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PREVALENCE AND PHYLOGENY OF PARASITIC DINOFLAGELLATES (GENUS *BLASTODINIUM*) INFECTING COPEPODS IN THE GULF OF CALIFORNIA

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ABSTRACT. Plankton samples collected from Bahía de La Paz, Baja California Sur, Mexico in June 2008 revealed infection of copepods by *Blastodinium* spp. Of eight copepod genera encountered in the samples, only one (*Paracalanus*) was parasitized by *Blastodinium*. Infection prevalence was low, 0.6 % to 2.0 %, with all parasitized individuals being females tentatively identified as *Paracalanus parvus*. All but one infected host examined during the study was parasitized by *Blastodinium crassum*, the sole exception being a *Paracalanus* cf. *parvus* infected by *Blastodinium contortum*. A phylogeny constructed using a dataset of ~1.3 Kb sequences of the small subunit ribosomal DNA gene of *B. contortum* and *B. crassum* from Bahía de La Paz and 36 other dinoflagellate sequences available in GenBank, including all available for *Blastodinium*, placed *Blastodinium* as a monophyletic clade deep within the Dinokaryota. Sequence divergence among *B. contortum* ex *Paracalanus* cf. *parvus* from Bahía de La Paz, *B. contortum* ex *P. parvus* from the Mediterranean Sea, *B. contortum* ex *Clausocalanus arcuicornis*, *B. crassum* ex *P*. cf. *parvus* from Bahía de La Paz, and *B. navicula* ex *Corycaeus giesbrechti* supported separation of the three parasites as distinct species.

Keywords: *Blastodinium*, copepod, dinoflagellate, parasite, molecular phylogeny

Prevalecencia y filogenia de dinoflagelados parásitos (género *Blastodinium*) que infectan copépodos en el Golfo de California

RESUMEN. Muestras de plancton recolectadas en junio de 2008 en la Bahía de La Paz, México, revelaron la infección de copépodos por *Blastodinium* spp. De ocho géneros de copépodos encontrados, solo *Paracalanus* estuvo parasitado por *Blastodinium*. El porcentaje de prevalecencia de infección fue bajo, de 0.6 % a 2.0 %. Todos los ejemplares parasitados fueron hembras, identificadas tentativamente como *Paracalanus parvus*. Los hospederos analizados durante este estudio, a excepción de uno, estuvieron parasitados por *Blastodinium crassum*, siendo la única excepción *Paracalanus* cf. *parvus*, el cual fue infectado por *Blastodinium contortum*. Se construyó la filogenia usando la base de datos de secuencias ~1.3 Kb de pequeñas subunidades de DNA ribosomal de los genes de *B. contortum* y *B. crassum* de La Bahía de La Paz, además de otras 36 secuencias disponibles en GenBank, incluyendo todo lo disponible de *Blastodinium* y colocando a *Blastodinium* como un grupo dentro de los Dinokaryota. La divergencia de las secuencias entre *B. contortum* ex *Paracalanus* cf. *parvus* de Bahía de La Paz, *B. contortum* ex *P. parvus* del Mar Mediterráneo, *B. contortum* ex *Clausocalanus arcuicornis*, *B. crassum* ex *P*. cf. *parvus* de Bahía de La Paz, y *B. navicula* ex *Corycaeus giesbrechti* respalda la separación de los tres parásitos como especies diferentes.

Palabras clave: *Blastodinium*, copépodo, dinoflagelado, parásito, filogenia molecular

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INTRODUCTION

The dinoflagellate genus *Blastodinium* contains 12 species and one subspecies, all of which inhabit the digestive tract of copepods (Shields, 1994). *Blastodinium* has long been assigned to the Blastodiniphyceae, a class of extracellular parasites whose members possess a dinokaryon only during part of their complex life cycles and reproduce by palisporogenesis or palintomy (Cachon & Cachon, 1987). The bi-flagellated dinospores of *Blastodinium* were originally reported to be athecate, gymnodinoid cells (Chatton, 1906; 1920; 1952). Recently, however, Skovgaard *et al.* (2007) demonstrated that the dinospores of *Blastodinium contortum* and *B. navicula* have thecal plates arranged in a Kofoidian series, with plate tabulation of the two *Blastodinium* species being characteristic of the Peridiniphycidae. He also used phylogenetic analysis of small subunit ribosomal gene sequences to place *Blastodinium* spp. as late-branching members of the Dinokaryota, thus drawing into question the validity of the class Blastodiniphyceae and the order Blastodiniales.

All but one species of *Blastodinium*, *B. hyalinum*, have plastids (Chatton, 1920) and appear to have a mixotrophic life-style, gaining part of their nutrition by photosynthesis and part by uptake of organic compounds from host digestive fluids (Pasternak *et al*., 1984). How these parasites gain entry into the host digestive system is uncertain, but infection is thought to occur through ingestion of dinospores (Chatton, 1920). Once inside the host, the parasite grows to produce a large (up to 700 µm long) trophont that divides to form a trophocyte and a gonocyte. The gonocyte undergoes rapid sequential divisions to produce non-flagellated spores, while the trophocyte continues to grow and divide, producing successive generations of gonocytes. Spores exit the host via the anus (Chatton, 1920), develop flagella, and swim away as dinospores, presumably in search of another host. Little is known about the biology of *Blastodinium* dinospores, although attempts to maintain them in culture as phototrophs have been unsuccessful (Skovgaard, 2005). Likewise, attempts to infect naive hosts using dinospores have failed (Skovgaard, 2005).

Infection by *Blastodinium* is not typically lethal, but does have a negative effect on host fitness. Reproductive castration of female copepods resulting from poor development or lack of gonads and oviducts has been reported by several investigators (Chatton, 1920; Jepps, 1937; Sewell, 1951), but does not appear to be a consistent feature of *Blastodinium* infections (Ianora *et al*., 1990). In the absence of food, infected hosts show reduced survival relative to uninfected copepods (Pasternak *et al*., 1984; Skovgaard, 2005), indicating some energetic costs due to parasitism, whether that be due to uptake of nutrients by the para- site, or inefficient digestion due to blockage of the host digestive tract (Shields, 1994). In rare instances, infection by *Blastodinium* may result in hemorrhoidal protrusion of the host intestine and gut (Skovgaard, 2004), a condition that results in death of the copepod.

While too few accounts are available to assess the biogeography of individual *Blastodinium* species, the genus appears to have a cosmopolitan distribution, with reports from the North Sea, the Skagerrak, the English Channel, the Mediterranean Sea, the Arabian Sea, Mutsu Bay, Japan, the Southeast Pacific Ocean, and the Weddell Sea (Apstein, 1911; Chatton, 1920; Lebour, 1925; Kofoid, 1931; Sewell, 1951; Vane, 1952; Pasternak *et al*., 1984; Øresland, 1991; Skovgaard, 2005). Infection prevalence for several *Blastodinum* species shows clear seasonal cycles with lowest occurrence in cooler months (Chatton, 1920; Ianora *et al*., 1990; Skovgaard & Saiz, 2006). Prevalence tends to be low even during warm months, typically having maximum values under 10%. Epizootic outbreaks with 20% - 60% of host showing *Blastodinium* infections, however, have been reported for some copepod species (Chatton, 1920, Cattley, 1948; Vane, 1952). Such outbreaks have the potential to influence host populations by reducing recruitment.

Here, we provide the first record of *Blastodinium* living in copepods of Gulf of California and report parasite prevalence for samples collected in Bahía de La Paz, México. We also use small subunit ribosomal DNA gene sequences of *B*. *contortum* and *B. crassum* infecting *Paracalanus* cf. *parvus* of the Gulf of

California to develop a phylogeny placing *Blastodinium* as a monophyletic clade within the Dinokaryota. We use “cf.” after the copepod genus to indicate tentative species identification.

MATERIALS AND METHODS

Stations and sampling protocol. Copepods were collected at three stations near La Paz, Baja California Sur, México during June 2008 (Fig. 1). Bahía de La Paz is the largest coastal water body on the peninsular side of the Gulf of California (Sea of Cortez). It has constant exchange of water with the Gulf of California via a northern and a southern mouth (Gómez-Valdés *et al*., 2003). The main northern mouth is wide and deep (up to 300 m), while the southern mouth is straight and shallow and associated with a shallow basin about 10 m in depth and a coastal lagoon, the Ensenada de La Paz, connected to Bahía de La Paz by a narrow inlet (1.2 km wide and 4 km long) having an average depth of 7 m. Station 1 (Sta 1) was located in the inlet at 24.16° N; 110.33° W, while station 2 (Sta 2: 24.21 N; 110.31 W) and station 3 (Sta 3: 24.23N; 110.34 W longitude) were located in the shallow basin of the southern most region of the Bahía de La Paz.

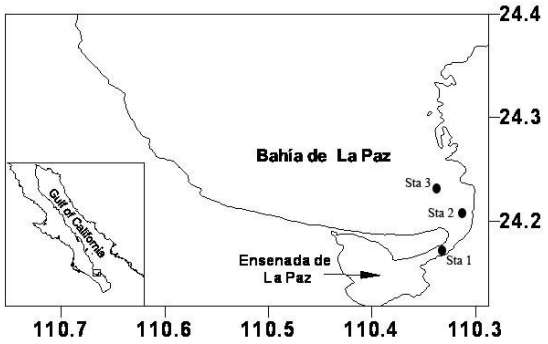


Figure 1. Location of stations sampled on June 9 - 11, 2008.

Near surface plankton tows were collected using a 50 cm diameter, 160 µm-mesh net. A portion of each net tow was immediately preserved with non-acid Lugol’s solution (final concentration 0.04% iodine and 0.06 % potassium iodide), with the remainder placed in a cooler for transportation to the lab. Preserved samples were stored at 4 °C, except during transport.

Parasite prevalence and morphology. Lugol’s preserved samples were used to enumerate copepods and to estimate the prevalence of *Blastodinium* spp*.* Prior to analysis, samples were destained for $\geq$ 1 hour by adding 5% sodium thiosulfate until the solution was no longer amber in color. Destained samples were placed in 55-mm diameter plastic Petri dishes and examined at 100 - 200 X magnification using an Olympus IX51 inverted microscope equipped with epifluorescence capabilities (WB cube for chlorophyll excitation). A Zeiss Axiocam interfaced with a PC running Axiovision software was used for image capture and morphological measurements. The first 500 copepods encountered for each sample were identified to genus and scored as infected or not infected. Copepods found to be infected by *Blastodinium* spp. were tentatively identified to species by consulting Razouls *et al*. (2005-2008). *Blastodinium* species were identified using the primary literature, in particular Chatton (1920).

For examination of parasite cytology, infected copepods and parasites dissected from the host intestine were destained as above, thoroughly washed with distilled water, and then placed in Bouin’s solution (Coats & Heinbokel, 1984) for $\geq$ 4 days. Specimens were then processed by the quantitative protargol staining method of Montagnes & Lynn (1993), examined using a Zeiss Axioscope, and photographed using Zeiss Axiovision as above. Data are reported in the text as mean $\pm$ standard error of the mean (SE).

DNA extraction, amplification, and sequencing. Unfixed material was examined within 6 hours using a Leica stereomicroscope to identify and isolate copepods infected by *Blastodinium* spp. Infected hosts were examined and photographed using a Zeiss Axiocam and Nikon CoolPix 5000 digital camera. Each specimen was then washed 6 times in 0.45 µm filtered seawater and placed into a sterile 1.5-ml microfuge tube containing 40 µL of non-acid Lugol’s solution. In some instances, infected copepods were dissected to remove the parasite, which was then washed and fixed

as above. Fixed specimens were stored at 4 °C, except during transport.

Specimens in microfuge tubes were washed 3 times with sterile distilled water (dH_20), placed into 50 µL dH_20 for individual parasites or 100 µL of dH_20 for whole copepods, and sonicated using a probe tipped sonicator (Heat Systems Ultrasonic, Inc. sonicator Model W-225R, Plain view, NY) set to a power level of 3 and a 30% duty cycle. The sonicator probe was immersed in the sample and three to five pulses of sonication were used over ~5 seconds. Between each sample, the probe was washed with 10% bleach solution, rinsed with dH_20, and wiped dry with a Kimwipe. Dummy samples without specimens were sonicated as above and used as negative controls.

To develop a *Blastodinium* specific amplification primer, ribosomal DNA regions were amplified from a few sonicated samples using Dino06F (Handy *et al*., 2008), a dinoflagellate specific primer, in combination with the general reverse primer 25R1 (Yamaguchi & Horiguchi, 2005). Polymerase chain reactions (PCRs) were run in 20-µL volumes, containing 500 mg/mL BSA (Sigma A2053), 50 mM Tris HCl (pH 8.3), 3 mM MgCl, 0.12 units of Promega Go-Taq DNA polymerase and 8 µL, 4 µL, or 2 µL of sample. Products from these reactions were precipitated using PEG (20% w/v polyethylene glycol, mw 8000 in 2.5 M NaCl solution), washed with 70% ethanol, briefly air dried, resuspended in 10 µL of dH_2O, and sequenced with the primers Dino06F, 25R1, and 25F (Yamaguchi & Horiguchi, 2005) using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, California) and an ABI 3730 sequencer. Data were edited to remove low quality bases, assembled using the program Sequencher 4.8 (Genecodes, Ann Arbor), and verified as *Blastodinium* by comparison with the GenBank database using BLAST (Altschulet *et al*., 1990). Ribosomal DNA sequences available in GenBank for *Blastodinium* and representative dinoflagellates were aligned using MacClade (Maddison & Maddison, 2002). This alignment was used to design a *Blastodinium* specific primer (BlastR) targeting the small subunit ribosomal DNA (SSU rDNA) gene. The nucleotide sequence for the amplification specific primer BlastR was AAACTGCCCTTGTTCCATTG.

For all subsequent analyses, sonicated samples were amplified using the *Blastodinium* specific primer (BlastR) in combination with the general eukaryotic primer EukA (Medlin *et al*., 1988). Resulting amplicons were sequenced using the primers EukA (Medlin *et al*., 1988), SR4, SR5, SR8, SR9 (Yamaguchi & Horiguchi, 2005), and EukB (Medlin *et al*., 1988) for complete double stranded coverage and compared to the GenBank database as above.

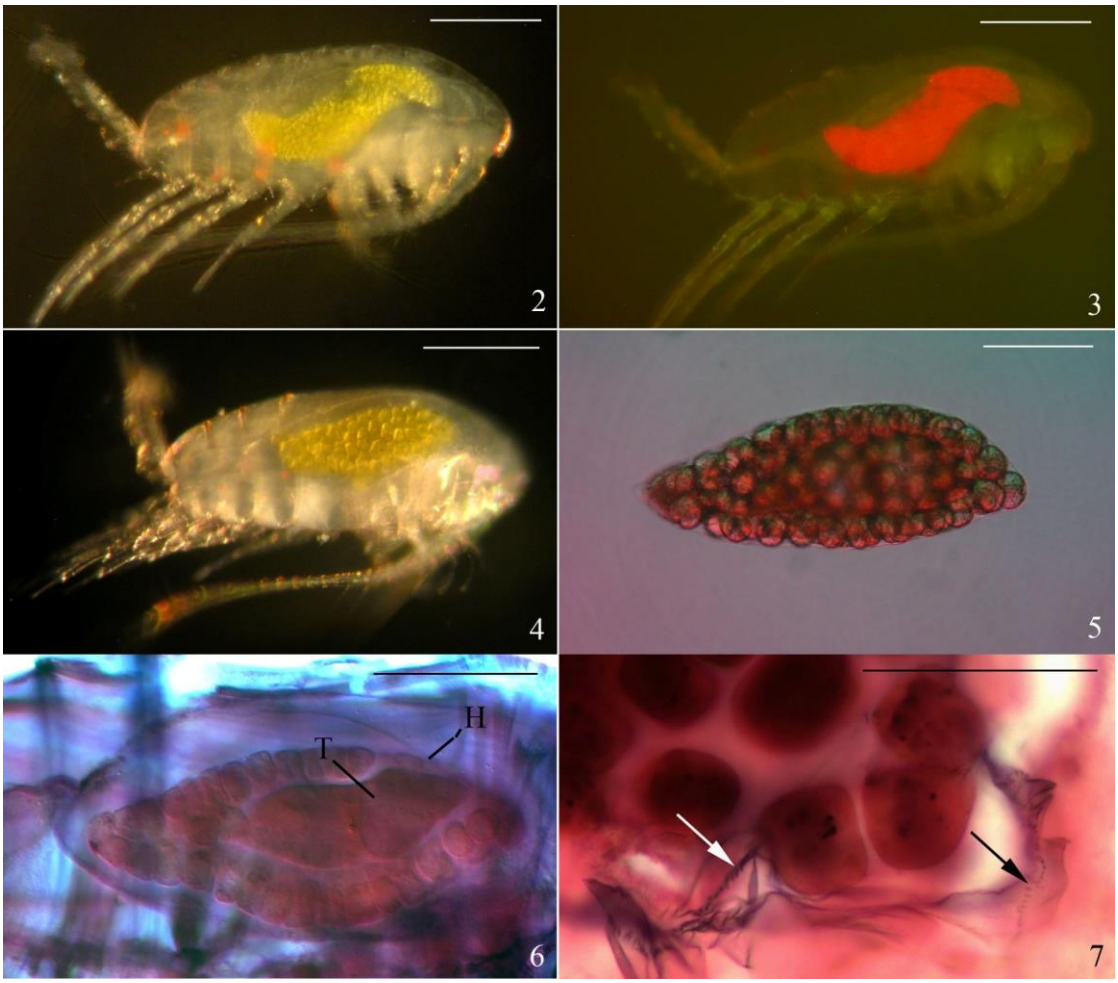
Phylogenetic analyses. Sequences available in GenBank for 36 dinoflagellates, plus a closely related alveolate as an outgroup (*Perkinsus* sp.), and two *Blastodinium* sequences from our samples were aligned using Muscle (Edgar, 2004), followed by hand editing using MacClade 4.08 (Maddison & Maddison, 2002); see Fig. 9 for names of species and GenBank accession numbers. The dataset contained ~1.3 Kb of sequence (partial 18S or SSU rDNA gene) for each entry.

Phylogenetic analyses were run using GARLI v. 0.951 (Zwickl, 2006) for maximum likelihood (ML), with PAUP* version 4b10 (Swofford, 2003) then used to visualize the trees. For the ML analyses, MrModeltest v. 2 (Nylander, 2004) was used to identify the GTR+I+Γ as the best-fit model. GARLI was run using the best-fit model and a random-starting tree. Runs were repeated using the previous ML tree until the maximum-likelihood value stabilized. Branch support was then assessed with 100 non-parametric bootstrap replicates using parameters from the best-fit model.

RESULTS

Parasite prevalence and morphology. *In vivo* examination of bulk net-tow samples collected June 9-11, 2008 revealed a single *Paracalanus* cf. *parvus* infected by *Blastodinium contortum* (Figs. 2 & 3) and numerous *Paracalanus* cf. *parvus* infected by *Blastodinium crassum* (Figs. 4 & 5).

Analysis of preserved samples showed *Paracalanus* to be the dominant copepod genus in most net tows (Table 1), forming 58% $\pm$



Figures 2-7. *Blastodinium* spp. infecting *Paracalanus* cf. *parvus.* Fig. 2. Host infected by *Blastodinium contortum*. Fig. 3. The same specimen as Fig. 2 viewed with epifluorescence microscopy to show chlorophyll autofluorescence of the parasite. Fig. 4. Host infected by *Blastodinium crassum*. Fig. 5. *Blastodinium crassum* removed from the host by dissection. Circular profiles within the parasite are daughter cells resulting from sporogenesis. Fig. 6. Protargol-stained host infected by *Blastodinium crassum* showing a single generation of spores surrounding the trophocyte (T) except at the hilum (H). Fig. 7. Protargol-stained *Blastodinium crassum* removed from the host. Note the rows of thin spines on cell surface (arrows). Scale bar = 200 µm in Figs. 2-4, 100 µm in Figs. 5-7, and 50 µm in Fig. 8.

9.3% of the community on average (Fig. 8). *Acartia* was the next most common genus, averaging 32% ± 10.7% of the community and forming a major fraction (60%) of the assemblage collected in the inlet connecting Bahía de La Paz to the Ensenada de La Paz (Sta 1). Five other genera and an unidentified member of the Family Pontellidae were also recorded, but formed a minor proportion of the copepod community (Table 1).

B. crassum occurred at low prevalence in *Paracalanus* from all samples, ranging from 0.6% to 2.0% and averaging 1.3 % ± 0.3% (n = 5). Infection prevalence showed no a clear pattern relative to station location or sampling data. All *Paracalanus* infected by *B. crassum* were females tentatively identified as *P. parvus*. Other copepod genera present in the samples were not infected.

The single specimen of *B. contortum* encountered *in vivo* (Figs. 2 & 3) had a sigmoid shape with a spiral twist to the body, was yellow-green, showed strong chlorophyll fluorescence, measured 357 µm in length x 100 µm maximum width, and appeared to have multiple generations of spores. Specimens of *B.*

Table 1. Number of individuals recorded for copepod taxa in samples of 500 specimens per date and station. Also indicated are percent of *Paracalanus* infected by *Blastodinium crassum*.

	9-June-08	10-June-08	11-June-08		
Copepod taxa	Sta 1	Sta 2	Sta 2	Sta 2	Sta3
Paracalanus	147	332	217	344	404
% infected	2.0	1.8	0.9	0.6	1.2
Acartia[1]	302	136	264	63	34
Centropages[1]	0	0	1	2	2
Corycaeus[1]	8	11	3	19	21
Euterpina[1]	15	4	4	48	6
Oithona[1]	28	15	2	21	30
Pontellidae[1]	0	2	0	0	0
Temora[1]	0	0	9	3	3

[1]No infected individuals.

crassum were broadly spindle-shaped, with a more pointed posterior end, as was particularly evident in parasites removed from the host by dissection (Fig. 5). *B. crassum* also showed yellow-green pigmentation (Fig. 4), had strong chlorophyll fluorescence, and possessed a single generation of spores. After destaining Lugol's preserved samples, *B. crassum* appeared brownish-green in color and exhibited slight chlorophyll fluorescence for several weeks when held at 4 °C in the dark. Specimens in the 2-cell stage measured 167 µm -194 µm in length by 43 µm - 70 µm in maximum width (n = 2), while those with spores ranged from 229 µm - 358 µm by 86 µm - 138 µm (mean 317 µm ± 17 µm by 113 µm ± 6 µm; n = 8). A hilum (region where spores do not encase the trophocyte) and a line of small spines spiraling over the cell surface (Figs. 6 & 7) were evident in protargol-stained specimens. The latter feature was not obvious when specimens were examined at intermediate magnification *in vivo* or following fixation, but was clearly evident in protargol-stained specimens.

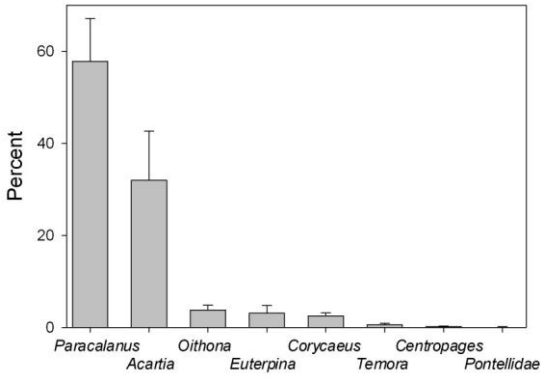


Figure 8. Proportional occurrence of copepods in samples collected June 9 - 11, 2008. Percents are means for five samples. Error bars indicating standard error of the mean.

Molecular analyses. SSU rDNA sequences obtained for *Blastodinium* species isolated from Bahía de La Paz were nested deep within the Dinokaryota and formed a monophyletic, albeit unsupported, clade with other *Blastodinium* sequences and an uncultured eukaryote sequence available in GenBank. The *B. contortum* sequence of *Paracalanus* cf. *parvus* from Bahía de La Paz clustered with *B. contortum* ex *Clausocalanus arcuicornis* and *B. contortum* ex *P. parvus*, both from the Mediterranean Sea. Divergence across the three *B. contortum* sequences was 0.07%, with the sequence for *B. contortum* ex *P. cf. parvus* from Bahía de La Paz being identical to that of *B. contortum* ex *Clausocalanus arcuicornis*. *Blastodinium crassum* ex *P.* cf. *parvus* from Bahía de La Paz grouped with uncultured eukaryote sequence SCM16C36 AY664982

from the Sargasso Sea, forming a clade sister to the three *B. contortum* sequences. Sequence divergence between *B. crassum* ex *P*. cf. *parvus* and the uncultured eukaryote was only 0.34%. *B. navicula* ex *Corycaeus giesbrechti* branched basally in the *Blastodinium* clade. The sequence for *B. crassum* ex *P.* cf. *parvus* differed from the three *B. contortum* sequences by 5.43% to 5.51 % and from that of *B. navicula* ex *Corycaeus giesbrechti* by 4.86%.

DISCUSSION

Eleven of the 13 morphotypes of *Blastodinium* were formally characterized by Chatton in a series of papers culminating in his 1920 monograph (Chatton 1906, 1908, 1911, 1912, 1920). In those works, Chatton progressively refined description of the species and their distinguishing characteristics, sometimes leaving margin of uncertainty about particular taxa. That uncertainty lead Sewell (1951) to suggest synonymy of *B. inornatum* with *B. crassum*, as he was unable to see the primary character used by Chatton to separate the two taxa, namely the presence of a spiraled row of spines over the surface of *B. crassum*, a feature lacking in *B. inornatum*. Sewell (1951) may not have detected the spines, as he worked exclusively with specimens that had been preserved for many years. We too were unable to see spines in living or fixed specimens examined at low to intermediate magnification, even when the parasite was removed from the host by dissection. The spines, however, were clearly visible in protargol-stained specimens and were distributed over the surface of the cell as described by Chatton (1920). Other features of our specimens, including size, shape, pigmentation, and presence of only one generation of spores were consistent with Chatton's descriptions of *B. crassum* (1908, 1920).

Sewell (1951) also believed that Chatton had mistakenly included multiple species under the name *Blastodinium contortum* and described two new species, *B. apsteini* and *B. chattoni.* Both of those species represented forms considered by Chatton (1920) under the name *B. contortum hyalinum* (Chatton, 1920), a subspecies now given species status as *B. hyalinum* (Shields, 1994). *B. hyalinum,* unlike *B. contortum,* lacks pigmentation, a trait that is very difficult to assess after years of preservation as in the material studied by Sewell. We have assigned our specimen to *B. contortum* because it conformed to the description given by Chatton, including the S-shaped profile, spirally twisted body, and yellow-green pigmentation. It also lacked the pronounced ventral protuberance characteristic of *B. apsteini*, as well as the untwisted body form of *B. chattoni* (Sewell, 1951). Further, the SSU rDNA gene sequence for our *B*. *contortum* was identical to that of *B. contortum* ex *Clausocalanus arcuicornis* (GenBank Assession DQ317537) and 99.9 % similar to that of *B. contortum* ex *Paracalanus parvus* (GenBank Assession DQ317536).

Approximately 50 species of copepods are known to host *Blastodiniuim*, the vast majority of which were recorded from warm waters of the Mediterranean Sea, 27 species, and the Arabian Sea, 26 species (Chatton, 1906, 1908, 1912, 1920, 1929; Sewell, 1951; Ianora *et al*., 1987, 1990; Skovgaard & Saiz, 2004; 2006; Skovgaard, 2005; Skovgaard *et al*., 2007). Only six host species are known for the northeast Atlantic Ocean (Apstein, 1911; Lebour, 1925; Jepps 1937; Cattley, 1948), two for the Pacific Ocean (Kofoid, 1931; Pasternak *et al*., 1984; Horiguchi *et al*., 2006), and one for the Antarctic (Øresland, 1991). The skewed distribution in host records clearly reflects uneven geographic sampling effort, but may also be influenced by differences in physiological tolerance of *Blastodinium* species that specialize on different host taxa. For example, *Blastodinium hyalinum* is the only species reported from colder waters of the northeast Atlantic and the North Sea, even though host taxa infected by other *Blastodinium* species inhabit those regions (Chatton, 1929). By contrast, 11 forms of *Blastodinium* (10 species and 1 subspecies) have been reported from the Mediterranean Sea (Chatton 1906, 1908, 1912, 1920, 1929; Ianora *et al*., 1987, 1990; Skovgaard & Saiz; 2004, 2006; Skovgaard, 2005; Skovgaard *et al*., 2007) and 10 species from the Arabian Sea (Sewell, 1951).

Infection of copepods by *Blastodinium* has previously been reported for the Pacific Ocean on three occasions. Kofoid (1931) and

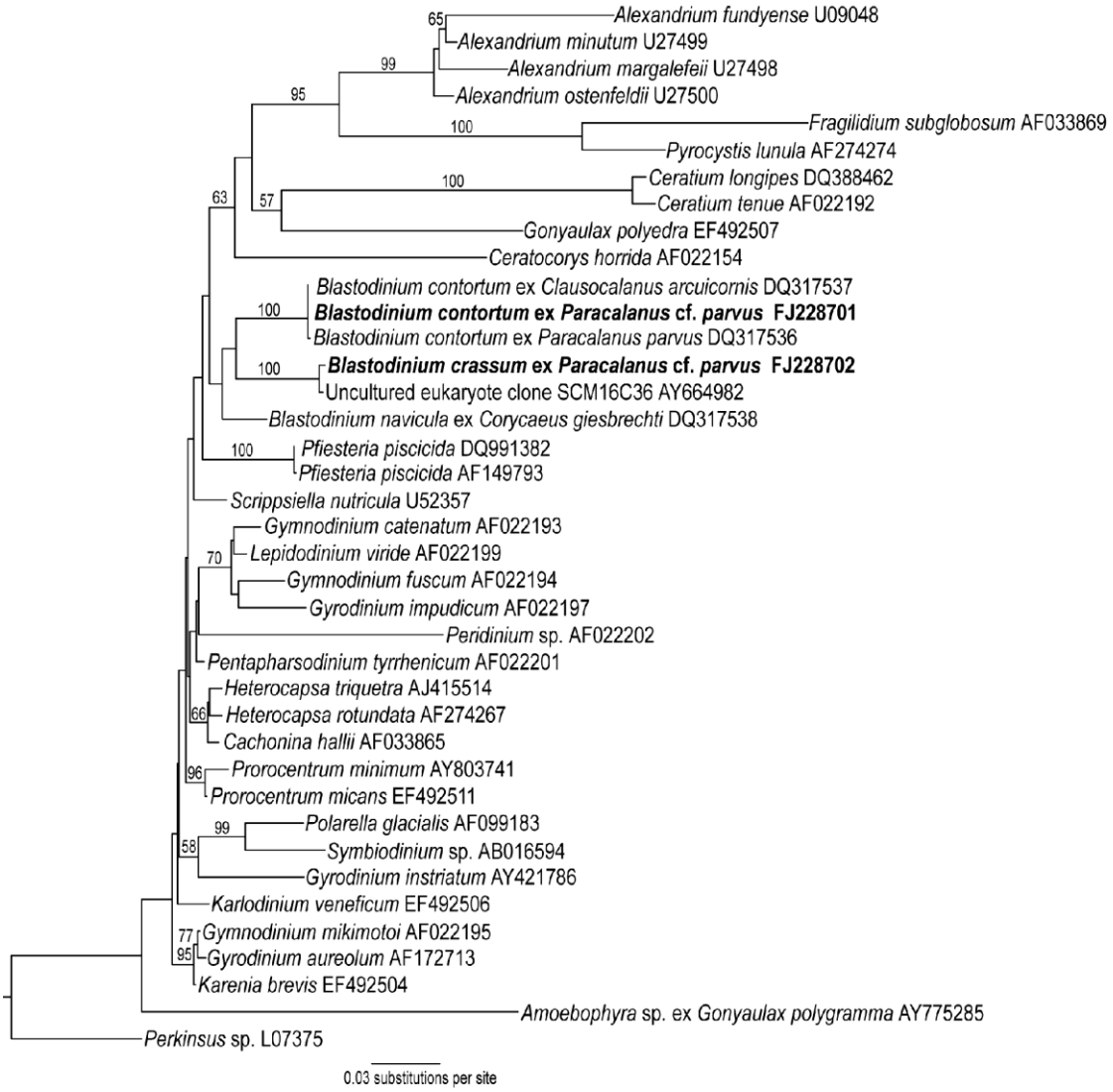


Figure 9. Maximum likelihood tree for 38 taxa of dinoflagellates, including two *Blastodinium* sequences from Bahia de La Paz. *Perkinsus* sp. served as the outgroup. Numbers at nodes are bootstrap values from 100 non-parametric replicates.

Horiguchi *et al*. (2006) found *B. crassum* in *Paracalanus parvus* collected in Japanese waters, while Pasternak *et al*. (1984) studied infection of *Eucalanus subtenuis* by an unidentified species of *Blastodinium* in the southeast Pacific. Our account thus represents the first record of *Blastodinium* in copepods of the Gulf of California and the first record of *B. contortum* from the Pacific Ocean. Given the apparent high diversity of *Blastodinium* species in subtropical waters, we expect further study of Bahía de La Paz would reveal additional host and parasite taxa. That only *Paracalanus* cf. *parvus* showed infection in our samples is not surprising, as most other copepod taxa formed a minor component of the community. *Acartia*, however, was as abundant as *Paracalanus* in two of our samples, but did not show infection. Interestingly, infection of *Acartia* by *Blastodinium* has only been observed on one occasion (Chatton, 1929), and in that case the host was *Acartia clausi*. The *Acartia* in our samples were mostly of the "*tonsa*-type" and may not have been suitable hosts.

Skovgaard *et al.* (2007) used phylogentic analysis of the SSU rDNA gene to place *Blastodinium* within the Dinokaryota, well removed from the group I and II alveolates that include parasitic dinoflagellates with which the genus had been previously associated. Our tree also placed *Blastodinium* within the Dinokaryota, but unlike Skovgaard's anlaysis showed the genus as a monophyletic group, with *Blastodinium contortum*, *Blastodinium crassum*, and *Blastodinium navicula* sorting on separate branches. That topology, along with the high sequence divergence across *Blastodinium* morphotypes ($\leq$ 95.1 % similarity) and low sequence divergence within morphotype ($\geq$ 99.9 % similarity for *B. contortum* from two different host species and two geographic locations), supports separation of *B. contortum*, *B. crassum*, and *B. navicula* at the species level. The large number of base differences among the three parasite morphotypes also suggests that *Blastodinium* is likely a more diverse genus than currently recognized.

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REFERENCES

Altschulet, S.F., W. Gish, W. Miller, E.W. Myers & D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.*, 215: 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

Apstein, C. 1911. Paraditen von *Calanus finmarchicus*. *Wiss. Meeresunters.*, Kiel, Abt., Kiel, N F, 13: 205-223.

Cattley, J.G. 1948. Sex reversal in copepods. *Nature*, 161: 937. https://doi.org/10.1038/161937a0

Cachon, J. & M. Cachon. 1987. Parasitic dinoflagellates, 571-610. *En*: Taylor, F. J. R. (Ed.). *The Biology of Dinoflagellates*, Blackwell Sci. Publ., Oxford.

Chatton, É. 1906. Les Blastodinides, ordre nouveau de Dinoflagellatés parasites. *C. R. Acad. Sci.*, *Paris*, 143: 981-983.

Chatton, É. 1908. Note préliminaire sur trois formes nouvelles du genere *Blastodinium* Chatton. *Bull. Soc. Zool.* France, 33: 134-137.

Chatton, É. 1911. Sur divers parasites de Copépodes pélagiques observés par M. Apstein. *C. R. Acad. Sci., Paris*, 153: 474-476.

Chatton, É. 1912. Diagnoses préliminaires de péridiniens parasites nouveaux. *Bull. Soc. Zool.* France, 37: 85-93.

Chatton, É. 1920. Les Peridiniens parasites: morphologie, reproduction, ethologie. *Arch. Zool. Exp. Gen.*, 59: l-475.

Chatton, É. 1929. Différenciation et isolement géographique d'une espèce (*Blastodinium hyalinum* Ch.) par substitution partielle des conditions ecologiques (parasitisme hétérotrophe) aux conditions cosmiques (autotrophisme) de son existence. *Archiv. Zool. Exp. Gén.*, 68: 1-7.

Chatton, É. 1952. Classe des Dinoflagellés ou Péridiniens, 309-390. *En*: Grassé, P. P. (Ed.). *Traité de Zoologie*, Masson et Cie, Paris.

Coats, D.W. & J.F. Heinbokel. 1982. A study of reproduction and other life cycle phenomena in planktonic protists using an acridine orange fluorescence technique. *Mar. Biol.*, 67: 71-79. https://doi.org/10.1007/BF00397096

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32: 1792-1797. https://doi.org/10.1093/nar/gkh340

Gómez-Valdés, J., J.A. Delgado & J.A. Dwora. 2003. Overtides, compound tides, and tidal-residual current in Ensenada de la Paz lagoon, Baja California Sur, México. *Geof. Inter.*, 42(4):623-634. https://doi.org/10.22201/igeof.00167169p.2003.42.4.316

Handy, S.M., E. Demir, D.A. Hutchins, K.J Portune, E. B. Whereat, C. E. Hare, J. M. Rose, M. Warner, M. Farestad, S. C. Cary & K.J. Coyne. 2008. Using quantitative real-time PCR to study competition and community dynamics among Delaware Inland Bays harmful algae in field and laboratory studies. *Harmful Algae*, 7: 599-613. https://doi.org/10.1016/j.hal.2007.12.018

Horiguchi, T. A. Harada & S. Otsuka 2006. Taxonomic studies on parasitic dinoflagellates in Japan. *Bull. Plank. Soc. Jap.*, 53: 21-29.

Ianora, A., M.G. Mazzocchi & B.S. di Carlo. 1987. Impact of parasitism and intersexuality on Mediterranean populations of *Paracalanus parvus* (Copepoda: Calanoida). *Dis*. *Aquat*. *Org*., 3: 29-36. https://doi.org/10.3354/dao003029

Ianora, A., B.S. di Carlo & M.G. Mazzocchi. 1990. Histomorphological changes in the reproductive condition of parasitized marine planktonic copepods. *J*. *Plank*. *Res*., 12: 249-258. https://doi.org/10.1093/plankt/12.2.249

Jepps, M.W. 1937. On the protozoan parasites of *Calanus finmarchicus* in the Clyde Sea Area. *Quart. Journal. Micr. Sci*., 19: 589-658. https://doi.org/10.1242/jcs.s2-79.316.589

Kofoid, C.A. 1931. Report on the biological survey of Mutsu Bay. 18. Protozoan fauna of Mustu Bay. Subclass Dinoflagellata; Tribe Gymnodinoidae, *Fac*. *Sci*. *Rep*. *Tohoku Imp*. *Univ*. *4th Ser*., *Biology*, 6: l-43.

Lebour, M.V. 1925. *The Dinoflagellates of Northern Seas.* The Mayflower Press, Williams Brendon & Son, Ltd., Plymouth, UK, 250 pp.

Maddison, W.P. & D.R. Maddison. 2002. MacClade: analysis of phylogeny and character evolution. Sinauer, Sunderland, Mass.

Medlin, L., H. J. Elwood, S. Stickel & M. L. Sogen, M.L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71, 491-499. https://doi.org/10.1016/0378-1119(88)90066-2

Montagnes, D.J.S. & D.H. Lynn. 1993. A quantitative protargol stain (QPS) for ciliates and other protists, 229-240, *En*: Kemp, P. F., B. F. Sherr, E. B. Sherr & J. J. Cole, (Eds.). *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton. https://doi.org/10.1201/9780203752746-28

Nylander, J. A.A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Øresland, V. 1991. Feeding of the carnivorous copepod *Euchaeta antarctica* in Antarctic waters. *Mar*. *Ecol*. *Prog*. *Ser*., 78: 41-47. https://doi.org/10.3354/meps078041

Pasternak, A.F., Y.G. Arashkevich & Y.S. Sorokin. 1984. The role of the parasitic algal genus *Blastodinium* in the ecology of planktic copepods. *Oceanology*, 24: 748-751.

Razouls C., F. de Bovée, J. Kouwenberg & N. Desreumaux. 2005-2008. Diversity and geographic distribution of marine planktonic copepods. http://copepodes.obs-banyuls.fr/en.

Sewell, R.B.S. 1951. The epibionts and parasites of the planktonic copepoda of the Arabian Sea. John Murray Exp., *Sci*. *Rep*. *Br*. *Mus*., (N.H.), 9: 255-394.

Shields, J.D. 1994. The parasitic dinoflagellates of marine crustaceans, *Ann. Rev. Fish Dis*., 4: 241-271. https://doi.org/10.1016/0959-8030(94)90031-0

Skovgaard, A. 2004. Tumour-like anomalies on copepods may be wounds from parasites. *J. Plank. Res.*, 26: 1129-1131. https://doi.org/10.1093/plankt/fbh100

Skovgaard, A. 2005. Infection with the dinoflagellate parasite *Blastodinium* spp. in two Mediterranean copepods. *Aq. Microb. Ecol.*, 38: 93-101. https://doi.org/10.3354/ame038093

Skovgaard, A. & E. Saiz. 2004. Pequeños parásitos del mar. *Investigación y Ciencia*, 329:38-39.

Skovgaard, A. & E. Saiz. 2006. Seasonal occurrence and role of protistan parasites in coastal marine zooplankton. *Mar. Ecol. Prog. Ser.*, 327: 37-49. https://doi.org/10.3354/meps327037

Skovgaard, A., R. Massana & E. Saiz. 2007. Parasitic species of the genus Blastodinium (Blastodiniphyceae) are peridinioid dinoflagellates. *J. Phycol*., 43: 553-560. https://doi.org/10.1111/j.1529-8817.2007.00338.x

Swofford, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, Massachusetts, USA.

Yamaguchi, A., & T. Horiguchi. 2005. Molecular phylogenetic study of the heterotrophic dinoflagellate genus *Protoperidinium* (Dinophyceae) inferred from small subunit rRNA gene sequences. *Phycol*. *Res*. 53: 30-42. https://doi.org/10.1111/j.1440-183.2005.00370.x

Vane, F.R. 1952. The distribution of *Blastodinium hyalinum* in the North Sea. *Challenger Soc*., 3: 23-24.

Zwickl, D.J. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.* Ph.D. Dissertation, The University of Texas at Austin.