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ORIGINAL PAPER

Parasitic infection of the hyperiid amphipod *Themisto libellula* in the Canadian Beaufort Sea (Arctic Ocean), with a description of *Ganymedes themistos* sp. n. (Apicomplexa, Eugregarinorida)

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Abstract Two parasites were found in the hyperiid amphipod *Themisto libellula* sampled with nets and collected by sediment traps over the annual cycle in the Canadian Beaufort Sea. The trophozoites of the newly described gregarine *Ganymedes themistos* sp. n. infected the digestive tract of 60.2% of the *T. libellula* analyzed from net collections. An unidentified ciliate infected the body cavity of 4.4% of amphipods. *G. themistos* possessed the ball-like structure at the anterior end and the cup-like invagination at the posterior end that are typical of the genus *Ganymedes*. The frequency and severity (number of parasites host^{-1}) of infection by *G. themistos* increased with the length of *T. libellula* in the range 8–20 mm, and leveled off at ca. 94% and 186 trophozoites host^{-1} on average in the range 20–34 mm. Spatially, gregarine infection was less severe (63 ± 100 *G. themistos* host^{-1}) on the Slope than on the Mackenzie Shelf (110 ± 160) and in the Amundsen Gulf (132 ± 157). No evidence of an impact of trophozoite infection on the feeding and sexual maturation of the host was found. For a given size of *T. libellula*, infection by both parasites was more frequent in the traps than in the nets (*G. themistos*: 91.0% vs. 82.7%; ciliates: 16.3% vs. 6%). The 2.7 times higher infection frequency in the traps suggested that the ciliate parasite may kill its host.

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Introduction

Parasites are known to infect diverse groups of marine crustaceans. Some crustacean hosts play a central role in trophic webs, and the impact of parasites on their ecology may shape entire ecosystems. For example, mass mortality of Antarctic krill (*Euphausia pacifica*, *Thysanoessa spinifera*, and *Thysanoessa gregaria*) has been attributed to a parasitic apostomatid ciliate of the genus *Collinia* (Gómez-Gutiérrez et al. 2003). In shrimp aquaculture, heavy gregarine infections may have economic impacts by reducing growth and causing mortality of the stocks (Jiménez et al. 2002 and references therein).

Themisto libellula (Lichtenstein 1822) is a 2–5-cm-long pelagic hyperiid amphipod widely distributed in Arctic and sub-arctic seas (Dunbar 1946; Bowman 1960). It is an important trophic link between its copepod preys that dominate secondary production in northern waters and vertebrates such as the Arctic cod (*Boreogadus saida*), seabirds, and seals (Welch et al. 1992; Koszteyn et al. 1995; Dalpadado 2002). *T. libellula* sampled in the Beaufort Sea were infected by two different parasites: *Ganymedes themistos* sp. n., a gregarine apicomplexan; and an unidentified ciliate. Gregarine apicomplexans are single-celled, host-specific parasites that reach remarkably large sizes in marine species (Leander 2008). Dunbar (1957) reported the presence of gregarines in the digestive system of *T. libellula*, but we found no mention of ciliate parasites of *T. libellula* in the literature.

In the present study, we describe the general morphology and molecular phylogeny of the gregarine parasite *Ganymedes themistos* sp. n., and investigate the impacts of gregarine infestation on the feeding and sexual maturation of *Themisto libellula* in different oceanographic provinces of the Beaufort Sea. Infection rates are contrasted between amphipods collected by plankton net (assumed to represent the population) and those collected in sediment traps (which would include weakened or dead animals sinking out of the water column), to test the hypothesis that *T. libellula* collected in the traps are weakened or killed by *G. themistos*.

Materials and method

Study area

Southeastern Beaufort Sea in the Canadian sector of the Arctic Ocean encompasses three biogeographical regions with different ocean and ice climates that underpin different primary production regimes and zooplankton assemblages (Barber and Hanesiak 2004; Arrigo and van Dijken 2004; Darnis et al. 2008). The shallow Mackenzie Shelf (Fig. 1) is typically covered by landfast ice from October until May to early August (Macdonald et al. 1995). The ice regime in the deeper Amundsen Gulf is dictated by the dynamics of the Cape Bathurst Polynya that varies considerably in timing and extent from year to year (Barber and Hanesiak 2004; Arrigo and van Dijken 2004). The deeper Slope of the shelf is covered by the mobile central ice pack of the Arctic Ocean. The Mackenzie Shelf and the Amundsen Gulf are influenced by the plume of the Mackenzie River (Macdonald et al. 1995).

Sampling

As a part of the Canadian Arctic Shelf Exchange Study (CASES), zooplankton including *Themisto libellula* was sampled at different times from September 2002 to August 2004. A preliminary fall survey of the study area was conducted from 18 September to 25 October 2002 on board the CCGS icebreaker *Radisson*. The 2003–2004 expedition of the CCGS research icebreaker *Amundsen* included a fall survey of the entire region from September to November 2003, over-wintering in Franklin Bay at 70°2.73′ N, 126°18.07′ W (station depth of 230 m) from December 2003 to May 2004, and a summer survey of the study area from June to August 2004.

Zooplankton was sampled using 3 different gears during the spatial surveys. At all stations, a Double Square Net (DSN) consisting of a rectangular metal frame carrying side by side two 6-m-long, 1-m^2-mouth aperture, square-conical nets with 750-µm mesh was deployed in a double oblique tow down to a depth of 47 ± 8 m (mean ± standard deviation) at a towing speed of 1 m s^{-1} (2 knots) and a cable angle of 60º on the horizon. With the ship stationary, a Kiel Hydrobios© multi-layer sampler carrying 9 nets (0.5 m^2 opening and 200 µm mesh size) was deployed from the bottom or a maximum depth of 200 m to the surface at

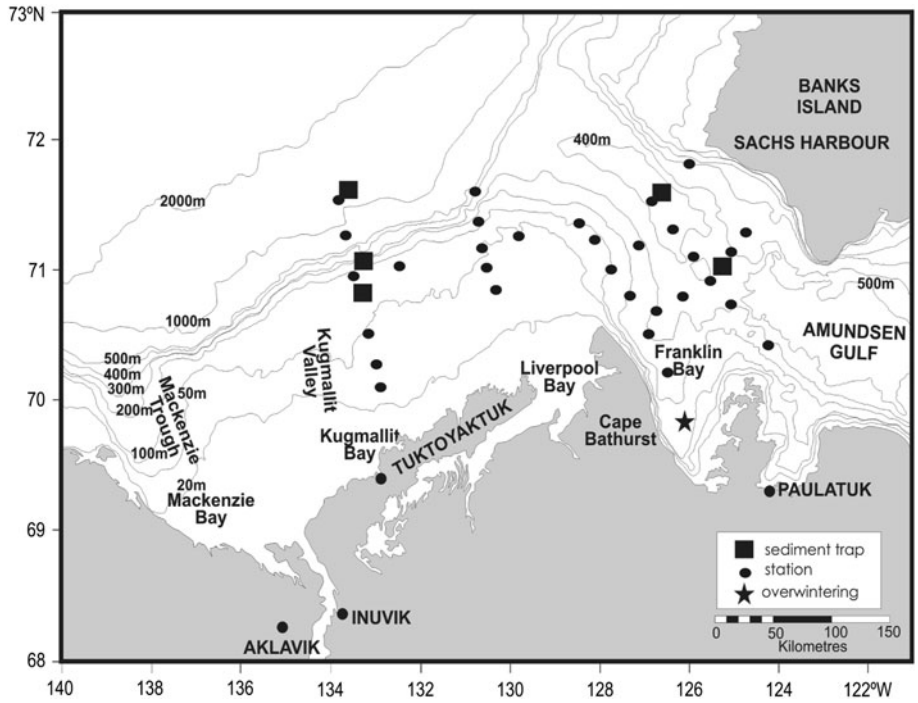

Fig. 1 Bathymetry of southeastern Beaufort Sea (Canadian Arctic Ocean) with location of the sampling stations in fall 2002 and in spring/summer 2004 (*dots*) and positions of the five time-sequential sediment traps deployed from October 2003 to July 2004 (*squares*). The overwintering station in Franklin Bay (winter 2003–2004) is indicated by a *star*

0.3 m s^{-1} to determine the vertical distribution of zooplankton. At selected stations, a 1-m^2-aperture E-Z-Net® multi-layer sampler equipped with nine sequentially opened and closed 6-m-long, 333-µm mesh nets was towed obliquely at 1 m s^{-1} in a stepwise manner from a maximum depth of 250 m to the surface.

During the over-wintering period, zooplankton was sampled through the *Amundsen*'s moonpool using the Kiel Hydrobios or a 1-m^2-aperture square 200-µm mesh net towed vertically from the bottom to the surface. Winter zooplankton was also sampled with the DSN towed horizontally at 1 m s^{-1} between two holes in the ice separated by a distance of 300 m, using a Bombardier BR180® tractor. Typically, a first 5-min tow was completed with two free-wheeling spherical buoys mounted on the frame to maintain the sampler at the ice–water interface and sample the 0.5- to 1.5-m-depth interval. After removing the buoys and lowering the sampler to a depth of 40 m, a second 5-min single oblique tow was completed from 40 m to the surface.

Additional *T. libellula* were collected by 5 sequential sediment traps deployed from October 2003 to July 2004 at 100- or 200-m depths at five locations in the study area (Fig. 1). Amphipods entering the traps were preserved by the 5% buffered formalin that filled the sample cups.

Themisto libellula morphometry, gut content, and parasites

Zooplankton samples were preserved in a 4% borax-buffered solution of formaldehyde in filtered seawater immediately upon collection. In the laboratory, *Themisto libellula* were sorted from the preserved samples. Specimens with segmented antennas were classified as males, those with signs of oostegites as females, and those with no distinct sexual differentiation as immatures (Dunbar 1957). Morphometric measurements and the presence of parasites were assessed using a stereomicroscope equipped with an ocular micrometer (±0.1 mm). When available, up to 15 specimens of each category (immatures, females or males) from each sampling month were analyzed. Amphipod length was measured from the anterior tip of the cephalon to the posterior tip of the telson. The stomachs of 896 *T. libellula* (640 from the nets and 256 from the traps) were dissected for gut content analysis under the stereomicroscope. The alimentary canal was opened along the ventral midline from the mesothorax to the seventh abdominal segment in a solution of glycerin in water. The entire gut was then removed, divided into three parts (front-gut, mid-gut, hind-gut) and parasites and prey were identified, measured, and counted. The body cavity of dissected specimens infected by ciliates was thoroughly rinsed to recover the parasites that were then counted in a sedimentation chamber to obtain a rough estimate of their number.

Themisto libellula specimens were classified into 2-mm-length classes. Infection frequency (percentage of hosts infected) and infection severity (number of parasites per host) were calculated for each length classes and then averaged over length classes.

Collection and isolation of parasites for microscopy and PCR

Ethanol- and formalin-preserved specimens of the gregarines and ciliates were isolated in seawater by teasing apart the gut of *T. libellula* under a dissecting microscope (Leica MZ6). The gut material was examined under an inverted microscope (Zeiss Axiovert 200) and parasites were removed by micromanipulation and washed three times in seawater. Differential interference contrast (DIC) light micrographs were produced by securing parasites under a cover slip with Vaseline and viewing them with an imaging microscope (Zeiss Axioplan 2) connected to a color digital camera (Leica DC500).

Ten of the formalin-preserved gregarines were prepared for scanning electron microscopy (SEM). Individuals were deposited directly into the threaded hole of a Swinnex filter holder, containing a 5-µm polycarbonate membrane filter (Millipore Corp., Billerica, MA), which was submerged in 10 ml of seawater within a small canister (2 cm diameter and 3.5 cm tall). A piece of Whatman filter paper was mounted on the inside base of a beaker (4 cm dia. and 5 cm tall) that was slightly larger than the canister. The Whatman filter paper was saturated with 4% OsO_4 and the beaker was turned over the canister. The parasites were fixed by OsO_4 vapors for 30 min. Ten drops of 4% OsO_4 were added directly to the seawater and the parasites were fixed for an additional 30 min. A 10-ml syringe filled with distilled water was screwed to the Swinnex filter holder and the entire apparatus was removed from the canister containing seawater and fixative. The parasites were washed then dehydrated with a graded series of ethyl alcohol and critical-point dried with CO_2. Filters were mounted on stubs, sputter coated with 5-nm gold, and viewed under a scanning electron microscope (Hitachi S4700). Some SEM images were presented on a black background using Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA).

DNA isolation, PCR, cloning, and sequencing

Thirty-four individuals of the ethanol-preserved *Ganymedes themistos* sp. n. were isolated from dissected hosts, washed three times in filtered seawater, and deposited into a 1.5-ml microfuge tube. DNA was extracted by using the total nucleic acid purification protocol as specified by EPICENTRE (Madison, WI, USA). The small subunit (SSU) rDNA was PCR amplified using puReTaq Ready-to-go PCR beads (GE Healthcare, Quebec, Canada).

The SSU rDNA gene from *Ganymedes themistos* sp. n. was amplified in one fragment using universal eukaryotic PCR primers F1 5′-GCGCTACCTGGTTGATCCTGCC-3′ and R1 5′-GATCCTTCTGCAGGTTCACCTAC-3′ (Leander et al. 2003) and internal primers designed to match existing eukaryotic SSU sequences (525F, NPF1, Nomet) F2 5′-AAGTCTGGTGCCAGCAGCC-3′; F3 5′-TGCGCTACCTGGTTGATCC-3′ and R2 5′-TTTAAGTTTCAGCCTTGCCG-3′; R3 5′-GCCTYGCGACCATACTCC-3′. PCR products corresponding to the expected size were gel isolated and cloned into the PCR 2.1 vector using the TOPO TA cloning kit (Invitrogen, Frederick, MD). Eight cloned plasmids were digested with EcoR1 and screened for size. Two clones were sequenced with ABI bigdye reaction mix using vector primers and internal primers oriented in both directions. The SSU rDNA sequence was identified by BLAST analysis (GenBank Accession number FJ976721).

Molecular phylogenetic analysis

The new sequence from *Ganymedes themistos* sp. n. was aligned with 54 alveolate SSU rDNA sequences using MacClade 4 (Maddison and Maddison 2000) and visual fine-tuning. Maximum likelihood (ML) and Bayesian methods under different DNA substitution models were performed on the 55-taxon alignment containing 1,146 unambiguous sites. All gaps were excluded from the alignments before phylogenetic analysis. The α-shape parameter was estimated from the data using the general-time-reversible (GTR) model for base substitutions (Posada and Crandall 1998) and a gamma-distribution with invariable sites (eight rate categories, $\alpha = 0.438$, fraction of invariable sites = 0.037). The ML bootstrap analyses were performed using PhyML* (Guindon and Gascuel 2003; Guindon et al. 2005) on 100 resampled data under an HKY model using the α-shape parameter and transition/transversion ratio (Ti/Tv) estimated from the original data set.

We also examined the 55-taxon data set with Bayesian analysis using the program MrBayes 3.0 (Huelsenbeck and Ronquist 2001). The program was set to operate with GTR, a gamma-distribution, and four Monte Carlo Markov chains (MCMC; default temperature = 0.2). A total of 2,000,000 generations were calculated with trees sampled every 100 generations and with a prior burn-in of 200,000 generations (2,000 sampled trees were discarded). A majority-rule consensus tree was constructed from 18,000 post-burn-in trees with PAUP* 4.0 (Swofford 1999). Posterior probabilities correspond to the frequency at which a given node is found in the post-burn-in trees.

Results

General morphology and surface ultrastructure of the gregarine parasite

Ganymedes themistos sp. n. is a gregarine belonging to the family Ganymedidae within the order Eugregarinorida. When the host is heavily infected, the midgut is filled with trophozoites (i.e., the feeding stage of gregarine lifecycles) (Fig. 2A). The trophozoites were elongated and cylindrical in shape (Fig. 2F). The anterior end was slightly tapered, while the posterior end was broader than the rest of the cell (Fig. 2B–E). The color of the trophozoites was light brown, suggesting an accumulation of amylopectin granules within the cytoplasm. In most cases, the nucleus was positioned within the posterior third of the cell (Fig. 2B–F). The nucleus was large and had an oval to rectangular shape (35×27 μm) with a prominent spherical nucleolus (8–11 μm). Some of the trophozoites terminated at the anterior tip with a ball-like bulge (Fig. 2C–E). The posterior end sometimes contained a cup-like invagination (Fig. 2C). The trophozoites varied considerably in size (Fig. 2B, E) and ranged from 130 to 820 μm (514 μm, $n = 10$) in length and 15–55 μm (37 μm, $n = 10$) in width, measured at the height of the nucleus. Several trophozoites were paired up in syzygy where the anterior gregarine, the so-called "primite", was usually bigger than the posterior trophozoite, the so-called "satellite" (Fig. 2E). Except for the mucron area (Fig. 3A–C), epicytic folds (Fig. 3D) were present over the entire cell surface and were observable under the light microscope (Fig. 2F). An indentation was present beneath the anterior bulge (Figs. 2C, D, 3A–C). The base of the mucron was inscribed with epicytic folds (Fig. 3A, B). Because the parasites were recovered from formalin and ethanol fixed hosts, the motility of the cells could not be observed. We assume that the cells were capable of gliding locomotion because of the numerous epicytic folds present (Leander 2008).

Molecular phylogeny of *Ganymedes themistos* sp. n. as inferred from SSUrDNA

The 55-taxon data set recovered a strongly supported clade of ciliates and two weakly supported clades: one consisting of dinoflagellates and *Perkinsus* and the other consisting of *Colpodella* species. An apicomplexan clade was also recovered with a poorly resolved backbone (Fig. 4). Within the apicomplexans, a clade consisting of piroplasmids and coccidians formed the sister group to a clade consisting of rhytidocystids, cryptosporidians, and gregarines. The new sequence from *Ganymedes themistos* sp. n. clustered within a weakly supported clade of mainly marine eugregarines.

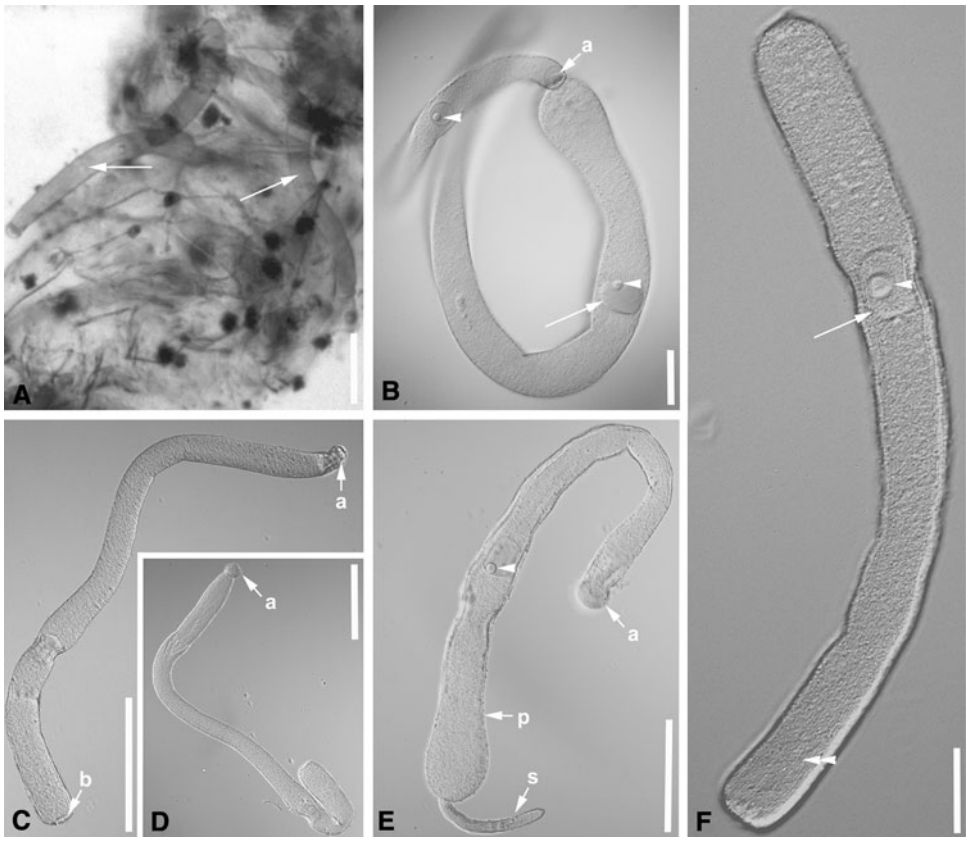

Fig. 2 Light micrographs of the intestinal gregarine *Ganymedes themistos* sp. n. **A** Midgut of *Themisto libellula* filled with trophozoites (*arrows*) of *Ganymedes themistos* sp. n. (*scale bar* = 120 μm). **B** Two trophozoites paired up in syzygy. The nucleus (*arrow*) and the nucleoli (*arrowheads*) are visible. The anterior trophozoite or primite is bigger than the posterior trophozoite or satellite. The ball-like bulge (*a*) at the anterior end of the primite is clearly visible (*scale bar* = 45 μm). **C** Long trophozoite with the cup-like invagination (*b*) at the posterior end and the ball-like bulge (*a*) at the anterior end of the cell (*scale bar* = 150 μm). **D** Trophozoite with a prominent ball-like bulge (*a*) (*scale bar* = 135 μm). **E** Two trophozoites in syzygy. The primite (*p*) is much bigger than the satellite (*s*), has a prominent ball-like bulge (*a*) at the anterior end and has a visible spherical nucleolus (*arrowhead*) (*scale bar* = 130 μm). **F** Trophozoite with visible epicytic folds (*double arrowhead*), and a large nucleolus (*arrowhead*) within the nucleus (*arrow*) (*scale bar* = 40 μm)

Like most other marine eugregarines, this sequence was highly divergent (as indicated by the very long branch in Fig. 4).

Species description

Family Ganymedidae Huxley 1910
Genus *Ganymedes* Huxley 1910
Ganymedes themistos sp. n. (Figs. 2A–F, 3A–D)

Hapantotype. Southeastern Beaufort Sea (132°–124° W; 70°–72° N) in 0–250 m depth. Parasites on gold sputter-coated SEM stubs have been deposited in the Beaty Biodiversity Research Centre (Marine Invertebrate Collection) at the University of British Columbia, Vancouver, Canada (Fig. 2C, D, F).

Etymology. The name of this species refers to the genus of the hyperiid amphipod type host, *Themisto libellula* (Lichtenstein 1822).

Type host. *Themisto libellula* (Lichtenstein 1822) (Arthropoda, Crustacea, Amphipoda, Hyperiidea, Hyperiidae).

Location in host. Mid-intestinal lumen.

Diagnosis. The overall cell morphology of this new species corresponds to that of other *Ganymedes* species; however, the new species is longer and the position of the nucleus is in the posterior third of the cell. In addition, the small subunit rDNA sequence GenBank Accession number FJ976721 and the host distinguish *G. themistos* from other known *Ganymedes* species.

Description. Body elongated (Fig. 2B–F), mean length 514 μm (130–820 μm), mean width 37 μm (15–55 μm), and light brown in color. Oval to rectangular nucleus (35 × 27 μm) in the posterior third of the cell. Anterior tip of most cells terminates in a ball-like bulge (Fig. 2A–D). Posterior end of some cells contains a cup-like invagination. Small subunit rDNA sequence GenBank Accession number FJ976721.

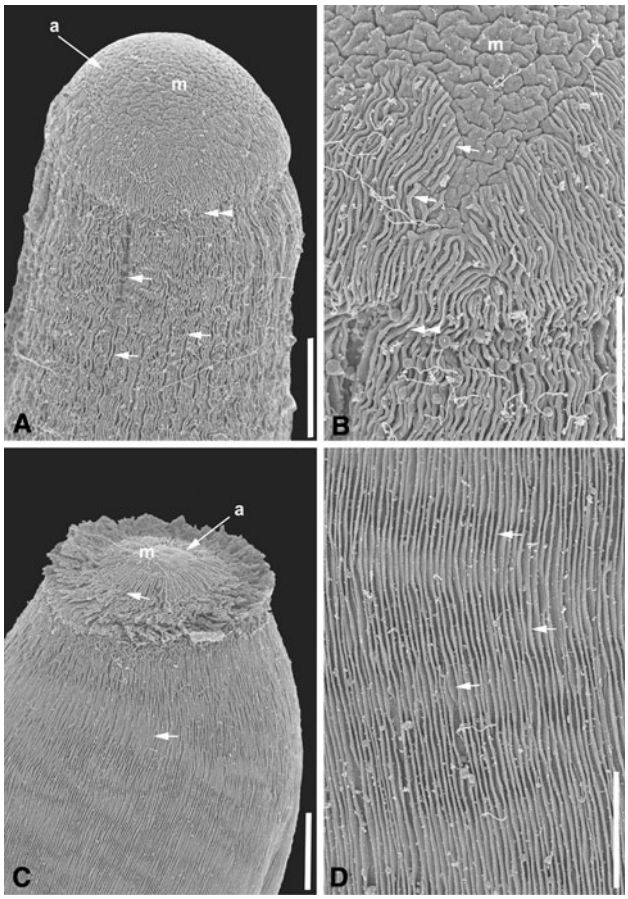

Fig. 3 Scanning electron micrographs of *Ganymedes themistos* sp. n. **A** The anterior end of *Ganymedes themistos* sp. n. showing a ball-like bulge (*a*) and the mucron (*m*). The entire body surface is inscribed with longitudinal epicytic folds (*arrows*). The *double arrows* indicate an indentation caused by the anterior bulge (*scale bar* = 10 μm). **B** High magnification view of the bulge. The epicytical folds (*arrows*) end shortly after the indention around the mucron (*m*). The mucron is inscribed with convoluted grooves. (*scale bar* = 5 μm). **C** Trophozoite with a flattened crown-shaped anterior end (*a*). The mucron (*m*) in the middle is free of epicytic folds (*arrows*) (*scale bar* = 10 μm). **D** High magnification view of the longitudinal epicytic folds (*arrows*) (*scale bar* = 5 μm)

Remarks. The gregarine described here infects a different host (*Themisto libellula*) in a different geographical region than any other described species of the genus *Ganymedes*: *Ganymedes anaspidis* is from *Anaspides tasmaniae* in Tasmania, Australia (Huxley 1910). No gregarines have previously been described from *T. libellula* (Fig. 5).

Frequency and severity of parasitic infection in *Themisto libellula*

A large fraction (60.2%) of the *Themisto libellula* collected by plankton nets in southeastern Beaufort Sea were parasitized by trophozoites of the gregarine *Ganymedes themistos* sp. n. (mean length of 310 ± 150 μm SD) located in the gut (Figs. 2, 3). The average number of *G. themistos* varied significantly among sections of the gut (ANOVA of log + 1 number of parasites, $F = 238.56$, $P < 0.0001$), with 17 ± 21 SD parasites in the foregut, 138 ± 151 in the midgut, 11 ± 29 in the hindgut.

The length of *Themisto libellula* was the main determinant of the severity of infection by *Ganymedes themistos* (Table 1). Infection frequency and severity increased with host length in the range 6–20 mm and then leveled off at around 94% and 186 trophozoites host^{-1} respectively at lengths >20 mm (Fig. 6). Once length was taken into account, differences in infection severity were not statistically significant among immature, male, and female *T. libellula* (Table 1).

The other identified factor that affected the severity of infection by *Ganymedes themistos* was oceanographic province as represented by station depth (Table 1). Average infection severity was significantly lower at stations corresponding to the Slope (>600 m, 63 ± 100 *G. themistos* host^{-1}) than on the Mackenzie Shelf (0–200 m, 110 ± 160 *G. themistos* host^{-1}) or the Amundsen Gulf (201–600 m, 132 ± 157 *G. themistos* host^{-1}) (Fig. 7). There was no clear influence of season, temperature or salinity on infection frequency and severity (data not shown).

Infection by an unidentified parasitic ciliate (mean length 130 ± 110 μm, Fig. 5) distributed in the body cavity was less common (4.4% of *Themisto libellula* collected in the nets) than infection by *Ganymedes themistos*. Among length classes, the average frequency of infection by ciliates increased linearly with the size of *T. libellula* captured in the nets ($r^2 = 0.71$).

Impact of *Ganymedes themistos* infection on the feeding and sexual maturation of *Themisto libellula*

Details of the diet will be reported elsewhere. *Themisto libellula* preyed essentially on large to medium-sized calanoid copepods such as *Calanus hyperboreus*, *C. glacialis*, and *Metridia longa*, and on other amphipods, including its own species. The number and average size of prey varied primarily with the length of *T. libellula* (Fig. 8). With length taken into account, variations in prey number and size among logarithmic classes of *Ganymedes themistos* infection severity were not significant (two-way ANOVA, $P \geq 0.07$).

Similarly, the severity of infection by *Ganymedes themistos* had no clear effect (two-way ANOVA, $P = 0.186$) on the development of oostegites in female *Themisto libellula* (Fig. 9a). Statistically, the length of the antennae of male *T. libellula* (an index of sexual maturity) tended to increase ($P = 0.009$) with infection severity (Fig. 9b). This marginal effect was clear only for the 16–22 mm size range of *T. libellula*, as sample sizes were low and categories of infection level were missing in the

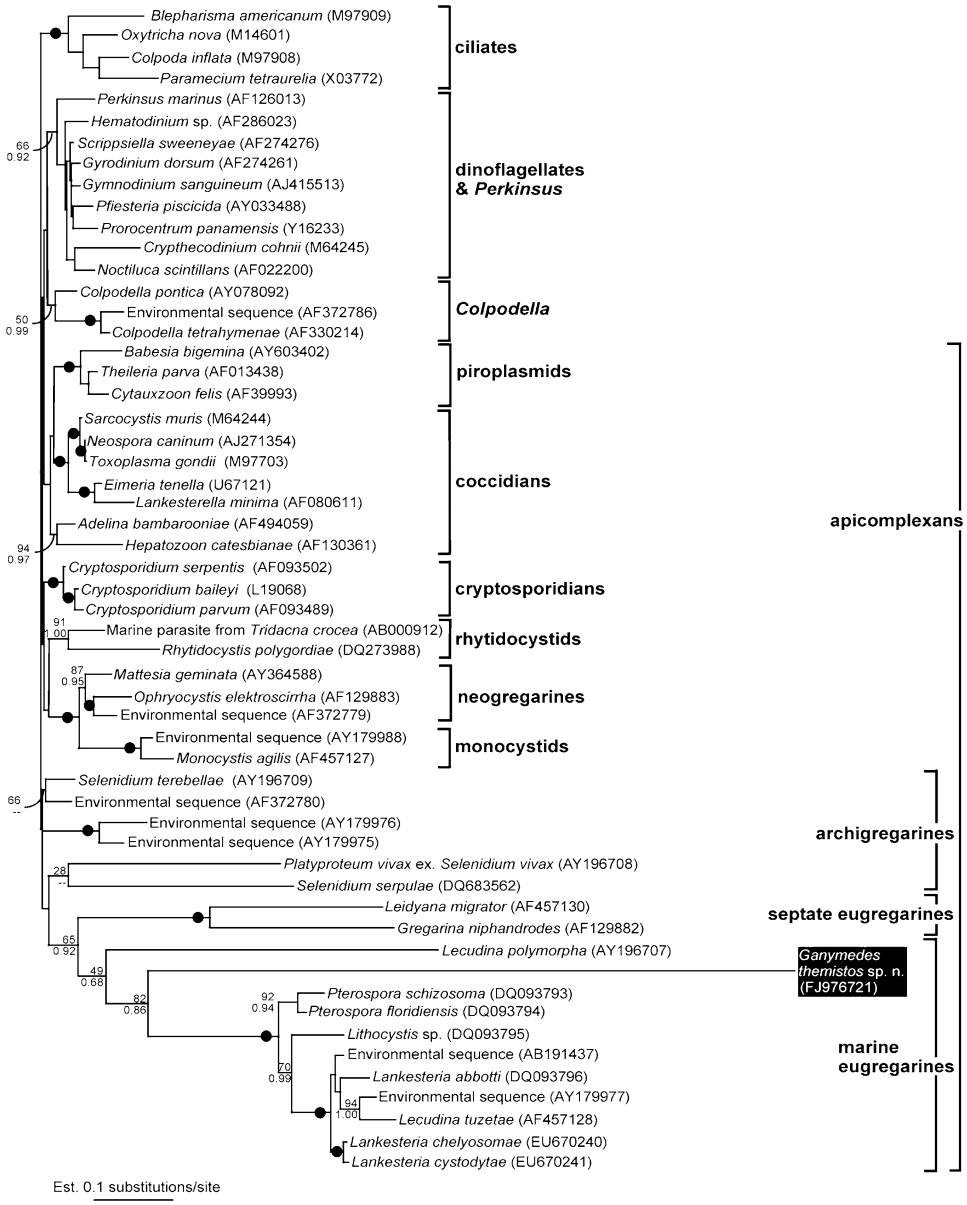

Fig. 4 Gamma-corrected maximum likelihood tree (−ln L = 16188.83549, α = 0.438, eight rate categories) inferred using the GTR model of substitution on an alignment of 55 SSU rDNA sequences and 1,146 unambiguously aligned sites. *Numbers* at the branches denote bootstrap percentage (*top*) and Bayesian posterior probabilities (*bottom*). *Black dots* on branches denote Bayesian posterior probabilities and bootstrap percentages of 95% or higher. The sequence of the species derived from this study is highlighted in the *shaded box*

higher size classes. The frequency of infection by ciliates was too low to carry statistical analyses of its impact on the feeding and sexual maturation of *T. libellula*.

Contrasting infection frequency and severity in plankton nets and sediment traps

The plankton nets captured *Themisto libellula* of all sizes and developmental stages while the traps collected essentially large males and females (Fig. 10). Over the host size range present in both samplers (12 to 28+ mm), the frequency of infection by *Ganymedes themistos* was marginally but significantly (*t*-test paired by size categories, $P = 0.032$) higher in the traps (91.0%) than in the nets (82.7%) (Fig. 11a). Infection by ciliates was more frequent (paired *t*-test, $P = 0.016$) in the traps (16.3%) than in the nets (6.0%) (Fig. 11b). Differences between the two samplers in the severity of infection by *G. themistos* (two-way ANOVA, $P = 0.743$) and by ciliates ($P = 0.140$) were not statistically significant. Combining data from nets and traps, the frequency of infection by ciliates was not significantly different (*t*-test paired by size categories, $P = 0.335$) between *T. libellula* infected (7.4%) and non-infected (4.7%) by *G. themistos*.

Discussion

Ganymedes themistos sp. n. (Apicomplexa, Gregarinia)

The genus *Ganymedes* belongs to the family Ganymedidae (Apicomplexa, Eugregarinorida) and consists of gregarine

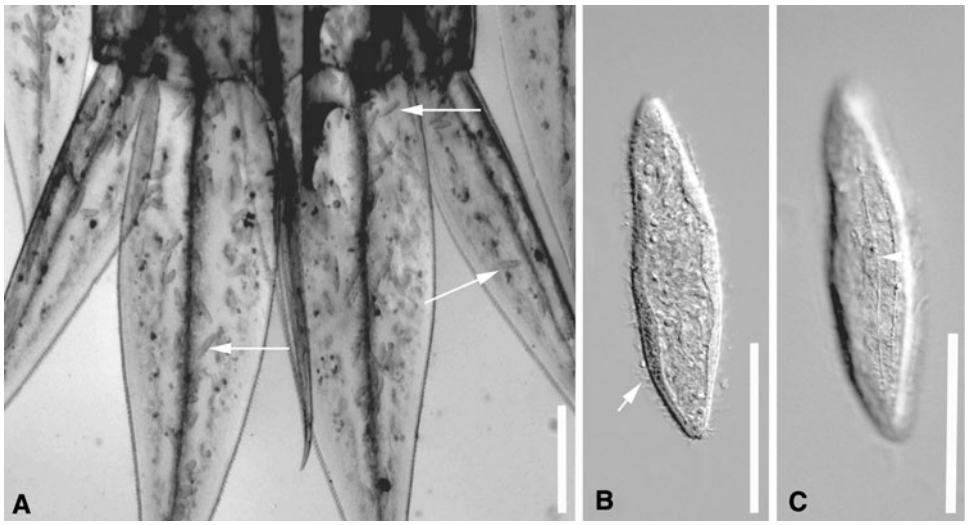

Fig. 5 Light micrographs of an unidentified parasitic ciliate in the body cavity of *Themisto libellula*. **a** Telson cavity filled up with ciliates (*arrows*) (*scale bar* = 360 μm). **b** Ciliate with visible cilia (*arrow*) at the outer rim of the cell (*scale bar* = 42.5 μm). **C** Different focal plane of the cell showing ciliary (longitudinal) kineties (*arrowhead*) (*scale bar* = 42.5 μm)

Table 1 ANOVA of the number of *Ganymedes themistos* trophozoites in the gut of *Themisto libellula* in relation to host length classes (2 mm from 4 to 28+ mm), oceanographic provinces (Shelf, 0–200 m: Amundsen Gulf, 201–600 m; Slope, >601 m), and host stage/sex (immature, male, female)

Source	*df*	Sum of squares	*F*	*P*
Length class	5	52.088377	15.0693	<0.0001
Oceanographic province	2	12.183269	8.8116	0.0002
Stage/sex	2	1.421893	1.0284	0.3584
Length class × stage/sex	10	4.226536	0.6114	0.8046
Length class × province	10	8.600977	1.2441	0.2603
Stage/sex × province	4	1.993090	0.7208	0.5781
Length class × stage/sex × province	20	12.537156	0.9068	0.5787

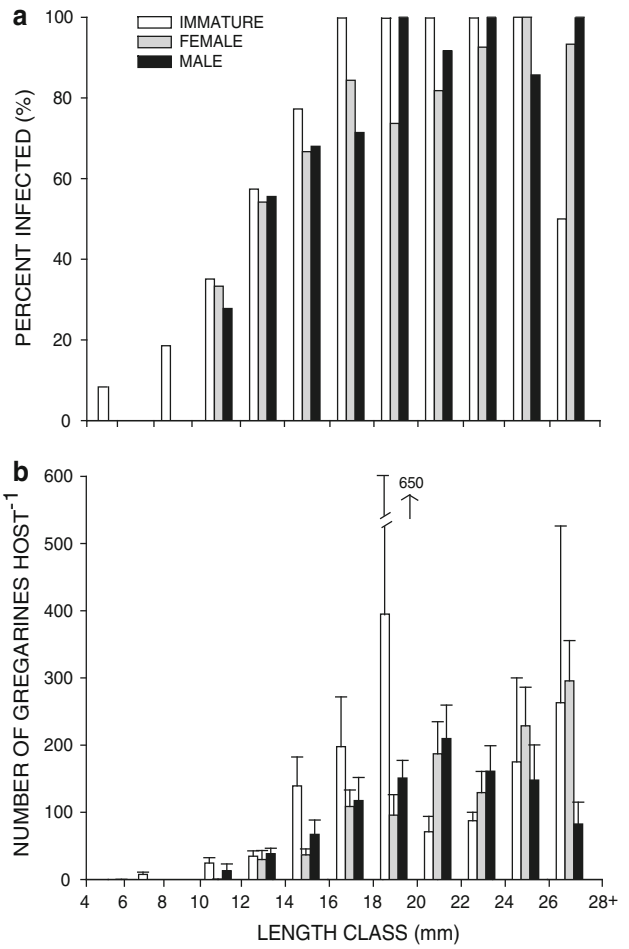

Fig. 6 **a** Percent infected and **b** average infection severity (mean number of *Ganymedes themistos* trophozoites ± standard error) by length classes for immature, female, and male *Themisto libellula* collected in the plankton nets

species that infect crustaceans. Seven species were formerly described in this genus: *Ganymedes anaspidis* (the type species) from *Anaspides tasmaniae* in Tasmania, Australia (Huxley 1910), *G. apsteini* from *Calanus gracilis* and *Clausocalanus arcuicornis* in Germany and France (Théodoridès and Desportes 1972), *G. eucopiae* from *Eucopia hanseni* and *G. korotneffi* from *Sergestes robustus* in Villefranche-sur-Mer, France (Théodoridès and Desportes 1975), *G. oaklandi* from *Gammarus fasciatus* in southern Michigan, USA (Jones 1968); *G. haeckeli* from *Sapphirina* spp. in Italy and *G. vibiliae* from *Vibilia armata* in Villefranche-sur-Mer, France (Théodoridès and Desportes 1972). Levine (1977) subsequently assigned four species (all but *G. eucopiae* and *G. korotneffi*) described in that genus, which lack the ball-like and cup-like structures to a new genus, namely *Paraophiodina*. However, according to Perkins et al. (2000), only the type species *G. anaspidis* remains within the genus *Ganymedes*. As the specimens found in *Themisto libellula* possess the ball-like structure at the anterior end, and some specimens displayed the cup-like invagination at the posterior end, we place this species within the *Ganymedes*.

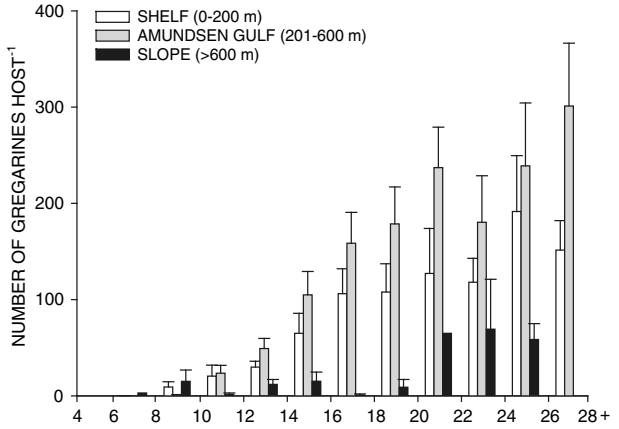

Fig. 7 Average infection severity (mean number of *Ganymedes themistos* trophozoites ± standard error) by length classes of the hyperiid amphipod *Themisto libellula* collected in the plankton nets for station depth categories corresponding to the Mackenzie Shelf (0–200 m), the Amundsen Gulf (201–600 m), and the Slope (>600 m) in the Canadian Beaufort Sea (Arctic Ocean)

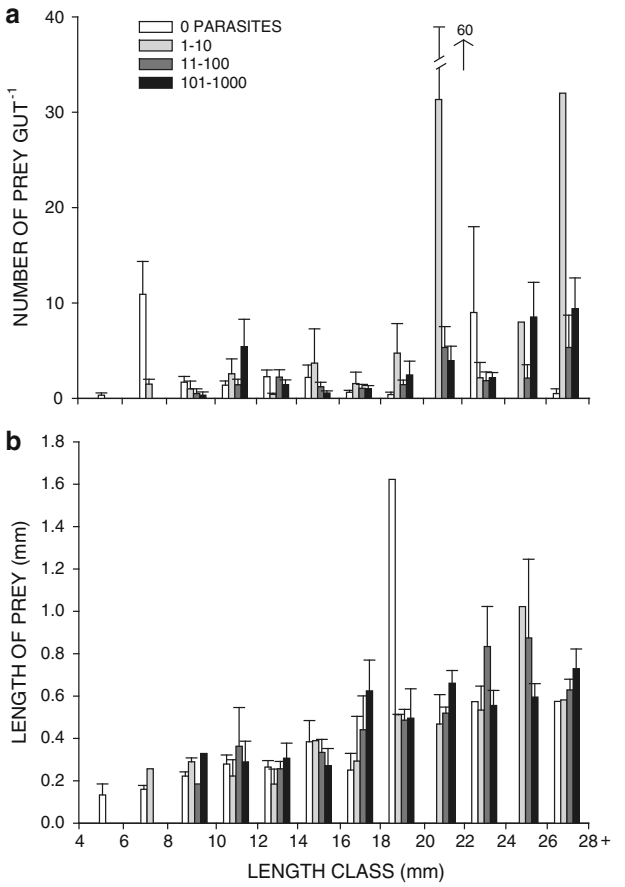

Fig. 8 Average (±standard error) prey number (**a**) and average (±standard error) prey length (**b**) by length classes of *Themisto libellula* collected in the plankton nets for logarithmic classes of infection severity (number of *Ganymedes themistos* trophozoites)

The phylogenetic position of *G. themistos* sp. n. within the marine eugregarines is uncertain, due to probable affects of long-branch attraction. The sequence from *G. themistos* sp. n. branches with low support as the sister lineage to other marine eugregarines. It does not specifically cluster with the septate gregarines of terrestrial arthropods (e.g., *Gregarina* and *Leidyana*), suggesting that there might be a distinct clade of marine arthropod gregarines. Further studies on the gregarines of marine arthropods should shed considerable light on their phylogenetic position within the eugregarines.

The determinants of *Themisto libellula* infection by *Ganymedes themistos*: host size, sex, and biogeography

In birds and mammals, the greater susceptibility of males to parasitism has been linked to the immunosuppressive effect of testosterone and other steroid hormones, an effect absent in invertebrates (Zuk 1990; Zuk and MacKean 1996). Studies of sexual biases in susceptibility to parasites in invertebrates are few. Sheridan et al. (2000) report no differences in parasitic infection between sexes in arthropods. Consistent with this, we found no significant differences in infection by *Ganymedes themistos* among male, female, and immature *Themisto libellula* of the same size. The length of *T. libellula* was the main determinant of infection severity by *G. themistos*. Based on the known host-specific life cycle of other gregarine parasites (see Leander 2008 for a review), *T. libellula* likely become infected by ingesting the oocysts of *G. themistos* released in the water column with the feces of parasitized amphipods. The trophozoites occurred in immature *T. libellula* as small as 6 mm, indicating that infection, sporulation, and the intra-cellular sporozoite phase that precedes the colonization of the intestine by trophozoites take place early during the ontogeny of the amphipod. Apparently, once infected, trophozoite numbers grow with time in parallel with the size of the host.

By affecting oocyst survival and sporulation (e.g., Clopton and Janovy 1993), ambient factors such as temperature or salinity could dictate differences in infection frequency and severity among oceanographic provinces. However, in the Beaufort Sea, the severity of *Themisto libellula* infection by *Ganymedes themistos* was not related to salinity or temperature at the time of capture. Hence, the higher infection severity in the Amundsen Gulf and on the Shelf relative to the Slope is not explained by the influence of the Mackenzie River plume. Thanks to local upwelling and the recurrent Cape Bathurst polynya, primary production is more intense in the Amundsen Gulf than on the Mackenzie Shelf (e.g., Arrigo and van Dijken 2004; Simpson et al. 2008; Tremblay et al. 2008). It is least intense over the nearly permanently ice-covered Slope. These regional differences in depth and microalgal production translate into different zooplankton assemblages, with prevalence in

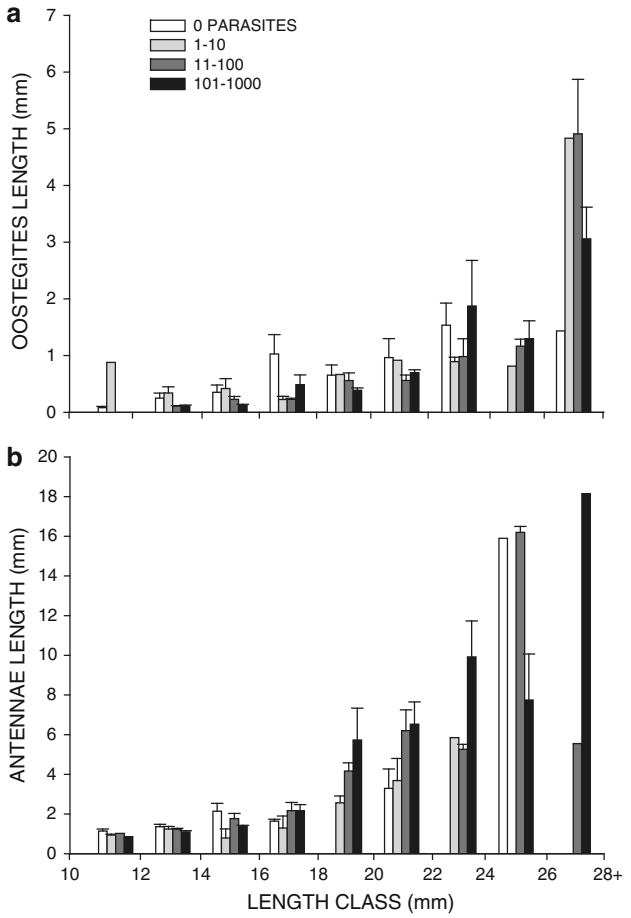

Fig. 9 Average length (±standard error) of oostegites by length classes of female *Themisto libellula* (**a**) and average length (±standard error) of antennae by length classes of male *Themisto libellula* (**b**) for logarithmic classes of infection severity (number of *Ganymedes themistos* trophozoites)

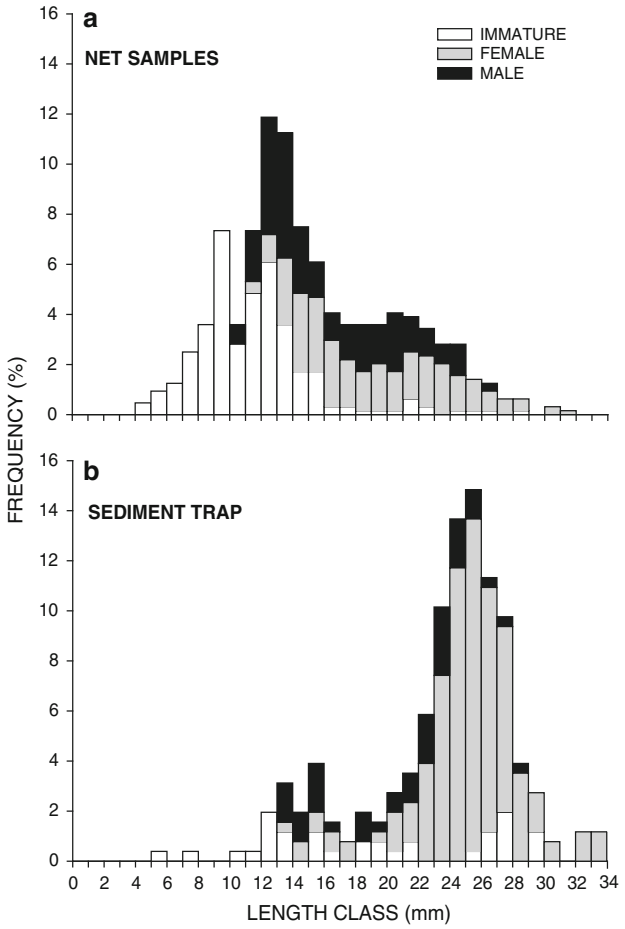

Fig. 10 Percent length frequency distribution of *Themisto libellula* in net and sediment trap collections

the Amundsen Gulf of the large calanoid copepods that are the preferred prey of *Themisto libellula* (Darnis et al. 2008). The general correspondence between infection severity and biological productivity among the three regions suggests that high availability of copepod prey, intense feeding, and the resulting increased broadcasting of *G. themistos* oocysts by *T. libellula* could explain the higher infection levels in the Amundsen Gulf and the Shelf than on the Slope.

Impact of parasites on *Themisto libellula*

The impacts of gregarine parasites on their host range in severity from harmless commensalism to death (Lightner 1993; Jiménez et al. 2002). Even at low abundances, gregarines can reduce a host's ability to assimilate food (Siva-Jothy and Plaistow 1999), delay development, and decrease body weight and longevity (Zuk 1987; Åbro 1996). However, in contrast to the intra-cellular sporozoites that colonize the tissues of the host, it is believed that the trophozoites of gregarines living in the lumen of the gut are relatively harmless (Jiménez et al. 2002; Takahashi et al. 2003).

Consistent with this notion that gregarine trophozoites are innocuous, we found no evidence for a significant impact of *Ganymedes themistos* trophozoites infection on the feeding and sexual maturation of *Themisto libellula*. Concerning survival, the initial linear rate of increase in trophozoite infection severity with length predicted average infection levels as high as 500 trophozoites host^{-1} in *T. libellula* 34 mm long (Fig. 6b). The leveling-off of average infection severity at ca. 200 trophozoites host^{-1} in *T. libellula* ≥20 mm suggested that beyond this level, *T. libellula* may be starved and killed by its gregarine parasite. Large *T. libellula* weakened or killed by *G. themistos* would be expected to sink out of the water column. Consistent with this scenario, the sediment traps collected primarily individuals >20 mm heavily infected by *G. themistos* (Fig. 10). If high levels of trophozoites or ciliate infestation actually killed large *T. libellula*, average infection

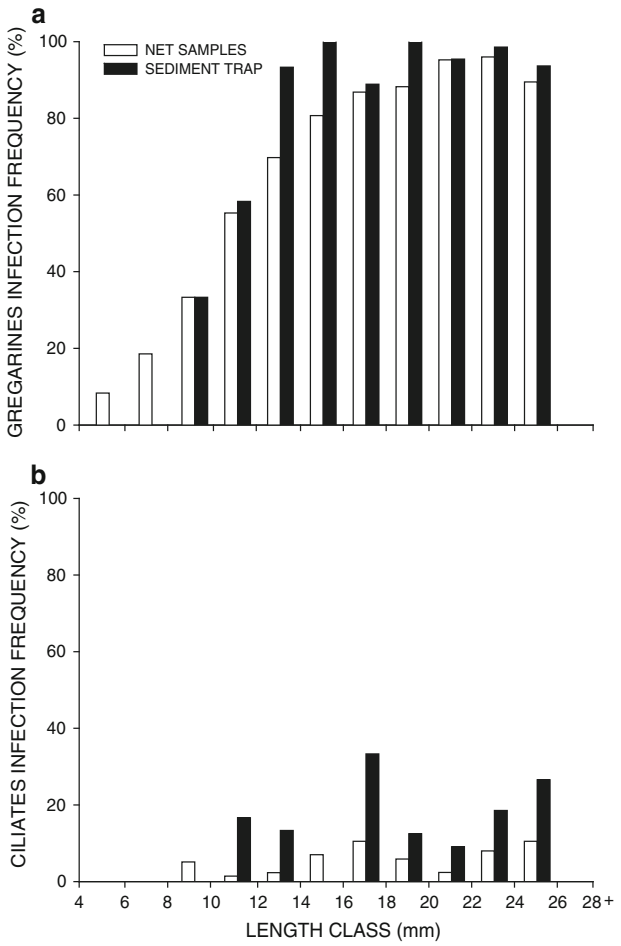

Fig. 11 Infection percent frequency by *Ganymedes themistos* trophozoites (**a**) and ciliates (**b**) by length classes of the hyperiid amphipod *Themisto libellula* for net and trap collections

frequency and severity would be expected to be higher in the traps than in the plankton. This prediction was not verified for infection severity (number of parasites per host) that did not differ significantly between the two samplers. However, infection of *T. libellula* was more frequent in the traps than in the nets by 8.3 and 10.3% for gregarine (91.0% vs. 82.7%) and ciliate (16.3% vs. 6%) parasites, respectively. The high and similar infection frequencies by *G. themistos* in the nets and the traps suggest that most *T. libellula* infected only by gregarine trophozoites were healthy animals that survived their parasite and accidentally entered the traps during their vertical migrations. By contrast, infection by ciliates was relatively rare and 2.7 times higher in the traps than in the nets. This suggests that amphipods infected by ciliates survived poorly and sank rapidly out of the water column.

In conclusion, we found no clear evidence of a negative impact of trophozoite infection on the feeding, sexual maturation, and survival of *T. libellula*. Further experimental work may be needed to assess the potential impact of infection by the sporozoite stage of *G. themistos* on the ecology of *T. libellula*. By contrast, comparing infection frequency in the nets and in the traps suggested that ciliates could actually kill their *T. libellula* hosts. If killed by their ciliate parasites before entering the sediment traps, *T. libellula* should be considered an integral part of the vertical particulate carbon flux (e.g., Sampei et al. 2009). Hence, death by parasitism could be an additional trophic process to consider in studying the contribution of zooplankton to particulate organic carbon fluxes in the Arctic Ocean.

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