European Journal of Taxonomy 930: 53–78
https://doi.org/10.5852/ejt.2024.930.2487

ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2024 · Lörz A.-N. *et al.*

Research article

urn:lsid:zoobank.org:pub:3677E539-4B17-4B37-A137-671AD52434EA

A new deep-sea species of *Halirages* Boeck, 1871 (Crustacea: Amphipoda: Calliopiidae) inhabiting sponges

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[3] urn:lsid:zoobank.org:author:26BB8830-FA36-4F87-B3DD-0C28C7F0C504
[4] urn:lsid:zoobank.org:author:5E724507-8435-44F8-843C-0F98D660C39B
[5] urn:lsid:zoobank.org:author:720F61F1-5EAC-42D2-A66C-B91B439DB2A1

Abstract. In the vast abyssal plains northwest of Iceland, white glass sponges of the genus *Caulophacus* Schulze, 1886 were inhabited by reddish *Bythocaris* G.O. Sars, 1870 shrimps and pinkish amphipods. After in situ observations at 3700 m depth, in -1°C waters by a remotely operated vehicle, members of this assemblage were collected and preserved for molecular studies. Based on integrative taxonomic analyses, the amphipods were identified as a new species of the genus *Halirages* Boeck, 1871 – *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg –, as described in detail below. Part of our integrative approach was to establish reference DNA barcodes for known species of *Halirages*. However, our investigation of material of Calliopiidae G.O. Sars, 1895 collected around Iceland and Norway revealed slight morphological discrepancies in all the described species of *Halirages*. Except for *Halirages fulvocinctus* (M. Sars, 1858), none of the encountered specimens of Calliopiidae fully matched a current species description. We illuminate the morphological characteristics of nine operational taxonomic units, which also represented clades in COI and 28S. We set the Icelandic samples in the context of *Halirages* from Canada and Norway. A key to the world species of *Halirages* is provided.

Keywords. Iceland, DNA barcoding, benthos, sponge habitat.

Lörz A.-N., Nack, M., Tandberg A.-H.S., Brix S. & Schwentner M. 2024. A new deep-sea species of *Halirages* Boeck, 1871 (Crustacea: Amphipoda: Calliopiidae) inhabiting sponges. *European Journal of Taxonomy* 930: 53–78. https://doi.org/10.5852/ejt.2024.930.2487

Introduction

Geomorphological features are known as habitat forming in the deep sea (Ramirez-Llodra *et al.* 2010). Seamounts, canyons, troughs, ridges, trenches, and abyssal plains are anticipated to represent distinct types of benthic habitats (Harris & Baker 2012). In addition to geomorphological features, reef-building corals or sponge grounds serve as a habitat themselves (biological habitats). This is known from arctic seamounts (Meyer *et al.* 2023) and large OSTUR aggregations (aggregations of large Demospongiae Sollas, 1885 off the Faroe Islands (Klitgaard & Tendal 2004)). The complexity of deep-sea habitats holds a huge number of undescribed macrofauna species, as well as in comparably well-sampled areas, such as the Norwegian Sea (Kürzel *et al.* 2021, Lörz & Horton 2021, Paulus *et al.* 2022). Surprisingly, even 'large' (visible by eye in ROV pictures) species were revealed to be new to science during the expedition of IceAGE 3 (SO276) in the frame of the IceAGE project (Icelandic Marine Animals: Genetics and Ecology). A remotely operated vehicle (GEOMAR ROV KIEL 6000) captured in situ video footage images of the abyssal plain at station 37 (3700–3450 m) from the sedimented plain in the median valley towards the rise of the Aegir Ridge System (Ránarhryggur) northwest of Iceland by RV Sonne in 2020 (Brix *et al.* 2020). The transect showed a highly sedimented habitat with sparse rocky outcrops in the abyssal plain area (Brix *et al.* 2022). Occasional rocks were colonized by the glass sponge *Caulophacus arcticus* (Hansen, 1885) (Brix *et al.* 2022). The colonies of *C. arcticus* provide a habitat for aggregations of caridean shrimp (*Bythocaris* cf. *leucopis* G.O. Sars, 1879), Pycnogonida Latreille, 1810 and amphipod species of different size classes, most prominently large amphipods from the family Calliopiidae, which Brix *et al*. (2022) identified as a species new to science needing further taxonomic attention (Fig. 1).

Calliopiidae is a species-rich hyperbenthic family that includes 105 species in 28 genera worldwide (Horton *et al*. 2023). Calliopiid amphipods are key players of the North Atlantic benthos (Lörz *et al.* 2021, 2022). They are bottom-dependent animals that have a strong swimming capacity, with some species migrating vertically, daily, or seasonally with varying regularity (Enequist 1949). Moreover, they are small- to medium-sized carnivores and pelagic suspension feeders living mainly from sublittoral to bathyal depths, but some species are found at abyssal depths (Barnard 1991). The Arctic and subarctic regions are particularly rich in species of Calliopiidae (Barnard 1991; Weisshappel 2001).

Nine species of *Halirages* Boeck, 1871 are known worldwide, all of which occur in the northeast Atlantic and Norwegian Arctic (Horton *et al*. 2021): *Halirages caecus* Kamenskaya, 1980, *Halirages cainae* d'Udekem d'Acoz, 2012, *Halirages fulvocinctus* (M. Sars, 1858), *Halirages gorbunovi* Gurjanova, 1946, *Halirages helgae* Ringvold & Tandberg, 2014, *Halirages mixtus* Stephensen, 1931, *Halirages nilssoni* Ohlin, 1895, *Halirages qvadridentatus* G.O. Sars, 1877 and *Halirages stappersi* d'Udekem d'Acoz, 2012.

Here we identify *Halirages* collected during recent expeditions to the North Atlantic, describe a species of *Halirages* new to science, set it in molecular context and present an updated identification key for the genus *Halirages*.

Material and methods

Amphipods were collected during IceAGE voyages to the deep sea off Iceland, and included 470 specimens belonging to the family Calliopiidae (Brix *et al.* 2018). Specimens were immediately sorted on deck, often photographed alive on board, initially preserved in 98% ethanol and later transferred to 70% ethanol. Specimens were examined and dissected using a Leica MZ9.5 stereo microscope at the Institute for Marine Ecosystems and Fishery Science, University of Hamburg, and drawn using a camera lucida attachment. Small appendages (mouthparts, uropods, telson) were temporarily mounted in glycerin, examined, and drawn using a compound microscope (Leica DM1000) fitted with a camera lucida. The body lengths of the examined specimens were measured by tracing the individual's mid-trunk lengths (tip of the rostrum to the end of the telson) using a camera lucida. All illustrations were inked electronically using a Wacom Intuous 3 Graphics Tablet and Adobe Illustrator CS2 following techniques in Coleman (2003, 2009). The type material is held at the Natural History Museum Vienna, Austria, and at the Museum of Nature, Leibniz Institute for the Analysis of Biodiversity Change, Hamburg, Germany.

Additionally, calliopiid amphipods from several Norwegian expeditions were identified and selected for sequencing at the University Museum of Bergen, and sequenced through BOLD via the NorBOL node. Relevant voucher information, taxonomic classifications, and sequences were merged into one dataset in BOLD (www.boldsystems.org) and are accessible through the public via "DS-HALIRAGE" (https://doi.org/10.5883/DS-HALIRAGE) (Ratnasingham & Hebert 2007).

DNA extraction, PCR amplification, sequencing and phylogensetic analyses

DNA was extracted using one leg, usually the first left pereiopod. Different DNA extraction methods were employed. The majority of extractions were performed following the HotSHOT protocol (Montero-Pau *et al.* 2008): the leg was incubated for 20 min at 96°C in 40 µl alkaline lysis buffer (NaOH 25 mM,

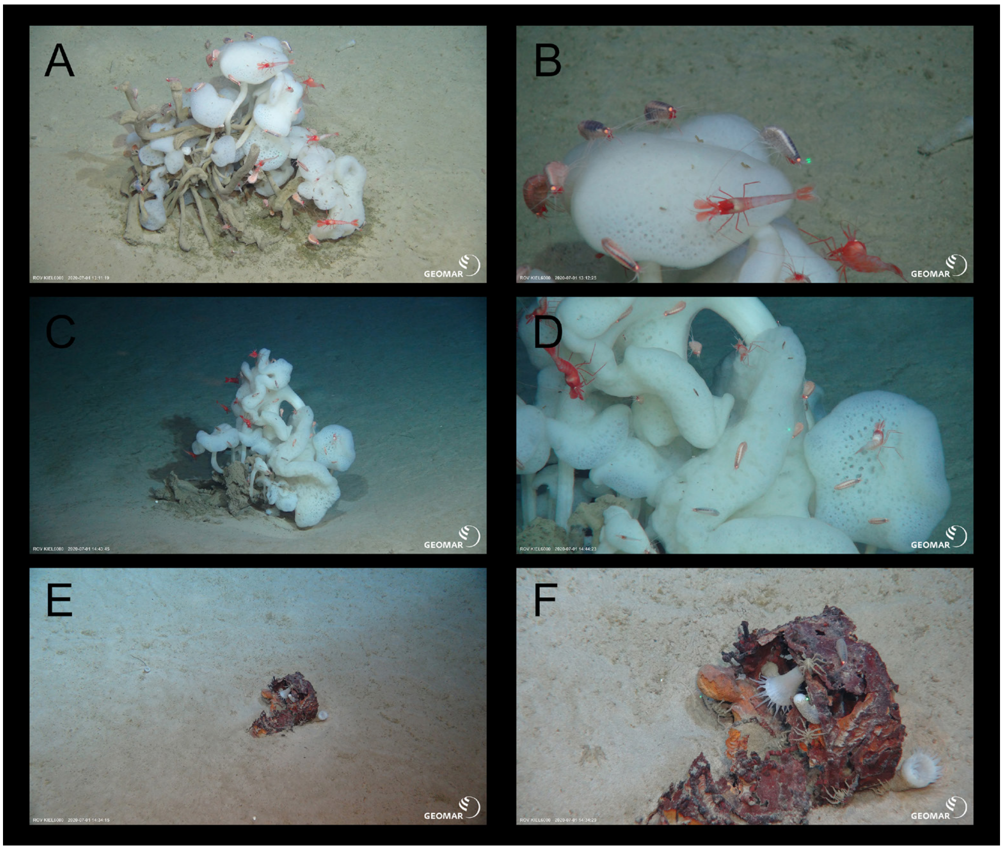

Fig. 1. Examples of ROV Kiel 6000 images at 3500 m depth northwest of Iceland at a water temperature of minus 1°C. **A–D**. Sponge aggregations of *Caulophacus arcticus* (Hansen, 1885) provide a habitat for caridean shrimp (*Bythocaris* cf. *leucopis* G.O. Sars, 1879) and *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg. **E–F**. On a rusted can (rubbish) during the same transect the same amphipod was found, next to anemones and Pycnogonida.

disodium EDTA 0,2 mM, pH 10.0), cooled down to 4°C for 4 min and then 40 µl of neutralizing buffer (Tris-HCL 40 mM, pH 5.0) were added. Alternatively, tissue was lysed with proteinase K and the DNA recovered using magnetic beads (AmpliClean$^{\mathrm{TM}}$), adding the 1 × volume of magnetic beads, incubating and ethanol washing the bound DNA following the manufacturer's protocol and eluting the DNA in 40 µl H_2O. Two genes were PCR-amplified, mitochondrial cytochrome c oxidase subunit I (COI) and nuclear 28S rDNA, using the primers LCO1490-JJ CHACWAAYCATAAAGATATYGG (Astrin & Stüben 2008) and HCO2198-jj (AWACTTCVGGRTGVCCAAARAATCA; Astrin & Stüben 2008) or BR2 (TCDGGRTGNCCRAARAAYCA; Elbrecht & Leese 2017) for COI and 28S-3311F (GGGACTACCACCCGCTGAACTTAAGCAT; Witt *et al.* 2006) and 28SR (GTCTTTCGCCCCTATGCCCAACTG; Hou *et al.* 2007) for 28S. PCR reactions comprised 2 µl DNA extract, 1.5 µl of each primer (10 mM), 0.12 µl dNTPs (25 mM each), 0.4 µl M_gCL_2 (50 mM), 1.5 µl 10x buffer 0.075 µl TopTaq DNA polymerase (Qiagen) and 7.9 µl H_2O. PCR cycling programs had 1 min initial denaturation step at 94°C, followed by 38 cycles (COI) or 40 cycles (28S) at 94°C for 1 min, 46°C (COI) or 50°C (28S) for 30 s and 72°C for 1 min, and a final elongation step at 72°C for 5 min. The success of PCR reactions was visualized on 1.5% agarose/TBE gels. Remaining primers and dNTPs were inactivated by incubating the PCR product for with 0.6 µl shrimp alkaline phosphatase (rSAP, NEB) and 0.3 µl exonuclease I (NEB) for 15 min at 22°C and 20 min at 85°C. The cleaned PCR products were bidirectionally sequenced using PCR primers with the Sanger sequencing service of Macrogen Inc., Netherlands. Electropherograms of both sequencing reactions were assembled in Geneious Prime 2022.2.2, sanitized by eye, and the resulting sequences were aligned by gene using the MUSCLE algorithm implemented in Geneious ver. 10.2.6 (Biomatters Ltd.). COI sequences of *Halirages* publicly available through the BOLD and NCBI databases were also included (Table 1). One individual of *Haliragoides* was included as the outgroup. COI alignment was checked for indels and stop codons in MEGA-X (Kumar *et al*. 2018). The sequences were uploaded to GenBank via BOLD and are available there under the following accession numbers: OR859294-OR859353 (COI) and OR859189- OR859220 (28S). All are available for download via the BOLD dataset https://doi.org/10.5883/DS-HALIRAGE. Species were delimited using a combination of DNA distance and phylogenetic analyses. Pairwise uncorrected *p*-distances were computed for COI using MEGA-X. Distance-based species delimitation was done with the web-version of the Assemble Species by Automatic Partitioning program (ASAP; Puillandre *et al*. 2021), using uncorrected *p*-distances and splitting groups below a probability of 0.01. Phylogenetic analyses were performed for four alignments: COI, 28S, COI + 28S (including all individuals), and COI + 28S (including only individuals for which both gene fragments were available). Each alignment was analysed using two phylogenetic methods: maximum likelihood and Bayesian Inference. Prior to phylogenetic analysis, the best-fitting substitution model was determined using MEGA-X following the AIC criterion. Maximum likelihood was run in MEGA-X using subtree-pruning regrafting (level 5) and 1000 bootstrap replicates. Bayesian Inference was run in MrBayes ver. 3.2.7a (Ronquist *et al*. 2012) with nruns 4, nchains 4 and $30*10^6$ generations, sampling every 1000 generations. The first 25% of generations were discarded as burn-in. The resulting trees were visualized using FigTree ver. 1.4.3.

Table 1 (continued next page). Overview of Calliopiidae G.O. Sars, 1893 examined for this study; BOLD IDs, museum accession numbers, sampling locality data and type status. GenBank numbers for sequence data can be accessed via the public BOLD dataset DS-HALIRA (https://doi.org/10.5883/DS-HALIRAGE).

Process ID (BOLD)	museum number	taxon			latitude	longitude	depth	type info
NACA001-23	SMF 52236_1	*Halirages*	*qvadridentatus* G.O. Sars, 1877	A	67.21383	-26.2075	716.5	
NACA002-23	SMF 52236_5	*Halirages*	*qvadridentatus*	A	67.21383	-26.2075	716.5	
NACA003-23	SMF 52236_6	*Halirages*	*qvadridentatus*	A	67.21383	-26.2075	716.5	
NACA004-23	SMF 52189	*Halirages*	*qvadridentatus*	A	62.15233	0.2585	587.4	
NACA005-23	SMF 52238	*Halirages*	*qvadridentatus*	A	62.15233	0.2585	587.4	
NACA006-23	SMF 52200	*Halirages*	*qvadridentatus*	A	62.15233	0.2585	587.4	
NACA007-23	SMF 52200_3	*Halirages*	*qvadridentatus*	A	62.15233	0.2585	587.4	
NACA008-23	SMF 52241_2	*Halirages*	*qvadridentatus*	A	69.111	-9.917	2202.8	
NACA009-23	SMF 52235_3	*Halirages*	*qvadridentatus*	A	67.2135	-26.24166	696.9	
NACA010-23	SMF 52235_4	*Halirages*	*qvadridentatus*	A	67.2135	-26.24166	696.9	
NACA011-23	ZMH K-64590	*Halirages*	*qvadridentatus*	B	65.93536	-3.52878	3702	
NACA012-23	NHMW-CR-28125	*Halirages*	*spongiae* sp. nov. Lörz, Nack & Tandberg		66.05157	-4.00203	3674	holotype
NACA013-23	NHMW-CR-28126	*Halirages*	*spongiae*		66.05157	-4.00203	3674	paratype
	ZMH K-64213	*Halirages*	*spongiae*		66.05157	-4.00203	3674	paratype
	ZMH K-64214	*Halirages*	*spongiae*		66.05157	-4.00203	3674	paratype
	ZMH K-64215	*Halirages*	*spongiae*		66.05157	-4.00203	3674	paratype
	NHMW-CR-28127	*Halirages*	*spongiae*		66.05157	-4.00203	3674	paratype
NACA014-23	SMF 52244_4	*Haliragoides*			62.15233	0.2585	587.4	
NACA015-23	ZMH K-64591	*Halirages*	*fulvocinctus* M. Sars, 1858		64.43656	-11.62897	415	
NACA016-23	ZMH K-64592	*Halirages*	*fulvocinctus*		64.55168	-11.59162	433	
NACA017-23	SMF 52240	*Halirages*	*cainae* d'Udekem d'Acoz, 2012		62.32883	-0.10166	1058.4	
NACA018-23	SMF 52239_2	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA019-23	SMF 52239_4	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA020-23	SMF 52239_5	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA021-23	SMF 52239_6	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA022-23	SMF 52239_8	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA023-23	SMF 52239_9	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA024-23	SMF 52202_1	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA025-23	SMF 52202_2	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA026-23	SMF 52202_3	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA027-23	SMF 52202_5	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA028-23	SMF 52202_6	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA029-23	SMF 52202_7	*Halirages*	*cainae*		62.32883	-0.10166	1058.4	
NACA030-23	SMF 52242_1	*Halirages*	*qvadridentatus*	B	67.60633	-7.00133	2372.6	

Table 1 (continued).

Process ID (BOLD)	museum number	taxon			latitude	longitude	depth	type info
NACA031-23	SMF 52242_2	*Halirages*	*qvadridentatus*	B	67.60633	-7.00133	2372.6	
NACA032-23	SMF 52242_4	*Halirages*	*qvadridentatus*	B	67.60633	-7.00133	2372.6	
NACA033-23	SMF 52242_5	*Halirages*	*qvadridentatus*	B	67.60633	-7.00133	2372.6	
NACA034-23	SMF 52206_3	*Halirages*	*qvadridentatus*	B	63.10166	-8.572	510.9	
NACA035-23	SMF 52244_7	*Halirages*	*qvadridentatus*	B	62.15233	0.2585	587.4	
NACA036-23	SMF 52237_2	*Halirages*	*qvadridentatus*	B	67.60633	-7.00133	2372.6	
NACA037-23	SMF 52203	*Halirages*	*qvadridentatus*	B	63.03133	-1.4985	1858.3	
NACA038-23	SMF 52188_1	*Halirages*	*qvadridentatus*	B	67.57816	-6.93466	2422.4	
NACA039-23	SMF 52241	*Halirages*	*qvadridentatus*	B	69.111	-9.917	2202.8	
NACA040-23	SMF 52241_3	*Halirages*	*qvadridentatus*	B	69.111	-9.917	2202.8	
NACA041-23	SMF 52241_4	*Halirages*	*qvadridentatus*	B	69.111	-9.917	2202.8	
NACA042-23	SMF 52241_5	*Halirages*	*qvadridentatus*	B	69.111	-9.917	2202.8	
NACA043-23	SMF 52241_6	*Halirages*	*qvadridentatus*	B	69.111	-9.917	2202.8	
NACA044-23	SMF 52241_7	*Halirages*	*qvadridentatus*	B	69.111	-9.917	2202.8	
NACA045-23	SMF 52241_8	*Halirages*	*qvadridentatus*	B	69.111	-9.917	2202.8	
NACA046-23	SMF 52235_2	*Halirages*	*qvadridentatus*	B	67.2135	-26.24166	696.9	
NACA047-23	SMF 52235_6	*Halirages*	*qvadridentatus*	B	67.2135	-26.24166	696.9	
NACA048-23	SMF 52236_9	*Halirages*	*stappersi* d'Udekem d'Acoz, 2012		67.21383	-26.2075	716.5	
NACA049-23	SMF 52235_1	*Halirages*	*stappersi*		67.2135	-26.24166	696.9	
NACA050-23	SMF 52190_6	*Halirages*	*fulvocinctus*		67.2135	-26.24166	696.9	
NACA051-23	ZMH K-64593	*Halirages*	*spongiae*		64.80858	-9.53027	2289	
AMPNB110-14	ZMBN_94859	*Halirages*	*qvadridentatus*	A	67.805	9.685	823	
AMPNB313-15	ZMBN_104491	*Halirages*	*qvadridentatus*	B	68.48	9.787	2569	
AMPNB315-15	ZMBN_104493	*Halirages*	*qvadridentatus*	A	68.188	10.356	890	
AMPNB349-15	ZMBN_104527	*Halirages*	*qvadridentatus*	A	67.805	9.685	809	
AMPNB375-15	ZMBN_104553	*Halirages*	*stappersi*		67.35	8.642	842	
AMPNB377-15	ZMBN_104555	*Halirages*	*qvadridentatus*	B	68.247	9.249	2338	
AMPNB530-17	ZMBN_112437	*Halirages*	*fulvocinctus*		79.724	11.093	216	
AMPNB534-17	ZMBN_112444	*Halirages*	*fulvocinctus*		80.152	16.935	363	
DECNB617-18	ZMBN_112670	*Halirages*	*fulvocinctus*		73.833	25.898	458	
ARBEN136-18	ZMBN_154282	*Halirages*	*fulvocinctus*		78.064	26.044	219	
BBAY057-12	BBAY013-01	*Halirages*	*fulvocinctus*	aff.	49.503	-57.818	200	
BBAY058-12	BBAY013-02	*Halirages*	*fulvocinctus*	aff.	49.503	-57.818	200	
BBAY059-12	BBAY013-03	*Halirages*	*fulvocinctus*	aff.	49.503	-57.818	200	
WW501-08		*Halirages*	*fulvocinctus*	aff.	47.691	-69.825	25	
WW502-08		*Halirages*	*fulvocinctus*	aff.	47.691	-69.825	25	

Results

Taxonomy

Order Amphipoda Latreille, 1816
Superfamily Eusiroidea Stebbing, 1888
Family Calliopiidae G.O. Sars, 1893

Genus ***Halirages*** Boeck, 1871

Halirages Boeck, 1871: 114.

Halirages – Boeck 1876: 337. — G.O. Sars 1893: 435. — Stebbing 1906: 290. — Stephensen 1931: 263. — Gurjanova 1951: 605. — Barnard 1969: 177. — Bousfield 1973: 80. — Barnard & Karaman 1991: 322. — Bousfield & Hendrycks 1997: 45. — d'Udekem D'Acoz 2012.
Halirhages – Stuxberg 1880: 23, 27, 28, 47, 68 (erroneous spelling).

Halirages spongiae sp. nov. Lörz, Nack & Tandberg
urn:lsid:zoobank.org:act:BEBF5415-BCDF-4CD2-8BD3-A89F20CB7067
Figs 1–9, Table 1–3

Etymology

'*Spongia'*: Latin for 'sponge'. The name originated from the habitat of the holotype. It was found on a sponge in an abyssal plain east of Iceland.

Type material

Holotype

ICELAND • ♂, 27.9 mm; North-Atlantic; IceAGE 3 cruise, SO276 station 37; 66°03.094′ N, 004°00.122′ W; depth 3674 m; 1 Jul. 2020; collected with the slurp-gun of the ROV from the sponge *Caulophacus arcticus* (Hansen, 1885); NHMW-CR-28125.

Paratypes

ICELAND • 1 ♂, 41.2 mm; same collection data as for holotype; NHMW-CR-28126 • 1 ♂, 40.3 mm; same collection data as for holotype; NHMW-CR-28127 • 1 ♂, 37.4 mm; same collection data as for holotye; ZMH K-64213 • 1 ♂, 45.0 mm; ZMH K-64214 • 1 ♂, 41.1 mm; ZMH K-64215.

Description

Measurements. Large animals up to 4.5 cm with whiplike antennae as long as body. Body length. 27.9–44.9 mm (average length of types 34.7 mm).

Dorsal ornamentation (Figs 2–3). Pereionite 7 and pleonites 1–2 each with strong posterodorsal tooth; pereionite 6 without posterodorsal tooth.

Head (Fig. 4A). Rostrum weak; anterior lobe of head blunt and square (corners rounded), followed posteriorly by narrow sinus; ventral lobe of head pointed, pointing anteriorly, smooth-edged, and not toothed; eye large and subreniform, with defined ommatidia, no pigmentation evident after fixation in alcohol.

Antennae (Figs 2, 4A–C). Antennae 1 slightly longer than Antenna 2, flagella long. Peduncular articles of antenna 1 progressively shorter, flagellum with more than 150 articles; Antenna 2 article 1 of

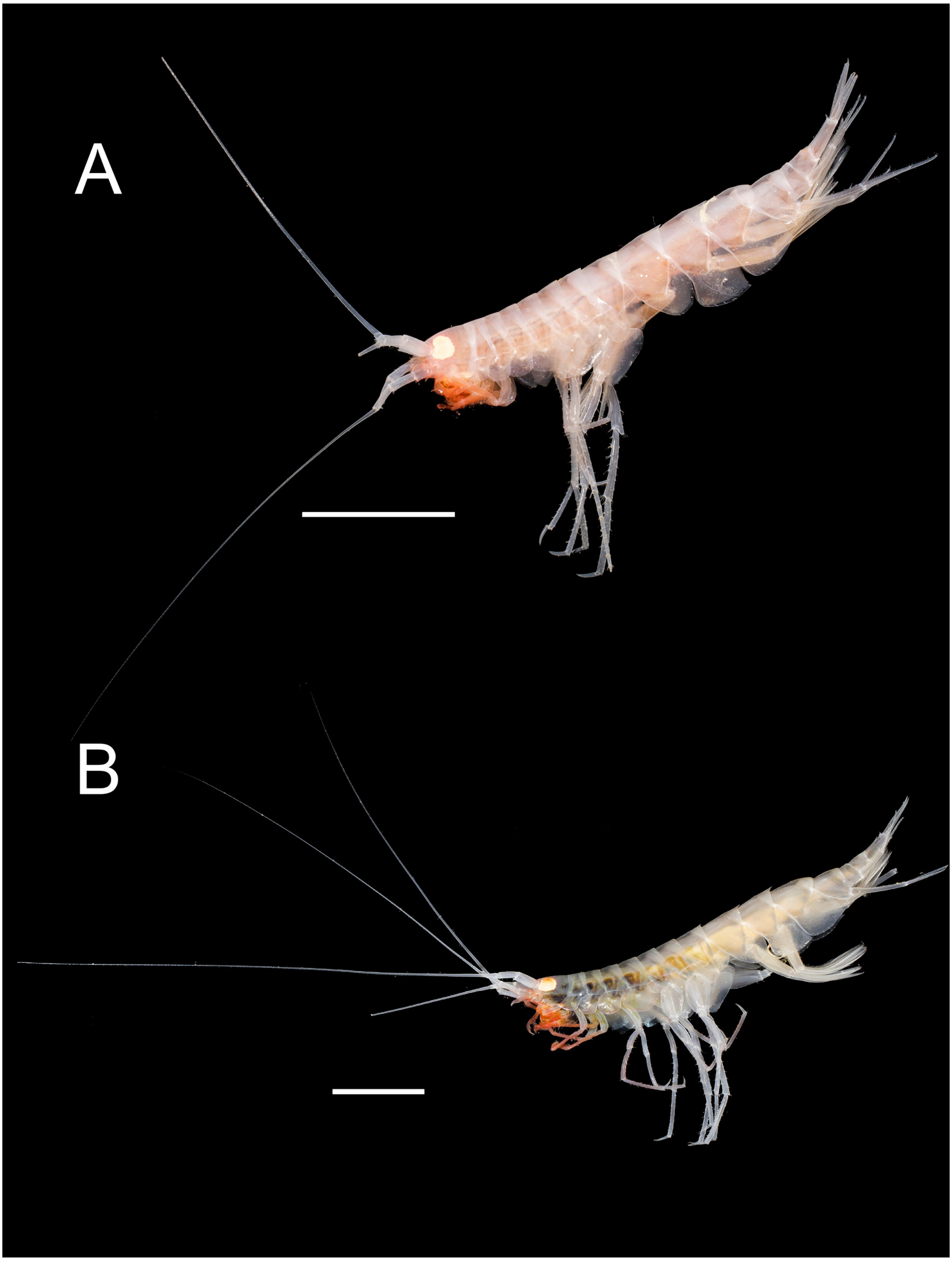

Fig. 2. Photos of live *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg taken on board shortly after sampling. **A**. Holotype, ♂ (NHMW–CR-2812). **B**. Paratype, ♂ (ZMH K-64213). Scale bars = 1 cm.

peduncle with 2 normally developed ventrolateral distal teeth, articles 4 and 5 subequal, flagellum with approximately 200 articles.

LOWER LIP (Fig. 4D). With narrow rounded mandibular processes, inner lobes present, broad outer lobes.

UPPER LIP (Fig. 4E). Apically rounded, entire.

MANDIBLE (Fig. 4F). Incisor process with 3 very blunt teeth; molar ridged, lateral margin, with a row of narrow spines, left molar with anterolateral longer seta; palp article 1 short, with one stronger seta and three short setae; articles 2 and 3 equal in length; article 2 strong, with a row of setae; article 3 falciform, with row of setae, with apical tuft of setae.

MAXILLA 1 (Fig. 4H). Inner plate with 13 pinnate spines, length varying making uneven fringe; outer plate with six cuspidate spines apically; palp well developed, with strong article 2, article 2 with row of long styliform marginal spines and setae (most forming a row) with three longer anterodistal spines (uppermost arising medially).

MAXILLA 2. Damaged.

MAXILLIPED (FIG. 4G). Maxilliped with inner and outer plates broad and subequal; palp of four articles, article 4 shorter than article 3, plates and all article of palp except for dactylus setose.

PEREIOPOD 1 (Fig. 5A). Coxa with anteroventral corner pointing forward, ventral margin with about 10 weak crenulations; carpus longer than wide, as long as basis, anterior margin smooth, posterior margin setose; propodus longer than wide and shorter than carpus; palm sharply dentate, with row of thin setae; dactylus dentate along entire posterior margin.

PEREIOPOD 2 (Fig. 5B). Coxa quadrate, with 10 weak crenulations along ventral margin; carpus about 5 × as long as wide, as long as basis, plumose setae at distal margin; palm sharply dentate, with row of thin setae; dactylus dentate along entire posterior margin; gill length of basis.

PEREIOPOD 3 (Fig. 5C). Coxa quadrate with about 10 weak to almost indistinct crenulations; leg only weakly setose; basis distinctly concave anteriorly and distinctly convex posteriorly, with setae on both margins.

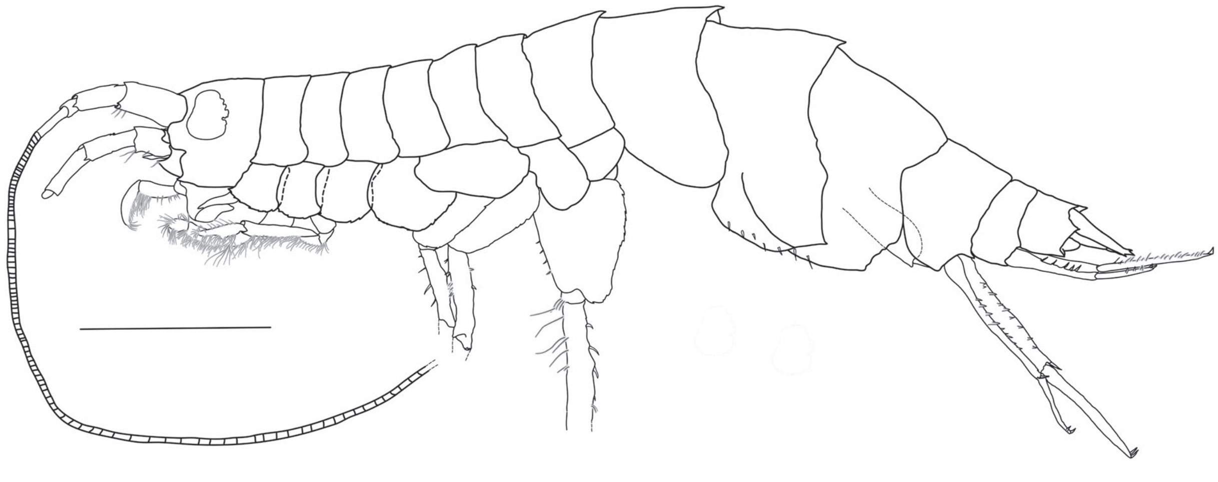

Fig. 3. Habitus of *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg, holotype, ♂ (NHMW-CR-28125). Scale bar = 5 mm.

Pereiopod 4 (Fig. 5D). Coxa wider than long and merging posteriorly into blunt slightly triangular projection, with ventral margin with 10 weakly developed serrations; leg slightly tapering, slightly longer than pereiopod 3; basis concave anteriorly and convex posteriorly, with setae on both margins.

Pereiopod 5 (Figs 2, 5E). Pereiopod 5 < pereiopod 6 < pereiopod 7; posterior lobe of coxa distinctly longer than anterior lobe; leg weakly setose; basis elliptic, anterior margin with spines and sparse

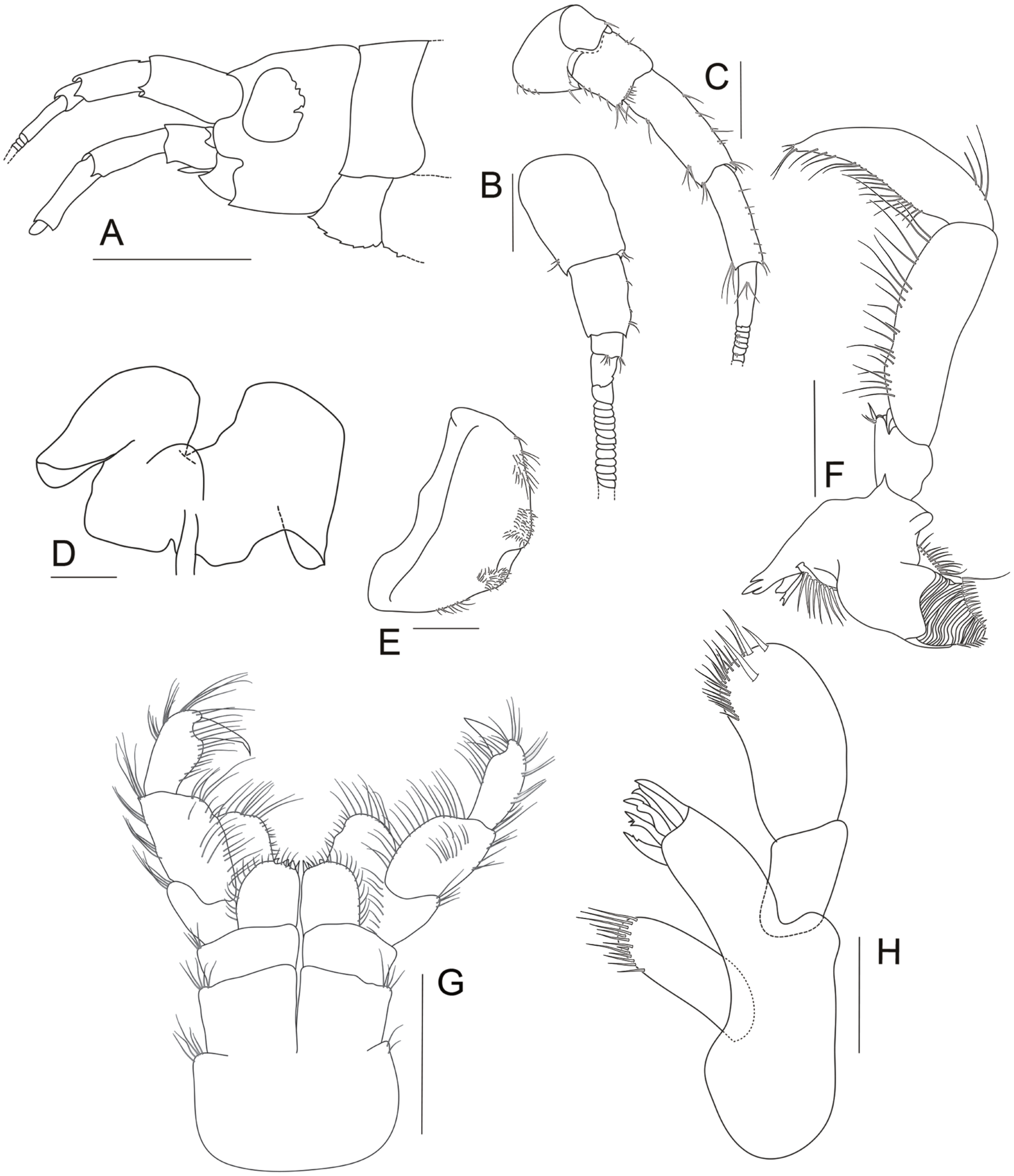

Fig. 4. *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg, holotype, ♂ (NHMW-CR-28125). **A**. Head and beginning of left antennae. **B**. Antenna 1. **C**. Antenna 2. **D**. Labium. **E**. Labrum. **F**. Mandible. **G**. Maxilliped. **H**. Maxilla 1. Scale bars: A = 2 mm; B–C, G = 1 mm; D–F, H = 0.5 mm.

thin setae, distally without tooth, posterior margin with very low to barely discernible crenulations, posterodistal margin rounded and smooth.

Pereiopod 6 (Figs 2, 5F). Posterior lobe of coxa distinctly longer than anterior lobe; basis elliptic, longer than wide, anterior margin with spines and sparse thin setae, posterior margin with indistinct to very low crenulations, posterodistal margin rounded and smooth.

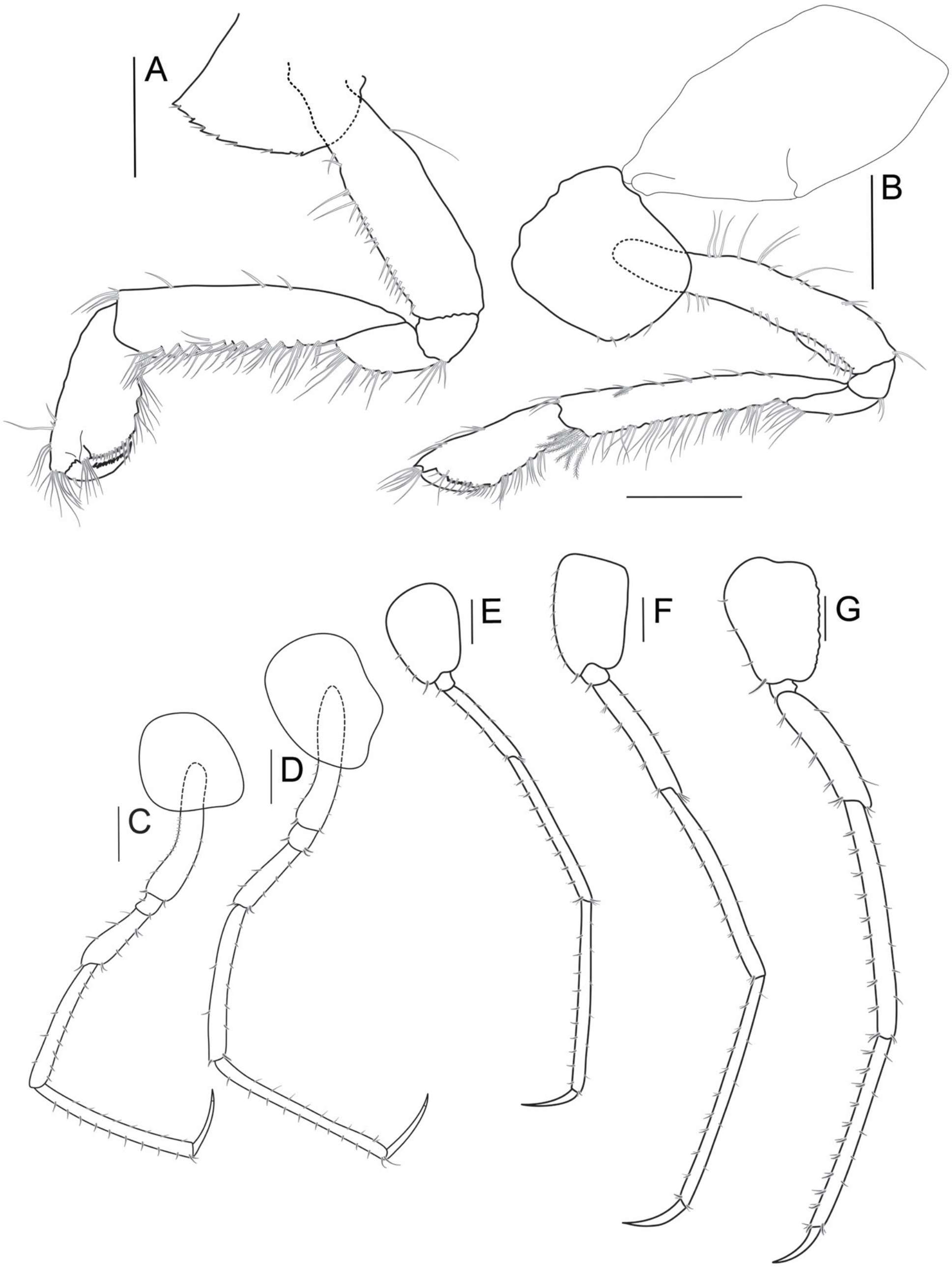

Fig. 5. *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg, holotype, ♂ (NHMW-CR-28125). **A**. Pereiopod 1. **B**. Pereiopod 2. **C**. Pereiopod 3. **D**. Pereiopod 4. **E**. Pereiopod 5. **F**. Pereiopod 6. **G**. Pereiopod 7. Scale bars = 1 mm.

Pereiopod 7 (Figs 2, 5G). Coxa rather small and elliptic in shape; leg weakly setose; basis with anterior and posterior margins straight and converging toward apex, longer than wide, anterior margin with five spines and few thin setae, posterior margin with 18 spines, posterodistal margin with three spines; transition between posterior and posterodistal margins obtusely angular.

Epimeral plate 1 (Fig. 6A). With four isolated spines, posterior border smooth and rounded with minuscle tooth.

Epimeral plate 2 (Fig. 6B). With five isolated spines, with very weak but acute posteroventral tooth, posterior border straight and smooth.

Epimeral plate 3 (Fig. 6C). With three isolated spines, weak posteroventral protrusion, posteroventral edge between the protrusion and posteroventral tooth straight and weakly crenulate.

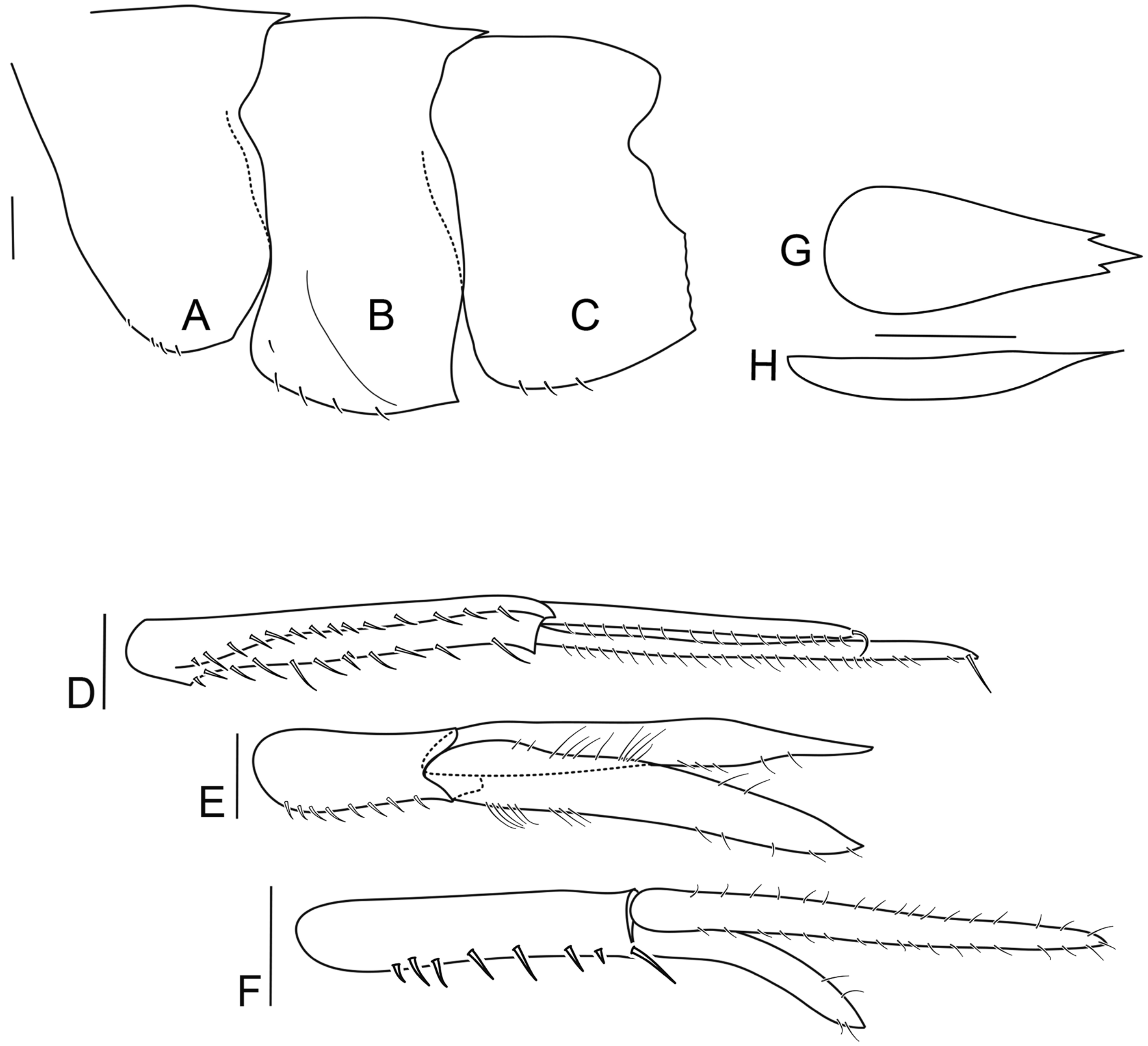

Fig. 6. *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg, holotype, ♂ (NHMW-CR-28125). **A**. Epimeral plate 1. **B**. Epimeral plate 2. **C**. Epimeral plate 3. **D**. Uropod 1. **E**. Uropod 2. **F**. Uropod. 3. **G**. Telson dorsal view. **H**. Telson lateral view. Scale bars = 1 mm.

Uropod 1 (Fig. 6D). Peduncle with dorsolateral and dorsomedial rather slender and irregularly large spines; outer ramus with dorsolateral spines of different sizes, with dorsomedial and apical spines; inner ramus as long as peduncle, with dorsolateral spines, with dorsomedial rather slender and very irregularly large spines; medial margin of inner ramus finely serrate. Apical seta on both rami stronger than other seta of the rami.

Uropod 2 (Fig. 6E). Peduncle with dorsolateral and dorsomedial rather slender, irregularly sized spines; outer ramus with dorsolateral, irregularly sized spines; inner ramus with dorsolateral and dorsomedial spines; medial margin of inner ramus minimally toothed.

Uropod 3 (Fig. 6F). Peduncle with eight distolateral dorsal spines; inner ramus almost twice length of outer ramus; outer ramus with several lateral irregularly sized spines (most small and slender); inner ramus, without distinct bulge, with several lateral spines.

Telson (Fig. 6G–H) Triangular, with convex margin, distally tridentate, without cusp.

Colour pattern (Fig. 2). Body uniformly light pink, mouthparts and pereiopods 1 and 2 distinctly more reddish, eyes white. In alcohol, red pigment remains longer on mouthparts and pereiopods.

Distribution

East off Iceland, 3674 m (currently known only from the type locality).

Remarks

Halirages spongiae sp. nov. Lörz, Nack & Tandberg is morphologically closest to *H. cainae* d'Udekem d'Acoz 2012 and *H. qvadridentatus* G.O. Sars, 1877, but differs in the following characteristics (see also Table 2): the dorsal ornamentation of *H. spongiae* and *H. cainae* show teeth on pereonite 7, pleonites 1

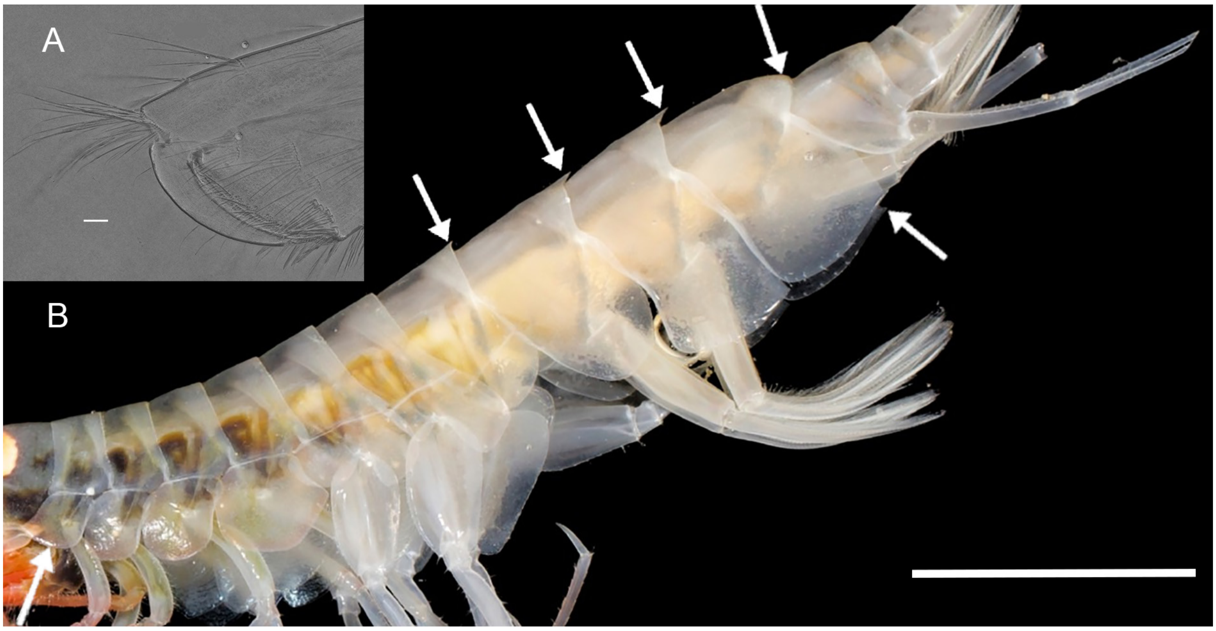

Fig. 7. *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg, paratype, ♂ (ZMH K-64213). **A**. Pereiopod 1, palm. **B**. Defining characters such as the posterodorsal ornamentation and pleon, the extension of the first coxa and the crenulation of the third epimeral plate mentioned in the text and Table 2 discussing the *Halirages* Boeck, 1871 OTUs. Scale bars: A = 100 μm; B = 1 cm.

and 2, whereas *H. qvadridentatus* shows the additional tooth on pereonite 6. The anteroventral corner of coxa 1 forms a square in *H. cainae* while in *H. spongiae* and *H. qvadridentatus* the corner is projected anteriorly. In addition, the species differ in their ornamentation of posterior border of basis of P7; in *H. cainae* this is distinctly serrate whilst in *H. spongiae* only weakly serrate. *Halirages spongiae* bears a small protrusion on the posterior border of epimeral plate three, whereas this is absent in *H. cainae* and *H. qvadridentatus*. Also, the tip of the telson differs; in *H. cainae* the telson forms a single tooth at the tip, while the telson of *H. qvadridentaus* and *H. spongiae* is tridentate.

Freshly caught *H. cainae* is dark red (d'Udekem d'Acoz 2012: fig. 1) whereas live *H. spongiae* sp. nov. Lörz, Nack & Tandberg is coloured light pink (Fig. 2).

Key to described *Halirages* species amended after d'Udekem d'Acoz, 2012 including *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg

1. Ep3 with strong posteromedian and posteroventral tooth (posterior border forming an angular protrusion) (Example A) 2
– Ep3 with posteroventral tooth only (posterior border rounded, but can have a minute posteromedian protrusion) (Example B) 4

Example A

Example B

2. Pereionite 7 with 0–1 posterodorsal tooth; pleonite 1 and 2 with 1 posterodorsal tooth 3
– Pereionite 7 and pleonites 1–2 with 3 posterodorsal teeth .... ***H. nilssoni*** Ohlin, 1895

3. Telson distally truncated and slightly concave; ventral border of coxae 1–2 smooth; posterior border of basis of P5–7 with very weak crenulations; carpus of Gn1–2 equal to propodus .... ***H. fulvocinctus*** (M. Sars, 1859)
– Telson pointed, with large distal tooth flanked by 2 pairs of small lateral subdistal teeth; ventral border of coxae 1–2 and posterior border of basis of P5–7 distinctly serrate; carpus of Gn1–2; distinctly longer than propodus .... ***H. stappersi*** d'Udekem d'Acoz, 2012

4. Ventral lobe of head produced into a sharp tooth; carpus of P1–2 about 4× as long as broad or narrower; at least some segments of pleon with posterodorsal tooth; telson pointed; bathyal and abyssal species 5
– Ventral lobe of head bluntly subquadrate; carpus of P1–2 a bit less than 2× as long as broad; pleon smooth, segments without posterodorsal tooth; telson truncated; infralittoral species .... ***H. mixtus*** Stephensen, 1931

5. Eyes present 6
– Eyes absent 8

6. Eye small and subreniform; coxa 1 with anteroventral corner forming a square angle; ventral border of coxa 1 with about 10 weak crenulations; posteroventral corner of basis of P7 bluntly angular; pereionite 7 (but never 6) and pleonites 1–2 with posterodorsal tooth; tip of telson with a single distal tooth .... ***H. cainae*** d'Udekem d'Acoz, 2012
– Eye large, tip of telson tridentate 7

Table 2. Synoptic overview of morphological characters in the *Halirages* Boeck, 1871 operational taxonomic units (OTUs) examined in this study.

taxa / characters	***H. cainae*** d'Udekem d'Acoz, 2012	***H.* aff *cainae***	***H. qvadridentatus*** G. O. Sars, 1877	***H.* aff *qvadridentatus* A**	***H.* aff *qvadridentatus* B**	***H. fulvocinctus*** (M. Sars, 1858)	***H. stappersi*** d'Udekem d'Acoz, 2012	***H.* aff *stappersi***	***H. spongiae* sp. nov.** Lörz, Nack & Tandberg
coxa 1 anteroventral corner	square	pointing forward	pointing forward	pointing forward	pointing forward	square	pointing forward	pointing forward	pointing forward
coxa 1 ventral ornamentation	weakly crenulate	serrate	strongly serrate	serrate	crenulate	smooth	serrate	crenulate	weakly crenulate
posterodorsal ornamentation on pereon (P) and epimeron (E)	teeth on P7, E1, E2, smooth E3	teeth on P7, E1, E2, hump on E3	teeth on P6, P7, E1, E2, flat hump on E3	teeth on P6, P7, E1, E2, hump on E3; on small specimens: smooth P6, hump P7, teeth E1, E2, smooth E3	teeth on P6, P7, E1, E2, hump on E3	teeth on P7, E1, E2, smooth E3	teeth on P7, E1, E2, smooth E3	thin teeth on P7, E1, E2, smooth E3	teeth on P7, E1, E2, small hump on E3
epimeral plate 3, posterior border	weakly rounded, lower half crenulate	weakly rounded, lower half crenulate	weakly rounded and weakly serrate	weakly rounded, lower half serrate	weakly rounded, lower half crenulate	border between teeth straight and serrate	border between teeth concave and serrate	border between teeth straight and serrate	weakly rounded and serrate
epimeral plate 3, midposterior extension	absent	absent	absent	absent	absent	tooth	tooth	tooth	rounded cup
epimeral plate 3, development of posteroventral tooth	weak tooh	weak tooth	weak but acute posteroventral tooth	weak but acute posteroventral tooth	tiny postroventral tooth	strong tooth	strong tooth	strong tooth	small tooth
tip of telson	with single distal triangular tooth	sharply tridentate	tridentate	large specimens: tridentate; small specimens: thin extended mid tooth, outer teeth rounded	sharply tridentate, all 3 teeth pointing backwards	gradually tapering, tip emarginate	triangular, distally 2 pairs of subdistal teeth	single styliform tooth	tridentate
depth range	2589–2615 m	1058 m	425–1435 m	587–2202 m	696–3702 m	5–670 m	207–1435 m	696–842 m	3674 m
distribution	North Atlantic, East Norwegian Sea	North Atlantic, Faroe Channel	North Atlantic, from Baffin Bay to Laptev Sea, Faroes	North Atlantic, on continental shelf breaks	North Atlantic, shelf to mid basins	Arctic and subarctic, probably circumpolar distribution	North Atlantic, North and West off Iceland and North off Faroe Islands	North Atlantic, on continental shelf breaks	North Atlantic, East off Iceland

7. Eye large and subreniform; coxa 1 with anteroventral corner pointing forwards; ventral border of coxa 1 with about 10 weak crenulations; posteroventral corner of basis of P7 angular; pereionite 7 (but never 6) and pleonites 1–2 with posterodorsal tooth; tip of telson tridentate .... ***H. spongiae* sp. nov.** Lörz, Nack & Tandberg
– Eye large and broad; coxa 1 with anteroventral corner pointing anteriorly; ventral border of coxa 1 with about 20 pronounced serrations; posteroventral corner of basis of P7 forming a sharp square angle; pereionite 7 (and often 6) and pleonites 1–2 with posterodorsal tooth; tip of telson tridentat .... ***H. qvadridentatus*** G.O. Sars, 1877

8. Ventral border of coxa 1–2 with pronounced serrations .... ***H. gorbunovi*** Gurjanova, 1946
– Ventral border of coxa 1–2 smooth or nearly so .... ***H. caecus*** Kamenskaya, 1980

Molecular genetics

Alignments, species delimitation and phylogenetics

The final COI alignment had a length of 468 bp (as several sequences were not obtained at full length, the alignment was shortened accordingly) without any indels or stop codons. The 28S alignment has a length of 1297 bp.

ASAP delimited seven putative species of *Halirages* with a COI threshold distances of 3.6% (gap ~3.0–7.6%) (ASAP score 2.0). These included *H.* aff. *stappersi*, two putative species in *H. fulvocinctus* (in the following *H. fulvocinctus* and *H.* aff. *fulvocinctus*), *H.* aff. *cainae,* two species within *H.* aff. *qvadridentatus* (in the following *H. qvadridentatus* A and B) as well as *H. spongiae* sp. nov. Lörz, Nack & Tandberg (Fig. 9). The second best scoring partition (ASAP score 3.0; threshold distance 12.4%) collapsed *H.* aff. *cainae*, *H.* aff. *qvadridentatus* A and *H.* aff. *qvadridentatus* B into a single putative species.

COI uncorrected *p*-distances between *H.* aff. *qvadridentatus* A and *H.* aff. *qvadridentatus* B were 9.7–10.9% and between these and *H.* aff. *cainae* 7.1–12.2% (*H.* aff. *qvadridentatus* A exhibiting the lower distances) (Table 3). *Halirages fulvocinctus* and *Halirages* aff. *fulvocinctus* are separated by uncorrected *p*-distances of 8.6–9.0% in COI; all other species pairs had distances > 16% (Table 3). 28S distances were generally lower, also here distances between putative species exceeded those observed within species. Intraspecific distances were ≤ 0.2% and interspecific 0.5–1.5% between the three geentically most similar species *H.* aff. *cainae, H.* aff. *qvadridentatus* A and *H.* aff. *qvadridentatus* B and ≥1.6% between all other species pairs (Table 3).

Halirages fulvocinctus and *H.* aff. *fulvocinctus* are geographically separated, with *H. fulvocinctus* occurring from east Iceland to Svalbard and *H.* aff. *fulvocinctus* B along eastern Canada (Fig. 8). *Halirages* aff. *qvadridentatus* A and *H.* aff. *qvadridentatus* B occur sympatrically over a wide region in the Arctic deep sea, ranging from Greenland to Norway. Moreover, they occur sympatrically with the closely related *H.* aff. *cainae* north of the Shetland Islands (Fig. 8).

Phylogenetic analyses of COI, 28S and COI+28S mirrored the overall ASAP results (Fig. 9). With a few exceptions, the seven species of *Halirages*, which had been suggested by ASAP, were each recovered as monophyletic groups with high support values. Only *H.* aff. *cainae* was non-monophyletic in the 28S analyses and the support values of *H.* aff. *cainae* was low in the Bayesian analyses of COI. However, all individuals of *H.* aff. *cainae* clearly grouped together and were genetically highly similar and distinguished from all other putative species (see also Table 3). *Halirages* aff. *qvadridentatus* A and *H.* aff. *qvadridentatus* B were always in sister-group relationships, though usually with rather low support and formed a highly supported clade with *H.* aff. *cainae* in all analyses. The newly identified *H. spongiae* sp. nov. Lörz, Nack & Tandberg and *H. aff. stappersi* were always in a sister group relationship, but their

Table 3. COI and 28S genetic distances. Top values are always COI and bottom values 28S uncorrected *p*-distances. Intraspecific distances are shown along the diagonal and interspecific distances above the diagonal.

	H. aff. *cainae*	*H.* aff. *qvadridentatus* A	*H.* aff. *qvadridentatus* B	*H.* aff. *fulvocinctus*	*H. fulvocinctus* (M. Sars, 1858)	*H.* aff. *stappersi*	*H.* **spongiae** sp. nov. Lörz, Nack & Tandberg
H. aff. *cainae*	0.0-1.8 0.0-0.2	7.1-8.3 1.0-1.4	11.1-12.7 0.5-0.7	23.1-23.7 11.8-12.6	23.7-24.7 NA	20.9-21.6 2.7-2.9	18.8-19.3 2.4-2.5
H. aff. *qvadridentatus* A		0.0-1.0 0.0-0.2	9.7-10.9 1.2-1.5	24.2-24.6 12.8-13.0	23.8-25.0 NA	19.5-20.0 3.7-3.8	18.1-18.5 3.2-3.4
H. aff. *qvadridentatus* B			0.0-0.8 0.0-0.2	24.0.-24.4 12.2-12.3	25.7-26.7 NA	19.7-20.4 3.2-3.3	19.0-19.6 2.9-3.1
H. aff. *fulvocinctus*				0.0 0.0-0.2	8.6-9.0 NA	23.7 12.4-12.5	23.1 11.9-12.0
H. fulvocinctus (M. Sars, 1858)					0.4 NA	23.9-24.3 -	23.7-23.9 -
H. aff. *stappersi*						0.2 NA	16.3-16.5 1.6
H. spongiae sp. nov. Lörz, Nack & Tandberg							0.2 NA

affinities were not consistently recovered (either closer related to *H.* aff. *qvadridentatus* and *H.* aff. *cainae* or to *H. fulvocinctus* and *H.* aff. *fulvocinctus*). *Halirages fulvocinctus* and *H.* aff. *fulvocinctus* were always sister species (it should be noted that no 28S sequences were available for *H.* aff. *fulvocinctus* B).

Morphological investigations of Halirages OTUs collected during recent expeditions to the North Atlantic

Calliopiid specimens from recent expeditions to the North East Atlantic of the genus *Halirages* were identified to the lowest taxonomic level possible (Table 1). Small morphological details differ compared to most of the described species in *Halirages*, these morphological characters are listed in Table 2 and in the text below, where we present the morphological characteristics of the Operational Taxonomic Units (OTUs) suggested by our molecular analyses.

Except for *Halirages fulvocinctus*, none of the encountered specimens of Calliopiidae fully matched a current species description. Most OTUs were morphologically highly similar to accepted species of *Halirages*, but differed in minute characteristics, which are listed below. Whether these minute morphological differences represent intraspecific variability of the respective species or indicate the presence of species new to science cannot be answered here. We highlight this taxonomic uncertainty by stating the affinity to the respective species instead of providing a definitive species identification. Figure 7 illustrates placement of dorsal teeth and hump discussed under the different OTUs. For geographic distribution of the OTUs, see Fig. 8 (map). Overview of OTUs encountered:

- *Halirages* aff. *cainae*: 13 specimens from IceAGE expeditions (one station in Faroe channel/ Storegga). Three sharp dorsal teeth starting on pereonite 7 and a round hump on epimeron segment 3; Coxa 1 forward pointing, contrasting the square angle of *H. cainae* d'Udekem d'Acoz, 2012, serrate; epimeral plate 3 with rounded serrations with a small distinctive tooth posterodistally; telson is sharply tridentate, whereas *H. cainae* d'Udekem d'Acoz, 2012 has a single dorsal tooth on its telson.
- *Halirages* aff. *qvadridentatus* A: ten specimens collected during IceAGE between Iceland and Greenland, north of Iceland, three specimens collected by the University of Bergen on shelf-break off Lofoten (N Norway). Four visible dorsal teeth starting from pereonite 6, on small specimens only two dorsal teeth starting on epimeron segment 1, together with a rounded hump on pereonite 7. Coxa 1 forward pointing, serrate; epimeral plate 3 rounded posterior margin, lower half serrate, very small tooth on posterodistal corner; telson tridentate, on smaller specimens with the outer teeth rounded around a longer and thin median tooth.
- *Halirages* aff. *qvadridentatus* B: 19 specimens collected during IceAGE between Iceland and Greenland, north and east of Iceland, two specimens collected by the University of Bergen from deeper stations off Lofoten than *H. qvadridentatus* aff A. Large specimens. Three sharp teeth starting from pereonite 7, on smaller specimens the first tooth is very small; coxa 1 rounded with a very slight direction forwards, much less than the forward-pointing coxae 1 in the other taxa, ventral margin crenulate; epimeral plate 3 very weakly crenulate lower half of posterior margin and a minuscule tooth posterodistally; telson sharply tridentate, with all three teeth pointing backwards.

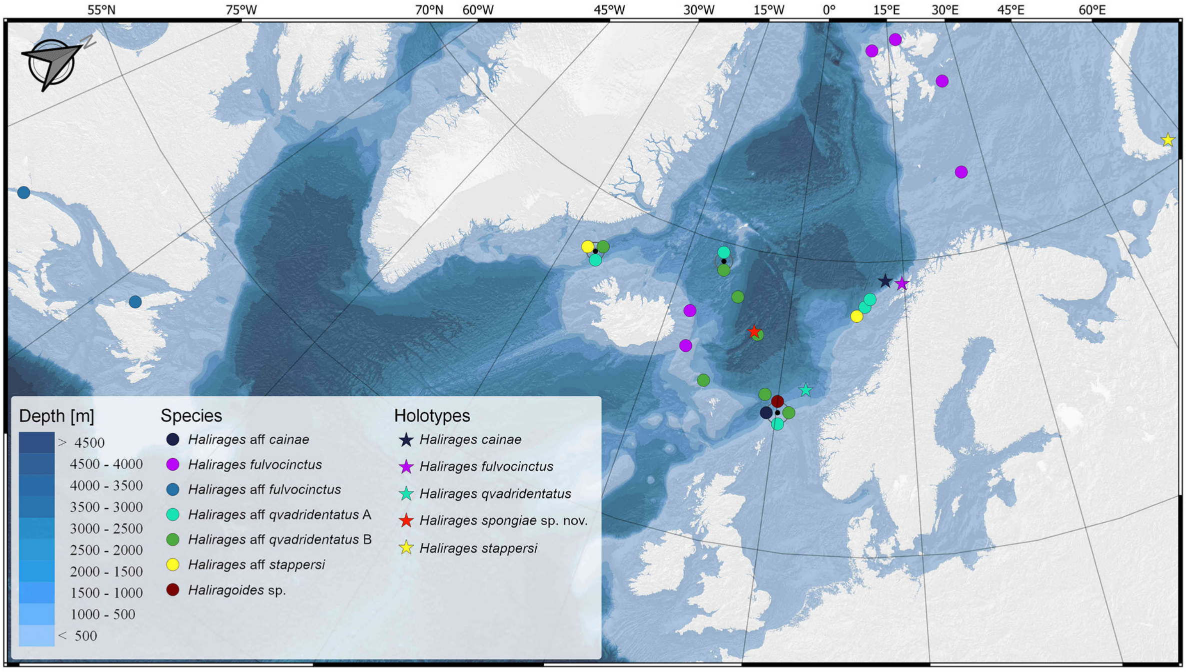

Fig. 8. Distribution of *Halirages* Boeck, 1871, opperational taxonomic units discussed in the present paper. Circles indicate specimens used in phylogenetic analysis, stars indicate type localities for the indicated taxa. (Apart from *Halirages* aff. *fulvocinctus*, from Canada, all specimens have been sampled by the authors.)

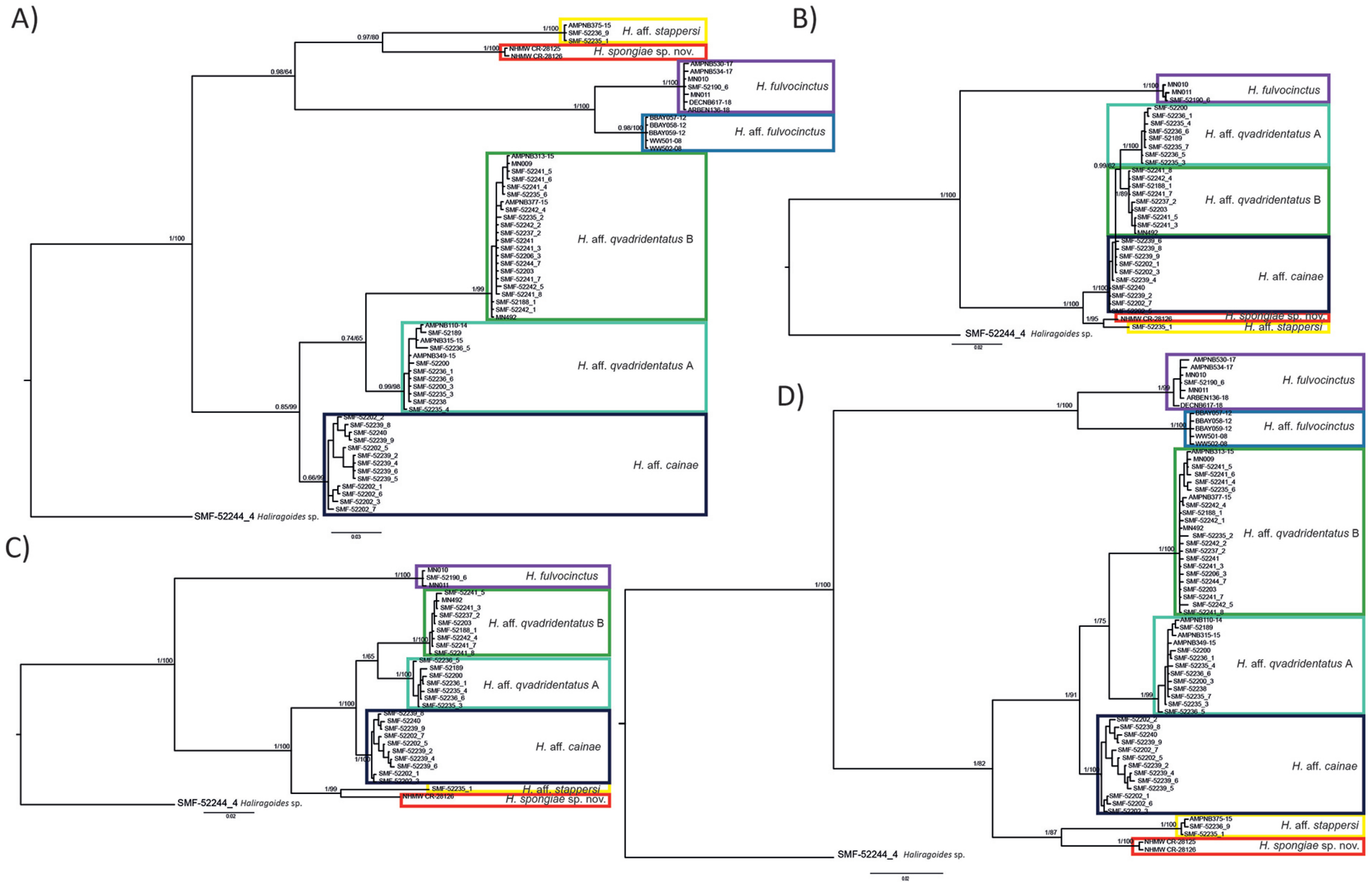

Fig. 9. Phylogenetic analyses of *Halirages* Boeck, 1871. **A**. COI. **B**. 28S. **C**. COI+28S (including only individuals for which both genes were available). **D**. COI+28S (including all specimens). The depicted topologies are based on the Bayesian inference analysis. Bayesian posterior probabilities and bootstrap support from the Maximum Likelihood analyses are shown for each node.

- *Halirages fulvocinctus*: three specimens collected during IceAGE east of Iceland, 4 specimens collected by the University of Bergen from Barents Sea and north and east of Spitsbergen. Three sharp teeth starting from pereonite 7, third epimeron with a rounded hump; coxa 1 smooth and rounded, not pointing forwards; epimeral plate 3 two posterior teeth, margin between teeth serrate, the lower posterodistal tooth strong; telson emarginate.
- *Halirages* aff. *fulvocinctus*: five COI sequences derived from BOLD, two locations in the western Atlantic, Bay of St Lawrence. Based on the images provided in BOLD the tooth on pereionite 7 is missing.
- *Halirages* aff. *stappersi*: two specimens collected during IceAGE between Iceland and Greenland, one specimen collected by University of Bergen from southwest of Lofoten. Dorsally smooth; coxa 1 strongly serrate; epimeral plate 3 with two small teeth on posterior margin, with crenulations between the teeth; telson with single styliform tooth, on one specimen the tooth is bifurcated.
- *Halirages spongiae* sp. nov. Lörz, Nack & Tandberg, six specimens, is described above.

Discussion

The entire Calliopiidae family is in need of revision (Ringvold & Tandberg 2014). D'Udekem d'Acoz (2012) stated that the differences between *Halirages* and *Apherusa* Walker, 1891 are blurred. With the exception of the presence (*Halirages*) or absence (*Apherusa*) of calceoli in terminal males, a careful comparison of illustrations of species within the two genera does not reveal any stable differential morphological character states. Character states previously considered as differential, such as the size and shape of article 3 of mandibular palp, the length of uropods and telson, proved to be too variable to be retained (d'Udekem d'Acoz 2012). We fully agree with the observations made by d'Udekem d'Acoz (2012). The phylogenetic separation of *Apherusa* and *Halirages* is beyond the scope of this study.

The genetic distances observed within and between the herein studied species of *Halirages* suggest the presence of hitherto unknown species. Genetic distances of ~7% in COI have been previously suggested as a suitable DNA barcoding threshold for species within most marine amphipod families (Tempestini *et al.* 2018; Jażdżewska *et al.* 2018; Lörz *et al.* 2018), which corresponds to the difference of ~7–9% observed between the two OTUs within *H.* aff. *qvadridentatus* (including the separation from the closely related and morphologically similar *H.* aff. *cainae)* and *H. fulvocinctus* as well as the difference of > 16% among all other species pairs. Relying solely on genetic distances in the mitochondrial and strictly maternally inherited COI can overestimate species diversities in evolutionary old and thus genetically highly diverse and divergent species. For example, for the freshwater amphipod *Echinogammarus sicilianus* Karaman & Tibaldi, 1972, multiple genetically highly divergent lineages (exceeding 10% genetic distance in COI) were identified (Hupało *et al.* 2022). However, nuclear data suggest that many of these divergent lineages are not reproductively isolated and thus belong to a single biological species (Hupało *et al.* 2022). The situation is very different for the two putative species differentiated in *H.* aff. *qvadridentatus* as well as the closely associated *H.* aff. *cainae*. Here, analyses of the nuclear 28S gene showed clear and consistent genetic differences between the three putative species. All three species were sympatrically recorded north of the Shetland Islands, and the two species of *H.* aff. *qvadridentatus* had widely shared or overlapping distributions in the North Atlantic deep sea. The mito-nuclear concordance in combination with sympatric occurrence is a very strong indicator of reproductive isolation between *H.* aff. *qvadridentatus* A*,* *H.* aff. *qvadridentatus* B and *H.* aff. *cainae* and thus for their status as distinct biological species (sensu Mayr 1942): the three OUT's have the possibility to inter-breed in their natural habitat but did not (see also Meier & Willmann 2000; Schwentner *et al.* 2011, 2015). These three OUTs were the genetically most closely related taxa in our study. Given their reproductive isolation, we are confident that all other species of *Halirages* studied here are reproductively isolated from each other, even if not all of them were recorded sympatrically.

The first morphological analysis of the specimens revealed subtle differences, allowing their separation into Operational Taxonomic Units (OTUs). These OTUs were molecularly supported and were candidates for full descriptions of species. However, for several reasons we hesitated to formally describe these species (except for *H. spongiae* sp. nov. Lörz, Nack & Tandberg, which was clearly a species new to science): we do not have samples from the original type localities, and for some of the species (notably *H. qvadridentatus*) the types are missing. The presented morphological differences are very subtle; therefore, we are not sure whether these are variations of previously described species or consistently different character states. Ontogenetic changes can also occur. For *Halirages* aff. *qvadridentatus* A and *Halirages* aff. *qvadridentatus* B we have described allometric characters, such as the dorsal dentation and shape of the telson tip. Because of the subtleness of morphological differences and the uncertainties regarding the morphological variations of the taxonomic units, we have not described the different OTUs as new species in the present paper. Some of these OTUs may represent species known to science, whereas others may be morphological variants of known species. Additional genetic data, ideally from morphologically unambiguously identified specimens collected closer to the type localities of the species, will be needed to solve this issue.

Sequences assigned to *H.* aff. *fulvocinctus* were obtained from the BOLD database. We were unable to assess detailed morphological differences between *H. fulvocinctus* and *H.* aff. *fulvocinctus*; but the images shown in BOLD for three of the five Canadian specimens show an indistinct or absent tooth on pereionite 7 contrasting the *H. fulvocinctus* from the eastern Atlantic, which do have a distinct tooth on pereionite 7. Based on their geographic distribution and type locality (northern Norway, at a depth of a few meters), we are convinced that the eastern species (occurring from Iceland to Norway) is the true *H. fulvocinctus* and the Canadian species *H.* aff. *fulvocinctus* is a morphologically similar new species.

Our analyses focusing on Icelandic waters include only one of the nine previously recognised species of *Halirages* (see d'Udekem d'Acoz 2012) with certainty. With this limited dataset, a large number of likely new species could be recognised, raising the number of observed (but not formally taxonomically described) species by about 70%.

Halirages fulvocinctus and *H.* aff. *fulvocinctus* might be another example of a species, that was believed to be geographically widely distributed, but actually a complex of two or more species with narrower geographical distributions. Although narrower than initially assumed for *H. fulvocinctus*, the established geographic distribution of *H. fulvocinctus* from eastern Iceland to Svalbard remains extensive. *Halirages* aff. *qvadridentatus* with the two OTUs A and B discussed in the present paper, however, exemplify that such morphologically close species are not necessarily geographically separated. Denser sampling might reveal additional species of the *Halirages* even in the relatively well studied waters of the North Atlantic.

The discovery that the new species uses sponges of *Cauliphacus* as habitat in the abyssal plain would not have been possible without designated ROV video transects for habitat description. Video- and image-based tools provide exclusive insights into the habitats and community structure of deep-sea ecosystems (Taylor *et al.* 2017; Morganti *et al.* 2021; Meyer *et al.* 2023). Thus, ROV-based sampling improves our understanding of community structure, species interactions, and biological habitats. This makes it increasingly clear that many deep-sea benthic species live in association with other specific species. Porifera Grant, 1836 are well known as habitat-builders, but the specificity of sponge species to its associated hyperbenthos in the North Atlantic is not as well described (but see Amsler *et al.* 2009). Since *Halirages* are predators not feeding on the sponge but using it as elevation to be higher in the current to catch prey, most likely any hard substrate sticking out of the softer surrounding sediments can be used, including a tin can (see Fig. 1E–F). The fact that our observations of this new species of *Halirages* comes from sponges of *Caulophacus* adds information about both species.

Acknowledgements

Sampling of the specimens of *Halirages sponigiae* sp. nov. Lörz, Nack & Tandberg was supported by the GEOMAR ROV team during Sonne cruise SO276 and possible due to the financial support of the German Science Foundation (grant number MerMet17-06 to SB) and the BMBF (The Federal Ministry of Education and Research). This publication is a contribution to the iAtlantic HORIZON2020 project based on the iAtlantic expedition SO276 (IceAGE3).

Anne-Nina Lörz was financed by the German Science Foundation project IceAGE Amphipoda (LO2543/1-1). Anne Helene S. Tandberg was financed through the Norwegian Biodiversity Initiative grant NorAmph (NBIC 46-15 and NBIC 16-18). Vivien Lukas Hartmann helped with the making of the map in QGIS.

We thank Dr Charles Oliver Coleman (Natural History Museum Berlin) for the introduction of Madita Nack to amphipod illustrations and the three anonymous reviewers for their helpful and constructive comments on an earlier version of the manuscript.

Karen Jeskulke, Antje Fischer (DZMB Hamburg), Kathrin Philipps-Bussau (LIB Hamburg), Prof. Angelika Brandt, Bianca Trautwein and Kristin Arnold (Senckenberg Museum Frankfurt) are thanked for the database, curation and loan of specimens.

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Manuscript received: 6 April 2023
Manuscript accepted: 6 December 2023
Published on: 28 March 2024
Topic editor: Magalie Castelin
Section editor: Fabio Stoch
Desk editor: Eva-Maria Levermann