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Article

First record of the halobiontic *Diplodontus semiperforatus* (Acari, Hydrachnidia) from North Khorasan, with notes on water mites from saline habitats of Iran

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ABSTRACT
We report the first record of *Diplodontus semiperforatus* (Walter, 1925) for Iran. Thus far, this halobiontic species was only known from the Western Mediterranean. We discuss the environmental characteristics of the sampling sites and present the first barcodes of *D. semiperforatus* and its sister species *D. scapularis* Dugès, 1834.

KEYWORDS: DNA barcoding, first record, Morocco, saline waters, Trombidiformes.

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INTRODUCTION

The genus *Diplodontus* Dugès, 1834 includes several species known from Europe, Asia, Africa and Australia (Smit 2020). In the Western Palaearctic region the genus is represented by two closely related species., i.e. *D. scapularis* Dugès, 1834, a species known from the SW Palaearctic, and *D. semiperforatus* (Walter 1925), recorded from Western Mediterranean saline inland waters (Di Sabatino *et al*. 2010). From other species of the genus, *D. scapularis* and *D. semiperfortaus* differs in having stout palps and a marked sexual dimorphism in the legs (in male legs, in particular the third and fourth leg, are robust and have strong shortened claws) and genital field (in male, genital flaps posteromedially extending into a distinct projection, see Fig. 1D). As showed by Moreno *et al*. (2008)

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these two species differ in salinity tolerance, with *D. semiperforatus* adapted to saline and hypersaline inland waters.

The objective of this work was to report the first occurrence of *D. semiperforatus* from northeastern Iran, provide the first DNA barcodes of *D. scapularis* and *D. semiperforatus* and discuss molecular and ecological data that effectively differentiate these two closely related species.

MATERIAL AND METHODS

All material was collected by hand netting and fixed in 96% EtOH for the purpose of the molecular analyses. The collected specimens were sent to the Canadian Centre for DNA Barcoding, Guelph, Canada for molecular analysis. In the latter institution, the specimens were sequenced for the barcode region of COI using standard protocols for DNA extraction (Ivanova *et al*. 2007), amplification (Ivanova and Grainger 2007a) and sequencing (Ivanova and Grainger 2007b). The DNA extracts were archived in −80 °C freezers at the Centre for Biodiversity Genomics (CBG; https://biodiversity genomics.net). After DNA extraction, the specimen vouchers were returned to the senior author for morphological examination. Some of these vouchers were dissected and slide mounted in Faure's medium, while the rest was transferred to Koenike-fluid.

The vouchered material will be deposited in Naturalis Biodiversity Center, Leiden (RMNH). DNA sequences prepared in the course of this study were deposited in the BOLD system (DNA Barcode of Life Data System) and GenBank (Table 1). Water mite specimens successfully barcoded in this study are listed in Table 1. Data related to each BIN, including the minimum *p*-distance to the nearest neighboring BIN, was estimated through BOLD.

Table 1. Details on barcoded specimens, including data and coordinates of sampling sites, the barcode index number and associated data obtained from BOLD. DNN = *p*-distance to nearest neighbor; NN BIN = nearest neighbor BIN; NN taxonomy = species assigned to nearest neighbor BIN. BOLD data presented here was last accessed on 1st October 2023.

Species	Locality	Coordinates	Voucher Code	BOLD/ GenBank	BIN BOLD	DNN (%)	NN BIN BOLD:	NN taxonomy
Family Hydryphantidae								
Diplodontus scapularis	Morocco, Oued Messoussate	35.0674° N, 2.9032° W	CCDB 38392 B09	DCBDJ021-21/ OR614831	BOLD:AEL3056	11.33	BOLD:AFG9586	*D. semiperforatus*
	Morocco, Messoussate, intermittent river	35.0682° N, 2.90114° W	CCDB 38560 B08 CCDB 38560 B09	NOVMC020-21/ OR614832 NOVMC021-21/ OR614830				
Diplodontus semiperforatus	Iran, Qareh Ghan, a pool of saline river	36.9358° N, 57.2071° E	CCDB_39399_C12	HYDIR036-23/ OR501657	BOLD:AFG9586	11.33	BOLD:AEL3056	*D. scapularis*
Family Hydrachnidae								
Hydrachna skorikowi	Iran, Qareh Ghan, a pool of saline river	36.9358° N, 57.2071° E	CCDB_39399_C11	HYDIR035-23/ OR501658	BOLD:AFF6278	11.11	BOLD:ACS0797	*H. skorikowi*

The final alignment for species delimitation in the *Diplodontus* dataset included 5 sequences of *D. scapularis* from Morocco (n = 3), *D. semiperforatus* from Iran (n = 1) and *D. haliki* Lundblad, 1947 from Australia (n = 1, AFWM105-21/OK042143.1 [BOLD/GenBank], STL44Acar2[sampleid]; Carew *et al*. 2022, Smit and Pešić 2023). *Hydodroma despiciens* (Müller, 1776) (NLACA210-15) from the Netherlands was used as an outgroup. The final alignment consisted of 658 nucleotide positions. DNA barcode sequences were aligned using MUSCLE alignment (Edgar 2004). Intra- and interspecific genetic distances were calculated in MEGA X (Kumar *et al*. 2018) employing the Kimura-2-Parameter (K2P) (Kimura 1980), and *p*-distance metrics. The latter software was used to

produce the Maximum Likelihood (ML) tree (models selected by the BIC (Bayesian Information Criterion) implemented in MEGA X: GTR + I with an initial Neighbour-Joining (NJ) tree and using the Subtree- Pruning-Regrafting - Extensive heuristic search (SPR level 5). The support for tree branches was calculated by the nonparametric bootstrap method (Felsenstein 1985) with 1000 replicates and shown next to the branches. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair.

RESULTS

Diplodontus scapularis Dugès, 1834 (Figs. 1C, D)

Material examined

Morocco: Oued Messoussate, 35.0674° N, 2.9032° W, 10 June 2020 leg. Mabrouki, 1♀ (sequenced; Table 1); environmental characteristics of the sampling site: pH 7.5, water temperature 24 °C, conductivity 541 µS/cm, dissolved oxygen 12 mg/L. Messoussate, intermittent river, 35.0682° N, 2.90114° W, 7 Oct. 2021 leg. Mabrouki, 1♂, 1♀ (sequenced; Table 1), ♀ dissected (palps and gnathosoma slide mounted, idiosoma conserved in Koenike fluid); environmental characteristics of the sampling site: pH 7.8, water temperature 23 °C, conductivity 597 µS/cm, dissolved oxygen 10 mg/L.

Molecular data

The Moroccan specimens molecularly analyzed in this study match the description of *D. scapularis*. These individuals form a unique BIN (BOLD: AEL3056), with the nearest neighboring BIN being BOLD: AFG9586, which consists of a specimen of *D. semiperforatus* from Iran (see below).

Habitat

Individuals collected in Morocco, here used for molecular analysis, were collected on two localities, a remnant pool of a summer-dry stream and a small pond fed by a spring. Both localities are characterized by low electrolyte concentrations (conductivity 541–597 µS/cm).

Distribution

Morocco, Croatia, France, the Netherlands, Portugal, Russia, Spain, Italy, Syria.

Diplodontus semiperforatus (Walter, 1925) (Figs. 1A, B, E)

Material examined

Iran, IR14-2022 North Khorasan Province, Qareh Ghan, a remnant pool of river crossing Feshanjerd-Gurpan-Esfarayen road, 36.9358° N, 57.2071° E, 2 Aug. 2022, leg. Pešić 1♀ (sequenced, BOLD/GenBank HYDIR036-23/OR501657; Table 1), ♀ dissected (one palp and chelicera slide mounted, idiosoma conserved in Koenike fluid); environmental characteristics of the sampling site: pH 7.79, water temperature 24 °C, conductivity 36.4 mS/cm, dissolved oxygen 15.97 mg/L.

Molecular data

A 658-bp fragment was successfully amplified (BOLD accession code: HYDIR036-23). The specimen shows a 11.5 % of divergence (uncorrected *p*-distance) to specimens of *D. s*capularis from Morocco, as well an 15.5% of divergence to specimens of *D. haliki* from Australia.

Remarks

The specimen from Iran molecularly analyzed in this study matches the description of *D. semiperforatus*. From its sister species *D. scapularis*, the female of *D. semiperforatus* can be

distinguished by the shape of the chelicerae (claw relatively longer, length ratio basal segment/claw < 1.7) (compare Figs. 1B and C).

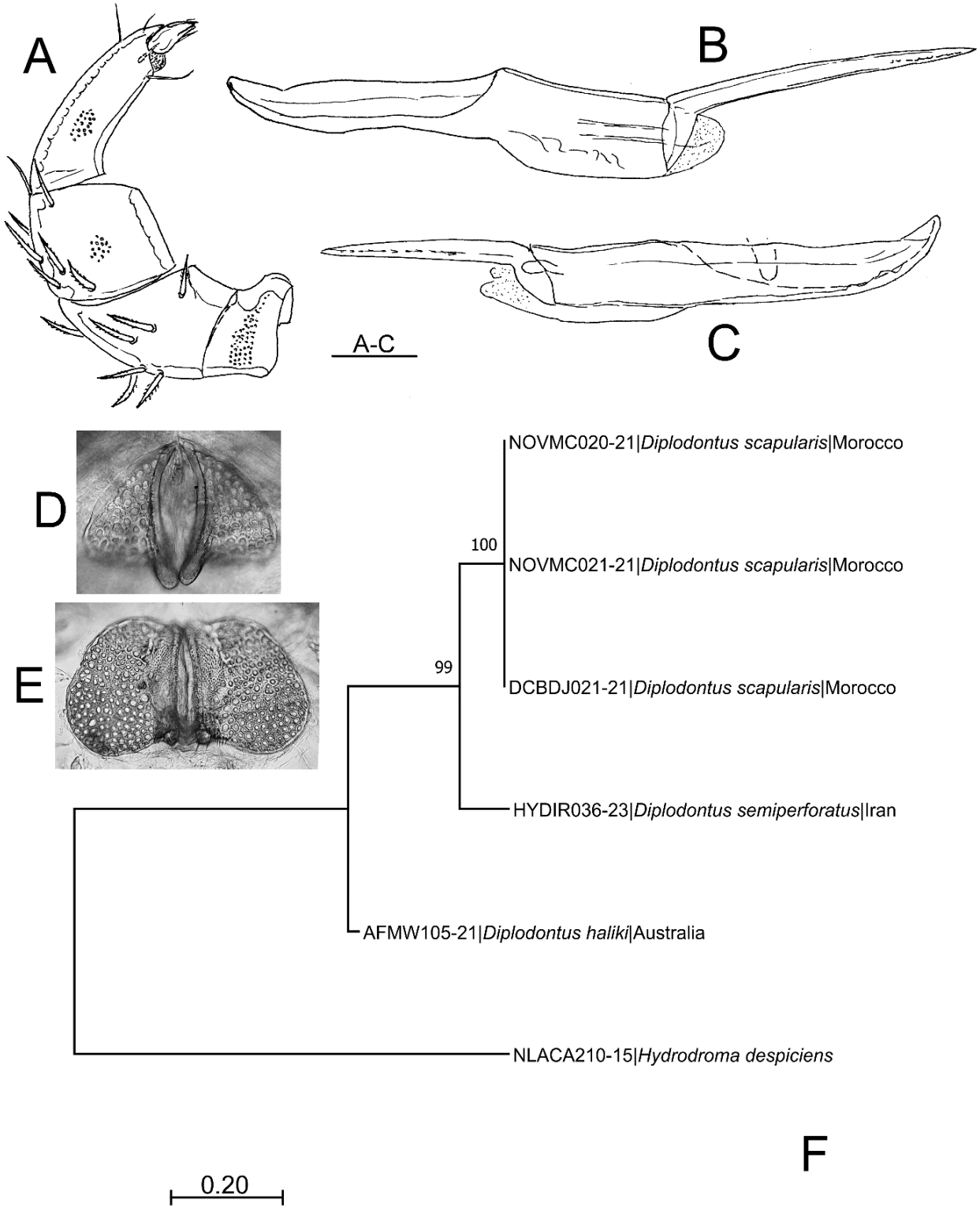

Figure 1. A-B, E: *Diplodontus semiperforatus* (Walter, 1925), ♀, Qareh Ghan, Iran – **A.** Palp, lateral view; **B.** Chelicera; **E.** Photograph of genital field. **C–D:** *Diplodontus scapularis* Dugès, 1834, Messoussate, Morocco (C – ♀, F – ♂) – **C.** Chelicera; **D.** Photograph of genital field. **F.** Maximum Likelihood tree (GTR+I model) of the genus *Diplodontus* obtained from 6 nucleotide COI sequences.

Figure 2. Photograph of sampling site (Qareh Ghan, North Khorasan Province) of *Diplodontus semiperforatus* in northeastern Iran. Photo by V. Pešić.

Habitat

The only site in northeastern Iran where *D. semiperforatus* was collected was a remnant pool of a saline river (Fig. 2) (conductivity 36.4 mS/cm). In shore areas of this river, the formation of salt crusts was noticeable. Bank vegetation was represented by a *Phragmites* belt, while the submerged vegetation consisted of *Chara* sp., which formed uniform mats over much of the bottom area. The soil mainly consisted of fine sediments, in which anaerobic conditions prevail.

Accompanied species

The single specimen of *D. semiperforatus* was collected together with a larger number of *Hydrachna* specimens that morphologically matched the description of *H. skorikowi* (Piersig, 1900), a species considered to be widespread in the Palaearctic. This species, which inhabits all kinds of standing and slow running waters, has been reported as tolerant against high salinity and can be found in water bodies with conductivity up to 8700 µS/cm (Gerecke 1991). Genetic data indicate that a single successfully barcoded specimen of *H. skorikowi* from northeastern Iran forms a unique cluster (BOLD: AFF6278) with the closest neighboring BIN is that of *H. skorikowi* (BOLD: ACS0797) which includes specimens from the Netherlands and Morocco. The high *p*-distance (11.11%) between these two BINs indicates the need for taxonomic revision of the *H. skorikowi* complex, indicating the presence of possibly undescribed cryptic species.

Distribution

Algeria, Morocco, Tunisia, Southern France (?), Italy (Sicily).

DISCUSSION

Our results support many previous observations that DNA barcodes can be a useful tool for efficiently differentiating closely related species (Pešić *et al*. 2017, 2019, 2020, 2022, Pešić and Esen 2022). The high genetic distance (11.3% *p*-distance) between *D. scapularis* and *D. semiperforatus* indicates a long independent history of these species. Moreno *et al*. (2008) suggested that both species probably evolved from a common stem species during the Messinian salinity crisis.

Our research confirms earlier ecological observations that *Diplodontus scapularis* prefers small water bodies with slightly saline water, while *D. semiperforatus* colonizes electrolyte-rich waters (saline and hypersaline water bodies). Beadle (1943) stated that *D. semiperforatus* is the most salt-tolerant water mite. A study by Gerecke (1991) on water mites inhabiting saline rivers in Sicily revealed that among Sicilian water mites, only *D. semiperforatus* is a true halobiont species, while all other species in waters with conductivity > 5 mS/cm can only be found sporadically as single individuals.

During our survey in northeastern Iran in 2022 (see Pešić *et al*. 2023), we took samples from a number of saline and hypersaline streams (most of them were intermittent streams) but we did not collect water mites. On the other hand, in several localities of flowing water with slightly increased conductivity (1–3 mS/cm), water mites were found. The diversity of mites in these localities was generally poor, and included species with a pronounced tolerance to slightly increased salinity; e.g. *Hygrobates persicus* Pešić & Asadi, 2017, *H. angustipalpis* K.O. Viets, 1982 and *Spechon papillosus* Thor, 1901.

Both *Diplodontus* species from our study, *Hydrachna skorikowi*, as well as *Limnesia* (*Halolimnesia*) *diversipes* Pešić, Smit & Saboori, 2012 (Limnesiidae), described from Hormozgan province of Southern Iran (Pešić *et al*. 2012), are lenitobionts, which mainly inhabit the remnant pools of saline, mostly summer-dry streams. For now, the only halobiontic species found in lotic habitats is *Haloaxonopsis salina* Pešić, Smit & Saboori, 2012 (Aturidae), which inhabits saline streams along the Gulf of Oman and Persian Gulf (Pešić *et al*. 2012). The unique wheel-like acetabula in the latter species, which was previously found only in the marine family Pontarachnidae, should be considered as an adaptation to the saline habitat.

As recently emphasized by Pešić & Glöer (2023), increased salinization of water bodies can be the main driver that affects the species composition and abundance of communities of aquatic snails, but also of water mites, which inhabit small water bodies in arid and semi-arid areas, especially in the context of the ongoing climate changes facing northeastern Iran.

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نخستین گزارش *Diplodontus semiperforatus* (Acari: Hydrachnidia) هالوبیونتیک از خراسان شمالی، همراه با یادداشت‌هایی در مورد کنه‌های آبزی از زیستگاه‌های شور ایران

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چکیده

نخستین بار *Diplodontus semiperforatus* (Walter, 1925) را برای ایران گزارش می‌شود. تا کنون، این گونه هالوبیونتیک تنها از غرب مدیترانه شناخته شده بود. ویژگی‌های محیطی محل‌های نمونه‌برداری را مورد بحث قرار گرفته است و نخستین بارکد *D. semiperforatus* و گونه خواهری آن *D. scapularis* Dugès, 1834 ارایه می‌شود.

واژگان کلیدی: نخستین گزارش، بارکد دی.ان.ای، مراکش، آب‌های شور، راستهٔ Trombidiformes.

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