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Authors: Kopecký, Oldřich, Husák, Tomáš, and Jablonski, Daniel

Source: Journal of Vertebrate Biology, 72(23039)

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/jvb.23039>

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Genetically verified record of non-indigenous turtle, *Pelodiscus sinensis* (Weigmann, 1835) in Central Europe

Oldřich KOPECKÝ^{1*}, Tomáš HUSÁK¹ and Daniel JABLONSKI²

¹ Department of Zoology and Fisheries, Faculty of Agriculture, Food and Natural Resources, Czech University of Life Sciences Prague, Praha 6 – Suchbátka, Czech Republic, e-mail: kopecky@af.czu.cz, husakt@af.czu.cz

² Department of Zoology, Comenius University in Bratislava, Bratislava, Slovakia; e-mail: jablonski.dan@gmail.com

► Received 17 April 2023; Accepted 26 June 2023; Published online 31 August 2023

Abstract. Biological invasions are among the major global causes of biodiversity loss. The pet trade is the main pathway by which reptiles are introduced into new environments. After the EU import ban on the pond slider (*Trachemys scripta*), other species of pet-traded freshwater turtles are more often reported from the wild. Here we report the first and genetically verified record of Chinese soft-shelled turtle (*Pelodiscus sinensis*) from the eastern part of the Czech Republic. The genus *Pelodiscus* has been reported from the Czech Republic and neighbouring countries; however, only as a visual record, which is insufficient for identification due to the complexity of the taxonomic status of this species. We physically captured the turtle and used genetic data (sequences of 16S rRNA and cytochrome *b*) to confirm the species affiliation as *P. sinensis*. The studied individual represents a lineage of *P. sinensis* that is widely bred on farms with a native range in North Vietnam, South and south-east China.

Key words: allochthonous species, biological invasions, Czech Republic, Trionychidae

Biological invasions are among the major global causes of biodiversity loss (Courchamp et al. 2016). The pet trade is the main pathway by which reptiles are introduced into new environments (Kraus 2009). For example, the pond slider (*Trachemys scripta*) invasion into the area of the European Union is well-known and documented (Cadi & Joly 2004, Polocavica et al. 2008). The import ban of *T. scripta* given by the European Commission Regulations opened the door for the increase in trade with other potentially invasive turtles and tortoises and their subsequent introduction (Ficetola et al. 2012, Kopecký et al. 2013). Several records of other non-native turtles throughout the EU have demonstrated these concerns as valid. These species originate predominantly from North

America and Asia (Pupins & Pupina 2011, Jelić & Jelić 2015, Jablonski et al. 2018).

The Chinese soft-shelled turtle (*Pelodiscus sinensis*) is one such species. *Pelodiscus sinensis* is a highly aquatic species not exceeding 350 mm in carapace length living in brackish- and (predominantly) freshwater, such as rivers, ponds, lakes, rice fields, swamps, and canals where it feeds on molluscs, fishes, crustaceans, insects and worms (Bonin et al. 2006). *Pelodiscus sinensis* is native to China, Taiwan, and North Vietnam, while other species of this genus live in other parts of East and south-east Asia (Farkas et al. 2019). Turtles of the *Pelodiscus* genus are traditionally bred on farms as a source of food, and due to their

* Corresponding Author



Fig. 1. Adult female of *Pelodiscus sinensis* (carapace length 230 mm, width 200 mm, overall weight 3,200 g) caught during a fish harvest of the Buňkov pond near the village of Břehy u Přelouče, Czech Republic in October 2018.

commercial importance (Gong et al. 2018), they are often translocated to farms in other countries such as Japan (Ota et al. 2004), Korea (Chang et al. 2012) or Philippines (Diesmos et al. 2008). Nevertheless, the pet trade remains the main reason for the transport of *Pelodiscus* turtles to Europe (Kraus 2009).

Members of the *Trionychidae* family were previously observed in the region of Central Europe in Vienna, Austria (R. Gemel pers. comm. in Brejcha et al. 2014) and in the Czech Republic near the city of Ostrava (Szymonik & Šandera 2014). Authors of both observations reported a probable sighting of *P. sinensis*; however, the identification of trionychid turtles without capturing is improbable (Lafleur et al. 1999), especially in the light of the recent taxonomical changes in the genus *Pelodiscus* (Gong et al. 2018, Farkas et al. 2019).

On 17th October 2018, during a fish harvest of the Buňkov pond (constructed in 1973 with an area of 53.3 ha; extensively used for fish farming of *Cyprinus carpio* and recreation) near the village of Břehy u Přelouče, Czech Republic, EU (GPS: 50°3'12" N, 15°35'31" E, elevation 210 m a.s.l.), we recorded one adult female of the genus *Pelodiscus* (Fig. 1). The second author of this paper was contacted by a local fisherman. The carapace length was 230 mm, the width of 200 mm, and weighed 3,200 g. In August 2019, the turtle laid 34 unfertilized eggs, which were transferred to an incubator. The turtle is fed on fish and is still alive as of the publication of this manuscript. The identification of the turtle was based on morphological features: plastron without femoral flaps, throat small with whitish spots and dark-edged markings, snow white to pinkish white plastron, and carapace without

medial keel (Lafleur et al. 1999, Farkas et al. 2019). The genetic affiliation was subsequently confirmed using the comparison of the newly generated sequences of 16S rRNA and cytochrome *b* sequences (*cytb*) of mitochondrial DNA. The laboratory work to generate *cytb* sequences followed Fritz et al. (2010) and Gong et al. (2018). For the 16S dataset, we amplified DNA using primers 16Sar-L and 16Sbr-H (the laboratory protocol of Palumbi et al. 1991). PCR products were sent to Macrogen Europe Inc. (Amsterdam, the Netherlands) for sequencing. The new sequences were checked via BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and compared with the published sequences.

Both sequences confirmed the affiliation of the captured individual with the genus *Pelodiscus*, where the cytochrome *b* sequence corresponds with the haplotype B2 (GenBank number FM999013-14; 100 % similarity). These sequences belong to the clade B (*sensu* Fritz et al. 2010 and Gong et al. 2018) of *P. sinensis*, which is widely bred on farms with a native range in North Vietnam, South and Southeast China. The 16S sequences also correspond with the material from China, particularly with sequences OQ236105-7 (99.47 % similarity; Chen et al. 2023). Our newly obtained sequences are available under GenBank accession numbers OR067156 (16S rRNA) and OR083068 (*cytb*).

According to the program Climatch, data from 16 climatic variables at the nearest climatic station a 40% consistent with the original climate of the native range of *P. sinensis* (O. Kopecký, unpublished data). This result suggests a low probability of potential long-term establishment of this species at the site of the record (Bomford 2008). However,



considering the whole area of Europe, *P. sinensis* is perceived as a species with an almost equal probability of establishment as *T. scripta* that has already established in the region (Kopecký et al. 2013); moreover, *P. sinensis* have better ecological and reproductive features facilitating their establishment than *T. scripta* (Masin et al. 2014). Based on the climate characteristics, its establishment is likely possible in South and South-East Europe (Kopecký et al. 2013, Masin et al. 2014), and some records of this species came from this area recently: Bosnia and Herzegovina, Croatia, Slovenia (Brejcha et al. 2014), Serbia (Urošević et al. 2016), Spain (Poch et al. 2020) and Romania (Iftime & Iftime 2021). However, individuals reported as *P. sinensis* were also recorded in Latvia (Pupins & Pupina 2011), France (OFB and UICN France in Koppetsch 2021), Switzerland and Germany (Koppetsch 2021).

The main threats to European wildlife include competition with native turtles, predation of protected

species, and possible transmission of various diseases (Bonin et al. 2006, Verneau et al. 2011). On the other hand, the low propagule pressure proven by the few reports of this species in the European landscape (usually in the form of a single individual per site) suggests that this species will likely cause fewer adverse effects than *T. scripta*.

Acknowledgements

The authors thank Dr Jaroslav Janosek for his valuable comments. English was corrected by Correcta. This study was supported by the Slovak Research and Development Agency under contract no. APVV-19-0076.

Author Contributions

O. Kopecký wrote the paper. D. Jablonski performed a DNA analysis. T. Husák discovered the turtle. D. Jablonski and T. Husák commented on preliminary versions of the manuscript.



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