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The illusion of rarity in an epibenthic jellyfish: facts and artefacts in the distribution of *Tesserogastria musculosa* (Hydrozoa, Ptychogastriidae)

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Abstract

Epibenthic and benthopelagic medusae are rarely collected by standard benthic or pelagic sampling methods, and many species are considered uncommon and geographically restricted. Peer-reviewed scientific literature contains only two records of medusae belonging to the monotypic genus *Tesserogastria* Beyer, 1958 since their original description, both from the vicinity of the type locality in Oslofjord, contributing to an illusion of extreme rarity and restricted distribution. Our analysis of fresh samples and a thorough evaluation of all previous records of this taxon from both peer-reviewed scientific sources and "gray" literature show that the species is both more common and widespread than suggested by the scant records in primary scientific literature, and represents an example of an overlooked taxon in the epibenthos. High numbers of medusae of *Tesserogastria musculosa* Beyer, 1958 were collected at Raunefjord in western Norway. New data, together with validated observations from fjords in western and eastern Norway as well as western Sweden, demonstrate that the species is much more common than is evident from published records. Data on the mitochondrial 16S ribosomal RNA and cytochrome oxidase I molecular markers for the species are provided for the first time, as well as new observations on the morphology of living animals. *Tesserogastria musculosa* constitutes an example of a hydrozoan species with a misleading reported distribution, a situation likely to occur in all members of family Ptychogastriidae and other delicate epibenthic invertebrates. Sampling techniques specifically targeting the epibenthos and careful processing of the samples are essential for correctly assessing the presence of the species, suggesting that the lack of records for this and other epibenthic medusae may in part be an artefact of the commonly used sampling methods. A comparison of molecular data for species and genus delimitation in Ptychogastriidae, presented here for the first time, highlights the need for a thorough taxonomic revision of the family.

Keywords: Hydromedusa, Trachylina, Soft bottom, DNA-barcoding, Benthic medusa

Background

Epibenthic and benthopelagic medusae are an important but easily overlooked component of neritic and oceanic bottoms around the world. More than 45 species of hydromedusae live either attached to or closely associated with the substrate [1], and many representatives of the families Cladonematidae, Olindiidae, Ptychogastriidae, and Rhopalonematidae are morphologically adapted to life in a benthic habitat [2]. Epibenthic medusae can be locally abundant, likely playing a significant role in bentho-pelagic coupling [3] but, for the majority of the species, only a handful of records exist and information on their distribution, population dynamics and ecology is lacking. The scarcity of records for epibenthic and benthopelagic medusae may be partially attributed to methodological limitations. Sampling with traditional

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plankton nets generally does not reach the epibenthos or the water column immediately above the bottom, while specimens are easily damaged beyond recognition during collection with dredges or bottom trawls, and their fragments are often discarded as planktonic contaminants.

The "mud jellyfish" *Tesserogastria musculosa*, the sole species in the genus and a member of the poorly known family Ptychogastriidae, is one of the lesser-known species of benthic hydromedusae. It was first collected in the mid-1950s by Beyer at Digerud in the Oslofjord [4]. Beyer later reported on *T. musculosa*'s local distribution and abundance, suggesting that these medusae could be used as bioindicators of pollution in the area [5]. Subsequent work published in 1971 by Hesthagen, one of Beyer's students, resulted in a complete account of the morphology and behaviour of the species, and also constitutes the third and last record of the species published in a peer-reviewed scientific journal and the last report on the taxon in English [6]. The presence of *T. musculosa* has since been documented in Oslofjord—where it has, at times, been one of the most abundant components of the hyperbenthos—as well as in other Norwegian fjords (e.g. Fensfjord, Fanafjord, Sognefjord) [7–9], but these data are only available in technical reports, unpublished theses, and personal observations included in the so-called "gray" literature. The low accessibility of these sources has led to a serious bias in the estimation of the current and historical distribution of the species, resulting in its categorization as a rare taxon known exclusively from Oslofjord. In this paper, we challenge this view by reporting on the occurrence of high numbers of *T. musculosa* in Raunefjord (western Norway) and critically evaluating the existing peer-reviewed and "gray" literature records for the species. We also provide the first COI and 16S sequences for this taxon, and argue on the importance of careful sampling and processing of specimens for correct assessment of the presence of this and other benthic jellyfish.

Methods

Live individuals of *T. musculosa* were collected in Raunefjord, western Norway on February 10th 2017, using a Rothlisberg and Pearcy (RP)-sledge [10] with a 500 μm plankton net affixed horizontally over a thick rubber mat behind a steel sampling box. With the start position 60° 16.950′ N, 5° 11.212′ E, and a towing-speed of 0.5 knots for an approximate of 20 min bottom-time in a southerly direction, we covered a roughly 600 m transect of sandy mud at depths ranging between 142 (start) and 170 (end) m. As the opening width of the sledge is 1 m, the sampled area is ~600 m^2, even though it should be taken into due consideration that the RP-sledge is a semi-quantitative

gear [11] as it is not possible to check if there are any "jumps" off the bottom during the transect.

Upon retrieving the sample, the cod-end was immediately separated from the net-contents, and carefully rinsed into a tub of seawater. The net-contents were carefully washed into a separate tub with seawater and kept under a covering of water until processing. The slow towing-speed and keeping the samples submerged in water at all times ensured that the many fragile hyper-benthic species were collected intact. The sample was slowly and repeatedly decanted over to a 500 μm sieve submerged in seawater. *Tesserogastria musculosa* medusae were individually handpicked from the sample over a light table with a broad-mouthed pipette prior to fixing the rest of the sample in 96% ethanol. In addition to the epibenthic sampling, a WP3 plankton net (780 μm mesh and non-filtering cod-end) coupled to a CTD instrument (SAIV sd200, frequency of measurement 1 s, parameters salinity, temperature, dissolved oxygen) was hauled from ca. 10 m above the bottom to the surface at a speed of 0.3 ms^{-1}. Medusae of *T. musculosa* were also searched for in this plankton sample, and the accompanying environmental data were used to characterize the sampled locality.

After collection, the medusae were brought to the laboratory and identified to species level with the aid of specialized literature [4, 12]. Specimens were then fixed either in 4% formalin (for morphological analysis) or 96% ethanol (for DNA barcoding), with the latter group of specimens photographically documented prior to fixation. Live and formalin-preserved specimens were examined under a stereomicroscope (Olympus SZX16), and their detailed morphology was observed using a compound microscope (Leica CTR 6000). A subsample of 50 randomly chosen formalin-fixed individuals were measured (bell height on the oral/aboral axis; from umbrella margin to apex), and their sex determined before being deposited as vouchers in the University Museum of Bergen (UMB, Norway, catalogue number ZMBN 123772). The size distribution and sex ratio of *T. musculosa* in Raunefjord were determined, and the skewness coefficient (g1) and associated standard error (SES) of the size frequency distribution calculated following Joanes and Gill [13], skewness being detected whenever the absolute value of g1/SES is > 2 or < − 2.

For DNA barcoding, a ~1 mm^3 tissue fragment was taken from the umbrella margin of three ethanol-fixed specimens. These samples were subsequently sent to the Canadian Centre of DNA Barcoding (CCDB) in Guelph for DNA extraction and sequencing according to their protocols [14]. Specimens were sequenced for both mitochondrial cytochrome oxidase I (COI) and 16S ribosomal RNA markers. The PCR primer pairs C_LepFolF/C_LepFolR (for COI), and 16SgaF/16SgaR (for 16S) were used in

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PCR [15]. The hydrozoan origin of the sequences was verified by BLAST searches against the GenBank database of the National Center for Biotechnology Information. High quality gene fragments resulting from assembly of forward and reverse sequences were labelled as barcode compliant according to the criteria of BOLDSYSTEM and are available, together with voucher pictures and metadata, at Boldsystems.org (sample ID HYPNO_488 to 490) and GenBank (accession numbers MG700373 to MG700375 for 16S, MG700376 to MG700378 for COI). An alignment was created independently for each of the markers using MUSCLE [16] as implemented in MEGA v.7.0.26 [17], including the sequences of *T. musculosa* and those of all other available members of family Ptychogastriidae (see taxa and GenBank accession numbers in Table 1). Pairwise Kimura 2-parameter distances were calculated based on these alignments to estimate genetic differentiation among species.

A molecular phylogeny of the Ptychogastriidae was estimated independently for the above 16S and COI alignments. The sequences were analyzed using a phylogenetic approach based on (a) maximum likelihood (ML) optimality criterion in PAUP* 4.0a [18] and (b) Bayesian inference using MrBayes v. 3.2.6 [19]. The best-fit model for each dataset was calculated using jModelTest (v. 2.1.5; [20]) with default settings and chosen using the Akaike Information Criterion (AIC). For the 16S dataset, the best-fit model was TIM2+I, while for the COI dataset the best fit model was GTR+G+I. In the ML analyses, a maximum likelihood consensus tree was generated for each marker in PAUP* by conducting a heuristic search and bootstrapping with 200 replicates. In the Bayesian analyses four parallel Markov chain Monte Carlo runs

were carried out for 500,000 generations. Trees were sampled every 100 generations, discarding the 0.25% of trees as burnin.

To evaluate the previous assumption of spatial and temporal rarity of *T. musculosa*, we estimated its distribution based on all published and peer-reviewed occurrence records and mentions in the scientific literature, as well as in the "gray" literature (i.e. publicly available documents with low accessibility, not subjected to peer-review, and often published in a language other than English). We validated these records by (1) examining the corresponding specimens deposited in the scientific collections of the natural history museums of the University of Bergen and the University of Oslo, and (2) contrasting the information contained in each of them against the original description of the species. Examined material included the holotype (a single medusa mounted on a permanent slide) and 16 individuals designated as paratypes, all deposited in the Natural History Museum of the University of Oslo (ZMO, catalogue number B 860 and B 861, respectively). In all, ca. 60 years of issues (1958–2017) of scientific and technical reports from local, regional and national sources (e.g. NIVA–Norwegian Institute for Water Research, BIBSYS Brage) were analyzed, and a comprehensive search for relevant unpublished thesis and dissertations from Scandinavian universities was performed through online repositories (e.g. DUO/University of Oslo, BORA/University of Bergen; GUTEA/University of Gothenburg; NTNU Open; UiT Open Research Data). Publicly available distributional and occurrence data were also compiled from the online repositories OBIS (Ocean Biogeographic Information System) and GBIF (Global Biodiversity Information

Table 1 Percentage distance[a] for mitochondrial 16S and COI between *Tesserogastria musculosa* and other ptychogastriids

	Ptychogastria polaris (U) KY077292	*Ptychogastria polaris* (NW) MH407650	*Glaciambulata neumayeri* (WS) KY421621	
16S				
Ptychogastria polaris (NW) MH407650	0.236			
Glaciambulata neumayeri (WS) KY421621	0.162	0.233		
Tesserogastria musculosa (NW) MG700373	0.277	0.241	0.260	
	Ptychogastria polaris (AP) KY072784	*Ptychogastria polaris* (JP) KY072787	*Ptychogastria polaris* (NW) MH407229	*Glaciambulata neumayeri* (WS) KY426133
COI				
Ptychogastria polaris (JP) KY072787	0.266			
Ptychogastria polaris (NW) MH407229	0.311	0.350		
Glaciambulata neumayeri (WS) KY426133	0.219	0.246	0.347	
Tesserogastria musculosa (NW) MG700376	0.241	0.258	0.335	0.269

[a] Calculated as Pairwise Kimura-2 parameter distances. Sampling localities for each specimen are indicated in parenthesis as either North Sea (NW), Japan Sea (JP), Antarctic Peninsula (AP), Weddell Sea (WS), or Unknown (U). GenBank accession numbers are underlined

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Facility), and from the online collections catalogues of the Natural History Museum London and the Royal Belgian Institute of Natural Sciences.
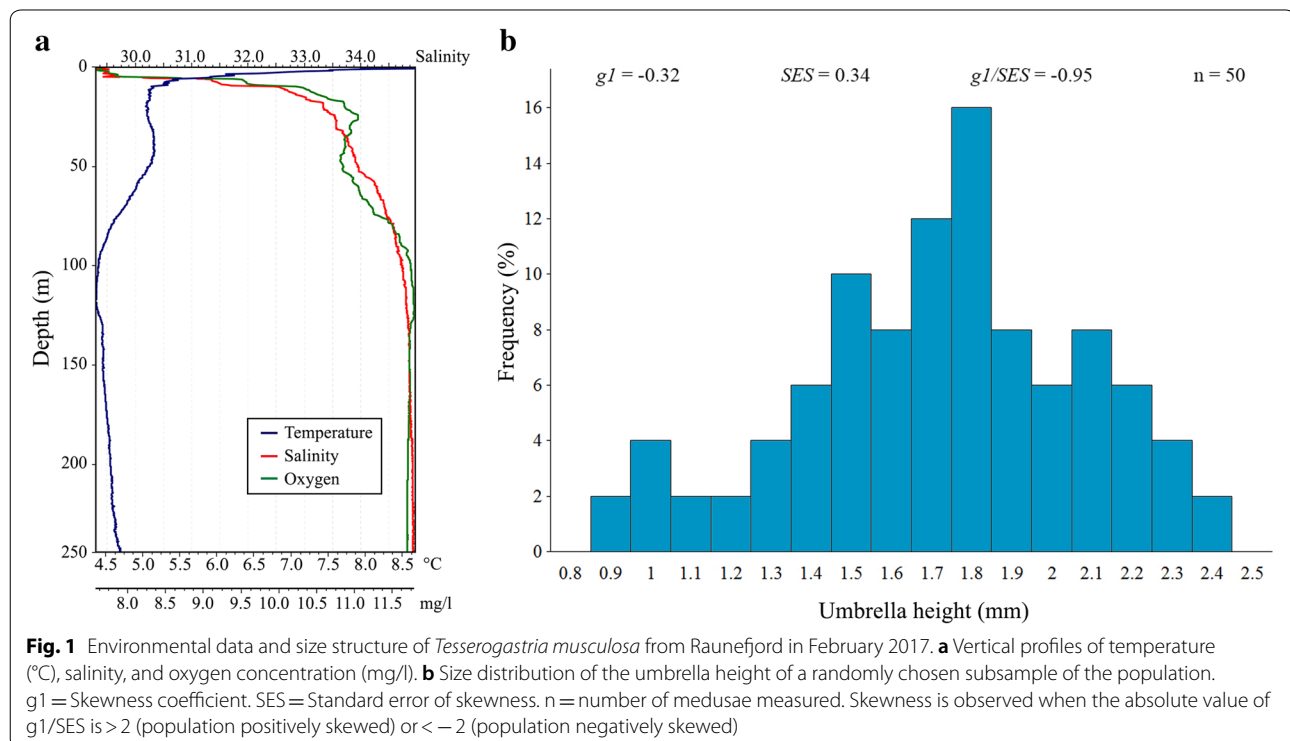
Results

A total of 260 individual medusae were collected in Raunefjord from an estimated sampled area of 600 m^2, corresponding to a density of approximately 0.43 ind/m^2. The sampled bottom consisted of sandy mud, with high amounts of organic degraded material. Our sample was dominated by various flatfish, holothurians and *Myxine glutinosa* L, 1758. Apart from the macrofauna, the main hyperbenthic taxa were *T. musculosa* and several crustaceans (i.e. Mysidacea, Amphipoda and juvenile Decapoda), with Polychaeta and Ophiuroidea present in large amounts in the heavy fraction remaining after the decanting. Within Amphipoda (a taxon with a close relationship to the substrate similar to that of *T. musculosa*) Oedicerotidae was the dominating family, as would be expected in a station with such soft, organic sediments. This is a family that is known for being fragile, with long thin legs that often break off during sampling. Stratification of temperature, salinity, and oxygen concentration was pronounced in the surface waters above the sampling locality, but conditions below ~130 m depth were relatively uniform (Fig. 1a). Mean values (±SD) of salinity, temperature, and dissolved oxygen in the water column

below 200 m depth were 34.93±0.010, 8.58±0.003 °C, and 7.81±0.036 mg/L, respectively.

The average umbrella height of *T. musculosa* in Raunefjord ranged from 0.9 to 2.4 mm, with a mean (±SD) of 1.71±0.37 mm. The majority of the medusae were in the intermediate size classes (1.5–1.9 mm). The skewness coefficient (g1/SES=−0.95) indicated that the size distribution in Raunefjord at the time of sampling was not significantly different from a symmetric/unimodal frequency distribution (Fig. 1b). Mature female medusae were readily recognized upon visual examination of the gonads through the umbrella, but the identification of males and immature females required assessment of histological preparations. The analysis of a subsample (n=20) showed that all individuals larger than 1.5 mm were mature, and the ratio of female to male in medusa larger than this size approached 1:1. The ratio of immature to mature medusae was 1:3.

The morphology of the collected *T. musculosa* is typical of the species, with a bell-shaped umbrella about as high as broad (0.9–2.4 mm), ending in a small, blunt apical projection surrounded by a circular depression (Fig. 2a, b). The otherwise thin umbrellar mesoglea is thickened at the margin in a belt of chordal cells, where up to 350 tentacles are inserted at 3–5 levels without any apparent grouping. Most of the collected specimens were completely devoid of tentacles, and only the drop-shaped scars left by shed tentacles remained (Fig. 2d, e).

Fig. 1 Environmental data and size structure of *Tesserogastria musculosa* from Raunefjord in February 2017. **a** Vertical profiles of temperature (°C), salinity, and oxygen concentration (mg/l). **b** Size distribution of the umbrella height of a randomly chosen subsample of the population. g1 = Skewness coefficient. SES = Standard error of skewness. n = number of medusae measured. Skewness is observed when the absolute value of g1/SES is > 2 (population positively skewed) or < − 2 (population negatively skewed)

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The few tentacles available for examination were all filiform, blunt, circular in transversal section, with evenly-distributed nematocysts (slightly more abundant in the tip), and were devoid of any adhesive structure. The manubrium is four-lobed, cross-shaped in transversal section (Fig. 2c), lacks mesenteries and a peduncle, and often extends beyond the bell margin before ending in a square mouth with four simple lips. Eight rather narrow radial canals connect the base of the manubrium to the broad circular canal situated next to the conspicuous nematocyst ring at the umbrellar margin (Fig. 2b). The velum is broad and strongly muscular. There are
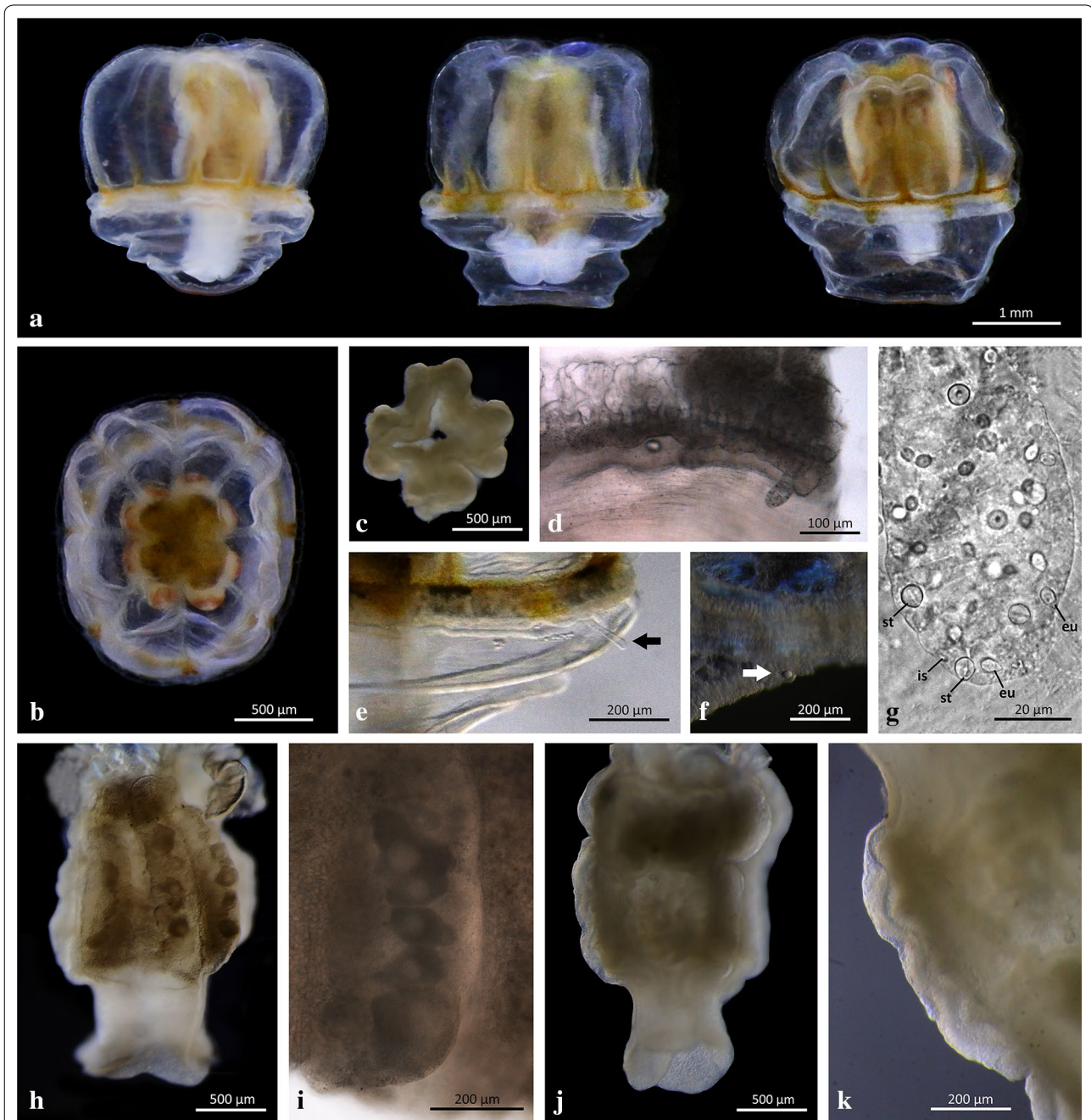
Fig. 2 *Tesserogastria musculosa* Beyer, 1958 from Raunefjord, Western Norway. **a**, **b** General morphology of the medusae in lateral and aboral view. **c** Cross-section of manubrium. **d**, **e** Tentacle stump (black arrow) and appearance of the marginal rim. **f** Statocyst with statolith (white arrow). **g** Undischarged nematocyst capsules: stenotele (st), microbasic eurytele (eu), and atrichous isorhiza (is). **h** Manubrium of a female mature medusa. **i** Close-up of developing eggs in gonadic tissue. **j** Manubrium of male mature medusa. **k** Close-up of homogeneous gonad

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eight short-stalked interradial statocysts, each with a single statolith (Fig. 2f). The gonads are organized in eight masses forming four perradial pairs (each pair corresponding to one manubrium lobe) and are evident in mature individuals: in females the eggs are arranged in 1–3 visible rows inside each gonadal mass (Fig. 2h, i), while in males the gonads appear homogenous (Fig. 2j, k). Three distinct nematocyst types, distributed in the umbrella margin and tentacles, were observed (Fig. 2g): almost spherical stenoteles [$(6–8) \times (6–7)$ μm], tear drop-shaped microbasic euryteles [$(6–7) \times (4–6)$ μm], and small atrichous isorhizas (1.5–2 μm). The umbrella of the living specimens is translucent, with 8 interradial yellow spots, roughly square or rectangular in shape, situated at its margin, corresponding with the statocysts. The distal portion of the radial canals is also pigmented, giving the appearance of faint and roughly-defined yellow triangles on the portion of the radial canals immediately above the intersections of the circular canal and the radial canals.

The sequenced products of COI from three analyzed individuals were identical to each other, as were the 16S products. All sequences were free of gaps and had a final length (after alignment and trimming) of 658 and 542 base pairs, respectively. When blasted against the Gen-Bank database, all *T. musculosa* sequences were shown to be most similar to other ptychogastriid sequences, confirming the lack of contamination. The genetic distances between *T. musculosa* and the rest of the genera in family Ptychogastriidae are presented in Table 1, evidencing the clear separation between the 16S and COI sequences of *T. musculosa* and all other analyzed taxa. Preliminary comparisons of 16S and COI sequence data from the three valid ptychogastriid genera are shown in Fig. 3, further confirming the separation of *T. musculosa*, but also suggesting the non-monophyly of *Ptychogastria polaris* Allman, 1878.
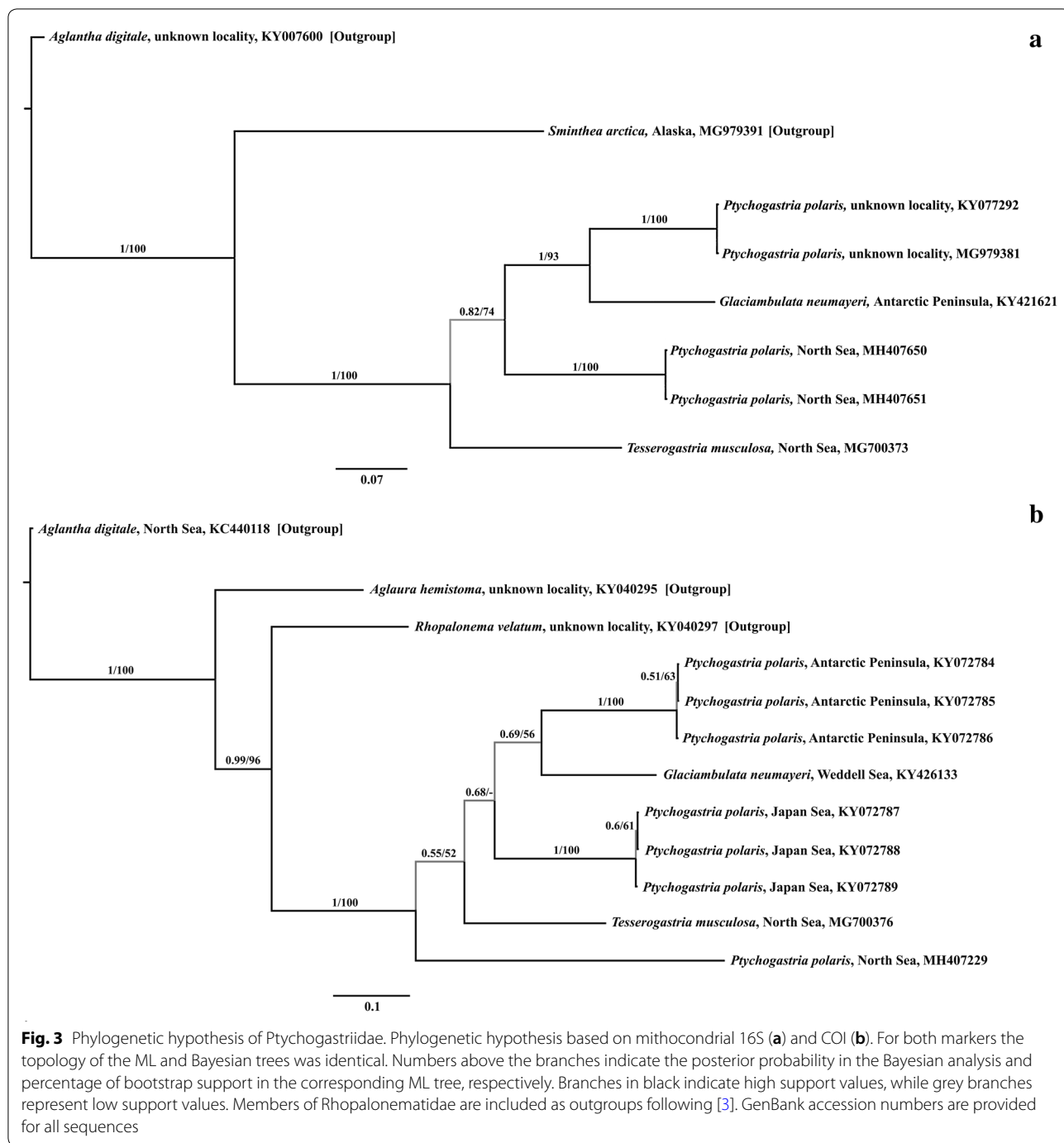
In all, 57 previous records of *T. musculosa* from 30 localities were validated (Fig. 4, Table 2, Additional file 1). Twenty-three different sources providing validated records were identified, 87% of them from "gray" literature. The known distributional range of the species is expanded to include fjords in the north-eastern section of the Skagerrak strait (numerous localities inside and around Oslofjord in eastern Norway, and Gullmar-fjord in western Sweden), and the northernmost sector of the North Sea (the western Norwegian fjords of Sog-nefjord, Fensfjord and Fanafjord), demonstrating that the presence of at times high numbers of *T. musculosa* has been continuously documented for almost six decades in the North Sea region, particularly in the Oslofjord, from 1958 to 2017.

Discussion

The distinctive morphology of the collected specimens prevented confusion with any other hydromedusa species in the area and allowed for a straightforward identification of the animals as *T. musculosa* Beyer, 1958, subsequently confirmed through a detailed comparison with the holotype of the species. The morphology of the medusae is in close agreement with both the original description by Beyer [4] and the subsequent account by Hesthagen [6], except for the coloration pattern. Previous accounts on the coloration of *T. musculosa* include the presence of white to yellow epidermal spots located at the perradial junctions of the radial canals with the ring canal [6]. In the individuals from Raunefjord, however, the entire ring canal and the distal parts of the radial canals were pigmented, with eight additional interradial pigment patches not associated with the radial canals present on the umbrella margin. A potential role of exumbrellar pigment spots as photoreceptors has been discussed previously for this species, since the medusae are known to spawn in response to strong illumination under the microscope in laboratory conditions [6], but further research is needed to determine the degree of interaction between light cues and ectodermal pigments in *T. musculosa*. When examined alive under the direct light of the microscope at different intensities, medusae from both Raunefjord (present study) and Oslofjord [6] did not show any phototaxic response, suggesting that these bottom-living medusae may not depend on light for orientation and movement. It has been suggested that the adult medusae permanently remain on the sea bottom, with the possible exception of swimming as a flight response [6, 8].
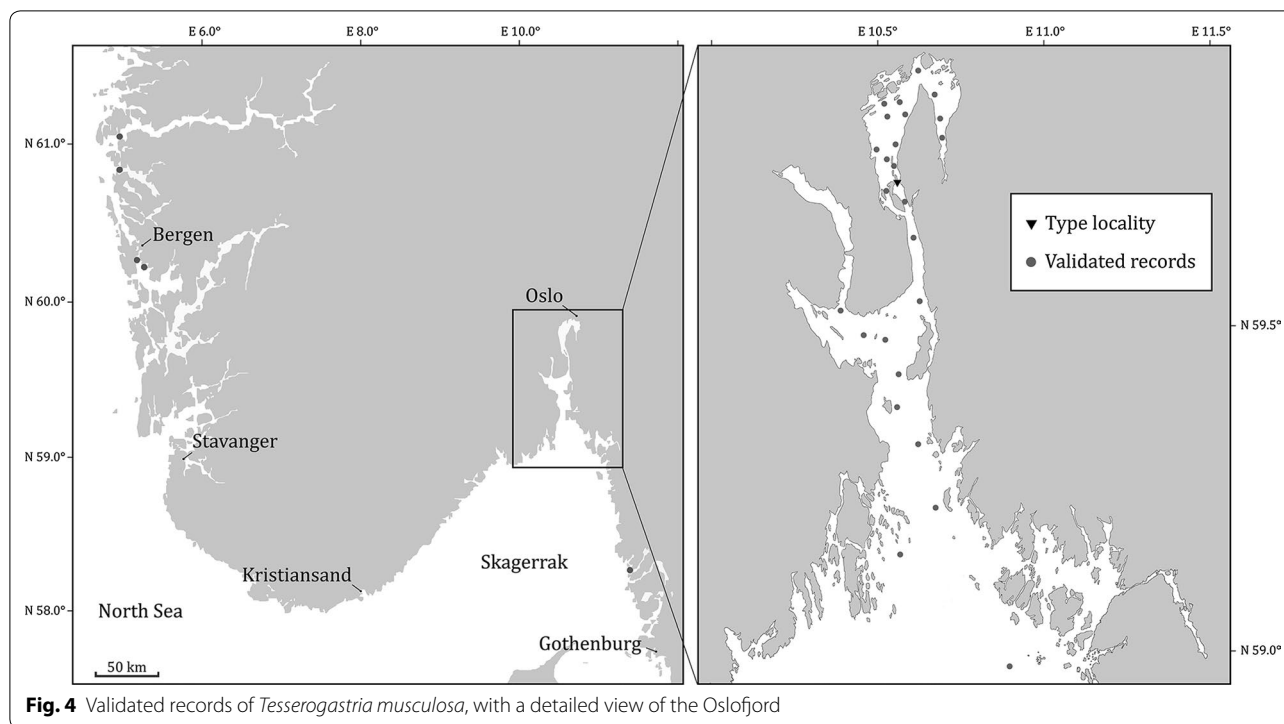
The number and position of the statocysts are diagnostic characters separating *Tesserogastria* from all other genera in family Ptychogastriidae [12, 21]. However, some confusion exists regarding the exact position of these structures in *T. musculosa*, as they have been alternatively described in scientific accounts as adradial [6] or interradial [12]. The original description of the species does not include information on the position and number of statocysts because Beyer was unable to find any in formalin-preserved animals, presumably due to the disintegration of the statoliths in this fixative [4]. Nevertheless, the statocysts from medusae from Raunefjord fixated with neutral-buffered formalin were evident even after 6 months of preservation, allowing us to confirm that in *T. musculosa* the statocysts are invariably interradial and can be used as a reliable character separating *Tesserogastria* from both *Ptychogastria* and *Glaciambulata*.

For both the 16S and COI markers, the sequences generated for this study show a large degree of differentiation (> 20%) between *T. musculosa* and individuals of the

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Fig. 3 Phylogenetic hypothesis of Ptychogastriidae. Phylogenetic hypothesis based on mithocondrial 16S (**a**) and COI (**b**). For both markers the topology of the ML and Bayesian trees was identical. Numbers above the branches indicate the posterior probability in the Bayesian analysis and percentage of bootstrap support in the corresponding ML tree, respectively. Branches in black indicate high support values, while grey branches represent low support values. Members of Rhopalonematidae are included as outgroups following [3]. GenBank accession numbers are provided for all sequences

other two species in the family for which genetic data are available (i.e. *Glaciambulata neumayeri* Galea et al., 2016 and *P. polaris*), highlighting the value of these two markers as DNA-barcodes for this species. At genus level, all three ptychogastriid genera show a similarly large degree of differentiation, which is comparable to the distances observed by Grange et al. between individuals identified as *P. polaris* from the Antarctic Peninsula and the Sea of

Japan [3]. Our *P. polaris* individuals from Hjeltefjord are again clearly different from both the Antarctic and Japanese specimens, and the preliminary phylogenetic analysis suggests that the taxonomy of the Ptychogastriidae, and in particular the hypothesis of monophyly of genus *Ptychogastria*, needs to be revised, as the intraspecific degree of differentiation within this genus appears comparable to the degree of differentiation between genera. It

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Fig. 4 Validated records of *Tesserogastria musculosa*, with a detailed view of the Oslofjord

is unlikely that DNA sequences from either the holotype or the paratypes of *T. musculosa* will be available in the near future, since in both cases the specimens have been subjected to fixation and long-term storage in formalin. The 16S and COI sequences provided here for *T. musculosa* constitute thus a useful tool for identification, as well as the first molecular evidence of the relationships of the genus with both *Glaciambulata* and *Ptychogastria*.

Tesserogastria musculosa was a common component of the epibenthic community in the sampled locality, with the estimated density (ca. 0.5 ind/m^2) similar to what has been reported for other ptychogastriid medusae in boreal waters (0.01–0.91 ind/m^2 for *P. polaris* in northeast Greenland, and 0.01–0.76 ind/m^2 for the same species in the Barents Sea) [22, 23]. The density of *T. musculosa* was however considerably lower than the maximum densities reported for ptychogastriid medusae in Antarctic waters (up to 13 ind/m^2 for *P. polaris* in Antarctic fjords) [3]. In Digerud and neighbouring localities, *T. musculosa* has reached maximum values of >10 ind/m^3, being the most abundant organism in at least one sampling station [5], while subsequent observations in Oslofjord have shown that the species is common and, at times, highly abundant in soft bottoms [9, 24–26]. The medusae from Raunefjord and Oslofjord shared similar size ranges and unimodal distribution of bell height, but the mean umbrella height in this study (1.71 mm) was higher than the mean height (0.96–1.03 mm) reported for the same

species in the type locality at any given time of the year [6].

The high numbers of medusae in some localities along Oslofjord allowed Beyer to analyze the local distribution of the species. This lead him to suggest that *T. musculosa* is an indicator of non-polluted bottoms, with the number of individuals rapidly increasing towards the open sea [5]. Later on, the consistent patterns of decreasing abundance towards the inner parts of the fjord caused Beyer and Indrehus to advocate the species as an indicator of oceanic and unpolluted conditions in the area [9]. Despite this general pattern, the population has fluctuated during the recent decades, with a dramatic population reduction in the inner fjord starting in the 1960s and continuing until the mid-1990s [9]. Remarkably high numbers of medusae were observed again in 1996 [26], and the species has since been recorded rather consistently in Oslofjord until as recently as 2012 [27], but without information on population density or abundance.

High densities of ptychogastriid medusae in soft sediments have been linked to high productivity in subantarctic fjords [3, 28], and to the accumulation of organic and inorganic debris in the seafloor of relatively isolated deep environments [4, 29]. Raunefjord's sediment characteristics and oceanographic processes (e.g. enhanced benthic productivity, vertical flux, trapping of detritus) are likely to provide a suitable habitat for epibenthic medusae in an analogous way to the observed abundance

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Table 2 Validated previous records of *Tesserogastria musculosa* Beyer, 1958 summarized by reference

References	Locality	Region/country	Collection date[a]	Abundance	Type of record/language
[4]	Digerud (type locality) and several others in Oslofjord	Skagerrak/Norway	May 1952; June 1949, 1952, and 1953	>4000 Medusae	Published scientific article/English
[5]	Steilene, Gåsöy, Lysakerfjord, and Helvik; Oslofjord	Skagerrak/Norway	January and October 1962, August 1965	Up to >1000 ind/m^3	Published scientific article/English
[6]	several stations inside Oslofjord	Skagerrak/Norway	September and December 1966, April 1967	N/D	Published scientific article/English
[7]	Fensfjorden and Sognefjorden	North Sea/Norway	N/D	N/D	Technical report/Norwegian
[8]	Fanafjord	North Sea/Norway	April, June, September and December 1979	Abundant	Thesis dissertation/Norwegian
[9]	several stations inside Oslofjord	Skagerrak/Norway	August 1981 to 1993. Unknown dates in 1983–1986	Common	Technical report/Norwegian
[24]	Gråøyrenna; Oslofjord	Skagerrak/Norway	May 1973	>1500 Medusae	Published scientific article/English
[25]	Gråøyrenna and Elle; Oslofjord	Skagerrak/Norway	December 1974, June 1975	Abundant	Thesis dissertation/Norwegian
[26]	Granerøstøa; Oslofjord	Skagerrak/Norway	June 1996	Abundant	Technical report/Norwegian
[27]	Elle; Oslofjord	Skagerrak/Norway	September 2012	Presence only record	Technical report/Norwegian
[39]	Elle and Gråøyrenna; Oslofjord	Skagerrak/Norway	September 2000	Presence only record	Technical report/Norwegian
[43]	Oslofjord	Skagerrak/Norway	N/D	Several medusae	Museum catalogue/French
[44]	Rauer	Skagerrak/Norway	November 1997	Presence only record	Technical report/Norwegian
[45]	Elle; Oslofjord	Skagerrak/Norway	September 2001	Presence only record	Technical report/Norwegian
[46]	Vesthullet; Oslofjord	Skagerrak/Norway	September 2002	Presence only record	Technical report/Norwegian
[47]	Elle and Steilene; Oslofjord	Skagerrak/Norway	September 2003 and 2004	Presence only record	Technical report/Norwegian
[48]	Steilene, Gråøyrenna, and Vesthullet; Oslofjord	Skagerrak/Norway	September 2005	Presence only record	Technical report/Norwegian
[49]	Gråøyrenna; Oslofjord	Skagerrak/Norway	September 2008	Presence only record	Technical report/Norwegian
[50]	Gråøyrenna; Oslofjord	Skagerrak/Norway	September 2009	1 Medusa	Technical report/Norwegian
[51]	Spro; Oslofjord	Skagerrak/Norway	October 1962	N/D	Museum catalogue/English
[52]	Essvik, and Kiuben; Gullmarfjord	Skagerrak/Sweden	October 1964	N/D	Museum catalogue/English
[53]	Digerud; Oslofjord	Skagerrak/Norway	N/D	N/D	Museum catalogue/English

N/D no data

[a] Exact position (latitude, longitude) of the sampled sites are not always available in the original sources. Sampling was conducted in all cases with a Beyer's 50 cm epibenthic closing net or a slightly modified version of the same

of *P. polaris* in subantarctic fjords [3], *Ptychogastria asteroides* (Haeckel 1879) in Mediterranean canyons [29], and *T. musculosa* in Oslofjord [4].

Contrary to the interannual variation, significant seasonal population fluctuations have not been observed for *T. musculosa* in its type locality, with high numbers of medusae collected throughout the year in seasonal surveys [6]. In West-Norwegian Fanafjord, however, strong seasonality in *T. musculosa* was reported by Kaartvedt, who observed the highest abundances in June and much fewer specimens in April, September and December [8]. Unpredictable, sporadic and episodic population fluctuations are widely documented for planktonic jellyfish [30, 31] and some benthic hydrozoans [32], but the

mechanisms behind these remain poorly understood [31, 33]. Episodes of bloom-and-bust have never been reported for benthic ptychogastriid hydromedusae and these organisms may not be part of the subset of medusozoan species with life-cycle attributes that predispose them to bloom [34]. Existing evidence appears to suggest that epibenthic medusae in genera *Ptychogastria* and *Tesserogastria* do not form blooms with abrupt periods of presence in the environment followed by completely disappearance, and instead maintain rather stable large populations in habitats such as the bottom of Boreal, Arctic and Antarctic fjords, and deep submarine canyons [3, 6, 22, 23]; although the observed variations in the abundance of *T. musculosa* in Fanafjord [8] could instead

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indicate that seasonal dynamics differ widely among populations of this species in different habitats.

For a relatively easy-to-identify species distributed in the well-studied vicinities of active marine biological stations in both Oslofjord and Raunefjord, *T. musculosa* has a puzzlingly low number of records in the published, peer-reviewed scientific literature. Subsequently, it has been generally considered a rare and geographically restricted species. Based on our data and a thorough review of the "gray" literature, this perception is most likely incorrect, and *T. musculosa* in fact appears to be a relatively common and widely distributed component of the epibenthos in several fjords along the North Sea coast. The lack of published records for *T. musculosa* does not result from the rarity of the species, neither can it be attributed to undersampling of its habitat, as numerous studies have been conducted on the benthic fauna of the areas surrounding both the Marine Biological Station in Drøbak and the Espeland Marine Biological Station in Raunefjord since their founding in 1894 and mid-1950s, respectively [35, 36].

More than with other epibenthic animals, our ability to detect the presence of *T. musculosa*—and most likely other gelatinous epibenthos—appears to depend on a combination of the chosen sampling gear and a careful processing technique. In the original description, Beyer stressed the importance of the sampling gear in finding *T. musculosa* in Oslofjord, stating that his specially designed sledge, known as the Beyer sledge, consistently yielded catches of *T. musculosa* [4]. In the localities where abundant medusae have been caught with sledges, sampling with plankton net hauls from above the bottom has never yielded a single specimen, either in Oslofjord [4] or Raunefjord and the surrounding fjords [37]. Beyer suggests that "the species has probably been caught many times in grab and Mortensen dredge samples, but has then been disregarded together with the plankton inevitably caught in these apparatus", thus highlighting the importance of gentle sampling, adequate sample processing techniques, and correct identification for observing this species [4]. Prior to the current study, all specimens of *T. musculosa* ever collected have been obtained with Beyer's epibenthic sledge (illustrated in [38]) with a 50 cm closing plankton net mounted on a steel toboggan, designed for the purpose of catching organisms on or immediately above the bottom [5, 6, 8, 9, 27, 39]. In the current study, a slightly less gentle and much larger Rothlisberg and Pearcy (RP) sledge was used, but this was compensated for by the careful processing of the samples, as well as the very slow transect and retrieval speed. It is not surprising that careful processing of samples will result in higher numbers of species found: in Kiel Bay, Remane found > 300 new species after modifying his sampling techniques and

adopting a more careful handling approach of the samples [40].

The population assessment of benthic ptychogastriid medusae has only recently become feasible thanks to improved sampling techniques and in situ observations by divers or ROVs, resulting in the description of new species and several new records for the already-known ones [3, 12]. Other multidisciplinary approaches (e.g. sediment traps) have also been successfully used for the collection of well-preserved ptychogastriid medusae in remote locations such as submarine canyons [29], and thus represent a potential source of valuable information for this taxon. Ptychogastriid medusae were rarely reported before the late 1990s [22, 23], partly due to the difficulties of sampling deep marine environments, but also because the medusae, often damaged beyond recognition, were readily considered planktonic contaminants. The standard processing of benthic samples with sieves often results in the gelatinous species getting extruded and destroyed, leading to them remaining unreported. The lack of records for *T. musculosa* and other benthic and benthopelagic medusae [41] may thus be an artefact of the commonly used sampling techniques.

A second issue contributing to the perceived rarity of *T. musculosa* is the poor accessibility of the bulk of the records, which consist of technical reports, unpublished theses, and museum collection catalogues. Many basic and applied studies in biogeography, phylogenetics, and ecology rely on species distribution data compiled exclusively from the published scientific literature but, for some species, such as *T. musculosa,* these data are incomplete and may contain serious biases. While the use of unverified anecdotal occurrences and unpublished observations for the assessment of the current and historical ranges of rare species can certainly lead to large errors of omission and commission [42], for some taxa, such as *T. musculosa,* inclusion of records from the "gray" literature is crucial for a realistic estimation of their distribution. Common complaints against the inclusion of "gray" literature data in distributional analyses include the low quality and low accessibility of the sources. While the latter is also an issue in the case of *T. musculosa* (e.g. all records are in languages other than English and several unpublished documents are not available online), the quality of the data they contain is in general quite high: all observations were made by trained professionals working in the field of marine biology/ecology, in many cases in collaboration with the original author of the species or with one of his students, using established and well documented sampling protocols.

In the particular case of *T. musculosa,* reviewing and including occurrence records from "gray" literature considerably alters the perception of its distribution and

commonness. Furthermore, due to the hyperbenthic habitat and the gelatinous morphology of the medusa, the species is unlikely to be collected or recorded by the most commonly used pelagic or benthic sampling methods. A similar methodological bias applies to other epibenthic and benthopelagic medusae, which remain a poorly known group [37]. Targeted sampling with suitable gear and processing protocols, or using ROVs and other optical platforms, is required to establish a better understanding of diversity and ecology of ptychogastriids and other gelatinous benthos.

In addition to updating our knowledge of the distribution of the species, the reconstruction of the taxonomic history of *T. musculosa* also allowed us to clarify the existing confusion regarding its date of description. Although the paper by Beyer was actually published in 1958, some authors have mistaken the date of this publication as 1959 [2, 12, 21] probably based on a series of copies of Vol. 6 of the Nytt Magasin for Zoology printed in that year. The error has become widespread in the peer-reviewed scientific literature, although it has not completely permeated the "gray" literature produced in Norway. Beyer himself reported the date as 1958 in subsequent works, as did his students and collaborators (e.g. Hesthagen clearly states that the species was first described by Beyer in 1958) [6]. An enquiry into the publications of the University of Oslo (publisher of Nytt Magasin for Zoology) has confirmed that the correct date of publication is 1958, the correct date for the genus is *Tesserogastria* Beyer, 1958, and the correct name of the species is *Tesserogastria musculosa* Beyer, 1958.

Conclusions

Tesserogastria musculosa is not restricted to its type locality; freshly collected samples and validated observations from fjords in western and eastern Norway as well as western Sweden demonstrates that the species is more common and widespread than is evident from published records. Our current ideas about the restricted distributions of other epibenthic medusae need to be reassessed, as they are likely to be similarly biased, and further sampling at additional localities will likely provide more records of these organisms, including *T. musculosa*. Sampling techniques specifically targeting the epibenthos and careful processing of the samples are essential for correctly assessing the presence of epibenthic medusae, and the lack of records for these organisms may in part be an artefact of the commonly used sampling methods, but thorough validation and reassessment of previous records, including those in "gray" literature, are also necessary to estimate the real distributional area of delicate epibenthic invertebrates. The comparison of molecular data for species and genus delimitation

in Ptychogastriidae, presented here for the first time, shed further insight into the diversity and distributional range of epibenthic trachyline medusae and highlighted the need for a thorough taxonomic revision within the family.

Additional file

Additional file 1. Validated records of *Tesserogastria musculosa* Beyer, 1958. List of all the records of *Tesserogastria musculosa* Beyer, 1958 validated for this study and used to generate Fig. 4.

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