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NOTE

Emerging infectious disease in sea stars: castrating ciliate parasites in *Patiria miniata*

J. Sunday*, L. Raeburn, M. W. Hart

Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada

ABSTRACT: *Orchitophrya stellarum* is a holotrich ciliate that facultatively parasitizes and castrates male asteriid sea stars. We discovered a morphologically similar ciliate in testes of an asterinid sea star, the northeastern Pacific bat star *Patiria miniata* (Brandt, 1835). This parasite may represent a threat to Canadian populations of this iconic sea star. Confirmation that the parasite is *O. stellarum* would indicate a considerable host range expansion, and suggest that *O. stellarum* is a generalist sea star pathogen.

KEY WORDS: Scuticociliate · *Asterias amurensis* · Biocontrol · Sperm

INTRODUCTION

The spread of infectious pathogens may be enhanced by direct human dispersal or through indirect facilitation such as climate change (Dobson & Foufopoulos 2001). Such emergent diseases have important implications for natural and managed ecosystems (Krkosek et al. 2007). When emergent diseases interact with invasive host species, pathogens may limit the spread of these hosts or merely use them as a reservoir for propagation into native host populations (e.g. Beard & O'Neill 2005, Garner et al. 2006).

The holotrich ciliate *Orchitophrya stellarum* Cépède, 1907 (order Scuticociliata) is a facultative pathogen of male sea stars (Bouland & Jangoux 1988, Clareboudt & Bouland 1994). These ciliates seasonally invade developing testes, consume the sperm, and ravage the germinal epithelium. Heavily infected males are functionally castrated, and high castration rates can result in effectively unisexual populations of females (Leighton et al. 1991, Byrne et al. 1997). Known hosts are mainly Japanese and north Atlantic species of *Asterias* Linnaeus, 1758 (family Asteriidae). Spread of *O. stellarum* to other habitats and host species could have significant consequences for ecologically important sea stars. In North America, recently described pathogenic infections of asteriid hosts (Leighton et al. 1991, Stickle et al. 2001, 2007a,b), including the keystone predator *Pisaster ochraceus* Brandt, 1835, have been attributed to inadvertent anthropogenic introduction of *O. stellarum*; some *P. ochraceus* populations in Canada have been devastated. Deliberate introduction of *O. stellarum* into Tasmania has been proposed as a biocontrol measure to eradicate invasive populations of *A. amurensis* Lütken, 1871 (Goggin & Bouland 1997), but the desirability of such an effort depends in large part on the potential effect of *O. stellarum* on native Tasmanian sea stars (Byrne 1996, Byrne et al. 1997), especially endangered and endemic species such as *Parvulastra vivipara* (family Asterinidae) that represent much of the asteroid diversity of Australia (Byrne 2006).

Here we report the first occurrence of a ciliate resembling *Orchitophrya stellarum* in an asterinid host species, the bat star *Patiria miniata*. Infection of *P. miniata* represents a significant phylogenetic extension of the host range of such parasites from asteriids (order Forcipulatida) to asterinids (order Valvatida). The generalist nature of host use by *O. stellarum*-like

*Email: sunday@sfu.ca

parasites strongly argues against their deliberate introduction into other faunas for biocontrol of invasive sea stars.

MATERIALS AND METHODS

As part of a continuing study of the reproduction and population structure of *Patiria miniata*, we routinely collect and spawn adults from 2 intertidal locations at Dixon Island and Grappler Inlet near the Bamfield Marine Sciences Centre (48° 50′ N, 125° 8′ W) on Vancouver Island, British Columbia, Canada. In June 2007, we observed several males that responded to a hormonal spawning inducer by producing seminal fluid that had a slightly darker color than the usual milky white appearance of sea star sperm. On examination, these spawns contained abundant small ciliates mixed with sperm. We confirmed these observations with 2 subsequent collections. In August 2007, we searched for ciliates on the external body wall of individuals by gently pipeting from the aboral surface around the gonopores, and then examining this material for ciliates that resembled those found in gonads. We then dissected the gonads to search for ciliates in thin smears of the gonadal tissue. We cultured ciliates collected from gonads at 12°C in 50 ml plastic Falcon tubes containing seawater with pieces of testis or aliquots of sperm from *P. miniata*. We photographed individual ciliates in thin wet mounts using bright field or differential interference contrast (DIC) microscopy. For DIC, ciliates were fixed in 95% ethanol and immediately photographed. In February 2008, we collected 18 adults from Dixon Island and injected them with 1 to 3 ml of 100 µM 1-methyladenine (1-MA), which induces maturation of oocytes (in females) and spawning (in both sexes, Strathmann 1987). We estimated the prevalence of infection by light microscopy of spawned material in wet mounts.

RESULTS

In August 2007, 50% of 12 males sampled from Grappler Inlet had ciliates resembling *Orchitophrya stellarum* on the external body around the gonopores. Ciliates were also identifiable in gonadal smears in 1 of these samples. In samples from Dixon Island (August 2007), 50% of 14 males and 66% of 9 females had *O. stellarum*-like ciliates from the aboral body wall. Four of the males sampled from Dixon Island (28%) had ciliate infection in gonadal smears. In a later collection from Dixon Island (February 2008), 8 males spawned in response to 1-MA injection. Of these, 6 (75%) had abundant ciliates in the spawn.

Pathological testes of infected males were small, dark yellow-brown in color, and yielded few or no sperm in comparison to healthy testes that were creamy white, rounded, and spontaneously released abundant swimming sperm (Fig. 1). This gross pathology was similar to previous descriptions of infected asteriid hosts (Bouland & Jangoux 1988, Byrne et al. 1997). In most cases, single testes of infected males included both pathological and healthy tissue, as in Fig. 1. In our 2007 samples, there was some gross pathology in 75% of males from Grappler Inlet and 50% of males from Dixon Island; in our 2008 sample from Dixon Island, 1 of 8 males (12%) had gross testis pathology.

Ciliates parasitizing male *Patiria miniata* varied in form (e.g. Stickle et al. 2007a), but generally resembled *Orchitophrya stellarum* described from asteriid sea star hosts, viz. prolate spheroidal cells approximately 45 µm long and 25 µm wide with cilia organized in longitudinal rows or kineties and with a prominent vacuole at one end (Fig. 2). A long caudal cilium was visible in some specimens. Ciliates swam actively among spawned sperm (Appendix 1, available as supplementary material at: www.int-res.com/articles/suppl/d081p173_video/). In some specimens, we could clearly distinguish a buccal cavity with specialized ciliation. Such forms had a more pronounced flattened aspect (nearer to disk-shaped than prolate), and seemed to be more common among ciliates collected from the exterior body wall than from the testes. We

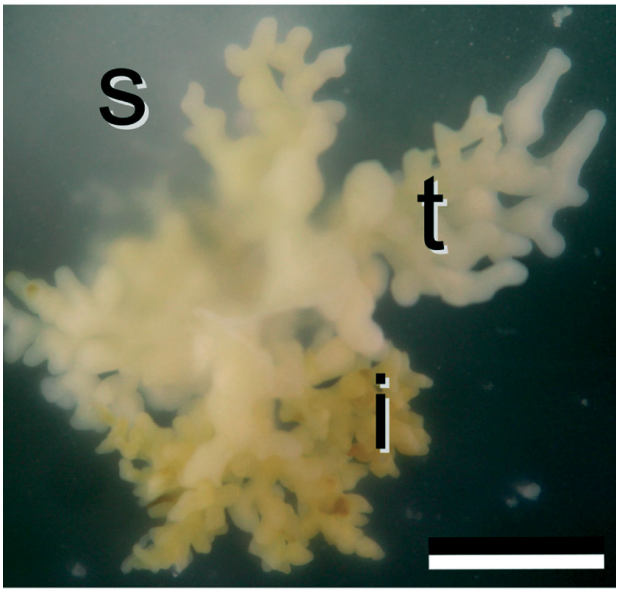

Fig. 1. *Patiria miniata* testis dissected from a male infected by ciliates (photographed in seawater). Healthy gonad tubules (t) are creamy white, and some have spawned a cloud of sperm (s). Infected tubules (i) are darker, yellow-brown, firm, and smaller in diameter. Scale bar: 500 µm

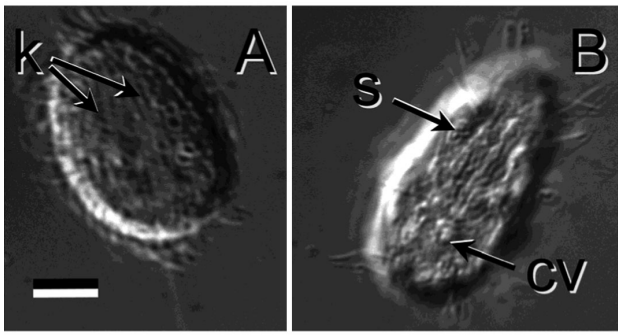

Fig. 2. *Orchitophyra* cf. *stellarun*. Differential interference contrast images of ciliate parasites from testes, with focal plane (A) at the surface and (B) through the centre of cells. Brightness and contrast in both cells were increased (approximately +40%) from the originals in Adobe Photoshop™. Morphological features similar to *Orchitophrya stellarum* include prolate shape, dimensions, tracks of cilia organized into kineties (k), ingested sea star sperm (s) in small phagosomes, and prominent contractile vacuole (cv). Scale bar = 10 µm

occasionally observed single ciliates capturing sperm and sweeping them toward the buccal cavity. We often observed sperm within small vacuoles in the ciliates or adhered to the outside of the ciliates (Fig. 2B).

DISCUSSION

The discovery of ciliates resembling *Orchitophrya stellarum* in the asterinid *Patiria miniata* greatly extends the known phylogenetic range of hosts for this pathogen. Although phylogenetic relationships among sea star orders are not fully resolved (Knott & Wray 2000), forcipulate sea stars (including ancestors of living asteriids) probably diverged from lineages that would give rise to valvatids (including *Patiria* and other asterinids) in the Jurassic (Blake 1987). This deep divergence of host lineages suggests that *O. stellarum*-like ciliates are much more generalist pathogens than previously realised.

Our identification of the *Patiria miniata* parasite as *Orchitophrya stellarum* is tentative because morphology appears to be highly variable within species (Goggin & Bouland 1997, Stickle et al. 2007a, E. Kozloff pers. comm.). Ultrastructural and genetic characterization of the *P. miniata* pathogen is desirable. Ribosomal DNA sequence markers (Goggin & Murphy 2000), mitochondrial DNA (Chantangsi 2007), and RAPD markers (Chen et al. 2000) might be used to determine whether the pathogens of asteriids and asterinids are one generalist parasite or several host-specific species or strains.

Emergent *Orchitophrya stellarum* infections have devastated some *Pisaster ochraceus* populations (Leighton et al. 1991), and might do so in *P. miniata* as well. We found many examples of gross testis pathology. More extensive surveys are needed to determine whether these infections are chronic, cause significant long-term pathology, and result in skewed sex ratios in *Patiria miniata* populations. Injection of 1-MA is an effective and non-invasive method for rapidly surveying sea star host populations for ciliate infections (Stickle et al. 2007a).

Byrne (1996) and Byrne et al. (1997) argued against the potential use of *Orchitophrya stellarum* as a biocontrol agent against invasive *Asterias amurensis* populations due to the non-specific nature of *O. stellarum* infections among asteriids. Our discovery of ciliates resembling *O. stellarum* in an asterinid host reinforces this argument. Use of such a generalist pathogen for biocontrol is more likely to threaten than to protect endemic or endangered asterinid (and other) sea star species.

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LITERATURE CITED

Beard KH, O'Neill EM (2005) Infection of an invasive frog *Eleutherodactylus coqui* by the chytrid fungus *Batrachochytrium dendrobatidis* in Hawaii. Biol Conserv 126: 591–595

Blake DB (1987) A classification and phylogeny of post-Paleozoic sea stars (Asteroidea: Echinodermata). J Nat Hist 21: 481–528

Bouland C, Jangoux M (1988) Infestation of *Asterias rubens* (Echinodermata) by the ciliate *Orchitophrya stellarum*: effect on gonads and host reaction. Dis Aquat Org 5: 239–242

Byrne M (1996) Starfish wanted, dead or alive. New Sci 2052:53

Byrne M (2006) Life history diversity and evolution in the Asterinidae. Integr Comp Biol 46:243–254

Byrne M, Cerra A, Nishigaki T, Hoshi M (1997) Infestation of the testes of the Japanese sea star *Asterias amurensis* by the ciliate *Orchitophrya stellarum*: a caution against the use of this ciliate for biological control. Dis Aquat Org 28: 235–239

Chantangsi C (2007) Barcoding protists: a trailblazing study on a ciliate genus of closely related species, *Tetrahymena*. J Eukaryot Microbiol 54:36S–37S

Chen Z, Weibo S, Warren A (2000) Studies on six *Euplotes* spp. (Ciliophora: Hypotrichida) using RAPD fingerprinting including a comparison with morphometric analyses. Acta Protozool 39:209–216

Claereboudt MR, Bouland C (1994) The effect of parasitic castration by a ciliate on a population of *Asterias vulgaris*. J Invertebr Pathol 63:172–177

Dobson A, Foufopoulos J (2001) Emerging infectious pathogens of wildlife. Philos Trans R Soc Lond Ser B Biol Sci 356:1001–1012

Garner TWJ, Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, Fisher MC (2006) The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana.* Biol Lett 2:455–459

Goggin CL, Bouland C (1997) The ciliate *Orchitophrya* cf. *stellarum* and other parasites and commensals of the northern Pacific seastar *Asterias amurensis* from Japan. Int J Parasitol 27:1415–1418

Goggin CL, Murphy NE (2000) Conservation of sequence in the internal transcribed spacers and 5.8S ribosomal RNA among geographically separated isolates of parasitic scuticociliates (Ciliophora, Orchitophryidae). Dis Aquat Org 40:79–83

Knott KE, Wray GA (2000) Controversy and consensus in asteroid systematics: new insights to ordinal and familial relationships. Am Zool 40:382–392

Krkosek M, Ford JS, Morton A, Lele S, Myers RA, Lewis MA (2007) Declining wild salmon populations in relation to parasites from farm salmon. Science 318:1772–1775

Leighton BJ, Boom JDG, Bouland C, Hartwick EB, Smith MJ (1991) Castration and mortality in *Pisaster ochraceus* parasitized by *Orchitophrya stellarum* (Ciliophora). Dis Aquat Org 10:71–73

Stickle WB, Weidner EH, Kozloff EN (2001) Parasitism of *Leptasterias* spp. (Echinodermata: Asteroidea) by the ciliated protozoan *Orchitophrya stellarum* (Scuticociliata). Invertebr Biol 120:88–95

Stickle WB, Kozloff EN, Henk MC (2007a) The ciliate *Orchitophrya stellarum* viewed as a facultative parasite of asteroid sea stars. Cah Biol Mar 48:9–16

Stickle WB, Kozloff EN, Story S (2007b) Physiology of the ciliate *Orchitophrya stellarum* and its experimental infection of *Leptasterias* sp. Can J Zool 85:201–206

Strathmann MF (1987) Reproduction and development of marine invertebrates of the northern Pacific coast. University of Washington Press, Seattle, WA

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