Received: 28 August 2020 | Revised: 6 September 2021 | Accepted: 11 October 2021
DOI: 10.1002/aqc.3748

SHORT COMMUNICATION

WILEY

The rare and enigmatic mayfly *Prosopistoma pennigerum* (Müller, 1785): Habitat characteristics, recent records from the Volga (Russia) and Vjosa (Albania) rivers, and a proposal for flagship species status

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Funding information
H2020 European Research Council, Grant/Award Number: ERC-STG 716196; www.theodorkoernerfonds.at, Grant/Award Number: Theodor Körner Förderpreis 2020, Klimaschutz-Preis; University of Natural Resources and Life Sciences Vienna (BOKU)

Abstract

1. Nymphs of all 29 described *Prosopistoma* species share a conspicuous synapomorphy: a round mesonotal shield, the carapace. They occur in the Palaearctic (nine species), the Oriental (12 species) and the Afrotropic as well as Australian regions (six and two species, respectively). Relatively little is known about their ecology, but past and extant distribution patterns indicate an association with undisturbed conditions.
2. *Prosopistoma pennigerum* is a rare European mayfly with conspicuous nymphs. Formerly common in large rivers, it has been extirpated from central Europe over the last century.
3. This study evaluated general habitat characteristics and human pressures for historical and current records of this rare species. *Prosopistoma pennigerum* is currently known from only three European rivers, all with gravel substrates, naturally dynamic discharge regimes, summer-warm water temperatures, and little human pressure.
4. This study showed that nymphs from the Vjosa and upper Volga rivers, two relatively natural watercourses 2,000 km apart, are morphologically indistinguishable, and show no variation across a ca. 600-bp fragment of the mitochondrial cytochrome c oxidase I gene.
5. Flagship species were first designated in the 1980s, when charismatic species with high habitat requirements such as the Bengal tiger or the giant panda, but also invertebrates are used to communicate conservation and protection needs. We propose that Europe's rarest mayfly *P. pennigerum*, with its unusual nymphs and remaining populations in naturally dynamic river courses, can serve as a flagship species promoting the preservation of ecological integrity in European rivers.

KEYWORDS
conservation evaluation, Europe, genetics, invertebrates, mayflies, rare species

1 | INTRODUCTION

Prosopistoma pennigerum is the only member of Prosopistomatidae (Ephemeroptera, Baetiscoidea) known from the European continent, where it was historically a typical component of the lower courses of large rivers, but most populations became extirpated by the 1950s (Schletterer & Füreder, 2009). Today, only three remnant populations of *P. pennigerum* are known: one in the Iberian Peninsula (Spain, River Cabriel) (Robles, Toro & Alba-Tercedor, 2002; Alba-Tercedor, 2008; Robles, personal communication), another in the East European Plain (Russia, Upper Volga River) (Schletterer & Kuzovlev, 2007; Schletterer & Füreder, 2009), and a new record from the Vjosa (Vjosë) catchment in the Balkan Peninsula (Albania, Greece) (Bauernfeind, 2018). Most records are based on collections of the highly distinctive nymphs and only a few adults have ever been found (Vayssière, 1881; Fontaine & Wautier, 1953; Degrange, 1955; Fontaine, 1982).

Nymphs of all 29 *Prosopistoma* species (Supporting Information 1) share a conspicuous synapomorphy: a round mesonotal shield, the carapace (Kluge, 2004; Schletterer, Bauernfeind & Lechthaler, 2016a; Kluge, 2020a; Kluge, 2020b). Biogeographically, Prosopitomatidae occur in the Palaearctic (nine species), Oriental (12 species) and Afrotropic as well as Australian regions (six and two species, respectively). Relatively little is known about their ecology, but past and extant distribution patterns indicate an association with undisturbed conditions. Because of its presence in near-natural stretches of the upper Volga River, *P. pennigerum* was suggested as a flagship species for intact European rivers (Schletterer et al., 2016b). Flagship species were first designated in the 1980s, when charismatic species with high habitat requirements such as the Bengal tiger or the giant panda – but also invertebrate species such as the monarch butterfly (*Danaus plexippus*), the Karner blue butterfly (*Lycaeides melissa samuelis*) (Guiney & Oberhauser, 2009) and dragonflies (*Leucorrhinia* spp.) that are typical for fens and peat bogs (Rúfusová, Beracko & Bulánková, 2017) – were used to communicate conservation and protection needs (Mittermeier, 1988; Guiney & Oberhauser, 2009; Barua, 2011; Barua et al., 2011). Although *P. pennigerum* may not be as charismatic as large or colourful species, its proposed status as a flagship species is connected to its potential for indicating near-natural conditions in large European rivers. Most large European rivers are affected by multiple stressors (Nijboer et al., 2004) and reference conditions remain to be defined for such rivers (Schletterer et al., 2014; Borgwardt et al., 2019; Leitner et al., 2021).

This article assesses and discusses potential factors that have led to the extirpation of *P. pennigerum* populations, and describes and compares habitat attributes of extant populations of *P. pennigerum* in the Volga and Vjosa rivers as well as all sites of historical occurrence. In addition, data on a partial mtDNA cytochrome c oxidase I (COI) sequence are provided to support biogeographical inferences and the development of molecular biomonitoring approaches.

2 | METHODS

2.1 | Research area and fieldwork

Sampling locations on the Volga and Vjosa rivers show minimal impact with regard to hydromorphology and hydrodynamics (Figure 1). The Volga River emerges at 228 m above sea level (a.s.l.) and is characterized by a low gradient channel in its upper course, with accompanying lowland forests for its first several hundred river kilometres. A viable population of *P. pennigerum* is known from a single stretch of about 2 km near Rzhev (Schletterer & Kuzovlev, 2007; Schletterer & Füreder, 2009) where a monitoring site

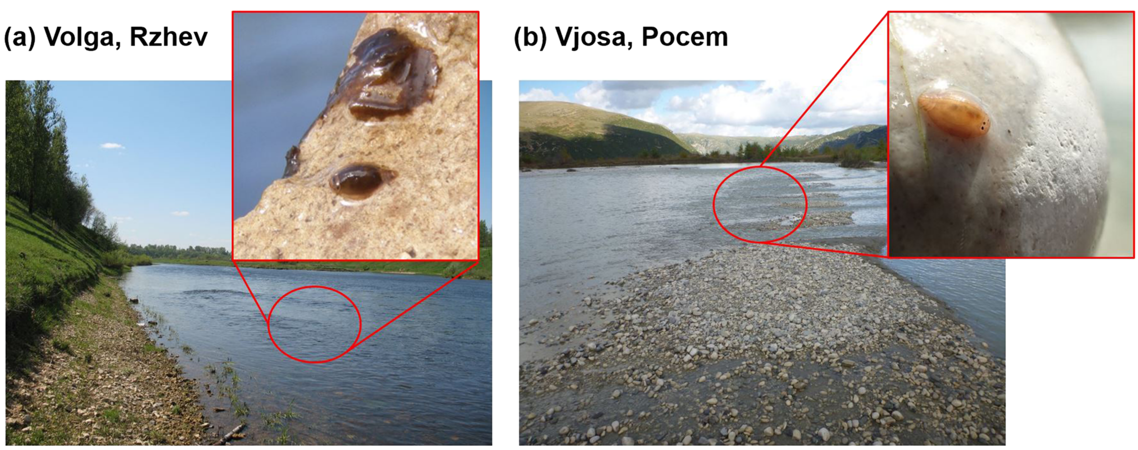

FIGURE 1 Habitats and habitus of *Prosopistoma pennigerum* at Rzhev (a) and Pocem (b)

has been sampled annually since 2006 (Schletterer et al., 2016b). The sampling site at Rzhev (upstream catchment area 12,200 km^2) is located 257 km from the source at 154 m a.s.l. (Schletterer et al., 2014). The Vjosa catchment covers 6,704 km^2 and the Vjosa River is formed at the Greek/Albanian border at the confluence of the Aoos and Sarantaporos rivers at 450 m a.s.l. from where it continues for ca. 180 km until its estuary into the Adriatic Sea. Sampling was conducted along the river in spring 2018, and *P. pennigerum* (Figure 2) was found along a ca. 65-km stretch between the tributaries Drinos and Shushica. Multi-habitat samples were collected with 500-μm nets and samples were preserved in ethanol (Volga) or 4% formaldehyde (Vjosa). For genetic analyses, specimens from the Vjosa River were collected in October 2017 near Poçem (Albania, Qark Fier) and preserved in 96% ethanol.

Based on hydro-chemical data, the ionic composition of the water from the two rivers was characterized according to Kurlov & Sobkevich (1921). Newly collected data from Rzhev were used for the Volga, and, for the Vjosa, published data from the lower Vjosa (bridge at the village of Mifol; summer 2009) (Abazi & Balliu, 2012) were used.

2.2 | Analysis of distribution and stressors

A distribution database for *P. pennigerum* was compiled based on Schletterer & Füreder (2009), supplemented with additional data. For the Vjosa, a single point near Poçem was considered. For each historical and present site of *P. pennigerum* occurrence, the type and number of human stressors was assessed, based on available literature and satellite images. Spatial queries were done in ArcGIS 10.7 (ESRI, 2011), intersecting points of occurrence with the river network layer of the Catchment Characterisation Model CCM2.1 (Vogt et al., 2007), the map of the European Inland Waterway Network (UNECE, 2018) and CORINE land cover 2018. Based on the CCM2.1 information was derived on catchment size, Strahler order (Strahler, 1957), elevation and mean temperature at the sites. The waterway map was used to describe the presence of navigation. For the land use evaluation, circular buffers with a radius of 5 km around the occurrence sites were created and the proportion in the buffers of the first level of the CORINE land cover classes (i.e. distinguishing artificial, agricultural, forest, and wetlands and water) was summarized.

FIGURE 2 Nymphs of *Prosopistoma pennigerum*: (a) in the field (photo: S. Vitecek) and (b) in the laboratory (photo: W. Graf)

2.3 | Genetic analyses

Total DNA was extracted from a leg, or whole specimen preserved in alcohol with a modified protocol based on Sambrook, Fritsch & Maniatis (1989). Two specimens were analysed for each river. A fragment of the mitochondrial COI gene approximately 600 bp long was amplified with the primers LCO1490 and HCO2198 following Folmer et al. (1994). Polymerase chain reactions (PCRs) contained 2 μl DNA template, 1 μl of 10× buffer, 0.75 μl of MgCl2 (50 mM), 0.8 μl of equimolar dNTP mixture (20 mM), 2 μl enhancer, 0.25 μl of each primer, 0.1 μl Taq (PeqLab) and 2.85 μl water to complete a 10 μl final reaction volume; PCR conditions were (5 min 94°C, 40× (30 s 94°C, 30 s 48°C, 60 s 72°C), 10 min 72°C). PCR products were purified with ExoSap-IT Enzyme (Amersham Biosciences). Sequencing products were obtained using the original PCR primers and a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instructions. Sequencing products were cleaned with Sephadex G-50 (Amersham Biosciences) and visualized on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Raw sequence data were aligned by eye in BioEdit v7.0.0 (Hall, 1999) together with two *Prosopistoma* sp. sequences (GenBank Accession number KR822423.1; BOLD-ID THMAY158–12), one *Prosopistoma annamense* sequence (THYMAY160–12) and one *Baetisca laurentina* (GenBank Accession number KR144660.1) outgroup sequence. The final alignment was imported into MEGA v7.0 (Kumar, Stecher & Tamura, 2015) to calculate p-distances.

3 | RESULTS

3.1 | Comparative description of the habitats in the Volga and the Vjosa rivers

The Volga River channel near Rzhev has a meandering character with sand and gravel bars, a few islands, predominantly sand at midchannel and small stones (6.3–20 cm) along the banks (moraine material from the Valdai glaciation). Annual mean flow is 94 $m^3 s^{-1}$ (1924–1985) with a maximum monthly discharge of 547 $m^3 s^{-1}$ in April and a summer low-flow period as well as higher discharge in autumn (pluvio-nival regime) (Schletterer et al., 2014; Schletterer et al., 2016b). During the sampling campaigns (2006–2019) summer water temperatures ranged from 15.5 to 24.8°C (mean = 19.7 ± 2.5°C) and dissolved oxygen ranged between 8.6 and 12.0 mg L^{-1} (mean = 10.6 ± 1.1 mg L^{-1}). Here, *P. pennigerum* occurred in fast-flowing habitats (about 0.6 m s^{-1}) in approximately 0.5 m depth (Figure 1).

During the summer low-flow period (July 2017), the ionic concentration of the Volga near the town of Rzhev is low (164 mg L^{-1} : long term range in summer 150–240 mg L^{-1}) and the hydrochemical type of the water is 'hydrogen carbonate calcium', with a measured conductivity of 195 μS cm^{-1} (Supporting Information 2). A specific feature of the upper reaches of the Volga is the significant content of organic matter throughout the year owing to the large extent of mires in the catchment (Zhenikhov et al., 2019). The concentration of nitrogen and phosphorus compounds in the warm summer low-water period decreases in the Volga to low values as a result of the photosynthetic activity of phytoplankton and macrophytes; for example, in July 2017: nitrate nitrogen ($N\text{-}NO_3$) – 0.17 mg L^{-1}; ammonium nitrogen ($N\text{-}NH_4$), 0.05 mg L^{-1}; phosphate phosphorus (P), 0.005 mg L^{-1}. In cold periods, the concentrations of those biogenic components are higher, for example, in March 2017: nitrate nitrogen ($N\text{-}NO_3$), 0.93 mg L^{-1}; ammonium nitrogen ($N\text{-}NH_4$), 0.15 mg L^{-1}; phosphate phosphorus (P), 0.016 mg L^{-1}.

In the Vjosa River, *P. pennigerum* was found in channel sections with a bar-braided and island-braided character (Schiemer et al., 2018), with substrates dominated by gravel and small stones. Annual mean flow at Poçem is about 141.5 $m^3 s^{-1}$, while maximum discharge between 500 and 600 $m^3 s^{-1}$ occurs between December and January with low flow in summer (Schiemer et al., 2018). In summer the water temperature at Poçem reaches ca. 25°C (Beqiraj, pers. comm.). In spring 2018, *P. pennigerum* was primarily found in fast-flowing sections (about 0.6 m s^{-1}) of the main river where temperatures ranged from 14.9 to 17.8°C and dissolved oxygen ranged between 9.79 and 10.41 mg L^{-1}. In the summer low-water period in 2009, the ionic concentration in the lower Vjosa River was 430 mg L^{-1} (according to Abazi & Balliu, 2012), and the hydrochemical type of the water is 'chloride-hydrocarbonate calcium–magnesium', with a calculated conductivity of 700 μS cm^{-1} (Supporting Information 2).

3.2 | Historical distribution and analyses of potential stressors

Historically, *P. pennigerum* had been reported from 33 rivers (Figure 3; Supporting Information 3, 5), with some records (e.g. in Algeria, Georgia and Turkey) potentially representing another species (Schletterer & Füreder, 2009). Records from 41 sites from 33 rivers (Supporting Information 3) were identified; calcareous geology dominated in 78% (n = 26) of these rivers. The analyses indicate that the species had colonized a wide range of river sizes (Strahler numbers 3–8 with a median of 6), catchments of various sizes (196–138,464 km^2 with a median of 11,000 km^2) and at elevations up to 1,000 m (with a median of 205 m; Table 1).

Rivers with extant populations of *P. pennigerum* share some habitat characteristics: (i) hydromorphology is minimally impaired in large continuous sections; (ii) there is no shipping; and (iii) the

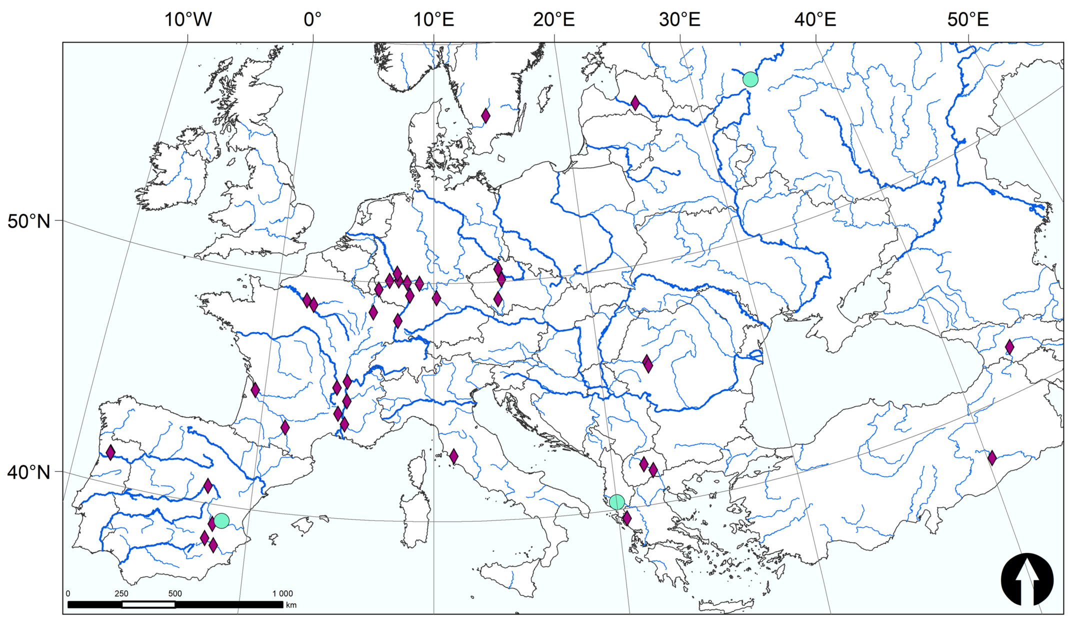

FIGURE 3 Distribution of *Prosopistoma pennigerum*: rhombus = historical sites, circle = recent sites (remark: owing to the scale, sites that are close together are only indicated once)

TABLE 1 Classification of the known sites (frequency analyses) related to Water Framework Directive typology (categories according to Solheim et al., 2019)

(a) Altitude (m above sea level)	<200 (lowland)		200–800 (mid-altitude)		>800 (highland)	
n	20		18		3	
(b) Strahler order (Strahler, 1957)	**3**	**4**	**5**	**6**	**7**	**8**
n	2	3	12	8	8	8
(c) Catchment area (km^2)	**<100 (very small–small)**		**100–10,000 (medium–large)**		**>10,000 (very large)**	
n	0		19		22	

free-flowing stretches are embedded in a landscape matrix of natural grasslands, forests and extensive agriculture where the only stressor that could be identified is (slight) organic pollution. In contrast, multiple stressors (three on average) affect historical sites that are now located in a mostly artificial and agricultural matrix, with hydromorphological impairment, shipping, damming and/or organic pollution as the most important stressors (Supporting Information 3).

3.3 | Genetic characterization of *P. pennigerum*

Approximately 612 bp of the mitochondrial COI gene was resolved. Sequences of the four specimens of *P. pennigerum* from the Vjosa and Volga rivers (two specimens from each river) were identical, and diverged significantly (17–21%, data not shown) from the other taxa involved. Sequences created in this study have been deposited in GenBank (Accession no. MZ707154 (Pp_Volga) and MZ707155 (Pp_Vjosa)).

4 | DISCUSSION

The habitat characteristics where *P. pennigerum* occurs in the Volga and Vjosa rivers are comparable in a very general sense and correspond to previous knowledge – extant populations occupy shallow, fast-flowing sites over gravelly substrate in generally larger, summer-warm river systems (Schletterer & Füreder, 2009). Historical distribution, however, indicates that the species also inhabited rivers quite diverging from these, but where, generally, habitat dynamics were high. The upper Volga and Vjosa rivers are also comparable in both having near-natural conditions, especially with respect to channel morphology and hydrological regime, now a rare characteristic among rivers in Europe. Beyond these minor similarities, however, there is nothing apparent, either at the catchment or reach scale of these two rivers that would allow further generalizations concerning the habitat requirements or limitations for *P. pennigerum*. Furthermore, our knowledge of the biology of *P. pennigerum* is extremely limited, and neither the occurrence of adult males nor sexual reproduction has been confirmed. Nonetheless, the extensive disappearance of the species requires an environmental explanation, which to some extent can be addressed by comparing its historical and extant occurrence.

The historical distribution of *P. pennigerum* across Europe involves many large rivers, some with lowland character such as the upper Volga, but also the lower courses of other systems, characterized by a relatively low gradient yet still exhibiting fast-flowing and shallow water over gravelly substrate. Collectively, most if not all such habitats in Europe suffer from a suite of human disturbances, historically dominated by organic pollution as well as hydromorphological changes caused by river channel engineering and dam construction (Schletterer & Füreder, 2009; Schiemer et al., 2020). The results on the presence of human pressures at sites of former occurrence reflect the notion that these disturbances may have played a major role in the extirpation of this species (Schletterer, Bauernfeind & Lechthaler, 2016a). Specific examples of extirpation following human disturbance include the construction of an irrigation reservoir in 1939 near Husinec on the Blanice River in the Czech Republic (Votruba & Brožа, 1989), where *P. pennigerum* was previously known but no longer occurs (Lafon, 1952), as well as irrigation flow disturbances on the Segura and Mundo rivers in Spain, where the species was historically recorded (Robles, Toro & Alba-Tercedor, 2002).

In addition to the sparse environmental and autoecological knowledge of *P. pennigerum*, the currently available genetic data are enigmatic. No sequence differences across a portion of the COI mitochondrial gene were found between specimens captured more than 2,000 km apart from the Volga and Vjosa rivers, and there was also no genetic divergence seen in specimens from the Volga River and the Segura River in Spain, using sequences from the 16S, 18S and Histone3 genes (Barber-James et al., 2015). Several evolutionary processes could have created such patterns – e.g., parthenogenesis in *Prosopistoma* (Campbell & Hubbard, 1998), efficient long-distance dispersal (Saito et al., 2016), extensive range losses (Bálint et al., 2012) or shared ancestral genomic characters (Theissinger et al., 2011). However, acknowledging limitations of our dataset, we refrain from any interpretation. The generated mitochondrial DNA sequence has already been used to develop a quantitative PCR monitoring protocol, to detect this mayfly by environmental DNA analysis (Martini et al., 2021).

Prosopistoma pennigerum is among the rarest mayfly species in Europe, and its occurrence in a few relatively undisturbed river courses, such as those found in the upper Volga and Vjosa catchments, as well as the Cabriel River in Spain, underline the importance of protecting these rivers from further human threats. The conservation value of the Vjosa River is at present gaining international attention, and a proposal to create Europe's first Wild River National Park has recently been submitted to the International Union for Conservation of Nature (EcoAlbania, 2021). The river has also been shown to harbour relatively healthy populations of the critically endangered European eel *Anguilla anguilla*, among many other faunal highlights (Meulenbroek et al., 2020; Schiemer et al., 2020). The free-flowing reaches of the upper Volga River, where *P. pennigrum* is found, may serve as reference conditions for large rivers (Schletterer et al., 2014), as needed in the context of the European Water Framework Directive (Council of the European Communities, 2000).

Reflecting on the unique river systems as well as the rarity and enigmatic nature of *P. pennigerum*, we suggest that this mayfly can serve as a flagship species promoting the maintenance of ecological integrity in some of the last free-flowing larger river courses on the European continent, following the recent example of the flagship mayfly species *Palingenia longicauda* (the Tisza mayfly, Cantonati et al., 2020), as well as the general trend to include more insects as flagship species (Barua et al., 2012; Schlegel, Breuer & Rupf, 2015). Specifically, indexing *P. pennigerum* into Red Lists (Albania, 2013; Orlov et al., 2016) and elaborating conservation strategies that prioritize large-scale habitat protection are essential to halt the extirpation of this aquatic insect and the communities that share its habitat. The species itself, however, requires considerably more research to understand its ecological needs including nutritional and micro-habitat preferences, as well as its life cycle. The continuing monitoring programmes on the Volga and Vjosa rivers should also support these more focused research questions to ensure the survival of *P. pennigerum* in Europe.

ACKNOWLEDGEMENTS

We would like to acknowledge Prof. Dr Y.N. Zhenikov and K.Y. Zhenikov for their help during fieldwork on the Volga River, which was carried out in the framework of the REFCOND_Volga project. Sampling at the Vjosa River in 2018 was funded by the European Research Council through the project FLUFLUX (ERC-STG 716196) awarded to Gabriel Singer. We also thank Olivia Wilfing as well as Roland Sumper for their support within this study. Sajmir Beqiraj supported the study with unpublished temperature data from the Vjosa River. The first author was supported by the prize of the Theodor Körner Foundation (Theodor Körner Förderpreis 2020, Klimaschutz-Preis). Finally, we thank Professor John Brittain from the University of Oslo as well as an anonymous referee for helpful feedback in the review process. Open access funding provided by University of Natural Resources and Life Sciences Vienna (BOKU).

CONFLICT OF INTEREST

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

DATA AVAILABILITY STATEMENT

Data available in a public repository (sequences created in this study have been deposited in GenBank) and / or Data available on request from the authors.

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How to cite this article: Schletterer, M., Weiss, S.J., Kuzovlev, V.V., Vitecek, S., Borgwardt, F. & Graf, W. (2021). The rare and enigmatic mayfly *Prosopistoma pennigerum* (Müller, 1785): Habitat characteristics, recent records from the Volga (Russia) and Vjosa (Albania) rivers, and a proposal for flagship species status. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(12), 3636–3643. https://doi.org/10.1002/aqc.3748