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ORIGINAL ARTICLE

First record of the Tunisian Bleak, *Tropidophoxinellus chaignoni* (Vaillant 1904) from Algeria (Teleostei: Cyprinidae)

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Abstract
The Tunisian Bleak *Tropidophoxinellus chaignoni* (Vaillant 1904), a species endemic to northeastern Tunisia, has recently been discovered in Wadi El Mellah, located in southern M'Sila, Algeria. Fish samples were collected on August 9, 2023, from the Wadi El Mellah in Algeria using a scoop net. Genetic pairwise distances between *Tropidophoxinellus chaignoni* haplotypes ranged from 0.007 to 0.028. The phylogenetic tree showed that the haplotype identified in Algeria belongs to the Tunisian Bleak clade and forms a monophyletic subclade with the *species* haplotype from the Abid River in Tunisia. This indicates the first known occurrence of the species in Algeria, highlighting a gap in research on the ichthyofauna of the country's continental freshwater systems. In this study, we document the presence of *Tropidophoxinellus chaignoni* in Algeria for the first time; using mitochondrial DNA cytochrome oxidase subunit I (COI) gene sequences as a DNA barcodeto confirm its identification.

Keywords: DNA Barcoding, *Tropidophoxinellus chaignoni*, First record, Algeria

INTRODUCTION

The species of the genus *Tropidophoxinellus* spp. (Stephanidis 1971) are freshwater fish belonging to the family Cyprinidae. The distribution area of the genus is primarily located in West Asia (Perea et al. 2010), although some species are also found in North Africa (Doadrio 1994).Recently, species of the genus *Pseudophoxinus* spp. (Bleeker, 1860) were reclassified under the genus *Tropidophoxinellus* spp. (Smith et al. 2006; Perea et al. 2010), including two species in North Africa (Ford et al., 2020), one of which occurs in Algeria (Ford et al. 2020). In North Africa, the only reported species are the Maghreb Bleak, *Tropidophoxinellus callensis*(Guichenot 1850), the Tunisian Bleak, *Tropidophoxinellus chaignoni* (Vaillant 1904), and *Tropidophoxinellus punicus*(Pellegrin 1920) (Doadrio 1994).The latter species is sometimes considered to be classified under a distinct genus that would still need tobe described, as the true *Tropidophoxinellus* spp. belong to a different lineage. Thus, *Tropidophoxinellus punicus*has recently been associated with the *Alburnus* and *Leucaspius* groups (Perea et al. 2010).

The Tunisian Bleakis regarded as an endemic species found in several rivers in Tunisia (Kraiem 1983; Doadrio 1994; Freyhof & Ford 2022). It is not listed in the inventories of freshwater fish in Algeria (Playfair & Letourneux 1871; Pellegrin 1920). Aside from *Tropidophoxinellus callensis*, no other species from this genus hasbeen reported in Algeria since then (Doadrio 1994). Furthermore, recent ichthyological surveys conducted in the country still do not mention this species (Bacha & Amara 2007; Kara 2012; Amalou&Moulai 2020; Baikeche et al. 2021).This species may be present in Algeria because of its morphological similarity to *Tropidophoxinellus callensis* and the overlap in their distribution ranges (Kraiem 1983; Doadrio 1994). However, its presence has yet to be confirmed, and its taxonomic status remains uncertain (Marfoua et al. 2017; Salhi et al. 2021).In this study, we present the first report of *Tropidophoxinellus chaignoni* in Algeria, specifically within the biogeographical region of the High Plateaux of El Hodna in M'Sila.

Fig.1. Wadi El Mellah, M'Sila, Algeria, habitat of *Tropidophoxinellus chaignoni* (©Tababouchet M.).

MATERIALS AND METHODS

Study area: Fish samples were collected on August 9, 2023, from the Wadi El Mellah using a scoop net. The Wadi El Mellah, which is a permanent watercourse (Fig. 1), is located about 100km from the capital of the wilaya of M'Sila and 14km north of the town of Ben-Srour (35˚04'50.6"N 004˚44'43.4"E; altitude: 518m a.s.l.) (Fig. 2). It is situated in an arid bioclimatic zone. Its depth ranges from a few centimeters to 2 meters (Marfoua et al. 2017).

Biometric data: After confirming the taxonomic status of this species, we consider it essential to present the available biometric data for Algeria. Five specimens of the species were collected using a scoop net and preserved in fresh ethanol for further analysis. Thirteen distinct measurements were recorded using a caliper.

DNA extraction and PCR: Molecular analysis was conducted at the Zoological Institute of the University of Basel, Switzerland. The specimens were identified based on COI (Cytochrome C Oxidase, subunit 1) barcoding. DNA was extracted from dorsal fin clips preserved in fresh ethanol using E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek, VWR Catalog Number: D3396-02) following the manufacturer's instructions. The COI gene was amplified by PCR (polymerase chain reaction) using universal fish barcoding primers: FishF1- (5'-TCAACCAACCACAAAGACATTGGCAC-3') and

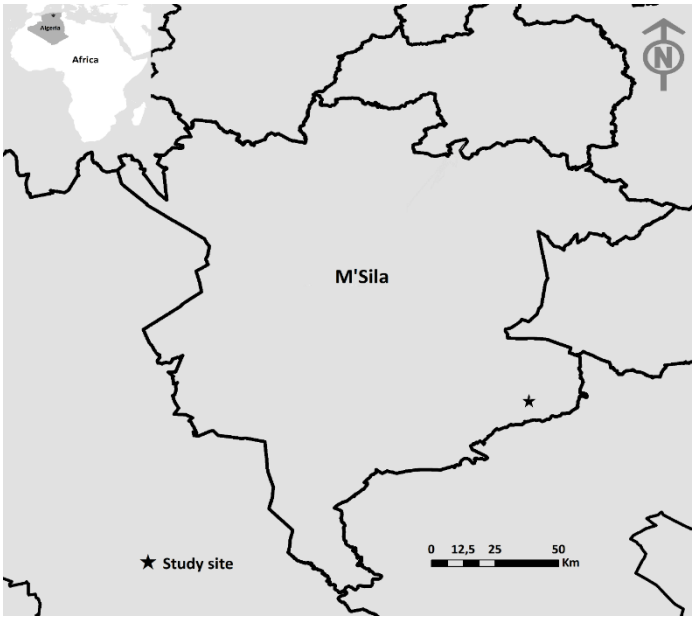

Fig.2. Study area and Wadi El Mellah localisation.

FishR1- (5'-TAGACTTCTGGGTGG CCAAAGAATCA-3'). Prior to sequencing, the PCR products were purified in order to remove excess primers and unincorporated nucleotides by digesting a small volume of PCR product with by ExoSAP- IT PCR Product Cleanup (Thermo Fisher Scientific) then sequenced by the Sanger cycle sequencing method with capillary electrophores at Microsynth (Balgach, Switzerland).

Molecular data analysis: The sequenced data was analyzed using BLAST (www.ncbi.nlm.nih.gov) search engine provided by the National Center for

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Fig.3. *Tropidophoxinellus chaignoni*, (PQ505104): 64mm ST, Wadi El Mellah, M'Sila, Algeria (©Tababouchet M.).

Table 1. Morphometric characteristics (mm) of *Tropidophoxinellus chaignoni* from Algeria. (-): missed data.

Morphometric traits	Specimen 1	Specimen 2	Specimen 3	Specimen 4	Specimen 5	Mean±SD
Total length	60.23	53.50	56.1	53.5	64	57.87±4.95
Forkedlength	54.03	47.57	51.1	48.5	58.1	51.86±4.30
Standard length	47.02	42.32	45.5	45.1	53.8	46.75±4.29
Head length	12.39	11.35	12.1	10.2	12.80	11.77±1.02
Body depth	11.84	10.67	11.20	10.68	11.97	11.27±0.62
Eeyediameter	4.24	3.54	3.70	3.50	4.30	3.86±0.39
Dorsal-fin height	10.55	9.72	11.2	9.9	10.87	10.45±0.63
Orsal-fin base length	4.50	3.83	6.1	4.9	4.64	4.79±0.83
Pectoral-fin lenght	9.89	5.5	8.1	9.5	9.90	8.58±1.87
Pectoral fin height	5.28	4.2	4.5	5.9	5.30	5.04±0.68
Pectoral-fin base length	2.39	2.1	3.1	1.9	2.42	2.38±0.46
Anal-fin height	8.30	-	9.5	8.5	8.35	8.66±0.56
Anal-fin base length	5.62	-	5.1	5.1	5.65	5.37±0.31

Biotechnology Information (NCBI), which is one of the main public repositories for DNA barcode sequences, in order to find highly similar sequences. Then, the sequence was deposited in GenBank with under the accession number PQ505104. The COI barcoding sequence and additional sequences obtained from GenBank were then aligned with Clustal Was implemented in MEGA 11 (Tamura et al. 2021).The two-parameter Kimura model (K2P) (Kimura 1980) was applied using the Molecular Evolutionary Genetic Analysis (MEGA 11) software (Tamura et al. 2021) to compare the sequence from specimen collected in Wadi El Mellah (Algeria) with two sequences of *Tropidophoxinellus chaignoni* from Tunisia, available on GenBank. The cladogram of the phylogenetic relationships of the identified *Tropidophoxinellus chaignoni* haplotype from Algeria, along with two other haplotypes of the species from Tunisia, was constructed using the Maximum Likelihood (ML) method with MEGA11 (Tamura et al. 2021).

RESULTS

The five sampled specimens of *Tropidophoxinellus chaignoni* from Wadi El Mellah (Algeria) have an average total length of 57.87mm (Fig. 3) and an average standard length of 46.75mm (Table 1). Other morphological measurements are provided in Table 1.

The DNA sequence obtained for the *Tropidophoxinellus chaignoni* population in Algeria (accession number: PQ505104) showed similarities of 99.03% and 97.06% with the sequences of *Tropidophoxinellus chaignoni* collected in Tunisia (accession numbers: HM560302 and HM560301, respectively). Genetic distances among pairs of the

Table 2. Genetic distances between and within haplotypes of eight *Tropidophoxinellus* spp. species and obtained haplotype from *Tropidophoxinellus chaignoni* in this study (PQ505104).

	Species	1	2	3	4	5	6	7	8
1	*Tropidophoxinellus chaignoni* PQ505104 (This study)	n/c							
2	*Tropidophoxinellus chaignoni* HM560302	0.007							
3	*Tropidophoxinellus chaignoni* HM560301	0.028	0.026						
4	*Tropidophoxinellus callensis* KJ554910	0.091	0.092	0.079					
5	*Tropidophoxinellus callensis* KJ554899	0.091	0.092	0.079	0.000				
6	*Tropidophoxinellus hellenicus* MG806909	0.094	0.095	0.092	0.075	0.075			
7	*Tropidophoxinellus hellenicus* MG806908	0.093	0.092	0.088	0.084	0.084	0.031		
8	*Tropidophoxinellus spartiaticus* HM560381	0.093	0.91	0.088	0.077	0.077	0.00	0.031	
9	*Tropidophoxinellus spartiaticus* KJ554869	0.093	0.094	0.091	0.075	0.075	0.00	0.031	0.000

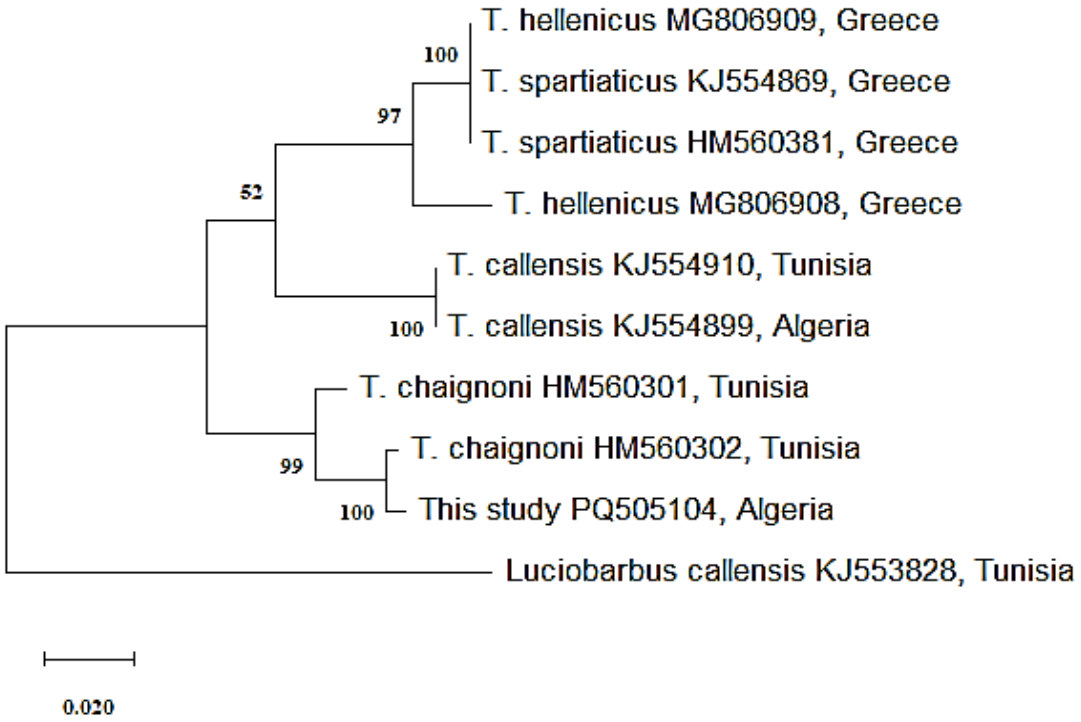

Fig.4. Maximum likelihood analysis based on the mitochondrial cytochrome c oxidase subunit I (COI) sequences of *Tropidophoxinellus chaignoni* from Wadi El Mellah in Algeria.

nine *Tropidophoxinellus* spp. haplotypes ranged from 0.007 to 0.095, while distances among the three *Tropidophoxinellus chaignoni* haplotypes ranged from 0.007 to 0.028 (Table 2). Excluding the outgroup clade (*Luciobarbus callensis*), two distinct clades are observed within the *Tropidophoxinellus* genus (Fig. 4). The three *Tropidophoxinellus chaignoni* haplotypes form one clade, with the identified haplotype (PQ505104) clustering more closely with the haplotype (HM560302) from Tunisia, forming a monophyletic subclade, compared to the haplotype (HM560301).

DISCUSSION

The discovery of *Tropidophoxinellus chaignoni* in Algeria indicates that its geographical distribution is more extensive than previously thought, surpassing the known historical boundaries, which were restricted to the rivers of northeastern Tunisia (Freyhof & Ford 2022). Previous ichthyological

surveys conducted by Playfair & Letourneux (1871) in the High Plateaux region of Algeria recorded the presence of a *Tropidophoxinellus callensis* population in Wadi El Mellah at M'Sila. However, the accurate identification of this species may now be reassessed and considered as *Tropidophoxinellus chaignoni*. In fact, the distribution of this species in Algeria could extend from Constantine, through M'Sila and Biskra, all the way to the Tunisian borders at Tébessa (Playfair & Letourneux 1871). It would be relevant to update the distribution map and reassess the conservation status of the species according to IUCN criteria, considering these new data.

The *Tropidophoxinellus chaignoni*from Wadi El Mellah exhibits a K2P genetic distance of under 3% when compared to specimens from Abid River in Tunisia (Perea 2010; Geiger et al. 2014), clearly indicating that they may be conspecific based on their COI sequences across the studied populations. Furthermore, the morphological measurements of the specimens analyzed in Wadi El Mellah correspond to those of *Tropidophoxinellus chaignoni* found in Tunisia. These morphological features are consistent with the range of values observed in related *Tropidophoxinellus chaignoni* populations in Tunisia, where Kraiem (1983) notes that the species can reach a size of up to 100mm.

The ca. upper 99% genetic similarity between the *Tropidophoxinellus* spp. from the High Plateaux in Algeria and *Tropidophoxinellus chaignoni* from Tunisia further supports their morphological similarity (Kraiem 1983; Marfoua et al. 2017).These similarities also support previous hypotheses about the presence of *Tropidophoxinellus chaignoni* in this region of Algeria (Playfair & Letourneux 1871), emphasizing the geographical link between these areas. Furthermore, the biogeographical similarities between southern Tunisia and the High Plateaux of Algeria may influence this genetic clustering.

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REFERENCES

Amalou, D. &Moulai, R. 2020. The fish fauna of the inland waters of the lower Kabylie (Bejaia, Northern Algeria): Diversity and spatial distribution. StudiaUniversitatisVasileGoldisSeriaStiin teleVietii (Life Sciences Series) 30(2):76-87.

Bacha, M. & Amara, R. 2007. Les poissons des eaux continentales d'Algérie. Étude de l'ichtyofaune de la Soummam. Cybium 31(3): 351-358.

Baikeche, L.; Lounaci, A.; Ford, M.; Lounaci-Daoudi, D. & Freyhof, J. 2021. Freshwater fishes of West Kabylia, Algeria. Biodiversity Journal 12(4): 957-966.

Doadrio, I. 1994. Freshwater fish fauna of North Africa and its biogeography. Musée Royal d'Afrique Centrale 8: 21-34.

Ford, M.; Brahimi, A.; Baikeche, L.; Bergner, L.; Clavero, M.; Doadrio, I.; Lopes-Lima, M.; Perea, S.; Yahyaoui, A. &Freyhof, J. 2020. Freshwater fish distribution in the Maghreb: a call to contribute. OSF Preprints. January 9.

Freyhof, J. & Ford, M. 2022. *Tropidophoxinellus chaignoni*. The IUCN Red List of Threatened Species 2022: e.T144643181A144655098. https://dx.doi.org/10.2305/IUCN.UK.2022-1.RLTS.T144643181A144655098.en

Geiger, M.F.; Herder, F.; Monaghan, M.T.; Almada, V.; Barbieri, R.; Bariche, M.; Berrebi, P.; Bohlen, J.;Casal-Lopez, M.; Delmastro, G.B.; Denys, G.P.;Dettai, A.; Doadrio, I.;Kalogianni, E.; Karst, H.; Kottelat, M.; Kovacic, M.; Laporte, M.; Lorenzoni, M.; Marcic, Z.; Ozulug, M.; Perdices, A.; Perea, S.; Persat, H.; Porcelotti, S.; Puzzi, C.; Robalo, J.; Sanda, R.; Schneider, M.; Slechtova, V.; Stoumboudi, M.; Walter, S. & Freyhof, J. 2014. Spatial heterogeneity in the Mediterranean Biodiversity Hotspotaffects barcoding accuracy of its freshwater fishes. Molecular Ecology Resources 14(6): 1210-1221.

Kara, H.M. 2012. Freshwater fish diversity in Algeria with emphasis on alien species. European Journal of Wild Life Research 58(1): 243-253.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111-120.

Kraiem, M.M. 1983. Les poissons d'eau douce de Tunisie: inventaire commenté et répartition géographique. Bull. Inst. Nain. Scient. Tech. Oceanogr. Pêche Salammbô 10:107-124.

Marfoua, M.; Souffi, I.; Chaibi, R. & Sibachir, A. 2017. Caractérisation biologique et écologique d'un poisson d'eau douce (genre *Pseudophoxinus*) dans l'Est Algérien. Journal Algérien des Régions Arides 12(2): 84-92.

Pellegrin, J. 1921. Les poissons des eaux douces de l'Afrique du Nord française. Maroc, Algérie, Tunisie, Sahara. Mémoire de la Société des Sciences Naturelles du Maroc 1, 2: 1-216.

Perea, S.; Böhme, M.; Zupančič, P.; Freyhof, J.; Šanda, R.; Özuluğ, M.; Abdoli, A. & Doadrio, I. 2010. Phylogenetic relationships and biogeographical patterns in Circum-Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. BMC Evolutionary Biology 10: 1-27.

Playfair, R.L. & Letourneux, M. 1871. XLVII.-Memoir on the hydrographical system and the freshwater fish of Algeria. Annals and Magazine of Natural History Series 4, 8, 48: 373-394.

Salhi, S.; Chaibi, R.; Badache, H.; Hamidouche, M. & Laouar, R. 2021. Seasonal variation in the diet and the morphometric parameters of the genus *Pseudophoxinus* sp. (Cyprinidae) in Eastern Algeria. Biosystems Diversity 29(4): 326-333.

Smith, K.G. & Darwall, W.R. 2006. The status and distribution of freshwater fish endemic to the Mediterranean Basin (Vol. 1). IUCN.

Tamura, K.; Stecher, G. & Kumar, S. 2021. MEGA11: moleculare volutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022-3027.

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مقاله کامل

اولین گزارش *Tropidophoxinellus chaignoni* (Vaillant 1904) از الجزایر (ماهیان استخوانی عالی: کپورماهیان)

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چکیده: *Tropidophoxinellus chaignoni* یک گونه بومی شمال شرقی تونس، اخیراً در وادی الملاه واقع در جنوب M'Sila، الجزایر کشف شده است. نمونه‌های ماهی در ۹ آگوست ۲۰۲۳ از وادی المله در الجزایر با استفاده از یک تور دستی جمع‌آوری شد. فواصل جفت ژنتیکی بین هاپلوتیپ‌های *Tropidophoxinellus chaignoni* از ۰/۰۰۷ تا ۰/۰۲۸ متغیر بود. درخت فیلوژنتیک نشان داد که هاپلوتیپ شناسایی شده در الجزایر متعلق به کلاد *Tropidophoxinellus chaignoni* است و با گونه هاپلوتیپ از رودخانه ابید در تونس یک زیرشاخه مونوفیلتیک تشکیل می‌دهد. این نشان‌دهنده اولین مشاهده حضور تأیید شده این گونه در الجزایر است و خلاء موجود در تحقیقات در مورد ماهیان سیستم‌های آب شیرین این کشور را مشخص می‌کند. در این مطالعه، ما برای اولین بار حضور *Tropidophoxinellus chaignoni* در الجزایر را مستندسازی می‌کنیم. توالی‌های ژن سیتوکروم اکسیداز I (COI) DNA میتوکندریایی به‌عنوان بارکد DNA، شناسایی آن را تأیید می‌کند.

کلمات‌کلیدی: بارکد DNA، *Tropidophoxinellus chaignoni*، اولین گزارش، الجزایر