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# First record of the Canestrini's goby *Ninnigobius canestrinii* (Ninni, 1883); a rare and endangered fish species from Slovenian waters

**Domen Trkov[1*] and Ana Fortič[1]**

[1] *Marine Biology Station, Piran, National Institute of Biology, Piran, Slovenia*

**Abstract**: The Canestrini's goby is a small goby endemic to the Adriatic basin, inhabiting brackish and fresh waters. The species is threatened by habitat destruction and pollution. For this reason, the goby is protected in part of its range, while its conservation status in other areas is not yet established. The detailed biogeography of the species should be known so that appropriate conservation measures can be taken. Despite extensive sampling of fish fauna in coastal wetlands, this species has not yet been detected in Slovenia. In this study, the first record of *Ninnigobius canestrinii* (Ninni, 1883) in Slovenian waters is reported along with a comprehensive review of documented records of the species.
***Keywords***: Gobiidae; endemic goby; habitat destruction; northern Adriatic basin; Slovenia

**Sažetak**: PRVI NALAZ GLAVOČIĆA CRNOTRUSA *NINNIGOBIUS CANESTRINII* (NINNI, 1883); RIJETKE I UGROŽENE VRSTE RIBE IZ SLOVENSKIH VODA. Glavočić crnotrus mala je i endemska jadranska vrsta glavoča koja naseljava bočate i slatke vode. Ovaj glavoč ugrožen je uslijed uništavanja staništa i zagađenja te je iz tog razloga zaštićen u jednom dijelu svog područja rasprostranjenosti, dok u ostalim područjima zaštita još nije uspostavljena. Detaljno poznavanje biogeografije vrste preduvjet je za uspostavljanje odgovarajućih mjera zaštite. Unatoč opsežnom uzorkovanju ihtiofaune u obalnim močvarnim područjima Slovenije, ova vrsta do sada nije bila zabilježena. U ovom radu opisan je prvi nalaz vrste *Ninnigobius canestrinii* (Ninni, 1883) u slovenskim vodama, zajedno sa sveobuhvatnim pregledom dosadašnjih dokumentiranih nalaza ove vrste.
***Ključne riječi***: Gobiidae; endemski glavoč; uništavanje staništa; sjevernojadranski bazen; Slovenija

## INTRODUCTION

Gobies are a family of small, mostly benthic fishes with low economic value but high ecological importance (Patzner *et al*., 2011). Because of their life history characteristics, including a short planktonic larval stage and strong association and specialization to specific microhabitats, gobies tend to have a high degree of local endemism as well as cryptic species (Neilson and Stepien, 2009; Patzner *et al*., 2011). One such species is *Ninnigobius canestrinii* (Ninni, 1883), a small brackish and freshwater goby with a total length (TL) of up to 6.7 cm that is endemic to the Adriatic basin and has a disjunct distribution (Miller, 2004; Dulčić and Kovačić, 2020). Although the species is currently classified as "Least Concern" by the IUCN (Freyhof, 2018), it is considered a threatened fish species throughout its range (Franco *et al*., 2005). The species is strictly protected by several European conventions and protocols, *e.g.*: Appendix II of the "Convention on the Conservation of European Wildlife and Natural Habitats", Bern 1979 (Council Decision 82/72/EEC); it is also considered "Endangered" or "Threatened" by the Barcelona Convention - "Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean" (1999/800/ EC); and as a species of Community Interest - "Council Directive on the conservation of natural habitats and of wild fauna and flora" (92/43/EEC). In Croatia, where most of its occurrences are known, it is listed as "Endangered" in the "Red Book of Freshwater Fishes of Croatia" (Mrakovčić *et al*., 2006). Moreover, the species is strictly protected in Croatia according to the "Regulation Designating the Wild Species Protected and Strictly Protected" (NN, 144/2013) in accordance with the "Environmental Protection Act" (NN, 80/2013) (Dulčić and Kovačić, 2020). One of the main reasons for this is the lack of suitable habitats, which are severely degraded by human activities (Kovačić, 2005). In Slovenia, no protection measures have been established for this species.

*Ninnigobius canestrinii* has been recorded so far in Italy, Croatia, and Bosnia and Herzegovina. The species is distributed from the Po Delta (Italy) in the north to the Neretva River Basin (Croatia and Bosnia and Herzegovina) in the south, with a gap in the northeastern Adriatic Basin, more specifically in the Istrian Peninsula (where it has been found only in the Mirna and Raša rivers) and

**Corresponding author:* domen.trkov@nib.si
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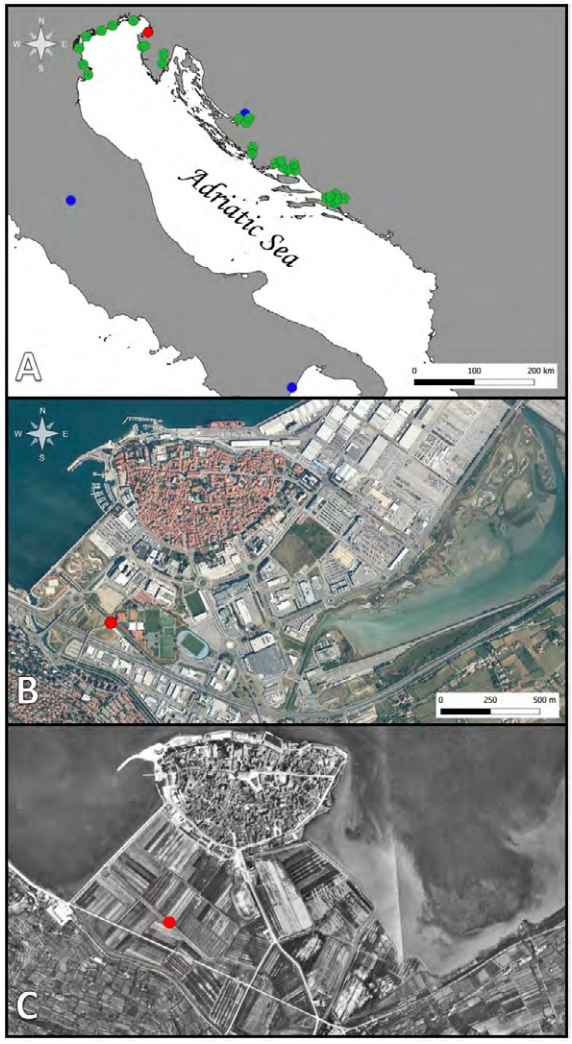


**Fig. 1**. Maps of occurrences of *Ninnigobius canestrinii* (Ninni, 1883): Presently known geographical distribution of the species (Gandolfi *et al*., 1982; Freyhof, 1988; Kovačić, 2005; Stelbrink and Freyhof, 2006; Šanda, and Kovačić, 2009; Tutman *et al*., 2013 and references therein; Dulčić and Kovačić, 2020; present study). The locations of previously known distribution are marked with ● and ● (occurrences which are probably due to a recent introduction) and location of the specimen found in the present study with ● (**A**); Location of *N. canestrinii* caught in Slovenian waters in the current land extent around the city of Koper (**B**); and same location in the land extent around Koper in 1954 (Surveying and Mapping Authority of the Republic of Slovenia) (**C**).

in the Kvarner Bay (Kovačić, 2005; Šanda and Kovačić, 2009; Tutman *et al*., 2013; Dulčić and Kovačić, 2020). The occurrence of the species in the Ionian Sea (Gulf of Taranto) (Gandolfi *et al*., 1982), Lake Trasimeno (Italy) (Freyhof, 1988), and Ričica Reservoir (Croatia) (Stelbrink and Freyhof, 2006) is likely due to recent introductions (Tutman *et al*., 2013; Dulčić and Kovačić, 2020) (Fig. 1A). The species lives in standing and flowing waters, where it inhabits unvegetated and vegetated muddy and sandy bottoms, but can also be found among stones (Miller, 2004; Dulčić and Kovačić, 2020). The depth distribution of the species ranges from 0.2 to 2 m. It is an annual species that reproduces from March to July (Dulčić and Kovačić, 2020).

## MATERIAL AND METHODS

On September 26, 2022, fish samples were collected from the artificial canal system in the city of Koper (45°32'29.259'' N, 13°43'30.298'' E; Fig. 1B), Slovenia. The canal system is connected to the small Badaševica River and the largest Slovenian brackish lagoon, which is part of the Škocjan Inlet Nature Reserve. The canal system is a part of a former wetland. Sampling was done in a 70 cm deep brackish water canal (Fig. 2A, B) on a muddy bottom where rocks, reefs with the non-native polychaetes *Ficopomatus enigmaticus* (Fauvel, 1923) and vegetation (*e.g. Ruppia cirrhosa* (Petagna) Grande, 1918) were observed. Surface salinity was 4.9 and bottom salinity was 18.0 at the time (10 am) of collection (measured with a multiparametric probe 3620 IDS, WTW). At the site, fish were caught using a hand net (20 x 20 cm).

The fish were identified on site and returned to the capture site. A single goby specimen, which we were unable to identify on site, was collected and transported in a 10-litre bucket of water to the laboratory of the Marine Biology Station (MBS) in Piran (National Institute of Biology). The fish was photographed alive in

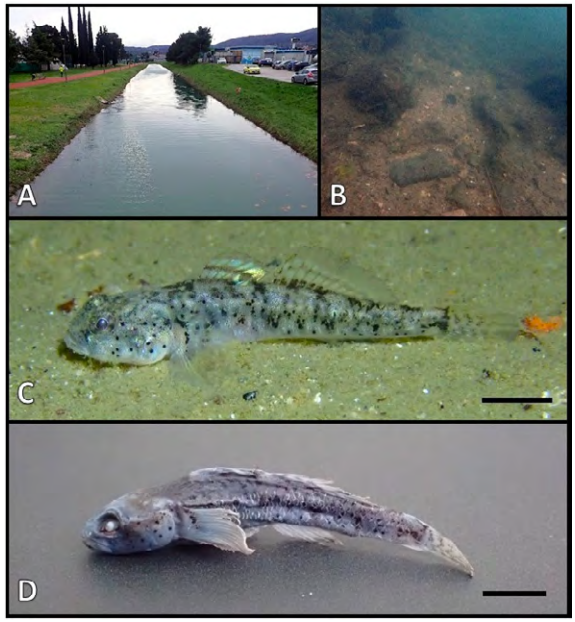


**Fig. 2**. Habitat and specimen of *Ninnigobius canestrinii* (Ninni, 1883) caught in Slovenian waters: Habitat where the specimen was found is an artificial brackish canal (**A**); The fish was caught on vegetated muddy bottom, with occasional rocks and reefs of *Ficopomatus enigmaticus* (**B**); Left side of the live specimen photographed *ex situ*. The black spots on the body are typical for this species. The scale bar is 5 mm long (**C**). Left side of the preserved specimen. The blue color of the fish is due to dyeing with cyanine blue for identification purposes (**D**). The scale bars (C and D) are 5 mm.

aquarium using Olympus TG-6. The specimen was then euthanized with an overdose of the anaesthetic Quinaldine (Sigma-Aldrich). The specimen was measured to the nearest 0.1 mm using callipers. To examine the fish scales and lateral line system of the head, specimen was preserved in 96% ethanol and reversibly stained in a 2% solution of cyanine blue and distilled water as described by Saruwatari *et al*. (1997). After staining, the lateral line system was examined under an Olympus SZx16 stereomicroscope with an Olympus DP74 camera. The specimen was identified according to Kovačić (2005, 2008, 2020) and the recommendations of publications of first records in ichthyology were followed, as suggested by Bello *et al*. (2014) (except for genetic sequence determination) and Kovačić and Svensen (2017). The sex of the fish was determined based on the shape of the urogenital papillae. The collected specimen was labelled and stored in the MBS collection.

## RESULTS AND DISCUSSION

A single male of *N. canestrinii* (Fig. 2C, D) was captured during a fish sampling along with *Knipowitschia panizzae* (Verga, 1841) and the non-native *Gambusia holbrooki* Girard, 1859. *N. canestrinii* is distinguished from all other marine and freshwater Mediterranean gobiid species by the following combination of characters: (1) suborbital row *a* present, with one to several transverse rows (4 in the present specimen), (2) suborbital series *c* with 5-6 transverse rows (6 in the present specimen), (3) head canals present, with single interorbital canal and single pore *λ*, (3) branchiostegal membrane attached to entire side of isthmus, (4) predorsal area, back at least to the third articulated ray of D2, and breast scaleless (scaleless to the 7th articulated ray of D2 in the present specimen), (5) suborbital row *cp* descending clearly below level of suborbital row *d*, (6) males with tiny intense black spots scattered on head and body and double dark spot on rear of the first dorsal fin.

Head canals are variably developed in this species and may be present or absent, although they may also be present as open furrows (Kovačić, 2005). In particular, our specimen lacks the posterior oculoscapular canal, which carries the *ρ1* and *ρ2* pores. The preopercular canal is present and bears all 3 pores *γ*, *δ*, and *ε*. The anterior oculoscapular canal, which carries pores *σ, λ, κ, α*, and *ρ*, is normally developed on the right side of the specimen, whereas on the left side of the specimen, it looks as if the canal is absent at the position of the *α* pore. It looks like an anomaly with the absence of the part of the anterior oculoscapular canal with the *α* pore, while there are two additional openings in the two parts of the anterior oculoscapular canal that look and function like irregular pores. The length, fin meristics, sex, and collection number of the specimen preserved in the MBS ichthyological collection (IC-MBP 334) are given in Table 1.

**Table 1**. The length, fin meristics, sex and collection number of *Ninnigobius canestrinii* (Ninni, 1883) caught in Slovenian waters.

| MORPHOMETRICS | |
|---|---|
| Total length (TL) | 30.2 mm |
| Standard length (SL) | 25.0 mm |
| MERISTICS | |
| First dorsal fin rays | VI |
| Second dorsal fin rays | I/10 |
| Pectoral fin rays | 16 |
| Anal fin rays | I/9 |
| SEX | male |
| COLLECTION NUMBER | IC-MBP 334 |

The morphological characteristics of the specimen were consistent with the description of the species (Miller, 2004; Franco *et al*., 2005; Kovačić, 2005; Kovačić, 2008; Kovačić, 2020). However, the specimen had one more soft fin ray in the second dorsal fin than indicated by Kovačić (2005), but this is consistent with Franco *et al*. (2005) who indicate that species can have up to 10 soft fin rays in the second dorsal fin. The size of our male specimen is similar to those of Kovačić (2005) (28.4 - 50.1 mm TL) and Tutman *et al*. (2013) (30.1 - 45.3 mm TL).

This finding is the first record for Slovenia. Although the species was expected to occur in Slovenia (Marčeta, 1999; Lipej *et al*., 2006), it was never found despite sampling of fish fauna in all three largest coastal wetlands in Sečovlje and Strunjan, as well as in waters in the wider surroundings of the city of Koper, including the Badaševica River and the Škocjan Inlet Nature Reserve (Žiža, 1997; Lipej and Mavrič, 2012; Mavrič *et al*., 2021; Trkov *et al*., 2022). This indicates that the species is probably rare in Slovenia and has a low population density. The presence of small populations with low density of the species has also been highlighted by other researchers (Gandolfi *et al*., 1982; Franco *et al*., 2005 and references therein). It is possible that a population of this species occurs in the Badaševica and Rižana river systems that feed the wetlands. However, there is also a possibility that the specimen migrated from neighboring areas (*e.g.* Italy, Croatia).

Habitat degradation in the Slovenian coastal zone is very problematic, only less than 20% of it is still in a natural state (Turk, 1999). In particular, the coastal plains are subject to strong anthropogenic influences (Lipej *et al*., 2006), resulting in degraded estuaries and coastal wetlands, where *N. canestrinii* was found in an artificial canal (Fig. 1B, C). In addition, anoxia occurs in the canal system and adjacent river in summer due to low water levels and low flow (Trkov *et al*., 2022). Several cases of pollution have been reported, resulting in fish kills in the Badaševica River (Trkov *et al*., 2022), to which the canals are connected, posing an additional threat to this species. The problem of habitat degradation and pollution has also been noted in other

places where the species occurs (Mrakovčić *et al*., 2004; Franco *et al*., 2005; Kovačić, 2005). In addition, the pressure of habitat degradation is exacerbated by the limited range of this species (Franco *et al*., 2005). These threats, as well as the risks of possible loss of the population of *N. canestrinii* in Croatia, were discussed by Kovačić (2005).

Recent molecular studies suggest that *N. canestrinii* is a complex of at least three species (Tougard *et al*., 2021). The importance of samples from Slovenia for comprehensive studies of endangered goby species in the Adriatic region has been highlighted, in particular, by Kovačić (2005). Therefore, further studies on this species are needed to investigate the study area through systematic fish sampling (taking into account fish communities and distribution structure of fish populations) in order to verify the existence of an established population of this species, clarify its status and define conservation measures.

## CONCLUSIONS

This finding of *N. canestrinii* is the first record for Slovenia, providing important information about the distribution of this rare and endangered fish species. Since the species lives in an environment influenced and degraded by humans, it is important to gain knowledge about its distribution and human influence in order to protect the species and improve the condition of the degraded habitats it inhabits. Therefore, further studies on this species are needed not only in Slovenia, but also in other parts of its range.

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