

Aphanius villwocki, a new species from the Sakarya River basin of central Anatolian plain, Turkey (Teleostei: Cyprinodontiformes)

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Aphanius villwocki, new species, is described from the upper Sakarya River basin of Anatolia, central Asiatic Turkey. It is distinguished from the other Anatolian *Aphanius* species by maximum size, adult color pattern and mitochondrial DNA sequences. Based on the phylogenetic analysis of molecular sequence data, the new species forms a sister taxon to *A. anatoliae* and *A. danfordii*.

Introduction

The extant and extinct species of the killifish genus *Aphanius* are widely distributed along the late period Tethys Sea coastlines. The present distribution includes coastal areas of the Mediterranean region and coastal areas from the Gir Peninsula of northwestern India to northeastern Somalia, including the Red Sea and the Persian Gulf. Inland distribution is restricted primarily to the Mediterranean and near eastern orogenic belt, including Turkey and Iran. The ability of species of this genus to tolerate hypersaline conditions has enabled them to occupy saline and bitter lakes, like those in central Anatolia, although most Anatolian populations occur in freshwater habitats. Distribution and taxonomy of *Aphanius* in Anatolia has been subject to several studies (e.g. Akşiray, 1948a-b; Villwock, 1964; Schöll et al., 1978; Grimm, 1979), and an over-

view of *Aphanius* species in Turkish waters, including a discussion on the generic name and a review of the available literature, has been given in Wildekamp et al. (1999).

In spite of previous studies, information about the presence of *Aphanius* in the upper Sakarya River basin, including Lake Çavuşçu, was limited (Pellegrin, 1928; Villwock, 1964; Erk'akan & Kuru, 1982), and the phylogenetic relationships of the Sakarya River basin populations to other Anatolian species and populations were unknown. The relationships between the Lake Çavuşçu population and other Anatolian *Aphanius* populations have only been studied by Villwock (1964). Although no clear relationship to the other populations could be established, the Lake Çavuşçu *Aphanius* was regarded by Villwock (1964) as *A. anatoliae* (Leidenfrost, 1912), a view shared by Wildekamp et al. (1999). In 1999 and 2000 we collected an *Aphanius* species from sev-

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eral localities in the upper Sakarya River system and from Lake Çavuşçu that differed in color, color pattern and maximum size from all other Anatolian species. A molecular phylogeny of the genus *Aphanius* (Hrbek & Meyer, in press) placed the upper Sakarya and Lake Çavuşçu *Aphanius* species as a sister taxon to *A. anatoliae* and *A. danfordii*, thus prompting us to reconsider the taxonomic status of the upper Sakarya River and Lake Çavuşçu populations.

Material and methods

The description is based on specimens collected in the field by the authors and others, and deposited at the Royal Museum of Central Africa, Tervuren, Belgium. Live specimens were maintained for the study of breeding biology, coloration, maximum size and general aquarium maintenance.

Measurements and counts follow Amiet (1987). Measurements, including sub-units of head, are presented as percentages of standard length (SL). Cross-bar count was taken by counting the number of dark bands in male on the mid-lateral series of scales of the left side.

Molecular data were obtained as part of an investigation of the phylogenetic relationships of the genus *Aphanius* (Hrbek & Meyer, in press). Six individuals representing three populations of the new species including specimens from the type locality as well as from Lake Çavuşçu were used in the analysis. DNA sequences included in the study consisted of genes encoding a portion of 12S rRNA, complete ND1 and ND2 genes, transfer RNAs coding for valine, leucine, isoleucine, glutamine, methionine, tryptophan, alanine, asparagine, cysteine and tyrosine, and the light-strand replication origin. The particulars of molecular methods and data analyses are described in detail in Hrbek & Meyer (in press).

For the purpose of the current analysis, we generated an alignment of 24 individuals of the central Anatolian *Aphanius* reported in Hrbek & Meyer (in press) representing three individuals of the new species, four individuals of *A. danfordii* and 12 individuals of *A. anatoliae* sensu lato and one individual of *A. asquamatus*. A total of 3409 molecular characters were included in the analyses; 935 of these characters were variable and 661 were parsimony informative. Seventy six of these character states formed molecular synapomor-

phies for the new species. All sequence data have been deposited in Genbank (www.ncbi.nlm.nih.gov) under numbers AF449287-AF449306, and AF449349-AF449368. Aligned sequence data are shown in Table 1, with a complete version available on line at www.cyprinodontiformes.org.

Institutional abbreviation: MRAC, Musée Royal de l'Afrique Centrale, Tervuren.

Aphanius villwocki, new species (Figs. 1-2)

Cyprinodon sophiae (non Heckel, 1846): Pellegrin, 1928: 113-115.

Aphanius anatoliae (non Leidenfrost, 1912): Villwock, 1964: 459.

Aphanius chantrei (non Gaillard, 1895): Erk'akan & Kuru, 1982: 17.

Holotype. MRAC A1-30-P-1, male, 48.1 mm SL; Turkey: Pınarbaşı, approximately 10.5 km east of Emirdağ, drainage canal of small spring pond; 39°02'53"N 31°19'38"E; W. Van Neer, F. Küçük, M. Ünlüsayın & R. Wildekamp, 5 Oct 1999.

Paratypes. MRAC A1-30-P-2-3, male, 43.4 mm SL, female, 41.0 mm SL; same data as holotype. – MRAC 99-072-P-0001-0006, 4 males, 23.5-20.2 mm SL, 2 females, 28.5-17.7 mm SL; Turkey: Pınarbaşı, spring pond in source area; 39°03'42"N 31°19'37"E; W. Van Neer, F. Küçük, M. Ünlüsayın & R. Wildekamp, 5 Oct 1999.

Additional material. MRAC A1-30-P-4-9, 3 males, 31.6-20.0 mm SL, 3 females, 34.3-22.4 mm SL; Turkey: Ahiler, Sakarya River and floodplain; 39°11'38"N 31°37'17"E; W. Van Neer, F. Küçük, M. Ünlüsayın & R. Wildekamp, 5 Oct 1999. – MRAC 99-072-P-0016-0023, 3 males, 23.1-18.8 mm SL, 5 females, 25.0-21.3 mm SL; Turkey: Küçükhasan River; 38°57'30"N 31°49'27"E; W. Van Neer, F. Küçük, M. Ünlüsayın & R. Wildekamp, 5 Oct 1999. – MRAC 99-072-P-0007-0015, 9 females, 32.0-15.6 mm SL; Turkey: Küçükhasan River near bridge; 38°57'28"N 31°49'53"E; W. Van Neer, F. Küçük, M. Ünlüsayın & R. Wildekamp, 5 Oct 1999. – MRAC 99-72-P-24, female, 33.4 mm SL; Turkey: Seydi creek, south west of Yıldızören; 39°11'38"N 31°37'17"E; W. Van Neer, F. Küçük, M. Ünlüsayın & R. Wildekamp, 6 Oct 1999. – MRAC A0-20-P-0001-0006 2 males, 33.3-19.0 mm SL, 4 females, 31.8-26.6 mm SL; Turkey: İmamoğlu, spring fed stream on road to Ishakuşağı; 38°53'36"N 31°58'30"E; T. Hrbek, K. Valkenburg, B. Vlijm, I. Wildekamp & R. Wildekamp, 26 Apr 2000.



Fig. 1. *Aphanius villwocki*, wild caught male, about 50 mm SL; Turkey; Ahiler, upper Sakarya River. Photograph taken about seven months after capture; not preserved.



Fig. 2. *Aphanius villwocki*, wild caught female, about 45 mm SL; Turkey; Pınarbaşı, drainage canal from spring pond, upper Sakarya River system (type locality); not preserved.

Diagnosis. Males of *A. villwocki* are distinguished from those of all other species of the genus by the presence of a small dark ocellus at the rear part of the dorsal-fin, even visible in subadults (vs. absence of ocellus). They are most similar to males of *A. danfordii*, but are further distinguished from them by a higher number of dark cross-bars (13-19 vs. 9-13), absence of cross-bars on caudal fin (vs. presence of 2-3 cross-bars), and by a narrow black margin on the dorsal fin (vs. an extremely

wide black margin covering almost the entire dorsal fin). They are distinguished from the morphologically highly variable but usually distinctly more slender males of *A. anatoliae* by a higher number of dark lateral cross-bars (13-19 vs. 5-14), lack of cross-bars on caudal-fin (vs. presence of 1-3 cross-bars), and the patterning of the dorsal fin (narrow black margin vs. almost completely black dorsal-fin).

Females of *A. villwocki* are distinguished from

is 8.8-10.0%, and between *A. villwocki* and *A. anatoliae* 7.4-9.7%. Within *A. villwocki* pair-wise sequence divergence is up to 2.1%.

Coloration. Living male (Fig. 1): Body and head scales silvery grey. Scales on back with narrow grey margins. From snout to dorsal-fin base, and on back and nape, a marbled pattern of dark grey spots. Thirteen to nineteen dark grey cross-bars on side, irregular in shape. Dark cross-bars usually less distinct on ventral part of side. Dorsal fin transparent yellowish grey with many small dark grey spots. Dorsal-fin margin black, narrower anteriorly than posteriorly, ending in small ocellus. Anal fin yellowish grey, usually without markings; narrow grey margin may be present. Caudal fin transparent pale grey with many small dark grey spots; spots may be arranged in a vertical or oblique direction. Pelvic fin yellowish grey. Pectoral fin hyaline. Breeding males with pale yellowish sheen over entire body.

Living female (Fig. 2): Head and upper body half light copper grey with irregularly spread dark grey markings. Lower body half pale silvery grey, usually with some grey markings. On mid-lateral line a vague grey band. Juvenile specimens usually with higher number of grey markings on side. Marking on lateral body axis arranged in a horizontal series. When aging, body spots are less distinct or disappear completely. Middle of caudal-fin base always with a distinct dark spot. All fins colorless. Iris silvery, upper part with dark grey spot.

Etymology. Named in honor of Wolfgang Villwock, in recognition of his contributions to our knowledge of the genus *Aphanius*.

Distribution and habitat. The type locality (Fig. 3) is the drainage canal of a more or less circular spring-fed pond near the village of Pınarbaşı, approximately 10.5 km east of Emirdağ. The type locality was visited on 5 October 1999 and 26 April 2000. In 1999 *A. villwocki* was found in the drainage canal together with *Alburnus escherichii* Steindachner, *Barbatula angorae* (Steindachner) and the introduced *Gambusia affinis* (Baird & Girard). In 2000 *A. villwocki* was collected from the spring-fed pond (39°02'39"N 31°19'32"E) where it was the only fish present. On both occasions the water was clear. In 1999, conductivity was 1200 µS, pH 6.4 and temperature, at approximately 15.30 h., 21.3 °C. In 2000 conductivity was 800 µS, pH 7.9 and temperature, at approximately 19.00 h., 16 °C. Aquatic vegetation consisted of clumps of *Potamogeton* sp. and *Nasturtium* sp. growing in all areas of the pond except on the spring-heads. At the edges dense stands of *Typha* sp. were present. The bottom of the pond as well as the drainage canal were covered with a mixture of coarse gravel and sand, the banks were more muddy.

Aphanius villwocki is only known from the basin of the upper Sakarya Nehri (river), including Lake Çavuşçu (Fig. 5). The Sakarya originates from a series of springs and small streams south and southeast of Eskişehir. Its major source is the Seydi Çayı (creek) which, after the connection

Table 2. Morphometrics of *Aphanius villwocki*. All measurements in percents of standard length, except standard length in mm. Mean values in brackets.

	holotype	paratypes (n = 8)	Additional material (n = 30)
Standard length	48.1	17.7-43.4	15.6-34.3
Total length	122.5	117.8-127.7 (123.1)	116.9-126.3 (122.4)
Body depth	28.3	26.6-36.1 (30.0)	25.5-33.8 (29.9)
Body width	18.7	17.8-22.2 (19.4)	16.5-23.4 (20.3)
Head Length	31.6	24.6-32.0 (29.5)	24.6-34.2 (28.8)
Eye diameter	10.2	8.4-11.3 (9.7)	8.2-11.6 (9.6)
Inderorbital width	13.1	11.3-14.0 (12.6)	10.8-14.7 (12.0)
Snout length	6.2	6.0-7.3 (6.5)	5.4-7.8 (6.5)
Pre-dorsal length	58.4	60.1-66.0 (62.7)	58.9-67.1 (64.0)
Pre-anal length	60.3	65.0-69.4 (66.4)	64.1-72.6 (68.1)
Pre-ventral length	49.3	48.0-54.5 (50.9)	49.0-54.7 (52.2)
Caudal length	21.4	21.0-25.4 (21.1)	19.5-24.2 (22.1)
Caudal depth	20.2	17.5-18.7 (18.6)	14.6-20.6 (17.1)



Fig. 3. Type locality of *Aphanius villwocki*. Drainage canal from a spring pond near Pınarbaşı, about 10.5 km east of Emirdağ, central Turkey; 5 October 1999.



Fig. 4. Lake Çavuşçu. Shallow south west bank, about 6 km north west of Ilgın, central Turkey. Photograph taken on 18 April 2000.

with the Badra creek, continues as Sakarya River in a southeastward direction. East of the village of Çakmak, it turns northward to meet the Porsuk River located northwest of the town of Polatlı. From that point it winds through the Sündiken Mountains and finally empties in the Black Sea near the town of Karasu. It appears that *A. villwocki* is restricted to the Sakarya River, upstream of the junction with Porsuk River. We did not find this species in the Porsuk and middle Sakarya Rivers. *Aphanius villwocki* was also found in Lake Çavuşçu (38°19'10"N 31°51'52"E) on 18 April 2000 (Fig. 4) and in a wetland northwest of Lake Çavuşçu (38°23'15"N 31°50'56"E) on 2 May 2000. Lake Çavuşçu is a part of the upper Sakarya River system; however, it is only connected to it during times of high rainfall through temporary rivers in the plain surrounding Yığar. The presence of *A. villwocki* in the northern part of the Yığar plain was confirmed on 26 April 2000; *A. villwocki* was found in a thermal creek on the northern edge of the village Imamoğlu (38°02'38"N 31°40'47"E).

Hanko (1924) reported *Cyprinodon sophiae* from Beilik. Neu (1937) suggested this locality to be Beylikahur or Beylik Köprü (= Beylik village), near the railway bridge over the Sakarya River, east of Eskişehir. None of these localities could be found on recent maps, although the railway crossing of the Sakarya River is a likely locality for *A. villwocki*. Erk'akan & Kuru (1982) mentioned *A. villwocki* (as *A. chantrei*) as a new record for the Sakarya basin from five localities in the upper Sakarya basin.

Discussion

Villwock (1964) was the first to study the genus *Aphanius* by means of hybridization. Using the principles of the biological species concept (Mayr, 1942), and conducting the necessary hybridization experiments, Villwock (1964) divided the populations into three groups, named Kızılırmak, central Anatolian and southwest Anatolian. The Kızılırmak group was reproductively completely isolated from the central Anatolian and southwest Anatolian groups; the central Anatolian and the southwest Anatolian groups showed partial reproductive isolation. Villwock (1964) concluded that the Kızılırmak River group represented a valid species *A. chantrei* (Gaillard, 1895), later synonymised with *A. danfordii* in Wildekamp et al. (1999). The central and southwest Anatolian groups were placed into *A. anatoliae*.

One notable exception to the above conclusions was the treatment of the Lake Çavuşçu population (called Ilgın population in Villwock, 1964) (Fig. 4). Although all hybridization experiments with the Lake Çavuşçu population resulted in the production of sterile offspring (the only exception being Ilgın × Obruk cross that resulted in F2 larval mortality), the Lake Çavuşçu fish was included in *A. anatoliae*. At the time of these experiments, other populations from the Sakarya River basin were not available, and this result was regarded an aberrant observation.

The rediscovery of the Lake Çavuşçu population and the discovery of new populations in the Sakarya River basin allowed the establishment of the relationship of these populations to other Anatolian *Aphanius* species (Hrbek & Meyer, in

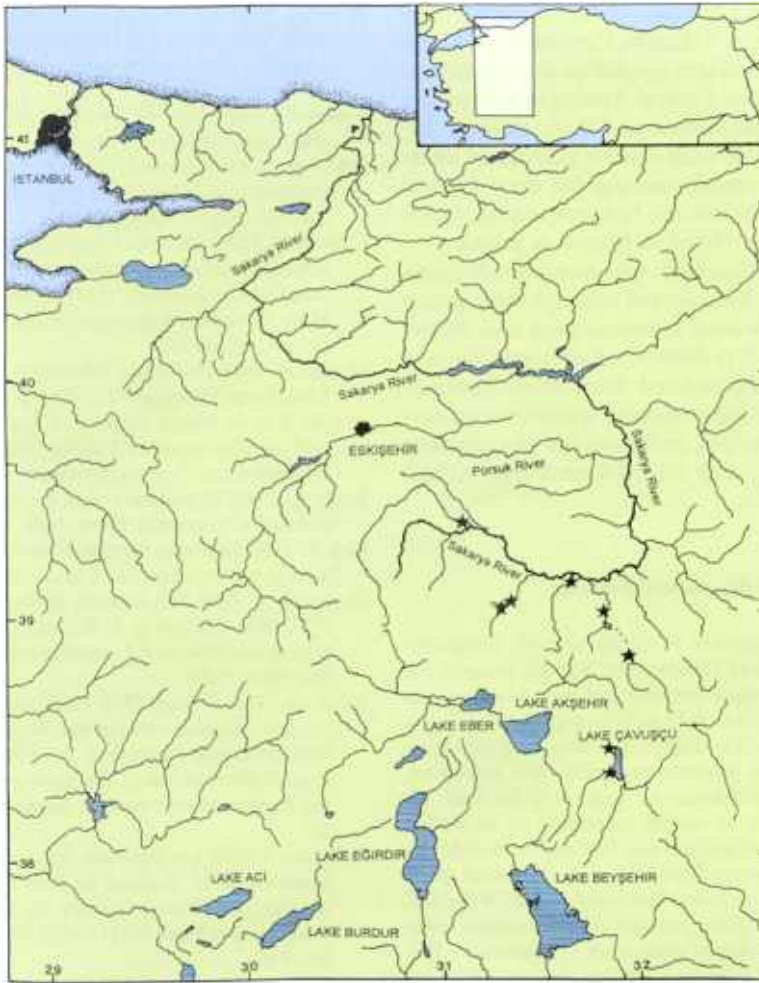


Fig. 5. Sketch map of the Sakarya River basin, western central Turkey. Stars indicate localities where *Aphanius villwocki* was collected by the authors.

press). The Lake Çavuşçu and Sakarya River basin populations, here described as *A. villwocki*, form a sister group to other members of the Central Anatolia clade. In addition to *A. villwocki* the Central Anatolian clade includes *A. danfordii* from the Kızılırmak and Yeşilirmak River drainages, and the Develi Depression, and *A. anatoliae* from the Tuz Gölü basin, from the Lakes District region and from the Büyük-Menderez River basin. Some morphological differences between clades of *A. anatoliae* also exist, and Villwock (1964) has noted partial reproductive isolation between fish from the Tuz Gölü basin and the Lakes District region. These clades may therefore represent more than just one biological species, however, this

hypothesis awaits further testing. All members of the Central Anatolian clade form the sister group to *A. asquamatus* from Lake Hazer. The Central Anatolian clade is monophyletic, and its monophyly is well supported (Hrbek & Meyer, in press).

The phylogenetic results are congruent with what is known of the geological history of Anatolia. While the initial agglomeration of Anatolia began as early as 35 million years ago, starting approximately 10 million years ago, extensive folding and mountain-building occurred in central Anatolia (Quennell, 1984; Steininger & Rögl, 1984). We hypothesize that this folding 10 million years ago was the driving force for the differenti-

ation of the major *Aphanius* clades in central Anatolia, including *A. villwocki*. Consistent with the hypothesis of vicariant speciation is the observation that the three Central Anatolian species are allopatrically distributed and their areas of distribution correlate with distinct geological units. The age of divergence among the three Central Anatolian species has also been calculated at least 10 million years (Hrbek & Meyer, in press).

The newly described *Aphanius villwocki* therefore forms a well supported monophyletic group diagnosed by several morphological and molecular characters. It is distributed allopatrically, and is reproductively isolated from other *Aphanius* species. Thus it forms a valid species under several species concepts, including the phylogenetic (Cracraft, 1983) and the biological (Mayr, 1942) species concepts.

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