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Maurice Kottelat, Rue des Rauragues 6
CH-2800 Delémont, Switzerland
Tel. +41 32 4623175 · Fax +41 32 4622259 · E-mail mkottelat@dplanet.ch

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Status of *Pseudophoxinus libani* and *P. kervillei*, two minnows from the Levant (Teleostei: Cyprinidae)

Michel Bariche* and Jörg Freyhof**

Prior to the current study, the Lebanese spring minnow *Pseudophoxinus libani* was considered an endemic species from a seasonal mountain lake within the Litani River drainage. New specimens were collected from nearby springs, 22 years after the species had been declared extinct. Morphological and molecular characters (COI) showed that *P. libani* and *P. kervillei* are conspecific. It is concluded that *P. libani* is a common species in the Orontes and Litani river drainages and that *P. kervillei* is a junior synonym of *P. libani*.

Introduction

Spring minnows of the genus *Pseudophoxinus* are divided into two phylogenetic groups, which correspond to different geographic distribution patterns (Hrbek et al., 2004; Geiger et al., 2014). One of them is distributed in Central Anatolia, where the genus has been intensively studied (Bogutskaya, 1992, 1997; Bogutskaya et al., 2006; Freyhof & Özuluğ, 2006, 2009a–b, 2010; Küçük, 2007; Küçük et al., 2013; Küçük & Güçlü, 2014).

The second group occurs in the Levant and the upper Euphrates, and there have been quite contradictory opinions on how many species of *Pseudophoxinus* should be recognized in it. *Pseudophoxinus kervillei* and *P. libani* were treated as valid species by Berg (1949), who placed *P. syriacus* and *P. drusensis* as synonyms of *P. libani*. Karaman (1972) considered *P. kervillei*, *P. libani*

and *P. syriacus* as subspecies of *P. zeregi* and treated *P. drusensis* as a synonym of *P. kervillei*. Goren (1972) treated *P. drusensis* as a synonym of *P. zeregi*. Bănărescu (1977) considered *P. kervillei* as a valid species and *P. libani*, *P. syriacus* and *P. rutiloides* as subspecies of *P. zeregi*. *Pseudophoxinus rutiloides* was considered a synonym of *P. kervillei* by Karaman (1972), Goren (1972) and Krupp (1985). Krupp (1985) was the last who reviewed the species from the Levant (as *Phoxinellus*). He recognized five valid species (*P. drusensis*, *P. kervillei*, *P. libani*, *P. syriacus* and *P. zeregi*) and two additional ones were left undescribed. Later on, one of these was described as *P. hasani* (Krupp, 1992) and the second species as *P. turani* (Küçük & Güçlü, 2014). Bogutskaya et al. (2006) also described two additional species from the Levantine species group: *P. zekayi* from the middle Ceyhan River and *P. firati* from the upper Euphrates in Turkey.

* American University of Beirut, Department of Biology, PO Box 11-0236, Beirut 1107 2020, Lebanon. E-mail: michel.bariche@aub.edu.lb

** German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5a, 04103 Leipzig, Germany (corresponding author). E-mail: joerg.freyhof@idiv.de



Pseudophoxinus libani was described by Lortet (1883) from the Lake Yammouneh in Lebanon. This lake basin was an endorheic karst polje situated within the Litani drainage. *Pseudophoxinus libani* (as *Phoxinellus libani*) was considered extinct by the Lebanese Ministry of Agriculture since 1985 (Khouzami et al., 1996). In 2007, new *Pseudophoxinus* specimens were collected from three isolated springs by one of the authors (MB) in the Yammouneh area and these fish were consequently identified as *P. libani*. Later, they were compared with superficially similar *Pseudophoxinus*, identified as *P. kervillei*, from the geographically adjacent Orontes and Litani drainages. *Pseudophoxinus kervillei* was described from the Orontes [Assi] River (Pellegrin, 1911), but is also widely distributed in the Litani and the upper Jordan River (Krupp, 1985).

All but one species (*P. firati*) from the Levantine group have been studied for their molecular characters (Geiger et al., 2014). In this study, DNA of *P. libani* and *P. kervillei* were sequenced from fishes caught at or close to their type localities and results showed that *P. libani* and *P. kervillei* shared the same COI sequence. The absence of differences in the COI gene raised the question of whether *P. kervillei* and *P. libani* might actually be the same species. Naturally, there is no threshold of a given percent differences in the COI gene, below which two species have to be automatically synonymized. According to Geiger et al. (2014), it is relatively common in Mediterranean freshwater fishes that distinct species may not be recognized by their COI sequences alone. Alternatively, the fishes sequenced by Geiger et al. (2014) might have been misidentified. They were collected from springs in Lake Yammouneh basin in Lebanon, despite the fact that *P. libani* was considered extinct (Khouzami et al., 1996). A possible introduction of *P. kervillei* individuals in the lake Yammouneh basin, after the extinction of *P. libani*, cannot be ruled out.

Here we compare two contradicting hypotheses: *P. kervillei* is a junior synonym of *P. libani* vs. *P. libani* is extinct and fish being present today in the Lake Yammouneh basin represent introduced *P. kervillei*.

Material and methods

After anesthesia, all fishes were fixed in 5 % formaldehyde and stored in 70 % ethanol, or directly fixed in 99 % molecular grade ethanol. Measurements were made with dial calliper and recorded to 1 mm. They were made point to point, never by projections. Methods for counts and measurements followed Kottelat & Freyhof (2007), with the exception of the midlateral row scales, which are counted from the anterior-most scale (posterior to opercular bone) to the posterior-most one on the caudal fin. The number of pored scales in lateral line were counted separately. Standard length (SL) was measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle was measured from behind the base of the last anal-fin ray to the end of the hypural complex, at mid-height of the caudal-fin base. Gill rakers were counted on the outer margin of the anterior gill arch.

Abbreviations used: SL, standard length; HL, lateral head length. Collection codes: AUBM, American University of Beirut Museum, Beirut; FSJF, Fischsammlung J. Freyhof, Berlin; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge; MHNG, Muséum d'Histoire Naturelle, Genève; MNHN, Muséum National d'Histoire Naturelle, Paris; SMF, Senckenberg Museum Frankfurt; SMNS, Staatliches Museum für Naturkunde, Stuttgart; and USNM, National Museum of Natural History, Washington.

Pseudophoxinus libani (Lortet, 1883)

(Figs. 1–7)

Material examined. LEBANON: YAMMOUNEH BASIN: SMF 804, syntypes, 3, 29–35 mm SL; Lake Yammouni. – AUBM OS3742, 7, 47–91 mm SL; Yammouneh, 34°07'20" N 36°01'30" E. – AUBM OS3641, 24, 44–75 mm SL; Yammouneh at Dar el Wasaa, 34°05'50" N 36°04'32" E. – AUBM OS3643, 11, 67–77 mm SL; Yammouneh at Nabaa el Mghor, 34°07'11" N 36°01'21" E. – FSJF 3263, 23, 44–95 mm SL; spring south of Yammouneh, 34°07'21" N 36°01'3" E.

LEBANON: LITANI DRAINAGE: AUBM OS3588, 11, 58–73 mm SL; Chmestar at Nabee Hoshba spring, 33°57'35" N 36°02'56" E. – AUBM OS3552, 45, 32–89 mm SL; Haouch el Nabee, 33°56'38" N 36°03'45" E. – AUBM OS3581, 7, 52–72 mm SL; Anjar, 33°44'42" N 35°55'18" E. – AUBM OS3584, 12, 47–57 mm SL; Hadath Baalbeck, 33°58'38" N 36°04'47" E. – AUBM OS3530, 76, 26–85 mm SL; spring Ras el Ain at Baalbek, 33°59'54" N 36°13'01" E.



– FSJF 3266, 7, 45–84 mm SL; small field canal north of Anjar, 33°44'08" N 35°56'08" E. – FSJF 3267, 30, 39–92 mm SL; spring canal at western margin of Ammik marshes north of Ammik, 33°43'57" N 35°47'04" E.

ORONTES DRAINAGE: SYRIA: MNHN 1910-0018, lectotype of *P. kervillei*, 36 mm SL; Orontes River near its outlet from Lake Homs. – FSJF 2644, 13, 39–72 mm SL; Orontes at Al Qusayr, 24°30'31" N 36°32'20" E. – FSJF 2645, 4, 44–47 mm SL; spring Al Fawwar north of Mashta Bayth Darwish, 35°32'00" N 36°15'10" E. – FSJF 2646, 1, 44 mm SL; Orontes at Shayzar, 35°16'18" N 36°33'46" E. – FSJF 2641, 26, 26–66 mm SL; Orontes north of Ain al Zarqa, 35°56'40" N 36°24'09" E. – FSJF 2642, 1, 66 mm SL; Orontes north of Ain al Zarqa, 34°57'27" N 36°47'07" E. – FSJF 2643, 15, 25–70 mm SL; spring south of Qala'at al Jarras, 35°19'49" N 36°18'38" E. – FSJF 2647, 6, 29–54 mm SL; Nahr al Barid at Nahr al Barid, 35°18'08" N 36°20'43" E. – FSJF 2756, 1, 35 mm SL; Orontes at Al Ghassaniyya, 34°35'38" N 36°32'01" E. – FSJF 2757, 9, 29–67 mm SL; Orontes at Mashr'a al Bouz, 35°57'03" N 36°23'45" E. LEBANON: AUBM OS3518, 53, 36–79 mm SL; Chouagheer, 34°24'25" N 36°26'09" E. – AUBM OS3520, 17, 58–92 mm SL; Ain el Zarqa spring, 34°21'09" N 36°22'25" E. TURKEY: FSJF 2391, 19, 39–68 mm SL; Orontes at Sinanli, 36°05.846' N 36°04.712' E.

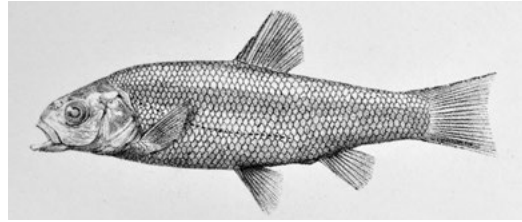


Fig. 1. *Pseudophoxinus libani*, syntype; Lebanon: Yammouneh basin; from Lortet (1883), right side, reversed.

Results

A total of 123 specimens (33 from springs in the Yammouneh basin, 60 from the Litani and 30 from the Orontes) were examined for 20 morphometric (Table 1) and nine meristic characters (Table 2). Other materials were examined for general body shape and position of fins. All these characters were found to be largely overlapping and no morphometric or meristic differences could be detected between the three populations examined. Furthermore, we found no difference in the head and body shape, position of fins or color patterns.

Table 1. Morphometric data of *Pseudophoxinus libani* from the Yammouneh basin (n=33), Litani River (n=60) and Orontes River (n=30). AUBM OS3518, 3520, 3530, 3552, 3581, 3584, 3588, 3641, 3643, 3742.

Drainages	Yammouneh			Litani			Orontes		
	range	mean	SD	range	mean	SD	range	mean	SD
Standard length (mm)	47.7–91.4			53.0–88.6			50.5–91.6		
In percent of standard length									
Head length	23.0–29.7	26.7	1.5	24.4–30.2	27.1	1.4	21.8–29.2	26.0	1.7
Body depth at dorsal-fin origin	27.6–33.1	30.7	1.5	27.0–33.2	29.9	1.7	27.6–34.9	31.2	1.7
Predorsal length	51.6–56.5	54.2	1.1	49.4–57.4	53.0	1.6	49.4–56.3	52.6	1.8
Prepelvic length	48.9–54.4	51.8	1.4	47.5–55.6	51.2	1.5	48.2–55.0	51.4	1.8
Preanal length	48.9–74.5	71.3	4.3	69.4–75.9	72.6	1.3	67.4–74.1	70.7	2.1
Body width at dorsal-fin origin	17.1–21.2	18.9	0.9	14.4–21.8	18.6	1.8	16.9–32.2	20.7	2.8
Depth of caudal peduncle	11.3–15.1	13.3	0.9	11.6–14.6	13.2	0.7	12.2–16.6	14.1	1.0
Length of caudal peduncle	16.1–21.6	19.1	1.2	15.2–24.2	19.3	1.7	15.5–22.0	19.3	1.5
Length of dorsal-fin base	10.5–14.1	12.5	0.8	9.1–14.0	11.7	0.9	9.9–14.5	12.0	1.2
Length of last unbranched dorsal-fin ray	17.9–22.4	20.0	1.2	15.0–21.9	18.7	1.7	16.5–23.8	20.2	2.0
Length of anal-fin base	9.0–11.6	10.3	0.8	7.1–13.4	9.4	1.1	7.9–12.0	10.0	1.1
Length of last unbranched anal-fin ray	9.4–16.5	13.7	1.5	7.1–14.6	12.2	1.4	10.3–17.5	13.9	1.7
Length of pectoral fin	15.7–20.7	18.2	1.4	13.7–20.8	16.7	1.5	14.8–22.5	18.3	1.8
Length of pelvic fin	14.1–19.1	16.2	1.0	12.8–17.3	15.0	1.1	13.8–19.2	16.2	1.5
In percent of head length									
Head depth	66–86	76.7	5.0	66–85	75.2	4.4	70–92	77.2	5.7
Snout length	23–33	28.0	2.2	23–31	26.5	1.7	23–37	28.0	2.8
Eye diameter	21–29	25.0	2.0	20–29	25.0	2.0	22–37	26.9	3.2
Interorbital width	31–42	34.8	2.6	28–38	32.2	2.3	28–38	33.7	2.0
Mouth width	24–35	29.3	2.2	25–33	28.7	2.0	26–37	30.8	2.4



Fig. 2. *Pseudophoxinus libani*, MCZ 25546, syntypes; Lebanon: Yammouneh basin. (Photograph from MCZ Ichthyology, © President and Fellows of Harvard College).

The syntypes of *P. libani* (SMF 804) were distorted and had been dry once. They look similar to the photograph available online at MCZ (MCZ 25546) database (Fig. 2). We did not examine all syntypes since their examination did not allow to distinguish them from the other studied specimens by any of the characters listed in Tables 1 and 2.

Discussion

Lake Yammouneh, the type locality of *P. libani*, was situated within the Litani drainage, but isolated from the Litani River itself. Water accumulated to form a temporary mountain lake of about 3 km length and 2 km width, at 1650 m altitude (Lortet, 1883). Lake Yammouneh drained from a sinkhole and existed for around five months a year (Abdul-Al, 1944; Joannon, 1969). *Pseudophoxinus libani* was the only native fish spe-



Fig. 3. *Pseudophoxinus libani*, MNHN-IC-1910-0018, lectotype of *P. kervillei*; Syria: Homs. (Photograph by C. Ferrara, MNHN, <http://coldb.mnhn.fr/catalognumber/mnhn/ic/1910-0018>, used under Creative Commons license CC BY-NC-ND).

cies in the lake where it was considered endemic (Lortet, 1883; Gruvel, 1931; Krupp, 1985). During the dry season, all fish accumulated in small ponds and nearby springs, and were captured by locals for consumption (Lortet, 1883; Gruvel, 1931). The species would reproduce very quickly the following spring and repopulate the entire water body in few months (Lortet, 1883). In 1937, an artificial tunnel was opened to allow water to drain towards the Al-Biq'a'a, a fertile valley situated east of Mount Lebanon (Joannon, 1969). Lake Yammouneh disappeared almost completely since then and only few small springs and ponds still have permanent water in the catchment basin.

The most important character states to distinguish *P. kervillei* from *P. libani* is the position of the dorsal fin and the number of scales along the longitudinal line (Pellegrin, 1911, 1923; Krupp, 1985). There is a large variability in the position of the dorsal- and pelvic-fin origins in fishes from the Orontes and Litani. The dorsal-fin origin is situated slightly behind the pelvic-fin origin in some individuals, but is usually clearly situated behind the pelvic-fin base.



Fig. 4. *Pseudophoxinus libani*, FSJF 2641, 36 mm SL; Syria: Orontes, north of Ain al Zarqa; juvenile showing position of dorsal-fin origin.



Fig. 5. *Pseudophoxinus libani*, FSJF 3263, about 90 mm SL; Lebanon: spring south of Yammouneh, male with long paired fins and subterminal mouth.



Fig. 6. *Pseudophoxinus libani*, FSJF 3267, 92 mm SL; Lebanon: spring canal at western margin of Ammik marshes, Litani drainage; female with short paired fins and slightly upwards directed mouth.



Fig. 7. *Pseudophoxinus libani*, FSJF 2644, 72 mm SL; Syria: Orontes at Al Qusayr.



According to Lortet (1883), the dorsal-fin origin is situated above the pelvic-fin origin in *P. libani*. See syntypes of *P. libani* for this character state on Figures 1 and 2. Pellegrin (1911) mentioned that the dorsal-fin origin is situated above the base of the pelvic-fin in *P. libani* while it is slightly behind the pelvic-fin base in *P. kervillei*. See Figure 3 for the lectotype of *P. kervillei*, designated by Krupp & Schneider (1989). In Krupp (1985), the measurements of the predorsal and prepelvic lengths of the syntypes of *P. libani* and *P. kervillei* from the Orontes and Litani showed a large overlap. This confirmed our results in which we find the dorsal-fin origin in the syntypes of *P. libani* not more anteriorly situated than is the case in at least some *Pseudophoxinus* individuals from the Orontes and Litani. The fish shown as *P. libani* by Lortet (1883: pl. 11 fig. 4) (reproduced here as Fig. 1) has the dorsal-fin origin slightly in

front of the pelvic-fin origin. All individuals from the Orontes and Litani as well as all those collected from the Yammouneh basin (Figs. 4–7), including the three syntypes of *P. libani* examined, have their dorsal-fin origin situated behind the pelvic-fin origin. In the three syntypes, the dorsal-fin origin is just slightly behind the pelvic-fin origin and this is the case also in some *Pseudophoxinus* of the same size from the Orontes and the Litani (Fig. 4).

A second character distinguishing *P. libani* from *P. kervillei* mentioned by earlier authors is the number of scales in midlateral row. Pellegrin (1911, 1923) counted 48–53 scales in midlateral row in *P. libani* and 37–42 scales in *P. kervillei*. According to Krupp (1985), the number of scales in midlateral row is 46–55 (n=19) in *P. libani*. Krupp (1985) gives different scale counts for *P. kervillei* collected from the Orontes (36–43), the

Table 2. Meristic data of *Pseudophoxinus libani* from the Yammouneh basin (n=33), Litani River (n=60) and Orontes River (n=30). AUBM OS3518, 3520, 3530, 3552, 3581, 3584, 3588, 3641, 3643, 3742.

	Number of total scales in midlateral row																	
	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	
Yammouneh	1	2	1	3	3	2	7	3	–	5	2	–	2	2				
Litani		1	–	3	4	5	5	5	3	8	6	3	4	7	2	3	1	
Orontes	1	–	1	–	6	6	2	5	1	4	1	2	–	–	–	1		
	Number of pored scales in lateral line																	
	0	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Yammouneh	9	1	2	1	1	1	4	3	3	3	1	1	1	2				
Litani	16				1	1	6	3	4	1	9	3	5	6	2	2	–	1
Orontes	3				1	1	1	2	4	3	5	5	1	1	2	1		
	Number of scales between dorsal-fin origin and pelvic-fin origin								Number of scales around caudal peduncle									
	14	15	16	17	18	19	20	21	18	19	20	21	22	23	24	25		
Yammouneh		3	8	12	3	5	1	1			9	5	6	7	4	2		
Litani		8	18	16	12	6					10	17	19	9	2	3		
Orontes	2	4	12	7	4	1				3	6	8	9	3	–	–	1	
	Number of branched dorsal-fin rays			Number of branched anal-fin rays			Number of pectoral-fin rays											
	6½	7½	8½	5½	6½	7½	11	12	13	14	15							
Yammouneh	1	30	2	1	32		4	13	12	4								
Litani	4	56		1	54	5	3	21	23	12	1							
Orontes	2	28		1	28	1	1	9	16	4								
	Number of pelvic-fin rays				Number of gill rakers (lower limb)													
	4	5	6	7	4	5	6	7										
Yammouneh			25	8		9	22	2										
Litani			40	20		13	42	5										
Orontes	1	–	17	12		3	19	8										



very upper Litani in Baalbek (Bal'abakk) (41–50) and the middle Litani (40–46). It is noteworthy that Krupp (1985) counted the scales on the body without those on the base of the caudal fin. Krupp (1985) also presented tables with measurements of morphometric characters and all of them largely overlap between *P. libani* and *P. kervillei*.

In this study, *Pseudophoxinus* collected from the Yammouneh basin, as well as from the Litani and the Orontes River drainages, cannot be distinguished from each other's or from the syntypes of *P. libani* by scale counts. Fishes from the Yammouneh have 42–55 scales in midlateral row. This is covering the full range of the scale count found in the syntypes of *P. libani* recorded by Pellegrin (1911, 1923) and Krupp (1985). Our fishes from the Litani have 43–58 and from the Orontes 42–57 scales in the midlateral row, not counting those on the caudal fin. Also, we find no additional indications in the study made by Krupp (1985), who examined all syntypes of *P. libani* (MCZ 25546 (28), MHNG 611.24 (10), SMF 804 (19), SMNS 3214 (several, not found), USNM 48012 (2)), on how to distinguish it from *P. kervillei* and by examining the syntypes, we also found no distinguishing characters not discovered before. Therefore, we conclude that only one species actually exist in the area. This also confirms the results of Geiger et al. (2014), based on molecular evidences.

As the recently collected fishes cannot be differentiated from the examined syntypes of *P. libani*, we reject the hypothesis that the species is extinct. Our results support the second hypothesis and *P. kervillei* becomes thus a junior subjective synonym of *P. libani*. There is no evidence, that the fish present today in the Lake Yammouneh basin are introduced; they are simply native populations of *P. libani* that had survived the drainage of the lake.

The taxonomy of the Lebanese spring minnow has remained unclear since its first record. It has been affected by a combination of poorly preserved type specimens, lack of specimens in collections, overlapping meristic and morphometric characters, and the fact that it was considered extinct. The discovery of new specimens and the development of molecular biology allowed to solve this question. This study showed also that hasty conclusions may be misleading and that on-site critical scientific studies are needed prior to declaring a species extinct.

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Liniparhomaloptera macrostoma (Photograph by Jiahu Lan)
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