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ONE OF THE WORLD'S WORST INVASIVE SPECIES, *CLARIAS BATRACHUS* (ACTINOPTERYGII: SILURIFORMES: CLARIIDAE), HAS ARRIVED AND ESTABLISHED A POPULATION IN TURKEY

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Background. Ornamental freshwater fish releases constitute a remarkable proportion of the 100 worst invasive species worldwide. Early detection and knowledge of likely introduction vectors and pathways of potentially invasive fishes into sensitive habitats are key for their proper management, hence rapid and correct identification of their occurrence is crucial. Therefore, we tested the hypothesis that a newly-discovered catfish population was that of *Clarias batrachus* (Linnaeus, 1758), and that this introduction might be of single origin released by aquarium pet fish owners.

Materials and methods. In total, 45 specimens of *C. batrachus* were captured during two electrofishing surveys on 9 and 15 March 2016 by three operators for morphometric and molecular examination. Additionally, 28 specimens were collected for assessing gonadal maturity and sex. They were also measured for standard length and total length and weighed before being dissected. We also produced COI sequences for molecular identification of the species and for tracing its origin.

Results. Morphological and molecular analyses indicated that the examined specimens belong to *C. batrachus*, and that they were likely introduced by aquarium hobbyists, and closest to Indonesian lineage. Successful reproduction and establishment of the species are demonstrated by the occurrence of ripe females and their young of the year and juvenile individuals in the catch.

Conclusion. Our findings confirmed the presence of *C. batrachus* in a region with extraordinarily high biodiversity, including the first evidence to indicate the successful establishment of this species in Turkey. An initial first policy and management step would be to ban the importation and keeping of this species in Turkey, thus reducing the risk of further releases. Increased public awareness for the detrimental impacts of non-native fishes would serve to support the policy and field-based management practices to control, and hopefully eradicate this highly invasive species.

Keywords: ornamental fish, aquatic invasion, walking catfish, Sakarya River, fishery, management

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INTRODUCTION

Global trade has increasingly facilitated biological organisms to spread beyond their natural range. Either deliberately or accidentally, human activities are responsible for the accelerated rate of introductions of non-native species into novel environments (Copp et al. 2005). When these introductions are successful and result in biological invasions, they have the potential to cause considerable economic and biodiversity losses, such as been observed worldwide (Pimentel et al. 2005), and they are thought to be the most significant factor in the loss of global biodiversity after habitat loss (Levine and D'Antonio 2003). Some of the several common vectors for movement of non-native fishes are: transport in ship ballast water (Ruiz et al. 1997, Rothlisberger et al. 2010), escape from aquaculture facilities (Naylor et al. 2001), intentional releases by pet fish owners (Copp et al. 2005, Duggan et al. 2006), and bait-bucket releases during sport fishing (DiStefano et al. 2009). The ornamental fish industry is rapidly growing worldwide and global exports of ornamental fish has grown from USD 181 million to USD 372 million between 2000 and 2011 (Ladisa et al. 2017). However, available information concentrates on North America, the European Union, and Japan (Rixon et al. 2005, Copp et al. 2007, Ladisa et al. 2017) and parts of Asia (Chan et al. 2019) and is poor for other regions, including Turkey. Owing to the lack of basic information on the distribution and potential ecological impacts of ornamental fishes, proper regulations in Turkey cannot be implemented despite tight controls at customs (Yoğurtçuoğlu and Ekmekçi 2018).

Many ornamental fishes cannot establish successful populations in the temperate zone due to the minimum water temperature that they require to reproduce. However, this is not necessarily an obstacle when hot water resources are available all year round. There are several examples of aquarium fishes released into such kind of natural hot water sources and resulted in a dramatic change in ecosystems and economic conditions (Tarkan et al. 2015, Emiroğlu et al. 2016). Indeed, ornamental species releases composed one-third of aquatic species listed in the 100 worst invasive species (Lowe et al. 2000) and nearly half of these species are freshwater fishes (Padilla and Williams 2004).

In Turkey, eight ornamental freshwater fish species have been reported in natural waters so far with four of them being established; goldfish, *Carassius auratus* (Linnaeus, 1758) (see Tarkan et al. 2015), vermiculated sailfin catfish, *Pterygoplichthys disjunctivus* (Weber, 1991), Amazon sailfin catfish, *Pterygoplichthys pardalis* (Castelnau, 1855) (see Emiroğlu et al, 2016), and guppy, *Poecilia reticulata* Peters, 1859 (see Türkmen 2019).

The walking catfish, *Clarias batrachus* (Linnaeus, 1758), is native to south-eastern Asia but has been introduced in many places in the world for aquaculture purposes (Das 2002). This species is also very popular in the aquarium fish trade and has widely spread all over the world through this pathway (Ng and Kottelat 2008). Although *C. batrachus* has become threatened in its native range due to habitat destruction, overfishing, and competition with alien fish species (Khedkar et al. 2010), it is a very robust species that can tolerate a wide variety of stressors such as low food availability and drought and is capable of surviving high turbidity, elevated pollution levels, and low-oxygen conditions (Verma et al. 2011). Assessment of *C. batrachus* using the Freshwater fish Invasiveness Screening Kit (FISK; Copp et al. 2009), has been widely used to assess the invasiveness risk of freshwater fish species, resulting in this species to be the fourth-highest scoring species globally (Vilizzi et al. 2019a). Using the FISK's replacement, the Aquatic Species Invasiveness Screening Kit (AS-ISK; Copp et al. 2016), *C. batrachus* received very high-risk scores specifically for the eastern Mediterranean region (Vilizzi et al. 2019b) and in particular for Turkey (Tarkan et al. 2017). Given that early detection and knowledge of likely introduction vectors and pathways of potentially invasive fishes into sensitive habitats are key for their proper management, rapid and correct identification of their occurrence is crucial. Thus, we tested the hypothesis that a newly-discovered catfish population was that of *C. batrachus*, and that this introduction might be of single origin released by aquarium pet fish owners.

MATERIALS AND METHODS

Study area. Fish were collected from two localities around the spring-fed watercourses in Pınarbaşı Creek (Porsuk River, Sakarya River drainage, Black Sea Basin, Central Anatolia; 39°48′48.60″N, 30°07′04.05″E–39°49′00.08″N, 30°07′53.90″E). The water source is a slow-flowing creek with a mean width of 2.5 m. Annual mean values (with min and max) of temperature, dissolved oxygen, pH, and electrical conductivity of water were 21.0ºC (16.4–24.8ºC), 8.1 (7.1–9.6) mg · L^{-1}, 7.3 (6.8–8.0), and 436.6 (420–460) µS · cm^{-1}, respectively. The depth of the creek varied from 30 to 150 cm and the bottom was covered by mud or soft sediment and submerged aquatic vegetation (Fig. 1). The Pınarbaşı Creek was also inhabited by six native fishes; Sakarya bleak, *Alburnus escherichii* Steindachner, 1897; Sakarya chub, *Squalius pursakensis* (Hankó, 1925); Villwock's killifish, *Anatolichthys villwocki* (Hrbek et Wildekamp, 2003); Sakarya spined loach, *Cobitis simplicispina* Hankó, 1925; Angora loach, *Oxynoemacheilus angorae* (Steindachner, 1897); Anatolian four-barbel scraper, *Capoeta tinca* (Heckel, 1843), and two non-native species (*Pterygoplichthys pardalis* and *Pterygoplichthys disjunctivus*).

Fish sampling and laboratory processing. In total, 45 specimens of *C. batrachus* were captured during two electrofishing surveys on 9 and 15 March 2016 by three operators for morphometric and molecular examination. Additional 28 specimens were captured by electrofishing on 5 April 2019 and stored on ice for transportation to the laboratory to determine assess gonadal development. After capture, all specimens were euthanized using an overdose of 2-phenoxyethanol, stored on ice, and transported to the laboratory.

Fig. 1. Study area (Pınarbaşı, Porsuk River, Sakarya Basin, Turkey)

The initial 45 specimens were subjected to morphological measurement using a point-to-point basis, and never by projections, then a sample of dorsal muscle tissue was taken from each specimen and stored in pure ethanol, frozen and stored at –20°C for molecular analysis. Methods for counts and measurements follow Teugels et al. (1990). Head measurements are presented as a proportion of head length (HL). All measurements including HL are given as proportions of standard length (SL), which along with total length (TL) were measured to the nearest tenth of an mm and weighed to 0.01 g. Some meristic characters, such as the number of branched fin rays of dorsal, pectoral, and ventral fin rays, were counted under a stereo-microscope. Vertebra counts were obtained from radiographs, and include the four Weberian vertebrae and the hypural complex following Bogutskaya and Coad (2009). These characters are among the most commonly used ones for differentiation within the family Clariidae (see Teugels et al. 1990). The unpreserved samples were photographed and then fixed in 5% formaldehyde solution, followed by storage in 70% alcohol. Fish specimens were diagnosed according to the keys in Ng (1999) and Ng and Kottelat (2008). Numbers in parentheses following a particular count are the numbers of examined specimens with that count.

The 28 specimens were collected for assessing the gonadal maturity and sex. They were also measured for their standard length and (SL) and total length (TL) to the nearest 0.1 mm and weight (to the nearest 0.1 g) before being dissected to determine gonad maturity stage, including the presence of ripening eggs.

Three additional samplings on 12 April 2018, 16 September 2018, and 9 June 2020 at the same stretch of the Pınarbaşı Creek were conducted to reveal relative abundances of resident fish species, which was calculated as the number of individuals per meter of the creek length, along 200 meters transect.

Molecular identification and molecular data analyses. The extraction of DNA from the tissue samples of 45 specimens was performed using Qiagen DNEasy Blood and Tissue Kit according to manufacturer protocol, except for the homogenization step, which instead used a bead-beating technique. The DNA concentration and purity of the samples were measured with a Qubit 3.0 fluorometer using a dsDNA kit and diluted to 50 ng · μL^{-1} for the standardization of PCR reactions. All samples were electrophoresed on 1% agarose gel for UV visualization of samples.

The PCR reactions were set according to 4 µL of 5× FIREPol Master Mix Ready to Load (12.5 mM $MgCl_2$)

(Solis BioDyne, Estonia), 0.5 µL of each primer (F, R), 1 µL of template DNA (50 ng·µL^{-1}) and 14 µL of ultrapure water (Keskin et al. 2016). Partial fragments of the COI gene were amplified using two universal primers (Ward et al. 2005) for fish DNA barcoding; FishF1: 5′-TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1: 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3. PCR thermal profile was set to pre-activation step at 95°C for 15 min, 30 cycles of denaturation step at 95°C for 15 s, annealing step at 54°C for 45 s, extension step at 72°C for 2 min, completed with a final extension step at 72°C for 10 min. Amplified PCR product size was confirmed on 2% agarose gel electrophoresis and UV visualization.

ExoSAP-IT PCR Product Clean up Reagent was used for enzymatic clean-up of the amplified PCR product. Products were marked using the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) and sequenced using an ABI 3730 capillary sequencer according to manufacturer's instructions. Nucleotide sequences were assembled and aligned using MEGAX (Kumar et al. 2018). CodonCode Aligner 8.0.2. was used for construction for consensus sequences. All sequences were trimmed to a minimum-uniform length of 548 base pairs without gaps and deposited in NCBI GenBank as two haplotypes under accession numbers MN663125-MN663126. GenBank and BOLD databases were used for the identification of samples. In order to quantify the proportion of correctly identified queries, TaxonDNA/SpeciesIdentifier v1.7.7 (Meier et al. 2006) was used according to Best Close Match (BCM), with a 3.0% threshold. Hasegawa-Kishino-Yano Gamma+ distribution (Hasegawa et al. 1985) was calculated with MEGAX (maximum likelihood fits of 24 different nucleotide substitution models) as the best substitution model according to lowest BIC and AICc values to be used in phylogenetic analysis. However, Hasegawa-Kishino-Yano model by using MEGAX was not allowed to construct a phylogenetic tree, so instead the second-best substitution model, Tamura 3 Parameter, was used to build a phylogenetic tree. Pair-wise genetic distances were also calculated using Tamura 3 Parameter+G (gamma distributed). The haplotype network tree was constructed to see relations between different sample sequences using Network 5.0 software.

RESULTS

Family CLARIIDAE

***Clarias batrachus* (Linnaeus, 1758)**

(Figs. 1–5, Table 1)

Description. Body cylindrical, becoming compressed towards caudal peduncle (Table 1). Dorsal profile rising gently from tip of snout to origin of dorsal fin and thereafter almost horizontal to end of caudal peduncle. Ventral profile slightly convex to middle of head and thereafter almost horizontal to end of caudal peduncle. Head dorsoventrally depressed; dorsal profile slightly convex and ventral profile almost straight. Snout narrow, lateral outline straight and anterior outline convex when viewed dorsally. Bony elements of dorsal surface of head covered with thick skin. Both fontanelles clearly seen, frontal fontanelle long and thin; anterior tip reaching just posterior to line through posterior orbital margin. Occipital process rounded (Fig. 2). Mouth narrow and sub-terminal, with fleshy, plicate lips. Barbels in four pairs; long and slender with thick fleshy bases. Maxillary barbel extending nearly to base of first dorsal-fin ray. Nasal barbel extending nearly to tip of occipital process. Inner mandibular barbel origin close to midline; barbel thicker and longer than nasal barbel and extending to base of pectoral spine. Outer mandibular barbel originating posterolateral of inner mandibular barbel, extending to tip of pectoral fin.

Total vertebrae 56 (3), 57 (2), 58 (3), 59 (2). Fin rays covered by thick layer of skin, dorsal and anal fins separated from caudal fin. Dorsal fin with 63 (1), 69 (1), 72 (1), 73 (1) ,74 (2), 77 (2), 78 (2) branched rays. Anal fin 46 (1), 50 (1), 52 (2), 55 (1), 56 (2), 58 (2) branched rays; margin straight and parallel to ventral edge of body. Caudal fin rounded with 18 (2), 19 (2), 20 (3), 21(2) rays. Pectoral fin with small spine, sharply pointed at tip, and 9 (6), 10 (3) rays. Anterior margin of spine rugose margin straight anteriorly, convex posteriorly. Pelvic fin origin at anterior third of body with 5 (4), 6 (5) branched rays and convex margin; tip of fin reaching base of first few anal-fin rays. Skin smooth. Lateral line complete and mid-lateral in position. All specimens were calico morph with spotted or particolored skin that is mostly white predominant. Fins are also calico colored in dark grey and white with the median fins that have very thin hyaline distal margin. Pectoral-fin rays, with hyaline interradial membranes. Pelvic fin hyaline.

Molecular identification. COI sequence similarities of the sequences generated by the presently reported study were between 99.82% and 100%. Only one specimen (No. 2; Fig. 3) showed an intraspecific distance of a single nucleotide with the remaining eight specimens. Possible stop codons were checked for confirmation of the data reliability using MEGAX software, but none were observed. Mean nucleotide compositions were calculated with nucleotide composition analysis and the values were 29.42% for A (Adenine), 26.10% for C (Cytosine), 16.08 for G (Guanine), and 28.50 for T (Thymine). For the entire data set, the mean A + T and C + G rates were 57.92% and 42.18%, respectively. The highest pair-wise genetic distance rate was 0.02% among specimen No. 2 and the remaining eight specimens. Analyses were done with an out-group sequence—African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), and eight different sequences, which belonged to different countries. The neighbor-joining tree included 17 sequences (Fig. 3). Samples were clearly separated on the country level except for Indonesian (KU692438) specimens. Mutational vectors were shown with a red circle and the populations were shown as a pie chart and yellow circle (Fig. 4).

Material examined and evidence for establishment. *Clarias batrachus*: FFR 05701, 10, 114–228 mm SL; Turkey: Eskişehir Province: Pınarbaşı Creek; Porsuk River, 39°48′48.60″N, 30°07′04.05″E–39°49′00.08″N,

Table 1

Morphometric characters and meristic counts of *Clarias batrachus* from Pınarbaşı, Porsuk River, Turkey (n = 10)

Character	Value	
	Range	Mean ± SD
Standard Length (SL) [mm]	114.0–228.0	175.7 ± 29.5
Predorsal length [%SL]	30.2–38.6	33.7 ± 2.1
Preanal length [%SL]	46.4–58.1	51.1 ± 3.0
Prepelvic length [%SL]	40.3–49.9	43.4 ± 2.5
Prepectoral length [%SL]	19.4–23.4	21.2 ± 1.3
Length of dorsal fin base [%SL]	58.0–76.0	66.4 ± 4.3
Anal fin length [%SL]	45.4–55.2	49.5 ± 2.6
Pelvic fin length [%SL]	9.0–11.5	9.9 ± 0.6
Pectoral fin length [%SL]	14.8–17.7	15.8 ± 0.8
Pectoral-spine length [%SL]	9.4–13.7	11.4 ± 1.5
Caudal fin length [%SL]	13.9–17.9	15.3 ± 1.2
Body depth at anus [%SL]	14.6–18.2	16.1 ± 1.2
Caudal peduncle depth [%SL]	5.6–7.3	6.6 ± 0.5
Distance between occipital process and dorsal fin [%SL]	5.4–7.5	6.4 ± 0.7
Head length (HL) [%SL]	25.3–31.1	27.4 ± 1.5
Head width [%HL]	89.9–100.1	95.1 ± 4.0
Head depth at eyes [%HL]	30.1–43.0	38.7 ± 3.7
Snout length [%HL]	30.0–35.0	32.7 ± 1.6
Interorbital distance [%HL]	44.0–47.0	44.9 ± 1.0
Eye diameter [%HL]	6.0–9.0	7.1 ± 0.8
Occipital process length [%HL]	21.0–33.0	28.5 ± 3.7
Occipital process width [%HL]	11.0–15.0	12.8 ± 1.0
Frontal fontanelle length [%HL]	17.0–23.0	19.8 ± 1.8
Frontal fontanelle width [%HL]	1.0–5.0	3.3 ± 1.1
Occipital fontanelle length [%HL]	3.0–8.0	5.1 ± 1.2
Occipital fontanelle width [%HL]	1.0–2.0	1.5 ± 0.4
Nasal barbel length [%HL]	56.0–77.0	65.6 ± 5.4
Maxillary barbel length [%HL]	87.0–116.0	98.2 ± 8.2
Inner mandbular barbel length [%HL]	69.0–95.0	84.0 ± 7.4
Outer mandibular barbel length [%HL]	58.0–77.0	65.5 ± 5.1

SD = standard deviation.

30°07′53.90″E. Comparative material: African sharptooth catfish, *Clarias gariepinus*: FFR 05702, 5, 200–350 mm SL; Turkey: Hatay Province: Asi River at Demirköprü, 36.2487°–36.3549°. *Clarias gariepinus*: FFR 05703, 12, 142–798 mm SL; Turkey: Sakarya Province: Sakarya River at Çifteler, 39.3617°–31.0600°.

Successful establishment of *C. batrachus* in the study area became apparent after examining 28 individuals of which 11 were females, 10 were males, and seven were juveniles. Length and weight of this sample varied from 42.5 to 323.0 mm TL and from 7.0 to 209.9 g, respectively, with values for seven juveniles ranging 42.5–129.0 mm TL and 7.0–15.8 g, respectively (Fig. 5). Four of the females >185 mm TL had ripening eggs (Fig. 5).

The relative abundance of *C. batrachus* in the ichthyofauna of the Pınarbaşı Creek was similar to other non-native species, *Pterygoplichthys disjunctivus* and some other native species, *Cobitis simplicispina*, *Oxynoemacheilus angorae*, *Capoeta tinca*, and *Squalius pursakensis* but considerably lower than two others, *Alburnus escherichii* and *Anatolichthys villwocki* (Table 2).

DISCUSSION

Clarias batrachus is similar in size and appearance to *C. gariepinus*, which also inhabits the Sakarya River basin. The former is distinguished from the latter by: the presence of a pointed-shaped occipital process (vs. rounded); narrower snout with angular lateral margins (vs. broader snout with rounded lateral margins); and fewer total number of vertebrae (54–59 vs. 56–63). Although most *C. batrachus* individuals are grey or grey-brown with small white spots, albino and calicomorph specimens are also possible (Teugels 1986). However, these color patterns are uncommon in the wild, but popular among aquarists. The individuals with calicomorph pattern (Fig. 2) in this study supported our molecular data that *C. batrachus* was introduced as a result of the release of aquarium fish, such as also reported for England (Zięba et al. 2010) and on the Island of Mauritius (Nunkoo et al. 2015).

Our molecular data also suggests that there is almost no intra-genetic variation among specimens (only a single nucleotide variation in one of nine specimens), with all specimens having the same population background.

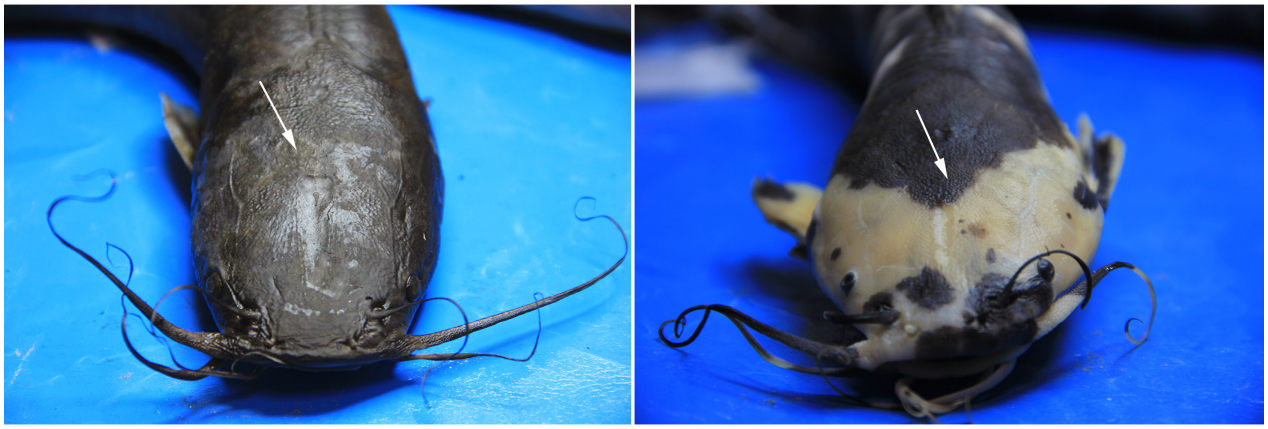

Fig. 2. Dorsal view of heads of *Clarias gariepinus* (left) and *Clarias batrachus* (right) collected at the Porsuk River, Sakarya Basin, Turkey; white arrows show occipital processes

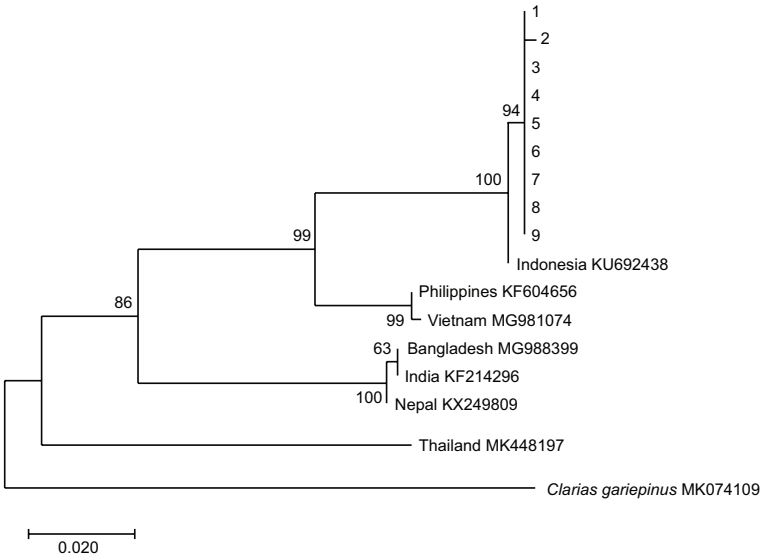

Fig. 3. Median-joining network tree of *Clarias batrachus* specimens from this study and reference sequences gathered from NCBI GenBank

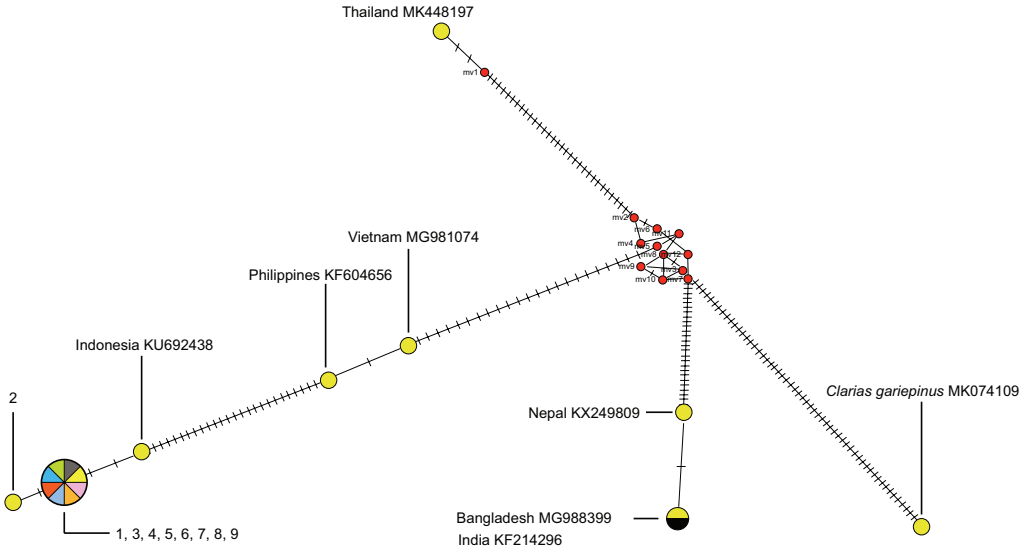

Fig. 4. Neighbor-Joining tree including 18 sequences (1–9 from this study, others taken as references from NCBI GenBank)

Fig. 5. *Clarias batrachus* ovaries with ripening eggs (above); a juvenile *Clarias batrachus* (below) caught at Pınarbaşı, Porsuk River, Sakarya Basin, Turkey

Table 2

The relative abundance of fish species collected at different dates from Pınarbaşı, Porsuk River, Turkey

Species	Relative abundance		
	12 Apr 2018	16 Sep 2018	9 Jun 2020
Pterygoplichthys disjunctivus	0.06	0.04	0.03
Anatolichthys villwocki	0.23	0.08	0.03
Squalius pursakensis	0.01	0.02	0.09
Alburnus escherichii	0.19	0.18	0.21
Cobitis simplicispina	0.04	0.02	0.01
Oxynoemacheilus angorae	0.03	0.06	0.01
Capoeta tinca	0.01	0.03	0.02
Clarias batrachus	0.04	0.03	0.02

Relative abundance was calculated as the number of individuals per meter of the river section.

The median-joining network indicated that specimens examined in the presently reported study belong to a genetic pool from Indonesia, Philippines, and Vietnam. It is known that there is no known aquaculture initiative for *C. batrachus* in Turkey, and aquarium hobby is quite common in some hot water resources in Turkey, where aquarists intentionally dump ornamental fishes into natural waters so the introduction of this species might have been through ornamental fish import/export activities.

The discovery of *C. batrachus* in Anatolia should be viewed with great caution, as this fish has a high potential to become invasive species (Tarkan et al. 2017), thus can threaten native species and ecosystems (Guerrero 2014). It has been reported by local inhabitants in the vicinity of the Sakarya River that abundance of *Clarias* species has substantially increased and outcompeted the native catfish, European catfish, *Silurus glanis* Linnaeus, 1758, which is in high demand in the region as a food fish (Emiroğlu et al.

2016). Further, *C. batrachus* has a relatively-high salinity tolerance (up to 18‰*) for a freshwater fish species (Sarma et al. 2013) and moderate tolerance to colder waters (lower lethal temperature of 9.8°C) for a tropical fish (Shahin et al. 2013). These factors facilitate the colonization success of *C. batrachus* into new environments, such as Anatolia, where the minimum water temperature is never <16°C due to available hot springs along the study basin (Emiroğlu et al. 2016). Additionally, the overwintering behavior of *C. batrachus*, which burrows itself into pond and stream beds during dry and cold winter months, makes it more resilient not only for hot water bodies but also for some other suitable regions across Anatolia.

The observed gonadal development, and the presence of young-of-the-year specimens of *C. batrachus*, gives evidence for the species' establishment success in Anatolia, such has been observed in other areas (e.g., Florida, USA) where its populations have become established within a few years of their initial introduction (Courtenay et al. 1984). Many countries have banned possession of this species, and it is considered illegal to have without a federal permit e.g., under the American Federal Register 67 FR 48855 under the Lacey Act (18 U.S.C. 42) (Patoka et al. 2018). However, in its native range, *C. batrachus* is threatened by its congener *C. gariepinus* (see Ng et al. 2014), which also co-occurs with *C. batrachus* in the Pınarbaşı Creek. In the Philippines, *C. batrachus* has been recorded to have displaced the native big catfish, *Clarias macrocephalus* Günther, 1864, where the former species has been introduced (Guerrero 2014). Potential ecological impacts due to a high abundance of *C. batrachus*, which has an opportunistic feeding behavior, includes egg predation of native fishes. This is of particular importance with regard to endemic species, which inhabit the same water bodies as *C. batrachus* in Anatolia (Emiroğlu et al. 2016).

In conclusion, our findings confirmed the presence of *C. batrachus* in a freshwater habitat in Anatolia, a region with extraordinarily high biodiversity, including the first evidence to indicate the successful establishment of this species in Turkey. An initial first policy and management step would be to ban the importation and keeping of this species in Turkey, thus reducing the risk of further releases. Eradication could also be applied where appropriate. However, it should be noted that any environmental factor, such as temperature, will not itself force the species to aggregate in certain places (e.g., hot springs) such as the case in more temperature dependent species (e.g., *Pterygoplichthys* spp.). Indeed, *C. batrachus* is known to disperse through entire river basins, especially during the increased temperatures of summer months (Emiroğlu et al. 2016). Thus, eradication efforts would probably fail not only for this reason but also owing to the species' survival capacity out of the water. A recent study on the eradication of *C. gariepinus* by rotenone revealed unexpected survival rates at relatively high treatment doses, post-treatment recovery, and avoidance response (Jordaan et al. 2017) suggesting that the management of *Clarias* species' invasion is likely to be complex and that introduction prevention in the first place is preferred. However, another alternative would be a control plan that includes comprehensive and detailed background information such as habitat vulnerability and inter-connectivity, propagule pressure, and impacts and biology of the species (Hill and Sowards 2015). Physical control by overfishing can be a partially-effective option when used on regular basis. Field-based management practices to control, and eradicate this and similar highly invasive ornamental species requires public embracement. Therefore, in the long term, increased public awareness for the detrimental impacts of non-native ornamental fishes would serve as a supportive policy.

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REFERENCES

Bogutskaya N.G., Coad B.W. 2009. A review of vertebral and fin-ray counts in the genus *Alburnoides* (Teleostei: Cyprinidae) with a description of six new species. Zoosystematica Rossica **18** (1): 126–173.

Chan F.T., Beatty S.J., Gilles A.S.jr., Hill J.E., Kozic S., Luo D., Morgan D.L., Pavia R.T.B.jr., Therriault T.W., Verreycken H., Vilizzi L., Wei H., Yeo D.C.J., Zeng Y., Zięba G., Copp G.H. 2019. Leaving the fish bowl: The ornamental trade as a global vector for freshwater fish invasions. Aquatic Ecosystem Health and Management **22** (4): 417–439. DOI: 10.1080/14634988.2019.1685849

Copp G.H., Bianco P.G., Bogutskaya N., Erős T., Falka I., Ferreira M.T., Fox M.G., Freyhof J., Gozlan R.E., Grabowska J., Kovač V., Moreno-Amich R., Naseka A.M., Peňaz M., Povž M., Przybylski M., Robillard M., Russell I.C., Stakėnas S., Šumer S., Vila-Gispert A., Wiesner C. 2005. To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology **21** (4): 242–262. DOI: 10.1111/j.1439-0426.2005.00690.x

Copp G.H., Templeton M., Gozlan R.E. 2007. Propagule pressure and the invasion risks of non-native freshwater fishes: a case study in England. Journal of Fish Biology **71** (sd): 148–159. DOI: 10.1111/j.1095-8649.2007.01680.x

Copp G.H., Vilizzi L., Mumford J., Fenwick G.V., Godard M.J., Gozlan R.E. 2009. Calibration of FISK, an invasiveness screening tool for nonnative freshwater fishes. Risk Analysis **29** (3): 457–467. DOI: 10.1111/j.1539-6924.2008.01159.x

* In the wake of the growing criticism of the Practical Salinity Scale concept (and especially "PSU" as a "unit"), Acta Ichthyologica et Piscatoria is in favor of expressing salinity in parts per thousand (‰), regardless if a direct or indirect method was employed to determine the water salinity.

Copp G., Vilizzi L., Tidbury H., Stebbing P., Tarkan A.S., Miossec L., Goulletquer P. 2016. Development of a generic decision-support tool for identifying potentially invasive aquatic taxa: AS-ISK. Management of Biological Invasions **7** (4): 343–350. DOI: 10.3391/mbi.2016.7.4.04

Copp G.H., Wesley K.J., Vilizzi L. 2005. Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): The human vector. Journal of Applied Ichthyology **21** (4): 263–274. DOI: 10.1111/j.1439-0426.2005.00673.x

Courtenay W.R., Hensley D.A., Taylor J.N., McCann J.A. 1984. Distribution of exotic fishes in the continental United States. Pp. 41–77. *In*: Courtenay W.R., Stauffer J.R. (eds.) Distribution, biology and management of exotic fishes. John Hopkins University Press. Baltimore, MD, USA.

Das S.K. 2002. Seed production of magur (*Clarias batrachus*) using a rural model portable hatchery in Assam, India—A farmer proven technology. Aquaculture Asia **7**: 19–21.

DiStefano R.J., Litvan M.E., Horner P.T. 2009. The bait industry as a potential vector for alien crayfish introductions: problem recognition by fisheries agencies and a Missouri evaluation. Fisheries **34** (12): 586–597. DOI: 10.1577/1548-8446-34.12.586

Duggan I.C., Rixon C.A.M., MacIsaac H.J. 2006. Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. Biological Invasions **8**: 377–382. DOI: 10.1007/s10530-004-2310-2

Emiroğlu Ö., Ekmekçi F.G., Aksu S., Başkurt S., Atalay M.A., Tarkan A.S. 2016. Introduction and establishment of tropical ornamental fish, *Pterygoplichthys* spp. (Actinopterygii: Siluriformes: Loricariidae) in hot springs: Aquarium trade as a potential risk for biodiversity in Turkey. Acta Ichthyologica et Piscatoria **46** (4): 351–356. DOI: 10.3750/AIP2016.46.4.07

Guerrero R.D. 2014. Impacts of introduced freshwater fishes in the Philippines (1905–2013): a review and recommendations. Philippine Journal of Science **143**: 49–59.

Hasegawa M., Kishino H., Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution **22** (2): 160–174. DOI: 10.1007/BF02101694

Hill J., Sowards J. 2015. Successful eradication of the non-native loricariid catfish *Pterygoplichthys disjunctivus* from the Rainbow River, Florida. Management of Biological Invasions **6** (3): 311–317. DOI: 10.3391/mbi.2015.6.3.11

Jordaan M.S., Dalu T., Wasserman R.J., Slabbert E., Wely, O.L. 2017. Unexpected survival of sharptooth catfish *Clarias gariepinus* (Burchell 1822) during acute rotenone toxicity trials will complicate management of invasions. Biological Invasions **19** (6): 1739–1744. DOI: 10.1007/s10530-017-1403-7

Keskin E., Unal E.M., Atar H.H. 2016. Detection of rare and invasive freshwater fish species using eDNA pyrosequencing: Lake Iznik ichthyofauna revised. Biochemical Systematics and Ecology **67**: 29–36. DOI: 10.1016/j.bse.2016.05.020

Khedkar G.D., Reddy A.C.S., Mann P., Ravinder K., Muzumdar K. 2010. *Clarias batrachus* (Linn. 1758) population is lacking genetic diversity in India. Molecular Biology Reports **37**: 1355–1362. DOI: 10.1007/s11033-009-9517-3

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution **35** (6): 1547–1549. DOI: 10.1093/molbev/msy096

Ladisa C., Bruni M., Lovatelli A. 2017. Overview of ornamental species aquaculture. FAO Aquaculture Newsletter No. 56: 38–39.

Levine J.M., D'Antonio C.M. 2003. Forecasting biological invasions with increasing international trade. Conservation Biology **17** (1): 322–326. DOI: 10.1046/j.1523-1739.2003.02038.x

Lowe C., Browne S., Boudjelas M., De S.M. 2000. 100 of the World's worst invasive alien species a selection from the Global Invasive Species Database. The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN).

Meier R., Shiyang K., Vaidya G., Ng P.K.L. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. Systematic Biology **55** (5): 715–728. DOI: 10.1080/10635150600969864

Naylor R.L., Williams S.L., Strong D.R. 2001. Aquaculture—A gateway for exotic species. Science **294** (5547): 1655–1656. DOI: 10.1126/science.1064875

Ng H.H. 1999. Two new species of catfishes of the genus *Clarias* from Borneo (Teleostei: Clariidae). Raffles Bulletin of Zoology **47**: 27–32.

Ng H.H., Kottelat M. 2008. The identity of *Clarias batrachus* (Linnaeus, 1758), with the designation of a neotype (Teleostei: Clariidae). Zoological Journal of the Linnean Society **153**: 725–732. DOI: 10.1111/j.1096-3642.2008.00391.x

Ng H.H., Low B.W., Kwik J.T.B., Yeo D.C.J. 2014. The tables are turned: An invasive species under potential threat. Biological Invasions **16**: 1567–1571. DOI: 10.1007/s10530-013-0618-5

Nunkoo I., Reed C., Kerwath S. 2015. First record of the Southeast Asian walking catfish, *Clarias batrachus* (Pisces: Clariidae), from the Island of Mauritius, south-western Indian ocean. African Zoology **50** (1): 73–75. DOI: 10.1080/15627020.2015.1022599

Padilla D.K., Williams S.L. 2004. Beyond ballast water: Aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Frontiers in Ecology and the Environment **2** (3): 131–138. DOI: 10.2307/3868238

Patoka J., Magalhães A.L.B., Kouba A., Faulkes Z., Jerikho R., Vitule J.R.S. 2018. Invasive aquatic pets: Failed policies increase risks of harmful invasions. Biodiversity and Conservation **27**: 3037–3046. DOI: 10.1007/s10531-018-1581-3

Pimentel D., Zuniga R., Morrison D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics **52** (3): 273–288. DOI: 10.1016/j.ecolecon.2004.10.002

Rixon C.A.M., Duggan I.C., Bergeron N.M.N., Ricciardi A., Macisaac H.J. 2005. Invasion risks posed by the aquarium trade and live fish markets on the Laurentian Great Lakes. Biodiversity and Conservation **14** (6): 1365–1381. DOI: 10.1007/s10531-004-9663-9

Rothlisberger J.D., Chadderton W.L., McNulty J., Lodge D.M. 2010. Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. Fisheries **35** (3): 121–132. DOI: 10.1577/1548-8446-35.3.121

Ruiz G.M., Carlton J.T., Grosholz E.D., Hines A.H. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. American Zoologist **37** (6): 621–632. DOI: 10.1093/icb/37.6.621

Sarma K., Prabakaran K., Krishnan P., Grinson G., Anand Kumar A. 2013. Response of a freshwater air-breathing fish, *Clarias batrachus* to salinity stress: an experimental case for their farming in brackishwater areas in Andaman, India. Aquaculture International **21** (1): 183–196. DOI: 10.1007/s10499-012-9544-2

Shahin M.I.H., Chandra K.J., Das D.R., Khalil S.M.I. 2013. Morphology and histopathology of alimentary canal of *Clarias batrachus* (Linnaeus) and *Heteropneustes fossilis* (Bloch). International Research Journal of Applied Life Science **2**: 11–20.

Tarkan A.S., Marr S.M., Ekmekçi F.G. 2015. Non-native and translocated freshwater fish species in Turkey. Fishes in Mediterranean Environments **3**: 1–28. DOI: 10.29094/fishmed.2015.003

Tarkan A.S., Vilizzi L., Top N., Ekmekçi F.G., Stebbing P.D., Copp G.H. 2017. Identification of potentially invasive freshwater fishes, including translocated species, in Turkey using the Aquatic Species Invasiveness Screening Kit (AS-ISK). International Review of Hydrobiology **102** (1–2): 47–56. DOI: 10.1002/iroh.201601877

Teugels G.G. 1986. A systematic revision of the African species of the genus *Clarias* (Pisces, Clariidae). Annals of the Central African Museum (Sc. Zool) **247**: 1–199.

Teugels G.G., Denayer B., Legendre M. 1990. A systematic revision of the African catfish genus *Heterobranchus* Geoffroy-Saint-Hilaire, 1809 (Pisces: Clariidae). Zoological Journal of the Linnean Society **98** (3): 237–257. DOI: 10.1111/j.1096-3642.1990.tb01209.x

Türkmen G. 2019. First record of the guppy (*Poecilia reticulata* Peters, 1859) in inland waters of Turkey. Ege Journal of Fisheries and Aquatic Sciences **36**: 397–400. DOI: 10.12714/egejfas.36.4.11

Verma V., Prasad Y., Singh B.R. 2011. Effect of pH and salinity on pathogenicity of *Flavobacterium columnare* and *Myxobacterium* sp. in Indian cat fish, *Clarias batrachus* (Linn.) and *Heteropneustes fossilis* (Bloch.). Journal of Environmental Biology **32**: 573–577.

Vilizzi L., Copp G.H., Adamovich B., Almeida D., Chan J., Davison P.I., Zeng Y. 2019a. A global review and meta-analysis of applications of the freshwater Fish Invasiveness Screening Kit. Reviews in Fish Biology and Fisheries **29** (3): 529–568. DOI: 10.1007/s11160-019-09562-2

Vilizzi L., Piria M., Tarkan A.S., Tricarico E., Vardakas L., Copp G.H. 2019b. Risk analysis tools for assessing the potential risks posed by non-native species in the eastern Mediterranean region. P. 2. *In*: Proceedings of the 14. International Congress on the Zoogeography and Ecology of Greece and Adjacent Regions, June 27–30, Thessaloniki, Greece.

Ward R.D., Zemlak T.S., Innes B.H., Last P.R., Hebert P.D.N. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences **360**: 1847–1857. DOI: 10.1098/rstb.2005.1716

Yoğurtçuoğlu B., Ekmekçi F.G. 2018. First record of the giant pangasius, *Pangasius sanitwongsei* (Actinopterygi: Siluriformes: Pangaiidae), from central Anatolia, Turkey. Acta Ichthyologica et Piscatoria **48** (3): 241–244. DOI: 10.3750/AIEP/02407

Zięba G., Copp G.H., Davies G.D., Stebbing P., Wesley K.J., Britton J.R. 2010. Recent releases and dispersal of non-native fishes in England and Wales, with emphasis on sunbleak *Leucaspius delineatus* (Heckel, 1843). Aquatic Invasions **5** (2): 155–161. DOI: 10.3391/ai.2010.5.2.04

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