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# Coupling molecular and risk analysis to investigate the origin, distribution and potential impact of non-native species: an application to ruffe *Gymnocephalus cernua* in Turkey

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## Abstract

Increased detrimental impacts of biological invasions have triggered the development of risk screening tools to streamline the identification of the invasive potential of organisms. These tools assess the biological and historical characteristics of non-native species and predict their invasiveness in a given geographical area. However, challenges for identifying such invasive potential often require a holistic approach, if more accurate predictions are needed. Here, a case study is provided that combines molecular analysis (mtDNA barcoding) with a risk screening (Aquatic Species Invasiveness Screening Kit: AS-ISK) and impact assessment scheme (Environmental Impact Classification for Alien Taxa: EICAT) to address the origin, distribution and potential impact of non-native ruffe *Gymnocephalus cernua* in Turkey. Recently, several populations of this species have been recorded from the Turkish region of Thrace (the European part of Turkey), which represents the species' southernmost geographic range of expansion in Europe, where it is non-native. Molecular data suggested the inclusion of populations from Thrace in a haplogroup widespread in eastern and western Europe from where they might have been introduced, as well as natural spread of the species into Thrace through connected river systems rather than by intentional transport. The AS-ISK scores, also accounting for the effects of climate change, indicated that *G. cernua* is likely to pose a high risk of being invasive in Thrace but a medium risk in Anatolia (the Asian part of Turkey), and the EICAT scores indicated “major” impact. This study suggests that, in the near future, *G. cernua* has a high potential to invade all suitable habitats in Thrace and establish viable populations in several Turkish freshwater ecosystems, including those in Anatolia.

**Keywords:** *Cytochrome c oxidase subunit 1*, *Aquatic Species Invasiveness Screening Kit*, *Environmental Impact Classification for Alien Taxa*, *Thrace*, *Anatolia*

## 1. Introduction

Identifying the invasive potential of non-native species (NNS) is a complex process that has become necessary for the prevention, control and management of biological invasions. Most of the toolkits developed for the risk screening of NNS rely on expert opinion and use a scoring system for risk categorisation (Pheloung et al. 1999; Copp et al.

2005, 2016). These tools provide a rapid decision support to policy makers (Copp et al., 2005; Copp et al. 2021) and address the initial step of a more comprehensive risk analysis (Hill et al. 2020). Whilst there are numerous risk screening applications (see Vilizzi et al. 2019, 2021), studies dealing with follow-up risk assessment are relatively scarce. A full risk assessment examines in detail the risks of

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introduction, establishment, dispersal and impacts, and is generally limited to those species screened as high risk due to the major economic commitment involved in the process (Copp et al. 2005, 2016). Yet, the need for such studies has become even more compelling as a result of climate change and increased human-induced introductions of NNS, which require accurate predictions on their potential dispersal routes and pathways of introduction, range of expansion and, ultimately, impact. In a similar way to climate-niche modelling, which has been recently used to achieve robust predictions of the future range of expansion of NNS (Yoğurtçuoğlu et al. 2021), recent advancements in molecular techniques have helped to uncover their potential pathways, dispersal routes and invasion history (Estoup & Guillemaud 2010; Cristescu 2015). Within this context, the integration of molecular techniques to risk assessment in studies on biological invasions is still at an inchoate stage. The present study aims to fill this research gap by investigating, as a case study, the origin and invasion history of an aquatic NNS, namely the ruffe *Gymnocephalus cernua*.

*Gymnocephalus cernua* is a freshwater fish native to the Caspian, Aral, Black, Baltic and North Sea basins as well as to water bodies of Siberia (Kottelat & Freyhof 2007). Since the 1950s, the species' range has largely expanded to France (Matthey 1966), the UK (Winfield et al. 1996) and the Great Lakes region of North America (Pratt 1988), mainly as a result of accidental introductions. *Gymnocephalus cernua* was also reported from the River Isonzo in Italy in the 1980s (Chiara 1986), and later in the year 2000 from the Hutovo Blato wetland in Bosnia and Herzegovina (Dulčić et al. 2005). One of the most recent records of this species has been from an artificial pond in northeast Algeria, which represents the southernmost point of the species' range of expansion (Arab et al. 2020). Whereas in Europe, the southernmost non-native range of expansion of *G. cernua* was first reported (Kuru 2004) and later confirmed (Çiçek et al. 2021) from the Thrace region of Turkey, and specifically from the delta of the River Maritza – a transboundary river between Turkey, Greece and Bulgaria, with the occurrence of *G. cernua* previously reported from the latter two countries (Popova et al. 1998; Petriki et al. 2014). This finding is noteworthy, as Thrace is known to be one of the main routes for the introduction and establishment of several non-native freshwater fish species into Anatolia – the Asian part of Turkey (Tarkan et al. 2015). However, none of the records of *G. cernua* in Anatolia has so far been confirmed, and especially so by molecular analysis, nor has

any study investigated the genomic origin of non-native species populations in this region except for the invasion history of topmouth gudgeon *Pseudorasbora parva* (Baltazar-Soares et al. 2020).

Similar to gibel carp *Carassius gibelio*, pumpkinseed *Lepomis gibbosus* and *Pseudorasbora parva* (Tarkan et al. 2015), *G. cernua* is known to be an invasive species that, once established, can disrupt interactions amongst native organisms (Savino & Kolar 1996), and ultimately cause adverse impacts (Selgeby 1998). This potential for exerting ecological impacts is due to the species' biological and ecological plasticity, which make it a sturdy and successful invader (e.g. Souchon & Tissot 2012). Indeed, *G. cernua* utilises a wide range of habitats including lakes, pools, estuaries, lagoons, and the margins of streams. Although this species is mainly found in waters up to 10 m depth with sand and gravel substrata, it is known to adapt to a wide range of depths (i.e. 0.25–85 m) in both mountainous and sub-mountainous zones as well as in oligotrophic and eutrophic water bodies (Bergman & Greenberg 1994). *Gymnocephalus cernua* is a successful invader also due to its remarkable reproductive potential (e.g. Kováč 1998; Ogle 1998), with relatively early sexual maturity (first year of life) and high egg production (up to 200,000 eggs in the first batch). The species' invasiveness is also a result of its ability to cope with a wide range of environmental conditions including salinity, temperature, turbidity and eutrophication (Rösch et al. 1996; Souchon & Tissot 2012). The species' opportunistic feeding behaviour, which includes the ability to feed at night or in highly turbid waters, gives it an inter-specific competitive advantage (Janssen 1997; Schleuter & Eckmann 2006). Also, several studies have suggested that *G. cernua* may consume fish eggs in both its native and non-native range (e.g. Adams & Tippett 1991; Hölker & Thiel 1998; Ogle 1998; Selgeby 1998) as well as prey upon young-of-the-year and small fishes (Kozlova & Panasenko 1977; Hölker & Thiel 1998; Popova et al. 1998). Globally, *G. cernua* has been reported to be the second most costly (i.e. US\$53 billion) invasive species with remarkable impacts on the abundance of economically valuable fish species (Cuthbert et al. 2021).

Preliminary risk screening of *G. cernua* using the Freshwater Fish Invasiveness Screening Kit (FISK: Copp et al. 2009) showed this species to pose a high risk of invasiveness in the Balkans region, the Iberian Peninsula, and Scotland (Vilizzi et al. 2019). Using the FISK's decision-support tool improvement represented by the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016; Vilizzi et al. 2021), *G. cernua* attracted a medium-risk

score of being invasive for Turkey (Tarkan et al. 2017b), but a high-risk score for the risk assessment areas represented by the River Neretva catchment in Bosnia-Herzegovina and Croatia (Glamuzina et al. 2017), Great Britain (Dodd et al. 2019), and Croatia and Slovenia (Radočaj et al. 2021). Of note, the risk screening for Turkey was made in the event that *G. cernua* would be introduced into the country, where the presence of the species had not yet been confirmed (see Çiçek et al. 2021).

The objective of this study was to identify the populations of *G. cernua* so far recorded from the Thrace region of Turkey by a molecular approach based on mtDNA barcode sequence analysis coupled with a risk screen and follow-up assessment. It was hypothesised that these populations either originated from a neighbouring geographical range, hence suggesting natural dispersal, or belong to a geographically remote lineage – an indicator of either intentional or unintentional introduction. Based on the premise that Thrace acts as a primary route for non-native freshwater fish introductions into Anatolia, it was predicted that the introduction of *G. cernua* into this region would be highly likely. For this reason, the invasiveness potential of this species in Thrace and Anatolia was evaluated through a risk screen followed by a risk assessment of its potential impacts. The management implications of the findings are discussed especially in view of the potentially rapid proliferation and possible related impacts of *G. cernua* on the native ecosystems of the region. Notably, this study exemplifies the use of an integrative and comprehensive (cf. holistic) approach to address the origin, distribution and potential impact of non-native species by complementing a DNA metabarcoding method with risk analysis.

## 2. Materials & methods

Specimens of *G. cernua* were collected from a wetland area in the delta of the River Maritza consisting of the three interconnected shallow lakes Gala, Pamuklu and Sığircı (40.78°E 26.28°N, 40.77°E 26.20°N and 40.85°E 26.32°N, respectively). Fish were caught with the help of fishers using gill nets of various mesh size (8–70 mm). After capture, all specimens were euthanised with an overdose of 2-phenoxyethanol, stored in ice or directly fixed in 99% ethanol, and transported to the laboratory for later processing.

### 2.1. Molecular analysis

Total DNA of *G. cernua* specimens was extracted from alcohol-fixed fin tissues according to the DNeasy Blood & Tissue Kit protocol (Qiagen,

Hilden, Germany). The vertebrate mitochondrial DNA barcode region of the cytochrome *c* oxidase subunit I (COI) gene was amplified using forward primer FishF1 5'-TCAACCAACCACAAAGACAT TGGCAC-3' and reverse primer FishR1 5'- TAGA CTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005). Polymerase chain reaction (PCR) was carried out as 50 µL total volume containing 5 µL of 10× reaction buffer, 5 µL MgCl<sub>2</sub> (25 mM), 6 µL dNTPmix (10 mM), 1 µL forward primer (10 pmol), 1 µL reverse primer (10 pmol), 0.2 µL Taq DNA polymerase (1 U), 2 µL of DNA template (50 ng/µL), and 29.8 µL sterilised pure water. The PCR was performed using a gradient thermal cycler Biorad T100™ (Bio-Rad, Hercules), with thermal cycling conditions as follows: one cycle at 95°C for 3 min followed by denaturation with 35 cycles at 95°C for 45 seconds, annealing at 60°C for 30 seconds, extension at 72°C for one minute, and one cycle at 72°C for five minutes. The PCR products were visualised on a UV illuminator system after electrophoresis in 1.2% agarose gel containing ethidium bromide (0.5 mg/mL) in Tris-Acetate-EDTA buffer and then purified using the QIAquick PCR Purification Kit (Qiagen) according to manufacturer's instructions. Bidirectional sequencing of the PCR products was performed with an ABI PRISM 3730 × 1 Genetic Analyser (Applied Biosystem; [www.appliedbiosystems.com](http://www.appliedbiosystems.com)) using a BigDye Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystem) at MacroGen Europe ([www.macrogen.com](http://www.macrogen.com)).

Mitochondrial COI barcode sequences of *G. cernua* were analysed via Basic Local Alignment Search Tool searches at the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>) and BOLDSYSTEMS Identification ([https://www.boldsystems.org/index.php/IDS\\_OpenIdEngine](https://www.boldsystems.org/index.php/IDS_OpenIdEngine)) websites. The sequences were aligned with the Clustal W method (Thompson et al. 1994) implemented in Bioedit 7.2.5 (Hall 1999). Sequences were then trimmed down to 606 bp and converted to Fasta and Nexus formatted files. All positions with less than 98% site coverage were eliminated (i.e. fewer than 2% alignment gaps), and missing data and ambiguous bases were allowed at any position (cf. partial deletion option). Haplotypes were defined using DnaSP v5 (Librado & Rozas 2009). Mean genetic distances were calculated using the Kimura 2-parameter (K2P: Kimura 1980) distance model in MEGA X software (Kumar et al. 2018). Phylogenetic relationships among *Gymnocephalus* species/populations were estimated using neighbour-joining (NJ) and maximum likelihood (ML) algorithms in MEGA X software. The

NJ tree was generated according to the K2P model that provided the best-fit evolution model for nucleotide substitution. The ML tree was generated according to the HKY+G model (Hasegawa et al. 1985), which is the most appropriate evolution model selected with the lowest Akaike Information Criterion (AIC) score in jModeltest 0.1.1 (Posada 2008). The NJ and ML trees were generated using 1000 bootstrap replicates to explore phylogenetic affinities of the mitochondrial lineages.

For phylogenetic analysis, five newly generated DNA barcodes of *G. cernua* from the Turkish Thrace region were used in addition to 31 already published sequences from the NCBI GenBank (Table I). DNA barcodes of one individual each of Danube ruffe *Gymnocephalus baloni*, *Gymnocephalus ambriaelacus* and schraetzer (striped ruffe) *Gymnocephalus schraetzer* (GenBank accession numbers: HM902262, KM286679, HM902260, respectively) were also included along with that of pike-perch *Sander lucioperca* (GenBank accession number: HQ960460) as an outgroup. The haplotype network was constructed with the Median-Joining network algorithm (Bandelt et al. 1999) implemented in PopART version 1.7.2 (Leigh & Bryant 2015). The COI barcode region sequenced from the five specimens of *G. cernua* and the corresponding sequences were deposited into GenBank accession numbers MW857189–MW857193.

## 2.2. Risk analysis

**2.2.1. Risk screening.** The AS-ISK decision-support tool was used to quantify the risk of invasiveness of *G. cernua* in both Thrace and Anatolia (hereafter, also referred to as the RA areas), with screenings carried out independently by AST and BY, hence resulting in four screenings in total. The AS-ISK is fully compliant with the “minimum standards” (Roy et al. 2018) for the assessment of non-native species according to the European Commission Regulation on the prevention and management of the introduction and spread of invasive alien species and has been used successfully to screen with a high level of accuracy potentially invasive non-native aquatic organisms in several RA areas worldwide (see Vilizzi et al. 2021).

The AS-ISK screening protocol consists of 55 questions (Copp et al. 2016). The first 49 questions comprise the Basic Risk Assessment (BRA) and are concerned with the biogeographical and biological aspects of the species being screened. The last six questions address the Climate Change Assessment (CCA) and require the assessor to evaluate how

future predicted climate conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. To achieve a valid screening, for each question the assessor must provide a response, a level of confidence in the response, and a justification. Upon completion of the screening, the species receives both a BRA score and a BRA+CCA (composite) score (ranging from –20 to 68 and from –32 to 80, respectively). Scores <1 suggest that the species is unlikely to become invasive and is therefore classified as “low risk” (Pheloung et al. 1999). Higher scores classify the species as posing either a “medium risk” or a “high risk” of becoming invasive. Distinction between medium-risk and high-risk levels depends upon setting a “threshold” value, which in this study was based on the calibrated BRA score = 27.75 for Turkey (Tarkan et al. 2017b) – noting that no calibrated BRA+CCA score was provided in that study. The ranked levels of confidence (1 = low; 2 = medium; 3 = high; 4 = very high) associated with each question-related response in the AS-ISK mirror the confidence rankings recommended by the International Programme on Climate Change (IPCC 2005). Based on the confidence level (CL) allocated to each response, the  $CL_{Total}$  (i.e. all 55 questions),  $CL_{BRA}$  and  $CL_{CCA}$  are computed.

**2.2.2. Environmental impact classification.** Recently, the International Union for Conservation of Nature (IUCN) Species Survival Commission in conjunction with the Invasive Species Specialist Group described a classification system known as Environmental Impact Classification for Alien Taxa (EICAT: [www.iucn.org/theme/species/our-work/invasive-species/eicat](http://www.iucn.org/theme/species/our-work/invasive-species/eicat)). The EICAT was developed for classifying non-native species in terms of magnitude of their detrimental impact on the environment in recipient areas (Blackburn et al. 2014). Implementation of the EICAT has been described in detail and framed into a guideline (Hawkins et al. 2015), and ultimately adapted as an IUCN’s impact scheme standard for the classification of non-native species impacts on the environment (IUCN 2020). The EICAT is based on the Generic Impact Scoring System of Nentwig et al. (2010).

Using five semi-quantitative categories (i.e. Minimal, Minor, Moderate, Major, Massive), the EICAT ranks the magnitude of a species’ environmental impacts and classifies such impacts from Minimal (species unlikely to cause deleterious impacts on the native biota or abiotic environment) to Massive (species likely to lead to the replacement and local extinction of native species and to produce irreversible changes in the structure of communities

Table I. Origin (country and location), GenBank or BOLD accession numbers and haplotype of the *Gymnocephalus* specimens analysed for molecular data.

Species	Country	Location	Accession no.	Haplotype	Source
<i>G. cernua</i>	Turkey	Lakes Gala, Pamuklu and Sığircı (Delta of River Maritza)	MW857189	Hap 7	This study
<i>G. cernua</i>	Turkey	Lakes Gala, Pamuklu and Sığircı (Delta of River Maritza)	MW857190	Hap 7	This study
<i>G. cernua</i>	Turkey	Lakes Gala, Pamuklu and Sığircı (Delta of River Maritza)	MW857191	Hap 7	This study
<i>G. cernua</i>	Turkey	Lakes Gala, Pamuklu and Sığircı (Delta of River Maritza)	MW857192	Hap 7	This study
<i>G. cernua</i>	Turkey	Lakes Gala, Pamuklu and Sığircı (Delta of River Maritza)	MW857193	Hap 7	This study
<i>G. cernua</i>	China	Unknown	KT716370	Hap 8	Yang et al. (2016)
<i>G. cernua</i>	Czechia	Svratka	HQ960461	Hap 1	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	Mydlovary	HQ960571	Hap 1	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	Pouzdransky pond	HQ960630	Hap 1	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	Mostiste	HQ960656	Hap 1	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	Orlice	HQ960766	Hap 1	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	Stara Dyje	HQ960775	Hap 4	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	Olesna Stream	HQ960868	Hap 3	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	River Morava	HQ961005	Hap 1	Halacka et al. (2010)
<i>G. cernua</i>	Czechia	Stipton hatchery	IFCZE825-11	Hap 1	Halacka et al. (2011)
<i>G. cernua</i>	Czechia	Hlohovecky pond	IFCZE843-11	Hap 4	Halacka et al. (2011)
<i>G. cernua</i>	Czechia	Sance dam	IFCZE976-12	Hap 1	Halacka et al. (2011)
<i>G. cernua</i>	France	River Rhone drainage	KJ553416	Hap 1	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KJ128506">https://www.ncbi.nlm.nih.gov/nuccore/KJ128506</a>
<i>G. cernua</i>	Germany	Between Egg Herrieden and Lake Island Constance	HM902266	Hap 1	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	River Pretzsch Elbe	HM902268	Hap 5	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	Muggelspre	KM286681	Hap 1	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	River Sieg	KM286685	Hap 1	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	Rheinsberger	KM286688	Hap 1	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	River Havel	KM286691	Hap 1	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	River Ren	KM286692	Hap 6	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	River Danube (Bavaria)	KM373650	Hap 1	Knebelberger et al. (2015)
<i>G. cernua</i>	Germany	Baden-Wuerttemberg, Lake Rhine, Lake Constance	KM373686	Hap 1	Knebelberger et al. (2015)
<i>G. cernua</i>	Norway	Akershus Hurdal	NOFIS129-18	Hap 2	Brabrand (2016)
<i>G. cernua</i>	Russia	Oka (River Volga, River Ugra)	JN026736	Hap 9	April et al. (2011)
<i>G. cernua</i>	Russia	River Don (Tsimlyansk Reservoir)	JN026737	Hap 10	April et al. (2011)
<i>G. cernua</i>	Russia	Lake Ilmen	JN026741	Hap 10	April et al. (2011)
<i>G. cernua</i>	Russia	Lake Pleschevo	KT989768	Hap 9	Karabanov and Borovikova (2016)
<i>G. cernua</i>	Slovakia	River Danube (downstream of Bratislava)	JN026729	Hap 1	April et al. (2011)
<i>G. cernua</i>	Sweden	Bjorkenas	KJ128506	Hap 2	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KJ128506">https://www.ncbi.nlm.nih.gov/nuccore/KJ128506</a>
<i>G. cernua</i>	Ukraine	Unknown	JN026734	Hap 1	April et al. (2011)
<i>G. cernua</i>	USA	Lake Superior	EU524645	Hap 1	Hubert et al. (2008)
<i>G. ambriaelacus</i>	Germany	Bavaria, Upper Bavaria, River Danube, Lake Ammersee	KM286679	–	Knebelberger et al. (2015)
<i>G. baloni</i>	Germany	River Danube (upstream of Vilshofen)	HM902262	–	Knebelberger et al. (2015)
<i>G. schraetser</i>	Germany	River Danube (upstream of Vilshofen)	HM902260	–	Knebelberger et al. (2015)

and the abiotic/biotic composition of ecosystems). This method also uses 12 classes of impact mechanisms (i.e. competition, predation, hybridisation, transmission of diseases, parasitism, poisoning/toxicity, biofouling, grazing/herbivory/browsing, chemical, physical, and structural impact on ecosystem and interaction with other alien species) associated

with a confidence level allocated as follows: high, medium and low (Blackburn et al. 2014). Species are assigned to an impact category based on the largest impact value recorded, whereas the overall confidence is taken from the impact category to which the highest confidence rank is attributed (Blackburn et al. 2014). In the case of *G. cernua*,

although the impacts of its invasive populations have been well documented throughout its non-native range, there has been no previous attempt to propose an EICAT categorisation for this species. In this study, an EICAT-based risk categorisation was therefore proposed for *G. cernua* in Europe and North America, where its non-native populations are well established. The EICAT-based assessment followed the risk screening process to evaluate more comprehensively the potentially adverse impacts of *G. cernua*.

### 3. Results

In total, 10 haplotypes were identified from the 36 specimens of *G. cernua* examined (Table I). Phylogenetic and network trees indicated the

existence of two haplogroups with a mean genetic distance of 2.03% (Figure 1). Populations of *G. cernua* from the Thrace region were nested within Haplogroup 1, which is widespread in central Europe (mainly, Germany and Czechia), with a similarity ratio >99%; whereas populations from Russia and China were nested within Haplogroup 2 (Figure 2). Molecular data also suggest that non-native *G. cernua* in Lake Superior (USA) belongs to Haplogroup 1.

Based on the calibrated threshold of 27.75, the BRA score for *G. cernua* fell within the high-risk category for Anatolia (28.5) and especially for Thrace (36.0) (Table II). After accounting for the potential effects of climate change, the BRA+CCA score for Anatolia decreased to 21.5, but it did not change for Thrace, hence reflecting a higher risk for the species

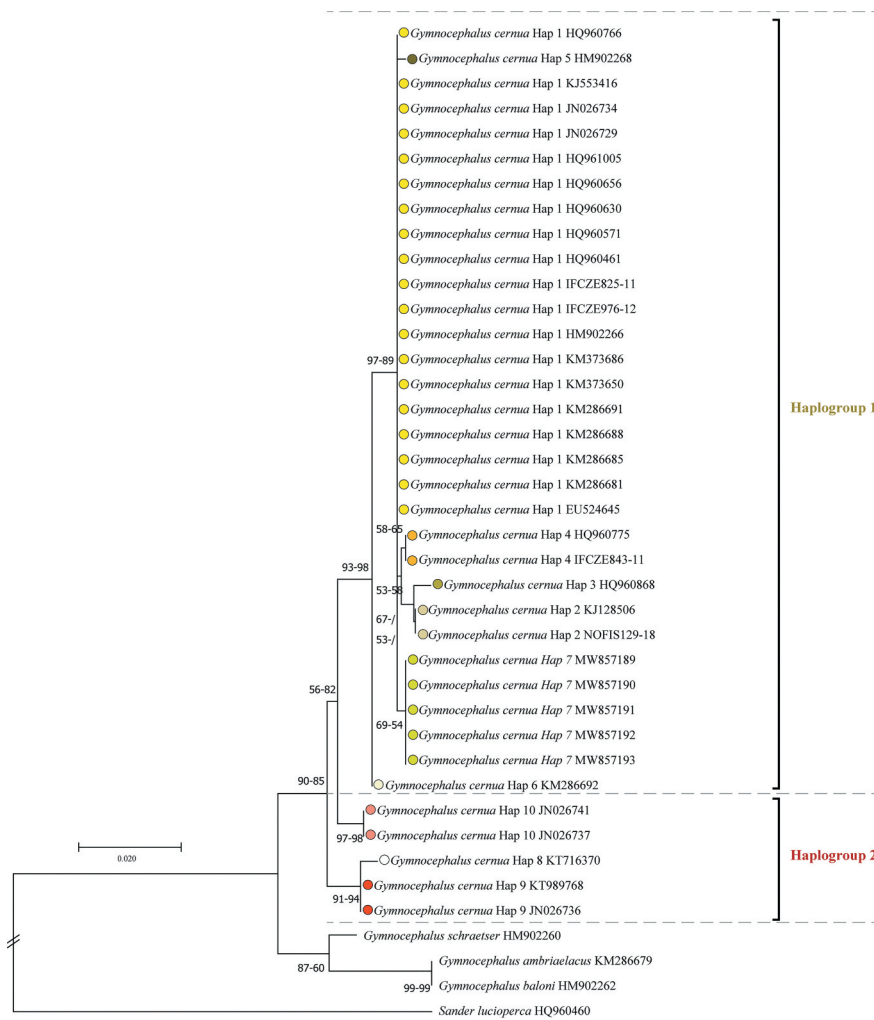


Figure 1. Neighbour-Joining (NJ) tree based on cytochrome *c* oxidase subunit I (COI) barcode gene sequences of ruffe *Gymnocephalus cernua*, Danube ruffe *Gymnocephalus baloni*, *Gymnocephalus ambriaelacus* and schraetzer (striped ruffe) *Gymnocephalus schraetzer*, plus pike-perch *Sander lucioperca* as an outgroup. Bootstrap values (maximum likelihood-NJ) are shown above nodes if 50% or higher in value.

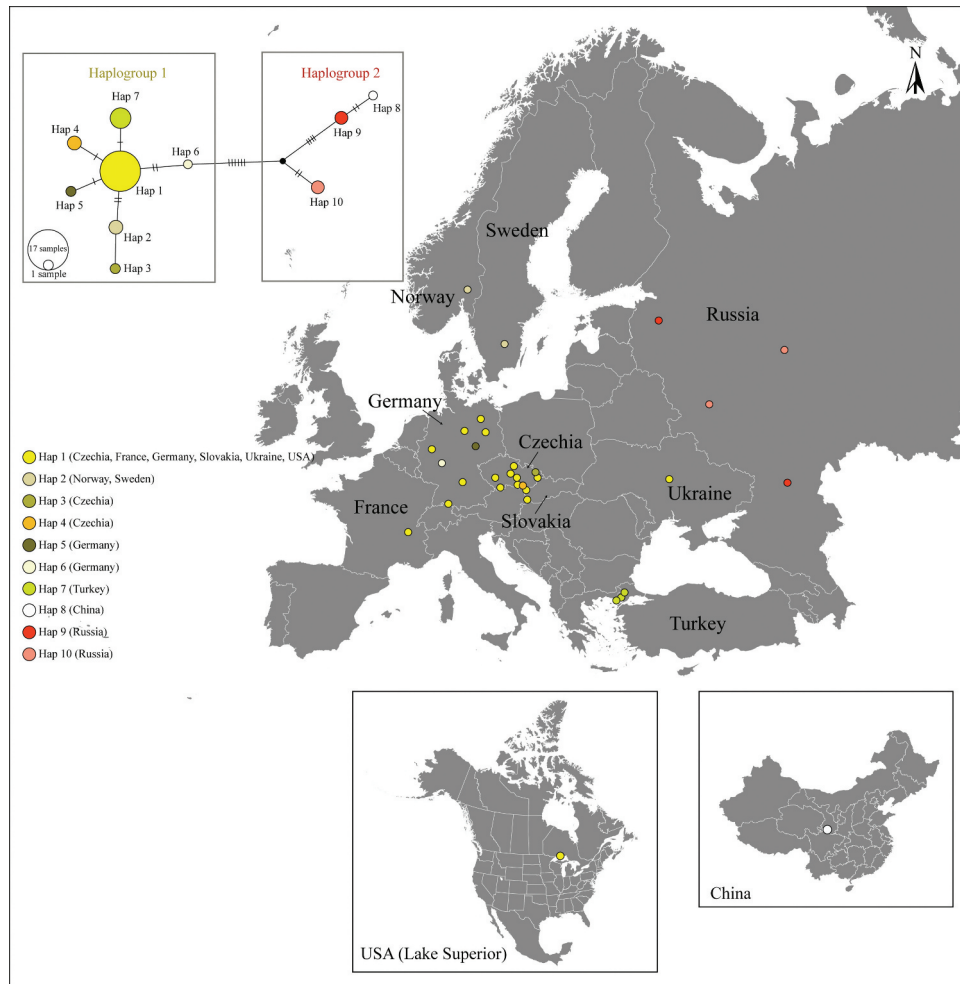


Figure 2. Median joining network tree of COI barcode haplotypes of *G. cernua*.

Table II. Scoring output (mean values from two assessors) from the Aquatic Species Invasiveness Screening Kit for ruffe *Gymnocephalus cernua* in the risk assessment areas of Thrace and Anatolia (Turkey).

Section/category	Thrace	Anatolia
Biogeography/historical	18.5	13.0
Domestication/cultivation	1.0	1.0
Climate, distribution and introduction risk	1.5	0.5
Invasive elsewhere	16.0	11.5
Biology/ecology	17.5	15.5
Undesirable (or persistence) traits	6.5	6.0
Resource exploitation	6.0	6.0
Reproduction	1.5	1.5
Dispersal mechanisms	-0.5	-2.0
Tolerance attributes	4.0	4.0
<b>BRA score</b>	<b>36.0</b>	<b>28.5</b>
Climate change	0.0	-7.0
<b>BRA+CCA score</b>	<b>36.0</b>	<b>21.5</b>

being invasive in this RA area under predicted climate change conditions (Table II). The factors and traits that contributed to the increase in the BRA score included a history of being invasive elsewhere, high climatic matching, parental care, relatively small size at maturity, opportunistic feeding behaviour, and an elevated likelihood of being illegally stocked; those that reduced the overall score included no likelihood of hybridisation with native species and low risk posed to native threatened or protected taxa. The mean CL for the responses to the questions contributing to the BRA, CCA and BRA+CCA were:  $CL_{BRA+CCA} = 2.45 \pm 0.14$  SE,  $CL_{BRA} = 2.51 \pm 0.14$  and  $CL_{CCA} = 1.92 \pm 0.08$ , for *G. cernua* in Thrace;  $CL_{BRA+CCA} = 2.44 \pm 0.16$  SE,  $CL_{BRA} = 2.52 \pm 0.15$  and  $CL_{CCA} = 1.75 \pm 0.25$ , for *G. cernua* in Anatolia. These values indicated medium-to-high confidence for the BRA but low-to-medium confidence for the CCA.



For both Europe and North America, the EICAT impact assessments resulted in “Major impacts”, with a high and medium confidence level, respectively (Table III). The paucity of information on the impacts of introduced populations in North America was a major contributor to the lower confidence ranking. Based on available information, competition and predation received the highest impact score for Europe, whereas for North America this was only true for predation.

#### 4. Discussion

The phylogenetic analysis of this study has supported the hypothesis that *G. cernua* may have entered Turkey through its invasive range in Europe. This was indicated by the haplotype identified for the *G. cernua* populations in the Turkish region of Thrace, which is quite similar to the haplotype for central European populations. Molecular data also showed that non-native *G. cernua* in Lake Superior (USA) shared the same haplotype (Hap 1) with the one widespread in Germany and Czechia. Based on these results and given the discordance between haplotype similarity and geographic distance in the available dataset, the most likely explanation for the non-native dispersion of *G. cernua* within Turkey’s Thrace appears to be natural spread through connected river systems (i.e. River Maritza). It is therefore unlikely that *G. cernua* was introduced by anglers or local fishers, as in the case of some other commonly translocated freshwater fishes in Turkish waters (e.g. perch *Perca fluviatilis*), because of the species’ relatively small size and likely damage to fishing nets (Edirne İpsala, Yenikarpuzlu Fishing Cooperative in Lake Gala: pers. comm.). Clearly, the current findings for the species’ partial invasion history would be supported by further research based on more DNA sequences being made available from the Balkan region.

Literature information on *G. cernua* was overall more widely available for the species’ non-native range in Europe (<https://www.cabi.org/isc/datasheet/80729>), where it has proven to be a powerful invader (Souchon & Tissot 2012). However, there have been only a few screening studies evaluating the species’ risk of invasiveness at the national (Tarkan et al. 2017b; Vilizzi et al. 2019), regional (e.g. river or lake basin: Glamuzina et al. 2017; Tarkan et al. 2017a) or climo-geographic level (Dodd et al. 2019), hence regardless of the species’ status as non-native or translocated. Also, and of note, the calibrated BRA threshold of 27.75 for Turkey (Tarkan et al. 2017b) used in the present study was based on a large sample of freshwater fish

species ( $n = 64$ ) screened for their invasiveness. This makes the threshold statistically highly accurate and robust even after accounting for the later confirmed record of *G. cernua* in Turkey (i.e. a re-screen of the entire dataset after accounting for the presence of *G. cernua* would not have changed the species’ outcome score in such a way to modify the threshold values). In the above risk screenings, the most influential factor for the invasiveness success of *G. cernua* was climate suitability, and especially the temperature regimes affecting the species’ successful dispersal. Although the BRA score in this study indicated a high risk of invasiveness in both risk assessment areas of Turkey (i.e. Thrace and Anatolia), when taken together with the CCA, *G. cernua* was classified as carrying a high risk of invasiveness only for Thrace, whereas for Anatolia, its risk level dropped to medium (Table II). The reason for this discrepancy is that, despite the species’ wide range of temperature tolerances (i.e. Köppen-Geiger climates Cf, Cs, Cw, Df, Ds, Dw: CABI, 2021), the Anatolian part of Turkey has a high variety of climatic regimes (i.e. including the arid types Bskand Bsh), whereas Thrace has a more homogeneous climate that provides suitable temperatures for the successful establishment and dispersal of *G. cernua* (Öztürk et al. 2017). Collectively, Thrace and Anatolia are very large geographic areas, and especially the latter should be evaluated with caution given its several climate types. In this regard, proper basin-level assessments (e.g. Dodd et al. 2019; Yoğurtçuoğlu et al. 2021) could provide for a better evaluation of the level of risk of this species throughout this RA area. This was clearly reflected in the relatively higher confidence levels for the BRA compared to the CCA, as future climate change scenarios predicting an overall increase in temperature (Collins et al. 2013) will likely not facilitate the dispersal and establishment of *G. cernua* in Anatolia.

Both risk screenings and impact assessments for the two RA areas highlighted some knowledge gaps in the environmental biology and impact mechanisms of *G. cernua*. These gaps appeared to be slightly more pronounced for the populations of North America (Table III). However, available literature data for the species clearly indicated its potential for invasiveness due to its high reproductive capacity with early maturity (e.g. Kováč 1998; Ogle 1998), tolerance of a wide range of environmental conditions (Rösch et al. 1996), and impacts mainly as a result of its opportunistic feeding behaviour (Schleuter & Eckmann 2006), with predation on fish eggs (Adams & Tippett 1991) and small fishes (Popova et al. 1998). Among these impact mechanisms by *G. cernua* reported for Europe and North

Table III. Outcomes of the environmental impact classification of Alien Taxa (EICAT; Blackburn et al. 2014) applied to *G. cernea* in its introduced ranges of Europe and North America.

Component	Europe			North America		
	Impacts	Uncertainty	Explanation	Impact	Uncertainty	Explanation
Competition	4	3	Competition for food showed in field	3	2	Laboratory and mesocosm experiments showing out-competition with native fish
Predation	4	3	Stomach content analyses revealed high predation including native fish egg	4	3	Stomach content analyses with different prey items
Hybridisation	1	2	No hybridisation reported	1	2	No hybridisation reported
Transmission diseases to native	1	2	No data available	1	2	No evidence
Parasitism	1	2	Transmissions to other species has not been documented	1	2	No impacts recorded
Poisoning/toxicity	1	3	No data available	1	3	No data available
Bio-fouling	1	3	No impacts reported	1	3	No impacts reported
Herbivory	1	3	No impacts reported	1	3	No impacts reported
Physical, chemical, structural	1	2	No data available	1	3	No data available
Interaction with other alien species	1	1	No impacts reported	2	2	Very weak competitive interaction with yellow perch
Final score	4			4		
Category	Major			Major		
Level of confidence	3 (High)			2 (Medium)		

America, predation on benthic species, insects and macroinvertebrates (e.g. Kováč 1998) could reduce the rich native biodiversity of both Thrace and Anatolia. This reduction could be more severe with high densities of *G. cernua*, particularly when pre-dating on young age groups of native organisms (e.g. Hölker & Thiel 1998). Also, competition for food and behavioural interference may contribute to the decrease in the abundance of native fish (Bergman 1991). More importantly, endemic species that are already endangered may be further threatened by *G. cernua* as a result of its opportunistic feeding behaviour. Considering the rich and unique ichthyofauna of Anatolia, the potential introduction of *G. cernua* should therefore be monitored with great caution. On the other hand, this species is known to co-exist not only with native species but also with some other non-native species (i.e. *Sander lucioperca*, *Perca fluviatilis*, *Lepomis gibbosus*, *Carassius gibelio* and *Pseudorasbora parva*) as observed in water bodies of Thrace where *G. cernua* has established populations (Çiçek et al. 2021). Yet, it is unclear how *G. cernua* may interact with these non-native fishes, which are also commonly found in Anatolian water bodies (Tarkan et al. 2015). Observations and anecdotal evidence from fishers in Lake Gala revealed that *Perca fluviatilis* outnumbered *G. cernua*, though further investigation is required on whether the co-occurrence of these non-native fish species in the same habitat would increase their likelihood of survival, hence ecological impacts and related magnitude – a phenomenon known as “invasion meltdown” (Montgomery et al. 2012).

In conclusion, the present findings based on coupling molecular and risk analysis represent a major step forward in understanding the origin, distribution and potential impacts of *G. cernua* in Turkey. Yet, further research is needed to better understand the species’ potential invasiveness and promote adequate management plans. In this respect, it is unknown whether predation pressure by native species would be efficient enough to control the abundance of *G. cernua*. Thus, contradictory results have been reported in which predation was unable to prevent the increase in the abundance of this species (Mayo et al. 1998), most probably due to selective predation by native species and their avoidance of *G. cernua*. Regardless, physical control by overfishing can be a partially effective option when employed on a regular basis, even though intense trawling on *G. cernua* in Lake Vortsjarv (southern Estonia) was reported to fail in contributing to the decrease in the abundance of this species (Pihu & Maemets 1982). This was attributed to the species’ adaptive capacity to compensate mortality by rapid growth, early maturity

and multiple spawning with high fecundity (Kováč 1998; Ogle 1998). Overall, the best management option for *G. cernua*, at least for Anatolia where the species has not yet been reported, would be by prevention or improvement of early detection mechanisms. These would consist of comprehensive and detailed background information such as habitat vulnerability and inter-connectivity, propagule pressure as well as impacts and biology of *G. cernua* (Hill & Sowards 2015). Increased public awareness of the detrimental impacts of *G. cernua* would further contribute to supportive policy.


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No potential conflict of interest was reported by the author(s).

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