

**Rapid Communication****Quietly invading the Balkans: *Economidichthys pygmaeus* (Holly, 1929) (Gobiidae) established in Northeastern Greece**

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**Abstract**

This study reports on a new translocated population of the Western Greece Goby (*Economidichthys pygmaeus* Holly, 1929) within the Thrace freshwater ecoregion in Northeastern Greece, approximately 370 km east of the species' native range (Ionian freshwater ecoregion; Western Greece). Specimens were identified by morphological examination and molecular confirmation. Based on a literature review and anecdotal information the Western Greece Goby was possibly introduced within the last two to five years, perhaps associated with fish farm imports from Western Greece. Population characteristics of the species in the new locations indicate that the species is fully established within an important biodiversity hotspot, the Aggitis sub-basin of the Strymon River, a transboundary river shared with Greece, Bulgaria and North Macedonia. This record represents the easternmost range expansion of the Western Greece Goby. In addition, it confirms a noticeable trend of expansion of this species since it has been also documented in the Southeastern Adriatic (Albania, North Macedonia) and the Western Aegean (southern Greece) during the last decade. This introduction may trigger ecological consequences, including the homogenization of formerly isolated ichthyofaunas.

**Key words:** alien species, East Mediterranean, gobiid fishes, translocated, endemic, molecular confirmation

**Introduction**

Non-native freshwater fishes can be separated into “alien species” and intra-country “translocated alien species”. Anthropogenic translocations of native freshwater fishes close to their local natural distributional ranges are often overlooked as an ecological concern contrary to alien species from other continents (Koutsikos et al. 2019; Hartman and Larson 2023). Translocated alien species refer to human-assisted transfers that have moved fishes out of their native range but within the same country or within a same geo-political region (Helfman 2007). Outside their natural distributional area, translocated alien species should be given special consideration as potential threats to biodiversity, as are other alien fish species (Tarkan et al. 2017). This issue is a growing concern since

transportation of water and aquatic biota beyond biogeographical boundaries is now frequent, especially with recent developments in aquaculture and transportation infrastructure throughout Europe.

During the last decade, several range-restricted native fishes, known to be endemic to specific river basins, have begun expanding due to human-induced transfers in the Balkan region (Koutsikos et al. 2019; Grapsi-Kotori et al. 2020). Some freshwater gobies originating from the Black Sea region are notorious for their recent expansion and several species are now widespread throughout central Europe where in several occasions they impose severe negative impacts to local ecosystems and native species (Gaye-Siessegger et al. 2022). In the Southern Balkans (i.e., south of the Danube River basin) the occurrence of alien gobies has only recently been documented (Apostolou et al. 2022), and some, formerly range-restricted endemic gobies have started to spread within this area (Barbieri et al. 2015; van Oosterhout et al. 2022).

The Western Greece Goby, *Economidichthys pygmaeus*, is a small-sized benthic goby, rarely exceeding 6 cm total length (TL), protected under Greek Law (L.67/1981) and with no direct commercial value (Economou et al. 2004; Barbieri et al. 2015). The species was originally confined as a native endemic to western Greece, found only within the Ionian freshwater ecoregion (Abell et al. 2008) and specifically in sections of lowland rivers, springs and wetlands, also including heavily modified canals and drainage ditch systems. The natural distributional range of the species includes wetlands and lowland rivers, from the Mornos River in southwestern Greece to the Kalamas River in the northwestern Greece, as well as the Ionian island of Lefkas. Nowadays however, the species' range has begun to expand outside of its natural distribution and it has been recently found in several areas in southern Greece (Barbieri et al. 2015) and in the Prespa Basin (Southeastern Adriatic freshwater ecoregion), while it was also recently been introduced into the territory of Albania and North Macedonia (van Oosterhout et al. 2022). This paper reports on the first documented case of this species in the eastern side of the Balkans, in the Thrace freshwater ecoregion, Northeastern Greece.

## Materials and methods

### *Field sampling procedure and study area*

During routine sampling for the Trans Adriatic Pipeline (TAP) monitoring, five sites were sampled within the Aggitis sub-catchment of the Strymon River basin in December 2022. Each site was sampled twice within two- or three-day intervals utilizing a standard electrofishing protocol aiming at monitoring the fish community composition. Electrofishing was performed using a battery-powered backpack device operating with unidirectional impulse current with a single anode (Smith Root 24L; DC pulsed, 1,5 KW,



**Figure 1.** Map of the five sampled locations, including sites 1 and 2 where the species was located. Inset orientation map shows original native range of *Economidichthys pygmaeus* (dark areas) and the rectangular area where this research took place.

35–100 Hz, max 980 v.). Sampling strategy followed the guidelines described in IMBRIW (2013) where at least a 100 m<sup>2</sup> area was electrofished at each site covering all representative aquatic habitat types (i.e., pools, glides, riffles, etc.). At two sites (Figure 1), a total of ten goby specimens were collected and photographed alive in field aquaria. All specimens were immediately identified as translocated Western Greece Gobies and they were collected for laboratory examination and molecular confirmation (see below). Specimens were euthanized with clove oil and kept in Eppendorf vials in 98% ethanol solution for laboratory analysis.

The two sites with gobies present were located approximately 10 km apart and adjacent to the large, drained swamp area of Tenaghi Philippon. The first site (Site 1), Kefalovrisso (40°59'51.78"N; 24°15'48.36"E) is a channelized perennial stream flowing from spring-fed waters into the topographic depression of Tenaghi Philippon. This sampled site is immediately below an impounded channelized section controlled by a weir and was densely vegetated with submerged aquatic vegetation during our samplings. The second site (Site 2), Kalampaki (41°3'15.34"N; 24°9'58.11"E) is a drainage-irrigation canal with regulated weir-controlled water flows. Both sites displayed similar geometric dimensions (i.e., wetted width, depth etc). Habitats at the Kalampaki site were predominately slow flowing in contrast with the Kefalovrisso site. In addition, the Kalampaki site was heavily silted with signs of organic pollution while it was also recently cleared of riparian vegetation (Figure 2). The other three sampled sites did



**Figure 2.** The two sites where the species was documented: Kalampaki, site 2 (Left) and Kefalovrisso, site 1 (Right) within the Tenaghi Phillipon basin of the Aggitis Drainage, Northeastern Greece. Photographs by Dimitris Zogaris and Alex Charlesworth.

not harbour any goby species; their locations, shown on the accompanied map, are as follows: Site 3 (41°1'56.53"N; 24°9'36.14"A); Site 4 (41°0'33.81"N; 24°13'58.50"E); Site 5 (41° 2'5.64"N, 24°4'30.72"E) (Figure 1).

#### *DNA extraction, amplification and sequencing of the COI gene*

Genomic DNA was extracted from the muscle tissue of a single individual using DNeasy Blood & Tissue Kit™ according to manufacturer's instructions. The DNA was eluted with 20 µL (first elution) followed by 40 µL (second elution) buffer. Specimens were retained for morphological examination. For the amplification of the Cytochrome oxidase subunit I coding area a set of two oligonucleotide primers was designed (F: GAT-CCG-TGC-AGA-GCT-TAG, R: CGG-TGA-GGA-GTA-TTG-TGA-TC). Both primers were designed to amplify a ~ 600 bp fragment. Candidate primers were tested for specificity through NCBI Blast, and through the web-based program Beacon Designer ([http://www.premierbiosoft.com/molecular\\_beacons/](http://www.premierbiosoft.com/molecular_beacons/)) for length, melting temperature, GC content, GC clamp and primer secondary structure. PCR reactions were performed in 20 µL reaction mixtures containing 10 ng template DNA, using the GoTaq® Green Master Mix (Promega, WI, USA). PCR amplification was applied under the following cycling conditions: an initial denaturation at 95 °C for 10 min followed by 35 cycles. Each cycle included the steps below: a denaturation at 95 °C for 45 s, an annealing at 50 °C for 45 s, and an extension at 72 °C for 1 min.

A final extension at 72 °C for 10 min was applied. The PCR amplification Cox1 products were separated in 1.5% (wt/vol) agarose gels using 1X Tris Borate EDTA (TBE) and photographed on a UV transilluminator. All sequences were



**Figure 3.** Specimens photographed in a field aquarium at Kefalovrisso site (Site 1): Adult male c. 4.3 cm TL (above) and subadult, c. 2.2 cm TL (below). Photographs by Dimitris Zogaris.

determined on an ABIPRISM® 3700 DNA Analyzer (Applied Biosystems). Each fragment used was sequenced in both directions in order to maximize the accuracy of the sequence. PCR products were analysed by eye through Alignment Viewer software (Larsson 2014).

## Results

### *Specimen identification*

The obtained sequence (Genbank accession number OQ791290) were matched with 100% identity with the sequence of *Economidichthys pygmaeus*. Therefore, the individual analyzed was confirmed as *E. pygmaeus*.

All goby specimens detected in the field displayed similar morphological characteristics. Morphological examination confirmed the identification of both large and smaller individuals collected at both sites (Figure 3). Body type and colour was similar to other small “sand goby” types with crossing darker lateral midline marks and a densely spotted rounded caudal fin. The dermal perianal organ was observed on the rear abdomen of larger males, which is a unique attribute of the gobies of the genus *Economidichthys* (Kottelat and Freyhof 2007). There is absolutely no possibility of confusion with the only other species of *Economidichthys* (*E. trichonis*) which is a much smaller fish with notable morphological differences and zebra-like patterning immediately evident in the field (Kottelat and Freyhof 2007).

**Table 1.** Abundance, density and size-class data of all species collected at the two sites in the Aggitis basin: Kefalovrisso (1) and Kalampaki (2). The following table provides total numbers caught on two samplings at each stream reach and total density (calculated as number of fishes per 100 m<sup>2</sup> area sampled). Size classes are recorded in five cm intervals.

Species	Status	Numbers	Density	Size Classes	Sites
<i>Alburnoides strymonicus</i>	Native	122	25.4	< 5 to 6–10	1
<i>Alburnus</i> sp. Volvi	Native	7	1.5	< 5 to 6–10	1
<i>Barbus strumicae</i>	Native	102	21.3	< 5 to 21–25	1
<i>Carassius gibelio</i>	Alien	1	3.4	16–20	2
<i>Chondrostoma vardarensis</i>	Native	2	0.4	11–15 to 16–20	1
<i>Cobitis punctilineata</i>	Native	8	1.7	6–10 to 11–15	1
<i>Economidichthys pygmaeus</i>	Translocated	1	3.4	< 5	2
<i>Economidichthys pygmaeus</i>	Translocated	9	1.9	< 5 to 6–10	1
<i>Gambusia holbrooki</i>	Alien	19	4	< 5	1
<i>Gambusia holbrooki</i>	Alien	2800	9380	< 5	2
<i>Lepomis gibbosus</i>	Alien	104	21.7	6–10 to 16–20	1
<i>Oxynoemacheilus bureschi</i>	Native	3	0.6	6–10	1
<i>Petroleuciscus borysthenticus</i>	Native	2	0.4	6–10	1
<i>Pseudorasbora parva</i>	Alien	35	7.3	< 5 to 11–15	1
<i>Rhodeus amarus</i>	Native	114	23.8	< 5 to 6–10	1
<i>Squalius orpheus</i>	Native	126	26.3	6–10 to > 40	1
<i>Squalius orpheus</i>	Native	3	10.1	21–25 to 31–40	2
<i>Vimba melanops</i>	Native	11	2.3	6–11 to 11–15	1

### Locality ichthyofaunal assemblage

The two sampled sites with gobies present (Figure 1) hosted very different fish assemblages. Kefalovrisso site (Site 1) was species-rich hosting 14 species, including rare and endemic species such as Aggitis Spined Loach *Cobitis punctilineata*. The site at Kalampaki (Site 2) had a depauperate species assemblage, with only four species captured, but with a very high number of one alien species, the Eastern Mosquitofish *Gambusia holbrooki* (Table 1).

### Discussion

In this study, we provide both molecular confirmation and photographic documentation of a newly established population of the Western Greece Goby, well outside of the species known native range. The Western Greece Goby shares morphological characteristics with other “sand goby” species (Kottelat and Freyhof 2007; Vanhove et al. 2012) and thus could be confused in the field with *Knipowitschia caucasica* which is a widespread native species in Northern Greece. No other gobiids are known to be present in the immediate study area; i.e., anywhere within the Aggitis subbasin or the Tenaghi Philippon area as evident in EU Water Framework Directive-WFD monitoring (HCMR records) and our personal former investigations. However, the Caucasian Dwarf Goby *Knipowitschia caucasica* is common in the main stem of the Strymon River, while the Western Tubenose Goby *Proterorhinus semilunaris* is known to inhabit a single spring-fed area in a tributary of the Strymon near the city of Serres (Economou et al. 2007; Barbieri et al. 2015).

The human-induced expansion of the Western Greece Goby has been documented since 1997 (Economou et al. 2007; Gkenas and Leonardos 2011).

The species was presumed to be introduced at Lake Pamvotis (Ioannina) through stocking of catfish in 1997 (Economou et al. 2004). Since the year 2010, the species has established reproducing populations at least in six more water bodies and two neighbouring countries. This expansion includes the Beotian Kiffisos and Lake Yliki in Beotia, Lake Taka in the Peloponnese (Barbieri et al. 2015), and Lake Marathon near Athens (D. Zogaris 2019, *personal obs.*). The species was discovered also at the Greek part of Lesser Lake Prespa in 2016 (van Oosterhout et al. 2022) and was soon documented in Greater Lake Prespa in Albania and North Macedonia (Shumka et al. 2020; Trajchevski et al. 2020). Our new finding constitutes the first record of this species in the Thrace freshwater ecoregion which is approximately 370 km eastwards of the species native range across the Pindos range, a very important biogeographic boundary in the Balkans (Vavalidis et al. 2021).

The anthropogenic transfer mechanisms responsible for this introduction remain unknown but the presumption that the fish are stowaways in batches of stocked fish such as carp or other fish from western Greece's fish farming facilities has been repeatedly stated (e.g. Economou et al. 2004). In nearly all cases where the species has spread after 2010, it co-occurs either with the Common Carp (*Cyprinus carpio*) or other translocated fishes; e.g. such as the Acheloos Roach *Rutilus rutilus*, also from Western Greece, translocated in Lake Taka, Peloponnese (Koutsikos et al. 2012). Other possible transfer pathways could be through angling or accidental transfers; e.g. possibly the species could be utilized as fish bait or within wet equipment transports. In any case, we hypothesize that the species has been established very recently in the Aggitis area, perhaps within the last two or five years (based on anecdotal information and monitoring reports by experienced scientists). We also deduce this assumption based on the absence of the species in re-current nation-wide surveys for the application of EU Water Framework Directive-WFD monitoring (HCMR records). Finally, there is no documented natural means for potential transport of this fish species so far from its native range; we are confident that the transfer mechanism is anthropogenic.

The established translocation of the Western Greece Goby in a new ecoregion and especially on a transboundary river basin, poses a potential threat to biodiversity, promoting a trend towards fish faunal homogenisation (Sommerwerk et al. 2017). At this early stage of research little is known of potential impacts the establishment of this species may have on native species. Since this benthic fish is carnivorous, feeding on crustaceans, insects and molluscs (Gkenas et al. 2012), it may compete for food resources with native species. Although the anthropogenic spread of species beyond their natural geographical boundaries may increase species richness within the basins, the result is an artificial novel fish community that has not evolved under the natural processes of regional biogeography

(Vavalidis et al. 2019). There is ample evidence that translocated fishes are expanding across the Balkans (Grapsi-Kotori et al. 2020; Koutsikos et al. 2021). The immediate risk is that most intra-regional translocated species have the ability to easily adapt to their recipient areas and become invasive due to similar environmental conditions with their source areas (Koutsikos et al. 2019).

The spread of Western Greece Goby is of particular conservation concern since the established population is located within an internationally important hotspot for freshwater fishes (Vavalidis et al. 2021). The Strymon is a transboundary river basin that hosts a total of 43 fish species (Economou et al. 2007) with nearly half of them present within the Aggitis subbasin. To the best of our knowledge there was no native goby species within the Aggitis subbasin in recent times, so this new addition certainly may change fish community structure in this area. Importantly, two threatened endemic species are known to be restricted to the lower Strymon with their largest populations hosted within the Aggitis subbasin, namely, the Aggitis Spined Loach *C. punctilineata* and the Greek Brook Lamprey *Caspiomyzon hellenicus*. (Economou et al. 2007). The Tenaghi Philippon area, within the range of the newly found goby population, has been designated a Natura 2000 site in 2017 specifically for this reason. The specific risks of the Western Greece Goby's establishment require further investigation. This important discovery supports the concern that the issue of intra-country translocated species is largely ignored in Greece (Koutsikos et al. 2021) and specific stress-points are transboundary areas and biodiversity hotspots.

### Authors' contribution

DZ – sampling design, data collection, interpretation, writing (original draft, editing); GG – methodology, interpretation, writing (review and editing); JS – methodology, laboratory support; NK and LV – research conceptualization, sampling design, data analysis and interpretation; writing (review and editing); AE – interpretation, writing (review and editing); funding provision, supervision.

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### Ethics and permits

The authors have complied with the institutional and/or national policies governing the humane and ethical treatment of the experimental subjects, and that they are willing to share the original data and materials if so requested. Ethics approval was not required since this work is under routine sampling surveys. Fish collections within this study were conducted within a research project contracted to the Hellenic Centre for Marine Research (HCMR), licenced by the Ministry of the Environment and Energy of Greece (no.173241/1497/27-8-2018).



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