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Pseudocollinia brintoni gen. nov., sp. nov. (Apostomatida: Colliniidae), a parasitoid ciliate infecting the euphausiid *Nyctiphanes simplex*

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ABSTRACT: A novel parasitoid ciliate, *Pseudocollinia brintoni* gen. nov., sp. nov. was discovered infecting the subtropical sac-spawning euphausiid *Nyctiphanes simplex* off both coasts of the Baja California peninsula, Mexico. We used microscopic, and genetic information to describe this species throughout most of its life cycle. *Pseudocollinia* is distinguished from other Colliniidae genera because it exclusively infects euphausiids, has a polymorphic life cycle, and has a small cone-shaped oral cavity whose left wall has a field of ciliated kinetosomes and whose opening is surrounded on the left and right by 2 'oral' kineties (or ciliary rows) that terminate at its anterior border. Two related species that infect different euphausiid species from higher latitudes in the northeastern Pacific Ocean, *Collinia beringensis* Capriulo and Small, 1986, briefly redescribed herein, and *Collinia oregonensis* Gómez-Gutiérrez, Peterson, and Morado, 2006, are transferred to the genus *Pseudocollinia*. *P. brintoni* has between 12 and 18 somatic kineties, and its oral cavity has only 2 oral kineties, while *P. beringensis* comb. nov. has more somatic kineties, including 3 oral kineties. *P. oregonensis* comb. nov. has an intermediate number of somatic kineties. *P. beringensis* comb. nov. also infects *Thysanoessa raschi* (a new host species). SSU rRNA and *cox1* gene sequences demonstrated that *Pseudocollinia* ciliates are apostome ciliates and that *P. brintoni* is different from *P. beringensis* comb. nov. High densities of rod-shaped bacteria (1.7 µm length, 0.2 to 0.5 µm diameter) were associated with *P. brintoni*. After euphausiid rupture, high concentrations of *P. brintoni* and bacteria cluster to form 3 to 6 cm long filaments where tomites encyst and transform to the phoront stage; this is a novel place for encystation. *P. brintoni* may complete its life cycle when the euphausiids feed on these filaments.

KEY WORDS: Euphausiids · Ciliates · Apostomatidae · *Collinia* · *cox1* · SSU rRNA · Mexico

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INTRODUCTION

Parasites regularly cause a gradient of damage to their hosts, ranging from almost innocuous to lethal effects (Bradbury 1994, Morado & Small 1995, Gómez-Gutiérrez et al. 2003, 2006, 2009, 2010a). Parasitoids are categorized as lethal when they must kill their host to complete their life cycle, with an ecological function analogous to predators (Lafferty & Kuris 2002).

Capriulo & Small (1986) described an endoparasitic ciliate of krill infecting the euphausiid *Thysanoessa inermis* Krøyer, 1846 in the Bering Sea, naming the species *Collinia beringensis* and assigning it to the family Colliniidae. A related species, named *Collinia oregonensis* Gómez-Gutiérrez, Peterson & Morado, 2006, was responsible for a mass mortality of the temperate euphausiid *Euphausia pacifica* Hansen, 1911, in Astoria Canyon, Oregon, USA, and was recognized as a parasitoid ciliate of euphausiids (Gómez-Gutiérrez et al. 2003). *Collinia oregonensis* also infects at least 2 other USA west coast euphausiid species, *Thysanoessa spinifera* Holmes, 1900, and *Thysanoessa gregaria* G. O. Sars, 1883 (Gómez-Gutiérrez et al. 2003, 2006).

In the southern part of the California Current System and Gulf of California, there is a diverse euphausiid assemblage, among which *Nyctiphanes simplex* Hansen, 1911 accounts for >90% of the euphausiid biomass and abundance in the neritic regions (Brinton 1962, 1981, Brinton & Townsend 1980, Lavaniegos 1994, Gómez-Gutiérrez 1995, Gómez-Gutiérrez et al. 1995). During a study of the fecundity and molting rates of *N. simplex* carried out along the southwest coast of the Baja California peninsula and Gulf of California, Mexico, Gómez-Gutiérrez et al. (2010b,c) discovered several adults infected with a novel apostome species similar to those previously assigned to the genus *Collinia* (Capriulo & Small 1986, Capriulo et al. 1991, Gómez-Gutiérrez et al. 2003, 2006, 2010a). Here, we formally establish the new genus *Pseudocollinia* gen. nov. and describe its type species as *Pseudocollinia brintoni* sp. nov., based on its morphology, its nuclear small subunit (SSU) ribosomal ribonucleic acid (rRNA) and mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene sequences. We provide the general symptoms of infection of this parasitoid, and we propose a hypothetical life cycle based on our field observational and experimental data.

MATERIALS AND METHODS

Euphausiid collection

Infected *Nyctiphanes simplex* euphausiids were collected during 3 oceanographic cruises carried out along the continental shelf and the middle part of Magdalena Bay, Baja California Sur, Mexico (BAMA cruises, March, July, and November 2004, 24° 30′ N, 112° 30′ W) (Fig. 1A,B) and during 4 oceanographic cruises carried out along the Gulf of California (GOLCA cruises, November 2005, January and July 2007, and March 2010, 24–30° N, 109–113.5° W) (Fig. 1C–E). In the September to October 2010 and November 2011 cruises in the same region, when euphausiids had unusually low abundance, no *Pseudocollinia*-infected euphausiids were collected (n = 18 and 13 zooplankton samples, respectively) (Tables 1 & 2). In the Bering Sea, additional infected *Thysanoessa inermis* and *Thysanoessa raschi* M. Sars, 1864 (new host species) euphausiids were collected during an oceanographic cruise carried out from 18 June to 10 July 2010.

Euphausiids were collected mostly at night using a drifting black 300 µm mesh net with a 1 m diameter mouth, 5 m length, and a PVC cod-end of 0.25 m diameter and 0.70 m length. In Mexico, this zooplankton net was equipped with an underwater lamp (Ikelite Pro-video-lite system, 50 W) to attract the euphausiids. Live zooplankton samples were obtained by lowering the net to the depth where the split-beam echosounder (SIMRAD EY-60, 120 kHz

Table 1. Total number of zooplankton samples collected during several oceanographic cruises using a drifting net, number of oceanographic stations where the euphausiid *Nyctiphanes simplex* were infected with the endoparasitoid ciliate *Pseudocollinia brintoni* gen. nov., sp. nov., and proportion (%) of oceanographic stations with infected krill. BAMA: Magdalena Bay; GOLCA: Gulf of California oceanographic cruises

Oceanographic cruise	Sampling stations	
	n	n with infected krill (%)
BAMA Mar 2004	32	3 (9.4)
BAMA Jul 2004	16	2 (12.5)
BAMA Dec 2004	35	6 (17.1)
GOLCA Nov 2005	26	1 (3.8)
GOLCA Jan 2007	18	4 (22.2)
GOLCA Jul 2007	26	1 (3.8)
GOLCA Mar 2010	16	2 (12.5)
GOLCA Sep–Oct 2010	18	0 (0)
GOLCA Nov 2011	13	0 (0)
Total (mean)	200	19 (9.5)

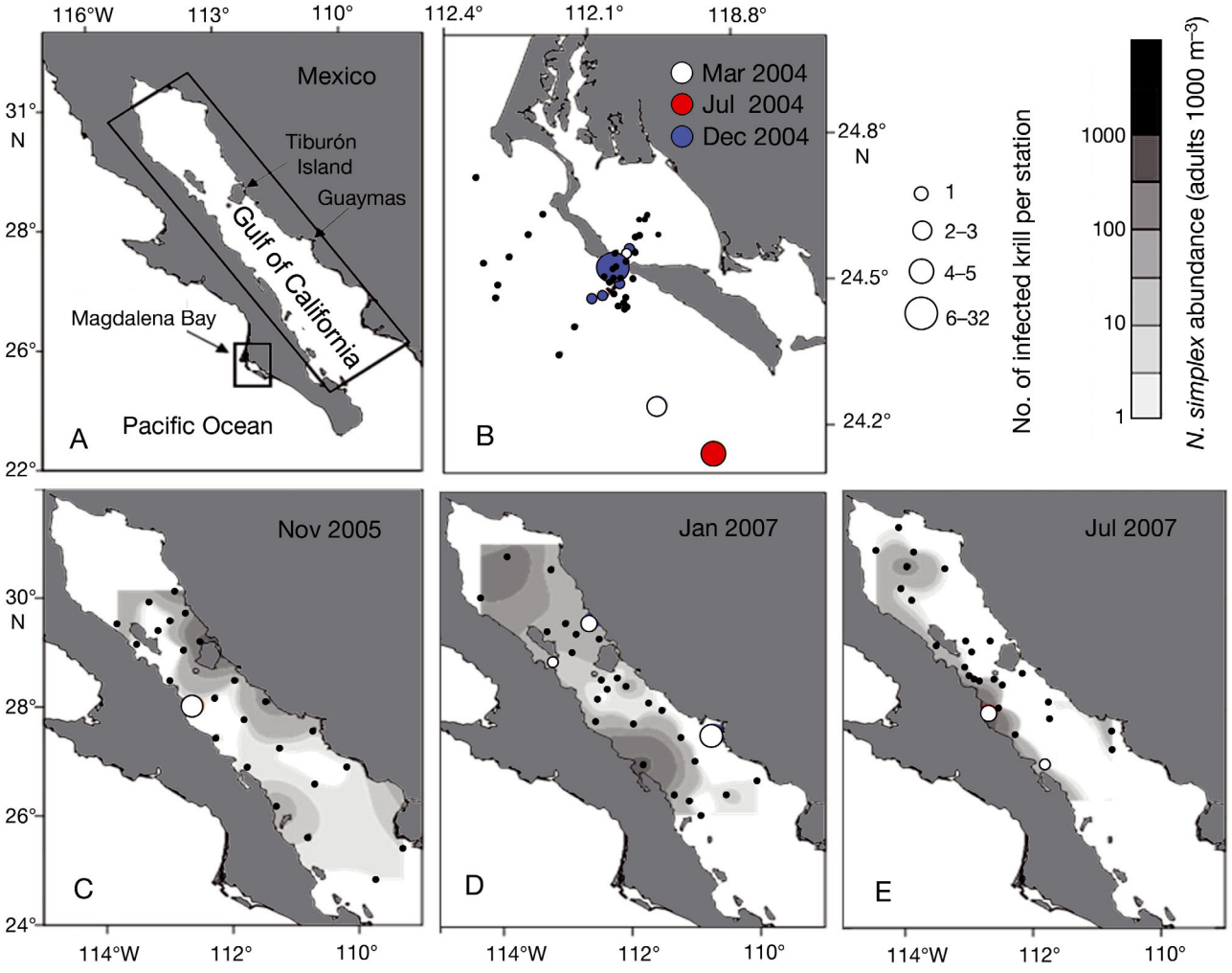

Fig. 1. Sampling stations (●) for krill in northwestern Mexico. (A) Areas of study and oceanographic stations along (B) the southwest coast of Baja California Peninsula (Magdalena Bay; Mar, Jul, and Dec 2004) and (C–E) Gulf of California during (C) Nov 2005, (D) Jan 2007 and (E) Jul 2007 where healthy (contour maps of abundance of krill >8 mm total length) and infected *Nyctiphanes simplex* with *Pseudocollinia brintoni* gen. nov., sp. nov. (○) were collected

frequency) and a submarine video camera (Deep Sea Power & Light) showed euphausiid aggregations. Sampling was conducted for 10 min while the ship was drifting. This methodology ensures negligible physical damage to the euphausiids.

Incubation of the infected euphausiid with endoparasitoid ciliates

Live, infected *Nyctiphanes simplex*, identified by having a swollen yellow-beige or orange cephalothorax, were transferred into 1 l bottles of surface seawater and incubated on board inside a cold room at 16 ± 0.5°C until they died due to ciliate infection. This temperature is near the mean temperature of the seawater where *N. simplex* is typically collected off both coasts of the Baja California peninsula (Table 2). One of the original research goals was to estimate the egg production and molting rates of *N. simplex* at each sampling station. Thus, we incubated >30 *N. simplex* adults with a healthy appearance individually in 1 l bottles for at least 48 h, plus ~200 adults in groups of 10 animals per 1 l bottle filled with seawater from 4 m depth sieved through a 20 µm mesh filter. After several hours of incubation in these bottles, some apparently healthy *N. simplex* specimens, based on observations at the beginning of the experiment, changed to a typically yellow-beige or orange infected coloration. Each infected specimen was isolated into a 1 l bottle for further observation. All of the infected euphausiids were monitored every 2 to 4 h during the

Table 2. Summary of the date and location where the parasitoid apostome ciliate *Pseudocollinia brintoni* gen. nov., sp. nov. was observed infecting the euphausiid *Nyctiphanes simplex* in Magdalena Bay, on the southwest coast of Baja California (BAMA) (n = 83 zooplankton samples) and Gulf of California (GOLCA) (n = 86 zooplankton samples), Mexico. All the infected euphausiids were adult females. SST: sea surface temperature during krill collection. During the Sep–Oct 2010 cruise (18 live-net zooplankton samples) and Nov 2011 cruise (13 live-net zooplankton samples), no infected krill were collected. Time: GMT – 7 h

Cruise	Date	Time	Latitude (N)	Longitude (W)	No. of infected specimens	No. of incubated euphausiids	Prevalence (%)	SST (°C)
BAMA 0304	25 Mar 2004	20:00	24° 24.33′	111° 57.13′	2	29	6.90	12.9
	30 Mar 2004	05:33	24° 32.99′	112° 01.17′	1	35	2.86	16.0
	30 Mar 2004	00:15	24° 33.28′	112° 01.15′	1	36	2.78	14.0
BAMA 0704	6 Jul 2004	00:38	25° 26.44′	113° 00.05′	1	30	3.33	17.8
	12 Jul 2004	01:08	24° 08.47′	111° 50.07′	3	43	6.98	21.0
BAMA 1204	5 Dec 2004	19:49	24° 29.09′	112° 03.39′	1	52	1.92	22.8
	9 Dec 2004	23:55	24° 29.73′	112° 04.12′	2	[a]	–	22.6
	10 Dec 2004	04:58	24° 25.61′	112° 00.30′	1	[a]	–	22.1
	12 Dec 2004	22:36	24° 31.17′	112° 02.47′	33	81	40.74	22.7
	12 Dec 2004	23:59	24° 33.45′	112° 00.93′	1	[a]	–	22.4
	15 Dec 2004	07:30	24° 27.47′	112° 05.51′	1	91	1.10	22.9
GOLCA 1105	23 Nov 2005	20:17	28° 00.91′	112° 39.11′	5	1000[b]	<0.001	21.3
GOLCA 0107	18 Jan 2007	02:46	29° 40.61′	112° 36.05′	3	2000[b]	<0.15	18.7
	19 Jan 2007	02:22	28° 53.22′	113° 14.15′	1	50	2.00	16.1
	22 Jan 2007	23:42	27° 33.15′	110° 43.97′	5	2000[b]	<0.25	17.2
	25 Jan 2007	06:28	28° 20.62′	112° 47.25′	4	900[b]	<0.44	15.5
GOLCA 0707	26 Jul 2007	02:00	26° 36.08′	111° 02.50′	1	36	2.78	25.1
GOLCA 0410	11 Mar 2010	20:50	28° 17.01′	112° 32.34′	2	57	3.51	17.4
	22 Mar 2010	00:43	28° 35.75′	112° 39.65′	1	2	50.00	17.1

[a]Zooplankton samples collected from net tow without shipboard incubation of euphausiids
[b]Field zooplankton samples with >1000 healthy euphausiids collected

night and every 6 h during the day to observe their behavior and the progression of infection and to detect when and how the euphausiids died. Euphausiids were sexed, and their total length (mm) was measured from the forward rim of the cephalothorax, located behind the base of the eyestalk, to the tip of the telson. Sequential photographs of the external appearance of the hosts were taken using an Olympus Camedia 3040 digital camera at 3.3×10^6 pixel resolution. We preserved the host and its ciliates with 4% (v/v) formaldehyde, or 2% (v/v) glutaraldehyde, for morphological analyses and 96% ethanol for genetic analyses, as the infection progressed through the following stages: (1) early infection stage (yellow-beige euphausiid carapace); (2) late infection stage (orange and swollen euphausiid carapace); (3) when the animal exploded and died; and (4) several hours after the host died and the ciliates formed <6 cm long sticky filaments of ciliate clusters. We used quantitative protargol staining on the ethanol-fixed ciliates (Montagnes & Lynn 1993) and scanning electron microscopy (SEM, Hitachi S-3000N) of sagittal cuts of the formaldehyde-preserved euphausiids to describe the different life-stages of these apostome ciliates inside *N. simplex* hosts.

When it was discovered through genetic analysis (see below) that this parasitoid ciliate from Mexican waters was indeed an apostome (order Apostomatida), samples of *Collinia beringensis* were also obtained from the type host *Thysanoessa inermis* (23.1 mm total length, Stn NP13, 23 June 2010) and a new host *T. raschi* (23.3 mm total length, Stn 29, 10 July 2010, at 70 m depth) during cruise KN195-10 (18 June to 10 July 2010) in the Bering Sea. Both host species were collected using a MOCNESS system and infected specimens (recognizable by their swollen orange cephalothorax) were sorted out alive and immediately preserved in 96% ethanol for ciliate protargol staining and genetic analyses.

DNA sequence analysis of ciliate mitochondrial *cox1* gene and SSU rDNA

Ciliates were obtained for genetic sequencing from 1 *Nyctiphanes simplex* specimen collected during the

BAMA 1204 cruise (12 December 2004) and from 2 specimens collected during the GOLCA 0410 cruise (11 March 2010). An infected *Thysanoessa inermis* and a *T. raschi* specimen collected from the Bering Sea (KN 195-10 cruise, 18 June to 10 July 2010) were also used for genetic sequencing. The cephalothorax of the ethanol-fixed krill was opened, and chunks of the infecting ciliate cells were transferred into 1.5 ml microcentrifuge tubes. The microcentrifuge tubes were left open for 30 min to let the ethanol evaporate. Subsequent DNA extraction was done using the DNEasy Blood & Tissue Kit (Qiagen), following the manufacturer's protocol, with the exception that the samples were incubated for 60 to 90 min and only 100 µl of AE buffer were used for elution.

PCR amplifications of the SSU rDNA and the *cox1* gene were performed in an ABI 2720 thermocycler (Applied Biosystems). The forward primers for the SSU rDNA were the universal eukaryote primer A (5′-AAC CTG GTT GAT CCT GCC AGT-3′) (Medlin et al. 1988) and the internal primer 300F (5′-AGG GTT CGA TTC CGG AG-3′) (Elwood et al. 1985). The universal eukaryote primer B (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) (Medlin et al. 1988) and the primers C (5′-TTG GTC CGT GTT TCA AGA C-3′) (Jerome et al. 1996) and LSUR (5′-GTT AGT TTC TTT TCC TCC GC-3′) (Bourland & Strüder-Kypke 2010) were used as reverse primers. The *cox1* gene was amplified using the forward primers F388dT (5′-TGT AAA ACG ACG GCC AGT GGW KCB AAA GAT GTW GC-3′) for *Pseudocollinia brintoni* and F298dT (5′-TGT AAA ACG ACG GCC AGT GCN CAY GGT YTA ATN ATG GT-3′) for *Collinia beringensis* and the reverse primer R1184dT (5′-CAG GAA ACA GCT ATG ACT ADA CYT CAG GGT GAC CRA AAA ATC A-3′) (Strüder-Kypke & Lynn 2010). The PCR products were purified with a MinElute gel purification kit (Qiagen) and sequenced in both directions with a 3730 DNA Analyzer (Applied Biosystems), using ABI Prism BigDye Terminator (ver. 3.1) and a Cycle Sequencing Ready Reaction kit. The sequences were imported into Sequencher ver. 4.0.5 (Gene Codes), trimmed at the ends, assembled into contigs, and checked for sequencing errors.

The *cox1* sequences were imported into MEGA ver. 4.1 (Kumar et al. 2008) and aligned based on the amino acid sequence. The SSU rDNA sequences were imported into an existing dedicated comparative sequence editor (De Rijk & De Wachter 1993) database and automatically aligned to other apostome ciliate sequences. Based on the secondary structure of the SSU rRNA molecule, we further refined the alignment.

Four different phylogenetic analyses were performed on the alignments of both genes to construct the trees: maximum likelihood (ML; PhyML) (Guindon et al. 2005), Bayesian inference (BI; MrBayes) (Ronquist & Huelsenbeck 2003), maximum parsimony (MP; PAUP ver. 4.10) (Swofford 2002), and neighbor joining (NJ; PHYLIP ver. 3.67) (Felsenstein 2005). Modeltest ver. 3.0 (Posada & Crandall 1998) was employed to find the model of DNA substitution that best fit our data. The general-time-reversible (GTR) model for nucleotide substitution, with gamma-distributed substitution rates and invariable sites, was determined as the best model for both of the genes. We included these parameters in PhyML and MrBayes. DNADIST was employed to calculate the genetic distances with the Kimura 2-parameter model (Kimura 1980), and the distance trees were constructed with NEIGHBOR (NJ) (Saitou & Nei 1987). The data were re-sampled 500 (PhyML) and 1000 (MP, NJ) times, respectively. The MP analysis was performed with a random addition (n = 5) of the species and the tree bisection-reconnection (TBR) branch-swapping algorithm in effect.

Experimental infection mechanism

In March and July 2004, individual live infected *Nyctiphanes simplex* collected in Magdalena Bay were transferred to a 0.25 l bottle with water previously filtered with 0.07 µm pore glass fiber filters (GF/F) to avoid plankton contamination. To determine the effect of just-released parasitoid ciliates on healthy euphausiids under laboratory conditions, several healthy *N. simplex* females were placed into the 0.25 l bottle and exposed to a recently exploded euphausiid that had released the tomite stage of the ciliate into the incubation bottle. The healthy euphausiids were monitored almost continuously until they died. The animals were preserved and dissected in the laboratory to search for ciliates inside the cephalothorax. In December 2004, we conducted similar experiments adding healthy specimens of *N. simplex*, an unidentified mysid species and the small planktonic decapod *Processa pippinae* Wickstein & Méndez, 1985 to previously filtered seawater with recently formed bacteria-ciliate filaments to test whether this ciliate species is specific to euphausiids or if it could infect other holoplanktonic crustaceans collected with the same gear and in the same areas where the infected *N. simplex* were collected. In December 2005, we made a complete set of experiments in the Gulf of California with healthy, mature *N. simplex* females exposed

initially to free-swimming tomite cells and also to bacteria-ciliate filaments.

RESULTS

From a total of 200 zooplankton samples collected on both coasts of the Baja California peninsula, Mexico, we found *Nyctiphanes simplex* infected with living ciliates in only 19 oceanographic stations (Table 1, Fig. 1B–E). The overall proportion of oceanographic stations with infected euphausiids varied among oceanographic cruises from 0 to 22.2% (mean = 9.5%) (Table 1). There was no significant difference in the prevalence of infected euphausiids among the oceanographic cruises during which infections were found (χ^2 = 0.046, p = 0.25, n = 7 cruises).

Prevalence of *Pseudocollinia brintoni* gen. nov., sp. nov.

During the BAMA cruises, the prevalence of infected euphausiids per station ranged between 1.1 and 40.7% (mean ± SE = 8.3 ± 8.4%, 95% CI = 0.00–31.5%, df = 10). From 83 oceanographic stations, 47 infected and 397 healthy euphausiids were collected for shipboard incubation (Tables 1 & 2). Infected animals were collected in waters with sea surface temperatures (SST) ranging from 12.9 to 22.9°C, indicating that infections occur during all seasons and at a wide range of SST (Table 2). The infected animals were usually collected at night in the mouth of Magdalena Bay at ~40 m depth near the seafloor and over the continental shelf (Fig. 1B).

During the GOLCA cruises, the prevalence of infected euphausiids per station ranged between <0.001 and 50% (mean ± SE = 7.4 ± 6.1%, 95% CI = 0.00–21.8%, df = 7). From 86 oceanographic stations, 22 infected and ~6000 healthy euphausiids were collected for shipboard incubation (Tables 1 & 2). Infected animals were collected in waters with SST ranging from 15.5 to 25.1°C (Table 2). The infected animals were typically collected where *Nyctiphanes simplex* had the highest abundances, along the east coast of the Gulf of California during November 2005 and July 2007 and along the west coast of the gulf in January 2007 and March 2010 (Fig. 1C–E). In the September to October 2010 and November 2011 oceanographic cruises, euphausiids were not abundant in night samples, and no infected *N. simplex* were collected in the drifting net.

Symptoms of infection by *Pseudocollinia brintoni* gen. nov., sp. nov.

Healthy euphausiids are typically transparent, and gonads are easily observed through the carapace (Fig. 2A,B). Infection appeared only in female adults of *Nyctiphanes simplex*, ranging from 9 to 19 mm TL (n = 69) with a yellow-beige to bright orange coloration and swollen cephalothorax (Fig. 2C,E, Table 2). At the station with the largest number of infected euphausiids in the southwest coast of the Baja California peninsula in December 2004 (33 infected out of 81 collected specimens, prevalence 41%), 3 animals with an initially healthy appearance (i.e. semi-transparent carapace and abdomen), incubated for egg production and molting rates, became orange (i.e. infected). The initial infection stage (i.e. yellow-beige carapace) developed in <12 h, the advanced infection stage (i.e. orange and swollen carapace) developed in <31 h, and death of these 3 euphausiids (i.e. exploding) occurred in <33 h.

The apostome endoparasitoid ciliate recorded in the hemocoel and abdomen of *Nyctiphanes simplex* showed all of the ciliate life-cycle stages known for apostome ciliates (i.e. phoront, trophont, tomont, and tomite in the host; and tomont, tomite, and phoront outside the host) with transitional stages between the excysting of the phoront–trophont, beginning of palintomy of the tomont (i.e. with a fission furrow in the center), nearing the end of the division of tomites (i.e. figure-8-shaped or an advanced fission furrow), and tomite–phoront transition during encystation (Table 3, Figs. 2 & 3). All of the excysted developmental stages (i.e. trophont, tomont, and tomite) had 12 to 18 kineties (ciliary rows) when observed using SEM. However, because one cannot count all of the kineties on each cell in SEM images, these numbers are only estimates (Table 3). The phoront stage, by definition encysted, did not have visible kineties. The life stages were sequentially separated according to different external host conditions and the degree of morphological variability observed within each ciliate developmental stage: a tomite semi-encysted, a phoront completely encysted, and a phoront starting excystation as a trophont — this last stage is already inside the cephalothorax of the krill host, in the hemocoel (Fig. 2C–H).

Pseudocollinia brintoni endoparasitoid phase

In the endoparasitoid phase, *Pseudocollinia brintoni* gen. nov., sp. nov. feeds, grows (i.e. trophont

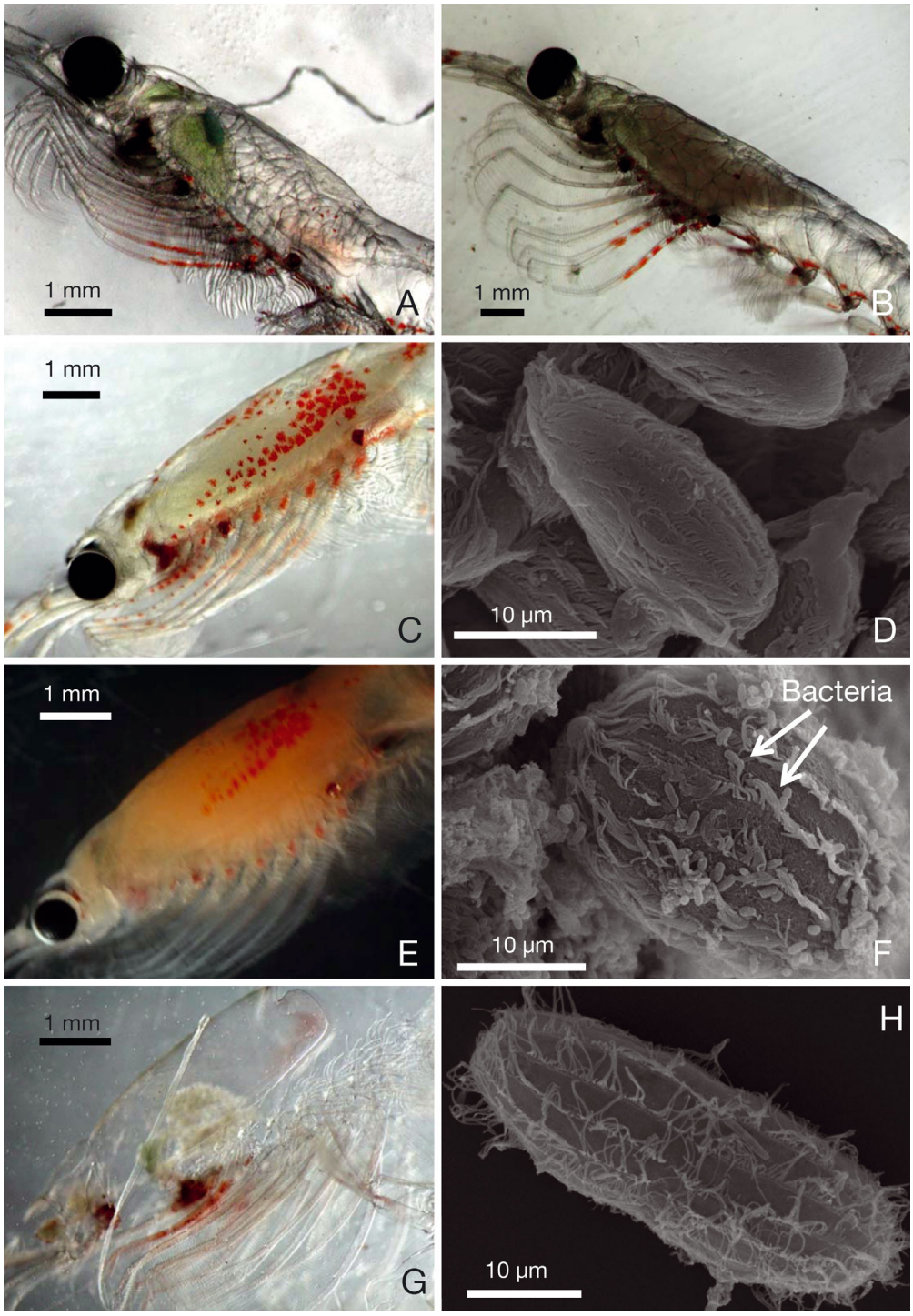

Fig. 2. (A,B) Healthy females of *Nyctiphanes simplex* with (A) transparent previtellogenic gonad and (B) pink gravid gonad. (C–H) Endoparasitoid phase of *Pseudocollinia brintoni* gen. nov., sp. nov. and chronological sequence of infection of *N. simplex*. (C) Female in early infection stage with yellow-beige opaque cephalothorax, ~12 h after the female had healthy appearance. (D) Trophont stage in early infection presumably just after phoront excystation. (E) Advanced stage of infection with bright orange cephalothorax, ~19 h after the carapace had yellow-beige coloration. (F) Mature trophont stage after feeding: cells appear almost spherical. In several host specimens, high abundances of rod-shaped bacteria (arrows; mean length 1.7 µm, width 0.63 µm) were observed on the ciliate's surface or in colonies on tissues in the euphausiid's hemocoel. (G) A euphausiid exploded after the ciliates had digested its internal organs, ~33 h after the animal had healthy appearance. (H) Reproductive ciliate or tomont stage starting multiple cell divisions without intervening growth (i.e. palintomy) to produce the dispersal stage (tomite)

Table 3. Biometry of the apostome ciliate *Pseudocollinia brintoni* gen. nov., sp. nov., an endoparasitoid of the subtropical sac-spawning euphausiid *Nyctiphanes simplex* along the west coast of Baja California, Mexico. Measurements are based on individuals observed using scanning electron microscopy (SEM). Accurately counting all kineties on a ciliate is impossible using SEM, so the number of kineties must be considered an estimate only. no: none observed

Ciliate stage	No. of kineties	Length (L, µm) Mean	SE	n	Width (W, µm) Mean	SE	n	L:W ratio	Observations
Yellow-beige krill									
Phoront	no	22.59	0.48	4	8.94	0.24	3	2.5	Encysted
Trophont	12	21.10	0.61	8	9.59	0.29	8	2.2	Semi-encysted
Trophont	12–18	22.21	0.32	25	8.93	0.16	25	2.5	Excysted (young and slender)
Orange krill									
Trophont	14–18	37.96	1.83	8	19.62	0.86	8	1.9	Excysted (young and slender)
Trophont	12–18	33.24	1.10	14	19.87	1.14	14	1.7	Excysted (mature)
Tomont	14–18	33.33	–	1	16.05	–	1	2.1	Excysted (slender)
Exploded krill									
Tomont	12–18	34.17	2.14	6	20.00	0.90	5	1.7	Spiral kineties
Tomont division	12–18	38.79	1.93	14	18.01	0.30	14	2.2	Palintomy
Tomites	12–14	29.99	5.32	4	20.00	0.67	4	1.5	Palintomy (daughter cells)
Tomite	12–14	36.97	2.36	4	21.24	0.78	4	1.7	Free-swimming, spiral kineties
Filament									
Tomite	12–14	25.16	1.35	8	12.66	0.44	8	2.0	Excysted, spiral kineties
Tomite–phoront transition	12	25.49	–	1	10.10	–	1	2.5	Semi-encysted
Phoront	no	29.87	1.37	5	9.26	0.50	5	3.2	Encysted

stage) and reproduces (i.e. tomont stage) in the hemocoel of *Nyctiphanes simplex*, producing fulminating infections that rupture the cephalothorax to release a large number of transmission stages (i.e. protomites and tomites) into the surrounding water. In early infection when the carapace of *N. simplex* has a yellow-beige coloration (Fig. 2C), most of the ciliates are young, encysted phoronts measuring on average 22.6 × 9 µm (length:width [L:W] ratio = 2.5) (Fig. 2D, Table 3). We observed phoronts in various excystation stages in the cephalothorax from a cell still encysted to a cell just excysted and showing 12 kineties (Table 3). The excysted phoronts, now trophonts, possess between 12 and 18 kineties with a similar L:W ratio as the phoronts observed in the host (Table 3). In the advanced infection phase, the euphausiid has a bright orange coloration and a swollen carapace (Fig. 2E). The trophonts are initially slender (L:W ratio = 1.9) and grow into a more elongated shape in the mature condition (33.2 × 19.9 µm, L:W ratio = 1.7) (Fig. 2F, Table 3). In the trophont stage, all of the kineties align from pole-to-pole without any apparent spiraling pattern. Two *N. simplex* with orange swollen cephalothoraxes collected in Magdalena Bay were examined with SEM and showed high densities of rod-shaped bacteria (mean length = 1.7 µm, mean width = 0.63 µm; n = 24) on the surface of the ciliates collected from the host's hemocoel. Most of the bacteria formed dense clusters in the hemocoel, but there were also high densities associated with the outer surface of the ciliates (Fig. 2F). Tomont stages were rarely observed in hosts with orange and swollen carapaces. Tomonts had a mean length × width of 33.3 × 16 µm with a slight dextral spiral (Table 3). When the euphausiid died, the ciliates exited from the juncture between the posterior part of the carapace and the first abdominal segment, leaving the carcass empty within a few minutes (Fig. 2G). Most of the tomont stages were observed once the euphausiid exploded, thus finishing the endoparasitoid phase; their average dimensions were 34.2 × 20 µm (L:W ratio = 1.7) (Table 3). The tomont stage begins to elongate when it undergoes normal apostome multiple binary fissions (i.e. palintomy) increasing the L:W ratio to 2.2 (Fig. 2H, Table 3).

Pseudocollinia brintoni ectoparasitic phase

The ectoparasitic phase starts when the ciliates exit from the host and, along with rod-shaped bacteria (discovered inside the euphausiid's hemocoel), form long filaments on which the ciliates encyst as the phoront stage. We speculate that a new crustacean host is infected by the ingestion of these filaments. The ectoparasitic phase of *Pseudocollinia brintoni* gen. nov., sp. nov. is composed of tomonts, a tomont-

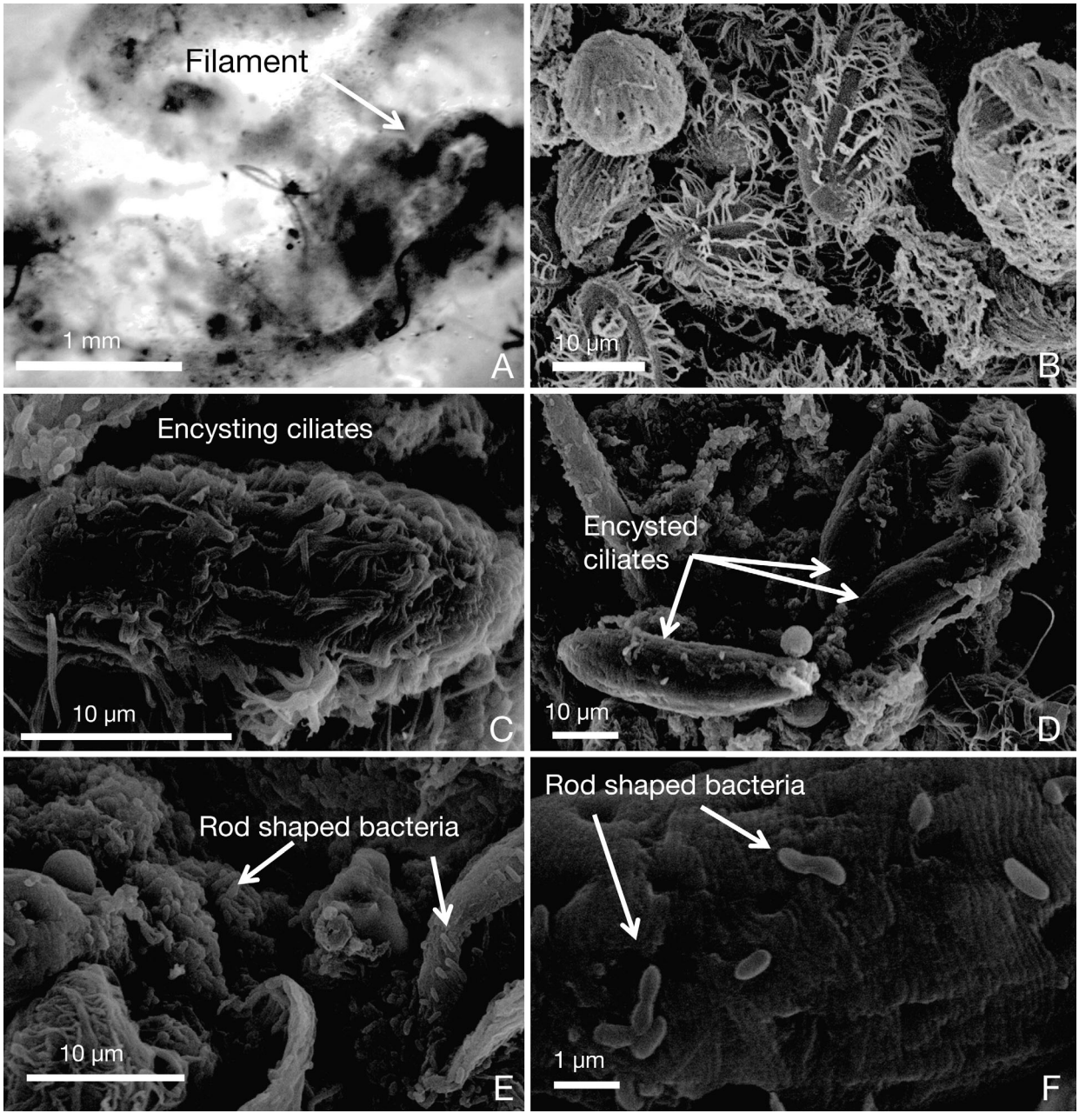

Fig. 3. Ectoparasitic phase of *Pseudocollinia brintoni* gen. nov., sp. nov. after infecting and killing *Nyctiphanes simplex*. (A) Micrograph of filaments formed several hours after the euphausiid's death. (B) High concentration of the dispersal tomite stage just attached to the filament; upper left corner possibly shows an encysted cell. (C) Ciliate encysting to transform to a phoront stage. (D) Ciliates completely encysted on the filaments; single rod-shaped bacteria are associated with both the ciliate's surface and the filament itself. (E,F) High concentrations of rod-shaped bacteria associated with several sections of the filaments. (B–F) are scanning electron micrographs

tomite transition during cell division, tomites, and phoronts. With each successive tomont division during palintomy, the kineties retain a slight spiral pattern. Free-swimming daughter cells or tomites are not necessarily of equal size, measuring 25.1 × 10 µm (L:W ratio = 2.5) (Table 3), and it is not known how many tomites are produced from each tomont. About 7 h after the euphausiid's rupture, the ciliates and bacteria in the seawater form sticky, mucus-like filaments, 3 to 6 cm long, to which clusters of ciliates, bacteria, and other particles adhere in high abundances (Fig. 3A). Sometimes these filaments were

attached to the krill carcass. When the tomites settle on the filament, the kineties still show a perceptible spiral pattern (Fig. 3B). The tomites beginning to encyst on the filaments (i.e. the tomite–phoront stage) have a L:W ratio of 2.5 (Fig. 3C). As encystment is completed, the cell shape elongates considerably, increasing the L:W ratio to 3.2 (Fig. 3D). The bacteria are also present in high densities on the newly formed filaments (Fig. 3E), where they actively reproduce likely by binary division (Fig. 3F). The bacteria from the filament are smaller than the bacteria found in the host (Table 4). It is unknown whether the ciliate, the bacteria or both form the filament.

Based on these observations, our proposed life cycle for *Pseudocollinia brintoni* gen. nov., sp. nov. infecting this sac-spawning euphausiid *Nyctiphanes simplex* is conceptually represented in Fig. 4. We still do not know how this species infects new hosts, but we speculate that it is by ingestion of filaments populated with parasitoid ciliates.

Tomite morphology of *Pseudocollinia brintoni* gen. nov., sp. nov.

Protargol staining of ethanol-fixed *Pseudocollinia brintoni* ciliates infecting *Nyctiphanes simplex* revealed mostly protomite–tomite stages (Figs. 5A–C, G & 6A–C). The cells were 28 to 35 µm long and 13 to 21 µm wide (average 31.1 × 16.6 µm; n = 33). They possessed a long and narrow macronucleus that stretched almost the length of the cell (18 to 28 µm long by 2 to 11 µm wide, average 22.8 × 5.1 µm, n = 32) (Figs. 5B & 6B); a micronucleus was not observed. There were on average 16 kineties (14 to 18, n = 33) that extended from pole to pole, except for 3 kineties (Figs. 5A,C & 6A,C). Two of these kineties curve around the oral cavity opening and terminate (hereafter referred to as 'oral kineties') and were numbered Kinety 1 (K1) and Kinety n (Kn) (Figs. 5A,C & 6A,C). The third kinety, Kinety n–1, curves anteriorly and right above the oral cavity before terminating in a non-ciliated region anterior to the oral cavity (Figs. 5A,C & 6A,C). The oral cavity, which is about one-third of the cell's length from the anterior end, appears cone-shaped and lined on its left wall with a dense field of ciliated kinetosomes or basal bodies (Figs. 5C,G & 6A,C). Kinety n–2 and occasionally Kinety n–3 terminate by abutting at the anterior end of Kinety 2 (Fig. 6A,C). Measurements were made on ciliates from a single *N. simplex* host because the ciliates from 2 other *N. simplex* hosts, which were confirmed genetically as *Pseudocollinia brintoni*, were not well stained. Nevertheless, a few individuals from these other 2 hosts showed identical characteristics.

Tomite morphology of *Pseudocollinia beringensis* comb. nov.

Protargol staining of ethanol-fixed *Collinia beringensis* (here proposed to be renamed *Pseudocollinia beringensis* comb. nov.) infecting *Thysanoessa inermis* and *T. raschi* euphausiids revealed protomite–tomite stages (Figs. 5D–F,H & 6D–F). The cells were 30 to 46 µm long and 16 to 27 µm wide (average 37.1 × 21.9 µm; n = 33). The cells possessed a long and wider macronucleus that stretched to almost the length of the cell (22 to 36 µm long by 5 to 14 µm wide, average 27.4 × 9.9 µm, n = 27) (Fig. 6E); a micronucleus was not observed. There were on average 19 kineties (16 to 20, n = 33) that extended from pole to pole, except for 4 kineties (Figs. 5D,E & 6D,F). Three oral kineties, numbered Kinety 1 (K1), Kinety n (Kn), and Kinety n–1, curve around the oral cavity opening and terminate (Figs. 5D,E & 6D,F). The fourth kinety, Kinety n–2, curves anteriorly and right above the oral cavity before terminating in a non-ciliated region anterior to the oral cavity (Figs. 5D,E & 6D,F). The oral cavity, which is about one-third of the cell's length from the anterior end, appears cone-shaped and lined on its left wall with a dense field of ciliated kinetosomes or basal bodies (Figs. 5D–F,H &

Table 4. Biometry of bacteria attached to the surface of the apostome ciliate *Pseudocollinia brintoni* gen. nov., sp. nov. infecting the subtropical euphausiid *Nyctiphanes simplex* along the west coast of Baja California, Mexico. All measurements were made using scanning electron microscopy images

Bacteria location	Diameter (µm)	Length (L, µm) Mean	SE	n	Width (W, µm) Mean	SE	n	L:W ratio	Observations
Orange krill	0.5	2.1	0.08	2	0.8	0	2	2.7	Dividing
Orange krill	0.4	1.8	0.14	7	0.7	0.1	7	2.8	Rod-shaped
Filament	0.2	1.4	0.17	7	0.5	0.01	7	3.0	Dividing
Filament	0.2	1.3	0.10	7	0.5	0	7	2.6	Rod-shaped

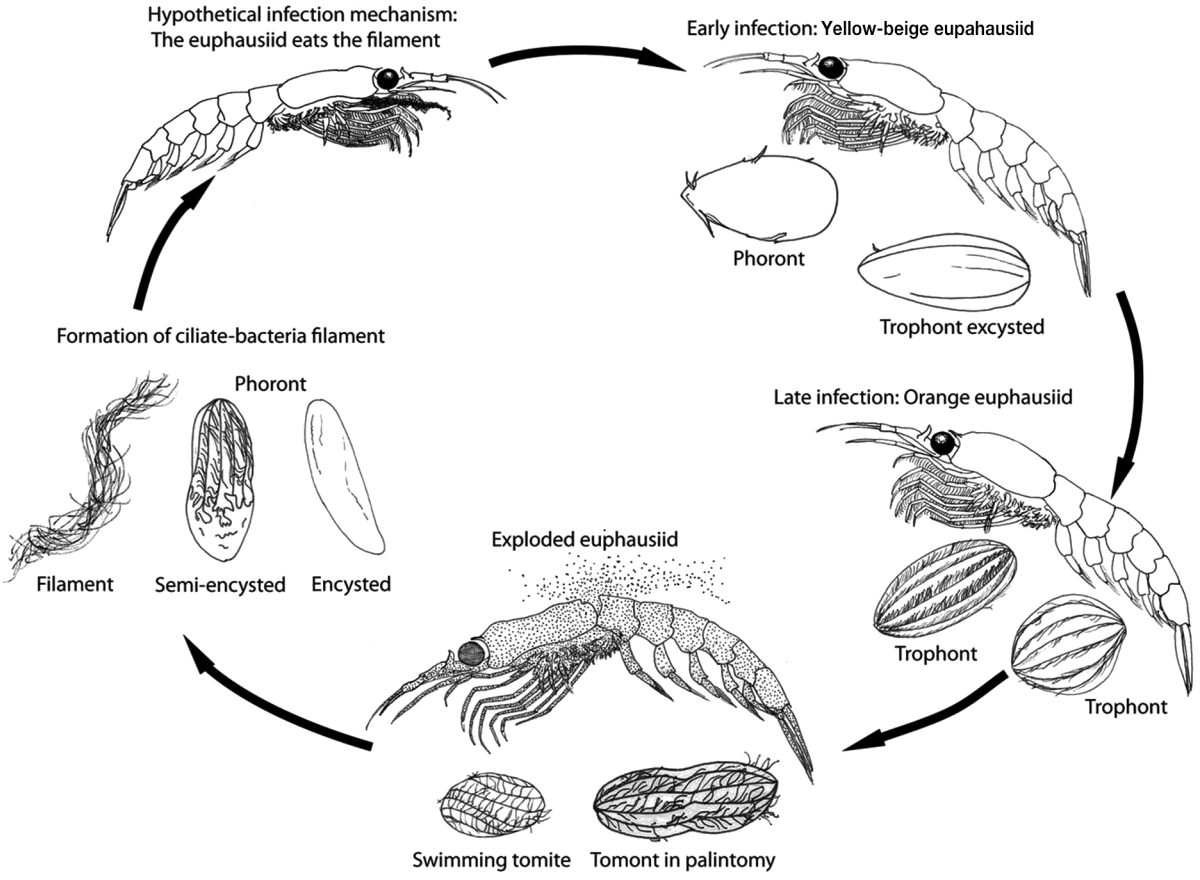

Fig. 4. Conceptual life cycle of the apostome ciliate *Pseudocollinia brintoni* gen. nov., sp. nov. infecting the subtropical sac-spawning euphausiid *Nyctiphanes simplex*. All life stages were observed and showed sequential development of the life-stages. The only hypothetical part of this life cycle is how the euphausiid becomes infected: we speculate that the host eats mucus-like filaments densely populated with the phoront stage

6D,F). Unlike *Pseudocollinia brintoni*, no kineties converge on each other at the anterior end (Fig. 6D,F). Measurements were made on ciliates from 1 host because the ciliates of 17 other hosts, which were confirmed genetically as *P. beringensis* comb. nov. (see genetic data for 2 of these host populations; other data will be presented in a separate publication), were either not well stained or represented different life cycle stages. One of these hosts had a few poorly stained trophont stages that measured 51 to 113 µm in length and 32 to 73 µm in width (average 83 × 58.1 µm; n = 17) with approximately 30 to 42 somatic kineties, although these were difficult to count accurately. Tomites from other hosts showed identical characteristics to those from the type host individual. Ciliates of *Collinia oregonensis* (here proposed to be renamed *Pseudocollinia oregonensis* comb. nov.) show similar morphological characteristics but an intermediate number of kineties (14 to 22 ciliate rows) (Fig. 5I).

Gene sequences of *Pseudocollinia* spp.

The complete SSU rRNA gene sequence of *Pseudocollinia brintoni* gen. nov., sp. nov. is 1730 nucleotides in length and has a GC content of 41%. Due to the different forward primers used, one of the isolates was only partially sequenced (i.e. 1379 nucleotides). For 2 of the isolates, the ITS1, 5.8S rRNA, ITS2, and partial large subunit (LSU) rRNA gene sequences were also obtained. The SSU rRNA gene sequences are identical for all 3 isolates, and the ITS1, 5.8S, and ITS2 gene regions of the 2 isolates are also identical. The complete SSU rRNA gene sequence of *Pseudocollinia beringensis* comb. nov. is 1746 nucleotides in length, also has a GC content of 41%, and is identical for the 2 isolates obtained. The ITS1, 5.8S rRNA, ITS2, and partial LSU rRNA gene sequences of the isolates of *P. beringensis* comb. nov. are identical. The divergence between the SSU rRNA gene sequences of the 2 species is 0.15%, while they show 4% divergence

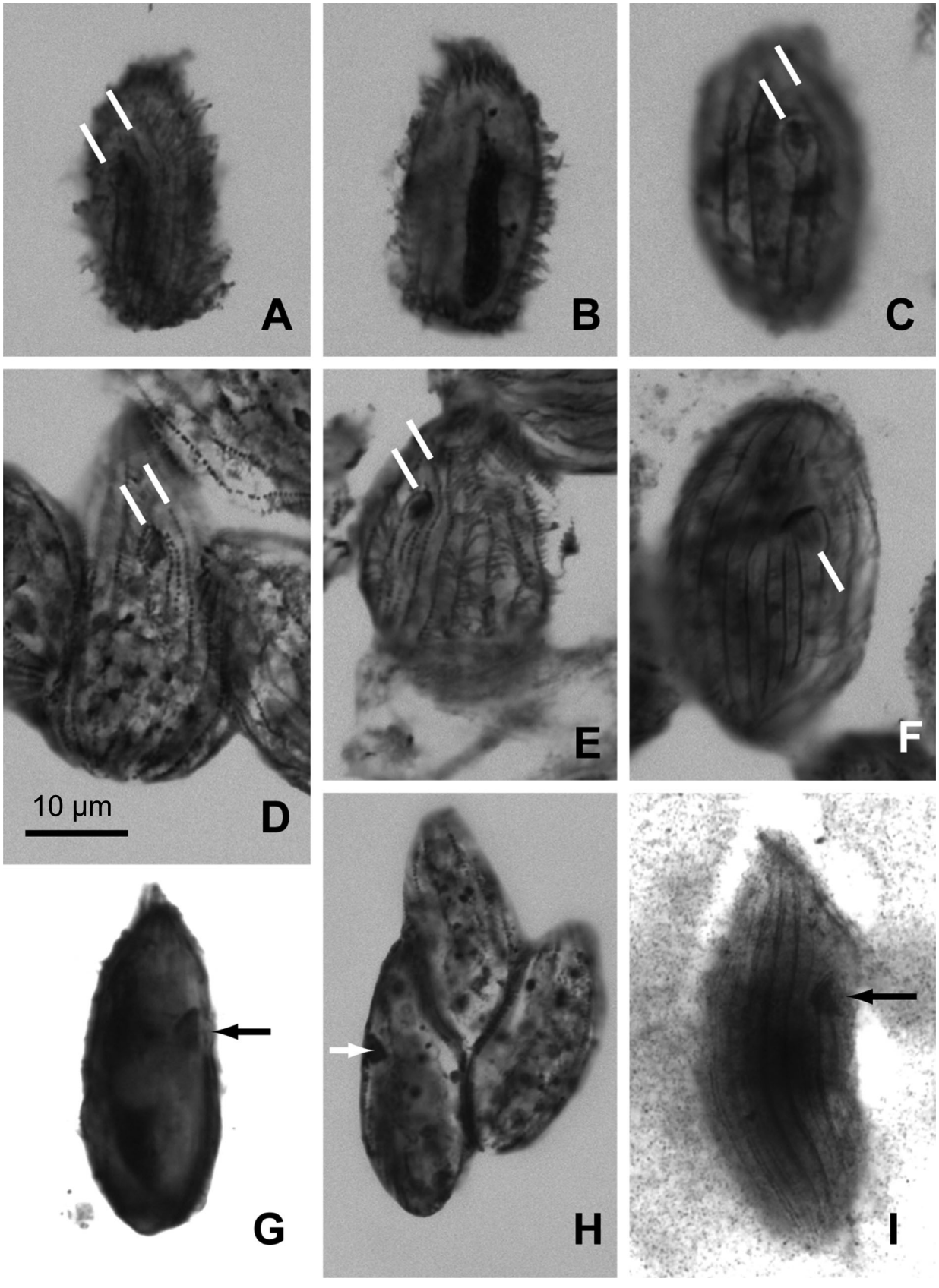
A
B
C
D
10 µm
E
F
G
H
I

Fig. 5. Protargol-stained protomite/tomite stages of *Pseudocollinia* species. (A–C,G) *Pseudocollinia brintoni* gen. nov., sp. nov. that infects *Nyctiphanes simplex*. (A) Ventral view of holotype showing dense ciliature with Kinety 1, the rightmost 'oral' kinety (left white bar) and Kinety n–2 (right white bar) indicated. Note that there are 2 oral kineties, Kinety 1 and Kinety n in this species (see Fig. 6C). (B) Macronucleus of the holotype. (C) Another individual with Kinety 1, the rightmost oral kinety (left white bar) and Kinety n–2 (right white bar) indicated and Kinety n in between. (G) Lateral view at sagittal plane showing cone-shaped oral cavity (arrow). (D–F,H) *Pseudocollinia beringensis* comb. nov. infecting *Thysanoessa inermis*. (D) Ventral view with Kinety 1, the rightmost oral kinety (left white bar) and Kinety n–3 (right white bar) indicated. This species has 3 oral kineties, Kinety 1, n and n–1 (see Fig. 6F). (E) Ventral view of another individual with Kinety 1, the rightmost oral kinety (left white bar), and Kinety n–3 (right white bar) indicated. (F) Ventral view with an indication that cilia may be extending from the oral cavity (white bar). (H) Lateral view at sagittal plane showing cone-shaped oral cavity (white arrow). (I) *Pseudocollinia oregonensis* comb. nov. infecting *Euphausia pacifica* showing a right lateral view of the cone-shaped oral cavity (arrow) of a specimen from the type slide. Scale bar = 10 µm in (D) applies to all photomicrographs

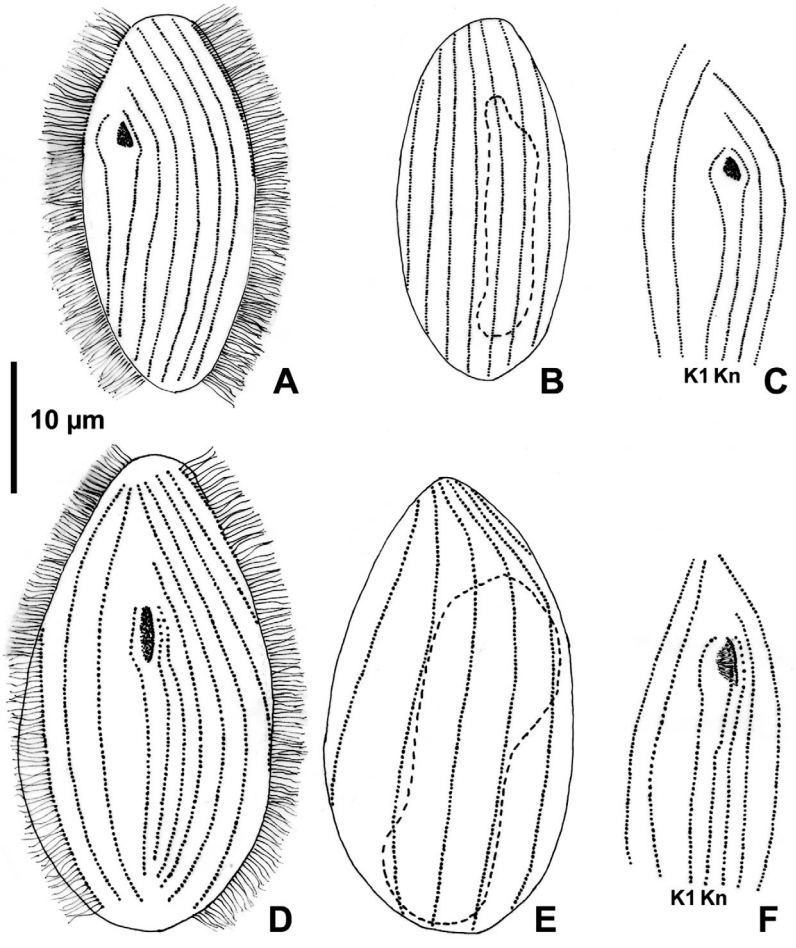

Fig. 6. Schematic drawings of *Pseudocollinia* species. (A–C) *Pseudocollinia brintoni* gen. nov., sp. nov. (A) Ventral view of holotype showing the dense ciliature and dense field of oral kinetosomes on the left side of the oral cavity. (B) Dorsal view of holotype showing elongated macronucleus (dashed line). (C) Ventral view of another individual showing the oral field surrounded by extensions of Kinety 1 (K1) and Kinety n (Kn). Note that Kinety n–2 terminates by abutting anteriorly onto Kinety 2. (D–E) *Pseudocollinia beringensis* comb. nov. (D) Ventral view showing the dense ciliature and the dense field of oral kinetosomes on the left side of the oral cavity. Note that all kineties extend to the anterior pole except the 4 adjacent to the oral region. (E) Dorsal view showing curvature of some kineties anteriorly and elongated macronucleus (dashed line). (F) Ventral view of another individual showing the oral field surrounded by extensions of K1, Kn, and Kinety n–1. Note that Kinety n–3 terminates at the anterior end (see D)

in their ITS and 5.8S gene regions. All of the sequences have been submitted to GenBank under the accession numbers HQ591468 to HQ591470, HQ591483, and HQ591484.

The mitochondrial *cox1* genes of *Pseudocollinia brintoni* sp. nov. and *Pseudocollinia beringensis* comb. nov. include inserts that are 440 and 473 nucleotides long, respectively. The inserts are typical for ciliates, but they are considerably longer than those of other ciliate species analyzed to date (Strüder-Kypke & Lynn 2010). The partial *cox1* gene sequences are 873 to 966 nucleotides in length due to the different primers used. As in all ciliates, the GC content is low, at 28.1 to 28.6%. The 3 isolates of *P. brintoni* were identical, while the 2 isolates of *P. beringensis* comb. nov. showed 0.59% divergence. Isolates of the 2 species were 47% divergent, mainly due to considerable differences in the extremely variable insert regions. The sequences have been submitted to GenBank under the accession numbers HQ591489 to HQ591491, HQ591496, and HQ591498. The assigned protein_id numbers are AER59686 to AER59688, AER59693, and AER59695.

The phylogenetic analyses place *Pseudocollinia* in a well-supported monophyletic clade with other apostome ciliates (Figs. 7 & 8). The 2 species are not well separated in the tree inferred from SSU rRNA gene sequences (Fig. 7). However, a distinct genetic distance of 0.47 separates them in the tree inferred from *cox1* gene sequences (Fig. 8).

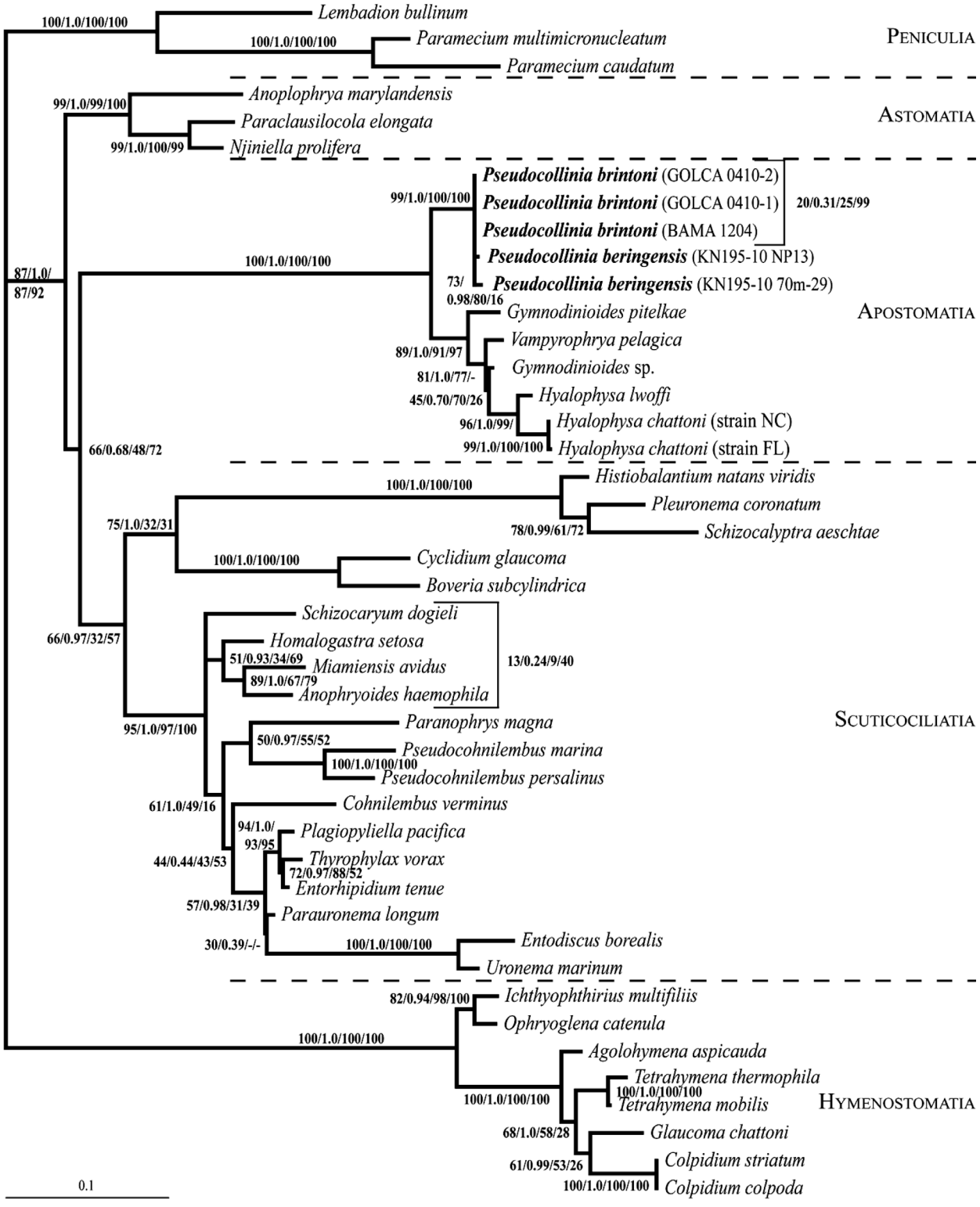

Fig. 7. Phylogenetic tree inferred from small subunit rRNA gene sequences, computed with PhyML (Guindon et al. 2005), based on a general-time-reversible (GTR) model with gamma distribution and an estimate of invariable sites. The first number at the nodes represents the bootstrap support for PhyML, the second number represents posterior probability values of the Bayesian analysis, and the third and fourth numbers represent bootstrap values for maximum parsimony and neighbor joining, respectively. New sequences for *Pseudocollinia* spp. in **bold** show unambiguously that they are apostome ciliates. Dashes indicate support values <20%. Scale bar = 10 substitutions per 100 nucleotides

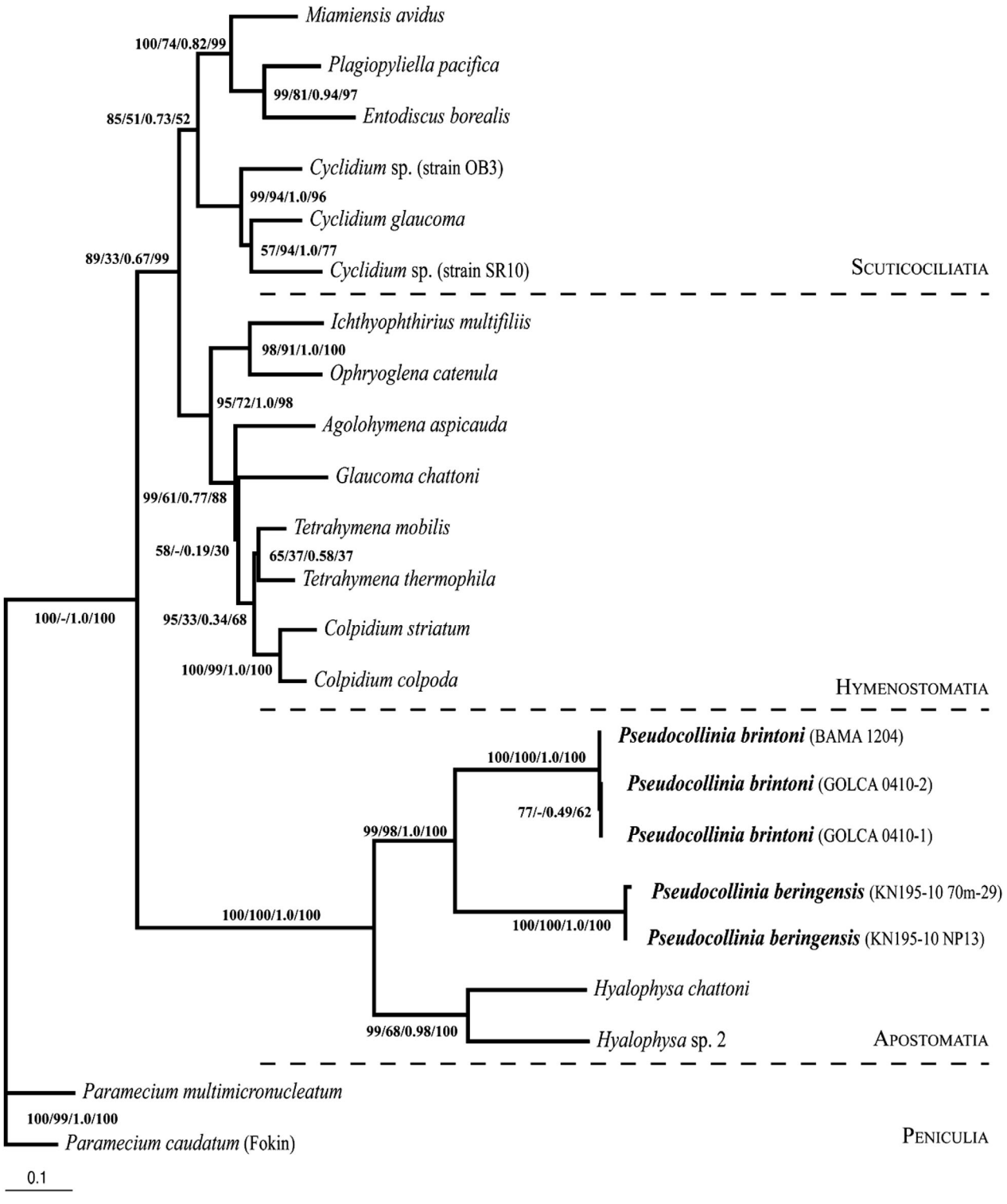

Fig. 8. Phylogenetic tree inferred from mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene sequences, computed with neighbor joining (Saitou & Nei 1987), based on the Kimura 2-parameter model (Kimura 1980). The first number at the nodes represents the bootstrap support for neighbor joining while the following numbers represent the support values of the PhyML, Bayesian inference, and maximum parsimony trees, respectively. New sequences for *Pseudocollinia* spp. in **bold** show unambiguously that they are apostome ciliates, and *P. brintoni* gen. nov., sp. nov. and *P. beringensis* comb. nov. are separated by 47% sequence divergence, which supports the hypothesis that they are different species. Dashes indicate support values <20%. Scale bar = 10 substitutions per 100 nucleotides

Pseudocollinia brintoni experimental infection

In March and July 2004, we incubated *Pseudocollinia brintoni* gen. nov., sp. nov. ciliates after they killed their euphausiid hosts. The ciliate and/or rod-shaped bacteria formed long filaments ~7 h after the rupture of the host at 16°C. The mechanism of formation of the filaments is unknown; perhaps they are formed from the host's digested material. To investigate the infection mechanism of *P. brintoni*, we exposed several healthy *Nyctiphanes simplex* females in individual jars in a 0.25 l bottle filled with ciliate-bacteria filaments. However, these potential hosts died without first showing symptoms of infection, appearing to have been semi-digested while still alive. Our interpretation is that the digestive enzymes (produced by ciliates or bacteria to feed on the euphausiid) become highly concentrated (not quantified), killing the euphausiids in the laboratory setting. In nature, the enzyme concentration is likely to be significantly lower, and the filament may be an evolutionary strategy to maintain high ciliate concentrations to produce a massive infection in a new euphausiid host.

During December 2004, we exposed healthy euphausiids (males, immature females, and gravid females) to ciliate-bacterial filaments in water previously filtered with GF/F filters. The euphausiids remained alive for a longer time, and any of the animals exposed to the filaments ate them as we expected. However, we also observed that when the euphausiid contacted the filaments, the filaments attached to the euphausiid body. The euphausiid eventually died in a very atypical way with the filaments attached to the krill's body, in contrast to the typical *Pseudocollinia* endoparasitoid infection in which the external body covering is apparently healthy. Identical experiments were made with females (n = 2) of the decapod *Processa pippinae*, some of which were ovigerous, and ovigerous female mysids (n = 3) of an unidentified species: they did not eat the filaments, and when we intentionally put them in physical contact with the filaments, they were completely unaffected, remaining alive after 8 d of incubation (until the oceanographic cruise ended).

DISCUSSION

Proposal of *Pseudocollinia* gen. nov. and morphological comparisons

Capriulo & Small (1986) first described apostome ciliates infecting the hemocoel of krill and assigned these ciliates to the genus *Collinia* because ciliates in this genus and family are characterized as parasitizing the hemocoel of crustaceans (Chatton & Lwoff 1935, Jankowski 2007, Lynn 2008). They included it in the genus *Collinia* using the following criteria: (1) the presence of a rosette structure and special kinetal *y* segment; (2) hemocoel-dwelling forms in crustaceans; (3) the unknown (at the time) complete life history of the forms; (4) major stages in which the mechanism of food ingestion is pinocytosis rather than phagocytosis; and (5) the characteristic of forms as apparently functional astomes with minimal apostome traits for the life history stages known then (Capriulo & Small 1986). Regrettably, there is no type slide for *Collinia beringensis*, the ciliate that Capriulo & Small (1986) described from *Thysanoessa inermis* in the Bering Sea (>55° N), so only the photomicrographs and drawings (Figs. 1 & 2 by Capriulo & Small 1986) can be used. A second species of krill-infecting ciliate, *Collinia oregonensis*, was also assigned to this genus by Gómez-Gutiérrez et al. (2006), who submitted 3 slides to the Smithsonian Institution (holotype USNM 1084004, paratype USNM 1084005, and non-type USNM 1084006). Although the specimens on these slides were not well stained, some do show a cone-shaped oral cavity (Fig. 5I), as reported above for *P. brintoni* and *P. beringensis* comb. nov.

Collinia species have been redescribed using modern cytological techniques (de Puytorac & Lom 1962, de Puytorac & Grain 1975). Based on this information and the earlier literature, Jankowski (2007) suggested that the genus must be split into 3 genera: (1) *Collinia* Cépède, 1910, whose type species *Collinia circulans* (Balbiani, 1885) Cépède, 1910, has large, astomatous trophonts with 10 spiral kineties, has a smaller tomite with short *x*, *y* and *z* kineties, and infects the freshwater isopod *Asellus aquaticus* Linnaeus, 1758; (2) *Paracollinia* Jankowski, 1980, whose type species *Paracollinia branchiarum* (Stein, 1852) Jankowski, 1980, has large astomatous trophonts with 55 to 60 straight kineties, has smaller flattened tomites with 9 kineties and short *x*, *y*, and *z* kineties, and infects the freshwater amphipod *Gammarus pulex* Linnaeus, 1758; and (3) *Metacollinia* Jankowski, 1980, whose type species *Metacollinia luciensis* (Poisson, 1921) Jankowski, 1980, has large astomatous trophonts with 65 to 70 slightly spiraling kineties with a non-ciliated dorsal stripe, has a smaller protomite-tomite with 9 kineties, short *x*, *y*, and *z* kineties, and a falciform field, and infects the marine amphipod *Orchestia littorea*, accepted as *Orchestia gammarellus* (Pallas, 1766), in Europe. Jankowski (2007) believed that *Collinia orchestiae* Summers & Kidder,

1936 redescribed by de Puytorac & Grain (1975) is likely conspecific with the type species for the genus *Metacollinia* (i.e. *M. luciensis*). This represents the diversity within the family Colliniidae, blood-parasites of crustaceans, when one excludes those infecting the blood of krill. Because none of the above has been reported to infect krill or to have a cone-shaped oral cavity covered on its left wall by a field of ciliated kinetosomes, we believe that the blood-parasites of krill represent a new genus. Because Capriulo & Small (1986) believed them close to *Collinia*, we suggest the name *Pseudocollinia* gen. nov.

Our results demonstrated that the ciliates found infecting krill in waters around Baja California and those infecting krill in the Bering Sea, the type locality for the first apostome ciliate species recorded to infect krill, share morphological and genetic similarities. Examination of the type slide of *Collinia oregonensis* (Gómez-Gutiérrez et al. 2006) convinces us that this krill-infecting species is also closely related to *P. brintoni* and *P. beringensis* comb. nov. Therefore, these 3 species should be placed in the new genus *Pseudocollinia* gen. nov.

The ciliates *Pseudocollinia brintoni* gen. nov. sp. nov. infecting *Nyctiphanes simplex* along the west coast of Baja California Sur (23°N) and Gulf of California have considerably fewer somatic kineties and smaller inter-stage variability in the number of kineties (12 to 18 kineties) than the other 2 apostome ciliates that infect euphausiids. *Pseudocollinia beringensis* comb. nov., which infects *Thysanoessa inermis* and *Thysanoessa raschi* in the Bering Sea, possesses 24 to 80 kineties with large inter-stage variability in the number of kineties (i.e. trophont = 80, tomont = 34, protomite I = 28, and protomite II = 24) (Capriulo & Small 1986). *Pseudocollinia oregonensis* infects 3 euphausiid species (*Euphausia pacifica*, *Thysanoessa spinifera*, and *Thysanoessa gregaria*) off the Oregon–Washington coast (42–46°N) and has 14 to 22 kineties and a moderate inter-stage variability in the number of kineties (i.e. trophont = 18–22; tomont = 16–20, protomite = 18–20, and tomite = 14–16) (Gómez-Gutiérrez et al. 2003, 2006). There is a clear latitudinal cline in the number of kineties of apostome ciliates along the Bering Sea and the California Current System, consistently decreasing from north to south.

Given the differences between these isolates, we hereby establish a new genus and species (i.e. *Pseudocollinia brintoni* gen. nov., sp. nov.) for the ciliates infecting the krill *Nyctiphanes simplex* in waters around Baja California. This species is characterized as follows: (1) this ciliate has a smaller number of kineties than the 2 previously described species that infect euphausiids (Capriulo & Small 1986, Gómez-Gutiérrez et al. 2006); (2) this ciliate has only 2 oral kineties surrounding the oral cavity, while *Pseudocollinia beringensis* comb. nov. has at least 3 oral kineties; (3) to date, this is the only apostome ciliate species that infects a euphausiid with a sac-spawning strategy; (4) this ciliate infects a krill species with a subtropical zoogeographic pattern, while other species infect euphausiids with arctic and subarctic distributions (Brinton 1962, 1981, Brinton et al. 2000); and (5) the genetic differences are sufficient to support a differentiation at the species level between *P. beringensis* comb. nov. and *P. brintoni*. Therefore, we propose to name this ciliate *Pseudocollinia brintoni* sp. nov. and designate it as the type species for the new genus. Given the morphological and genetic similarities of *Collinia beringensis* Capriulo & Small, 1986, with *P. brintoni* gen. nov. sp. nov., we hereby transfer the former species, making the new combination *Pseudocollinia beringensis* (Capriulo & Small, 1986) comb. nov. On the same grounds, we hereby transfer *C. oregonensis* Gómez-Gutiérrez et al., 2006, making the new combination *Pseudocollinia oregonensis* comb. nov. We are currently undertaking morphological and genetic research on *P. oregonensis* to provide details of its infraciliature and genetics to determine how closely it is related to *P. brintoni* and *P. beringensis* comb. nov. Given that our research on krill-infecting apostomes is relatively new, it is highly likely that these *Pseudocollinia* species will be found infecting other krill species and that more *Pseudocollinia* species may soon be discovered. Future SSU rRNA and *cox1* sequences for any other *Collinia* species infecting other crustaceans will confirm the proposed designation of *Pseudocollinia* as a new genus of parasitoid ciliates in the family Colliniidae.

Life cycle of endoparasitoid apostome ciliates

The endoparasitic infection of *Nyctiphanes simplex* by *Pseudocollinia brintoni* gen. nov., sp. nov. showed many similarities with the ciliate infections of the euphausiids along the Oregon coast (Gómez-Gutiérrez et al. 2003, 2006). In both regions, the trophont stage exclusively infects, in high numbers, adult female specimens. Larvae and juveniles seem to be inadequate hosts for this type of endoparasite, possibly because they have low biomass, low lipid content, and a relatively short developmental duration (Gómez-Gutiérrez et al. 2010c). The hypothesis that larvae and juveniles are infected with parasitoid cells but the

symptoms manifest once the host reaches a critical body-weight threshold, although unlikely, should be tested with experimental and observational work. Assuming that the life span of *N. simplex* is ~245 d (i.e. 17 mm total length, estimated using the Von Bertalanffy model $K = 0.010\ d^{-1}$ and asymptotic length = 18.6 mm; 1 yr old individuals should be considered exceptionally long-lived) (Lavaniegos 1992) and that the age of first maturity of this species is ~66 d (~9 mm total length) (Lavaniegos 1992, 1995, Gómez-Gutiérrez 1995), the period within the life span that is unlikely to be infected is short (<66 d; ~24% of the complete life span). This finding suggests that *P. brintoni* can infect *N. simplex* adults over more than two-thirds of their life span. The endoparasitoid phase of *P. brintoni* is on the order of days, considering that the time from healthy host appearance to host death is <33 h and the quick formation of filaments with encysted phoronts in an ectoparasitic phase is < 7 h. The relatively short infection-to-death period for these parasitoid ciliates (33 to 72 h) potentially enables them to infect and kill large numbers of euphausiids of the same swarm. It is obvious that either sex or any life stage of *N. simplex* could potentially ingest *P. brintoni*, but to date, only *N. simplex* females have been found to be infected. *N. simplex* produces ovigerous sacs every 7 to 15 d (Gómez-Gutiérrez & Robinson 2005, Gómez-Gutiérrez et al. 2010c). Thus, the endoparasitoid phase of *P. brintoni* should be shorter than the observed krill interbrood period.

The tomont stage of *Pseudocollinia brintoni* reproduces dramatically inside the host, producing thousands of transmission stages (i.e. tomites). This intense infection kills and ruptures the host, releasing the actively swimming ciliates. The exoparasitic phase of *Pseudocollinia* spp. is considerably less known (Gómez-Gutiérrez et al. 2006). Originally, it was thought that tomites of the genus *Pseudocollinia* infected the euphausiids by attaching to the external appendages because the phoront stages of other apostomes commonly attach to euphausiid appendages (Lindley 1978, Landers et al. 2006, 2007) and because it is the most common life-cycle mode of other well-studied apostome ectoparasites of crustaceans (Bradbury 1966, 1994, Bradbury & Clamp 1973, Bradbury & Goyal 1976). However, Landers et al. (2006, 2007) discovered that the commonly encysted phoronts attached to euphausiid appendages are actually epibiotic exuviotrophic ciliates of the genus *Gymnodinoides* that infest >70% of the krill population, rather than *Pseudocollinia* species.

Gómez-Gutiérrez et al. (2006) suggested that *Collinia* species (now *Pseudocollinia*) must be eaten to explain the large number of ciliates observed in early infection stages. However, our experimental laboratory efforts provide no conclusive evidence to show how euphausiids could filter such high densities of ciliates. Once *Pseudocollinia brintoni* escape from the infected euphausiids, they swim for a while and aggregate, forming filaments, several centimeters long, to which the tomite stages attach and encyst as phoront stages. Similar filament formation was also observed in infected euphausiids from the Oregon region, but at that time, the filaments were not observed using SEM techniques (Gómez-Gutiérrez et al. 2003, 2006). Rod-shaped bacteria also adhere in high densities on the body surfaces of *P. brintoni* and these filaments. Because bacteria are not always observed associated with the ciliates and because we have invariably observed filament formation, we strongly believe that the ciliates form the structural base of the filament for cell attachment, perhaps through exocytosis of extrusomes. We observed incompletely encysted ciliates (i.e. a tomite–phoront transition) on just-formed filaments, and we also observed completely encysted phoront stages from recently infected *Nyctiphanes simplex* (krill with a yellow-beige-colored carapace) and also ciliates incompletely excysted (i.e. phoront–trophont) inside the carapace. Thus, we assume that the more likely and parsimonious link between both events is that the euphausiids eat the filaments bearing high ciliate densities. Although we performed several shipboard laboratory incubations to test this hypothesis, we were unable to obtain direct evidence about this part of the life cycle of *P. brintoni*.

Can euphausiids actually eat drifting filaments in nature? *Euphausia pacifica* eats marine snow particles >0.5 mm diameter, even when other sources of food are provided (Dilling et al. 1998, Dilling & Brzezinski 2004). Thus, it is likely that euphausiids can ingest filaments with high numbers of encysted *Pseudocollinia* and bacteria in the field. *E. pacifica* is also able to fragment a single aggregate of marine snow into multiple, smaller aggregates by the fluid stress created around the appendages during swimming (Dilling & Alldredge 2000, Goldthwait et al. 2004). Thus, a single ciliate-bacterial filament may be fragmented and potentially infect multiple euphausiids within the swarm. This infection mechanism of feeding on ciliate-bacteria filaments could be morphologically analogous to feeding on marine snow, explaining the initially high density of trophont stages in the early stages of infection. We demonstrated that apostome endoparasitic ciliates are capable of forming filaments, and they may mimic marine snow. Gómez-Gutiérrez

et al. (2006) discussed why *Collinia* (now *Pseudocollinia*) infection on krill cannot be explained by infection mechanisms previously described for other apostome ciliates as follows. (1) Other apostome species perforate the cuticle of the gills with a secretion produced by the ciliate, thus dissolving a passage through the cuticle (Bradbury 1994, Bradbury & Goyal 1976); however, close examination of live infected krill did not reveal perforations in the cuticle. (2) Other apostome species penetrate the exoskeleton when it is soft after molting (Grimes & Bradbury 1992); however, none of the krill infected by *P. brintoni* had molted prior to infection. (3) Other apostome species infect their host after it has suffered a predatory attack by another metazoan predator (Grimes & Bradbury 1992); however, we can definitively state that infected krill observed by us showed no evidence of a previous predatory attack.

Crustaceans eat ciliates in nature as part of their diet (Zeldis et al. 2002), and some of these ciliates could be prey, endosymbionts, parasites, and/or parasitoids. Kucera (1992) suggested a passive transmission of virus-like particles from shrimp-to-shrimp when the hosts consume the exuviotrophic apostome ciliate *Hyalophysa chattoni*, which possesses the virus in their contractile vacuole. We were unable to infect live healthy euphausiids exposed to free-swimming and filament-attached ciliates. Induced infection of healthy *Acartia tonsa* by viruses has proved highly complex and has also been unsuccessful under laboratory conditions (Drake & Dobbs 2005). This suggests that infection by *Pseudocollinia* may require a combination of favorable conditions not completely met under our laboratory conditions or that the parasite/parasitoids have complex infection mechanisms and perhaps intermediate hosts not facilitated or explored under laboratory conditions.

It was notable that several healthy, mature females of *Nyctiphanes simplex*, introduced individually in the high-density ciliate seawater just after the original *N. simplex* exploded in a 0.25 l container, were killed and semi-digested a couple of hours after being exposed. This exogenous digestion of euphausiids must be a laboratory artifact because such high tomite ciliate concentrations are unlikely to occur in nature. However, this experiment suggests that ciliates can secrete high concentrations of enzymes that digest even live euphausiids. Experiments introducing healthy but starved females to previously filtered sea water with bacteria-ciliate filaments never showed a yellow-beige or orange cephalothorax (infection symptom), but the filament became attached to the surface of the euphausiids, and they eventually died. Local zooplanktonic female decapods and mysids undergoing similar experiments with filaments were not affected, suggesting highly specific infection with euphausiids. So far, no other euphausiid species from either coast of the Baja peninsula have been observed infected with *Pseudocollinia* (Gómez-Gutiérrez et al. 2010a).

Ecological impact of endoparasitoid ciliates

Because *Nyctiphanes simplex* accounts for >90% of the euphausiid standing stock from southern California (31° N) to Cape San Lucas (21° N) and the Gulf of California, it plays a significant role in the food web of this region (Brinton 1962, Brinton & Townsend 1980, Lavaniegos 1995, Gómez-Gutiérrez et al. 1995). Several top predators, such as the blue whale *Balaenoptera musculus*, fin whale *Balaenoptera physalus*, whale shark *Rhincodon typus*, manta rays *Mobula japonica* and *Mobula thurstoni*, and juveniles of the jumbo squid *Dosidicus gigas* feed almost exclusively on euphausiid swarms (Notarbartolo-di-Sciara 1988, Gendron 1992). This lethal ciliate may have a significant impact on the population structure, distribution, and secondary productivity of this euphausiid species, virtually competing with their better known predators. Infected euphausiids were recorded in ~9.5% (range 4 to 22%) of the sampled oceanographic stations. Those prevalence rates were similar to the proportion recorded along the Bering Sea and Oregon–California coast for other species of *Pseudocollinia* (Capriulo et al. 1991, Gómez-Gutiérrez et al. 2003, 2006).

To date, apostome ciliate infection has been discovered in 6 out of 86 euphausiid species currently described: *Thysanoessa inermis* (Capriulo & Small 1986, Capriulo et al. 1991) and *Thysanoessa raschi* in the Bering Sea (present study), *Euphausia pacifica*, *Thysanoessa spinifera*, and *Thysanoessa gregaria* in the Oregon-California coast (Gómez-Gutiérrez et al. 2003, 2006), and *Nyctiphanes simplex* in the northwest region of Mexico (present study). It is likely that other euphausiid species distributed around the world have similar ciliate parasitoid–host associations. In such serendipitous events, we recommend preserving the krill hosts in >80% ethanol, if possible with the cuticle perforated with a needle, so that the ciliates may be studied for both morphology and genetics. Formalin (4 to 5%) with sodium borate preservation is also adequate for ciliate morphological analysis. These infections may deserve more of our attention because parasitoids are thought to play

a significant regulatory role in the population dynamics of their hosts (Anderson & May 1978, Bradbury 1994, Wearing et al. 2004). The study of the metabolism of this ciliate (i.e. feeding and growth rates) and the detection of the exoparasitic phase of *Pseudocollinia* spp. *in situ* and the infection mechanism are the main scientific challenges for future studies.

Taxonomic summary

Subclass Apostomatia Chatton & Lwoff, 1928
Order Apostomatida Chatton & Lwoff, 1928
Family Colliniidae Cépède, 1910

Pseudocollinia Gómez-Gutiérrez, Strüder-Kypke, Lynn, Shaw, Aguilar-Méndez, López-Cortés, Martínez-Gómez, Robinson, gen. nov.

Diagnosis: Blood-infecting parasitoid ciliates of krill with a polymorphic life cycle, including trophont, tomont, protomite, tomite, and phoront stages. Ciliates contain a cone-shaped oral cavity in the anterior one-third of body with the left wall of the oral cavity covered by a field of ciliated kinetosomes and the oral cavity opening bordered by at least 2 oral kineties.

Type species: *Pseudocollinia brintoni* sp. nov.

Etymology: The genus name is given due to the presumed similarities to apostome ciliates of the genus *Collinia*, hence 'pseudo' from *pseudes* (Gr., false). Gender: female.

Pseudocollinia brintoni Gómez-Gutiérrez, Strüder-Kypke, Lynn, Shaw, Aguilar-Méndez, López-Cortés, Martínez-Gómez, Robinson, sp. nov.

Diagnosis: All life cycle stages possess 12 to 18 somatic kineties, with at least the anterior end of Kinety n–2 abutting on Kinety 2; the oral cavity is bordered by 2 oral kineties; the ciliate infects a sac-spawning euphausiid with subtropical and neritic distribution.

Type host: *Nyctiphanes simplex* Hansen, 1911

Type location: Northeast Pacific, southwest coast of Baja California peninsula, México, at the continental shelf off Magdalena Bay (24° 24.33′ N, 111° 57.13′ W) collected on 25 March 2004 (Table 3).

Endoparasitoid stage: In the hemocoel and inside the abdomen and appendages of the host krill.

Ectoparasitic stage: Forming clusters of phoronts on filaments, frequently associated with bacterial colonies.

Type material: A female *Nyctiphanes simplex* (11.2 mm total length) infected with trophont and tomont stages of *Pseudocollinia brintoni* gen. nov., sp. nov. located inside the cephalothorax and abdomen (paratype USNM 1154442) and a protargol-stained slide of *P. brintoni* gen. nov., sp. nov. cells in the tomite stage (holotype USNM 1156976) were deposited in the International Protozoan Type Slide Collection of the Department of Invertebrate Zoology of the National Museum of Natural History, Smithsonian Institution. The holotype (Figs. 5A,B & 6A,B) is circled in black on the underside of the slide.

Gene sequences: Gene sequences of *P. brintoni* gen. nov., sp. nov. were deposited in GenBank with the accession numbers HQ591468 to HQ591470 (SSU rRNA) and HQ591489 to HQ591491 (*cox1*).

Etymology: The species name is given in memory and honor of Professor Edward Brinton (1924–2010), Scripps Institution of Oceanography (San Diego, USA) who was an outstanding expert on the taxonomy, ecology, population biology, zoogeography, and evolution of Euphausiacea.

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LITERATURE CITED

Anderson RM, May RM (1978) Regulation and stability of host–parasite population interactions. I. Regulatory processes. J Anim Ecol 47:219–247

Balbiani EG (1885) Sur un infusoire cilie parasite du sang de l'Asselle aquatique (*Anoplophyra circulans*). Zool Suisse 2:277–305

Bourland WA, Strüder-Kypke MC (2010) *Agolohymena aspidocauda* nov. gen., nov. spec., a histophagous freshwater tetrahymenid ciliate in the family Deltopylidae (Ciliophora, Hymenostomatia), from Idaho (northwest U.S.A.): morphology, ontogenesis and molecular phylogeny. Eur J Protistol 46:221–242

Bradbury PC (1966) The life cycle and morphology of the apostome ciliate, *Hyalophysa chattoni* n. g., n. sp. J Protozool 13:209–225

Bradbury PC (1994) Parasitic protozoa of mollusks and crustacea. In: Kreier JP (ed) Parasitic protozoa, 2nd edn. Academic Press, San Diego, CA, p 139–263

Bradbury PC, Clamp JC (1973) *Hyalophysa lwoffi* sp. n. from the fresh-water shrimp *Palaemonetes paludosus* and revision of the genus *Hyalophysa.* J Protozool 20: 210–213

Bradbury PC, Goyal V (1976) The fine structure of a parasitic ciliate *Terebrospira* during ingestion of the exoskeleton of a shrimp *Palaemonetes.* Tissue Cell 8:573–582

Brinton E (1962) The distribution of Pacific euphausiids. Bull Scripps Inst Oceanogr Univ Calif 8:51–270

Brinton E (1981) Euphausiid distributions in the California Current during the warm winter-spring 1977-1978, in the context of a 1949-1966 time series. Calif Coop Ocean Fish Invest Rep 22:135–154

Brinton E, Townsend AW (1980) Euphausiids in the Gulf of California—the 1957 cruises. Calif Coop Ocean Fish Invest Rep 21:211–236

Brinton E, Ohman MD, Townsend AW, Knight MD, Bridgeman AL (2000) Euphausiids of the world ocean. World Biodiversity Database CD-ROM Series for [Microsoft] Windows version 1.0. Expert Center for Taxonomic Identification, Amsterdam

Capriulo GM, Small EB (1986) Discovery of an apostome ciliate (*Collinia beringensis* n. sp.) endoparasitic in the Bering Sea euphausiid *Thysanoessa inermis.* Dis Aquat Org 1:141–146

Capriulo GM, Pedone MJ, Small EB (1991) High apostome ciliate endoparasite infection rates found in the Bering Sea euphausiid *Thysanoessa inermis.* Mar Ecol Prog Ser 72:203–204

Chatton E, Lwoff A (1935) Les ciliés apostomes. 1. Aperçu historique et general étude monographique des genres et des espèces. Arch Zool Exp Gen 77:1–453

de Puytorac P, Grain J (1975) Étude de la tomitogenèse et de l'ultrastructure de *Collinia orchestiae*, cilié apostome sanguicole, endoparasite du crustacé *Orchestia gammarella* Pallas. Protistologica 11:61–74

de Puytorac P, Lom J (1962) La tomitogenèse des *Collinia* ciliés apostomes sanguicoles endoparasites des crustacés. Ann Parasitol Hum Comp 37:195–209

De Rijk P, De Wachter R (1993) DCSE, an interactive tool for sequence alignment and secondary structure research. Comput Appl Biosci 9:735–740

Dilling L, Alldredge AL (2000) Fragmentation of marine snow by swimming macrozooplankton: a new process impacting carbon cycling in the sea. Deep-Sea Res I 47: 1227–1245

Dilling L, Brzezinski MA (2004) Quantifying marine snow as a food choice for zooplankton using stable silicon isotope tracers. J Plankton Res 26:1105–1114

Dilling L, Wilson J, Steinberg D, Alldredge A (1998) Feeding by the euphausiid *Euphausia pacifica* and the copepod *Calanus pacificus* on marine snow. Mar Ecol Prog Ser 170:189–201

Drake LA, Dobbs FC (2005) Do viruses affect fecundity and survival of the copepod *Acartia tonsa* Dana? J Plankton Res 27:167–174

Elwood HJ, Olsen GJ, Sogin ML (1985) The small-subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata.* Mol Biol Evol 2:399–410

Felsenstein J (2005) PHYLIP (Phylogeny Interference Package) version 3.6a2. Distributed by the author, Department of Genome Sciences, University of Washington, Seattle, WA. Available at http://evolution.genetics.washington.edu/phylip.html

Gendron DL (1992) Population structure of daytime surface swarms of *Nyctiphanes simplex* (Crustacea: Euphausiacea) in the Gulf of California, Mexico. Mar Ecol Prog Ser 87:1–6

Goldthwait S, Yen J, Brown J, Alldredge A (2004) Quantification of marine snow fragmentation by swimming euphausiids. Limnol Oceanogr 49:940–952

Gómez JG (1995) Distribution patterns, abundance and population dynamics of the euphausiids *Nyctiphanes simplex* and *Euphausia eximia* off the west coast of Baja California, Mexico. Mar Ecol Prog Ser 119:63–76

Gómez-Gutiérrez J, Robinson CJ (2005) Embryonic, early larval development time, hatching mechanism and interbrood period of the sac-spawning euphausiid *Nyctiphanes simplex* Hansen. J Plankton Res 27:279–295

Gómez-Gutiérrez J, Palomares-García R, Gendron D (1995) Community structure of the euphausiid populations along the west coast of Baja California, Mexico, during the weak ENSO 1986-87. Mar Ecol Prog Ser 120:41–51

Gómez-Gutiérrez J, Peterson WT, De Robertis A, Brodeur RD (2003) Mass mortality of krill caused by parasitoid ciliates. Science 301:339

Gómez-Gutiérrez J, Peterson WT, Morado FJ (2006) Discovery of a ciliate parasitoid of euphausiids off Oregon, USA: *Collinia oregonensis* n. sp. (Apostomatida: Collinidae). Dis Aquat Org 71:33–49

Gómez-Gutiérrez J, Kawaguchi S, Nicol S (2009) Epibiotic suctorians and enigmatic ecto- and endoparasitoid dinoflagellates of euphausiid eggs (Euphausiacea) off Oregon, USA. J Plankton Res 31:777–786

Gómez-Gutiérrez J, Robinson CJ, Kawaguchi S, Nicol S (2010a) Parasite diversity of *Nyctiphanes simplex* and *Nematoscelis difficilis* (Crustacea: Euphausiacea) along the northwestern coast of Mexico. Dis Aquat Org 88: 249–266

Gómez-Gutiérrez J, Tremblay N, Martínez-Gómez S, Robinson CJ, Del Ángel-Rodríguez J, Rodríguez-Jaramillo C, Zavala-Hernández C (2010b) Biology of the subtropical sac-spawning euphausiid *Nyctiphanes simplex* in the northwestern seas of Mexico: vertical and horizontal distribution patterns and seasonal variability of brood size. Deep-Sea Res II 57:606–615

Gómez-Gutiérrez J, Rodríguez-Jaramillo C, Del Ángel-Rodríguez J, Robinson CJ, Zavala-Hernández C, Tremblay N, Martínez-Gómez S (2010c) Biology of the subtropical sac-spawning euphausiid *Nyctiphanes simplex* in the northwestern seas of Mexico: interbrood period, gonad development and lipid content. Deep-Sea Res II 57:616–630

Grimes BH, Bradbury PC (1992) The biology of *Vampyrophrya pelagica* (Chatton and Lwoff, 1930), a histophagous apostome ciliate associate with marine calanoid copepods. J Protozool 39:65–79

Guindon S, Lethiec F, Duroux P, Gascuel O (2005) PhyML Online — a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res 33:W557–W559

Jankowski AW (1980) Conspectus of a new system of the phylum Ciliophora. Trudy Zool Inst Akad Nauk SSSR 94: 103–121

Jankowski AW (2007) Phylum Ciliophora Doflein, 1901. In: Alimov AF (ed) Protista, Part 2, Handbook on Zoology. Russian Academy of Sciences, Zoological Institute, St. Petersburg, p 415–993 (in Russian with English summary)

Jerome CA, Simon EM, Lynn DH (1996) Description of *Tetrahymena empidokyrea* n. sp., a new species in the *Tetrahymena pyriformis* sibling species complex (Ciliophora, Oligohymenophorea), and an assessment of its phylogenetic position using small-subunit rRNA sequences. Can J Zool 74:1898–1906

Kimura M (1980) A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kucera FP (1992) Virus-like particles associated with the apostome ciliate *Hyalophysa chattoni*. Dis Aquat Org 12: 151–153

Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9:299–306

Lafferty KD, Kuris AM (2002) Trophic strategies, animal diversity and body size. Trends Ecol Evol 17:507–511

Landers SC, Gómez-Gutiérrez J, Peterson WT (2006) *Gymnodinioides pacifica*, n. sp., an exuviotrophic ciliated protozoan (Ciliophora, Apostomatida) from the euphausiids of the North Pacific. Eur J Protistol 42:97–106

Landers SC, Gómez-Gutiérrez J, Peterson WT (2007) The fine structure of the phoront of *Gymnodinioides pacifica*, a ciliated protozoan (Ciliophora, Apostomatida) from euphausiids of the Northeastern Pacific. Eur J Protistol 43:239–249

Lavaniegos EB (1992) Growth and larval development of *Nyctiphanes simplex* in laboratory conditions. Calif Coop Ocean Fish Invest Rep 33:162–171

Lavaniegos EB (1994) Dispersion and development patterns in larvae of *Nyctiphanes simplex* (Euphausiacea) in the upwelling region off Baja California. Mar Ecol Prog Ser 106:207–225

Lavaniegos EB (1995) Production of the euphausiid *Nyctiphanes simplex* in Vizcaíno Bay, western Baja California. J Crustac Biol 15:444–453

Lindley A (1978) Continuous plankton records: The occurrence of apostome ciliates (Protozoa) on Euphausiacea in the North Atlantic Ocean and North Sea. Mar Biol 46: 131–136

Lynn DH (2008) The ciliated protozoa: characterization, classification, and guide to the literature, 2nd printing [2010]. Springer, Dordrecht

Medlin L, Elwood HJ, Stickel S, Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71:491–499

Montagnes DJS, Lynn DH (1993) A quantitative protargol stain (QPS) for ciliates and other protists. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 229–240

Morado JF, Small EB (1995) Ciliate parasites and related diseases of Crustacea: a review. Rev Fish Sci 3:275–354

Notarbartolo-di-Sciara G (1988) Natural history of the rays of the genus *Mobula* in the Gulf of California. Fish Bull 86:45–66

Poisson R (1921) Sur un infusoire du genre *Balantidium*, parasite du tube digestif d'*Orchestia littorea*. CR Seances Soc Biol Paris 84:333–335

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818

Ronquist F, Huelsenbeck J (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Stein F (1852) Neue Beiträge zur Kenntniss der Entwicklungsgeschichte und des feineren Baues der Infusionsthiere. Z Wiss Zool 3:475–509

Strüder-Kypke MC, Lynn DH (2010) Comparative analysis of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene in ciliates (Alveolata, Ciliophora) and evaluation of its suitability as a biodiversity marker. Syst Biodivers 8:131–148

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony, 4.0b10 edn. Sinauer, Sunderland, MA

Wearing HJ, Sait SM, Cameron TC, Rohani P (2004) Stage-structured competition and the cyclic dynamics of host-parasitoid populations. J Anim Ecol 73:706–722

Zeldis J, James MR, Grieve J, Richards L (2002) Omnivory by copepods in the New Zealand subtropical frontal zone. J Plankton Res 24:9–23

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