of specimens from the entire distribution area must be examined to exclude or confirm the existence of cryptic taxa.

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