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Rediscovery of *Rutilus virgo* in the River Dyje, Czech Republic

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Abstract. Here we report the catching of a single specimen of a rare native fish species, the cactus roach *Rutilus virgo* (Heckel), by rod and line on 31 January 2016 at Břeclav (r.km 25.6), on the main channel of the River Dyje (right tributary of the River Morava, Danube basin). This report comes 62-years after the last confirmed record in the Czech Republic, the species having been reported as extinct in the country since then. The morphometric and genetic characteristics of the specimen are presented and evaluated in light of the previous report.

Key words: cactus roach, Danube basin, rare native species

Introduction

The cactus roach (*Rutilus virgo*) is presently found along the River Danube and its larger tributaries from the German town of Ulm to the Iron Gates Dam on the Serbian-Romanian border (Kottelat & Freyhof 2007). Lacépède originally described roach from the lakes of northern Italy and the Danube as one species: Pigo *Rutilus pigus* (Lacépède, 1804). Later, Heckel (1852) differentiated species from the Danube as a completely separate species, *R. virgo*. Berg (1932), however, described the Danubian specimens as a sub-species, *R. pigus virgo*. Mišík (1957), on the other hand, after analysing the morphometric characteristics of 69 specimens from the Slovak Danube and its tributaries, found no difference from the nominotypical form. Despite this, Kottelat (1997) postulated the existence of two endemic species, with roach occupying the Danubian basin indicated as *R. virgo* and roach from northern Italy as *R. pigus*. In analysing the phylogenetic relationship between Mediterranean and Central European roaches, Ketmaier et al. (2008) found a clear split between haplotypes into two allopatric groups, one from the Padano-Venetian district and one from the Danubian district, thus supporting the taxonomic arrangement of Kottelat (1997).

In the Czech Republic, Jeitteles (1863) reported *R. virgo* as occurring in the River Morava (a tributary of the River Danube) up to the town of Olomouc

(r.km 235) in the second half of the 19th century; though he obtained just four specimens, suggesting it was rare even then. The species was still present in the river in the 20th century, with Oliva et al. (1968) mentioning *R. virgo* as occurring near the town of Lanžhot (r.km ca. 80); though again, just one specimen was documented. In 1954, a male had been caught on the River Dyje (a main tributary of the River Morava) below a weir (r.km 26.7). This latter specimen was later analysed morphometrically by Hochman (1955). Since then, *R. pigus* has officially been classified as extinct in the Czech Republic (Hanel & Lusk 2005). In November 2015, a single specimen of what was highly probably *R. virgo* (total length 42 cm) was caught by an angler on the River Dyje. Unfortunately, the fish was returned to the river before any detailed identification could take place. A photo was taken, however, and presented on an angler's website (MRK. cz; <http://www.mrk.cz/rybarska-vidia.php?id=39287>). This note documents the recent catching of *R. virgo* on the River Dyje, 62-years after its last confirmed record in the Czech Republic on the same river. In addition, we present findings on its morphometric and genetic features.

Material and Methods

On 31 January 2016, one specimen of *R. virgo* was caught by the second author in the River Dyje

(right tributary of the River Morava, Danube basin; current fast, mean depth 1 m) in the town of Břeclav (r.km 25.6; 48.7575508 N, 16.8854878 E) using rod and line with maggots as bait. The specimen was subsequently frozen and given to the laboratory of the Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic. After defrosting, the specimen was measured (standard and total length; nearest 0.1 mm), weighed (nearest 1 g) and sex determined by examination of the gonads. The digestive tract was removed and the contents identified to the lowest taxonomic level. The contents of the digestive tract were weighed and the index of fullness (IF) calculated as the ratio between diet weight (w) and eviscerated fish weight (W_{evi}): $IF = 10^{4*}(w/W_{evi})$. Twenty-four plastic and eight meristic characters were measured according to the guidelines set by Holčík (1989). All measurements were expressed as a percentage of standard length, except for cranial characteristics which were expressed as a percentage of cranial length. During dissection, fragments of muscle and fin were taken and fixed in 98 % ethanol. Total DNA was extracted and the entire mitochondrial cytochrome *b* (mt *cytb*) gene amplified using polymerase chain reaction and sequenced directly. The resultant sequence was compared with previous records for the region using BLAST software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequence has now been submitted to GenBank (accession no. KU950742). The fish was later deposited in the collection of the National Museum in Prague (no. NMP P6V144119).

Results and Discussion

Based on gonad morphology, the *R. virgo* specimen was identified as a male, measuring 410/340 mm (total length/standard length) and weighing 822 g. Scale examination indicated an age of nine years. Meristic characteristics were as follows: D II 9, A III 11, P I 17, V II 8; scales in lateral line 46, above 7 and below 4. No significant differences were found between meristic and plastic (as percentage of standard length and head length, Table 1) measurements for the Dyje specimen and those published by Hochman (1955) for the River Dyje and Mišík (1957) for samples caught along the Slovakian stretch of the rivers Danube and Dyje.

BLAST indicated a 100 % homologous alignment between the mt *cytb* sequence of the new Dyje specimen and sequences from the Slovakian stretch of the rivers Danube (accession nos. FJ025064 and FJ025065) and Drava in Slovenia (accession nos.

Table 1. A comparison of the morphometric characteristics of cactus roach *Rutilus virgo* caught in the River Dyje in 2016 and 1954 (Hochman 1955).

Parameter	Břeclav 2016	Hochman (1955)
Total length	410	355
Standard length	340	293
As % of standard length		
Head length	19.9	19.7
Preorbital distance	6.04	
Diameter of eye	3.8	
Postorbital distance	10.3	
Head depth	15.5	
Head width	11.5	
Interorbital distance	8.1	
Predorsal distance	51.5	52.5
Preventral distance	49.4	51.2
Preanal distance	71.2	74.0
Body depth	30.7	29.3
Body width	14.5	12.9
Caudal peduncle length	18.2	16.0
Caudal peduncle depth	13.5	
Minimal body depth	9.9	10.2
P-V distance	29.8	30.6
V-A distance	22.9	24.2
Length of D	14.5	
Length of A	12.1	
Length of P	15.5	16.4
Length of V	16.4	14.3
Length of C	20.1	20.4
Depth of D	20.5	21.2
Depth of A	13.0	12.9
As % of head length		
Preorbital distance	30.3	27.5
Diameter of eye	18.9	19.9
Postorbital distance	51.6	51.6
Head depth	76.2	79.3
Head width	57.5	60.2
Interorbital distance	40.6	44.8

FJ025075 and FJ025076) (Ketmaier et al. 2008; Note: these sequences are still registered in GenBank under their older name of *R. pigus*). Homology with true *R. pigus* from Italy (accession nos. FJ025063, FJ025081 and FJ025082) was 92-93 %.

The digestive tract of the new Dyje specimen was full of algae (*Vaucheria* sp.; Xanthophyceae) and epiphytic diatoms of the genus *Gomphonema* (almost solely). Contents of the digestive tract weighed 20.9 g, resulting in an IF of 254%₀₀₀. The dietary components

found are typical for *R. virgo* in winter as the species switches to a vegetarian diet over the colder months. Despite this, the diet is fairly high in energy due to the rich oil content of the diatoms.

All indications suggest that the *R. virgo* specimen from the River Dyje had spread upstream along the River Morava naturally. As with other fishes of the genus *Rutilus*, *R. virgo* is a shoaling species; hence, it is highly likely that more specimens will be caught by anglers in the lower Dyje soon.

A number of native Danubian fish species had disappeared from Czech waters by the middle of the 20th century. By the 1990s, however, many of these species had begun to return as water quality improved (Jurajda et al. 2008), with formerly rare species such as the ziege (*Pelecus cultratus*), zingel (*Zingel zingel*), streber (*Zingel streber*) and others all making a comeback (Jurajda et al. 1992, Jurajda et al. 1994, Lusk & Jurajda 1995). The cactus roach *R. virgo* is almost the last of these native Danubian fish species to return to Czech waters. However, some anadromous species, such as the beluga (*Huso huso*), are unlikely ever to return due to the installation of major migration barriers, such as the Iron Gates and Gabčíkovo dams. As a result, *H. huso* is officially classified as extinct in the Czech Republic (Hanel & Lusk 2005). At present, therefore, aside from *H. huso*, almost all fish species historically

occurring along the lower Morava and Dyje have now returned and there is no doubt that the confluence of the rivers Dyje and Morava now represents an exceptional region, with the highest fish species diversity in the Czech Republic. Over recent years, fish passes have been built on three weirs along the lower Dyje; hence, there is the potential for a continuous population from the River Danube right up to the Nové Mlýny reservoirs (r.km 42.5; 48.8579225 N, 16.7319114 E), about 112 km from the Morava's confluence with the Danube. While two weirs (r.kms 77.9 and 92.8) were removed from the lower Morava in 2015, two further weirs and one rocky chute remain along the lower Czech-Slovak stretch of the River Morava and a large weir at the town of Hodonín (r.km 114.5; 48.8424389 N, 17.1425978 E), effectively preventing any future migration up this river (for further details see Lusk & Jurajda 1995). Aside from some form of anthropogenic transfer, therefore, the range of *R. virgo* in the Czech Republic appears to be limited to the area around the Dyje's confluence with the Morava for the foreseeable future.

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