

***Aphanius almiriensis*, a new species of toothcarp from Greece (Teleostei: Cyprinodontidae)**

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***Aphanius almiriensis*, a new species of toothcarp from Greece (Teleostei: Cyprinodontidae).** - *Aphanius almiriensis*, new species, is described from a brackish water spring and from a lagoon (and its inflowing freshwater spring) in the Peloponnese (Greece). It is distinguished by the yellowish caudal fin of the male that has a wide faint grey margin and by the colour pattern of the female (7-11 dark, roundish blotches on the side, more or less connected by an irregular dark midlateral stripe). *Aphanius almiriensis* is critically endangered; it is possibly extinct at the type locality and the second locality is much impacted. The identity, type material and type locality of *A. fasciatus* are discussed, and a neotype is designated. Several species are possibly confused under the name *A. fasciatus*.

Keywords: fish - Cyprinodontidae - *Aphanius* - Peloponnese - extinction.

INTRODUCTION

Tooth carps of the genus *Aphanius* are small fishes that inhabit fresh to hypersaline waters around the Mediterranean and Red Seas and the coasts of the Indian Ocean eastwards to Pakistan and southwards to Somalia. Several endemic species are also known from inland waters in arid areas, many of them in Turkey on the Anatolian plateau (Vilcock, 1984; Wildekamp *et al.*, 1999; Hrbek & Wildekamp, 2003). The small size of these fishes, their low dispersal ability, the isolation of their populations, the small size of the habitats and the even smaller size of the areas of occupancy, lead to the evolution of very localised endemics. A few species, usually coastal ones, have a distribution range that appears very wide, e.g., *A. fasciatus* (Valenciennes) that is recorded around most of the Mediterranean and its islands. In fact, the distribution of the latter is better described as a long succession of more or less isolated populations (Bianco & Taraborelli, 1988; Wildekamp, 1993; Bianco *et al.*, 1996). While a number of studies were performed on the life history, ecology and genetics of local *Aphanius* populations (often within a national framework) (e.g., Kiener & Schachter, 1974; Boumaiza, 1980; Tigano, 1982; Ferrito *et al.*, 2003), a large scale comparison of

populations throughout the range of the species is still missing. Such a comparison is likely to reveal that several species have been confused under the name *A. fasciatus*. We describe here a new species of *Aphanius* from Greek waters, firstly discovered in 1997 in a brackish spring. We also clear the nomenclature and the identity of the ‘real’ *A. fasciatus*.

There have been arguments in recent years on the name to be used for the current genus *Aphanius*, with Lazara’s (1995) attempt to pose *Lebias* as the valid name. To change the name of a genus, whose species are listed as endangered in numerous national and international legal instruments, was unfortunate, especially since a critical re-analysis of the case demonstrated this decision to be based on misinterpreted data (Kottelat & Wheeler, 2001). To close the case, Kottelat & Wheeler (2001) requested a ruling from the International Commission of Zoological Nomenclature [ICZN]. The ruling (ICZN, 2003) definitively conserves *Aphanius* and places *Lebias* on the list of names not available for the zoological nomenclature.

METHODS

Ideally, we tried to adopt the Phylogenetic Species Concept (Cracraft, 1989; Kottelat, 1997, 1999) as an operational tool for the evolutionary species concept (Wiley & Mayden, 2000; Mayden, 2002), which is closest to our perception of what a species is. The reality is that outside the academic world it is seldomly possible to investigate the phylogeny of a given group with enough detail to demonstrate the monophyly of all the included taxa. In the context of biodiversity inventories and conservation, alpha-level taxonomy is facing priorities strikingly different from those of academic research. The goals are different, and the approach has to be adjusted. Therefore, we often have to resort to the ‘Pragmatic Species Concept’ (Kottelat, 1995; see also Kottelat & Ng, 1994).

Methods for counts and measurements follow Kottelat (2001). Standard length (SL) is measured from the tip of the snout to the end of the hypural complex. Length of caudal peduncle is measured from behind the base of the last anal-fin ray to the end of the hypural complex, at the mid-height of the caudal-fin base. Scales on lateral series are counted from the anteriormost scale (the first one to touch the shoulder girdle) to the posteriormost one (at the end of the hypural complex). Scales on the caudal fin itself are indicated by “+”. Transverse scales are counted as the number of longitudinal scale rows. The scale row on the dorsal and ventral mid-lines is noted as “1/2”. All measurements are made point to point.

Abbreviations used: CMK, collection of first author; FSJF, collection of Jörg Freyhof, Berlin; HCMR, Hellenic Centre for Marine Research (formerly National Centre for Marine Research), Athens; MHNG, Muséum d’histoire naturelle, Genève; MNHN, Muséum National d’Histoire Naturelle, Paris.

COMPARISON MATERIAL

Aphanius fasciatus: Italy: Sardinia: MNHN 2005-1975, neotype, 39.5 mm SL; CMK 18600, 85, 32.9-47.8 mm SL; Italy: Sardinia: Cagliari, stagno di Cagliari, Primo Bacino (salt-works); A. Cau, 22 March 2005. – MNHN A.3968, male, 29.1 mm SL; MNHN 187, 3 syntypes of *Poecilia calaritana*, 33.9-35.7 mm SL; MNHN 92, 1, 37.5 mm SL; CMK 18599, 17, 29.2-

35.4 mm SL; CMK 18786, 3, 36.3-41.6 mm SL; FSJF 209, 6, 10.9-26.7 mm SL; Cagliari. – FSJF 183, 3, 18.1, 24.3 & 27.9 mm SL; Arbarax.

Italy: MNHN 197, 1 female, 43.7 mm SL, possible syntype of *Lebias flava*; Napoli. – MNHN 1888-112-115, 4, 31.8-33.0 mm SL; MNHN 1899-249, 5, 40.7-46.0 mm SL; Venezia.

France: Corsica: MNHN 1985-204, 1 (female), 44.2 mm SL. – MHNG uncat., 8, 26.1-42.0 mm SL; MHNG uncat., 2, 35.1-43.3 mm SL; MHNG 1321.34-38, 5, 26.8-29.2 mm SL; Bonifacio.

Greece: CMK 17015, 17; CMK 16976, 6; Messolonghi. – HCMR 1471, 14; CMK 18452, 2; Kato Basiliki. – HCMR 1121, 14; CMK 18541, 2; Prokopos. – HCMR 1188, 7; Kaiafa lake. – HCMR 1318, 170; Thermissia lagoon. – HCMR 1319, 48; Sachtouri lagoon. – HCMR 1321, 74; Metochi lagoon. – HCMR 1390, 16; Aitoliko. – HCMR 1418, 29; Gialova lagoon.

Turkey: MNHN 1927-79-80, females, 31.9-54.1 mm SL; MNHN 1927-78, 20 males, 23.5-48.9 mm SL; MNHN A.3796, 10, 25.9-42.0 mm SL; Izmir. – MNHN 1928-210-211, 23; Konya.

Israel: MNHN A.2809, 13, 15.4-24.9 mm SL; Ramle.

Egypt: MNHN A.5254, 6, 25.6-41.1 mm SL; no locality. – MNHN 4991, 1 male, 35.0 mm SL; Suez.

Tunisia: MNHN 1904-48, 8, 28.9-40.0 mm SL; no locality. – MNHN 1883-1031, 8, 26.7-36.4 mm SL.; Blidet Ahmar.

Algeria: MNHN 4392, 8 syntypes of *Cyprinodon doliatus*, males, 22.8-34.7 mm SL; MNHN 3218, 5 syntypes of *C. cyanogaster*, females, 24.9-32.6 mm SL; Biskra. – MNHN A.2367, 1 female, 36.7 mm SL; Touggourt. – MNHN 1999-542, 2 females, 19.0-31.6 mm SL; Annaba.

Aphanius sp.: MNHN 1923-29, 1 male, 28.4 mm SL; MNHN 1922-72, 1 female, 37.6 mm SL; Tunisia: Gabes.

RESULT

Aphanius almiriensis, new species

Fig. 1

HOLOTYPE: MHNG 2654.087, 35.1 mm SL; Greece: Peloponnese: Korinthia Distr.: brackish water spring Kokosi at southern end of Almiri beach, at Kato Almiri, about 4 km south of Loutra Elenis; 37°50'32"N 23°00'58"E; R. Barbieri & A. Economou, 13 May 1997.

PARATYPES: HCMR 1064, 14, 24.5-38.8 mm SL; CMK 17296, 3, 25.9-29.8 mm SL; same data as holotype. – HCMR 1473, 5, 13.4-22.1 mm SL; same locality as holotype; R. Barbieri & M. Tsatsas, 26 Oct 2003.

ADDITIONAL MATERIAL (NON TYPES): HCMR 1478, 12, 14.9-29.4 mm SL; Greece: Peloponnese: channel close to sea at east side of Meligou Lagoon; 37°23'07"N 22°45'02"E; R. Barbieri & M. Stoumboudi, 29 July 2004. – HCMR 1314, 190, 20.9-37.7 mm SL; MHNG 2654.088, 3, 23.4-30.2 mm SL; CMK 18370, 11, 19.1-33.1 mm SL; same locality; R. Barbieri & A. Economou, 13 Oct 1998. – HCMR 1477, 36, 9.4-26.0 mm SL; Greece: Peloponnese: freshwater spring at edge of Meligou Lagoon; R. Barbieri & M. Stoumboudi, 29 July 2004.

DIAGNOSIS: *Aphanius almiriensis* is distinguished from all other species of *Aphanius* in Europe by the yellow caudal of the male, with a wide faint grey margin (vs. hyaline, greyish, greyish blue, or bright yellow with or without a black subdistal bar) and by the colour pattern of the female (7-11 dark, roundish blotches on the side, more or less connected by an irregular dark midlateral stripe). Additional characters useful to distinguish the species (but not unique to it) are: male with 6-10 dark, broad, regularly set bars on the body; neuromasts in interorbital area in deep open grooves; 25-28+2 scales in lateral series on body; and 15-16 pectoral rays.

DESCRIPTION: Main morphometric data of the holotype and 13 paratypes are given in Table 1. General appearance is shown in Fig. 1. Head with relatively massive appearance. Dorsal profile of head and body convex, with a slight nuchal concavity in largest males. Mouth subterminal, oriented upwards, lower jaw almost vertical.

TABLE 1. Morphometric data of holotype and 13 paratypes of *Aphanius almiriensis* from type locality and of neotype of *A. fasciatus*. Holotype data included in range of female values.

	<i>A. almiriensis</i>			<i>A. fasciatus</i>
	female holotype	males (n= 7) range	females (n=7) range	male neotype
Standard length [mm]	35.1	24.5 - 28.5	25.2 - 38.8	39.5
Total length [mm]	43.2	30.5 - 35.8	31.1 - 46.6	48.2
In percents of standard length				
Head length	31.9	30.1 - 32.1	29.9 - 31.9	29.4
Predorsal length	64.1	60.4 - 62.0	61.0 - 64.7	61.0
Prepelvic length	52.0	50.2 - 53.1	52.0 - 54.9	52.9
Preanal length	70.8	64.3 - 65.9	67.6 - 70.8	62.8
Body width	23.5	20.4 - 22.1	22.1 - 23.5	17.5
Head depth	19.1	18.6 - 19.8	19.1 - 19.2	21.0
Body depth	28.3	24.9 - 27.8	25.6 - 28.6	28.9
Depth caudal peduncle	16.5	16.0 - 17.6	15.2 - 16.5	17.0
Length caudal peduncle	22.8	23.4 - 25.3	22.2 - 24.1	23.8
Depth of 1st simple dorsal ray	14.7	15.0 - 16.5	13.1 - 15.5	13.9
Length of middle caudal rays	21.9	24.2 - 26.2	20.2 - 23.9	23.5
In percents of head length				
Head depth	59.8	58.6 - 63.8	59.8 - 63.8	71.6
Eye diameter	27.7	30.9 - 33.6	27.6 - 31.1	25.9
Snout length	32.6	28.4 - 30.5	29.7 - 32.6	34.5
Interorbital width	49.6	47.3 - 52.6	47.7 - 51.0	39.7

Posterior extremity of maxilla under tip of snout. Eye diameter about equal to snout length. Depth of caudal peduncle 1.35-1.55 times in its length.

Dorsal-fin origin in front of anal-fin origin and behind midlength of body. Dorsal fin with 9-11 (modally 10) rays; anal with 10-11 (modally 10) rays, pectoral with 15-16 rays. 25-28+2 scales in lateral series, $1/2$ 8-9 $1/2$ in transverse row between dorsal origin and ventral midline in front of pelvic-fin base; $1/2$ 6-7 $1/2$ on caudal peduncle. Small ctenii along posterior edge of scales on caudal peduncle in largest males. Cephalic neuromasts in interorbital area in deep open grooves (Fig. 2).

Males slightly more robust than females, up to 28.5 mm SL in available material. Dorsal rounded, anal and caudal truncate. Females larger than males, up to 38.8 mm SL, body less laterally compressed. All unpaired fins rounded. Anus and genital openings separated.

COLOUR PATTERN: Based on the type series: Males with 6-8 dark bars on body, wider above than below, more or less reaching ventral midline and more or less fused along dorsum in front of dorsal-fin origin. Caudal and anal fins yellow, caudal fin with a greyish distal band. Dorsal fin yellowish, with an obvious black margin along anterior and upper margins, and a few dark dots near its base. Females with 7-11 dark, roundish blotches on side, more or less connected by an irregular dark midlateral stripe; no remains of other colour marks.

No observation on life colour of material from Almiri, but unpaired fins of males probably brighter yellow in life than in Fig. 1. In material from Meligou, all adult males with yellow caudal and anal fins.



FIG. 1

Aphanius almiriensis; Greece: Kato Almiri; CMK 17296, paratype, male, 29.8 mm SL (above); holotype, MHNG 2654.087, 35.1 mm SL (below).

VARIATION: Material from Meligou lagoon generally agrees with the above description, except for a single very large female (37.7 mm SL) that has the flank colour pattern consisting in slightly elongated bars. Most males have a few broad bars, but there are a few individuals with a few more and narrower bars (7-10). We do not adopt the hypothesis that these individuals may be hybrids between *A. almiriensis* and *A. fasciatus*, because the latter (although it is widely distributed along the eastern coast of the Peloponnese) was never observed at Meligou lagoon despite intense search for it.

DISTRIBUTION: Greece, only two populations are known to date, in the spring near Almiri (Saronikos Gulf) and in the Meligou lagoon (Argolikos Gulf). In 1997-2004, we searched about fifteen springs and brackish water systems along the coast of the Peloponnese and Southwestern Greece and found *Aphanius* in eleven (Fig. 3). Only *A. fasciatus* was observed and/or collected in all but the two aforementioned localities, including the Thermisia and Metochi lagoons that are located between the Almiri spring (Fig. 4) and the Meligou lagoon.

HABITAT: Almiri spring is a permanent brackish water spring (23‰) adjacent to the sea, which it joins after a few meters. The spring itself is now contained by a concrete wall and the enclosure is filled with typical halophytic aquatic vegetation.

Meligou lagoon is a permanent brackish marsh (11-25‰). Until the second half of the 20th century it occupied an area of about 2.6 km². Later on, reclamation works

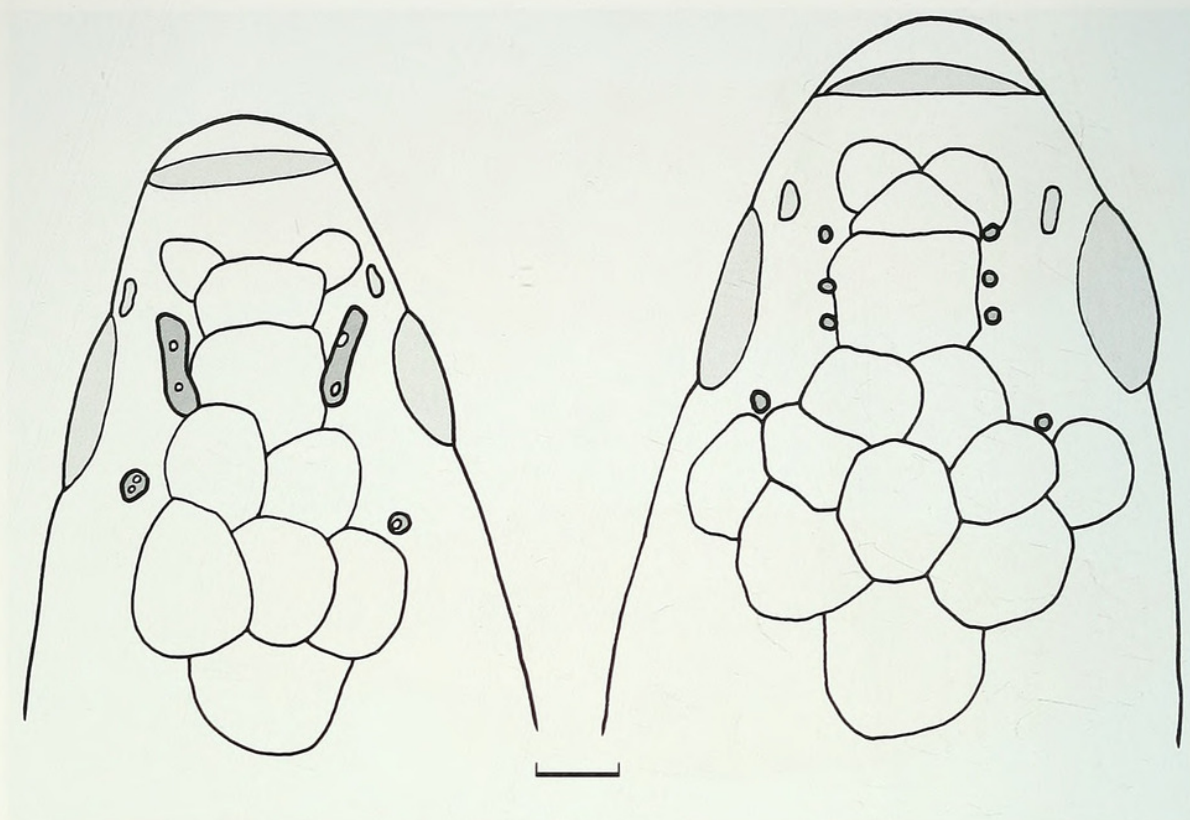


FIG. 2

Dorsal view of head (schematised) of: *Aphanius almiriensis*, CMK 17269, 29.6 mm SL (left); *A. fasciatus*, CMK 17015, 35.8 mm SL (right). Dark grey: canal and pores in interorbital area. Scale bar 1 mm.

reduced its surface area to 1.5 km². There is a spring along the west side of the lagoon, with permanent freshwater discharge.

BIOLOGY: *Aphanius almiriensis* is benthopelagic, observed in shallow areas with slow current, among vegetation, as well as in the water column. It is a very fast swimmer and forms schools. It is a short-lived species and a fractional spawner that breeds in late spring - early summer, spawning 1-3 eggs a time. However, larvae were collected in Meligou in September 1998 (HCMR 1314), showing that the reproductive period may be prolonged until late summer.

CONSERVATION STATUS: *Aphanius almiriensis* was discovered in 1997 in the Almiri spring, which at the time released brackish water of constant salinity. During various visits at this location, between 1997 and 2000, the observed salinity was 23‰. However, in 2002 the spring was releasing pure seawater and the only fish observed there were mugilids. At the same time, the spring was occupied by unidentified sea grasses and sea anemones. The species was then believed to be extinct, but a visit in October 2003 yielded 5 juveniles 13.4-22.1 mm SL (HCMR 1473). In July 2004, no *A. almiriensis* were observed in Almiri despite intensive search.

After realizing the distinctness of the Almiri population, older collections were re-examined for possible additional localities with *A. almiriensis* and a sample obtained in Meligou lagoon in October 1998 was discovered. In that sampling

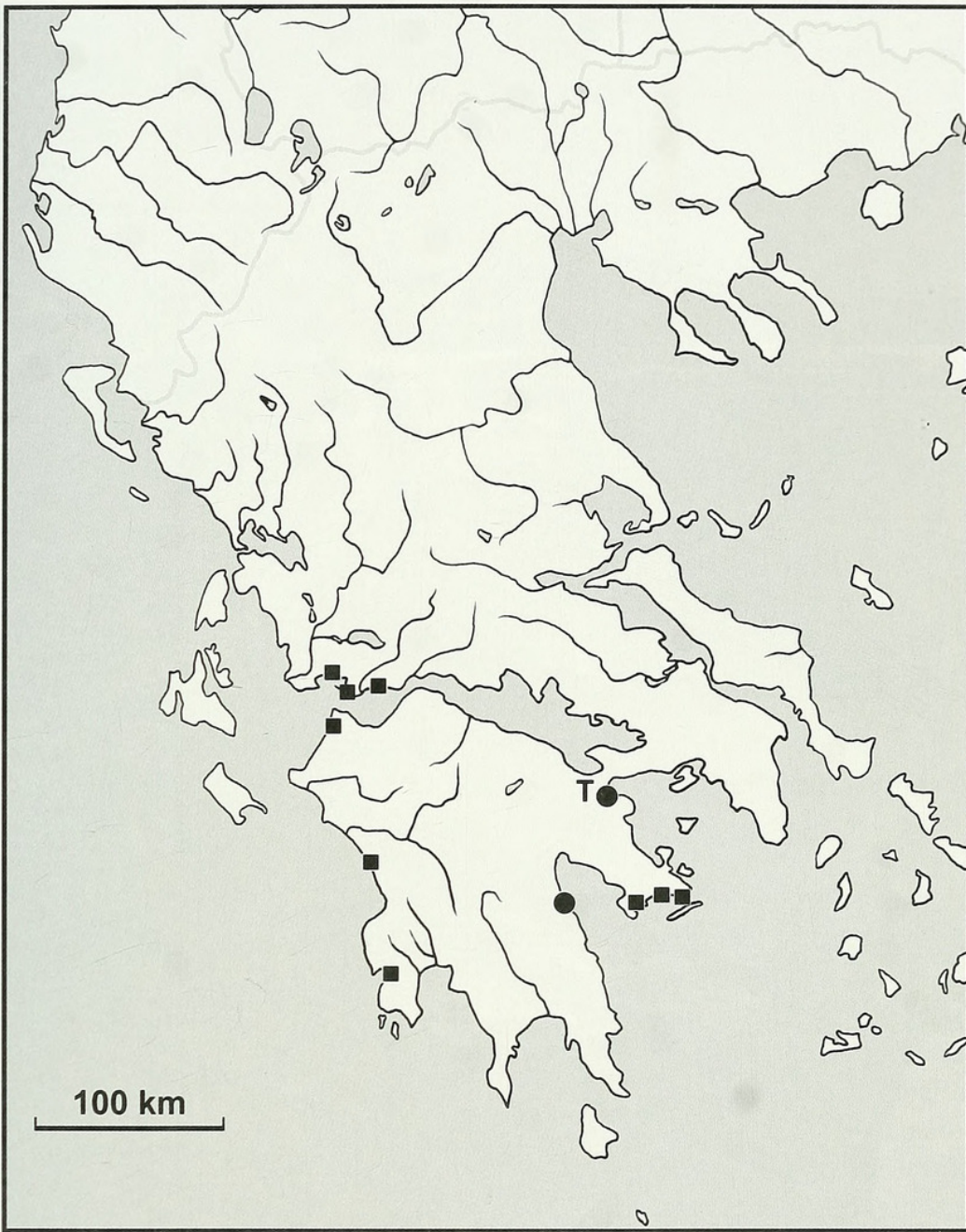


FIG. 3

Distribution of *Aphanius almiriensis* (circles) and *A. fasciatus* (squares) in the water systems investigated along the coast of Peloponnese and southwestern Greece. T, type locality.

occasion, *A. almiriensis* was estimated as abundant in the main freshwater spring supplying the lagoon at its NW side. However, recently, this spring was dammed with rocks, to create a “natural swimming pool”. During a visit in July 2004, no fish were present in the modified spring area. However, *A. almiriensis* was collected from different areas of the lagoon.

According to the above, the species is considered to be critically endangered (CR A2ace+3ce; B1ab(i,ii,iii,iv,v)+B2ab(i,ii,iii,iv,v)c(iv) according to IUCN criteria.



FIG. 4

Brackish water spring at Kato Almiri (Greece), type locality of *Aphanius almiriensis*.

DISCUSSION

COMPARISON WITH *A. FASCIATUS*: *Aphanius fasciatus* has a wide distribution, occurring along the northern coast of the Mediterranean from the Rhône delta (France) and Corsica eastwards, and along the southern coast from Algeria eastwards. It is

missing in France west of Camargue, along the Spanish coast and in western Algeria (Wildekamp, 1993: 48; Römer, 1991). The species is reported to be variable in shape, colour pattern and coloration (e.g., Wildekamp, 1993), but our observations (unpublished) show that part of this variability in fact should be re-interpreted as inter-specific differences.

The aim of the present paper is the description of *A. almiriensis*, which urgently needs a formal name. Conservation measures for a critically endangered species are impossible to be implemented if this does not have a formal name. A species without a name simply does not exist for policy makers, even though scientists recognise it. Our aim is not to revise *A. fasciatus* sensu lato, which we reserve for a later opportunity. In this context, we compare *A. almiriensis* with the populations of *A. fasciatus* present in coastal waters adjacent to the range of *A. almiriensis*, and with the population at the type locality of *A. fasciatus* (with the available data, these populations of *A. fasciatus* are conspecific). Our logic in doing so is that, as discussed below, we doubt the conspecificity of several of the populations usually referred to *A. fasciatus*. At the present stage what matters is to show that *A. almiriensis* is distinct from the species in adjacent waters, distinct from topotypical *A. fasciatus*, and distinct from the nominal species placed in the synonymy of *A. fasciatus*. To resolve these problems, a comparison of *A. almiriensis* with remote populations (species) (e.g., from France, Algeria, Egypt) is irrelevant, whatever the academic interest might be.

For the present discussion, data on *A. fasciatus* are based on material obtained in Greece (Messolonghi, Kato Basiliki, Prokopos) and Sardinia (Cagliari; type locality), unless otherwise stated.

Three valid species of *Aphanius* have so far been recorded in European waters and *A. almiriensis* is easily distinguished from them. In *A. iberus* (Valenciennes) from Spain, the caudal fin of the male is hyaline to bluish-grey, with 2-5 dark grey bars; the female has numerous dark brown spots on the side and the back. In *A. baeticus* Doadrio, Carmona & Fernández-Delgado, also from Spain, the caudal fin of the male is dark grey, with one distinct submarginal bar and 4-5 vertical rows of hyaline dots sometimes organised in regular bars; the female has a few large black spots on the side, usually organised in two rows, one on the middle of the flank, one at the level of the pectoral-fin base. In *A. fasciatus*, the male has 8-15 dark bars on the body; the female has 11-17 narrow, elongated dark bars occupying about half of the body depth on the side of the body, over a faint greyish midlateral stripe (Fig. 5b).

The males of *A. almiriensis* and *A. fasciatus* share the yellow caudal fin, but in *A. almiriensis* the caudal fin has a greyish distal edge, while in *A. fasciatus* the caudal fin is plain yellow (bright yellow in life) with or without a black subdistal bar (Figs 5a, 6). The colour pattern of the female *A. almiriensis* is not observed in other peri-Mediterranean *Aphanius*. The female colour pattern of many populations referred to *A. fasciatus* remains undescribed or only succinctly described, and reported as very variable. Our examination of material from all around the Mediterranean shows that while there is some intra-population variability (as for any other character), this intra-population variability is limited, and that the inter-population variability is not random but corresponds to a zoogeographic pattern and defines a number of (diagnosable) clusters of populations. The above-mentioned *A. fasciatus* female colour pattern is that of the



FIG. 5

Aphanius fasciatus, CMK 18451; Greece: Prokopos lagoon; male, 35.7 mm SL (above); female, 40.6 mm SL (below).

populations of the Tyrrhenian, Adriatic, Ionian and western Aegean basins. The eastern Mediterranean populations also differ from *A. almiriensis*; the female colour pattern consists, among others, in a row of very short, narrow, black, vertical bars.

Aphanius almiriensis also differs from *A. fasciatus* by its general appearance (compare Figs 1 and 5), with *A. almiriensis* having the dorsal head profile less convex than *A. fasciatus*, the ventral head profile more angular, the dorsal and anal fins of the male not reaching the caudal-fin base (vs. reaching), a quadrangular caudal fin (vs. somewhat trapezoidal), all characters difficult to quantify. Further, *A. almiriensis* has a stouter caudal peduncle (depth 1.4-1.6 times in its length, vs. 1.4-1.9), a wider inter-orbital (14.6-16.9% SL, vs. 11.6-14.4; 47-53% HL, vs. 40-47) and a larger eye (diameter 27-34% HL, vs. 24-32). The largest examined specimen of *A. almiriensis* is 38.8 mm SL, while the largest recorded size of *A. fasciatus* in Greek waters is 75.5 mm TL (estimated 60 mm SL) (Leonardos & Sinis, 1999). This is an unusually large size, and most adult specimens are in the size range 35-50 mm SL. The above comparison is based on specimens of similar sizes (*A. almiriensis* 24.5-38.8 mm SL, *A. fasciatus* 25.2-42.3 mm SL) and excludes the hypothesis that the characters distinguishing *A. almiriensis* from *A. fasciatus* are juvenile characters or size-dependant.

We also observe differences between the two species in the number and organisation of the neuromasts in the interorbital areas. In *A. almiriensis*, there are 2 neuromasts on each side, located in deep grooves (Fig. 2a). In *A. fasciatus* from Greece and the Adriatic and Tyrrhenian basins (including Sardinia and the neotype), there are three neuromasts in closed canals pierced with 3 pores (Fig. 2b). A single specimen of

A. almiriensis (the largest female, 38.8 mm SL) has the grooves partly closed, with two pores. In *A. fasciatus*, only few of the smallest males (less than about 30 mm SL) have the three neuromasts in wide, shallow grooves (vs. conspicuous, deep grooves in *A. almiriensis*). In juveniles of the eastern Mediterranean populations, there are three neuromasts in shallow grooves, which start closing anteriorly at about 30 mm SL; in most large specimens the posterior half of the groove remains open. All conditions are present in the material from Tunisia and Algeria.

Examination of type material, topotypes or original descriptions of all nominal species usually listed in the synonymy of *A. fasciatus* (see, e.g., Kottelat, 1997) shows that *A. almiriensis* is distinct from all of them. Their identities are discussed below.

ON THE IDENTITY AND TYPE LOCALITY OF *A. FASCIATUS*: Although it is not within the scope of the present paper to revise *A. fasciatus* sensu lato, it is necessary to briefly discuss the diversity within this 'species', as most *Aphanius* populations from the eastern Mediterranean drainages have been at some point referred to *A. fasciatus*. Also it needs to be ascertained that *A. almiriensis* is distinct from all the nominal species listed as synonyms of *A. fasciatus* (see Kottelat, 1997).

There has been a number of publications describing or discussing variability within *A. fasciatus*. We will not discuss them in detail, for most suffer from being only of limited, local interest, neglecting other populations or with problematic methodologies. Frequent problems are the use of morphometrics and meristics without defining the methods used, and misunderstanding of taxonomic procedures.

Aphanius fasciatus was originally described (as *Lebias fasciata*) by Valenciennes (in Humboldt & Valenciennes, 1821: 160, pl. 51 fig. 4). Valenciennes indicated neither the origin nor the number of his specimens. Kottelat (1997: 161) commented that Valenciennes (in Cuvier & Valenciennes, 1846: 156) later stated that these specimens were from Cagliari (Sardinia). In fact, the case is more complex. Valenciennes' 1846 account is somewhat ambiguous and it appears that the species was most likely based on a single specimen (thus holotype) of unknown origin and that in 1846 Valenciennes re-described *A. fasciatus* on the basis of additional (non-type) material from Cagliari obtained by Bonelli and Marmora. After describing the Cagliari specimens, Valenciennes stated (in Cuvier & Valenciennes, 1846: 158) that he compared them with the specimen (singular) "preserved for a long time in ... the museum". In 2005 we searched for the holotype in MNHN but it is no longer extant.

Valenciennes further commented that this specimen has broken dorsal and anal fins and that the artist should have made them higher, implying that this very specimen is the model of the plate. Valenciennes' 1821 description indicates 10-12 whitish bars while the figure shows only 8, but in 1846 he commented that the colour pattern too is not correctly depicted and that the artist had "exaggerated the bars, especially the anterior ones, believing that they were less contrasted because of the effect of alcohol".

Valenciennes (in Cuvier & Valenciennes, 1846: 159) commented "[in 1821] I gave only the description and figure of these two species [*L. fasciata* and *L. rhomboidalis*], whose origin I ignored. By doing this, I only wanted to complete what Mr. Cuvier [1816] had left to be done in the first edition of his *Règne animal*, where the only two species of the genus then known are designated as new". In a footnote to the

very brief diagnosis of his *Lebias* [a vernacular name], Cuvier (1816: 199) had merely mentioned that he knew two species of this genus, both new, but he neither named them nor provided any data. The meaning of Valenciennes' last sentence may seem ambiguous, but the context and his comments pp. 146-150 make it clear that he described the two unnamed species mentioned by Cuvier [implying he named them on the basis of the material on which Cuvier based his statement].

Identification of *Lebias fasciata* as the species presently called *Aphanius fasciatus* goes back to Valenciennes (in Cuvier & Valenciennes, 1846: 158) who simply stated that he compared the holotype with specimens from Cagliari and that he was "convinced of their being the same". (It is puzzling that on p. 146 Valenciennes mentioned "one of them [...] is my *Lebias fasciata*, which the late Mr. Delalande very recently brought back from the vicinity of Rio de Janeiro". The meaning of this sentence should not be misunderstood as 'the specimen being brought by Delalande from Brazil', but that Delalande had since brought specimen(s), which Valenciennes considered conspecific with his *L. fasciata*. Delalande [1787-1823] collected in Brazil in 1817 [Cuvier, 1828: 254; Bauchot *et al.*, 1990: 72], which was too late for him to have collected the holotype that was already examined by Cuvier before 1816. It is difficult to understand how Valenciennes could identify his *L. fasciata* being identical on the one hand with a Brazilian species and on the other hand with a Sardinian fish; this may relate with the small size of these fishes, the optical equipment, the state of preservation, etc.)

As the holotype was already in the MNHN in 1816, its origin might be speculated to be in areas from which the museum had received fish collections before that date. Noteworthy within the distribution range of *A. fasciatus* s.l. are the collections obtained by Geoffroy Saint-Hilaire in Egypt in 1798-1799 and by Delalande in Toulon (France) in 1813.

In conclusion, the type locality of *A. fasciatus* remains unknown and, as the holotype is now missing, it will remain impossible to establish from the original description either its identity or its type locality. This problem can be solved only by a neotype designation. We designate here MNHN 2005-1975, 39.5 mm SL (Fig. 6), as neotype. This specimen was collected in the salt-works of Cagliari in 2005, at the same locality as the material of Bonelli and Marmora, on which Valenciennes based his 1846 description. As this locality has long been erroneously considered as type locality of *A. fasciatus* (see above), the present neotype designation best creates stability in definitively linking the name to this locality. Morphometric data of neotype are listed in Table 1. Meristic data: 10 dorsal-fin rays; 9 anal-fin rays; 12 pectoral-fin rays; 26+2 scales in midlateral series; $1/2$ 10 $1/2$ scales in transverse row between dorsal origin and ventral midline in front of pelvic-fin base; $1/2$ 7 $1/2$ scales in transverse row on caudal peduncle.

WESTERN MEDITERRANEAN *A. FASCIATUS* S.L.: The material presently available to us and the published information are still insufficient to satisfactorily answer questions like the number of species hiding under the name *A. fasciatus*, their distribution and their diagnostic characters. Nevertheless, it is possible to comment on some populations and on the nominal species traditionally placed in the synonymy of *A. fascia-*

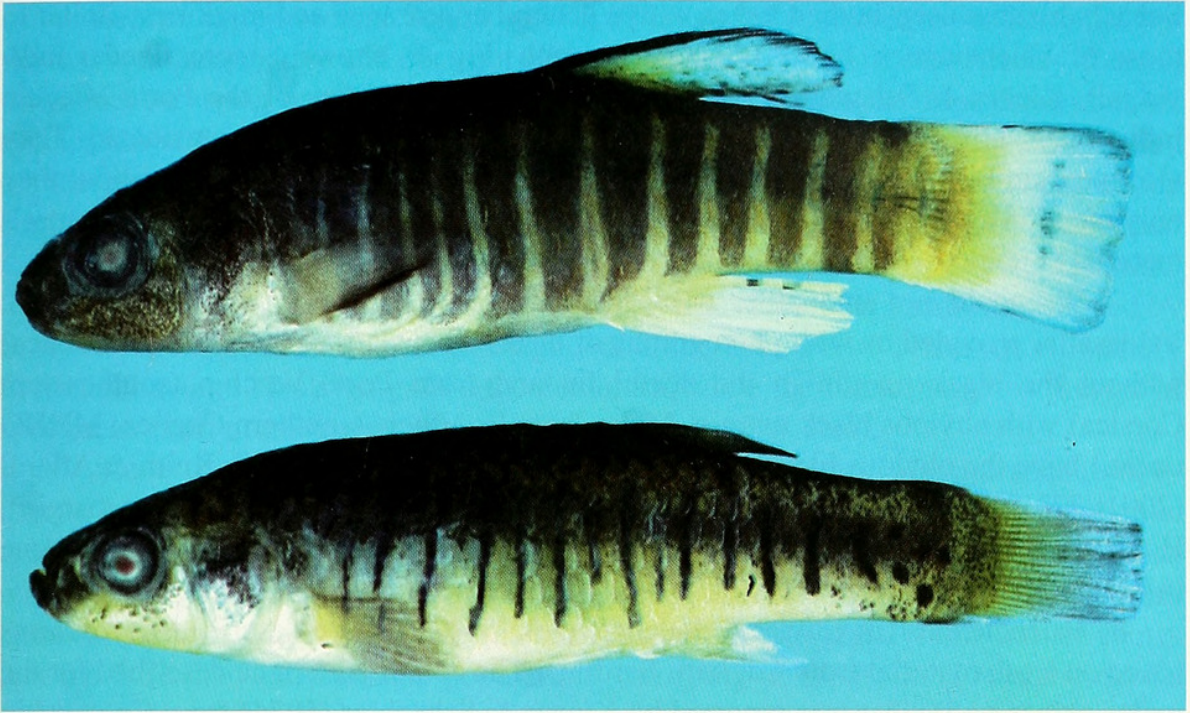


FIG. 6

Aphanius fasciatus, Italy: Sardinia: Cagliari; MNHN 2005-1975, neotype, male, 39.5 mm SL (above); CMK 18600, female, 42.6 mm SL (below).

tus. Up to now, attention has focussed on the western Mediterranean populations (Corsica, Sardinia, Sicily, Tyrrhenian slope of Italy, Malta, Tunisia). The different studies have been based only on a few populations and a few characters and therefore there are no characters with data homogeneously available for all populations. The colour pattern, however, is described or figured for a number of populations large enough to allow a comparison.

Valenciennes' figure (in Humboldt & Valenciennes, 1821: pl. 60) of *A. fasciatus* depicts a male, as shown by the conspicuous dark bars. An interesting character shown on the figure is the absence of the black margin of the dorsal fin. Even if the distal part of the fin was missing (as stated by Valenciennes, in Cuvier & Valenciennes, 1846), the membranes between the anterior rays should have been coloured dark or black. A perusal through the pictures of *A. fasciatus* in the literature shows all males to display this marking, except for material from Corsica in Kiener & Schachter (1974: 323), from Sardinia in Wagner (1828: 1055, pl. 12) and a population from Tunisia in van der Zee & Vonk (1991).

Kiener & Schachter (1974) figured specimens from 3 populations in Corsica (Diana and Biguglia in northeastern Corsica; Porto-Vecchio in southeastern Corsica) and compared them with material from Comacchio (northern Italy, Adriatic coast). They mention having seen males from Corsica up to 65 mm TL (that is, large adults) and state that the most salient features of the northeastern individuals when compared to the Comacchio ones are the shorter dorsal fin, a less vivid coloration and the absence of the black stripe in the anterior part of the dorsal fin. The drawings in Kiener &

Schachter show deep-bodied fishes with a general appearance and markings similar to those of Valenciennes' figure (their photograph -Fig. 6- shows a more slender individual). Kiener & Schachter (1974: 324) comment that a few of the Porto-Vecchio individuals show the characters of the Comacchio population, albeit attenuated. They also mention having seen material from Sardinia, but do not mention coloration; they only mention a shorter dorsal fin than the Comacchio specimens. Kiener & Schachter's material is not known to have been preserved; it is not in MNHN collections.

We could not find other published figures of Corsican populations, but S. Valdesalici provided us with photographs of fishes from Furiani (northeastern Corsica) without the black margin in the dorsal fin and from Porto-Vecchio (southeastern Corsica) with obvious black margin. Males from Bonifacio (southern Corsica; MHNG uncat.) have the obvious black margin. The account of *A. fasciatus* in Keith & Allardi (2001: 278) shows a fish with a faint dark distal margin (but no dark anterior margin). However, we cannot give any value to this drawing as it is obviously based on the one in Maitland (1976), itself based on unknown sources and not very realistic.

Valenciennes (in Cuvier & Valenciennes, 1846: 156) re-described *A. fasciatus* based on fresh material from Cagliari, Sardinia. The colour pattern he described for the males agrees with what was reported by Kiener & Schachter (1974) from Comacchio and by van der Zee & Vonk (1990) as 'short' form from Tunisia.

Valenciennes (in Cuvier & Valenciennes, 1846: 151) also described a *Cyprinodon calaritanus* from the same swamp in Cagliari as the neotype of *A. fasciatus*, which is the female of his 1846 *A. fasciatus* (confirmed by examination of 3 syntypes MNHN 187). He recorded the presence of narrow bars on the body and one or two, sometimes three, black spots at caudal-fin base, which is also agreeing with the Comacchio females figured by Kiener & Schachter and others. (*Lebias calaritana* is a name available from a very brief diagnosis by Cuvier [1829: 280], from which one deducts that the name is based on material from Sardinia sent by Bonelli and that most likely is the material used by Valenciennes in 1846.)

Wagner (1828) figured individuals from Cagliari, Sardinia (his figures show his *Lebias sarda* and *L. lineopunctata*, unambiguously male and female, respectively, of the same species). The type material of both nominal species is lost (Kottelat, 1997). The male has a relatively deep body and a short dorsal fin. There is no indication of a black anterior margin in the dorsal fin. The female has very narrow bars on the body, the posteriormost ones replaced by a number of small spots over the whole depth of the caudal peduncle. This pattern is also distinct in the Corsican females figured by Kiener & Schachter (1974) and in some populations (not all) from Tunisia figured by Boumaiza (1980: 87) (the figured Tunisian males have a large dorsal fin with a black margin).

The variability among the different accounts of material from Cagliari is intriguing, but can only be interpreted after more populations can be examined. Comparing Valenciennes' and Wagner's descriptions and figures and our material from Cagliari remains inconclusive. It might be that Wagner's figure is faulty or based on a not well preserved specimen; or it might mean that two 'forms' or species are (or were) present in Sardinia; or, that Wagner might have based his descriptions on material with erroneous locality data.

Lebias nigropunctata Schinz (1840: 334) is also described from Sardinia, but the text is uninformative and does not allow to objectively identify the species; there is no known surviving type material (Kottelat, 1997). The identity of these three nominal species based on non-extant Sardinian material can only be cleared by the designation of neotypes. We designate MNHN 2005-1975 (the neotype of *A. fasciatus*) as the common neotype of *Lebias sarda* Wagner, 1828, *L. lineopunctata* Wagner, 1828, and *L. nigropunctata* Schinz, 1840. This makes them objective synonyms of *A. fasciatus*.

We have seen photographs of *A. fasciatus* from Sardinian localities provided by S. Valdesalici. Adult males from Stagno di Pilo, near Porto Torres (northwestern Sardinia) have no black margin on the dorsal fin, while the black margin is present in specimens from northeastern Sardinia (no precise locality data).

Some data on the genetic diversity within the western Mediterranean populations is available, however in a format presently not compatible with a taxonomic analysis. Nevertheless, they somehow provide information paralleling the observations on the colour pattern. An electrophoretic analysis based on 11 populations from Corsica, Sardinia, Sicily, the Tyrrhenian and Adriatic coasts of Italy, and Tunisia shows that the 3 Sicilian and Tunisian populations constitute one unit, the 2 Adriatic ones a second unit, and the Tyrrhenian, Sardinian and Corsican ones a third unit (Maltagliati, 1999). Within that last unit, the mainland and Corsican populations are closer to each other than to the Sardinian ones. Another allozyme study of 20 Corsican and Sardinian populations shows that the Corsican populations have closest similarity with some populations from the northeastern tip of Sardinia (Maltagliati, unpublished, pers. comm.). Tigano *et al.* (2004) noted differences in the number and location of NOR loci on chromosomes of Adriatic and Sicilian populations.

Tigano *et al.* (1999) report differences in the morphology of pharyngeal bones between Sicilian and Adriatic populations. Ferrito *et al.* (2003) compared some morphological traits and allozymes of two Sicilian, one Sardinian and one Adriatic populations. Although the number of populations is limited, the three units reported by Maltagliati (1999) also show up in these studies.

Wildekamp (1993: 50) figures a specimen from Malta with more and narrower bars than the Adriatic ones, probably similar to the Tunisian ones. (Gulia [1861: 11] made a laconic description of two new species from Malta, *Micromugil timidus* and *M. macrogaster*, apparently the male and the female; there is no known type material.) Material from Sicily is figured by Wildekamp *et al.* (1999: 35) and its fins by Tigano (1982: 175). Material from Tunisia is figured by Boumaiza (1980) and van der Zee & Vonk (1991; see below). In all, the pre-dorsal bars tend to be vertically split in at least their lower extremity, and there is a tendency to have very thin bars intercalated between larger ones. This is not the case in material, figures and photographs we have seen from the Adriatic, Greece and Turkey (pers. obs.; Wildekamp, 1993 and unpublished photographs); in these populations, the bars tend to be fewer, wider and more regular.

Cyprinodon cyanogaster and *C. doliatus* were both described by Guichenot (1859: 379) from Biskara in northeastern Algeria. They are respectively female and male, and apparently conspecific (8 syntypes of *C. doliatus* MNHN 4392 and 5 syntypes of *C. cyanogaster* MNHN 3218 examined). Presently, we are unable to distin-

guish them morphologically from the Sardinian populations of *A. fasciatus* and we consider them as conspecific. The Algerian and most of the Tunisian material that we examined, as well as that figured by Boumaiza (1980) and van der Zee & Vonk (1990; 'short form'), also are conspecific. As observed in *A. fasciatus*, the syntypes of *C. doliatus* (males) have the black margin in the dorsal fin and the black subdistal bar on the caudal fin and the syntypes of *C. cyanogaster* (females) have the small spots on the caudal peduncle.

Van der Zee & Vonk (1990) have photographs of live specimens of an *Aphanius* from Tunisia with a deep body, short dorsal fin, pallid coloration and without dark margin in the dorsal fin. It differs from *A. fasciatus*, as now fixed by the neotype, in having more dark bars (about 20 vs. 8-15). Most interesting is that van der Zee & Vonk report the presence of two 'forms' of *A. fasciatus* in sympatry in Lake Kelbia drainage, the one mentioned above, which they call normal form (their figure 2), and a more stocky one, which they call 'short' form with a vivid coloration, fewer bars and a very conspicuous dark margin in dorsal fin. The two 'forms' reportedly also differ in their way of swimming (Wildekamp, 1993: 50). This is strongly suggesting that two species are involved: the 'short' form with an appearance identical to that of the Sicilian populations, and the 'normal' form possibly endemic. We had the possibility to examine only two specimens that possibly belong to this 'normal' form (Gabes, Tunisia; MNHN 1922-72, 1923-29).

Concluding, the data and material presently available to us justify investigating the possible existence of at least two species within *A. fasciatus* s.l. in the Western Mediterranean: 1) *A. fasciatus* from the Tyrrhenian basin, Sicilia, Malta, Tunisia and Algeria; and 2) an unnamed species from Tunisia. The status of some Corsican and Sardinian populations (without the black margin on the dorsal fin) also requires investigation. There are no available names for the Tunisian species or the last mentioned Sardinian-Corsican populations.

EASTERN MEDITERRANEAN *A. FASCIATUS* S.L.: Data on the Eastern Mediterranean populations are less numerous and less detailed. The earliest record of *Aphanius* from the Adriatic is apparently by Nardo (1827: 488) who recorded *A. fasciatus* and described a new species (*A. nanus*). Nardo did not give precise locality data, but his introductory paragraph makes it clear that his work was based mainly on his observations along the shores of Istria and additional observations in Dalmatia and Romandiola (Ravenna Province, Italy). Although brief, his account of *A. fasciatus* refers to 8-9 bluish bars on body and dorsal fin black anteriorly. His *A. nanus* is diagnosed by having 12-13 irregular black bars and black spots and refers to the female.

Lebias flava Costa (1838: fasc. 19: 35, pl. 17 fig. 1) was described from lake Varano, on the Adriatic shore of southern Italy. Valenciennes (in Cuvier & Valenciennes, 1846: 159) saw no difference between Costa's plate and his material from Sardinia.

Wildekamp (1993: 48) figures a male from Dalmatia with a colour pattern similar to that of the Comacchio (near Ravenna, Italy) material figured by Kiener & Schachter (1974). The material of *A. fasciatus* (of unknown origin) figured by Gandolfi *et al.* (1991: 617) shows the same colour pattern.

Cyprinodon desioi described by Gianferrari (1932: 214, fig. 1) from north-eastern Libya has been considered as a synonym of *A. fasciatus* (e.g. by Wildekamp, 1993: 49). Neither the description nor the figure mention dark markings in the dorsal fin. The figure does not show any pattern on the caudal fin, but the description mentions that there are one, two or no brown bars. With the presently available material and data, we are unable to distinguish any of these population and nominal species from *A. fasciatus* as recognised above.

Cyprinodon hammonis Valenciennes (in Cuvier & Valenciennes, 1846: 169) (type locality: Egypt [Siwa oasis, along the Libyan border] and Syria) is listed as a synonym of *A. fasciatus* by Wildekamp (1993: 48), who comments that the Syria specimens are *A. dispar*. Valenciennes' description of colour pattern (dorsal, caudal and pectoral fins blackish, without bars or spots) does not seem to be based on *A. fasciatus*.

Hrbek & Meyer (2003) examined the phylogeny of *Aphanius* using mtDNA. Their study included 7 populations that they identified as *A. fasciatus*; 6 of them pooled closely together (Prokopis [Prokopos], Greece; Ravenna and Palermo, Italy; Tarsus, Turkey; Corsica; Malta) and one slightly more distant (Lake Bafa, Turkey), but the data cannot be used to reach taxonomic conclusions.

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