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Rediscovery of Pamvotis Chub in Corfu Island after 63 Years

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Abstract: This study documents the presence of a chub population on Corfu (Kerkyra) Island, based on collected material through electrofishing in spring 2024, coexisting with *Pelasgus thesproticus* and *Telestes pleurobipunctatus*. Morphometric measurements, meristic characteristics and genetic assessment identified the collected specimen as *Squalius pamvoticus*. This rediscovery of the species fills a 63-year gap in the ichthyological literature and advances the knowledge on the already diverse freshwater ichthyofauna of the island. Moreover, it calls for immediate conservation actions to protect this unique insular population and its habitats.

Key words: *Squalius pamvoticus*, Kerkyra Island, Messonghi River, taxonomy, DNA barcoding

Introduction

The taxonomy and distribution of chubs in Greece and particularly in the Ionian biogeographic region is still not resolved. Countrywide, ten species are tentatively recognized as distinct (Barbieri et al. 2015, Tsoupas et al. 2022; but see also Zupančič et al. 2010): *Squalius felowesii* (Günther, 1868), *Squalius keadicus* (Stephanidis, 1971), *Squalius moreoticus* (Stephanidis, 1971), *Squalius orpheus* Kottelat & Economidis, 2006, *Squalius pamvoticus* (Stephanidis, 1939), *Squalius peloponensis* (Valenciennes, 1844), *Squalius prespensis* (Fowler, 1977), *Squalius vardarensis* Karaman, 1928, *Squalius cf. cii* (Richardson, 1856) and *Squalius* sp. Evia. The challenge of species distinction has attracted considerable genetic efforts (Imsiridou et al. 1997, 1998, 2000, Doadrio & Carmona 1998, Durand et

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al. 1999, 2000, Zardoya et al. 1999, Sanjur et al. 2003, Geiger et al. 2014, Perea et al. 2016, Tsoupas et al. 2022). However, according to Kottelat & Freyhof (2007), operational taxonomic outcomes were in most cases absent due to the lack of conceptual tools and therefore taxonomic research is still urgently needed.

This is particularly true for the Ionian River basins. During his pre–World War II PhD thesis, Stephanidis (1939) based on samples collected during 1937–1938, considered the chub populations present in the Aoos, Kalamas, Arachthos, Louros and Evinos rivers in northwestern/western mainland Greece to belong to *Squalius peloponensis* (Valenciennes, 1844) (mentioned as *Leuciscus peloponensis* CUV. et VAL. 1844). In the same study, he also examined specimens collected from Messonghi River in the southern part of Corfu (Kerkyra) Island and assigned them to the same species. Another chub species in the Ionian ichthyogeographic region, *Squalius pamvoticus* (Stephanidis, 1939), was originally regarded as confined to Lake Pamvotis, in which is still present (Leonardos et al. 2008, Vardakas et al. 2022). It was initially described by Steindachner (1895) as *Squalius cephalus* sp. L. var. *albus*, followed by Stephanidis (1939) who described it as *Leuciscus cabeda* var. *pamvoticus* n. var. Subsequently, Economidis (1973) regarded all Epirus/Corfu populations reported in previous published sources, including those by Schmidt–Ries (1943), Belloc (1948), Stephanidis (1948), Stephanidis (1962) and Oliva (1965) as collectively belonging to *Leuciscus cephalus albus* Bonaparte 1838. Economou et al. (2007) acknowledged this taxonomic confusion, stating that the taxonomy and distribution of *Squalius* species require immediate attention; accordingly, they listed chub populations in most Epirus River drainages as *Squalius* cf. *peloponensis* (with a provisional operational name meaning that identification is not completely certain). During the same year, Kottelat & Freyhof (2007) published a wider distribution range for *S. pamvoticus*, including nearly all the rivers of Epirus (Kalamas, Acheron, Louros, Arachthos–excluding Aoos which belongs to a different ichthyogeographic region). This wider range was accepted by the most recent comprehensive checklist of freshwater fish species in Greece (Barbieri et al. 2015), reiterating that the species inhabits clear streams and it reaches a maximum size of 30 cm TL. Outside Greece, the species has only been confirmed as native in Pavllo River (also known as Pavla or Ksanthos river in its Greek segment), which flows into the Butrint lagoon (southernmost coastal part of Albania; Shumka et al. 2023).

Fig. 1. Map of the hydrographic network and range limits (based on IUCN Red List) of *Squalius pamvoticus* in the continental part of NW Greece and Southern Albania, with indication (red arrow) of the Messonghi River segment in Corfu Island, where its population was rediscovered.

Concerning Corfu Island, the last study was by Oliva (1965) who reported a single specimen from an unidentified location in 1961, referring to it as *Leuciscus cephalus albus*. Economou et al. (2007) listed *Squalius* cf. *peloponensis* as having confirmed presence on the island but with doubtful taxonomic status of the population. However, this listing was not substantiated by collected material or related to specific location(s). Notably, neither Kottelat & Freyhof (2007), nor Barbieri et al. (2015) mentioned the existence of insular *Squalius* populations and, according to IUCN (Ford 2024a,b), both species are currently regarded as extant only to mainland Greece.

The present study aims to shed light on the presence of chub on Corfu Island and resolve associated taxonomic uncertainties based on morphometric/meristic and genetic analyses.

Materials and Methods

Sampling was performed on April 26, 2024 using a backpack electrofishing device (IG200/2, 250W, Hans Grassl GmbH) in a lowland segment of the Messonghi River in the southern part of Corfu (Kerkyra) (39.4767371N, 19.9118517E), just north of Vraganiotika village (Fig. 1, 2).

The Messonghi River has a total length of 7.51 km, a catchment area of 39.84 km2 and mean annual

Fig. 2. Photo of the sampling site in Messonghi River (Corfu Island).

flow of 16,910,000 m3. The sampling was conducted based on a modified protocol described in IMBRIW (2013). The total sampling length covered 160 m, with a total fished area of 512 m2. Basic physico-chemical measurements of water (i.e. water temperature, dissolved oxygen, conductivity, salinity) were measured with a HACH HQ40D polymeter. Additional environmental variables, such as depth, width, water velocity, and substrate composition, were also recorded. The abundance and relative density of the chub population were calculated, and one specimen was photographed and immediately preserved in 95% ethanol for further measurements and genetic analyses (Fig. 3, 4). Identification followed the Squalius species key for Europe and the diagnostic morphological, morphometric, and meristic traits described by Kottelat & Freyhof (2007) (Table 1).

Genetic identification of a collected chub specimen was performed using DNA barcoding. DNA was extracted from fin tissue preserved in 95% ethanol using a commercial extraction kit (QIAamp DNA mini kit Extraction Kit, Qiagen, Hilden, Germany). The primers FISHCOILBC_ts and FISHCOIHBC_ts, were used to amplify a partial mitochondrial cytochrome c oxidase I (COI) gene segment (Handy et al. 2011). PCR products were visualized on agarose gels, purified, and sequenced by GENEWIZ Germany GmbH (part of Azenta Life Sciences). The resulting sequence was processed using Geneious Prime software and was compared with those in the BOLD (Barcode of Life Data) database for species identification and was deposited in GenBank under accession number PV053164. To further validate the genetic identification, a maximum likelihood phylogenetic tree was constructed using MEGAX, incorporating *Squalius* spp. sequences from Greece retrieved from the database.

Fig. 3. Specimen of *Squalius pamvoticus* in Messonghi River (Corfu Island).

Finally, the visible and potential threats to the chub population and its habitat were recorded *in situ* for further assessment.

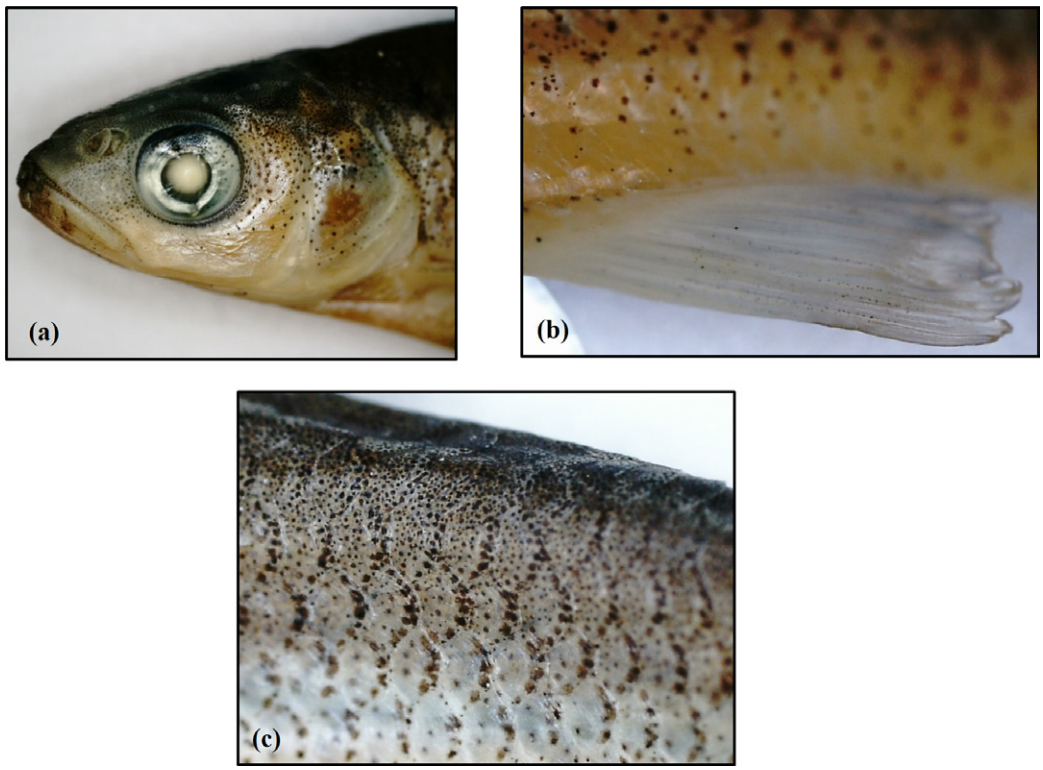

Fig. 4. Ethanol-preserved specimen of *Squalius pamvoticus* from Messonghi River. (a) Head morphology, (b) anal fin with black spots on anterior half; and (c) scales of dorsal half of flank with black spotted margin on posterior edge. Total weight (TW): 2.430 g; Total length (TL): 62.08 mm; Forked length (FL): 55.85 mm; Standard length (SL): 49.32 mm; Head length (HL): 13.75 mm; Body depth (BD): 13.50 mm; Interorbital distance (IOD): 5.22 mm; Eye diameter (ED): 3.65 mm; Caudal peduncle length (CPL): 9.40 mm.

Table 1. Key and basic diagnostic characters (morphological/morphometric/meristic) of the collected *Squalius pamvoticus* specimen in Messonghi River (Corfu Island). Key characteristics are highlighted in grey.

Characteristics of *Squalius pamvoticus* after Kottelat & Freyhof (2007)	**Measurement or criteria fulfillment**
Upper lip projecting beyond lower jaw	Yes
Mouth subinferior	Yes
Distal margin of anal fin straight	No
Distance between tips of first and last branched anal rays (with fin stretched out) less than depth of caudal peduncle	Yes
Head length 26-27% of SL	27.88% SL
Body depth 22-25% of SL	27.37% SL (BD/SL) 1.019 (HL/BD)
Eye diameter 1.4-1.7 times in interorbital distance	1.43 (IOD/ED)
43-47 + 2-3 scales on lateral line	43+2
Caudal peduncle length 18-21% SL	19.06% SL
Scales of dorsal half of flank with a black margin on posterior edge and no or very few pigment on scale pocket	Yes, but with sparse spots on the central part of the scales.
Dorsal fin usually with 8½ branched rays	8½
Anal fin usually with 9½ branched rays	9½

Table 2. Hydromorphological and physicochemical data of the sampling site in Messonghi River (Corfu Island).

Environmental parameter	Site
Altitude (m)	7
Slope (%)	0
Distance from the sea (m)	1,987 (straight line)/ 2,400 (river course)
Conductivity (μS/cm)	693
Dissolved oxygen (mg/l)	9.87
pH	7.35
Water temperature (°C)	18.3
Salinity (psu)	0.39
Shadedness (%)	25
Wet width (m)	4.0
Width of left bank (m)	2.0
Width of right bank (m)	2.0
Maximum depth (m)	0.5
Mean depth (m)	0.25
Pebbles (16-54 mm, %)	20
Gravel (2-16 mm, %)	30
Sand (<2 mm, %)	30
Silt (%)	20
Turbidity	Transparent
Water velocity (m/sec)	<0.1
Pools-deep/still (%)	10
Glide-shallow/move (%)	90
Riparian helophytes	Sparse (reeds)
Bottom vegetation	Abundant (various filamentous algae)

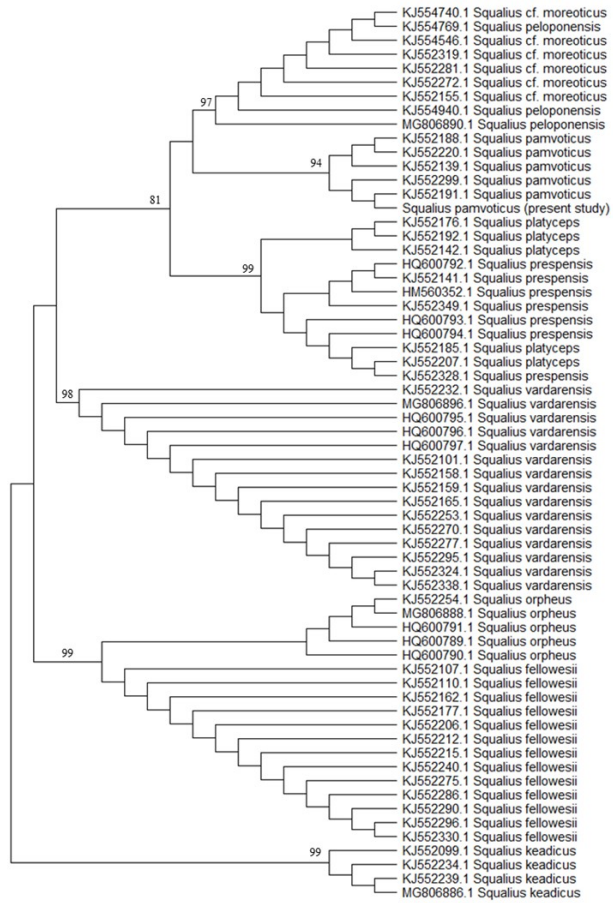

Fig. 5. Maximum likelihood phylogenetic tree based on mitochondrial COI sequences, showing the position of the collected *Squalius pamvoticus* specimen within the *Squalius* genus. *Squalius* sequences from GenBank originating from Greece are included. Bootstrap values are shown.

Results

The key hydromorphological and physicochemical characteristics of the sampled segment of the Messonghi River are presented on Table 2. These features are typical of a low-altitude, coastal and short length insular river of the Ionian Islands.

Chubs were observed in both flowing and still (pools) habitats of the river, coexisting with *Pelasgus thesproticus* (Stephanidis, 1939) and *Telestes pleurobipunctatus* (Stephanidis, 1939). The sampled chub population consisted of 26 individuals, with total length ranging from 5 cm to 8 cm, and an estimated population density of 0.051 inds/m^2. The collected specimen (Fig. 3) was tentatively identified as *Squalius pamvoticus*, based on the *Squalius* key and the relevant morphological/morphometric/meristic characters (Fig. 4, Table 1).

DNA barcoding analysis confirmed this identification, as the generated 554 bp COI sequence exhibited 100% similarity to previously deposited *S. pamvoticus* sequences in the BOLD database, fully validating the morphological assessment. Phylogenetic analysis further supported the identification of the collected chub specimen as *S. pamvoticus* (Fig. 5), as the generated sequence grouped within a well–supported clade containing *S. pamvoticus* sequences from GenBank originating from Greece.

The primary observed and potential anthropogenic impacts on the sampled river segment were

Table 3. Summary of anthropogenic pressures on and overall ecological condition of the segment in Messonghi River (quality ranking: high–1, good–2, moderate–3, inadequate–4, bad–5). Total score ranges from 12 (pristine/undisturbed habitat) to 60 (extremely disturbed habitat with degraded water quality).

Anthropogenic pressures	**Channelization**	**Aquatic habitats**	**Artificial embankment**	**Riparian vegetation**	**Obstacles above the sampling site**	**Obstacles below the sampling site**	**Obstacles in the catchment area**	**Water abstraction**	**Artificial control of water flow**	**Hydrological modifications**	**Artificial still waters**	**Pollution**	**Total score**
Sampling site/ catchment area	2	2	3	2	2	2	2	5	2	3	2	4	**23**

related to urban development, agriculture, pollution from waste and sewages, structural modifications of the riverbanks and water abstraction through pumping installations. Water abstraction and pollution emerged as the main pressures, followed by artificial embankment and hydrological modifications (Table 3). Additionally, bird predation by eagles was also seen in the river pools shortly before sampling. The overall score for all anthropogenic pressures in the respective river segment was 23, suggesting a good ecological condition.

Discussion

Freshwater ecosystems worldwide are facing increasing threats from anthropogenic pressures and climate change, making biodiversity assessments and conservation actions more critical than ever. Corfu Island, located in the Ionian Sea, hosts a unique and diverse freshwater ichthyofauna; however, its freshwater ecosystems remain largely underexplored. This knowledge gap is evident by previous rediscoveries of *Valencia letourneuxi* (Sauvage, 1880) in 2009 after 21 years (Giakoumi et al. 2010) and *Knipowitschia goerneri* Ahnelt, 1991 in 2014 after 31 years (Vukić et al. 2016). Both species were erroneously listed as extinct on the island by the IUCN Red List (Zogaris 2016). Similarly, no records of *Squalius* species have been documented on Corfu Island for over six decades, leading to the assumption that the genus was absent from the island. The present study provides concrete evidence, by using traditional ichthyological assessments and DNA barcoding, of the existence of *Squalius pamvoticus* on Corfu Island, making it the first confirmed record of the species on the island after 63 years.

Knowledge gaps regarding the taxonomic status of *Squalius* species and their phylogenetic relationships in Greece still exist (Barbieri et al. 2015, Vavalidis et al. 2019). The current study justifies the need for combining conventional ichthyological/taxonomic approaches with genetic tools, as field identification of chub species based solely on morphology is practically impossible. Even with the application of taxonomic keys, a definitive species–level identification was not possible, as the collected specimen exhibited overlapping characteristics and measurements with *S. peloponensis* (for comparison see Kottelat & Freyhof 2007). However, DNA barcoding unambiguously identified the species as *S. pamvoticus*, highlighting the critical role of genetic techniques in distinguishing closely related taxa. Furthermore, the discovery of *S. pamvoticus* on Corfu Island raises intriguing questions regarding its biogeographic history. Given the insular nature of its habitat, it remains unclear whether the population has persisted in isolation for an extended period or if periodic dispersal events from the mainland contributed to its presence. Further phylogeographic studies are necessary to assess genetic differentiation between Corfu Island and mainland populations, which could provide insights into historical connectivity and colonization patterns. In any case, this finding significantly extends the biogeographic range of *S. pamvoticus*, beyond its previously documented distribution in the continental areas of the Epirus Region (northwestern Greece) and southern Albania.

The Messonghi River segment, where *S. pamvoticus* was rediscovered is an important habitat for two additional fish species of the island *P. thesproticus* and *T. pleurobipunctatus*. While both species have been previously recorded from the island (Economou et al. 2007), their populations in the Messonghi River remain largely unstudied. Interestingly, they could be tentatively regarded as dwarf populations given that large size specimens were totally absent during our sampling efforts. Data on the biology of all these species are extremely scarce. Particularly, in the case of *S. pamvoticus* are totally absent (Kottelat & Freyhof 2007) and for now they can only be inferred from work on *S. peloponensis* (Barbieri et al. 2002). Given that sampling occurred within the seasonal range of the spawning period of leucicids in Greece (late March–May), we cannot exclude the possibility that larger adult specimens migrate upstream to spawn in less silty river segments with predominantly gravel bottom, a typical spawning substrate of congeneric species (Barbieri et al. 2002).

Corfu Island faces a range of anthropogenic pressures due to its highly developed tourism industry and seasonal population surges, particularly during the spring and summer periods. This, in turn, is associated with a range of complex impacts on water resources. Water abstraction for irrigation and potable supply reduces the available freshwater flow, while pollution from solid waste mismanagement, sewage discharge, and agricultural runoff further degrades water quality. Habitat modifications, such as embankments and the destruction of riparian zones, contribute to ecosystem disruption. Additionally, the introduction of invasive species, particularly *Gambusia holbrooki* (Girard, 1859), poses a significant threat, as it aggressively competes with native fish species (Kalogianni et al. 2022, Kapakos et al. 2024). All these impacts are further exacerbated by the deterioration of natural habitats within the wider context of climatic crisis which is evident through the increased severity of droughts and the increased intensity and frequency of floods.

The rediscovery of *S. pamvoticus* on Corfu Island highlights an urgent need for conservation action. Although the species is currently classified as Near Threatened (NT) on the IUCN Red List (Ford 2024a) and Least Concern (LC) in the Greek Red List of Endangered Species (Barbieri 2024), the restricted distribution of the Corfu population makes it particularly vulnerable to local extirpation. The estimated population density suggests a potentially healthy population; however, its current population trend is unknown as well as the level of intrapopulation genetic variation which is a critical issue demonstrated in other range–restricted species/populations (e.g. Vogiatzi et al. 2014).

Failure to implement conservation measures may result in the loss of the only known insular population of *S. pamvoticus*, though the presence of additional populations of the species on the island cannot be excluded. Corfu freshwaters have over 20 autonomous river basins (Zogaris 2016), many of which maintain perennial flowing reaches, and therefore, suggesting the possibility of undiscovered populations. Therefore, ichthyological surveys should be urged and supported; this pertains to specific initiatives to inventory small water bodies that are often unmonitored and unexplored. The rediscovered population on the island should be treated as priority and flagship population for protection. An encouraging aspect is the good quality of the Messonghi river system based on semi–quantitative assessment, although there is a variety and differentiated intensity of threats posed on all freshwater resources of the island. Habitat protection priorities should be focused on ensuring the minimum ecological discharge of at least 30% of the mean summer discharge measured in undisturbed segments. Water abstraction should be strictly managed at a sustainable level. Urban and agricultural pollution and littering should be also controlled and any actions affecting the hydrological, geomorphological and riparian integrity of river systems should be avoided and considered in all restoration initiatives. Moreover, the introduction and translocation of non-native species should be strictly prohibited for the entire watercourses of the island as well as any sort of recreational or professional fishing targeting such range restricted species. Recent studies (Kalogianni et al. 2025) suggest that eradication of invasive species in short–length watersheds can be successful, and similar efforts should be considered for Corfu's freshwater systems. To further safeguard the population, human assisted translocation based on strict criteria and feasibility assessment (see Kalogianni et al. 2024) into water bodies within the species potential native range on the island, should be also considered. It may be preferable to introduce the species into waterbodies that are depauperate in species in order to avoid any unnecessary potential negative interactions with other native fishes (i.e. predatory behavior by *Squalius* against the rare and localized *V. letourneuxi* populations). Beyond scientific efforts, effective conservation requires broad public engagement. Awareness campaigns, community and citizen science involvement, and collabo-

ration with regulatory agencies, conservation bodies, and local schools will be essential in fostering stewardship of Corfu's freshwater ecosystems. Only through coordinated, science–driven, and community–supported actions will we be able to ensure that valuable freshwater habitats and their species will overcome the anthropogenic challenges posed in the adverse context of climatic crisis events.

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