

Tube-nose goby *Proterorhinus semipellucidus* continues its spread in the Baltic Sea basin: first record from the temperate salmonid stream (Estonia)

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Abstract. The capture of the non-indigenous tube-nose goby *Proterorhinus semipellucidus* in the Pada River (Baltic Sea basin) is reported. Previous records about the occurrence of this species in this region are restricted to the brackish coastal surf-zone area. The finding implies that in the Baltic Sea region *P. semipellucidus* has started to invade from brackish coastal waters to freshwater tributaries of the Gulf of Finland.

Keywords: biological invasions, molecular analysis, Ponto-Caspian gobies, *Proterorhinus nasalis*, invasive species, Gulf of Finland.

INTRODUCTION

Tube-nose goby *Proterorhinus semipellucidus* is native to the Caspian Sea basin and the Volga River delta (reviewed in Kvach et al. 2021; see also Zarei et al. 2022). The species is invasive (Parin et al. 2014; reviewed in Kvach et al. 2021; see also Truuverk et al. 2021) in the Volga River basin and in the European Northern Invasion Corridor *sensu lato* (*sensu* Bij de Vaate et al. 2002). *P. semipellucidus* invasion into the Baltic Sea was confirmed in 2020, when several individuals were caught from the brackish coastal area of Narva Bay in Estonia (Truuverk et al. 2021). Truuverk et al. identified the species as *P. nasalis* (De Filippi, 1863). However, the latter species is currently considered as a junior synonym to *P. semipellucidus* in the northern and central Caspian Sea basin/Volga River/Baltic Sea regions (Zarei et al. 2021,

2022; Fricke et al. 2023). To date, the occurrence of this species in the Baltic Sea is exclusively related with the brackish surf-zone environment (Truuverk et al. 2021). This study reports the first observation of *P. semipellucidus* from a small freshwater tributary of the Baltic Sea.

MATERIALS AND METHODS

Three *Proterorhinus sp.* individuals were electrofished from the Pada River (59°29'44" N, 26°45'9" E; Fig. 1) during the annual national salmonid parr density survey on 31 August 2021. The study site was located in a riffle-pool sequence approximately 2 km upstream from the river mouth. The riffle-pool sequences in the lower reaches of the Pada River consist of a variety of depths, velocities and substrates. This area is a well-known spawning and nursery habitat for the anadromous brown trout *Salmo trutta* (ICES 2021). In the sampling biotope,

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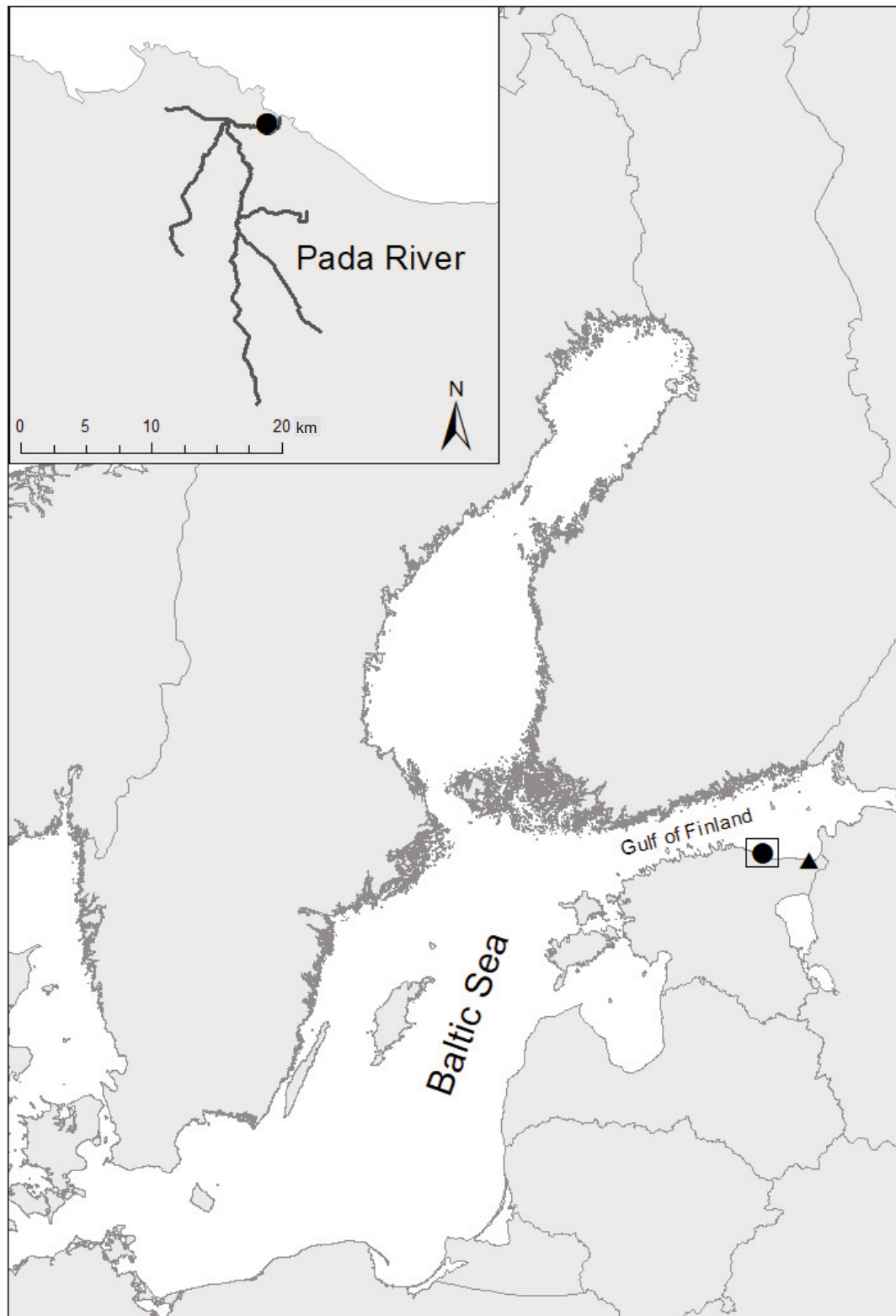


Fig. 1. Distribution of *P. semipellucidus* in the Baltic Sea basin. Circle – current study, triangle – records from Truuverk et al. (2021).

the average dominating depth was <0.3 m, and water velocity was moderate. The river bottom consisted of gravel and stones with scarce vegetation.

For all caught individuals, total length (TL) and body mass (TW) were recorded. All specimens were preserved in ethanol (96.5% vol), and DNA samples were collected for molecular species determination, as gobies of the genus *Proterorhinus* Smitt, 1900 are difficult to distinguish morphologically (e.g. Freyhof and Naseka 2007; Neilson and Stepien 2009). The mitochondrial cytochrome oxidase subunit I (COI) gene was amplified via the polymerase chain reaction (PCR) using goby-specific primers (for laboratory procedures, see Truuverk et al. 2021). DNA barcodes of the caught specimens were cross-checked against the Barcode of Life Data Systems (Ratnasingham and Hebert 2007) and determined to the species level following the taxonomy proposed by the international database of the Eschmeyer's Catalog of Fishes (Fricke et al. 2023). All original sequences were deposited in GenBank, and their accession numbers are listed in Table 1.

RESULTS

The size of tubenose goby individuals PADA1, PADA2, PADA3 (for voucher data, see Table 1) from the Pada River was 65 mm, 60 mm and 30 mm (TL), respectively, and their weight was 2.7 g, 2.2 g and 0.2 g (TW), respectively. Full 1271 bp COI sequences were successfully obtained for all three specimens and compared to selected *Proterorhinus* species from Truuverk et al. (2021) (Table 1). The evolutionary history of the analysed *Proterorhinus* sp. was inferred using MEGA X (Kumar et al. 2018), and the round goby *Neogobius melanostomus* was used as an outgroup (no bootstrapping was applied) by using the Neighbor-Joining (NJ) method (Saitou and Nei 1987). The NJ phylogenetic tree was constructed (Fig. 2) with a topology that corresponds with the findings of Neilson and Stepien (2009) and Truuverk et al. (2021). Three monophyletic clades represented the *Proterorhinus* species, whereas all three analysed specimens nested within *P. semipellucidus*. Thus, phylogenetical analyses clearly indicated that the *Proterorhinus* sp. individuals caught

Table 1. Species, voucher data and GenBank accession numbers of the specimens. Note that *Proterorhinus* cf. *semipellucidus* and *P. nasalis* are considered as junior synonyms to *P. semipellucidus* in the northern and central Caspian Sea basin/Volga River/Baltic Sea regions (Zarei et al. 2021, 2022; Fricke et al. 2023)

Species	Specimen ID	DNA ID	GenBank accession number
<i>Proterorhinus semipellucidus</i>	TUZ700175	PADA1	PP301897
<i>Proterorhinus semipellucidus</i>	TUZ700176	PADA2	PP301896
<i>Proterorhinus semipellucidus</i>	TUZ700177	PADA3	PP301895
<i>Proterorhinus nasalis</i>	TUZ700166	Goby1	MW984354
<i>Proterorhinus nasalis</i>	TUZ700167	Goby2	MW984353
<i>Proterorhinus nasalis</i>	TUZ700168	Goby3	MW984352
<i>Proterorhinus nasalis</i>	TUZ700169	Goby4	MW984351
<i>Proterorhinus nasalis</i>	TUZ700170	Goby5	MW984350
<i>Proterorhinus nasalis</i>	TUZ700171	Goby6	MW984349
<i>Neogobius melanostomus</i>	TUZ700173	Goby8	MW984348
<i>Proterorhinus nasalis</i>	TUZ700174	Goby9	MW984347
<i>Proterorhinus</i> cf. <i>semipellucidus</i>	PseAKP1	PseAKP1	EU444675
<i>Proterorhinus</i> cf. <i>semipellucidus</i>	PseAKP4	PseAKP4	EU444676
<i>Proterorhinus</i> cf. <i>semipellucidus</i>	PseALS1	PseALS1	EU444678
<i>Proterorhinus</i> cf. <i>semipellucidus</i>	PseALT1	PseALT1	EU444679
<i>Proterorhinus</i> cf. <i>semipellucidus</i>	PseALU1	PseALU1	EU444680
<i>Proterorhinus</i> cf. <i>semipellucidus</i>	PseAMK1	PseAMK1	EU444685
<i>Proterorhinus semilunaris</i>	PseAGN1	PseAGN1	EU444674
<i>Proterorhinus semilunaris</i>	PseAKP7	PseAKP7	EU444677
<i>Proterorhinus semilunaris</i>	PseAMF2	PseAMF2	EU444683
<i>Proterorhinus semilunaris</i>	PseAML1	PseAML1	EU444686
<i>Proterorhinus semilunaris</i>	PseAOC2	PseAOC2	EU444690
<i>Proterorhinus semilunaris</i>	PseAQE1	PseAQE1	EU444691
<i>Proterorhinus marmoratus</i>	PmaAMD1	PmaAMD1	EU444681
<i>Proterorhinus marmoratus</i>	PmaAME1	PmaAME1	EU444682
<i>Proterorhinus marmoratus</i>	PmaAMG1	PmaAMG1	EU444684
<i>Proterorhinus marmoratus</i>	PmaAMM1	PmaAMM1	EU444687
<i>Proterorhinus marmoratus</i>	PmaAMR1	PmaAMR1	EU444689

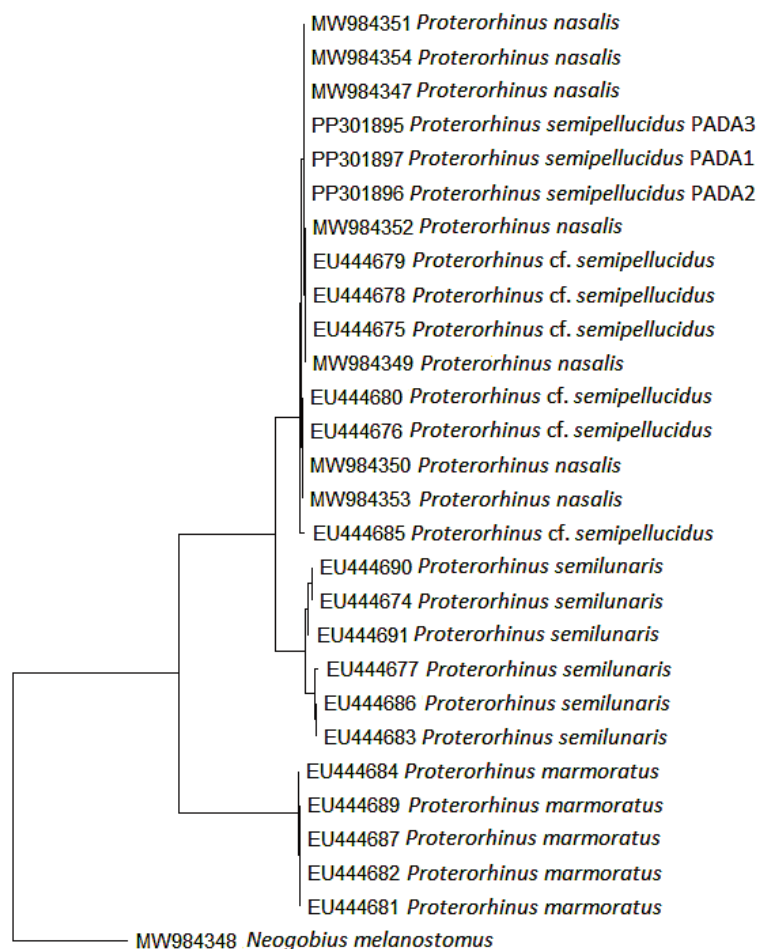


Fig. 2. Molecular phylogenetic analysis by the Neighbor-Joining method. Note that *Proterorhinus* cf. *semipellucidus* and *P. nasalis* are considered as junior synonyms to *P. semipellucidus* in the northern and central Caspian Sea basin/Volga River/Baltic Sea regions (Zarei et al. 2021, 2022; Fricke et al. 2023).

from the Pada River belong to the species *P. semipellucidus* (Fig. 2).

DISCUSSION AND CONCLUSIONS

To the authors' knowledge, the current study is the first to document the upstream invasion of *P. semipellucidus* into the tributaries of the Baltic Sea. Moreover, the findings of this study also indicate that the distribution of *P. semipellucidus* is expanding westwards (Fig. 1) in the Baltic Sea. According to Kottelat and Freyhof (2007), *P. semipellucidus* inhabits a variety of freshwater and brackish habitats. Thus, it may be possible for *P. semipellucidus* to thrive in riverine habitats in the Baltic basin.

Another invasive species of the *Proterorhinus* genus, *Proterorhinus semilunaris*, has shown a potentially negative impact on aquatic invertebrate density and community

composition in the Dyje River, Czech Republic (Mikl et al. 2017). Moreover, Van Kessel et al. (2011) suggested that competition for shelter is likely to occur in rivers invaded by *P. semilunaris* at sites where shelter is limited. Thus, the invasion of *P. semipellucidus* in the Baltic Sea basin rivers could also result in a potentially increased competition for food and habitat with several native benthic riverine species, including bullhead *Cottus gobio* (*C. gobio* listed in Annex II of the Habitats Directive (Council Directive 92/43/EEC)) and stone loach *Barbatula barbatula*.

The very fast colonisation of riverine habitats (this study) after initial detection of the invasion (Truuverk et al. 2021) indicates that tubenose gobies are rapidly increasing their distribution area in the Baltic Sea basin. However, the exact mechanisms of the initial regional invasions and potential factors associated with this fast colonisation speed of the species need to be addressed in the future.

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Ida-lontmudil *Proterorhinus semipellucidus* levib Läänemere vesikonna vooluveekogudesse

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Läänemere piirkonnas on invasiivset võõrliiki ida-lontmudilat *Proterorhinus semipellucidus* varem registreeritud vaid Soome lahe riiimveelises kaldavööndis. Käesolevas uuringus näidatakse esimest korda, et see liik on tunginud mageveekogudesse ka Läänemere vesikonnas. Riiklikel lõhe ja meriforelli noorjärkude katsepüükidel registreeriti ida-lontmudil Pada jões, ligikaudu 2 km kaugusel merest. Leid näitab, et oma looduslikul levialal ka vooluveekogusid asustav ida-lontmudil võib tulevikus Läänemere vesikonna jõgedes muutuda püsiasukaks ning seeläbi mõjutada kohalikke jõelisi ökosüsteeme.