Scientific paper

Barbatula leoparda (Actinopterygii, Nemacheilidae), a new endemic species of stone loach of French Catalonia

by

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Abstract. – This study described a new stone loach species in France, *Barbatula leoparda*, which is endemic to French Catalonia (Têt and Tech river drainages). Seven specimens were compared to 49 specimens of *B. barbatula* (Linnaeus, 1758) and 71 specimens of *B. quignardi* (Băcescu-Meşter, 1967). This new species is characterized by the presence of blotches on the belly and the jugular area in individuals longer than 47 mm SL and by a greater interorbital distance (35.5 to 41.8% of the head length). We brought moreover the sequence of two mitochondrial markers (COI and 12S, respectively 652 and 950 bp) of the holotype, which are well distinct from all other species, for molecular identifications. This discovery is important for conservation.

Résumé. – *Barbatula leoparda* (Actinopterigii, Nemacheilidae), une nouvelle espèce endémique de loche franche en Catalogne française.

Cette étude décrit une nouvelle espèce de loche franche en France, *Barbatula leoparda*, qui est endémique à la Catalogne française (bassins de la Têt et du Tech). Sept spécimens ont été comparés à 49 spécimens de *B. barbatula* (Linnaeus, 1758) et 71 spécimens de *B. quignardi* (Băcescu-Meşter, 1967). Cette nouvelle espèce est caractérisée par la présence de taches sur le ventre et dans la partie jugulaire pour les individus d'une taille supérieure à 47 mm LS et par une plus grande distance inter-orbitaire (35,5 to 41,8% de la longueur de la tête). Pour les identifications moléculaires, nous décrivons également la séquence de deux marqueurs mitochondriaux (COI et 12S, respectivement 652 et 950 pb) pour l'holotype, qui sont bien distincts des autres espèces. Cette découverte est importante pour la conservation.

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Key words
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INTRODUCTION

The biogeographical context of French Catalonia stands out among the French drainages. It is composed by three main coastal rivers (Agly, Têt and Tech) formed during the second part of the Miocene and particularly with the relief accentuation of the Pliocene and Quaternary, which has isolated them from the Segre catchment (see Persat and Keith, 2011). The rivers Têt and Tech both start at high altitudes (> 2000 m) in the Eastern Pyrenees and flow eastwards over 115 and 84 km, respectively, to the Mediterranean Sea close to the city of Perpignan. They were isolated from other French southern catchments like Garonne, and might be linked to Spanish Catalonian and Western French Mediterranean catchments in the downstream part during the last glaciations (Persat and Keith, 2011).

For these reasons, the ichthyofauna of these rivers is peculiar: the most common fish species in the Têt and Tech rivers are *Gobio occitaniae* Kottelat & Persat, 2005, *Phoxinus septimaniae* Kottelat, 2007, *Barbus meridionalis* Risso, 1827 and *Squalius laietanus* Doadrio *et al*., 2007 (Kottelat and Freyhof, 2007; Keith *et al*., 2011; Denys *et al*., 2013; Arsento *et al*., 2018). In higher altitude (> 100 m), stone loaches identified as *Barbatula quignardi* (Băcescu-Meşter, 1967) (Kottelat and Freyhof, 2007) are usually found in sympatry with *Salmo trutta* Linnaeus, 1758 (Blanc and Beaudou, 1998). *Gobio occitaniae, P. septimaniae* and *S. laietanus* were just recently described from French Catalonia (Kottelat and Persat, 2005; Kottelat, 2007; Doadrio *et al*., 2007).

The loaches of the rivers Têt and Tech were already identified as *B. quignardi*, a species known from the Lez River in southern France near Montpellier, but they also had

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been classified as *B. barbatula* (Linnaeus, 1758), which is believed to be widespread all over Europe (Kottelat, 1997) from North East of Spain to Northern Caucasus (Keith *et al.*, 2011). Kottelat and Freyhof (2007) revalidated *B. quignardi*, and extended its distribution from the Lez River to the whole South-West France (Garonne-Adour catchments) and French Mediterranean drainages up to North-Eastern Spain. However, the stone loaches have a settled behavior in the rivers, as they are not good at swimming long distances (Smyly, 1955). Thus, the populations of the rivers Têt and Tech could really be isolated and not the result of a recent dispersion of populations from other Mediterranean catchments.

Here we present the results of the morphological analysis including specimens from the rivers Têt and Tech as well as the two other species, *B. quignardi* and *B. barbatula*. The aim of this study is to analyse the stone loach populations of the rivers Têt and Tech, providing morphological and molecular data for a comparison with other French *Barbatula* species.

MATERIAL AND METHODS

Abbreviations used: Associations Agréées de Pêche et de Protection des Milieux Aquatiques, France (AAPPMA); Fédération Départementale des Associations Agréées de Pêche et de Protection des Milieux Aquatiques, France (FDAAPPMA); Muséum national d'Histoire naturelle, Paris, France (MNHN), Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); Muzeul Național de Istorie Naturală Grigore Antipa, Bucharest, Romania (MGAB); Office National de l'Eau et des Milieux Aquatiques, France (ONEMA); Claude Bernard Lyon 1 University, Villeurbanne, France (UCBLZ).

Samples were collected by electrofishing between 2010 and 2016 with the collaboration of the ONEMA. Specimens were fixed and preserved in 95% EtOH by using progressive concentration of EtOH over a few hours in order to lower the body shrivelling induced by osmotic shock.

A total of 128 specimens from 44 French and Spanish locations were examined. Counts and measurements were taken from the left side following Kottelat and Freyhof (2007). Measurements were taken using an electronic caliper. All measurements were made point to point, never by projection. Kováč *et al.* (1999) noticed in their study two steps of allometric growth with the transformation of the head (26-35 mm SL), and the fin shape and size as well as the body form (36-47 mm SL). Then, only the individuals longer than 35 mm SL were measured. For smaller ones (9 specimens), only meristic data were counted. Finally, we analysed separately the measurements for specimens shorter than 47 mm SL (27 specimens) called sub-adults in this study, and longer ones (92 specimens) called adults. The last dorsal and anal fin rays were not counted because they are connected to the previous one. Sex was determined observing the second branched pectoral-fin ray, which is longer than the third pectoral-fin in males (Băcescu-Meşter, 1967).

According to Prokofiev (2010, 2015), external characters were also observed such as the spacing between the anterior and posterior nostrils, the medial incision of the upper lip and the mental lobes of the lower lip, in addition to the external coloration of the body.

Thus, a total of 26 morphological characters were observed: standard length (SL), head length (HL), predorsal fin length, postdorsal fin length, prepectoral fin length, prepelvic fin length, preanal fin length, caudal-peduncle length, caudal-peduncle depth, body depth, pectoral fin length, pelvic fin length, dorsal fin length, anal fin length, snout length, eye diameter, postorbital length, interorbital width, dorsal fin rays, pectoral fin rays, pelvic fin rays, anal fins rays, spacing between the anterior and posterior nostrils, medial incision of the upper lip, mental lobes of the lower lip and external coloration of the body.

Two mitochondrial markers common for molecular identification were used for molecular description: the cytochrome oxidase subunit 1 (COI) for DNA barcoding *sensu* Hebert *et al.* (2003) and the 12S rDNA for environmental DNA (*e.g.* Valentini *et al.*, 2016). Complete 12S rDNA and the Folmer barcode region (start to position 650) of the COI were obtained for the type specimen from a fin-clip stored in ethanol. DNA was extracted following a chloroform isoamyl alcohol protocol from Winnepenninckx *et al.* (1993). Amplification and NGS sequencing followed Hinsinger *et al.* (2015). The reads were assembled using Geneious 10.2.2 (Kearse *et al.*, 2012) as in Hinsinger *et al.* (2015). The consensus was controlled, and using BLAST similarity search on the complete NCBI nucleotide (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and BOLDsystems (http://www.boldsystems.org/) databases. The sequences were labelled according to the nomenclature of Chakrabarty *et al.* (2013) and deposited in the BOLD (accession FFFR1019-16) and NCBI nucleotide database (accession MK518369 and MK518372).

Comparative material

Barbatula barbatula (Linnaeus, 1758)

Loire drainage: MNHN 2010-0991, 2, 61.7-72.9 mm SL ♂, France: Dept. Puy-de-Dôme: Ance du Nord at Sauvessanges: N 45.385 E 3.894, 13 Sep. 2010, Denys and ONEMA coll.; MNHN 2011-0822, 3, 33.3-54.1 mm SL, France: Dept. Haute-Loire: Allier at Saint-Haon: N 44.839 E 3.737, 7 Sep. 2011, Denys and ONEMA coll. Meuse drainage: MNHN 2018-0229, 5, 60.9-74.3 mm SL, France: Dept. Ardennes: Houille at Givet: N 50.129 E 4.840, 16 Jul. 2013, Persat coll. Rhine drainage: MNHN 2016-0055, 1, 48 mm SL, France: Dept. Bas-Rhin: Schaftheu at Sundhouse: N 48.253 E 7.675, 3 Sep. 2013, Denys and ONEMA coll.; Rhône drainage: MNHN 2013-0826, 4, 65.8-72.9 mm SL, France:

Dept. Jura: Clauge at La Loye: N 47.051 E 5.589, 1 Jul. 2013, Denys and ONEMA coll.; MNHN 2014-2806, 2, 49.7-55.4 mm SL, France: Dept. Gard: Luech at Chamborigaud: N 44.328 E 3.959 , 3 Jun. 2014, Denys and ONEMA coll.; UCBLZ 2012.9.298, 7, 30.4-48.2 mm SL, France: Dept. Vaucluse: Toulourenc at Veaux: N 44.215 E 5.214, 3 Nov. 2004, Persat coll.; UCBLZ 2012.9.309, 2, 34.2-37.8 mm SL, France: Dept. Hautes-Alpes: Buech at Aspremont: N 44.491 E 5.728, 3 Nov. 2004, Persat coll.; UCBLZ 2012.9.310, 7, 57.6-72.5 mm SL, France: Dept. Haute-Savoie: Fornant at Frangy: N 46.017 E 5.943, 14 Jul. 2009, Persat coll.; UCBLZ 2012.9.345, 2, 77.9-79.1 mm SL, France: Dept. Savoie: Gelon at Villard-Mougin: N 45.507 E 6.170, 17 Dec. 2003, Persat coll.; UCBLZ 2012.9.347, 1, 79 mm SL, France: Dept. Saône-et-Loire: Grosne at Brandon: N 46.351 E 4.569, 14 Oct. 2003, Persat coll.; Scheldt drainage: MNHN 2016-0350, 1, 47.8 mm SL, France: Dept. Nord: Lys at Erquinghem-Lys: N 50.678 E 2.840, 20 Sep. 2016, Denys and ONEMA coll.; Seine-Normandy drainage: MNHN 2011-0267, 6, 45-68.5 mm SL, France: Dept. Manche: Saires at Brillevast: N 49.611 W 1.406, 30 Jun. 2011, Denys and ONEMA coll.; MNHN 2011-1142, 1, 41.6 mm SL, France: Dept. Eure: Epte at Guerny: N 49.218 E 1.688, 20 Oct. 2011, Denys and ONEMA coll.; MNHN 2014-2811, 3, 68-81.5 mm SL, France: Dept. Nièvre: Beuvron at Ouagne: N 47.400 E 3.495, 24 Jun. 2014, Denys and ONEMA coll.; MNHN 2016-0348, 2, 46.9-63.3 mm SL, France: Dept. Aube: Seine at Marnay-sur-Seine: N 48.515 E 3.559, 16 Sep. 2016, Denys and ONEMA coll.

Barbatula quignardi (Băcescu-Meşter, 1967)

Lez drainage: MGAB77, holotype, 52 mm SL ♂, France: Dept. Hérault: Lez near from Montpellier, Jul. 1962, Băcescu coll.; MGAB78, allotype, 56.5 mm SL ♀, collected with holotype; MNHN 2010-1064, 6, 31.2-41.7 mm SL, France: Dept. Hérault: Lez at Prades-le-Lez: N 43.700 E 3.856, 24 Nov. 2010, Denys and ONEMA coll.; Adour drainage: MNHN 2010-0483, 3, 47.9-52.3 mm SL, France: Dept. Hautes-Pyrénées: Echez at Larreule: N 43.445 E 0.029, 23 Jun. 2010, Persat, Denys and Delacoste coll.; MNHN 2010-0488, 1, 55.2 mm SL, France: Dept. Hautes-Pyrénées: Adour at Tarbes: N 43.231 E 0.089, 23 Jun. 2010, Persat, Denys and Delacoste coll.; MNHN 2010-1034, 9, 55-66 mm SL, France: Dept. Pyrénées-Atlantique: Saison at Menditte: N 43.163 W 0.896, 8 Oct. 2010, Denys and ONEMA coll.; MNHN 2016-0067, 2, 41.2-42.8 mm SL, France: Dept. Hautes-Pyrénées: Adour at Estirac: N 43.503 E 0.030, 29 Aug. 2013, Denys and ONEMA coll.; Agly drainage: MNHN 2013-0667, 4, 38.3-62 mm SL, France: Dept. Aude: Torgan at Padern: N 42.870 E 2.656, 13 May 2013, Persat and Denys coll.; Artibai drainage: MNCN 279188-279189, 1, 70.5 mm SL, Spain: Vizcaya: Artibai at Amalloa: N 43.283 W 2.450, 12 Oct. 2009, Doadrio, Garzon, Pedraza and Ornelas coll.; Bidassoa drainage: MNCN 279064-279125, 3, 45.8-66.2 mm SL, Spain: Prov. Navarra: Bidassoa at Arrayoz: N 43.141 W 1.567, 12 Oct. 2009, Doadrio, Garzon, Pedraza and Ornelas coll.; Butron drainage: MNCN 279280-279297, 3, 39.4-59.5 mm SL, Spain: Prov. Vizcaya: Butroe at Fruiz: N 43.327 W 2.786, 13 Oct. 2009, Doadrio, Garzon, Pedraza and Ornelas coll.; Ebre drainage: MNCN 281596-281602, 3, 46.2-65.7 mm SL, Spain: Prov. Aragon: Aragon at Jaca: N 42.567 W 0.550, 3 Oct. 2009, Doadrio, Garzai, Solis and Polo coll.; MNCN 281832-281840, 3, 39.4-56.5 mm SL, Spain: Prov. La Rioja: Cidacos at Arnedo: N 42.228 W 2.100, 4 Oct. 2009, Doadrio, Garzai, Solis and Polo coll.; MNCN 275902-275919, 3, 60.2-69.2 mm SL, Spain: Prov. La Rioja: Najerilla at Uruñuela: N 42.442 W 2.707, 5 Oct. 2009, Doadrio, Garzai, Solis and Polo coll.; MNCN 276098-276119, 3, 60.5-67.6 mm SL, Spain: Prov. La Rioja: Tirón at Herramelluri: N 42.502 W 3.020, 5 Oct. 2009, Doadrio, Garzai, Solis and Polo coll.; MNCN 283034-283037, 3, 45.5-49.2 mm SL, Spain: Prov. Alava: Zadorra at Nanclares de la Oca: N 42.819 W 2.813, 19 Oct. 2009, Gonzalez, Prieto, Pedraza and Ornelas coll.; MNCN AT4364, 3, 47.9-61.6 mm SL, Spain: Prov. Teruel: Matarraña at Valderrobles: N 40.717 W 0.150, 23 Oct. 2009, Doadrio, Garzón and González coll.; Garonne drainage: MNHN 2010-0492, 2, 46.8-60.9 mm SL, France: Dept. Ariège: Douctouyre at Dun: N 43.038 E 1.793, 24 Jun. 2010, Persat and Denys coll.; MNHN 2010-0505, 2, 77.2-77.4 mm SL, France: Dept. Ariège: Salat at Rivèrenert: N 42.856 E 1.177, 24 Jun. 2010, Persat and Denys coll.; MNHN 2011-0279, 1, 62.7 mm SL, France: Dept. Haute-Garonne: Pique at Cierp-Gaud: N 42.912 E 0.638, 5 Jul. 2011, Denys and ONEMA coll.; MNHN 2014-0052, 2, 56.1-60.9 mm SL, France: Dept. Lot: Dordogne at Carennac: N 44.921 E 1.730, 27 Aug. 2013, Denys and ONEMA coll.; MNHN 2014-2814, 3, 67.8-77.6 mm SL, France: Dept. Gironde: Ciron at Escaudes: N 44.320 W 0.190, 2 Jun. 2014, Denys and ONEMA coll.; Leyre drainage: MNHN 2013-0829, 2, 54.1-55.7 mm SL, France: Dept. Gironde: Grande Leyre at Bélin-Béliet: N 44.474 W 0.795, 8 Jul. 2013, Denys and ONEMA coll.; Nivelle drainage: MNCN 278716-278735, 3, 57.2-70.9 mm SL, Spain: Prov. Navarra: Ugarana at Dantxarinea: N 43.291 W 1.506, 11 Oct. 2009, Doadrio, Garzon, Pedraza and Ornelas coll.; Oka drainage: MNCN 279227-279231, 3, 39.7-60.5 mm SL, Spain: Prov. Vizcaya: Oka at Mugica: N 43.321 W 3.126, 12 Oct. 2009, Doadrio, Garzon, Pedraza and Ornelas coll.; Ter drainage: MNCN 275355, 1, 46.2 mm SL, Spain: Prov. Girona: Ter at Ripoll: N 42.201 E 2.190, 4 Oct. 2009, Gonzalez, Cunha and Cuerva coll.

Barbatula leoparda n. sp.

(Figs 1-3; Tab. I)

Material examined

Holotype. – MNHN 2018-0228, 62.6 mm SL ♀, France: Dept. Pyrénées-Orientales: Têt River at Nefiach: N 42.697 E 2.663, 7 Sep. 2016, Denys and ONEMA coll.;

Paratypes. – MNHN 2016-0347, 4, 32.4 mm SL ♂, 36.84 mm SL ♂, 44.5 mm SL ♂, 61 mm SL ♂, collected with holotype; MNHN 2010-0997, 1, 48.2 mm SL ♂, France: Dept. Pyrénées-Orientales: Tech River at Elne: N 42.584 E 2.970, 14 Sep. 2010, Denys and ONEMA coll.; MNHN

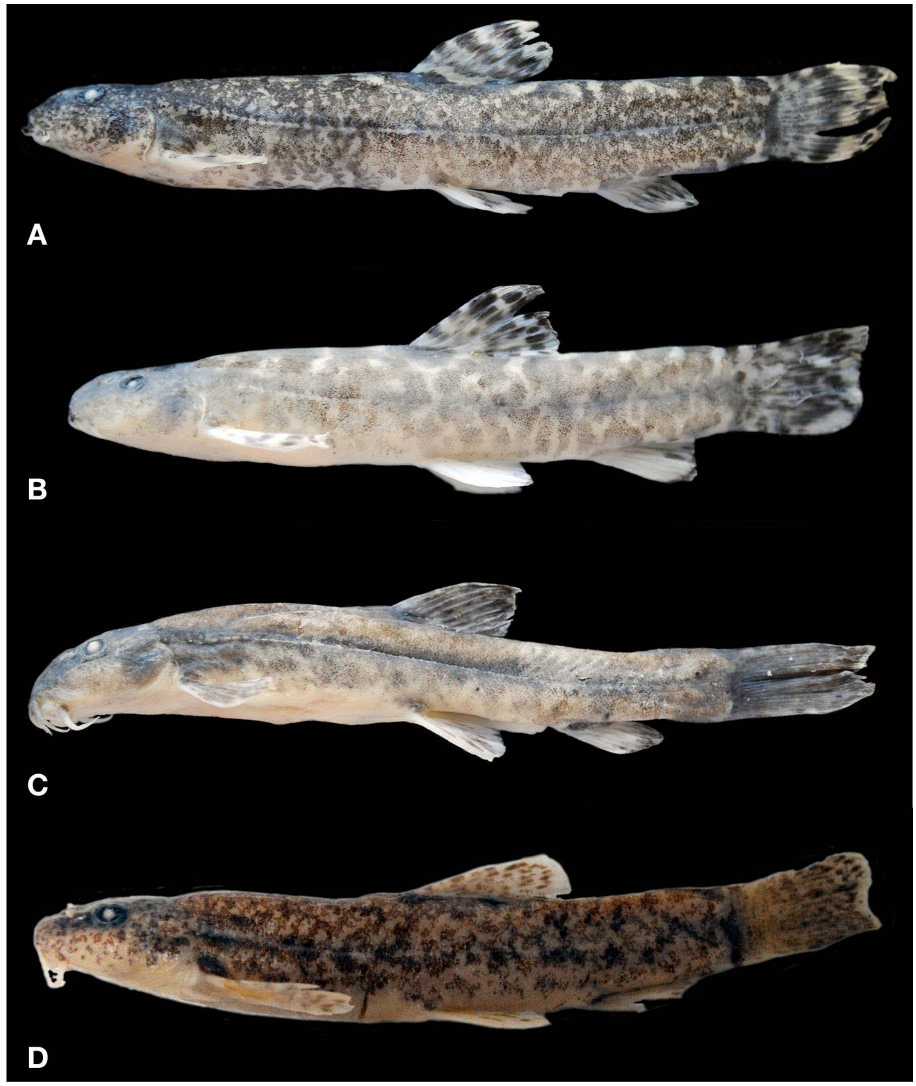

Figure 1. – Specimens of *Barbatula* spp. in lateral view. **A**: Holotype of *Barbatula leoparda* MNHN 2018-0228, 62.6 mm SL ♀, Têt River at Nefiach, 7 Sep. 2016, Denys and ONEMA coll.; **B**: Paratype of *Barbatula leoparda* n. sp. MNHN 2016-0351, 46.5 mm SL ♀, Tech River at Céret, 9 Sep. 2016, Denys, Hautecœur and ONEMA coll; **C**: *Barbatula barbatula* MNHN 2013-0826, 72.9 mm SL ♂, stream Clauge (Rhône drainage) at La Loye, 1 Jul. 2013, Denys and ONEMA coll.; **D**: Holotype of *Barbatula quignardi* MGAB77, 52 mm SL ♂, Lez River, Jul. 1962, Băcescu coll.

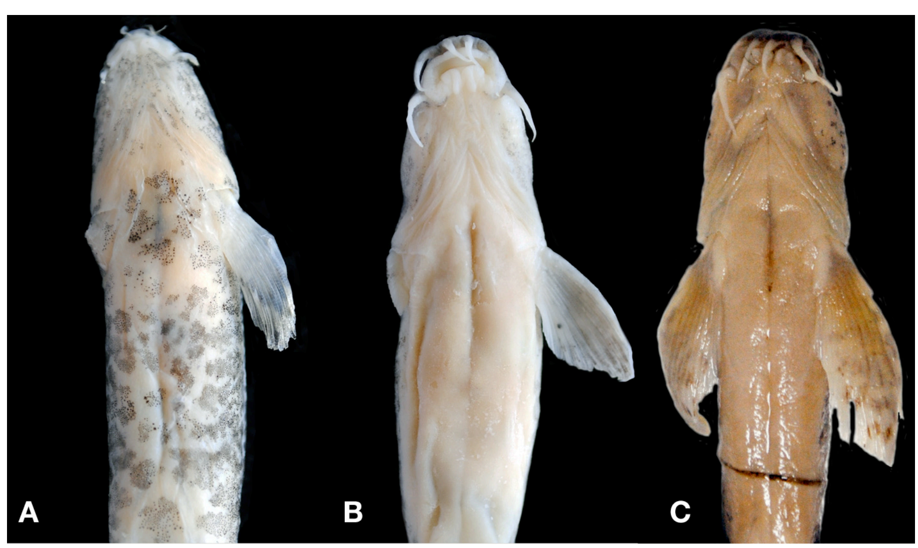

Figure 2. – Specimens of *Barbatula* spp. in ventral view. **A**: Holotype of *Barbatula leoparda* MNHN 2018-0228, 62.6 mm SL ♀, Têt River at Nefiach, 7 Sep. 2016, Denys and ONEMA coll.; **B**: *Barbatula barbatula* MNHN 2013-0826, 72.9 mm SL ♂, stream Clauge (Rhône drainage) at La Loye, 1 Jul. 2013, Denys and ONEMA coll.; **C**: Holotype of *Barbatula quignardi* MGAB77, 52 mm SL ♂, Lez River, Jul. 1962, Băcescu coll.

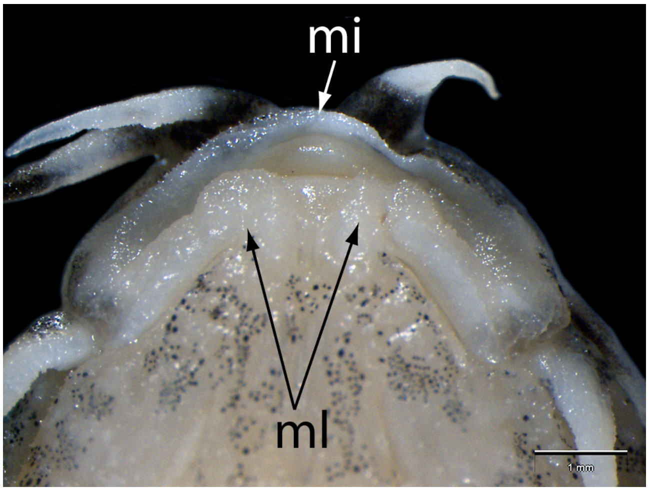

Figure 3. – Mouth of holotype of *Barbatula leoparda* MNHN 2018-0228, 62.6 mm SL ♀, Têt River at Nefiach, 7 Sep. 2016, Denys and ONEMA coll. Abbreviations: medial indentation of upper lip (mi), mental lobe of lower lip (ml). Scale bar = 1 mm.

2016-0351, 1, 46.5 mm SL ♀, Tech River at Céret: N 42.496 E 2.755, 9 Sep. 2016, Denys, Hautecœur and ONEMA coll.

Material for molecular references

Holotype. – MNHN 2018-0228, France: Dept. Pyrénées-Orientales: Têt River at Nefiach: N 42.697 E 2.663, genseq-1 COI (GenBank accession MK518369, 650 bp, average coverage of 218, minimum coverage 164)), genseq-1 complete 12S rDNA (GenBank accession MK518372, 950 bp, average coverage of 305, minimum coverage 93)).

Diagnosis

Barbatula leoparda larger than 47 mm SL is distinguishable from *B. barbatula* of the same size by the presence of dark-brown blotches on the belly and the jugular area (*vs.* absence of pigmentation) (Fig. 2), a larger interorbital width (35.6 to 41.8% HL, *vs.* 23.0 to 38.1% HL; Fig. 4A), a deeper caudal peduncle (12.1 to 13.0% SL, *vs.* 8.0 to 12.4% SL; Fig. 4C), and a fewer ratio length/depth of the caudal peduncle (1.30 to 1.48, *vs.* 1.38 to 2.56; Fig. 4D). Sub-adult specimens (36 to 47 mm SL) are diagnosable by a larger interorbital width (35.5 to 40.5% HL, *vs.* 28.7 to 38.4% HL; Fig. 4B), a fewer ratio length/depth of the caudal peduncle (1.41 to 1.45, *vs.* 1.45 to 2.15; Fig. 4E) and longer pelvic fins (15.8 to 16.5% SL, *vs.* 12.4 to 15.5% SL; Fig. 4G).

Barbatula leoparda larger than 47 mm SL is distinguishable from *B. quignardi* of the same size by the presence of dark-brown blotches on the belly and the jugular area (*vs.* absence of pigmentation) (Fig. 2), a larger interorbital width (35.6 to 41.8% HL, *vs.* 21.6 to 36.0% HL; Fig. 4A) and a shorter dorsal-fin length (17.1 to 20.1% SL, *vs.* 18.9 to 23.9% HL; Fig. 4F). Subadult specimens are diagnosable

Table I. – Morphometry of *Barbatula leoparda*, *B. barbatula* and *B. quignardi* for the two sizes classes (36 to 47 mm SL and > 47 mm SL). Values in parentheses: mean. Values of holotype are included in range. Bold mean values highlight morphometric differences (see Fig. 4).

	Barbatula leoparda n. sp.			*Barbatula barbatula*		*Barbatula quignardi*	
	36-47 mm SL	> 47 mm SL	Holoype	36-47 mm SL	> 47 mm SL	36-47 mm SL	> 47 mm SL
N	3	3		8	37	15	52
Standard length SL (mm)	36.6-46.5 (42.6)	48.2-62.6 (57.3)	62.6	37.8-46.9 (41.5)	47.1-81.5 (63.6)	36.4-46.8 (42.4)	47.8-77.6 (59.8)
In percent of SL							
Head length HL	22.8-23.4 (23.1)	22.1-23.4 (22.7)	22.1	21.7-25.0 (23.8)	20.6-24.9 (22.7)	22.4-26.7 (24.5)	19.8-26.5 (23.2)
Predorsal length	50.4-53.7 (52.3)	50.5-53.8 (52.2)	52.3	51.4-54.4 (53.3)	50.6-55.8 (52.7)	49.5-56.4 (52.9)	48.7-56.0 (52.5)
Postdorsal length	31.7-37.7 (35.6)	34.1-38.6 (35.6)	34.1	33.0-38.1 (36.5)	33.9-42.8 (38.1)	32.8-39.4 (36.1)	32.7-39.6 (36.5)
Prepectoral length	22.7-26.4 (24.4)	22.8-24.8 (23.6)	22.8	23.7-28.0 (25.7)	19.7-27.6 (23.3)	21.1-29.1 (24.9)	20.4-26.4 (23.8)
Prepelvic length	53.4-56.2 (55.2)	51.1-56.1 (53.7)	56.1	51.9-58.0 (54.9)	49.1-61.6 (53.5)	51.9-57.8 (54.3)	50.9-56.7 (53.9)
Preanal length	76.3-80.3 (78.5)	74.0-79.5 (76.7)	76.8	74.1-79.6 (76.7)	74.1-80.1 (76.1)	75.4-83.1 (77.8)	74.7-79.6 (77.1)
Caudal peduncle length CPL	16.6-17.9 (17.2)	17.0-17.8 (17.3)	17.0	15.0-20.2 (17.5)	15.0-20.6 (17.7)	13.1-20.4 (16.7)	14.2-19.7 (17.3)
Caudal peduncle depth CPD	11.7-12.3 (12.1)	**12.1-13.0 (12.7)**	12.9	9.3-12.0 (10.5)	**8.0-12.4 (10.8)**	9.5-13.2 (11.4)	10.0-14.0 (12.2)
Body depth	14.1-14.9 (14.4)	13.4-15.8 (14.5)	14.2	13.8-15.8 (14.6)	10.6-18.1 (15.0)	13.2-16.7 (14.5)	12.2-19.8 (15.1)
Pectoral-fin length	18.9-23.0 (20.5)	16.0-21.2 (18.4)	16.0	17.0-23.1 (19.3)	13.4-23.0 (17.9)	17.9-26.0 (20.5)	16.0-23.9 (19.9)
Pelvic-fin length	**15.8-16.5 (16.3)**	13.5-16.0 (15.1)	13.5	**12.4-15.5 (14.5)**	11.1-16.7 (14.0)	13.9-18.2 (15.8)	12.7-18.5 (15.8)
Dorsal-fin length	20.9-22.7 (21.8)	**17.1-20.1 (19.0)**	17.1	18.7-22.5 (20.2)	16.0-24.2 (19.2)	18.6-25.6 (21.9)	**18.9-23.9 (21.6)**
Anal-fin length	15.2-17.0 (15.9)	14.2-17.2 (15.4)	14.2	14.0-17.0 (15.6)	10.0-17.9 (14.8)	13.9-19.0 (16.6)	13.4-19.4 (16.4)
Ratio CPL/CPD	**1.40-1.45 (1.43)**	**1.30-1.48 (1.37)**	1.32	**1.45-2.15 (1.68)**	**1.38-2.56 (1.66)**	1.26-1.77 (1.47)	1.14-1.71 (1.42)
In percent of HL							
Snout length	41.4-48.6 (44.3)	39.5-48.3 (44.5)	45.8	39.7-53.8 (45.2)	39.4-50.2 (44.3)	36.0-47.9 (43.1)	38.0-51.7 (44.0)
Eye diameter	15.6-19.5 (17.8)	16.0-19.3 (17.8)	18.0	15.9-24.6 (18.6)	12.8-23.9 (16.3)	14.9-21.9 (18.9)	13.5-21.0 (17.6)
Postorbital length	46.0-51.6 (48.3)	45.7-47.0 (46.5)	46.9	45.7-54.2 (47.8)	40.7-53.5 (46.9)	38.4-48.8 (42.2)	37.3-56.3 (43.9)
Interorbital width	**35.5-40.5 (37.6)**	**35.6-41.8 (38.0)**	41.8	**28.7-38.4 (33.3)**	**23.0-38.1 (30.9)**	**21.4-35.6 (29.4)**	**21.6-36.0 (29.4)**

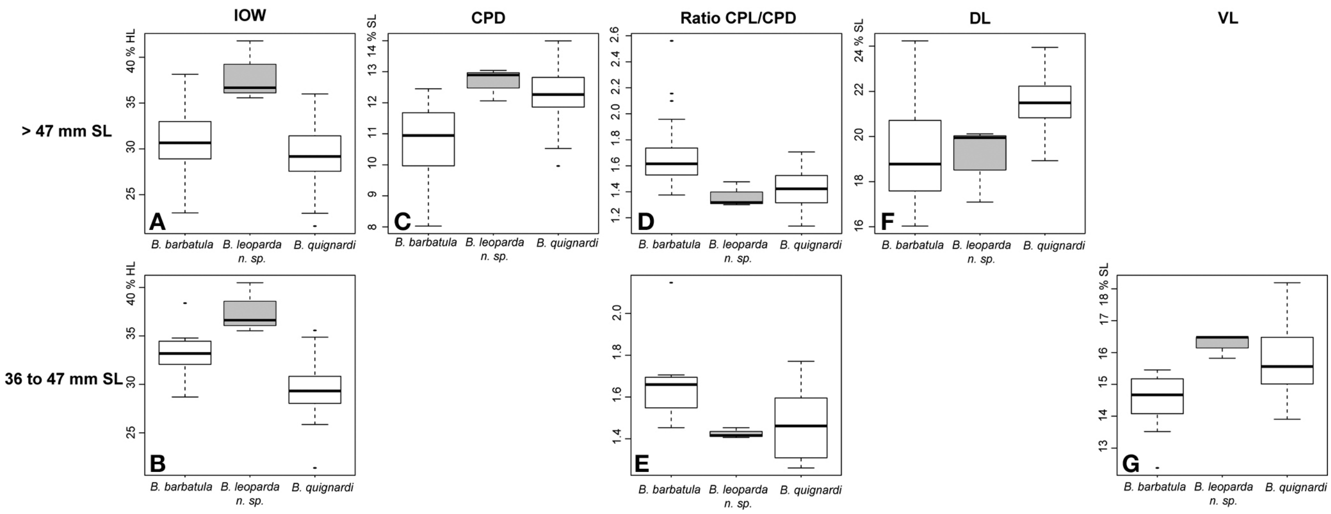

Figure 4. – Boxplots highlighting morphometric differences between *Barbatula leoparda* (in grey) and the two other species *Barbatula barbatula* and *Barbatula quignardi*, for the two sizes classes (36 to 47 mm SL and > 47 mm SL).

only by a larger interorbital width (35.5 to 40.5% HL, *vs*. 21.4 to 35.6% HL; Fig. 4B).

Description

For general appearance, see figure 1; morphometric data are provided in table I. Medium sized species. The stone loaches belonging to *B. leoparda* present a marbled pigmentation on the body with brown-dark blotches on the belly and in the jugular area for specimens larger than 47 mm SL (Fig. 2). The lateral profile is straight and the head is rounded. The body is cylindrical in front of the dorsal-fin origin and the tail is laterally compressed. The body width is equal on all its length, except for the head. The anterior and posterior nostrils are close to each other without any space between them. The mouth bears 3 pairs of barbels, and the last one located at the corners does not reach the posterior part of the eye. The upper lip is slim with a medial incision not exceeding the half of the width of the upper lip (Fig. 3). The mental lobes on the lower lip are not well marked (Fig. 3). This species has III simple and 7 to 8 branched dorsal fin rays (holotype III7), III simple and 5 to 6 branched anal fin rays (holotype III5), I simple and 9 to 11 branched pectoral fin rays (holotype I11), I simple and 6 to 7 branched pelvic fin rays (holotype I7), and 14 to 18 branched caudal-fin rays (holotype 18). The lateral line is complete. Specimens larger than 47 mm SL have a proportionally deeper caudal peduncle and a smaller dorsal fin than smaller specimens. Largest known specimen 62.6 mm SL.

Coloration after fixation

The body presents pale background coloration with a brownish-gray marbled pigmentation (Fig. 1). Adult specimens (> 47 mm SL) have also brown-dark blotches on the

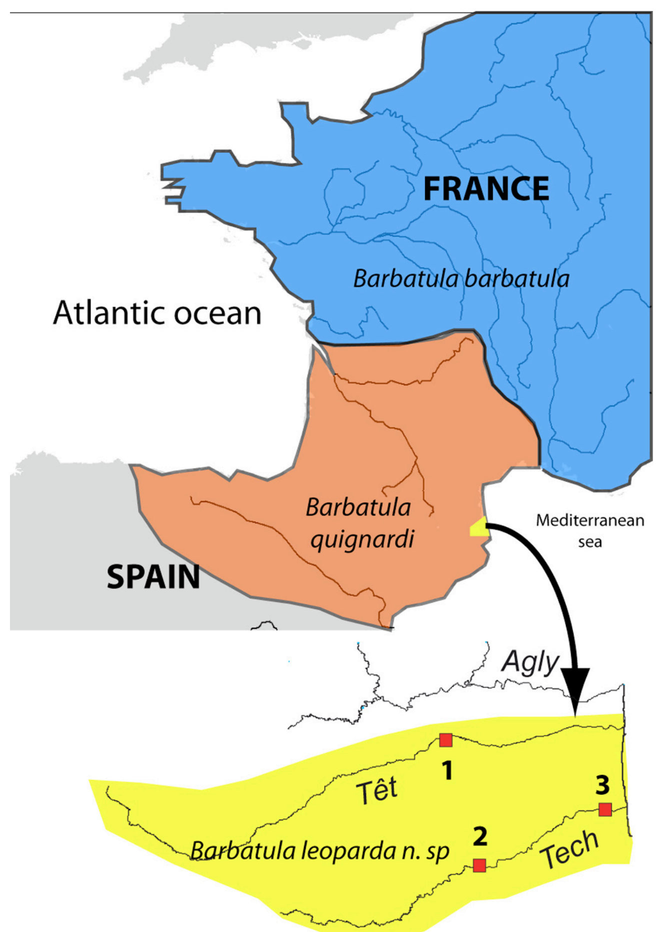

Figure 5. – Distribution map of the three recognized *Barbatula* spp. in France and Spain adapted and modified from Kottelat and Freyhof (2007): *Barbatula leoparda* in yellow, *Barbatula barbatula* in blue and *Barbatula quignardi* in orange. Locations: Têt River at Nefiach (1), Tech River at Céret (2) and at Elne (3).

Figure 6. – Type locality of *Barbatula leoparda*, the Têt River at Nefiach.

belly and in the jugular area (Fig. 2), smaller specimens have a pale yellowish belly with sometimes few little brownish blotches. The pattern of pigmentation is variable and depends on the habitat. All fins present dark blotches: two-three bands on the pectoral and caudal fins, one band on the pelvic fins, three to four bands on the dorsal fin.

Distribution and habitat

Barbatula leoparda is located in the rivers Têt and Tech in southern France (Fig. 5). It occurs principally in foothill section (> 100 m of altitude) where the populations are more abundant than in lowland section. This area is characterized by a predominantly Mediterranean rainfall regime with two noticeable low rain periods (a large one in summer, and a small one in the heart of the winter), as well as periods of high water during snowmelt and in autumn with possibilities of "cévenol" type flash floods (Curt and Davy, 1990). This species lives in streams under stones in the running waters (Fig. 6). Thus, this species is certainly threatened by the habitat fragmentation and the lowering of the water level resulting from the different dams and weirs built to fight the floods and to increase water abstraction for irrigation. They are also impacted by sand and gravel extraction as well as agricultural pollution (Desailly, 1999; Masson and Buquet 2000; Courtois *et al*., 2001).

Etymology

The name is derived from the Latin *leoparda* because of the ventral blotches on adult specimens (> 47 mm SL) conferring to a leopard-like coat.

Vernacular name

Leopard stone loach (English), Loche léopard (French).

Molecular characterization

The genseq-1 12S sequence (950 bp) has 99.37% of similarity being 6 bases of difference with the *B. barbatula* complete mitogenome sequence (GenBank Accession NC_27192), and average 98.91% of similarity being 10-11 bases of differences with the two 12S sequences of *B. quignardi* (GenBank Accession MK518370 and MK518371) (Tab. II). The genseq-1 sequence of the COI Folmer (652 bp) has 97.39% of similarity being 15 bases of difference with the *B. barbatula* complete mitogenome sequence (GenBank Accession NC_27192), and 97.55% of similarity being 18 bases of differences with the two COI sequences of *B. quignardi* (GenBank Accession MK518367 and MK518368) (Tab. III).

DISCUSSION

The examination of 128 specimens of stone loaches collected from 44 French and Spanish locations has allowed the distinction of a new species occurring in French Catalonia, *Barbatula leoparda*. It is distinct from the two other species *B. barbatula* and *B. quignardi* by the presence of dark-brown blotches on the belly and the jugular area for specimens longer that 47 mm SL, and also by a longer interorbital width (35.5 to 41.8 % HL). The molecular data on the COI marker highlighted

Table II. – Diagnostic sites for *Barbatula leoparda* (genseq-1 12S, MNHN 2018-0228), *Barbatula barbatula* and *Barbatula quignardi* (MNHN 2010-1064) on the 12S marker.

	GenBank Accession	37	134	178	363	384	385	477	616	639	642	875	889	890
Barbatula leoparda	MK518372	A	G	G	G	A	C	G	A	A	G	T	C	A
Barbatula barbatula	NC_27192	.	A	.	A	.	.	A	G	.	A	.	T	.
Barbatula quignardi	MK518370	G	.	A	A	G	T	A	G	G	A	C	.	G
Barbatula quignardi	MK518371	G	.	A	A	G	T	A	G	G	A	.	.	G

Table III. – Diagnostic sites for *Barbatula leoparda* (genseq-1 COI, MNHN 2018-0228), *Barbatula barbatula* and *Barbatula quignardi* (MNHN 2010-1064) on the Folmer region of the COI marker.

	GenBank Accession	10	61	64	124	238	250	265	268	287	313	347	370	403	409	484	526	541	565	574	595	604	650	652
Barbatula leoparda	MK518369	A	T	A	C	G	A	A	T	C	G	T	A	C	C	G	C	A	T	A	A	G	T	A
Barbatula barbatula	NC_27192	G	C	.	.	A	.	.	A	T	.	G	G	.	T	A	.	G	.	G	G	A	C	T
Barbatula quignardi	MK518367, MK518368	.	C	G	T	.	A	A	A	.	A	G	G	T	T	A	T	.	G	G	G	A	C	.

a divergence > 2%, that is most often observed in teleosts for different species (*e.g.* Ward *et al.*, 2009; Dettai *et al.*, 2011; Geiger *et al.*, 2014), not within a species. This paper brings then data for morphological identifications, as well as molecular characters from reference material (holotype), which will allow the molecular determination *via* DNA barcoding (COI; *e.g.* Ward *et al.*, 2009) and environmental DNA (12S; *e.g.* Valentini *et al.*, 2016).

According to Kottelat (2012), *Nemacheilus barbatulus* forma *hispanica* Băcescu-Meşter, 1967 is a junior synonym of *Barbatula quignardi* (Băcescu-Meşter, 1967). This taxon does not correspond to *B. leoparda* because its type localities are the Nervion River at Durango (Prov. Vizcaya) and the Tajo River in Spain. Moreover, our examined material around the Nervion River (MNCN 279188-279189, 279227-279231 and 279280-279297) does not present any blotch on the belly and the jugular area (Fig. 2), but presents longer dorsal-fin lengths (20.8 and 23.8% HL, *vs.* 17.1 to 20.1% SL for *B. leoparda*), and interorbital widths (25.9 to 33.7% SL) are inferior to those of *B. leoparda*. Thus, they stay *B. quignardi*.

By the same way, adult specimens from the Agly drainage do not present any blotch on the belly and the jugular area (Fig. 2), but present longer dorsal-fin lengths (20.8 and 21.6% SL) and shorter interorbital widths (28.6 and 36.0% HL, *vs.* 35.6 to 41.8% HL), as well as subadult specimens of Agly and Ter rivers have shorter interorbital widths too (21.4 to 32.2% HL, *vs.* 35.5 to 40.5% HL), so they belong to *B. quignardi* too.

According to Kottelat and Freyhof (2007), *B. quignardi* would be distinct from *B. barbatula* by a length of caudal peduncle 1.1 to 1.6 (usually 1.3 to 1.4) times in its depth (*vs.* 1.4 to 2.2 (usually 1.6 to 2.0)). Our results are in agreement with their diagnosis (1.1 to 1.7 for *B. quignardi* and 1.4 to 2.6 for *B. barbatula*) (Tab. I). Nevertheless, this character varies a lot in function of populations within both species, which could be explained by the presence of other undescribed species of *Barbatula*. This is supported by recent molecular studies in Europe revealing several distinct evolutionary lineages within *B. barbatula* (Culling *et al.*, unpubl. data; Šedivá *et al.*, 2008; Knebelsberger *et al.*, 2015; Behrmann-Godel *et al.*, 2017; Norén *et al.*, 2017). These demonstrated the weakness of our taxonomical knowledge on this genus, a problem shared with many other European freshwater fish genera.

A taxonomical revision for the whole of European stone loaches with an integrative taxonomy approach (*i.e.* Padial *et al.*, 2010) is then sorely needed.

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