BioInvasions Records (2022) Volume 11, Issue 2: 537–546

Research Article

The Yucatan molly *Poecilia velifera* (Regan, 1914) (Cyprinodontiformes: Poeciliidae): an invasive species in the Mediterranean lagoon of Ayn Zayanah (Benghazi, Libya)

Houssein Elbaraasi[1,*], Mohamed Atta Mohamed[1], Osama Elsilini[1], Hussein Jenjan[1], Cinzia Corinaldesi[2], Emanuela Buschi[3] and Ernesto Azzurro[4,5]

[1]*Department of Zoology, Faculty of Science, University of Benghazi. P.O. Box: 9480, Libya*
[2]*Department of Materials, Environmental Sciences and Urban Planning, Polytechnic University of Marche, 60131, Ancona, Italy*
[3]*Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131, Ancona, Italy*
[4]*CNR-IRBIM, National Research Council, Institute of Biological Resources and Marine Biotechnologies, 60125 Ancona, Italy*
[5]*SZN, Stazione Zoologica di Napoli, 80122, Naples, Italy*

**Corresponding author*
E-mail: *albrasi2000@gmail.com*

Citation: Elbaraasi H, Mohamed MA, Elsilini O, Jenjan H, Corinaldesi C, Buschi E, Azzurro E (2022) The Yucatan molly *Poecilia velifera* (Regan, 1914) (Cyprinodontiformes: Poeciliidae): an invasive species in the Mediterranean lagoon of Ayn Zayanah (Benghazi, Libya). *BioInvasions Records* 11(2): 537–546, https://doi.org/10.3391/bir.2022.11.2.26

Received: 22 July 2021
Accepted: 29 September 2021
Published: 21 February 2022

Handling editor: Michel Bariche
Thematic editor: Andrew David

Abstract

The Yucatan molly *Poecilia velifera* (Regan, 1914) is a popular aquarium fish, endemic to the Yucatan Peninsula, Mexico. It has been introduced to many countries worldwide. In this paper, we report the occurrence of Yucatan molly in the Mediterranean lagoon of Ayn Zayanah (Benghazi, Libya). Eighty-two individuals (22 males and 60 females) were collected in July 2020 during a field survey. All individuals were identified as *P. velifera* based on morphological characters and molecular analyses, providing the first evidence of a Yucatan molly invasion into the Mediterranean region.

Key words: coastal lagoons, alien fish, mollies, North Africa, aquarium release

Introduction

The Yucatan molly, *Poecilia velifera* (Regan, 1914) belongs to a large group of live-bearer species (family Poeciliidae, subfamily Poeciliinae) broadly distributed throughout the Americas. The Poeciliidae today, comprises a total of 216 described species (Froese and Pauly 2021), and the subfamily Poeciliinae includes well-known aquarium fishes such as the guppies, mosquito fishes, swordtails, platys, and species of the genus *Poecilia*, also known as mollies. Mollies are popular ornamental aquarium fishes because of their attractive coloration, short generation time and great adaptability to aquarium conditions (Nelson et al. 2016; Moyle and Cech Jr. 2004). Many of these species have been deliberately introduced into various aquatic ecosystems of the world either via aquarium release (Crossman and Cudmore 1999) or as biological control agents of insects (Juliano et al. 1989; Yamamoto and Tagawa 2000).

The Yucatan molly *Poecilia velifera* is endemic to the Yucatan Peninsula, Mexico (Hankison et al. 2006), where it is found in coastal environments

subject to constant variations in salinity, such as cenotes (sinkholes), tidal pools, and salt marshes (Neves et al. 2019). It is a small fish, which reaches a maximum length of 15 cm (Robins and Ray 1986; Rohde et al. 1994), and is characterized by a strong sexual dimorphism. Males typically display a single spineless dorsal fin with 16–20 fin rays, colored with a series of black bars toward the outer half, dark lines and spots near the base, and an orange edge. Females lack this bright coloration and the elongated dorsal fin (Miller 1983; Miller et al. 2005).

Invasive populations of *Poecilia velifera* have been introduced to the freshwater systems worldwide, and are reported from countries such as Thailand, Colombia, Peru, Taiwan, Vietnam, Singapore, and Israel (Sa-Nguansil and Lheknim 2010). For the first time, we document the occurrence of this species in a Mediterranean lagoon.

Materials and methods

Site description and field sampling

Specimens were collected in the Ayn Zayanah lagoon, located 15 km east of the Benghazi city center, between latitude 32°21′N and longitude 20°15′E (Figure 1). Today, this wetland is listed among the *Ramsar sites*, an area of recognized international importance for waterbirds (EGA-RAC/SPA waterbird census team 2012). The Ayn Zayanah lagoon is about 1.5 km long and 700 mwide with an average depth of 2.50 m and a salinity ranging seasonally between 14 and 20 ppt. Water temperature varies from 14 °C in the winter to 32 °C in the summer, and pH ranges from 7.8–8.8 (Al-Faitouri et al. 2018). The lagoon is connected to the Mediterranean Sea by a natural canal made by water overflow. Both native (i.e. the Flathead grey mullet *Mugil cephalus*, the Mediterranean killifish *Aphanius fasciatus*, fingerlings of Mediterranean fishes) and introduced (i.e. the Nile Tilapia *Oreochromis niloticus*, the Redbelly tilapia *Tilapia zillii*, the Eastern mosquitofish *Gambusia holbrooki*) fishes are known to occur in the lagoon, but very limited knowledge on their biology is currently available (Amera and El-Toumi 2018).

During a field survey on July 27, 2020, we collected a total of 82 individuals (22 males and 60 females) of the Yucatan molly using a dip net. Sampling was conducted in very shallow waters along the southwestern sector of the lagoon. At the sampling site, salinity was measured to be 16 ppt, while water temperature was 30.0 °C. Immediately after capture, the fish were transferred to the Aquaculture and Fisheries Laboratory of the Department of Zoology, Faculty of Science, University of Benghazi (Libya). The sex of each individual was identified, and morphometric and meristic analyses were performed. Tissue samples of two specimens (one male and one female) were fixed in 95% ethanol and employed for molecular analyses. Finally, 10 individuals were preserved in formalin and deposited at the Museum Collection of the Department of Zoology, Faculty of Science, University of Benghazi under the reference number: Z9351.

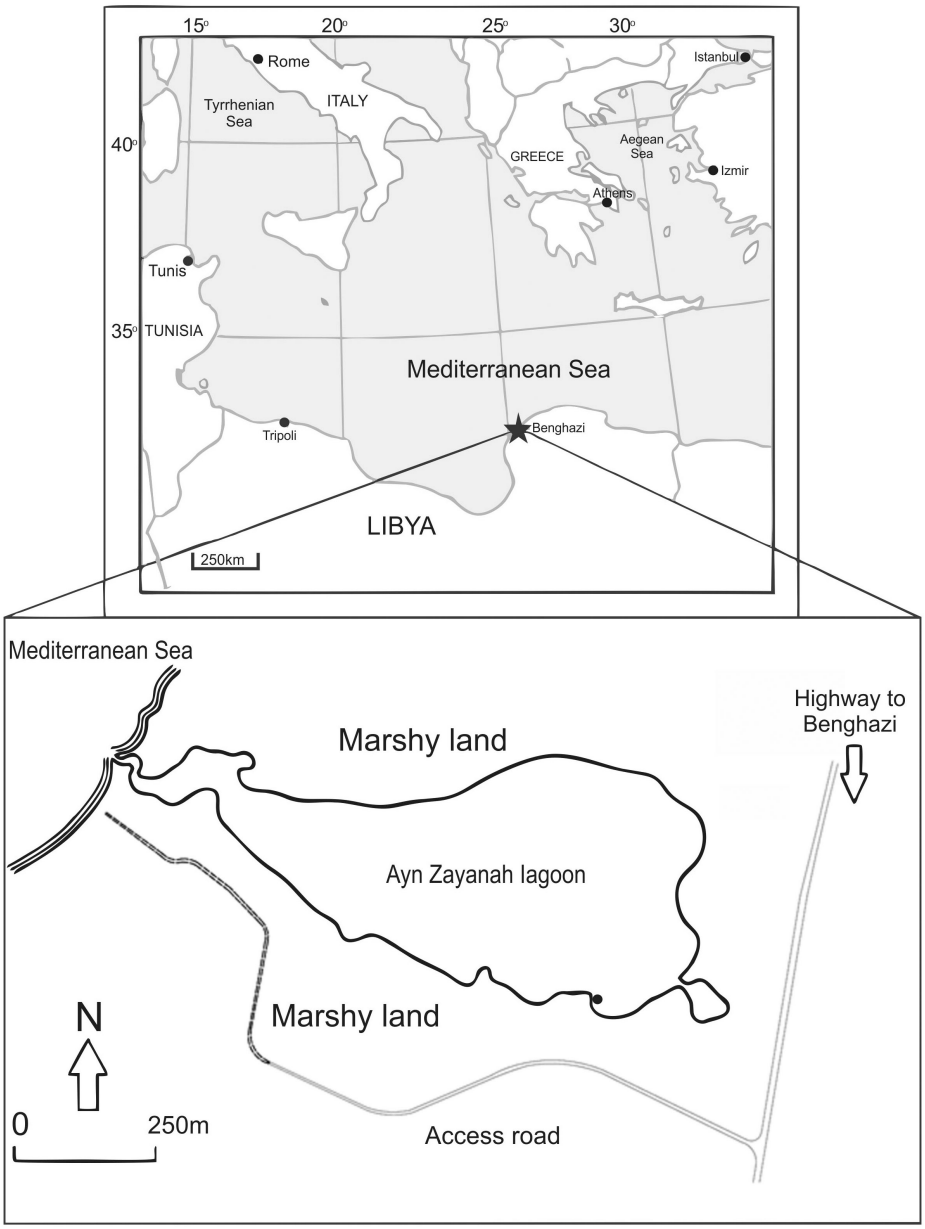

Figure 1. Map of Ayn Zayanah lagoon (Benghazi, Libya) showing the site of sampling of the Yucatan molly *Poecilia velifera*.

Additional information on the occurrence and abundance of the Yucatan molly in Ayn Zayanah lagoon was obtained by face-to-face interviews with five local fishermen operating in the same area. Before questioning the respondents, we first explained the purposes of the survey and then verified their ability to correctly recognize *P. velifera*. Finally, we asked about the first year they observed the species in the lagoon and the current level of perceived abundance.

Morphological analysis

Collected individuals were weighed to the nearest 0.1 g and measured to the nearest mm (see Table 1). Only three main meristic characters, which can effectively discriminate between shortfin mollies and various species

Table 1. Morphological and meristic characters of the Yucatan molly *Poecilia velifera* from Ayn Zayanah lagoon. Counts and measurements collected from 80 individuals (20 males and 60 females) are expressed as ranges of values.

	Males	Females
Weight (gr)	0.4–5.1	1.6–10.3
TL (cm)	4.5–8.8	6.2–11.0
SL (cm)	3.8–6.5	5.2–8.5
Head length (% of SL)	24–22	21–23
Body depth (% of SL)	29–35	31–33
Pre-dorsal length (% of SL)	29–32	38–34
Meristic characters		
N of dorsal fin rays	17–19	13–15
N of scales LL	26–28	27–29
N of scales CP	18–20	13–17

belonging to the sailfin molly complex (Koutsikos et al. 2017) were considered: the number of dorsal fin rays, lateral line scales, and scales around the caudal peduncle, as well as the position of the dorsal fin with respect to the anal, pelvic and pectoral fins (Table 1).

Genomic DNA isolation

Total genomic DNA was extracted from a portion of lateral muscle tissue of the specimens, using the Qiagen DNeasy Blood and Tissue Kit, following the manufacturer's instructions with proteinase K incubation at 56 °C overnight. Genomic DNA quantification and quality control were performed using a NanoDrop TM 1000 Spectrophotometer from Thermo Fisher Scientific.

Mitochondrial gene selection and PCR

The phylogenetic relationship among individuals was performed using two mitochondrial markers: rRNA 12S and a 655 bp portion of cytochrome oxidase I (COI) (Ward et al. 2005). rRNA 12S was selected for the efficient discrimination of the species, while COI is the most widely accepted marker in the Barcode of Life Data System (BOLD). COI is a fast-evolving gene and exhibits a high degree of genetic distance within species. Amplifications of mitochondrial 12S and COI genes were performed using the set of primers L1085 (5'-TAAACCAGGATTAGATACCC-3') and L1478 (5'-GAGAGTGACGGGCGATGTGT-3'; 375 bp; Miya and Nishida 2000), as well as FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'; 655 bp; Ward et al. 2005), respectively.

The reaction mixtures consisted of 5 µl of 5x MyTaq™ Reaction Buffer (Bioline), 0.5 µl of each primer (20 µM), 0.5 µl of MyTaq™ HS DNA Polymerase (5 U/µl concentration, Bioline), 1 µl of DNA template, and autoclaved Milli-Q water to bring the final volume up to 25 µl. The thermal cycling profiles consisted of an initial denaturation for 3 min at 95 °C, followed by 35 cycles for 30 s at 95 °C (12S) or at 94 °C (COI), 30 s at 50 °C (12S)

or at 48 °C (COI), 45 s at 72 °C, with a final extension of 10 min at 72 °C. The DNA amplification products were checked with 1% agarose gel electrophoresis using 10.000x GelRed® Nucleic Acid Gel Stain (Biotium), 0.4 g of agarose, 40 ml of TE Buffer for the gel preparation, as well as 2 µl of 5x GelPilot® DNA Loading Dye, (Qiagen), 2 µl of GeneRuler™ 1 kb DNA Ladder (Thermo Fisher Scientific) for the electrophoresis. PCR products were purified using QIAquick® PCR Purification Kit (QIAGEN).

Sequencing of mitochondrial 12S and COI genes

Sanger sequencing (Sanger et al. 1977) was performed on both DNA strands using the same primers as in the PCR protocols, through an Applied Biosystems 3730 DNA Analyzer 48 capillaries (Life Technologies).

Phylogenetic analysis

The sequences obtained were analyzed using the software Geneious 7.1.9 (Kearse et al. 2012). The terminal sections of the sequences, including low-quality reading and primers, were removed before assembling the two strands into consensus sequences. Multiple alignments for each marker were performed using MAFFT algorithm (Katoh et al. 2002). Additional sequences were downloaded from the National Center for Biotechnology Information (NCBI) GenBank database to build a more complex phylogeny. Phylogenetic analyses were conducted in IQ-Tree (Nguyen et al. 2015) for the separate rRNA 12S and COI datasets. For each dataset, the phylogenetic relationships were inferred by using the Maximum likelihood method applying the best-fit nucleotide substitution model: Kimura 2-parameter model (Kimura 1980) for rRNA 12S and Tamura–Nei model (Tamura and Nei 1993; Hasegawa et al. 1985) for COI markers. The support values to the phylogenetic trees were expressed in bootstrap (%) and shown next to the tree nodes. The new sequences of *Poecilia velifera* were deposited in GenBank (12S gene: OK041514 and OK041516; COI gene: OK036872 and OK036873).

Results

Morphological description of the collected individuals

Males: (Figure 2A) ranging between 4.5 to 8.8 cm in total length; body colored light blue to gray/olive, rows of light brown to orange spots laterally along the body; large dorsal fins, sail shaped with a row of dark blotches, characterized by distinctive orange spots which combine notably on the upper part of the fin to give the appearance of long stripes; caudal fin characterized by orange spots and black stripes on the lower part of the fin. In males, the origin of the dorsal fin was located anteriorly to the origin of the anal, pelvic and pectoral fins. Further morphological details of collected individuals are given in Table 1.

Figure 2. Specimens of the Yucatan molly *Poecilia velifera* collected in the Ayn Zayanah lagoon. A. Male, B. Female. Photo by H. Elbaraasi.

Females: (Figure 2B) ranging between 6.2 to 11.0 cm in total length; no sail-like dorsal fin with less extensive coloration and spotting on body and fins; dense regular brown to orange blotches on the caudal fin and body sides. Further morphological details are also presented in Table 1.

Molecular analyses

Primer sets used to amplify the mitochondrial rRNA 12S and COI genes provided good quality DNA sequences for both markers in the two individuals investigated (272 bp and 567 bp for the rRNA 12S gene, 552 bp and 628 bp for the COI gene, for the individual 1 and 2, respectively).

The alignment of rRNA 12S sequences and the resulting phylogenetic tree, supported by a total of 7 nucleotide informative positions, revealed that the sequences of the two specimens (Ind.1_Libya and Ind.2_Libya) were identical to the unique sequence of *Poecilia velifera* present in GenBank (*P. velifera*, collected in Australia; KJ774894), with a high bootstrap support (91.5%; Figure 3). Similar results were also obtained with the alignment and phylogenetic tree of COI, supported by a total of 56 nucleotide informative positions: the sequences of the two specimens (Ind.1_Libya and Ind.2_Libya) were identical to six sequences of *P. velifera* collected in different parts of the world, which are available on GenBank (KJ669591; KU568973; GU807009; JQ667581; JQ667585; JQ667583), with a high bootstrap support (83.2%; Figure 4). As highlighted by the phylogenetic trees (Figures 3 and 4), both Libyan specimens clustered separately from individuals of other species within the genus *Poecilia*, including *P. latipinna*.

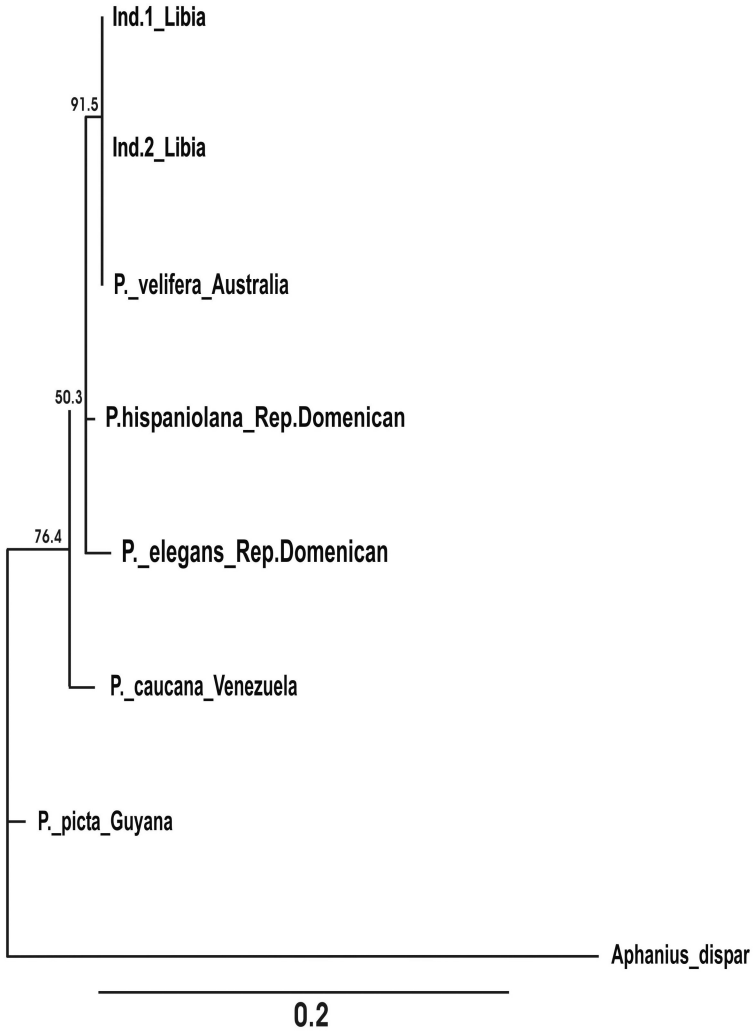

Figure 3. Phylogenetic tree based on the rRNA 12S mitochondrial gene inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. Bootstrap values (%), supporting the clusters of the tree, are shown next to the tree nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Discussion

Due to the overlapping morphological characters, mollies have undergone various re-classification (Lucinda and Reis 2005). Misidentification has often occurred in the past (e.g., Rosen and Bailey 1963; Miller 1983), with new species regularly being described (e.g., Kallman et al. 2004; Schories et al. 2009; Rodriguez-Silva and Weaver 2020). Moreover, the possibility of introgressive hybridization further complicates the taxonomy of these species (Kittel et al. 2005; Hankison et al. 2006). According to our morphological analyses, the range of dorsal fin ray counts (16–19) in our 31 specimens collected from the Ayn Zayanah lagoon is almost consistent with the range of the Yucatan molly *P. velifera* reported by Miller et al. (2005). The only slight discrepancy was that we counted 26–28 scales in the lateral series, whereas Miller et al. (2005) stated that the lateral-line scales usually 26 or 27. Other morphological characters, such as the distinctive coloration, enlarged sail-like dorsal fin, number of dorsal fin rays and position of the dorsal fin

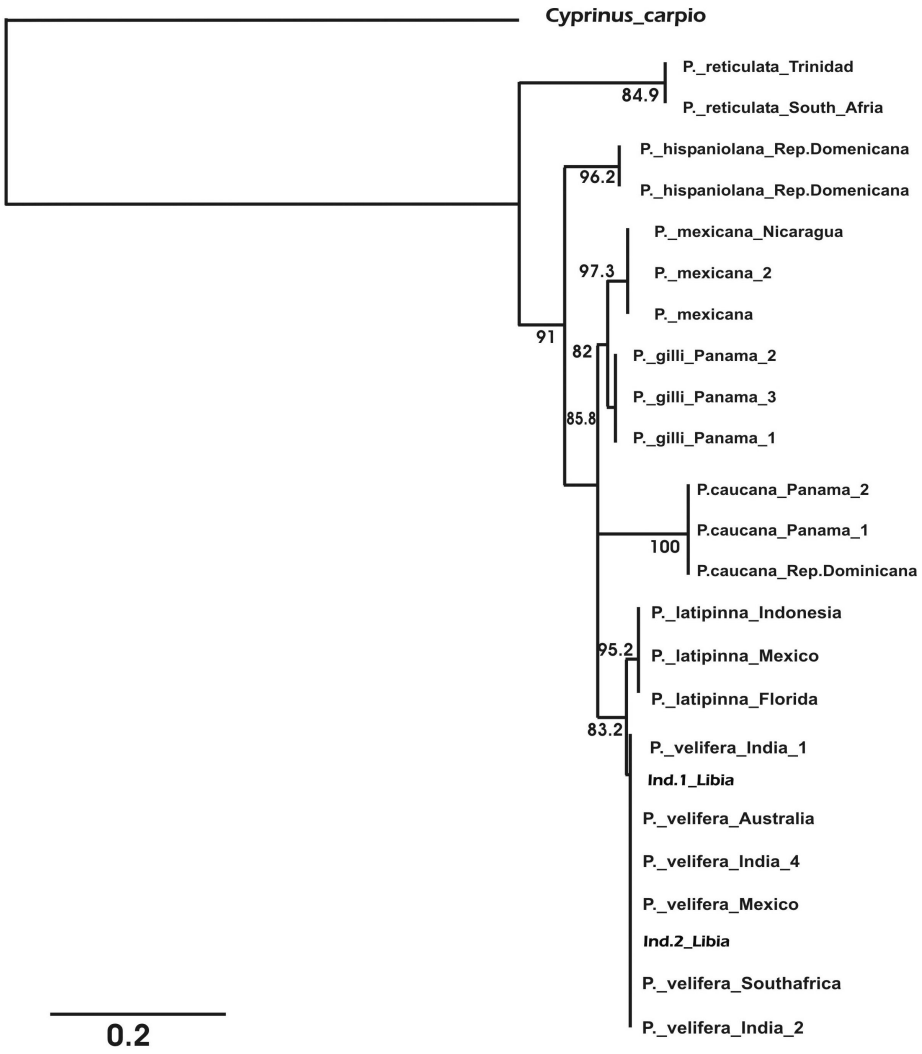

Figure 4. Phylogenetic tree based on the mitochondrial COI gene inferred by using the Maximum Likelihood method based on the Tamura-Nei model. Bootstrap values (%), supporting the clusters of the tree, are shown next to the tree nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

relative to anal, pelvic, and pectoral fins, are considered to be typical of this species (Miller 1983; Miller et al. 2005).

The identification of *P. velifera* was further confirmed by molecular analyses. Indeed, mitochondrial rRNA 12S and COI sequences of Ayn Zayanah lagoon individuals were different from those of other sailfin mollies such as *P. latipinna,* which has been already reported from the Mediterranean region (Koutsikos et al. 2017).

According to the interviewed fishers, the first individuals of *Poecilia velifera* appeared in Ayn Zayanah lagoon in summer 2019. The fishers reported that the population was well established and abundant, especially in the southern and western parts of the lagoon. They often observe the Yucatan molly when fishing for tilapia and other fish species. Considering the current trade of this ornamental fish all over the world (Sa-Nguansil and Lheknim 2010), its occurrence in the Ayn Zayanah lagoon is likely the result of one or more deliberate aquarium releases made by residents. The

species could have either been released directly into the lagoon, or in one of many underground channels connecting the lagoon with the surrounding urban centers (the hills of the southern parts of Benghazi city and the mountains to the east).

The sailfin molly *Poecilia latipinna* (Lesueur 1821) has recently been reported from a Mediterranean country (Papavlasopoulou et al. 2013; Koutsikos et al. 2018), but to the best of our knowledge, *P. velifera*, has not been reported before from Libya, nor from a Mediterranean lagoon (Kara and Quignard 2019). Further studies are needed to evaluate the expected ecological impacts of this invasive species and its possible occurrence in other freshwater systems of Libya.

Acknowledgements

We thank the fishermen of the Ayn Zayanah for providing additional information on the occurrence of the Yucatan molly in the lagoon. Many thanks to the reviewers and the editor for their constructive comments that helped improve the manuscript.

References

Al-Faitouri M, Salloum F, Muftah A (2018) Determination of water type in Benghazi Plain aquifers by chemical and statistical methods. *Journal of Science* special issue: 610–619

Amera A, El-Toumi F (2018) Taxonomical study on macroinvertebrates in Ain-Zayanah lagoon, Benghazi-Libya. *Journal of Pure and Applied Sciences* 7(1): 433–438

Crossman EJ, Cudmore BC (1999) Summary of North American introductions of fish through the aquaculture vector and related human activities. In: Claudi R, Leach JH (eds), Nonindigenous Freshwater Organisms: Vectors, Biology, and Impacts. Lewis Publishers, Boca Raton, Florida, pp 297–304

EGA-RAC/SPA waterbird census team (2012) Atlas of wintering waterbirds of Libya, 2005-2010. COTIM, Tunisia press, 153 pp

Froese R, Pauly D (2021) FishBase. www.fishbase.org (accessed February 2021)

Hankison SJ, Childress MJ, Schmitter-Soto JJ, Ptacek MB (2006) Morphological divergence within and between the Mexican sailfin mollies, *Poecilia velifera* and *Poecilia petenensis*. *Journal of Fish Biology* 68: 1610–1630, https://doi.org/10.1111/j.0022-1112.2006.001051.x

Hasegawa M, Iida Y, Yano T, Takaiwa F, Iwabuchi M (1985) Phylogenetic relationships among eukaryotic kingdoms inferred from ribosomal RNA sequences. *Journal of Molecular Evolution* 22: 32–38, https://doi.org/10.1007/BF02105802

Juliano RO, Guerrero R, Ronquillo I (1989) The introduction of exotic aquatic species in the Philippines. In: De Silva SS (ed), Exotic aquatic organisms in Asia. Proceedings of the Workshop on Introduction of Exotic Aquatic Organisms in Asia. Asian Fisheries Society Special Publication, pp 83–90

Kallman KD, Ronald BW, Donald CM, Steven K (2004) Two New Species of *Xiphophorus* (Poeciliidae) from the Isthmus of Tehuantepec, Oaxaca, Mexico, with a Discussion of the Distribution of the *X. clemenciae* Clade. *American Museum Novitates* 3441: 1–34, https://doi.org/10.1206/0003-0082(2004)441<0001:TNSOXP>2.0.CO;2

Kara MH, Quignard JP (2019) Fishes in Lagoons and Estuaries in the Mediterranean 3A: Migratory Fish. John Wiley and Sons, London, 302 pp, https://doi.org/10.1002/9781119452782

Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast fourier transform. *Nucleic Acids Research* 30: 3059–3066, https://doi.org/10.1093/nar/gkf436

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649, https://doi.org/10.1093/bioinformatics/bts199

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120, https://doi.org/10.1007/BF01731581

Kittel M, Harvey M, Contreras-Balderas S, Ptacek B (2005) Wild-caught hybrids between sailfin and shortfin mollies (Poeciliidae, Poecilia): morphological and molecular verification. *Hidrobiologica* 15(2): 131–137

Koutsikos N, Economou AN, Vardakas L, Kommatas D, Zogaris S (2017) First confirmed record of an established population of sailfin molly, *Poecilia latipinna* (Actinopterygii: Cyprinodontiformes: Poeciliidae), in Europe. *Acta Ichthyologica et Piscatoria* 47: 311–315, https://doi.org/10.3750/AIEP/02234

Koutsikos N, Vardakas L, Kalogianni E, Economou AN (2018) Global distribution and climatic match of a highly traded ornamental freshwater fish, the sailfin molly *Poecilia latipinna* (Lesueur, 1821). *Knowledge and Management of Aquatic Ecosystems* 419: 1–11, https://doi.org/10.1051/kmae/2018014

Lucinda PHF, Reis RE (2005) Systematics of the subfamily Poeciliinae Bonaparte (Cyprinodontiformes: Poeciliidae), with an emphasis on the tribe Cnesterodontini Hubbs. *Neotropical Ichthyology* 3: 1–60, https://doi.org/10.1590/S1679-62252005000100001

Miller RR (1983) Checklist and key to the mollies of Mexico (Pisces, Poeciliidae, *Poecilia*, Subgenus *Mollienesia*). *Copeia* 3: 817–822, https://doi.org/10.2307/1444354

Miller RR, Minckley WL, Norris SM (2005) Freshwater Fishes of Mexico. University of Chicago Press, Chicago, 490 pp

Miya M, Nishida M (2000) Use of mitogenomic information in teleostean molecular phylogenetics: a tree-based exploration under the maximum-parsimony optimality criterion. *Molecular Phylogenetics and Evolution* 17: 437–455, https://doi.org/10.1006/mpev.2000.0839

Moyle PB, Cech JJ Jr. (2004) Fishes: an Introduction to Ichthyology. 5th Edition. Prentice-Hall, Upper Saddle River, NJ, 750 pp

Nelson JS, Grande TC, Wilson MVH (2016) Fishes of the World. 5th Edition, John Wiley and Sons, Hoboken, New Jersey, 752 pp, https://doi.org/10.1002/9781119174844

Neves LD, Cipriano F, Silva Lorenzini JP, Cipriano KS, Gonçalves Junior LP, Nakayama CL, Luz RK, Miranda Filho KC (2019) Effects of salinity on sexual maturity and reproduction of *Poecilia velifera*. *Aquaculture Research* 50: 2932–2937, https://doi.org/10.1111/are.14247

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274, https://doi.org/10.1093/molbev/msu300

Papavlasopoulou I, Vardakas L, Perdikaris C, Kommatas D, Paschos I (2013) Ornamental fish in pet stores in Greece: a threat to biodiversity. *Mediterranean Marine Science* 15: 126–134, https://doi.org/10.12681/mms.484

Robins CR, Ray GC (1986) A field guide to Atlantic coast fishes of North America. Houghton Mifflin Company, Boston, 354 pp

Rodriguez-Silva R, Weaver PF (2020) A new livebearing fish of the genus *Limia* (Cyprinodontiformes: Poeciliidae) from Lake Miragoane, Haiti. *Journal of Fish Biology* 96: 1360–1369, https://doi.org/10.1111/jfb.14301

Rohde FC, Arndt R G, Lindquist DG, Parnell JF (1994) Freshwater fishes of the Carolinas, Virginia, Maryland and Delaware. University of North Carolina Press, Chapel Hill, 222 pp

Rosen DE, Bailey RM (1963) The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. *Bulletin of the American Museum of Natural History* 126(1): 1–176

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences* 74: 5463–5467, https://doi.org/10.1073/pnas.74.12.5463

Sa-Nguansil S, Lheknim V (2010) The occurrence and reproductive status of Yucatan molly *Poecilia velifera* (Regan, 1914) (Poeciliidae; Cyprinodontiformes): an alien fish invading the Songkhla Lake Basin, Thailand. *Aquatic Invasions* 5: 423–430, https://doi.org/10.3391/ai.2010.5.4.12

Schories S, Meyer MK, Schartl M (2009) Description of *Poecilia* (*Acanthophacelus*) *obscura* n. sp., (Teleostei: Poeciliidae), a new guppy species from western Trinidad, with remarks on *P. wingei* and the status of the "Endler's guppy". *Zootaxa* 2266: 35–50, https://doi.org/10.11646/zootaxa.2266.1.2

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1847–1857, https://doi.org/10.1098/rstb.2005.1716

Yamamoto MN, Tagawa AW (2000) Hawaii's native and exotic freshwater animals. Mutual Publishing, Honolulu, Hawaii, 200 pp