



Comparative molecular and morphological species delineation of *Halammohydra* Remane, 1927 (Hydrozoa)—with the description of four new species

Lenke Tödter¹ · Katrine Worsaae² · Andreas Schmidt-Rhaesa^{1,3}

Received: 21 July 2022 / Accepted: 27 January 2023 / Published online: 1 March 2023
© The Author(s) 2023

Abstract

Whereas most cnidarians are macrofaunal, a few microscopic lineages have evolved, and some of them inhabit marine sediments. The meiofaunal genus with the most species is *Halammohydra*, comprising nine nominal species. Species are described with high intraspecific variability in, e.g., the number of tentacles and statocysts and the shape and length of tentacles and body, complicating morphological identification to species level. Additionally, there is not much molecular data available. This study aims to revise already described species with morphological and molecular methods, as well as, to delineate potential new species answering questions about their geographical distribution. For this, specimens were sampled at 16 locations in the Northwest Atlantic and two localities in the East Atlantic, documented with light microscopy, and fixed individually for sequencing (16S, 18S, and CO1). Herewith, morphological characters were linked to a specific sequence, enabling the testing of character variation within one molecular phylogenetic group. Phylogenetic analyses were conducted (Bayesian Interference and Maximum Likelihood) in combination with species delimitation tests (ABGD, GMYC, and bPTP). Four already described species were identified in the data sets, and all of these were found at multiple localities. Four new species are described. Overall, the combined molecular and morphological data acquisition revealed multiple new species and a high degree of sympatry in *Halammohydra*. This, together with the confirmed excessive intraspecific variation in morphological traits, underlines the necessity of molecular sequencing for the taxonomy and species identification of *Halammohydra*.

Keywords Integrative taxonomy · Species delimitation · Meiofauna · Sanger sequencing · Cnidaria · *Halammohydra*

Introduction

The meiofauna is a highly diverse assemblage of animals across several phyla inhabiting sediments from the intertidal zone to the deep-sea, sharing a microscopic body size (Giere, 2009). Other features, such as elongated body shape,

reduction of long appendages, or the presence of special adhesive structures, help these animals to move between the sand grains in the interstitial environment and even withstand stronger currents. Because of their small body size and general lack of pelagic larvae, the dispersal ability is expected to be rather low (Giere, 2009). Nevertheless, several studies found a broad geographical range for some species, up to a cosmopolitan distribution (e.g., Bik et al., 2010; Boeckner et al., 2009; Cerca et al., 2018; Faurby et al., 2011; Fontaneto, 2019; Guil, 2011; Hagerman & Rieger, 1981; Schmidt & Westheide, 2000; Worsaae et al., 2019a). This contradiction is called the “meiofauna paradox,” and several hypotheses have been put forward to its explanation (see, e.g., Sterrer, 1973; Westheide, 1991; von Soosten et al., 1998; Giere, 2009). Over the last decades, molecular studies discovered a high amount of cryptic diversity in some meiofaunal animals (e.g., Fontaneto et al., 2009; Jörger et al., 2012; Leasi et al., 2016; Tessens et al., 2021; Todaro et al.,

✉ Lenke Tödter
l.toedter@leibniz-lib.de

¹ Museum of Nature – Zoology, Center for Taxonomy and Morphology, Leibniz Institute for the Analysis of Biodiversity Change (LIB), Zoological Museum, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

² Marine Biological Section, Department of Biology, University of Copenhagen, Universitetsparken 4, 2100 Copenhagen O, Denmark

³ University Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

1996; Worsaae et al., 2019b), which resulted in a taxonomic revision and the splitting of previously considered cosmopolites into taxonomic units with more restricted ranges. Population genetic methods may not provide the complete solution to the meiofauna paradox but especially in meiofauna with somewhat limited identification features, it is of major importance (Cerca et al., 2018; Fontaneto et al., 2015). Hence, more species revisions and, or in combination with, molecular data are needed.

Halammohydra Remane, 1927, is a genus of interstitial cnidarians with nine accepted nominal species (Schmidt-Rhaesa et al., 2020), whose morphological identification is notoriously difficult. Most species are described and recorded from Europe, and three were described exclusively from India (Schmidt-Rhaesa et al., 2020). The majority of European records are from Germany (Clausen, 1967; Ehlers, 1993; Polte & Schmidt-Rhaesa, 2011; Remane, 1927; Schmidt, 1969; Schulz, 1952; Tödter & Schmidt-Rhaesa, 2022) and France (d'Hondt, 1968; Renaud-Debyser, 1964; Swedmark & Teissier, 1967, 1957a, 1959; Teissier, 1950). Other locations are Sweden (Boaden, 1960; Dahl, 1953), the UK (Boaden, 1961, 1963, 1966; Gray, 1971; Harvey & Wells, 1961; Moore, 1979), Norway (Clausen, 1963, 1967, 1991, 2000, 2004), Netherlands (Wolff et al., 1974), Adriatic Sea (Salvini-Plawen, 1991), Azores (Tödter & Schmidt-Rhaesa, 2021), and Spain (Martínez et al., 2009, 2019). Outside of Europe, most records are from India (Altaff et al., 2005; Janakiraman et al., 2016; Mohan & Dhivya, 2010; Nagabhushanam, 1972; Rao, 1975, 1978, 1993; Rao & Ganapati, 1965, 1966; Rao & Misra, 1980; Salvini-Plawen & Rao, 1973; Sugumaran & Padmasai, 2019; Sugumaran et al., 2009; Varadharajan & Soundarapandian, 2013) and only a few from the Western Atlantic and Caribbean Sea (Bush & Zinn, 1970; Calder & Kirkendale, 2005; Garraffoni et al., 2017; Hochberg et al., 2014; Jörger et al., 2014; Kånneby et al., 2014). *Halammohydra* is exclusively reported from interstitial environments of sandy sediments, ranging from fine to coarse sand or shell gravel.

In the Northwest Atlantic, *Halammohydra* has mainly been sampled in the vicinity of marine biological research stations, especially in Helgoland (Germany), Roscoff (France), and Bergen (Norway). Five of the six European species were recorded around the small island of Helgoland, some of them in sympatry. Taken the relative scarcity of records and species, this diversity of species within localities is surprising. Moreover, the general scarcity of diagnostic morphological traits contradicts the sympatric distribution of several species, indicating physiological rather than morphological specializations.

Halammohydra is a modified medusa with a completely reduced umbrella (Remane, 1927). The main body is a gastric tube, or manubrium, with a mouth opening. On the aboral end, the diameter decreases and forms a neck connecting to

an aboral cone, the reduced umbrella. One whorl of statocysts and two whorls of tentacles connect to the aboral cone, and the aboral tip bears a special adhesive structure, the so-called adhesive organ. The whole body is ciliated. Due to its unique morphology, *Halammohydra* has been placed in various positions of the cnidarian phylogenetic tree. Remane (1927) placed it in Trachylinae (Hydrozoa) because of the lack of a pelagic phase and the type of statocysts, and further into Narcomedusae because of similarities to larval stages of other species herein. Swedmark and Teissier (1958) described another fully ciliated medusa in a new monotypic genus *Otohydra*. Remarkably, they chose to erect the name Otohydridae for this genus, rather than referring it to Halammohydridae (only containing *Halammohydra*), despite their proposed close relationship of these two families in the group Actinulida (Swedmark & Teissier, 1959). The exact placement of *Halammohydra* remained unclear for a long time, until Collins et al. (2008) conducted a molecular study of Trachylinae including sequences of an unidentified species of *Halammohydra*. This study indicated that *Halammohydra* has an origin within the family Rhopalonematidae in Trachymedusae (Trachylinae) rather than within Narcomedusae (Trachylinae).

No further molecular data are available, especially no sequences of individuals identified to species level. This is problematic, because the identification of species in this genus is rather difficult. The size and shape of the whole body and the aboral cone, the number and length of the tentacles in each whorl, and the shape of the tentacle bases connecting to the aboral cone are the main diagnostic characters. In some cases, the cnidome is also used for identification. The assessment of these characters is difficult because of their morphological variability, which is exacerbated by the contractibility of the animals. Moreover, some characters, such as the number of tentacles, have a wide range, which overlaps with other species and thus makes the identification of a single specimen challenging. For example, the total number of tentacles in *Halammohydra schulzei* Remane, 1927, was described as 14 to 26 (Remane, 1927; Swedmark, 1957; Swedmark & Teissier, 1957a) and in *Halammohydra octopodides* Remane, 1927, as 12 to 18 (Clausen, 1963, 1967; Rao & Ganapati, 1966; Remane, 1927; Renaud-Debyser, 1964; Swedmark, 1957; Swedmark & Teissier, 1957a). In both these species, the morphological variation in characters such as tentacle number or the cnidome appears to be considerable and led to the description of different types (= varieties) (Clausen, 1963, 1967; Swedmark, 1957).

This study aims to clarify the identification and delimitation of *Halammohydra* species by examining the variability of their morphological characters and combining this with molecular sequences. Species delimitation analyses of material from 18 localities revealed four described and four new species, as well as several unidentified species. Since there

are no sequences for specimens identified to species level so far, the data and species delimitation analyses of this study will also provide a base line for future taxonomic studies of *Halammohydra*.

Material and methods

Field work and extraction

A total of 302 specimens of *Halammohydra* were collected between 2011 and 2021. Sampling efforts focused on European-type localities and locations with previously published findings. Additional specimens were found in the context of a summer school on the Azores (Jörger et al., 2021; Tödter & Schmidt-Rhaesa, 2021) (Table 1). Most specimens originated

from two locations: 79 specimens from the subtidal station of Helgoland next to the Youth Hostel and 71 from “Basse Plate” in Roscoff. Intertidal samples were collected with a shovel and plastic bags or containers, by removing the upper centimeters of the sediment at several positions for investigation. Subtidal samples were collected in similar manner by scuba diving or research vessels using a Van Veen Grab or dredge.

To extract the animals from the sediment, the anesthesia-decantation-method was used (Higgins & Thiel, 1988). Specimens of *Halammohydra* were sorted using a dissecting scope and investigated in detail alive with a compound microscope (Leica DM2500), documenting morphology and behavior with a mounted camera (Sony Handycam and Canon 6D Mark II with AMScope adapter). Specimens were fixed individually in 100% ethanol to link photographic

Table 1 Localities of samples used for this study

Locality	Date	GPS latitude	Longitude	Sediment	Depth (m)	Sampling method
Denmark Noth Sealand Ellekildehage	26.08.2011; 30.06.2014; 05.11.2015	56°5'9.6"N	12°31'26.4"E	coarse sand	7–9	mini van veen grab
Germany Sylt Beach of Hörnum	20.- 29.05.2019	54°45'21.117"N	8°17'39.951"E	coarse sand	beach	with shovel
Helgoland Pier at the Youth Hostel	16.- 27.09.2019	54°11'18.7908"N	7°53'7.368"E	medium sand	app. 3–5	van veen grab
“Dune,” Northern beach	10.- 15.08.2020	54°11'23.8812"N	7°54'44.5212"E	shelly sand	0.5	with shovel
France Roscoff Basse Plate	14.- 23.09.2020	48°44'17.5812"N	4°2'25.62"W	medium sand	21	dredging
Trezen ar Skoden		48°44'20.58"N	4°2'36.5388"W	medium sand	29	dredging
Chenal l' Ile de Verte		48°45'34.2612"N	4°5'38.4"W	coarse shell	48	dredging
Banc de Bistarz		48°43'44.4"N	3°59'13.2"W	coarse sand	0	at low tide, shovel
Ognon Bazin Malvog		48°43'57"N	3.59'9.6"W	coarse sand	0	at low tide, shovel
Arcachon Plage d'Arcachon	30.06.2014	48°44'9.1752"N	4°1'41.0268"W	medium sand	18	at low tide, shovel
		48°44'10.968"N	4°0'21.924"W	medium sand	7	dredging
Spain Tenerife Los Abades	31.- 04.09.2021	28°8'25.836"N	16°26'1.14"W	-	19–22	diving
Arcos de Playa San Juan		28°8'24.756"N	16°26'25.944"W	coarse sand	4–5	diving
		28°10'43.536"N	16°49'0.66"W	coarse sand	20–25	diving
Portugal Azores (Sao Miguel)	15.- 24.07.2019	37°44'24.061"N	25°34'29.82"W	sand	app. 8–12	diving, with shovel and plastic containers
Lagoa		37°49'28.048"N	25°26'44.3508"W	sand	app. 3–6	
Praia dos Moinhos		37°50'9.8664"N	25°29'2.7996"W	coarse sand	app. 1–4	
Riberinha		37°50'10.2156"N	25°29'2.1876"W	coarse sand	app. 1–4	
		37°50'11.3784"N	25°29'0.7296"W	coarse sand	app. 1–4	
		37°50'8.2716"N	25°28'56.5032"W	medium sand	app. 1–4 app. 2–5	
Cuba Gibara Playa Cal-etones	18.11.2014	21°12'40.6"N	76°14'30.1"W	medium-coarse sand	17–18	diving
Brazil Fernando de Noronha Ilha Rata	19.10.2012	3°48'57.7"S	32°23'29.1"W	coarse sand	10–12	diving

documentation and sequencing data. For four of the 18 localities (Denmark, France (Arcachon), Cuba, Brazil), detailed microscopic examinations were only conducted on fixed material, limiting the amount of morphological information.

The occurrence of *Halammohydra* was highly patchy. Even in the most reliable localities, we sampled buckets of sediment with no specimens in it. Wherever any specimens were found, usually there were dozens of them. This sometimes complicated a detailed investigation, because specimens did not survive for very long after the extraction from the sample. In vivo investigations were needed to document the behavior and all characters, because the animals tended to contract when dying, which made the investigation of detailed characters difficult.

Specimen identity was analyzed at the laboratory of the Leibniz Institute for the Analysis of Biodiversity Change (LIB) using the images for measuring body size and tentacle lengths with Adobe Photoshop. Morphological and behavioral characters with diagnostic potential were analyzed in combination with the molecular data.

Molecular methods

For the DNA extraction, entire fixed specimens were digested individually in proteinase K (50 µl mixture of 45 µl Tris HCl with pH 7.5 and 5 µl proteinase K, 20 mg/ml) for 24 h at 50 °C and purified using magnetic beads (AmpliClean). A total of 100 µl of magnetic beads were added to each sample and incubated for 10 min at room temperature. DNA in the sample adhered to the beads, which were separated from the liquid with a magnet. After discarding the liquid, two washing steps with ethanol followed, and 20 µl of water was added to resolve the DNA from the beads. The magnetic beads were discarded.

Three genes were amplified, two mitochondrial (16S and COI) and one nuclear gene (18S), using polymerase chain reaction (PCR) with primers from the literature (Table 2). Thermo-cycler programs were as follows: 94 °C/5 min (94 °C/50 s, 48 °C/50 s, 72 °C/1 min; 35 cycles), 72 °C/5 min for 16S; 94 °C/4 min (94 °C/20 s, 57 °C/20 s, 72 °C/1 min 45 s; 35 cycles, 72 °C/7 min for 18S;

and 94 °C/5 min (94 °C/45 s, 45 °C/50 s, 72 °C/60 s, 38 cycles, 72 °C/5 min for COI). The results of the PCR were checked using gel-electrophoresis. Successfully amplified samples were purified and then sent to MacroGen Europe B. V. (Netherlands) for Sanger sequencing.

Sequence analysis and species delimitation methods

Forward and reverse reads of each gene were quality-checked and assembled using MEGA X (Kumar et al., 2018). The resulting sequences were checked with BLASTn to ensure that no contamination happened during the process. Sequences for an outgroup of species within Trachylinae close to *Halammohydra* and the only present sequences EU293991 (16S) and EU301622 (18S) of *Halammohydra* (Caribbean Sea/Panama) were downloaded from NCBI GenBank using the study of Collins et al. (2008) as a guideline (see SI 1). All newly sequenced gene fragments are uploaded to GenBank (see SI 2). Alignments for each gene were created using MAFFT (Katoh et al., 2019) with the default settings, checked visually, and conserved positions were identified with Gblocks 0.91b (Dereeper et al., 2008) in the default settings (final length without gaps of 16S: 480 bp, 18S: 1562 bp, COI: 810 bp, concatenated matrix: 2852 bp). The alignments were analyzed individually and in a concatenated supermatrix of all three genes. It is important to notice that the 18S sequence EU301622 from GenBank is about 370 bp shorter than the sequences acquired in this study.

Phylogenetic analyses were performed using Bayesian inference (BI) in MrBayes (Ronquist et al., 2012) and maximum likelihood (ML) in the IQ tree (Trifinopoulos et al., 2016). For BI, PartitionFinder2 (Lanfear et al., 2016) was used to find the best substitution model for 16S and 18S and the 1st, 2nd, and 3rd codon position of COI employing the corrected Akaike information criterion (AICc) and a greedy search scheme. Results were implemented in the settings for MrBayes and the analysis ran for 15 mill. generations with a burnin of 10%. ML analyses were done with default settings, an automatic search for the best substitution model and 1000 ultrafast bootstrap repeats. Log files were inspected in Tracer v1.7.1 (Rambaut et al., 2018) for convergence and effective

Table 2 Primers used for the PCR and their source

	Primer		Source
16S	SF2 SR2	TCGACTGTTTACCAAAAACATA ACGGAATGAACTCAAATCATGTAAG	Allen G. Collins, pers. commun
18S	Forward reverse	CCGAATTCGTCGACAACCTGGTTGATCCTG CCAGT CCCGGGATCCAAGCTTGATCCTTCTGCAGG TTCACCTAC	(Medlin et al., 1988)
COI	LCO MedCoir	GGTCAACAAATCATAAAGATATTGG GGAAGTCTATAATCATAGTTGC	(Folmer et al., 1994, Ortman et al., 2010)

sample size. If these values were insufficient, the number of generations was increased, and the analysis was performed again. The resulting tree files were edited with FigTree v1.44 and Adobe Illustrator.

The data sets were also used for species delimitation analyses. Three different approaches were performed to determine molecular operational taxonomic units (mOTUs): Automatic Barcode Gap Discovery (ABGD), Generalized Mixed Yule Coalescent (GMYC), and Poisson-Tree-Process (bPTP). For ABGD analyses (Puillandre et al., 2012), the web-server <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html> was used with default settings. GMYC analyses are based on ultrametric trees, which were obtained with Beast v2.6.2 (Bouckaert et al., 2014) using a strict clock, the substitution model GTR, and a Monte Carlo Markov chain length of 10 mill., sampling every 1000th generations. Results were checked with Tracer v1.7.1, and consensus trees were built with TreeAnnotator v2.6.2 with a 10% burnin. The GMYC analyses were done with R (R Core Team, 2013) following Michonneau (2017). The last analysis performed was bPTP (Zhang et al., 2013). Trees obtained by MrBayes were uploaded to the web-server <https://species.h-its.org/ptp/>, and the analyses ran for 100 000 generations with default settings. Genetic divergence, or k2p values, were obtained with the program MEGA X. Sequences were grouped according to the cluster in the tree, and the species delimitation tests and analyses were done between and within groups.

Results

Halammohydra of the Northwest (and East) Atlantic were investigated from 18 subtidal and intertidal locations with medium to coarse sand or shell gravel (Table 3). We examined 302 specimens and included the previously published records of *Halammohydra* from the Azores (Tödter & Schmidt-Rhaesa, 2021) and two here recorded specimens from Cuba and Brazil. *Halammohydra vermiformis* Swedmark & Teissier, 1957 is the most abundant and geographically dispersed species of this study (see Table 3).

Species delimitation tests result in mOTUs. We call mOTUs with single sequences singletons and groups of sequences clusters. Some clusters are identified as species in combination with the morphological data (Table 4). We assigned 234 animals to a species or cluster by morphology and/or obtained DNA sequences, leaving 68 unidentified. Sequences from 161 specimens were used to reconstruct a phylogenetic tree (Fig. 1). Topologies of both phylogenetic analyses (BI/ML) are mostly consistent, only differing in a different position of *H. joergerae* n. sp. and the singleton R115 in the ML analysis. The combined analyses of all

genes (Fig. 1) show similar results as single gene analyses (<https://doi.org/10.5061/dryad.ksn02v778>).

Species delimitation tests are conducted on sequences of each gene individually and on the concatenated matrix to find distinct mOTUs (Fig. 1, SI 2). The three gene sequences are not available for each specimen, but most specimens could be assigned to a mOTU with high support. Combined species delimitation analyses result in 16 clusters and seven singletons (23 mOTUs). Out of the 16 clusters, seven are assigned to four known species, with two species (*H. vermiformis* and *H. octopodides*, Fig. 1) having multiple clusters. Of the remaining nine clusters, four are described here as new species, and the remaining five are left undescribed due to insufficient information. The results supporting the four known species, four new species, and five undescribed clusters are described below.

Due to the smaller variation among the 18S sequences, ABGD and GMYC analyses of 18S tend to find fewer mOTUs, with 11 (ABGD) and 10 (GMYC) mOTUs. ABGD of 18S is able to distinguish between “Tenerife 1,” *H. adherens*, *H. coronata*, “Helgoland/Sylt,” and “Azores.” All other sequences of 18S are grouped together in one mOTU. GMYC of 18S distinguishes further which is grouped by ABGD but still groups *H. vermiformis* 1 and 2, *H. swedmarki* n. sp. and *H. teissieri* n. sp., *H. octopodides* 1, 2 and 3, and *H. kerblae* n. sp. and “Roscoff” with three singletons. Other resulted in joined mOTUs (*H. adherens* and *H. coronata*, and “Azores” and “Helgoland/Sylt”). In contrast, bPTP of 18S predicts several mOTUs within “Helgoland/Sylt,” “Azores,” *H. swedmarki* n. sp., *H. octopodides* 1, and “Tenerife 1” with a total of 30 mOTUs (SI 2).

Mean k2p values of 16S and CO1 sequences within each cluster are noticeably lower than between each cluster, with 0.003 ± 0.001 (0.000–0.027) in 16S and 0.006 ± 0.002 (0.000–0.017) in CO1 within each cluster and 0.221 ± 0.023 (0.012–0.448) in 16S and 0.241 ± 0.015 (0.053–0.394) in CO1 between each cluster. There is much less difference in variability between clusters of 18S sequences (mean k2p 0.017 ± 0.003 , 0.001–0.050) and values for “Tenerife 1” are the highest (mean k2p 0.035 ± 0.005). The mean variability within each cluster of 18S sequences is 0.002 ± 0.001 (0.000–0.008) (SI 3).

Description of the four known species

Halammohydra vermiformis Swedmark & Teissier, 1957

A total of 105 specimens are identified as *Halammohydra vermiformis* by morphological and molecular methods. Fifteen specimens are from Sylt, 76 from Helgoland (45 from “Pier at the Youth Hostel” and 31 from the “Northern

Table 3 Number of specimens of every species and cluster (identified by morphology and DNA sequences) found at each locality, displayed as total number of specimens/number of sequenced specimens used in molecular analyses

Species/ cluster	NW Atlantic												E Atlantic				
	DK			Germany			France			Spain			Portugal			E Atlantic	
	North Sea- land/ Ellekil- dehage	Sylt/ Hör- num	Helgo- land/ Pier at Youth Hostel	Helgo- land/ North- ern Beach	Roscoff/ Basse Plate	Roscoff/ Chenal l'Île de Verte	Roscoff/ Banc de Bistarz	Roscoff/ Ognon	Roscoff/ Bazin Malvog	Arca- chon/ Plage d' Arca- chon	Tenerife/ Los Abades	Ten- erife/ Arcos de Playa San Juan	Azores/ Piscina Lagoo	Azores/ Praya dos Moínhos	Riber- inha	Cuba/ Gibara, Playa Cal- etones	Brazil/ Ferro- nando de Noronha
<i>H. vermi- formis</i>	8/2	15/10	45/28	31/17	1/0	1/0	1/0	-	3/2	-	-	-	-	-	-	1/0	-
<i>H. octopo- dides</i>	-	-	2/2	-	21/15	9/3	1/1	1/0	-	-	-	-	-	-	-	-	-
<i>H. coro- nata</i>	-	-	8/8	-	1/1	1/1	1/0	-	-	-	-	-	-	-	-	-	-
<i>H. adther- ens</i>	-	-	-	-	-	6/4	-	1/1	-	-	-	-	-	-	-	-	-
<i>H. teissieri</i> n. sp.	-	-	6/5	-	3/3	1/1	1/1	4/4	-	-	-	-	-	-	-	-	-
<i>H. swed- marki</i> n. sp.	-	-	-	-	2/2	-	-	4/4	-	-	-	-	-	-	-	-	-
<i>H. kerblae</i> n. sp.	-	-	-	-	4/4	-	1/1	-	-	-	-	-	-	-	-	-	-
<i>H. joergene</i> n. sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	13/13	-	-	-
"Helgol/ Sylt"	-	1/1	8/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
"Roscoff"	-	-	-	-	10/9	-	-	-	-	-	-	-	-	-	-	-	-
"Azores"	-	-	-	-	-	-	-	-	-	-	-	-	2/2	-	-	-	-
"Tenerife 1"	-	-	-	-	-	-	-	-	-	2/2	2/1	-	-	-	-	-	-
"Tenerife 2"	-	-	-	-	-	-	-	-	-	4/3	-	-	-	-	-	-	-
singletons	-	-	-	-	2/2	2/2	-	1/1	-	-	-	-	-	-	-	-	1/1
unidenti- fied	3/0	3/0	10/0	-	27/0	5/0	2/0	2/0	2/0	-	1/0	2/0	-	12/0	-	-	-
Total	11/2	19/11	79/46	31/17	71/36	25/11	6/4	5/1	8/6	3/2	6/5	3/1	2/0	2/2	25/13	1/0	1/1

Table 4 Summarized characters of species/clusters of *Halammohydra*

Species/cluster	Tentacles: number		Shape	Thickening at bases		Length differences		Separation of whorls			
	Total	Per Whorl		Aboral	Subaboral	One Long tentacle	Within whorl	Between whorls			
<i>H. vermiformis</i> 1 & 2	7–8	3(4)+4	slender	slender, slightly thicker or irregular surface	no	slight bulb or club-shaped	yes, 2–3 times longer	no	diverse	yes	
<i>H. octopodides</i> 1, 2 & 3	8–14	even	slender	slender, some with irregular surface	no	no, some club-shaped	yes, 2 times longer	subaboral unequal	diverse	no	
<i>H. coronata</i>	10–15	uneven, mostly 4 in aboral whorl	slender	slender	no	no	some, 1.5 times longer	subaboral unequal	diverse	yes	
<i>H. adherens</i>	11–>25	-	slender	slender	no	no	no	-	-	no	
<i>H. teissleri</i> n. sp.	10–19	even	slender	slender	no	no	no	no	aboral tentacles 1.5 times longer	no	
<i>H. swedmarki</i> n. sp.	10–20	even	slender	thicker	no	no	no	both unequal	aboral tentacles 2 times longer	no	
<i>H. kerblae</i> n. sp.	11–12	uneven, 4+7/8	slender	irregular surface	no	bulb or club-shaped, some no	no	no	no	yes	
<i>H. joergerae</i> n. sp.	10–14	even	slender	slender, some with irregular surface	no	no, some club-shaped	no	subaboral unequal	diverse	no	
“Helgoland/Sylt”	9–16	even	slender	slender, slightly thicker or irregular surface	no	sometimes slight bulb or club-shaped	no	no	no	no	
“Roscoff”	10–18	even	slender	thicker or irregular surface	no	bulb or club-shaped	no	subaboral unequal	aboral tentacles longer	no	
“Azores”	16, 18	even	slender	thicker	no	bulb	no	no	no	no	
“Tenerife 1”	12, 14	even	slender, irregular surface	slender, irregular surface	no	no	no	-	-	no	
“Tenerife 2”	19–21	even	slightly thicker	slightly thicker	no	no	no	no	-	no	
Species/cluster	Aboral cone		Statocysts		Gastric tube		Behaviour		Other		Environment
<i>H. vermiformis</i> 1 & 2	conical	diamond shaped/inversed cone	45 ± 7 (1) 60 ± 12 (2)	4	round to elongated	209 ± 75 (1) 298 ± 215 (2)	free-swimming	subtidal and intertidal, marine and brackish			
<i>H. octopodides</i> 1, 2 & 3	conical or round	cub-shaped	54 ± 10	4–5	ovoid	175 ± 72	adhesive	subtidal			
<i>H. coronata</i>	cylindrical or slightly round	-	56 ± 7	4–5	ovoid to elongated	292 ± 96	free-swimming	subtidal			
<i>H. adherens</i>	-	-	-	4–5	ovoid	-	very adhesive	elongated nematocysts			

Table 4 (continued)

Species/cluster	Aboral cone		Adhesive organ		Statocysts		Gastric tube		Behaviour	Other	Environment
	Shape	Length (in μm)	Number	Shape	Length (in μm)	Length (in μm)					
<i>H. teissieri</i> n. sp.	-	-	5–8	ovoid	172 \pm 45	adhesive	tapering or square mouth opening	subtidal			
<i>H. swedmarki</i> n. sp.	* flat	* cup-shaped	5 or 7	variable	-	adhesive	-	subtidal and intertidal			
<i>H. kerblae</i> n. sp.	trapezoid or conical	* cup-shaped	4–6	ovoid or slightly elongated	159 \pm 47	free-swimming	-	subtidal			
<i>H. joergerae</i> n. sp.	conical or cylindrical	cup-shaped	5–6	ovoid	348 \pm 117	adhesive	tapering or square mouth opening	subtidal			
“Helgoland/ Sylt”	-	-	4–8	ovoid, sometimes elongated	227 \pm 37	adhesive	-	subtidal and intertidal			
“Roscoff”	-	-	5–7	ovoid	211 \pm 52	adhesive	-	subtidal and intertidal			
“Azores”	* conical	* cup-shaped	8, 9	ovoid	255 or 301	adhesive	-	subtidal			
“Tenerife 1”	-	-	6–7	elongated	-	adhesive	-	subtidal			
“Tenerife 2”	-	-	6–7	-	-	adhesive	-	subtidal			

[†]missing data

* data only from one specimen

Beach”), two from Roscoff, and 12 from field trips lacking detailed morphological examination of live animals (eight from Denmark, three from France, Arcachon and one from Cuba; Table 3).

Molecular analyses result in two clusters of this species, *H. vermiformis* 1 and 2. Both clusters comprise several specimens and support values of 100 in both phylogenetic analyses. Species delimitation tests group them in two clusters with comparably many sequences in each gene (Figs. 1, SI 2). 18S sequences do not resolve both groups in ABGD and GMYC, but do resolve them with a low support in bPTP (SI 2). K2p values between both clusters are 0.149 ± 0.019 (16S), 0.003 ± 0.001 (18S), and 0.207 ± 0.020 (CO1) and thus lower than the mean values of all clusters (SI 3). Both clusters differ in the sampling locality. *H. vermiformis* 1 was sampled exclusively at the subtidal station of Helgoland (“Pier at the Youth Hostel”), *H. vermiformis* 2 at several intertidal locations, except for the ones from “Ellekildehage” (Denmark), which was at seven to nine meters depth but in brackish shallow water.

The clear separation into two clusters recognized in molecular analyses is not reflected in the morphology (Table 4). Both groups have a variety of body shape, ranging from completely round to elongated, with the elongated form dominating (Fig. 2a). *Halammohydra vermiformis* 2 is slightly larger (but with higher variation) with a mean gastric tube length of $298 \pm 215 \mu\text{m}$ ($n = 19$) compared to $209 \pm 75 \mu\text{m}$ ($n = 31$) in *H. vermiformis* 1 in the length of the gastric tube. Most specimens have a conical aboral cone, with a higher length than the width (Fig. 2b). The aboral cone of *H. vermiformis* 2 is slightly larger again ($60 \pm 12 \mu\text{m}$ ($n = 13$) in mean length and $46 \pm 8 \mu\text{m}$ ($n = 10$) in mean width), compared to *H. vermiformis* 1 ($45 \pm 7 \mu\text{m}$ ($n = 21$) in mean length and $37 \pm 9 \mu\text{m}$ ($n = 28$) in mean width). Whenever it was possible to investigate the adhesive organ, it showed a cup- or diamond-/inversed cone shape in the aboral cone (Fig. 2b). All specimens have 4 statocysts.

The total number of tentacles is usually 7 (3 aboral + 4 subaboral, Fig. 2a), with the exception of a few animals from Sylt with 8 (4 + 4) tentacles. No special structure or thickening is documented for the bases of aboral tentacles, and a few specimens have a slight thickening or club-shaped base in the subaboral whorl (Fig. 2b). Aboral tentacles are of slender shape. Subaboral tentacles are slightly thicker or with an irregular surface. Within a whorl, the tentacle length is roughly the same, except for the one long tentacle present in the subaboral whorl. It is about two to three times the length of the other tentacles and has a thicker appearance and sometimes a bulb at the base (Fig. 2a and b). In some cases, it is coiled up. Aboral tentacles are directed aborally and subaboral tentacles orally (Fig. 2a). This was clearly visible while the animals were swimming. In general, specimens observed were less adhesive than other species and

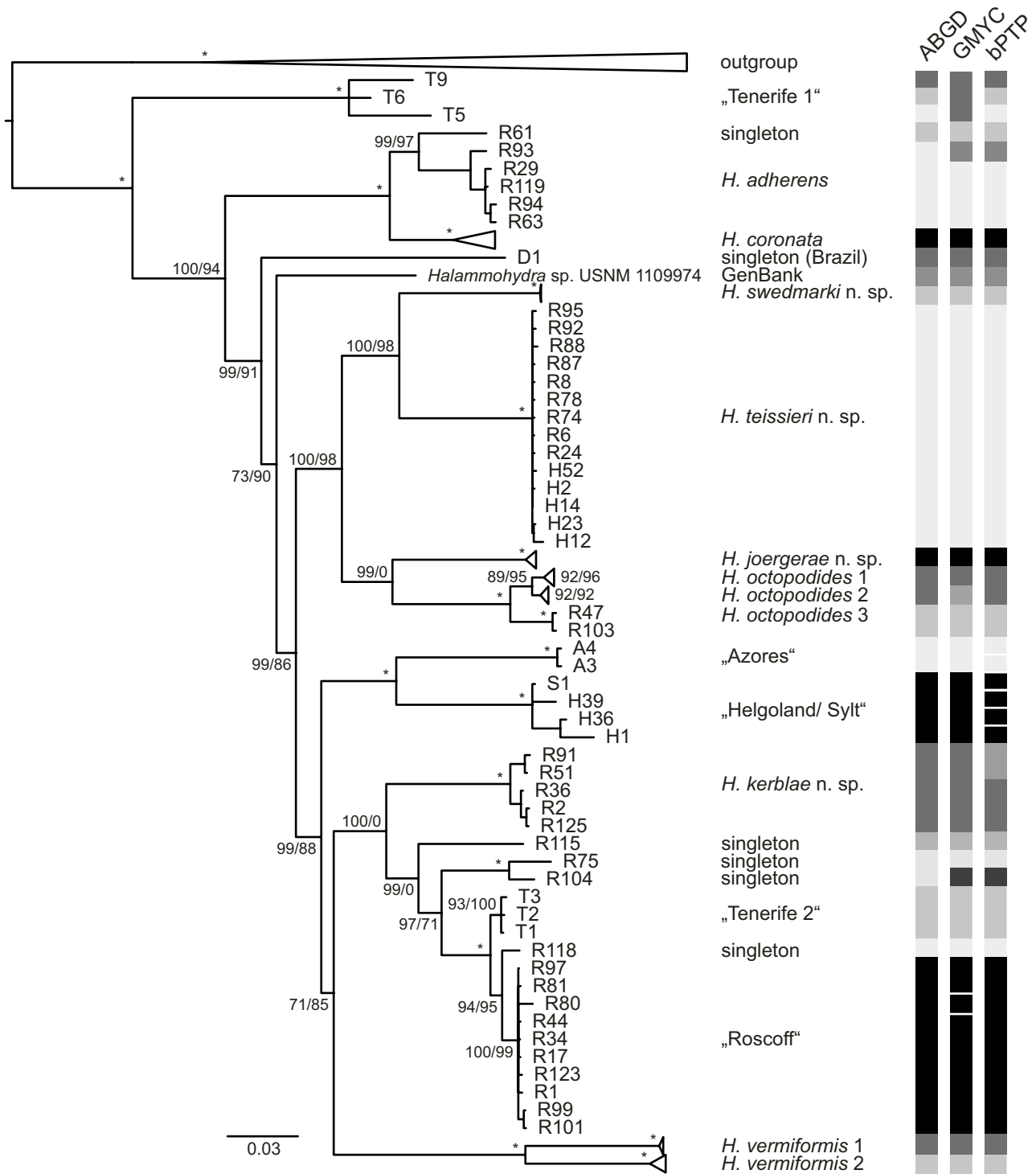


Fig. 1 Phylogenetic tree of all three genes concatenated with support values of BI/ML (posterior probability/bootstrap value). Nodes with an * have a support of 100/100. Some clusters are collapsed. Sum-

marized species delimitation of ABGD, GMYC, and bPTP results are shown for each cluster

mainly found free-swimming with the aboral cone in the direction of movement. Tentacles of the aboral whorl were bent to the oral end while swimming, but the separation of whorls was still visible.

***Halammohydra octopodides* Remane, 1927**

Most of the specimens of *Halammohydra octopodides* are from Roscoff (32 specimens), particularly from the station

“Basse Plate” (21 specimens). Two are from the “Pier at the Youth Hostel” of Helgoland (Table 3). Molecular analyses separate this species into three clusters, *H. octopodides* 1, 2, and 3. The two specimens from Helgoland are positioned in the *H. octopodides* 1 clade. Node support values are high for all three mOTUs (Fig. 1). ABGD and bPTP analyses group *H. octopodides* 1 and 2 together and GMYC separates *H. octopodides* 1 and 2. All analyses distinguish *H. octopodides* 1 and 2 from 3, except bPTP of 18S, which groups *H. octopodides* 2 and 3 together with a low support (SI 2). There are no CO1 sequences available for *H. octopodides* 2 and 3. K2p values of 16S between *H. octopodides* 1 and 2 are 0.012 ± 0.005 and thus lower than between *H. octopodides* 1 and 3 (0.051 ± 0.011) or 2 and 3 (0.049 ± 0.011 , SI 3).

Specimens from all three groups have a similar morphology (Table 4). The ovoid gastric tube is $175 \pm 72 \mu\text{m}$ ($n=24$) in length (Fig. 2c). In some specimens, the body is slightly elongated. The aboral cone is triangular or round and $54 \pm 10 \mu\text{m}$ ($n=16$) in length and $47 \pm 6 \mu\text{m}$ ($n=10$) in width. Specimens are very adhesive, which complicated the documentation of the adhesive organ. Whenever it was possible to investigate, the adhesive organ was cup-shaped and sometimes reached deep into the aboral cone.

The total number of tentacles varies from 8 to 14, mostly 10, evenly distributed in both whorls. In contrast to *H. vermiformis*, there is no clear separation of the direction of both tentacle whorls. Neither of the tentacle whorls has a visible bulb at the base (Fig. 2c). Some specimens have a slightly club-shaped base in the subaboral whorl. All tentacles are of

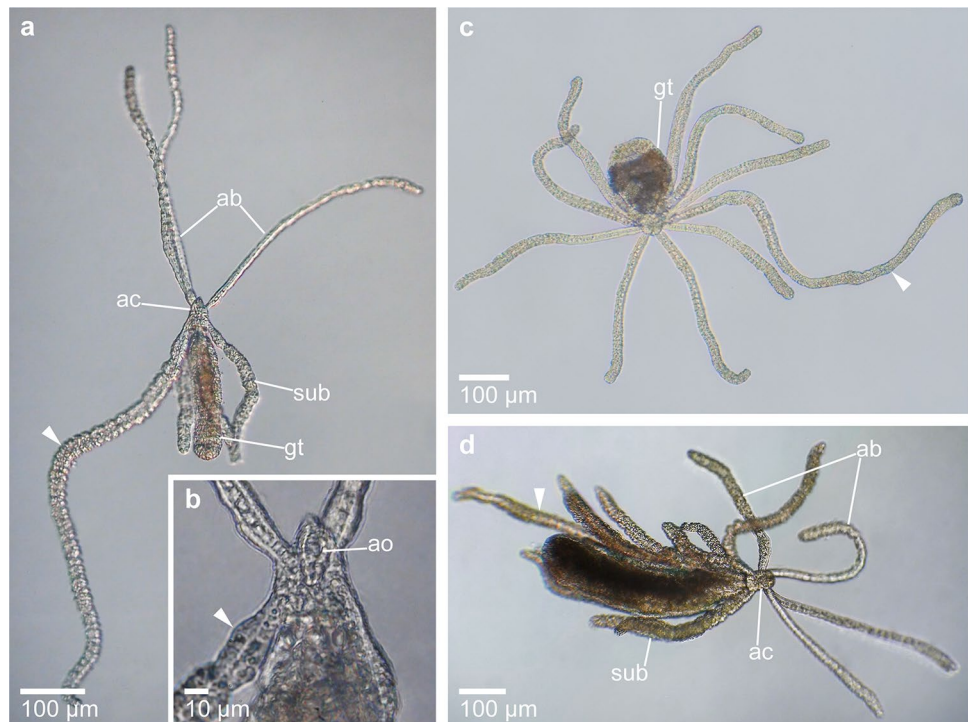
slender shape, whereas in the subaboral whorl, they sometimes have an irregular surface. One tentacle (in four specimens two) of the subaboral whorl is about two times longer than the other tentacles (Fig. 2c). Most of the specimens have no thickening at the base in this long tentacle, and the structure is the same as in the subaboral whorl. Tentacles in the subaboral whorl are of unequal length. The number of statocysts is 4 or 5.

Halammohydra coronata Clausen, 1967

Eight specimens of *Halammohydra coronata* are from the “Pier at the Youth Hostel” of Helgoland, and three are from Roscoff (Table 3). All species delimitation tests and node values of both phylogenetic analyses support the cluster of this species well (Figs. 1, SI 2). The ovoid to the elongated gastric tube is $292 \pm 96 \mu\text{m}$ ($n=9$) long and connects to a cylindrical and slightly round aboral cone, measuring $56 \pm 7 \mu\text{m}$ ($n=4$) in length and $46 \pm 6 \mu\text{m}$ ($n=3$) in width (Fig. 2d). Unfortunately, most specimens adhered to the slide during the investigation, which made it impossible to observe the adhesive organ in detail. The number of statocysts is 4 or 5.

In total, specimens have 10 to 15 tentacles, distributed unevenly in the two whorls (Fig. 2d). The number of aboral tentacles is lower, mostly 4, than of subaboral tentacles. When the animals were swimming, this uneven number was best visible, because the tentacles of the aboral and subaboral whorl are held in different directions (Fig. 2d). There is no thickening at the tentacle bases, and the tentacles are

Fig. 2 Light microscopy images of *Halammohydra* species. **a** *H. vermiformis* with a 3 times longer tentacle (arrowhead) in the subaboral whorl. **b** Magnification of aboral cone in **a** showing a thickening at the tentacle bases of the subaboral tentacles (arrowhead). **c** *H. octopodides* with 2 times longer tentacle (arrowhead). **d** *H. coronata* with 1.5 times longer tentacle (arrowhead) in the subaboral whorl. ab, aboral tentacles; ac, aboral cone; ao, adhesive organ; gt, gastric tube; sub, subaboral tentacles



slender in both whorls. In some cases, one tentacle is about one and a half to two times longer than the others, and it has no bulb at the base. Tentacles in the subaboral whorl have an unequal length (Fig. 2d, Table 4).

In this group, some specimens are clearly identified as *H. coronata*, whereas others are not, due to the lower difference in structure between the tentacles of both whorls, some damage, and the adhesion to the slides. The identification of the specimens with unclear characters is based solely on molecular analyses.

***Halammohydra adherens* Swedmark & Teissier, 1967**

All seven animals identified as *Halammohydra adherens* are from Roscoff, six of them from the station “Trezen ar Skoden” (Table 3). Genetically, this species is closely related to *H. coronata* and GMYC analysis of 18S even groups these two species into one cluster (Fig. 1). It is important to notice that there is only one sequence of 18S and no CO1 sequence available for *H. adherens*. The cluster of this group is mostly based on 16S sequences. Node support values, ABGD implies an affiliation of the specimen R93 from “Bazin Malvog” to this species, but GMYC and bPTP exclude it (SI 2). K2p values between R93 and *H. adherens* are 0.018 ± 0.006 and between *H. adherens* and *H. coronata* 0.090 ± 0.014 (SI 3).

Specimens of *H. adherens* have an ovoid body shape and are very adhesive (Fig. 3). The structure of the aboral cone and adhesive organ could not be investigated due to this behavior (Table 4). The tentacles of both whorls are not separated and are of slender shape. The number of tentacles was 11, 16, or 22 (Fig. 3a). One specimen even has more than 25 tentacles (Fig. 3b). This specimen is exceptionally large, and the amount of tentacles obscured further observation of the aboral cone or the tentacle bases. The other specimens have no special structure at the tentacle bases, and 4 or 5 statocysts are visible. All specimens of this group have elongated nematocysts, which are even observable in lower magnification (Fig. 3c and d). Tentacles of the largest specimen are fully packed with these nematocysts (Fig. 3d), whereas in the smaller ones, they are less densely concentrated (Fig. 3c).

Description of the four new species

***Halammohydra teissieri* n. sp**

Zoobank: urn:lsid:zoobank.org:act:7DBB8CE1-F9A8-4844-96BB-1BBAD9EC44D9.

Etymology This species is dedicated to Georges Teissier, who contributed much information about *Halammohydra*.

Material examined *Holotype*: “Pier at the Youth Hostel” on Helgoland, Germany, 16 Sep 2019, subtidal, medium sand, L. Tödter. *Paratype*: “Basse Plate” in Roscoff, France, 14 Sep 2020, 21 m depth, medium sand, A. Kerbl and L. Tödter. Images taken with a compound microscope and 16S, 18S, and CO1 sequences of the holotype and 16S and 18S sequences of the paratype obtained. Entire specimens used for DNA extraction, frozen DNA solutions deposited in the Museum of Nature–Zoology in Hamburg (LIB, Biobank) under Sample ID H2.40 (holotype) and R95 (paratype).

Description of the holotype ovoid gastric tube with square mouth opening and adhesive behavior. 16 tentacles, evenly distributed in both whorls, aboral tentacles 1.5 times longer than subaboral ones, slender shape, no thickening at the bases and no pronounced long tentacle in subaboral whorl. 8 statocysts.

Remarks in total, 15 animals from the “Pier at the Youth Hostel” on Helgoland (6 specimens) and four stations in Roscoff (“Basse Plate,” “Trezen ar Skoden,” “Ognon,” and “Bazin Malvog,” 9 specimens) are genetically assigned to this species (Table 3). All species delimitation tests, except for ABGD and GMYC of 18S, support this cluster, as well as phylogenetic analyses (Fig. 1). bPTP of CO1 overestimates this cluster creating three mOTUs with high support (SI 2).

Some morphological characters vary within this group compared to the holotype. The body has a length of $172 \pm 45 \mu\text{m}$ ($n=9$; Fig. 4a). Tentacles vary from 10 to 19 in number, which are evenly distributed in both whorls. The number of statocysts varies from 5 to 8, corresponding to the number of tentacles in each whorl (Table 4). Characters are similar to the ones of *H. schulzei*, but without a pronounced thickening of the tentacle bases, longer tentacles in the aboral whorl, slightly lower tentacle number, and different shape of mouth opening.

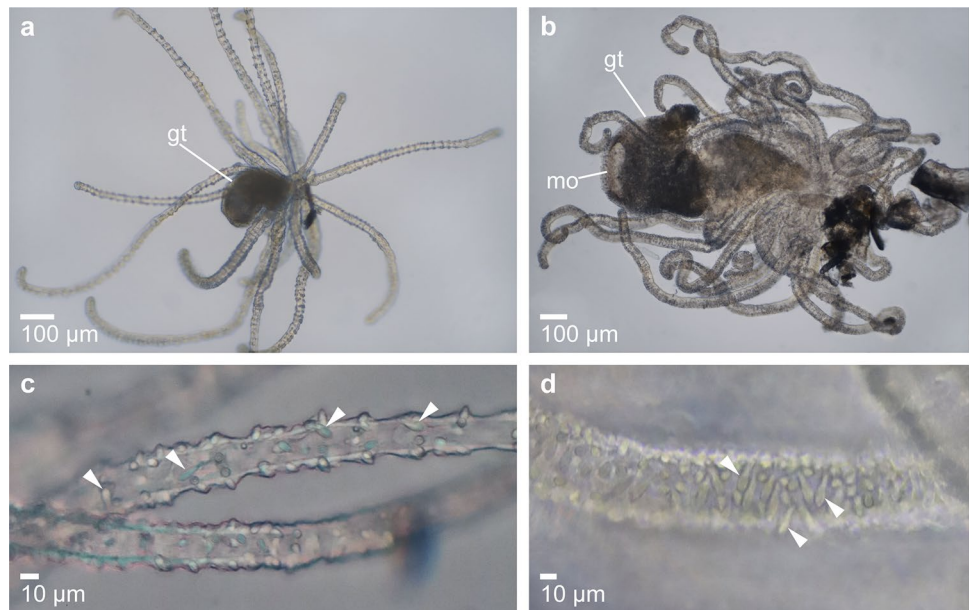
***Halammohydra swedmarki* n. sp**

Zoobank: urn:lsid:zoobank.org:act:9AE64D1D-BC85-4B78-84DF-2B7F58BEA49C.

Etymology this species is dedicated to Bertil Swedmark, who contributed much information about *Halammohydra*.

Material examined “Chenal l’Ile de Verte” in Roscoff, France, 16 Sep 2020, 0 m depth, at low tide, coarse sand, A. Kerbl and L. Tödter. Images taken with a compound microscope and 16S and CO1 sequences of the holotype obtained. Entire specimens used for DNA extraction, frozen DNA solutions deposited in the Museum of Nature–Zoology in Hamburg (LIB, Biobank) under Sample ID R71 (holotype).

Fig. 3 Light microscopy images of *Halammohydra adherens*. **a** Smaller variant with less tentacles. **b** Larger variant with more tentacles. **c** Magnification of a tentacle of **a** with a few elongated nematocysts (arrowheads). **d** Magnification of the tentacle of **c** filled with elongated nematocysts (arrowheads). gt, gastric tube; mo, mouth opening



Description of the holotype gastric tube with variable shape, adhesive behavior, and a flat aboral cone with a cup-shaped adhesive organ (half the depth of aboral cone). 16 tentacles, equally distributed in both whorls, no separation between whorls and slender aboral tentacles about 2 times longer than thicker subaboral ones, both without a thickening at the base and of unequal length. No prominent longer tentacle. 7 statocysts.

Remarks in Roscoff, four specimens of this group were found at “Chenal l’Ile de Verte” and two at “Basse Plate” (Table 3). Phylogenetic analyses of all four specimens (Fig. 1) and all species delimitation test, except for 18S, support this cluster well (SI 2). Variation of morphological characters is in the number tentacles and statocysts. The number of tentacles varies from 10 to 16, and one specimen even has a minimum of 20 tentacles. Statocysts are either 5 or 7 (Fig. 4b, Table 4). Differences to other species are the aboral tentacles are longer, tentacles of both whorls have unequal lengths and the variable body shapes.

Halammohydra kerblae n. sp

Zoobank: urn:lsid:zoobank.org:act:C8494CF2-1C1A-4FF3-92CD-00ADE9C11CE8.

Etymology this species is dedicated to Alexandra Kerbl, who was part of the field trip to Roscoff and was a valuable help in finding the specimens.

Material examined *Holotype and paratype*: “Basse Plate” in Roscoff, France, 14 Sep 2020, 21 m depth, medium sand, A. Kerbl and L. Tödter. Images taken with a compound

microscope and 16S sequence of the holotype and 16S, 18S, and CO1 sequences of the paratype obtained. Entire specimens used for DNA extraction, frozen DNA solutions deposited in the Museum of Nature–Zoology in Hamburg (LIB, Biobank) under Sample ID R51 (holotype) and R2 (paratype).

Description of the holotype ovoid to a slightly elongated gastric tube and trapezoid aboral cone. 12 tentacles, with 4 slender aboral and 8 irregular shaped subaboral ones, club-shaped bases on the subaboral tentacles, no pronounced long tentacles and both whorls pointing in different directions. Statocysts 4.

Remarks Four specimens of this group are from “Basse Plate” and one from “Ognon” in Roscoff (Table 3). This cluster is well supported by phylogenetic analyses and all species delimitation tests, except for ABGD and GMYC of 18S and bPTP results in two mOTUs (Fig. 1, SI 2). Characters of this group varying are the size, the number of tentacles, and the statocysts. The gastric tube has a mean length of 159 ± 47 μm ($n=3$; Fig. 4c) and is connected to an aboral cone of a trapezoid or conical shape with a mean length of 48 ± 8 μm ($n=3$; Fig. 4d). Tentacles are 11 or 12 in number, and unevenly distributed between the two whorls: the aboral whorl always has 4 tentacles and the subaboral 7 or 8 (Fig. 4c). Statocysts are 4 to 6 (Table 4). Characters are similar to the ones of *H. coronata*, but with pronounced thickenings at the bases of subaboral tentacles and a lacking long tentacle.

Halammohydra joergerae n. sp

Zoobank: urn:lsid:zoobank.org:act:D83A943A-EB86-49B9-9C09-01C68E93CE72.

Etymology this species is dedicated to Katharina Jörger for organizing of the summer school (“Meiozores 2019”) on the Azores.

Material examined “Riberinha” on the island Sao Miguel (Azores), Portugal, 22 Jul 2019, medium coarse sand, K. Jörger and F. Goetz. Images taken with a compound microscope and 18S and CO1 sequences of the holotype obtained. Entire specimens used for DNA extraction, frozen DNA solutions deposited in the Museum of Nature–Zoology in Hamburg (LIB, Biobank) under Sample ID A17 (holotype).

Description of the holotype ovoid gastric tube, square mouth opening, adhesive behavior, and cylindrical aboral cone. 12 tentacles, equally distributed in both whorls (6+6), slender tentacles and subaboral ones with unequal lengths and irregular surface. No long tentacles. Statocysts 6.

Remarks all 13 specimens are from the station “Riberinha” on the Azores (Table 3) and form a cluster, which is well supported by phylogenetic analyses and all species delimitation tests, except for 18S in ABGD (Fig. 1, SI 2). In this group, morphological characters varying are the body size, aboral cone, number of tentacles and statocysts, and tentacle bases. The gastric tube measures $348 \pm 117 \mu\text{m}$ ($n=5$) in length (Fig. 4e). Whenever the aboral cone was visible, it had a conical or cylindrical shape and a cup-shaped adhesive organ reaching in half of the depth of the aboral cone. The tentacle number ranges from 10 to 14, and they are equally distributed in the two whorls. Some specimens have a club-shaped base in the subaboral whorl, but most have no thickening. Statocysts are 5 or 6 (Table 4).

Characters of undescribed groups

Helgoland/Sylt

Nine specimens are assigned to this group morphologically and genetically, eight from the “Pier at the Youth Hostel” of Helgoland and one from Sylt (Table 3). This cluster is supported by both phylogenetic analyses and all species delimitation tests (Fig. 1), except for GMYC of 18S, which groups this cluster together with “Azores,” and bPTP, which separates this group in four mOTUs with low support. The analysis is based mostly on 18S sequences, since there are only two 16S and one CO1 sequences (SI 2).

Specimens of this cluster have an ovoid, sometimes elongated, gastric tube with a mean length of $227 \pm 37 \mu\text{m}$ ($n=6$, Fig. 4f). All animals were very adhesive, so the aboral cone could not be investigated. The number of tentacles varies between 9 and 16, evenly distributed in both whorls. Aboral

tentacles are slender, whereas subaboral ones are sometimes slightly thicker or with an irregular surface. There is no bulbous base documented in aboral tentacles, but some specimens have a club-shaped or slight thickening in the subaboral tentacles. There is no longer a tentacle on the subaboral whorl, and no striking length differences are noticed. The number of statocysts varies between 4 and 8 and is the same as tentacles in the subaboral whorl (Table 4).

Roscoff

Animals of this cluster are from the stations “Basse Plate” (10 specimens) and “Banc de Bistarz” (3 specimens) in Roscoff (Table 3). “Roscoff” is well supported by high node values and all species delimitation tests, except for 18S. ABGD of CO1 grouped “Roscoff” together with the singleton R118 and “Tenerife 2” (SI 2), but the support values of the tree and the other analyses clearly separate “Tenerife 2” and the singleton from the other clusters (Fig. 1).

The ovoid gastric tube has a length of $211 \pm 52 \mu\text{m}$ ($n=7$), and the animal is very adhesive, which is why the aboral cone could not be investigated (Fig. 4g). Tentacles vary from 10 to 18 in number and are equally distributed between the two whorls. Aboral tentacles have no thickening at the bases and are slender and longer than subaboral ones (Fig. 4g). Subaboral tentacles have a pronounced bulb or club-shaped base and are thicker with an irregular surface and unequal in length (Fig. 4h). There are no noticeably longer tentacles in the subaboral whorl. The number of statocysts range from 5 to 7 (Fig. 4h, Table 4).

Azores

Two specimens of the station “Praya dos Moinhos” on the Azores are assigned together (Table 3). Phylogenetic analyses, as well as, species delimitation tests support this cluster (Fig. 1). GMYC of 18S groups it together with “Helgoland/Sylt,” but all other genes not (SI 2).

Specimens have an ovoid gastric tube with 255 or 301 μm in length (Fig. 4i). Only one aboral cone could be investigated, since the animals were very adhesive. It is conical with a length of 41 or 50 μm , and the adhesive organ is cup-shaped and reaches half the depth into the aboral cone. Tentacles are 16 (8+8) and 18 (9+9) with slender aboral tentacles and thicker subaboral ones (Fig. 4i). There was no long tentacle in the subaboral whorl, but a thickening at the bases. The number of statocysts is 8 and 9 (Table 4).

Tenerife 1

Four animals of the station “Los Abades” on Tenerife group together morphologically and genetically (Table 3). There are only 18S sequences available of “Tenerife 1.” GMYC



Fig. 4 Light microscopy images of *Halammohydra* clusters and new species. **a** *H. teissieri* n. sp., **b** *H. swedmarki* n. sp. with cup-shaped adhesive organ (arrowhead), **c** *H. kerblae* n. sp., **d** magnification of aboral cone in **c** showing a club-shaped thickening (arrowhead) at the tentacle bases of subaboral tentacles. **e** *H. joergerae* n. sp., **f** “Helgoland/Sylt,” **g** “Roscoff,” **h** magnification of aboral cone in **g** showing a thickening at the tentacle bases of subaboral tentacles. **i** “Azores” (image source Tödter & Schmidt-Rhaesa, 2021). ab, aboral tentacles; ac, aboral cone; gt, gastric tube; mo, mouth opening; st, statocysts; sub, subaboral tentacles

and the node values support this group, but ABGD and bPTP separate all specimens with high support values (Figs. 1, SI 2). They have an elongate gastric tube and are adhesive. It was not possible to investigate the aboral cone and thus the adhesive organ. The number of tentacles is 12 or 14 with 6 or 7 in each whorl. The tentacles are slender with an irregular surface and no thickening at the base. There is no unusually long tentacle documented. The number of statocysts is the same as that of the tentacles in each whorl, 6 or 7 (Table 4).

Tenerife 2

Two specimens of the station “Los Abades” and two of the station “Arcos de Playa San Juan” on Tenerife group together morphologically and genetically (Table 3). All species delimitation tests, as well as the support values of the tree support this group (Fig. 1). There is only one CO1 sequence available and no 18S sequences. In ABGD and bPTP, this CO1 sequence contradicts the clustering of the 16S sequences (SI 2). There is not much information about the body because the animals were sticking strongly to the slides. The tentacle number varies between 19 and 21 and is evenly distributed in each whorl. There is no thickening at the base but the tentacles themselves are slightly thicker than in other groups. There is no prominent long tentacle documented, and the number of statocysts is 6 or 7 (Table 4).

Singletons

There are seven singletons present in the analyses, which are not assigned to any of the clusters. R61 from the station “Basse Plate” in Roscoff is positioned close to *H. adherens* in the tree (Fig. 1). It is an adhesive animal with 11 tentacles and an elongated gastric tube. Tentacles are of slender shape, unequal length, and have no thickening at the bases. There are no elongated nematocysts visible in light microscopy, as in *H. adherens*.

The second singleton D1 is placed and isolated to other sequences (Fig. 1). This is a fixed specimen from Brazil. Hence, there are no detailed morphological characters. It is a rather small specimen with an ovoid body and 14 tentacles (7+7), where the aboral tentacles are longer and the subaboral are of unequal length. There is no noticeably

long tentacle. As well as D1, the GenBank sequences from Panama are placed isolated (Fig. 1) with no morphological information.

Three singletons from Roscoff are placed between *H. kerblae* n. sp. and the cluster of “Tenerife 2” and “Roscoff” (Fig. 1). R115, found at “Basse Plate,” has an ovoid body and 15 tentacles of slender shape and no thickening at the base. Tentacles in the subaboral whorl have an irregular surface as well. There are four statocysts, and no long tentacle is visible. The other two singletons, R75 (“Bazin Malvog”) and R104 (“Trezen ar Skoden”), are placed closer together and ABGD analysis even groups them in one MOTU. Both are juveniles with 12 slender tentacles and no thickening at the bases.

The last singleton R118 from “Trezen ar Skoden” in Roscoff is nested between “Tenerife 2” and “Roscoff” (Fig. 1). Unfortunately, it was very contracted during the investigations, which complicated the detailed documentation. It has an ovoid body with at least 14 tentacles and adheres to the slides.

Topology

Bayesian Interference and Maximum Likelihood analyses result in trees with a similar topology and nodes with mostly high support values (Fig. 1). “Tenerife 2” and “Roscoff” create a clade with the singleton R118 nested within it and a sister relation with the clade consisting of the two singletons R104 and R75. Together with the singleton R115 and *H. kerblae* n. sp. it creates a sister clade of the two clusters of *H. vermiformis*. All mentioned groups from a clade in sister relation to a clade of the two clusters “Azores” and “Helgoland/Sylt.”

H. swedmarki n. sp. and *H. teissieri* n. sp. are a sister group of *H. joergerae* n. sp. and the monophyletic group of *H. octopodides*, whereas the clusters in each group are in a sister relation as well. This is supported well by the node values, except for the ML value of the second clade (Fig. 1). Together, they form a sister clade to the former described clade. This relation contains the majority of the sequences.

Close to the base of the tree is a clade containing the singleton R61 and *H. adherens*, creating a sister clade to *H. coronata*. In between this clade and the majority of the sequences, the singleton D1 from Brazil and the GenBank sequences are positioned in isolation. “Tenerife 1” is located at the base of the tree.

Discussion

This is the first study investigating sequences of several species of the interstitial cnidarian *Halammohydra* in combination with morphological characters. The addition

of molecular data to the traditional identification helped to verify previously described species and to delimit new species or mOTUs within this genus as well. In addition, it was possible to assign individuals to a clade, which could not be identified morphologically because of damage or an unusual shape due to the contraction of the animal. The integrative approach of this study permitted to document of the morphology as well as DNA sequences for every specimen, which was useful to document the variability of the characters within a mOTU.

To ensure to have all morphological features for the combined analysis, images were taken of every specimen. This was not always easy to achieve. *Halammohydra*, like other meiofaunal animals, has a patchy occurrence, even in reliable locations. If they were found, they came in comparably high numbers at once. This led to time pressure in the detailed investigation because the animals did not survive for too long after the extraction. Additionally, not every character was fully visible due to the behavior (very adhesive or contracted animals) and thus, some information could not be documented. On the other hand, obtaining the genetic material was rather difficult. The animals were very small and did not contain a high amount of DNA, which restricted the number of attempts for the PCR. Nevertheless, it was possible to gain enough information for the identification by combining both methods.

Three genes (16S, 18S, and CO1) were used, but only 16S and CO1 gave detailed results on the species level. The 18S gene was able to identify five clusters with ABGD and GMYC and grouped the rest of the sequences together. This is a common result for the 18S gene, as it is a slow-evolving gene (Hillis & Dixon, 1991) and does not always contain phylogenetic information on the species level (Fontaneto et al., 2015; Tang et al., 2012). In general, the topology of the trees and the species delimitation test show similar results, differing only in a few parts. The interpretation of the validity of a group became difficult, when there was only the 18S sequence available, e.g., in “Tenerife 1,” or when one species delimitation test shows a different result, e.g., GMYC of 16S separated *H. octopodides* 1 and 2.

The utility of the CO1 gene for species delimitation in Cnidaria has been debated, since there is a slow evolutionary rate in most Anthozoa (Hellberg, 2006; McFadden et al., 2010; Shearer et al., 2008). However, in Medusozoa, CO1 was considered useful on the species level (Bucklin et al., 2011; Ortman et al., 2010), but the conducted study by Ortman et al. (2010) contained only a few representatives across Cubozoa, Scyphozoa, or Hydrozoa (except Siphonophora), with groups of Hydrozoa being the best represented. In the present study, CO1 was useful to discriminate species of *Halammohydra* and showed evolutionary rates similar to 16S sequences. Additionally, the

alignment process of CO1 sequences was easier than of 16S sequences, because of the common presence of indels (insertions/deletions) in the latter.

In this study, four already described species were identified morphologically and characterized at the molecular level. The highest number of specimens was attributed to *Halammohydra vermiformis*. The species was described from Roscoff (Swedmark & Teissier, 1957a), but in our analysis, only two morphologically identified individuals were from Roscoff. The majority of specimens were from two locations on Helgoland. Here, at least from the subtidal location at the Youth Hostel, *H. vermiformis* has been found before (Clausen, 1967). There is no record of *H. vermiformis* from the “Dune” of Helgoland so far, but Remane (1927) mentioned in his description of *H. octopodides* small individuals with only 7 tentacles, 3 aboral and 4 subaboral from this location. It is possible that he already found *H. vermiformis*, but did not identify them as a different species.

Halammohydra vermiformis has a very special morphology with low variation in the characters. This makes it easy to identify. Most specimens have 7 tentacles, and few have 8 but not more (Swedmark & Teissier, 1957b). The latter individuals can be confused with *H. octopodides*, especially if the gastric tube is not as elongated as in other individuals, i.e., due to contraction or a variation in shape. *Halammohydra octopodides* mostly have an ovoid to the slightly elongated gastric tube. A molecular investigation can differentiate between them with certainty, as it was the case for some individuals from Sylt with eight tentacles. *Halammohydra vermiformis* was not reported from this location before, but *H. octopodides* (Polte & Schmidt-Rhaesa, 2011) and *H. schulzei* (Schmidt, 1969).

Interestingly, in the molecular analysis, there was a separation of morphologically similar individuals of *H. vermiformis* in two clusters. The only difference between these clusters is the slight size difference, which can be a result of a sampling or measuring bias, and, much more important, the habitat. One group was found exclusively in the “Pier at the Youth Hostel” of Helgoland, whereas the other group was from several locations. Most of them were in the intertidal, except the station in Denmark, but here the specimens were found above the halocline. At this station, the mechanical influence of the waves is less, but there is a lower salinity, which is also found at times in intertidal locations, especially at the station of Sylt. Here, animals inhabit the moist sand of the beach at low tide without water covering the sediment, and variations in salinity are very frequent. Clusters of *H. vermiformis* are in a sister group relationship, but one group potentially tolerates higher variability in abiotic factors, such as salinity.

Halammohydra octopodides on the other hand separated into three clusters without an obvious relation to environmental factors. Morphologically, they are similar and fit in

the range of descriptions and locations of previous records (Clausen, 1963, 1967; Remane, 1927; Renaud-Debyser, 1964; Swedmark, 1957; Swedmark & Teissier, 1957a). Although they were first described on Helgoland (Remane, 1927), only two specimens were found in this study there. The majority came from Roscoff. Species delimitation tests grouped *H. octopodides* 1 and 2 together (except GMYC), which indicates them as one species. *Halammohydra octopodides* 3 is separate in every analysis but there are no morphological characters separating them, and they are from the same stations as the animals of groups 1 and 2. Additionally, *H. octopodides* 3 contains only two individuals with features falling in the range of morphological characters of *H. octopodides*. However, since we only have limited molecular data and insignificant morphological variation, we follow a conservative approach and regard all three clusters as one species. The “Roscoff—type” with a higher number of tentacles and slight differences in the cnidome described by Swedmark (1957) was not found in this study and thus, could not be tested.

For *Halammohydra coronata* a smaller and a larger form was described at Helgoland (Clausen, 1967). Specimens found in this study were of the smaller form, which corresponds to the location and morphological characters described. The larger form was from the “Amphioxus”—flat near Helgoland (Clausen, 1967). One feature not described before was the one long tentacle in the subaboral whorl of some individuals. The description of the smaller form of this species is based on only three specimens, which can be a reason that not all characters could be documented. There is some variation in this feature, as it only occurred in a few animals here. This is the first record of *H. coronata* from Roscoff. Before it was reported only from Helgoland (Clausen, 1967) and the Delta area in the Netherlands (Wolff et al., 1974).

Halammohydra coronata was described to be closely related to *H. adherens* because of the similar cnidome, at least in the larger forms (Clausen, 1967). This similarity could not be tested, since we did not find the larger form here, but the relationship between both species was confirmed in the molecular analyses. Most animals of *H. adherens* were found in the shell gravel of the station “Trezen ar Skoden” in Roscoff, similar to what is reported in the literature (Swedmark & Teissier, 1959, 1967). *H. adherens* was described as being large (about 800 µm), with 12–14 tentacles in each whorl (Swedmark & Teissier, 1967). Such character combination was documented only for one animal in our investigation, assigned to *H. adherens*. Other specimens of *H. adherens* were much smaller and had less tentacles, so there appears to be a higher variability in characters than previously reported. One special feature, compared to other species, is the cnidome. In light microscopy, nematocysts of an elongated shape, possible macrobasic euryteles,

are visible. Swedmark and Teissier (1967) described micro- and macrobasic euryteles with seemingly the same shape for this species. However, this cannot be stated with certainty, because no detailed investigation was done, but no other group showed these noticeable nematocysts in light microscopy. This raises the question, if the described larger form of *H. coronata* is actually *H. adherens* or an individual species and a potential intermediate between *H. coronata* and *H. adherens*.

Two species recorded in Europe were not identified in our study or not with sufficient certainty. One is *H. intermedia* (Clausen, 1967), which combines characters of both *H. schulzei* and *H. octopodides* and was described from Norway and also reported from Helgoland (Clausen, 1967). The other is *H. schulzei*, a species with records from many different locations: Helgoland (Clausen, 1967; Remane, 1927), Sylt (Schmidt, 1969), Western Baltic Sea (Schulz, 1952), Roscoff (Swedmark, 1957; Swedmark & Teissier, 1957a, 1957b; Teissier, 1950), Marseille (Swedmark, 1957), United Kingdom (Boaden, 1961), Norway (Clausen, 1963, 1967) and one record from the Western Atlantic (Bush & Zinn, 1970). These records give the impression of a broad distribution and high numbers of specimens for *H. schulzei*, but this could not be confirmed in our investigations. In this study, specimens of “Roscoff” had characters closest to the description, except for the lower number of tentacles and statocysts. *Halammohydra schulzei* was described with 14 to 24 (Remane, 1927) and up to 26 tentacles (Swedmark, 1957; Swedmark & Teissier, 1957b) and 12 statocysts (Remane, 1927). There is no information about the tentacle length within one whorl and between the two whorls in previous records. Aboral tentacles of “Roscoff” were longer than subaboral ones, which were of unequal length. When comparing our observations with the few images available in the literature, these length differences are not visible there. This can be due to the absence of these characters in the described specimens of *H. schulzei* or because of the choice of pictures in the publications.

Another cluster with a similar character combination is “Azores.” The characters here observed fall in the range of variation described for *H. schulzei*, thus the specimens were preliminary assigned to this species (Tödter & Schmidt-Rhaesa, 2021). Molecular analyses placed both clusters, “Roscoff” and “Azores,” in different positions of the tree, indicating no close relationship and complicating the identification. Since *H. schulzei* was described from Helgoland, a closer look into the cluster “Helgoland/ Sylt” is needed. It is in a sister relation to “Azores” but lacking the pronounced bulb at the tentacle bases in the subaboral whorl and the number of tentacles is too low in some specimens. There are records of *H. schulzei* from Roscoff (Swedmark, 1957; Swedmark & Teissier, 1957a, 1957b; Teissier, 1950), but it is surprising not to find this species at its location

of description on Helgoland (Remane, 1927). Hence, the molecular identification of *H. schulzei* remains unclear and needs more data to conclude. Additionally, as for *H. octopodides*, Swedmark (1957) described a “Roscoff—type” for *H. schulzei*, which was not documented in this study and thus could not be tested here.

Besides the identified species, there are four clusters, for which support values and species delimitation tests suggest that they are separate species. These clusters are *H. teissieri* n. sp., *H. swedmarki* n. sp., *H. kerblae* n. sp. and *H. joergerae* n. sp.. They are described as new species here (see results). *Halammohydra teissieri* n. sp. resembles *H. schulzei* in many aspects but is lacking the pronounced thickening at the tentacle bases and aboral tentacles are longer than subaboral ones. *Halammohydra swedmarki* n. sp. has not many similarities to described species. One striking feature is the length of the tentacles. They appear noticeably longer than in other species. *Halammohydra kerblae* n. sp. on the other hand has similarities to *H. coronata*. The differences are the thickening of the tentacle bases and the lacking long tentacle in the subaboral whorl. Specimens of *H. joergerae* n. sp. have different character combinations than the other species and clusters. Additionally, there is one singleton from Brazil and the GenBank sequences (Panama) in isolated positions of the tree. All analyses support them to be a separate species, but since no detailed morphological data could be obtained from the fixed material or the downloaded sequences, no new species are described here.

For the further clusters or singletons, support to separate species is less strong or morphological data are lacking and therefore we do not describe them as new species. In Tenerife at least two species occur. “Tenerife 2” is positioned close to “Roscoff,” but the three species delimitation tests separate them in two clusters. Additionally, there are some differences in the morphology, like the lacking thickening in the tentacles of the subaboral whorl in “Tenerife 2.” Due to the geographical distance, it is possible, that “Tenerife 2” and “Roscoff” are different species, but there is not enough data available to reliably characterize both as new species. The singleton R118 from “Trezen ar Skoden” is nested between them but is not clustered to one of the two groups by species delimitation tests. Since no useful morphological data were documented, no further conclusions can be made. “Tenerife 1” consists of three specimens, but this cluster is only supported by 18S sequences, which does not give reliable results on species level and even ABGD and bPTP of 18S separated all three specimens, therefore it is not confirmed that the three specimens even belong to the same species. Given the position of “Tenerife 1” as a sister clade to all remaining species of *Halammohydra*, further investigations on these specimens is very interesting.

The three singletons positioned between *H. kerblae* n. sp. and the clade of “Tenerife 2” and “Roscoff” have more

morphological similarities to the latter clade than *H. kerblae* n. sp. but are lacking a thickening at the bases of the subaboral tentacles. Additionally, R104 and R75 were juveniles and thus cannot be used for comparison. It is possible, that these are additional species, but there is not enough data to reliably describe new species.

Previously only nine species of *Halammohydra* were described, mostly from Europe (e.g., Clausen, 1967; Remane, 1927; Swedmark & Teissier, 1957a) and India (e.g., Rao, 1978; Rao & Ganapati, 1966; Rao & Misra, 1980), but this is likely a result of a sampling bias. Most of the early meiofaunal research was done in Europe (Giere, 2009), hence the high amount of records. Additional previous findings in the Western Atlantic and the Caribbean Sea (e.g., Bush & Zinn, 1970; Calder & Kirkendale, 2005; Garraffoni et al., 2017) and on the Azores (Tödter & Schmidt-Rhaesa, 2021) suggest a broader distribution than expected. Nonetheless, it is interesting, that Europe, or single localities within, harbor such a high number of different species, especially compared to other genera of meiofaunal Cnidaria. For example, five out of the six species described in Europe were found at Helgoland, while this study adds at least one new species for this location. Another species-rich locality is Roscoff. Four species and two “types” were found in Roscoff and this study reveals that there are at least three new species present in the Roscoff area and adds the finding of *H. coronata* as a new record. Our study shows that the species diversity in Europe is distinctly higher than previously assumed, with four new species described from here and several potential new species, for which further investigation is needed.

Conclusions

This study contributes to the molecular database by providing species-specific sequence information of three genes, which is useful for future biodiversity studies. The combination with morphological investigations shows the different variability of characters within one species and the sometimes overlapping features between species. Identification of specimen of *Halammohydra* in the field remains difficult for some species, but due to the information about the range of characters, it can be narrowed down to a few species. However, to ensure a correct identification, 16S or CO1 sequence data are essential.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13127-023-00606-x>.

Acknowledgements We thank the multiple people and funding agencies supporting our field trip sampling: Alexandra Kerbl, during the Roscoff sampling (supported by AssemblePlus_20190215), participants of “Meiozoos 2019” on the Azores (supported by the VW

Foundation N° 96493 and), Jan Raeker and Alejandro Martínez in Tenerife (expedition supported by the DFG grant SCHM 1278/20-1), Maikon Di Domenico in Brazil (Permit Number: 34686-1 (ICMBIO/IBAMA—Brasil—Parque Nacional Marinho de Fernando de Noronha; supported by FAPESP 2012/08581-0), Maickel Armenteros, Erik García-Machado in Cuba (expedition supported by the Carlsberg Foundation grant 2013_01_0779). We thank Wolfgang Sterrer and Nicolas Bekkouche for providing us a specimen from Arcachon. We moreover thank Simon Bober and Oliver Hawlitschek for their support in the lab.

Funding Open Access funding enabled and organized by Projekt DEAL. This study was funded by Deutsche Forschungsgemeinschaft (DFG) with the grant number SCHM 1278/18–1 and conducted at the University of Hamburg and the Leibniz Institute for the Analysis of Biodiversity Change (LIB) in Hamburg (Museum of Nature—Zoology) as part of a PhD project. Additionally, the expedition to Roscoff was funded by AssemblePlus with the grant number Assemble-Plus_20190215 at the Station Biologique de Roscoff.

Data availability All sequences generated and analyzed during the current study are available in GenBank,

Declarations

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study since experimental work was conducted on an unregulated invertebrate species.

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Altaff, K., Sugumaran, J., & Naveed, M. S. (2005). Impact of tsunami on meiofauna of Marina beach, Chennai. *India. Current Science*, 89(1), 34–38.
- Bik, H. M., Thomas, W. K., Lunt, D. H., & Lamshead, P. J. D. (2010). Low endemism, continued deep-shallow interchanges, and evidence for cosmopolitan distributions in free-living marine nematodes (order Enoplida). *BMC Evolutionary Biology*, 10(1), 1–10. <https://doi.org/10.1186/1471-2148-10-389>
- Boaden, P. (1960). Three new gastrotrichs from the Swedish west coast. *Cahiers De Biologie Marine*, 1, 397–406.
- Boaden, P. (1961). Littoral interstitial species from Anglesey representing three families new to Britain. *Nature*, 191, 512.
- Boaden, P. (1963). The interstitial fauna of some North Wales beaches. *Journal of the Marine Biological Association of the United Kingdom*, 43(1), 79–96. <https://doi.org/10.1017/S0025315400005270>
- Boaden, P. (1966). Interstitial fauna from northern Ireland. *Veröffentlichungen Des Instituts Für Meeresforschung in Bremerhaven*, 2, 125–136.
- Boeckner, M. J., Sharma, J., & Proctor, H. C. (2009). Revisiting the meiofauna paradox: Dispersal and colonization of nematodes and other meiofaunal organisms in low- and high-energy environments. *Hydrobiologia*, 624(1), 91–106. <https://doi.org/10.1007/s10750-008-9669-5>
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. -H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS computational biology*, 10(4), e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>
- Bucklin, A., Steinke, D., & Blanco-Bercial, L. (2011). DNA barcoding of marine metazoa. *Annual Review of Marine Science*, 3, 471–508. <https://doi.org/10.1146/annurev-marine-120308-080950>
- Bush, L., & Zinn, D. J. (1970). *Halammohydra schulzei*: First actinulid recorded from Western Atlantic. *Transactions of the American Microscopical Society*, 89(3), 431–433.
- Calder, D. R., & Kirkendale, L. (2005). Hydrozoa (Cnidaria, Hydrozoa) from shallow-water environments along the Caribbean coast of Panama. *Caribbean Journal of Science*, 41(3), 476–491.
- Cerca, J., Purschke, G., & Struck, T. H. (2018). Marine connectivity dynamics: Clarifying cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox. *Marine Biology*, 165. <https://doi.org/10.1007/s00227-018-3383-2>
- Clausen, C. (1963). The hydrozoan *Halammohydra* found in Norway. *Sarsia*, 11(1), 17–20. <https://doi.org/10.1080/00364827.1963.10410280>
- Clausen, C. (1967). Morphological studies of *Halammohydra* Remane (Hydrozoa). *Sarsia*, 29(1), 349–370. <https://doi.org/10.1080/00364827.1967.10411094>
- Clausen, C. (1991). Differentiation and ultrastructure of nematocysts in *Halammohydra intermedia* (Hydrozoa, Cnidaria). In *Coelenterate biology: recent research on Cnidaria and Ctenophora* (pp. 623–628): Springer.
- Clausen, C. (2000). Light and ultrastructural observations on a microsporidium in the hydrozoan *Halammohydra intermedia* (Cnidaria). *Sarsia*, 85, 177–180. <https://doi.org/10.1080/00364827.2000.10414568>
- Clausen, C. (2004). A new species of *Acanthodasys* (Gastrotricha: Thaumastodermatidae) from the west coast of Norway. *Sarsia*, 89(2), 137–141.
- Collins, A. G., Bentlage, B., Lindner, A., Lindsay, D., Haddock, S. H. D., Jarms, G., Norenburg, J. L., Jankowski, T., & Cartwright, P. (2008). Phylogenetics of Trachylina (Cnidaria: Hydrozoa) with new insights on the evolution of some problematical taxa. *Journal of the Marine Biological Association of the United Kingdom*, 88(8), 1673–1685. <https://doi.org/10.1017/S0025315408001732>
- d'Hondt, J. -L. (1968). Gastrotriches et Halammohydrines des côtes Flamandes et Picardes. *Bulletin Du Muséum National D'histoire Naturelle*, 2, 214–227.
- Dahl, E. (1953). The Narcomedusa *Halammohydra octopodides* Remane new to Sweden. *Kungl. Fysiografiska Sällskapet I Lund Förhandlingar*, 22(18), 1–2.
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J., Guindon, S., Lefort, V., & Lescot, M. (2008). Phylogeny.fr: Robust phylogenetic analysis for the non-specialist. *Nucleic acids research*, 36(suppl_2), W465–W469. <https://doi.org/10.1093/nar/gkn180>
- Ehlers, U. (1993). Ultrastructure of the spermatozoa of *Halammohydra schulzei* (Cnidaria, Hydrozoa): The significance of acrosomal structures for the systematization of the Eumetazoa. *Microfauna Marina*, 8, 115–130.
- Faurby, S., Jørgensen, A., Kristensen, R. M., & Funch, P. (2011). Phylogeography of North Atlantic intertidal tardigrades: Refugia,

- cryptic speciation and the history of the Mid-Atlantic Islands. *Journal of Biogeography*, 38(8), 1613–1624. <https://doi.org/10.1111/j.1365-2699.2011.02533.x>
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Fontaneto, D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Movement Ecology*, 7(1), 1–10. <https://doi.org/10.1186/s40462-019-0155-7>
- Fontaneto, D., Flot, J. -F., & Tang, C. Q. (2015). Guidelines for DNA taxonomy, with a focus on the meiofauna. *Marine Biodiversity*, 45, 433–451. <https://doi.org/10.1007/s12526-015-0319-7>
- Fontaneto, D., Kaya, M., Herniou, E. A., & Barraclough, T. G. (2009). Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution*, 53(1), 182–189. <https://doi.org/10.1016/j.ympev.2009.04.011>
- Garraffoni, A. R., Di Domenico, M., & Hochberg, R. (2017). New records of marine Gastrotricha from São Sebastião Island (Brazil) and the description of a new species. *Marine Biodiversity*, 47(2), 451–459. <https://doi.org/10.1007/s12526-016-0486-1>
- Giere, O. (2009). *Meiobenthology: The microscopic motile fauna of aquatic sediments*. Springer.
- Gray, J. S. (1971). Occurrence of the aberrant bryozoan Monobryozoon ambulans Remane, off the Yorkshire coast. *Journal of Natural History*, 5(1), 113–117.
- Guil, N. (2011). Molecular approach to micrometazoans. Are they here, there and everywhere. In D. Fontaneto (Ed.), *Biogeography of microscopic organisms: Is everything small everywhere* (Vol. 79, pp. 284–306): Cambridge University Press.
- Hagerman, G. M., & Rieger, R. M. (1981). Dispersal of benthic meiofauna by wave and current action in Bogue Sound, North Carolina, USA. *Marine Ecology*, 2(3), 245–270. <https://doi.org/10.1111/j.1439-0485.1981.tb00099.x>
- Harvey, L., & Wells, J. (1961). Occurrence of a species of Halammothra on the Isles of Scilly. *Nature*, 189(4768), 944–944.
- Hellberg, M. E. (2006). No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology*, 6(1), 24. <https://doi.org/10.1186/1471-2148-6-24>
- Higgins, R. P., & Thiel, H. (1988). *Introduction to the study of meiofauna*. Washington D.C.: Smithsonian Institution Press.
- Hillis, D. M., & Dixon, M. T. (1991). Ribosomal DNA: Molecular evolution and phylogenetic inference. *The Quarterly Review of Biology*, 66(4), 411–453.
- Hochberg, R., Atherton, S., & Kieneke, A. (2014). Marine Gastrotricha of Little Cayman Island with the description of one new species and an initial assessment of meiofaunal diversity. *Marine Biodiversity*, 44(1), 89–113. <https://doi.org/10.1007/s12526-013-0186-z>
- Janakiraman, A., Naveed, M., Sheriff, M. A., & Altaff, K. (2016). Meiofaunal response to the tidal exchange and domestic sewage in the Adyar Estuary, Chennai. *India. Indian Journal of Geo-Marine Science*, 45(10), 1341–1348.
- Jörger, K. M., Álvaro, N. V., Andrade, L. F., Araújo, T. Q., Aramayo, V., Artois, T., Ballentin, W., Bergmerier, F. S., Botelho, A. T., Buckenmeyer, A., Capucho, A. T., Cherneva, I., Curini-Galletti, M., Davison, A. M., Deng, W., Di Domenico, M., Ellison, C., Engelhardt, J., Fais, M. et al. (2021). Meiozoos 2019 - Exploring the marine meiofauna of the Azores. *Açoreana*, 17–41.
- Jörger, K. M., Norenburg, J. L., Wilson, N. G., & Schrödl, M. (2012). Barcoding against a paradox? Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal sea slugs. *BMC Evolutionary Biology*, 12(1), 1–18. <https://doi.org/10.1186/1471-2148-12-245>
- Jörger, K. M., Stoschek, T., Migotto, A. E., Haszprunar, G., & Neusser, T. P. (2014). 3D-microanatomy of the mesopsammic *Pseudovermis salamandrops* Marcus, 1953 from Brazil (Nudibranchia, Gastropoda). *Marine Biodiversity*, 44(3), 327–341. <https://doi.org/10.1007/s12526-014-0224-5>
- Kånneby, T., Atherton, S., & Hochberg, R. (2014). Two new species of *Musellifer* (Gastrotricha: Chaetonotida) from Florida and Tobago and the systematic placement of the genus within Paucitubulatina. *Marine Biology Research*, 10(10), 983–995. <https://doi.org/10.1080/17451000.2013.872797>
- Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, 20(4), 1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34(3), 772–773. <https://doi.org/10.1093/molbev/msw260>
- Leasi, F., Andrade, S. C. D. S., & Norenburg, J. (2016). At least some meiofaunal species are not everywhere. Indication of geographic, ecological and geological barriers affecting the dispersion of species of Ototyphlonemertes (Nemertea, Hoplonemertea). *Molecular ecology*, 25(6), 1381–1397. <https://doi.org/10.1111/mec.13568>
- Martínez, A., Di Domenico, M., Leasi, F., Curini-Galletti, M., Todaro, M. A., Zotto, M. D., Gobert, S., Artois, T., Norenburg, J., Jörger, K. M., Núñez, J., Fontaneto, D., & Worsaae, K. (2019). Patterns of diversity and endemism of soft-bodied meiofauna in an oceanic island, Lanzarote. *Canary Islands. Marine Biodiversity*, 49(5), 2033–2055. <https://doi.org/10.1007/s12526-019-01007-0>
- Martínez, A., Palmero, A. M., del Carmen Brito, M., Núñez, J., & Worsaae, K. (2009). Anchialine fauna of the Corona lava tube (Lanzarote, Canary Islands): Diversity, endemism and distribution. *Marine Biodiversity*, 39, 169–182. <https://doi.org/10.1007/s12526-009-0023-6>
- McFadden, C. S., Benayahu, Y., Pante, E., Thoma, J. N., Nevarez, P. A., & France, S. C. (2010). Limitations of mitochondrial gene barcoding in Octocorallia. *Molecular Ecology Resources*, 11(1), 19–31. <https://doi.org/10.1111/j.1755-0998.2010.02875.x>
- Medlin, L., Elwood, H. J., Stickel, S., & Sogin, M. L. (1988). The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71, 491–499. [https://doi.org/10.1016/0378-1119\(88\)90066-2](https://doi.org/10.1016/0378-1119(88)90066-2)
- Michonneau, F. (2017). Using GMYC for species delineation. *Zenodo*. <https://doi.org/10.5281/zenodo.838260>
- Mohan, P., & Dhivya, P. (2010). Diversity of meiobenthos of the Andaman and Nicobar waters. In R. Raghunathan & C. Sivaperuman (Eds.), *Recent Trends in Biodiversity of Andaman and Nicobar Islands* (pp. 89–104). Kolkata: Zoological Survey of India.
- Moore, C. G. (1979). The distribution and ecology of psammolittoral meiofauna around the Isle of Man. *Cahiers De Biologie Marine*, 20(4), 383–415.
- Nagabhushanam, A. (1972). Studies on the marine intertidal ecology of the Orissa coast. *Zoological Survey of India*, 38, 308–315.
- Ortman, B. D., Bucklin, A., Pages, F., & Youngbluth, M. (2010). DNA barcoding the Medusozoa using mtCOI. *Deep Sea Research Part II: Topical Studies in Oceanography*, 57(24–26), 2148–2156. <https://doi.org/10.1016/j.dsr2.2010.09.017>
- Polte, S., & Schmidt-Rhaesa, A. (2011). Immunohistochemical investigations of the interstitial cnidarian *Halammothra octopodides* (Hydrozoa). *Meiofauna Marina*, 19, 17–32.
- Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, automatic barcode gap discovery for primary species delimitation.

- Molecular Ecology*, 21(8), 1864–1877. <https://doi.org/10.1111/j.1365-294X.2011.05239.x>
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67(5), 901. <https://doi.org/10.1093/sysbio/syy032>
- Rao, G. C. (1975). The interstitial fauna in the intertidal sands of Andaman and Nicobar group of Islands. *Journal of the Marine Biological Association of India*, 17(2), 116–128.
- Rao, G. C. (1978). On a new species of *Halammohydra* (Actinulida, Hydrozoa) from Andamans, India. *Bulletin of Zoological Survey of India*, 1(2), 147–149.
- Rao, G. C. (1993). Littoral meiofauna of little Andaman. *Zoological Survey of India*, 155.
- Rao, G. C., & Ganapati, P. (1965). On a new species of *Sphaerosyllis* (Polychaeta) from the beach sands of Waltair coast. Paper presented at the Proceedings of the Indian Academy of Sciences-Section B.
- Rao, G. C., & Ganapati, P. (1966). A report on the occurrence of an aberrant cnidarian *Halammohydra octopodides* Remane. *Indian Waters. Current Science*, 35(5), 129–130.
- Rao, G. C., & Misra, A. (1980). On a new species of *Halammohydra* (Actinulida, Hydrozoa) from Sagar Island, India. *Bulletin of Zoological Survey of India*, 3(1 & 2), 113–114.
- R Core Team. (2013). R: A language and environment for statistical computing.
- Remane, A. (1927). *Halammohydra*, ein eigenartiges Hydrozoon der Nord-und Ostsee. *Zeitschrift Für Morphologie Und Ökologie Der Tiere*, 7(4), 643–677.
- Renaud-Debyser, J. (1964). Note sur la faune interstitielle du bassin d'Arcachon et description d'un Gastrotriche nouveau. *Cahiers De Biologie Marine*, 5, 111–123.
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3), 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Salvini-Plawen, L. (1991). *Pseudovermis thompsoni* new species (Nudibranchia: Aeolidioidea) from the Northern Adriatic sea. *Journal of Molluscan Studies*, 57(Supplement_Part_4), 179–187. https://doi.org/10.1093/mollus/57.Supplement_Part_4.179
- Salvini-Plawen, L., & Rao, G. C. (1973). On three new mesopsammbiotic representatives from the Bay of Bengal: Species of *Anthohydra* gen. nov. (Hydrozoa) and of *Pseudovermis* (Gastropoda). *Zeitschrift für Morphologie der Tiere*, 74(3), 231–240. <https://doi.org/10.1007/BF00375786>
- Schmidt-Rhaesa, A., Pyataeva, S., & Collins, A. G. (2020). Cnidaria. In A. Schmidt-Rhaesa (Ed.), *Guide to the identification of marine meiofauna* (pp. 33–43). Pfeil-Verlag.
- Schmidt, H., & Westheide, W. (2000). Are the meiofaunal polychaetes *Hesionides arenaria* and *Stygocapitella subterranea* true cosmopolitan species?—results of RAPD-PCR investigations. *Zoologica Scripta*, 29(1), 17–27. <https://doi.org/10.1046/j.1463-6409.2000.00026.x>
- Schmidt, P. (1969). Die quantitative Verteilung und Populationsdynamik des Mesopsammons am Gezeiten-Sandstrand der Nordsee-Insel Sylt II. Quantitative Verteilung und Populationsdynamik einzelner Arten. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, 54(1), 95–174. <https://doi.org/10.1002/iroh.19690540104>
- Schulz, E. (1952). *Halammohydra schulzei* Remane in der westlichen Ostsee. *Faunistische Mitteilungen Aud Norddeutschland*, 2, 6–7.
- Shearer, T. L., van Oppen, M. J. H., Romano, S. L., & Würheide, G. (2008). Slow mitochondrial DNA sequence evolution in Anthozoa (Cnidaria). *Molecular Ecology*, 11(12), 2475–2487. <https://doi.org/10.1046/j.1365-294X.2002.01652.x>
- Sterrer, W. (1973). Plate tectonics as a mechanism for dispersal and speciation in interstitial sand fauna. *Netherlands Journal of Sea Research*, 7, 200–222. [https://doi.org/10.1016/0077-7579\(73\)90045-8](https://doi.org/10.1016/0077-7579(73)90045-8)
- Sugumaran, J., Naveed, M., & Altaff, K. (2009). Diversity of meiofauna of Chennai, East coast of India. *Journal of Aquatic Biology*, 24, 31–34.
- Sugumaran, J., & Padmasai, R. (2019). Meiofaunal diversity and density of Manamelkudi – an intertidal sandy beach of Palk Bay, India. *Research Journal of Life Science, Bioinformatics, Pharmaceutical and Chemical Science*, 5(2), 31–46. <https://doi.org/10.26479/2019.0502.03>
- Swedmark, B. (1957). Variation morphologique des différentes populations régionales d'*Halammohydra*. *Année Biologique*, 33, 3–4.
- Swedmark, B., & Teissier, G. (1957a). *Halammohydra vermiformis* n. sp. et la famille des Halammohydridae Remane. *Bulletin de la Société de Zoologie de France*, 82(1), 38–49.
- Swedmark, B., & Teissier, G. (1957b). Organisation et développement des *Halammohydra* (Hydrozoaires). *Comptes Rendus Des Séances De L'académie Des Sciences*, 244, 501–504.
- Swedmark, B., & Teissier, G. (1958). *Otohydra vagans* n. g., n. sp., hydrozoaire des sables apparenté aux Halammohydridae. *Comptes rendus des séances de l'Académie des Sciences*, 247(2), 238–240.
- Swedmark, B., & Teissier, G. (1959). *Halammohydra* et *Otohydra*, hydrozoaires de la microfaune des sables et l'ordre des Actinulidés. *Proceedings of the XVth International Congress of Zoology*, 4(15), 330–332.
- Swedmark, B., & Teissier, G. (1967). Structure et adaptation d'*Halammohydra adherens*. *Cahiers De Biologie Marine*, 8, 63–74.
- Tang, C. Q., Leasi, F., Obertegger, U., Kieneker, A., Barraclough, T. G., & Fontaneto, D. (2012). The widely used small subunit 18S rDNA molecule greatly underestimates true diversity in biodiversity surveys of the meiofauna. *Proceedings of the National Academy of Sciences*, 109(40), 16208–16212. <https://doi.org/10.1073/pnas.1209160109>
- Teissier, G. (1950). Notes sur quelques hydrozoaires de Roscoff. *Archives De Zoologie Expérimentale Es Générale*, 87, 1–10.
- Tessens, B., Monnens, M., Bäckeljau, T., Jordaens, K., Van Steenkiste, N., Breman, F. C., Smeets, K., & Artois, T. (2021). Is 'everything everywhere'? Unprecedented cryptic diversity in the cosmopolitan flatworm *Gyratrix hermaphroditus*. *Zoologica Scripta*, 50(6), 837–851. <https://doi.org/10.1111/zsc.12507>
- Todaro, M., Fleeger, J., Hu, Y., Hrinkevich, A., & Foltz, D. (1996). Are meiofaunal species cosmopolitan? Morphological and molecular analysis of *Xenotrichula intermedia* (Gastrotricha: Chaetonotida). *Marine Biology*, 125(4), 735–742. <https://doi.org/10.1007/BF00349256>
- Tödter, L., & Schmidt-Rhaesa, A. (2021). First record of *Halammohydra* (Cnidaria, Hydrozoa) on the Azores. *Acoreana Special Volume*, (11), 97–102.
- Tödter, L., & Schmidt-Rhaesa, A. (2022). Ultrastructural organization of *Halammohydra vermiformis* Swedmark & Teissier, 1957 (Cnidaria: Hydrozoa). *Zoomorphology*, 1–17. doi:<https://doi.org/10.1007/s00435-022-00560-w>
- Trifinopoulos, J., Nguyen, L. -T., von Haeseler, A., & Minh, B. Q. (2016). W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, 44(W1), W232–W235. <https://doi.org/10.1093/nar/gkw256>
- Varadharajan, D., & Soundarapandian, P. (2013). Meiofauna distribution from Arukkattuthurai to Aiyampattinam. *South East Coast of India*, 2(682), 2.
- von Soosten, C., Schmidt, H., & Westheide, W. (1998). Genetic variability and relationships among geographically widely separated populations of *Petitita amphophthalma* (Polychaeta: Syllidae). *Results from RAPD-PCR Investigations. Marine Biology*, 131(4), 659–669. <https://doi.org/10.1007/s002270050358>

- Westheide, W. (1991). The meiofauna of the Galapagos. In M. J. James (Ed.), *Galápagos marine invertebrates* (Vol. 8, pp. 37–73). Springer.
- Wolff, W., Sandee, A., & Stegenga, H. (1974). *Halammohydra vermiformis* and *H. coronata* (Hydrozoa), new to the fauna of the Netherlands. *Netherlands Journal of Sea Research*, 8(4), 407–409. [https://doi.org/10.1016/0077-7579\(74\)90008-8](https://doi.org/10.1016/0077-7579(74)90008-8)
- Worsaae, K., Kerbl, A., Vang, Á., & Gonzalez, B. C. (2019a). Broad North Atlantic distribution of a meiobenthic annelid – against all odds. *Scientific Reports*, 9(1), 15497. <https://doi.org/10.1038/s41598-019-51765-x>
- Worsaae, K., Mikkelsen, M. D., & Martínez, A. (2019b). Description of six new species of Mesonerilla (Nerillidae, Annelida) and an emended description of *M. intermedia* Wilke, 1953, from marine and cave environments. *Marine Biodiversity*, 49(5), 2141–2165. <https://doi.org/10.1007/s12526-019-00984-6>
- Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29(22), 2869–2876. <https://doi.org/10.1093/bioinformatics/btt499>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.