Another cryptic invasion? The highly toxic clinging jellyfish *Gonionemus* sp. (Hydrozoa, Limnomedusae) on the Swedish west coast

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Abstract

The clinging jellyfish *Gonionemus* sp. is a small hydromedusa species known historically from the Swedish west coast but not reported in recent times. This species is thought to be native to the northwest Pacific where it is notorious for causing severe stings in humans and is considered invasive or cryptogenic elsewhere. This year, unlike in the past, severe stings in swimmers making contact with *Gonionemus* sp. medusae occurred in Swedish waters from a sheltered eelgrass bed in the inner Skagerrak archipelago. To the best of our knowledge, this is only the second sting record of *Gonionemus* sp. from the Northeast Atlantic - with the first record occurring off the Belgian coast in the 1970s. Stinging *Gonionemus* sp. medusae have also been recently reported from the northwestern Atlantic coast, where, like on the Swedish coast, stings were not reported in the past. We analyzed sea surface temperature data from the past 30 years and show that 2018 had an exceptionally cold spring followed by an exceptionally hot summer. It is suggested that the 2018 temperature anomalies contributed to the Swedish outbreak. An analysis of mitochondrial COI sequences showed that Swedish medusae belong to the same clade as those from toxic populations in the Sea of Japan and northwest Atlantic. *Gonionemus* sp. is particularly prone to human - mediated dispersal and we suggest that it is possible that this year's outbreak is the result of anthropogenic factors either through a climate-driven northward range shift or an introduction via shipping activity. We examined medusa growth rates and details of medusa morphology including nematocysts. Two types of penetrating nematocysts: euryteles and b-mastigophores were observed, suggesting that *Gonionemus* sp. medusae are able to feed on hard-bodied organisms like copepods and cladocerans. Given the now-regular occurrence and regional spread of *Gonionemus* sp. in the northwest Atlantic, it seems likely that

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outbreaks in Sweden will continue. More information on its life cycle, dispersal mechanisms, and ecology are thus desirable.

Introduction

There is increasing concern over the highly toxic cryptogenic clinging jellyfish *Gonionemus* sp. (Hydrozoa, Limnomedusae) due to outbreaks in scattered temperate coastal areas worldwide, where the jellyfish are either previously unrecorded, or where they have not been observed for decades (Rodriguez et al., 2014; Govindarajan & Carman, 2016; Gaynor et al., 2016; Govindarajan et al., 2017; Marchessaux et al., 2017). These hydromedusae can have a potent sting that causes severe pain and other symptoms to humans (Pigulevsky & Michaleff, 1969; Otsuru et al., 1974; Yakovlev & Vaskovsky, 1993; Govindarajan & Carman, 2016; Marchessaux et al., 2017). As well, they can be lethal to their predators (Carman et al., 2017).

It appears likely that the current *Gonionemus* outbreaks are facilitated by anthropogenic transport (citations). The adult *Gonionemus* medusae which reach approximately 3 cm in diameter, have adhesive structures positioned towards the distal ends of their tentacles (citations), which they use to cling to the eelgrass such as *Zostera marina* (citations). Thus while the medusa occasionally swim out of the eelgrass meadows, natural or anthropogenic medusa dispersal, while possible, may not be the primary mechanism for its spread. *Gonionemus* sp. has a complex life history that includes minute benthic asexual stages (Perkins, 1902; Kakinuma, 1971; Uchida, 1976) that may be amenable to human-mediated transport on ship hulls (Tambs-Lyche, 1964), shellfish (Edwards, 1977), and debris (Choong et al., 2018).

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An understanding of the dispersal history and spread of clinging jellyfish has been hampered by a complex taxonomic history. The name *Gonionemus vertens* Agassiz, 1862 has been used recently to refer to “clinging jellyfish” from throughout the northern hemisphere but was originally described from material collected in Puget Sound, the North East Pacific (citations). In the Atlantic the clinging jellyfish were originally described as *Gonionemus* *murbachii* Mayer, 1901, and were considered distinct from *G. vertens* (citations). They were later synonymised (Kramp, 1959) and the Atlantic populations were hypothesized to have been founded by anthropogenic introductions from the Pacific (Tambs-Lyche, 1964; Edwards, 1977; Bakker, 1980), although this was not accepted by all. Based on consistent morphological characters, some authors either maintained the *murbachii* name (Rottini, 1979) or considered the two forms to be *subspecies* (Naumov, 1960). Govindarajan et al. (2017) found that differences in mitochondrial COI sequences were also consistent with the *vertens* - *murbachii* forms; but noted that these differences do not correspond to the Atlantic - Pacific division suggested by Naumov (1960). Owing to their episodic nature and the lack of continuity in observations of late 19th and early 20th century *G. murbachii* and contemporary populations in the *G. murbachii* type locality, Govindarajan et al. (2017) conservatively referred to the more toxic, putative *murbachii* lineage as *Gonionemus* sp. until the taxonomy can be further clarified.

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Gonionemus sp. has been previously reported from Scandinavian and North Sea waters (reviewed in Bakker, 1980; Wolff, 2005), but we are not aware of any stings associated with past observations. Here, using morphological and molecular evidence, we document blooms of the highly toxic lineage *Gonionemus* sp. in the summer of 2018 associated with a sheltered eelgrass (*Zostera marina*) bed on the Swedish west coast. We report the first case of a *Gonionemus* sp. envenomation in Scandinavian waters and discuss the possible origins of these apparently new and highly toxic *Gonionemus* sp. populations. We also suggest that warmer than average sea surface temperatures may have contributed to the 2018 *Gonionemus* sp. outbreaks.

Materials and Methods

Sample collection and field observations

The first reports of an unknown stinging medusae came from swimmers through media on 27th July 2018 (SVT, 2018). Several swimmers had been stung at Knuten on the northeastern (leeward) side of the island Tjörn (58.0782°N; 11.7065°E, Fig. 1). On two occasions, the 2nd and 18th of August, we sampled medusae by snorkeling in the eelgrass bed with a small hand held net (120x150 mm, mesh size?, JBL GmbH & CO, Neuhofen Germany). Medusae were transported live in aerated tanks to the laboratory for analysis and maintained on a diet of copepods and frozen *Artemia* larvae. Samples were preserved in 96% ethanol for DNA analysis.

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Medusa size and nematocyst identifications

The diameter of the *Gonionemus* medusae was measured by imaging the uncontracted medusae when resting on the bottom of a white plastic box with 50 mm of natural sea water using a DSLR camera (Nikon D7100, Nikon Corporation, Japan). A plastic millimeter ruler in the box was used for reference to measure the bell diameter in Image J (Schneider et al., 2012). Some of the *Gonionemus* medusae and their nematocysts were examined and photographed with a Leitz DMRBE stereomicroscope and with a Leica MZI6A light microscope (LM) (company and country) equipped with interference-contrast optics, 100x/1.30 PL, fluotar objectives. Both microscopes were connected to the digital photo equipment Leica application suite, version 3.8 (LAS V3.8). Measurements on different medusa structures were made in the stereomicroscope, and measurements on the nematocysts were made from the light microscope. All pictures and measurements are from living hydromedusae.

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The nematocysts were identified by size, structure and shape of their undischarged and discharged capsule and shaft, and on the spine-pattern of the shaft. The classification system and nematocyst nomenclature of Östman (2000, and references therein) was used.

Temperature record

Sea surface temperature data was downloaded from the Swedish repository for environmental monitoring data (SMHI, 2018). The closest monitoring station with sufficient resolution and duration was “Åstol”, 23 km from the collection site (57.922°N; 11.590°E, Fig. 1). Sea surface temperature from 1986 to 2018 was binned into monthly averages. The monthly mean temperatures for 2018 were graphically superimposed to identify anomalies.

Phylogeographic analysis

Molecular procedures and analyses were conducted at the Woods Hole Oceanographic Institution (Woods Hole, MA, USA) except where indicated. Genomic DNA was extracted from 15 preserved hydromedusae collected from the leeward side of Tjörn Island, Skåpesund (Fig. 1) using DNe~~E~~asy Blood & Tissue? ~~Extraction ~~K~~k~~its (Qiagen, Germany?) according to the manufacturer’s protocol. A ~650 base pair portion of the mitochondrial COI gene was amplified and sequenced using primers from Folmer et al. (1994) using the approach described in Govindarajan et al. (2017). PCR conditions were 3 minutes at 95°C; 35 cycles of 95°C 30s; 48°C 30s, 72°C 1 min; and 5 minutes at 72°C. PCR products were visualized on a 1% agarose gel stained with GelRed, purified with QIA~~ia~~quick PCR P~~p~~urification K~~k~~its (Qiagen, Germany?) according to the manufacturer’s protocol, and quantified using a NanoD~~d~~rop 2000 spectrophotometer (Thermo Fisher Scientific, US). Purified products were sequenced in both directions (Eurofins, https://www.eurofins.com/). An additional specimen was amplified using a similar protocol in Sweden and sent for sequencing using the GATC LightRun Barcode service (www.eurofinsgenomics.eu). Sequence chromatograms were evaluated and assembled using

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Geneious version 9.0.5 (https://www.geneious.com/). Assembled sequences were aligned with sequences representing the 7 haplotypes in Govindarajan et al. (2017; Table 1). Representatives of additional haplotypes from *Gonionemus* sp. sequences that were deposited on GenB~~b~~ank after Govindarajan et al. (2017) were identified in a preliminary alignment, and were then added to the alignment dataset with the Swedish sequences. Alignments were conducted using ClustalW (Larkin et al., 2007) in the Geneious platform with default parameters. The alignments were confirmed by eye and the ends were trimmed to 501 base pairs to standardize sequence length and facilitate a direct comparison with the analysis conducted by Govindarajan et al. (2017) and the new GenB~~b~~ank sequences that were also that length. Neighbor-joining trees based on Kimura 2-parameter distances (to be consistent with previous analyses; Zheng et al., 2014; Govindarajan et al., 2017) were constructed the PAUP* 4 (Swofford, 2003) accessed through Geneious.

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Results

Sample collection and field observations

Medusae were collected at two occasions from the Skåpesund location (Fig. 1) and identified morphologically as *Gonionemus* sp. (Fig. 2). Medusae possessed adhesive pads characteristic of the genus *Gonionemus* located towards the distal ends of their tentacles (Fig. 2-3, more detailed morphology in Supplementary Figs 1-6), which allow them to “cling” to the eelgrass. Similar to *Gonionemus* sp. from the Northwest Atlantic, Northwest Pacific, and the Sea of Japan (Govindarajan et al., 2017), medusae were relatively flat and had relatively thin, dull orange - brown gonads.

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One of us (BK) was stung several times while skin diving on the first sampling date. The stings left red marks at the site of contact and produced marked pain for several hours and feelings of unease throughout the first night afterwards. As reported elsewhere (e.g. Pigulevsky & Michaleff, 1969) stinging sensations where felt throughout the night even at places on the body where no direct contact had occurred. Local newspapers also reported stings in other swimmers, with similar outcomes and in a few cases the victims had strong reactions that demanded medical attention (Aftonbladet, 2018).

Medusa size distributions

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The *Gonionemus* sp. population showed slight growth over the 16-day period between sampling dates ($p < 0.001$, Fig. 2). At the initial time point, the mean diameter was 9.8 ± 2.7 mm. Sixteen days later, at the second time point, the mean size was 11 ± 1.8 mm suggesting an average growth rate of 0.08 mm per day.

Detailed medusa and nematocyst morphology

Gonionemus sp. possesses a well-developed transparent velum (inward projecting rim of tissue; Figs. 3A-3D). Four narrow radial canals form a noticeable cross centrally inside the dorsal bell cavity (Figs. 3B, 3C). The stomach with connecting manubrium (tube-like projection with the mouth) is centrally attached to the cross-region of the radial-canals (Figs. 3B-3D). The gonads are arranged along most of the length of the radial canals (Figs. 3A-3B, 3D). Mature female gonads are light yellow-brown; each gonad is folded into 6-8 broad bulbs (Fig. 3A). The male gonads are darker brownish-red and each folded into 9-13 smaller bulbs (Figs. 3B, 3D).

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Around 45 - 58 slender tentacles are attached to the ventral side of the bell rim close to the ring canal, which surrounds the bell close to the velum (Figs. 3A-3G). Contracted tentacles are stubby (Fig. 3D) and are less than half the length of extended tentacles (Figs. 3F, 3G). Close to

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or at a short distance from the tentacle tip, a small bending is present on each tentacle, caused by the presence of an adhesive pad (Figs. 3D, 3F, 3G). The adhesive pad is located to one side of the tentacle and causes the tentacle to bend, thus pointing outwards (Figs. 3A, 3G, detailed view in Fig. 4B).

The gracile tentacles are in their mid-region black colored along most of their length (Figs. 4A-C) and are armed with ring-shaped nematocyst batteries. Batteries with closely packed nematocysts form rings around the tentacle (Fig. 4A). Small patches of nematocysts are scattered between the nematocyst rings, most clearly visible on the black pigmented mid-streak of a tentacle (Figs. 4A, 4B).

Two nematocyst types, microbasic euryteles and microbasic b-mastigophores, are present in the nematocyst batteries around the tentacles (Figs. 4D-4K). The euryteles are larger and by far the more abundant. Some small microbasic b-mastigophores were loosely scattered among the euryteles. The capsules of both euryteles and the b-mastigophores are broad, rounded basally and slightly narrower apically (Fig. 4E). The inverted eurytele shaft is broad, rod-shaped with pointed apical tip. The pattern of the shaft is caused by its long, inverted spines, all pointing towards the apical capsule opening with its lid. The inverted tubule makes slightly oblique coils to the long capsule axis and almost fills the whole capsule, except for its basal end. The narrow shaft of the small microbasic b-mastigophore was slightly bent, following the convex capsule side (Figs. 4E-4K). Discharged eurytele shaft was broad, rod-shaped with distal swelling armed with long spines (Figs. 4H, 4I, inset). The prominent rounded lid at apical capsule, and the difference of the diameter of shaft and distal tubule were obvious (Figs. 4I, 4J). On discharged microbasic b-mastigophores no clear spine-pattern on the narrow shaft and no obvious difference between the diameter of distal tubule and shaft tubules were visible (Figs. 4I, 4K).

Additional morphological details are presented in Supplementary Figures 1-6.

Temperature

The 2018 spring and summer temperatures in Åstol were anomalous relative to the previous 28 years (Fig. 5). The spring temperatures were approximately 2°C cooler than during 1986-2018; while the summer temperatures were approximately 3°C warmer than 1986-2018.

Phylogeographic analysis

DNA Sequences were obtained for 16 Swedish *Gonionemus* sp. medusae and submitted to GenB~~b~~ank (accession numbers MK158929-MK158944). These 16 sequences comprised three haplotypes. Nine medusae possessed one haplotype, 6 medusae possessed a second haplotype, and a single medusa possessed a third haplotype. The Swedish sequences were aligned with representatives of each of the *Gonionemus* sp. haplotypes described in Govindarajan et al. (2017) and additional haplotypes found in subsequently available GenB~~b~~ank sequences. These newer GenB~~b~~ank sequences included one representative from New Jersey on the USA mid-Atlantic coast (accession number KY451454; Gaynor et al., 2016) and 104 sequences from three Chinese locations (accession numbers MH020640 - MH020743; Liu & Dong, unpublished).

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An initial alignment and neighbor-joining tree of the new Chinese sequences from GenB~~b~~ank showed that they comprised 9 haplotypes (Fig. 6). Haplotypes were labeled by number following Govindarajan et al. (2017) and new haplotypes were given new numbers. Of the 9 haplotypes, one matched Haplotype 9, one matched Haplotype 4, and 7 were unique for Sweden. One sequence representing each of the 7 unique haplotypes were selected for the

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analysis with the Swedish unique sequences. The single New Jersey sequence matched one of the Swedish haplotypes, as described below.

A neighbor-joining tree of the Swedish sequences and the unique COI haplotypes was generated (Fig. 6). We found that the most abundant Swedish haplotype (found in 9 out of 16 specimens) exactly matched Haplotype 4 from Govindarajan et al. (2017) that was possessed by medusae from the Northwest Atlantic (from the states of Connecticut, Rhode Island, Massachusetts, and New Hampshire along the northeastern USA coast) and the Northwest Pacific (including the highly toxic Vladivostok-area populations from the Sea of Japan). The second Swedish haplotype, termed “Haplotype 9” here and found in 6 out of 16 specimens, matched the haplotype from New Jersey.

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Discussion

We documented a bloom of the highly toxic clinging jellyfish *Gonionemus* sp. associated with severe stings to humans on the Swedish west coast. To our knowledge, this is the first record of clinging jellyfish envenomations to humans from this region. The symptoms reported by one of the authors (BK) are consistent with those described from the Northwest Atlantic and Sea of Japan (citations).

Clinging jellyfish have been previously reported from European Atlantic, North Sea, and Mediterranean coasts, as well as the northwestern Atlantic, the northwestern Pacific and Sea of Japan (citations). The records of clinging jellyfish in Europe are sporadic. In Atlantic coastal waters, observations date back to the early 1900s (Bakker, 1980), and in the Mediterranean possibly back to the 1870s (as *Cosmotira salinarium*; Duplessis, 1879). *Gonionemus* sp. has also been reported from several aquaria with Atlantic and Mediterranean source water (reviewed in

Edwards, 1976 and Bakker, 1980). However, in contrast to western Pacific and Sea of Japan populations, where there is a long record of severe stings, stings to humans have not been reported to our knowledge from European populations until 2016 (from the French Mediterranean coast; Marchessaux et al. 2017).

The history of clinging jellyfish in European Atlantic waters is comparable to that along the Northwest Atlantic US coast and may similarly indicate a new, cryptic invasion of a more toxic form. Both regions have a history of episodic clinging jellyfish sightings, but no record of stings until recently. However, the existence of multiple species and highly episodic life cycle make drawing conclusions difficult. Our genetic analysis confirmed our morphological identification, placing the Swedish form into the *Gonionemus* sp. clade that includes with the highly toxic phenotype. Our morphological observations are consistent with historical European observations of the apparently less toxic form (*Gonionemus murbachii*), but it seems likely that toxicity varies within the *Gonionemus* sp. clade (Govindarajan et al., 2017) so this discrepancy does not rule out a new introduction.

Additional sampling and analysis of nuclear markers will be required to fully solve the *Gonionemus* “zoogeographic puzzle”. However, our COI data provide several new insights. One of the three Swedish haplotypes (Haplotype 4 in Fig. 6) is also found in the northern Northwest Atlantic and the western Pacific/Sea of Japan regions which contain highly toxic individuals, and may indicate a common origin. Another of our haplotypes (Haplotype 9 in Fig. 6) has also been reported from New Jersey, USA, which is in the mid - Northwest Atlantic region. *Gonionemus* sp. was first reported in New Jersey in 2016 (Gaynor et al., 2016). Our analysis could indicate an independent origin of the New Jersey population relative to the northern Northwest Atlantic populations. Our third haplotype (Haplotype 8 in Fig. 6), found in only one individual, was unique.

Interestingly, our haplotype tree also shows that the Pacific region contains the greatest number of haplotypes (10) but only one of these (Haplotype 4) is found outside of the region. This result is consistent with a scenario where a subset of the ostensibly native Pacific diversity inoculated other regions. However, the observation of several haplotypes in Sweden and elsewhere that have not yet been found in the Pacific, in combination with the historical record of sightings from these same regions, suggests that we cannot rule out that the Northwestern Atlantic and Mediterranean regions contain native diversity, either instead of or in addition to introduced lineages.

There are ample pathways and opportunities for *Gonionemus* sp. to be introduced to the Swedish coast. The life cycle of *Gonionemus* sp. includes minute polyp and cyst stages (Perkins, 1902; Kakinuma, 1971; Uchida, 1976) that could have easily arrived unnoticed. In a genetic survey of epifauna, *Gonionemus* sp. was recently identified from the North American Pacific coast on tsunami debris originating from Japan (Choong et al., 2018). This suggests that polyp, frustule, and or cyst stages are capable of long-distance transport on anthropogenic surfaces. There are two larger international harbors near our study site *Gonionemus* sp., Wallhamn and Stenungsund (Fig. 1); thus, it is quite possible that a highly toxic lineage arrived via one of these stages on ship hulls most likely as polyps. Furthermore, there are many records of *Gonionemus* sp. occurring in public aquaria, where they presumably establish from polyp stages accompanying materials brought to the aquaria (Tambs-Lyche, 1964).

Another factor that may have played a role in the 2018 Swedish clinging jellyfish outbreak is temperature. Water temperature is a critical factor initiating seasonal hydrozoan polyp activity (Calder, 1990). The year of the outbreak (2018) was exceptional in that it had both an approximately two degrees colder than average spring and a two degrees warmer than average summer. Either or both of these anomalies could have facilitated the *Gonionemus* sp. outbreak. *Gonionemus* sp. medusae are produced by polyps, which may arise from frustules or cysts (Perkins, 1903; Uchida, 1976). It is not known what environmental factors or the time scales on which they operate trigger these resting stages to become active. Both temperature and salinity have been implicated in affecting Russian Sea of Japan populations (Yakovlev & Vaskovsky, 1993).

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It seems probable that, similar to the US Atlantic coast, *Gonionemus* sp. will spread to new sites along the North Sea coast and potentially pose hazards to both humans and ecosystems. Regular monitoring and surveys will be crucial for providing warnings to protect bathers and others from potentially harmful interactions. Our growth rate data showed a ~12% increase in size over 16 days. Temperature and food availability are likely factors affecting growth rate and should be explored.

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More information is also needed to understand the impact of *Gonionemus* sp. in eelgrass communities, but the morphological features we observed provide some insight into their ecological roles. *Gonionemus* sp. medusae spend much of their time “clinging” to eelgrass with their adhesive pads. Free (unattached) tentacles are often extended into the ambient current. This pattern of passive drifting of tentacles is typically seen in hydromedusae that are ambush predators (Madin, 1988; Colin et al., 2003). Tethering to the seagrass will also increase encounter rate with prey if there is a current passing the tethered medusa. The nematocyst types we observed, euryteles and microbasic b-mastigophores, and their arrangement in raised clusters on the tentacles suggest that *Gonionemus* sp. feeds on hard-body prey such as crustaceans

(Purcell & Mills, 1988). This is consistent with reports that *Gonionemus* sp. medusae feed on small zooplankton such as copepods (Mills, 1983 (for *G. vertens*)) and observations in laboratory cultures that they feed on copepods and *Artemia* nauplii (AFG, CÖ, pers. obs.).

Intriguingly, *Gonionemus* sp. may mediate the interactions of other species and cause mortality in non-prey organisms. For example, along the Northwest Atlantic coast in Massachusetts, Carman et al. (2017) found that *Gonionemus* sp. was consumed by a native spider crab but not by the invasive green crab. The authors also found that *Gonionemus* ingestion resulted in crab death when large numbers of jellyfish were consumed. Thus, *Gonionemus* sp. may potentially impact native ecosystems via differential predation by a native species (spider crabs) that may lead to a decline of that species, while avoidance of *Gonionemus* by a destructive invasive species could potentially facilitate its dominance.

Acknowledgements

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References

Aftonbladet. 2018. https://www.aftonbladet.se/nyheter/a/0EABM2/ovanlig-manet-upptackt-i-bohuslan--kan-orsaka-kramp-och-hjartrusning

Bakker C. 1980. On the distribution of “*Gonionemus vertens*” A. Agassiz (Hydrozoa, Limnomedusae), a new species in the eelgrass beds of Lake Grevelingen (S.W. Netherlands). *Hydrobiological Bulletin* 14(3):186–195.

Broch H. 1929. Craspedote Medusen. Teil II: Trachylinen (Trachymedusen und Narcomedusen). *Nordisches Plankton* 12:481-539.

Calder DR. 1990. Seasonal cycles of activity and inactivity in some hydroids from Virginia and South Carolina, USA. *Canadian Journal of Zoology* 68(3):442-50.

Carman MR, Grunden DW, Govindarajan AF. 2017. Species–specific crab predation on the hydrozoan clinging jellyfish *Gonionemus* sp. (Cnidaria, Hydrozoa), subsequent crab mortality, and possible ecological consequences. *PeerJ* 5:e3966.

Choong HH, Calder DR, Chapman JW, Miller JA, Geller JB, Carlton JT. 2018. Hydroids (Cnidaria: Hydrozoa: Leptothecata and Limnomedusae) on 2011 Japanese tsunami marine debris landing in North America and Hawaii, with revisory notes on *Hydrodendron* Hincks, 1874 and a diagnosis of Plumaleciidae, new family. *Aquatic Invasions* 13(1):43-70.

Colin SP, Costello JH, Klos E. 2003. In situ swimming and feeding behavior of eight co-occurring hydromedusae. *Marine Ecology Progress Series* 253:305-309.

Duplessis G. 1879. Étude sur la *Cosmetira salinarum* nouvelle Méduse paludicole des environs de Cette. *Bulletin de la Société Vaudoise des Sciences Naturelles* 16(81):39-45.

Edwards C. 1977. A study in erratic distribution: the occurrence of the medusa *Gonionemus* in relation to the distribution of oysters. *Advances in Marine Biology* 14:251–284.

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(5):294-299.

Gaynor JJ, Bologna PA, Restaino D, Barry CL. 2016. First occurrence of the invasive hydrozoan *Gonionemus vertens* A. Agassiz, 1862 (Cnidaria: Hydrozoa) in New Jersey, USA. *Bioinvasion Records* 5(4):233-237.

Govindarajan AF, Carman MR. 2016. Possible cryptic invasion of the Western Pacific toxic population of the hydromedusa *Gonionemus vertens* (Cnidaria: Hydrozoa) in the Northwestern Atlantic Ocean. *Biological Invasions* 18(2):463–469.

Govindarajan AF, Carman MR, Khaidarov MR, Semenchenko A, Wares JP. 2012. Mitochondrial diversity in *Gonionemus* (Trachylina: Hydrozoa) and its implications for understanding the origins of clinging jellyfish in the Northwest Atlantic Ocean. *PeerJ* 2017 5:e3205.

Gulliksen B. 1971. A new record of *Gonionemus vertens* Agassiz (Limnomedusae) in Norway. *Det Konlige Norske Videnskabers Selskab* 12:1–4.

Kakinuma Y. 1971. Life cycle of a hydrozoan, Gonionema oshoro Uchida. *The bulletin of the Marine Biological Station of Asamushi, Tohoku University* 14:91-97.

Kramp P. The Hydromedusae of the Atlantic Ocean and adjacent waters. *Dana Report*, 46, pp.1–289.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947-2948.

Commented [R25]: Check publish year in the text. Not 2017 but 2016.

Leentvaar P. 1961. Two interesting invertebrates, *Limnadia lenticularis (*L.) (Crustacea Phyllopoda) and *Gonionemus vertens* A. Agassiz (Limnomedusae), found in the Netherlands *Zoologische Mededelingen* 37:225-230.

Lönnberg E. 1930. Några notiser från Kristinebergs Zoologiska station sommaren 1930. *Fauna och Flora* 25:165–174.

Madin LP. 1988. Feeding behavior of tentaculate predators: in situ observations and a conceptual model. *Bulletin of Marine Science* 43(3):413-429.

Marchessaux G, Gadreaud J, Martin-Garin B, Thiéry A, Ourgaud M, Belloni B, Thibault D. 2017. First report of the invasive jellyfish *Gonionemus vertens* A. Agassiz, 1862 in the Berre Lagoon, southeast France. *BioInvasions Records* 6(4):339–344.

Mills CE. 1983. Vertical migration and diel activity patterns of hydromedusae: studies in a large tank. *Journal of plankton research* 5(5):619-635.

Naumov DV. 1960. Hydroids and hydromedusae of the USSR. Keys to the Fauna of the USSR, Zoological Institute of the Academy of Sciences of the USSR, 70. Translated from Russian by the Israel Program for Scientific Translations.

Otsuru M, Sekikawa H, Hiroh Y, Suzuki T, Sato Y, Shiraki T, Nagashima Y. 1974. Observations on the sting occurring among swimmers in the rocky seashore. *The Japan Society of Medical Entomology and Zoology* 24(3):225-235 (English summary).

Perkins HF. 1902. The development of *Gonionema murbachii*. Proceedings of the academy of Natural Sciences of Philadelphia. 1:750-90.

Pigulevsky SV, Michaleff PV. 1969. Poisoning by the medusa *Gonionemus vertens* in the sea of Japan. *Toxicon* 7(2):145–149.

Purcell JE, Mills CE. 1988. The correlation between nematocyst types and diets in pelagic hydrozoa. In: Hessinger DA and Lenhoff HM (eds). The biology of nematocysts. Academic Press, Elsevier. pp. 463-485.

Rodriguez CS, Pujol MG, Mianzan HW, Genzano GN. 2014. First record of the invasive stinging medusa *Gonionemus vertens* in the southern hemisphere (Mar del Plata, Argentina). *Latin American Journal of Aquatic Research* 42(3):653-657.

Rottini L.1979. *Gonionemus murbachi* Mayer (Hydrozoa, Limnomedusae) nella Laguna di Grado (Golfo di Trieste). *Bollettino della Società Adriatica di Scienze (Trieste)* 63:39-43.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9:671-675.

SMHI 2018. Online marine monitoring data. https://www.smhi.se/klimatdata/oceanografi/havsmiljodata/marina-miljoovervakningsdata (Accessed on 2018-10-01)

SVT. 2018. Swedish Broadcasting Corporation. News on July 31, 2018. https://www.svt.se/nyheter/lokalt/vast/ny-brannmanet-forokar-sig-pa-vastkusten

Swofford DL. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tambs-Lyche H. 1964. *Gonionemus vertens* L. agassiz (limnomedusae) — A zoogeographical puzzle. *Sarsia* 15(1):1–8.

Uchida T. 1976. A new sporozoan-like reproduction in the hydromedusa, *Gonionemus vertens*. *Proceedings of the Japan Academy* 52(7):387-388.

van Duren JA, Knook HL. 1979. Ein “nieuwe” stekende kwal in Nederland, *Gonionemus vertens* L. Agassiz. *Nederlands Tijdschrift voor Geneeskunde* 123(50):2151–21.

Wolff WJ. 2005. Non-indigenous marine and estuarine species in The Netherlands. *Zool Meded Leiden* 79:1–116.

Yakovlev YM, Vaskovsky VE. 1993. The toxic krestovik medusa *Gonionemus vertens*. *Russian Journal of Marine Biology* 19(5-6):287-294.

Zheng L, He J, Lin Y, Cao W, Zhang W. 2014. 16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China. *Acta Oceanologica Sinica* 33(4):55-76.

Östman, C. 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. *Scientia Marina* 64(S1):31–46.

Figure legends

Figure 1. Map of the study area indicating new records of *Gonionemus* sp.
Red dots are records associated with stings. Historical records of *Gonionemus* sp., which are not associated with stings, indicated by blue dots. The blow up shows the area on the Swedish coast where stinging *Gonionemus* were found during 2018. Near the location where they were found are two international harbors, shown as black filled triangles. The sea surface temperature monitoring station in Åstol is indicated by a star.

Figure 2. Size distribution of *Gonionemus* on August 2 and August 18.
Size increase by 1.2 mm overt the 16 days, corresponding to a growth rate of 0.08 mm d^{-1} ($p<0.05$). Each histogram contains the bell diameters of 120 individuals.

Figure 3. Macromorphology of medusae and tentacles.
Medusae in dorsal (A-C), lateral (D,E) and ventral view (F,G), showing gonads, radial-canals, ring-canal, bell-rim flaps, statocysts, manubrium, tentacles with nematocyst batteries and adhesive pads, tentacle base tentacle with tentacle-canal and yellow streak, and velum. Abbreviations: *arrow*s, point at adhesive pads; *brf*, bell-rim flap; *gd*, developing gonad; *gf*, female gonad; *m*, manubrium; rac, radial-canal; *ric* ring-canal; *s*, statocyst; *sto*, stomach; *stoa*, stomach attachment; *t*, tentacle; *tb*, tentacle base; *tc*, tentacle-canal; *v*, velum; *ys*, yellow streak.

Commented [R26]: apical
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Figure 4. Micromorphology of tentacles and nematocysts.
(A-C) Tentacle parts with nematocyst batteries and adhesive pads. Note dark pigmented mid-line in tentacles, yellow-reddish pigments in batteries and around adhesive pad. (Inset B) LM.

Euryteles, note shaft and tubule. (D-K) LMs. Tentacle nematocysts. Undischarged and discharged microbasic euryteles and small microbasic b-mastigophores. Note shaft, tubule, lid and apical capsule opening (*). (E) Microbasic eurytele with broad, rod-shaped shaft, pointed apically and microbasic b-mastigophore with slightly bent, narrow shaft, following the convex capsule side. (F,G) Microbasic b-mastigophores. Note shaft and tubule pattern. (H-J, inset) Discharged microbasic euryteles. Note broad shaft with spined distal swelling, rounded lid, difference in diameter of shaft and distal tubule. (J) Note spine pattern on distal tubule. (I,K) Microbasic b-mastigophores. Narrow shaft with unclear spine-pattern. Abbreviations: *, marks apical capsule opening*;* *ap*, adhesive pad*;* *b*, *b-mast*, microbasic b-mastigophore; *dt*, distal tubule*;* *eu*, eurytele; *l*, lid; *nb*, nematocyst battery; *sh*, shaft; *sp*, spines; *tu*, tubule.

Figure 5. Temperature data from Åstol, adjacent to the locations where *Gonionemus* sp. was found in 2018.

The black line shows the monthly mean temperature ± standard deviations (shaded area) from 2000 - 2018. The red line with open circles shows the monthly mean temperature during 2018.

Figure 6. Neighbor-joining tree of COI haplotypes based on Kimura 2-parameter distances. Haplotype numbering for haplotypes 1 - 7 corresponds to those in Govindarajan et al. (2017). Haplotypes 8 - 16 are newly presented here based on Swedish specimens and GenB~~b~~ank (Table 1).

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