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NOTE

First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia

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ABSTRACT: The monogenean *Neobenedenia melleni* (MacCallum, 1927) Yamaguti 1963 is a well-known and virulent pathogen in culture conditions recorded from the skin of many teleost fish species worldwide. Until now, *N. melleni* has not been reported from wild or cultured fish in Australian waters. This study documents a recent outbreak of *N. melleni* that occurred on *Lates calcarifer* (barramundi) cultivated in sea cages in Hinchinbrook Channel between Hinchinbrook Island and mainland Queensland, Australia, which resulted in the loss of 200 000 fish (50 tonnes). The origin of this outbreak is unclear because *N. melleni* has not been recorded from any wild host species in Australia and strict quarantine regulations exclude the possibility of its introduction on imported fish. We propose that *N. melleni* occurs naturally on wild populations of some teleost species in Australian waters and that the few surveys of wild fish conducted along the east coast have failed to report this species. The possibility that uncharacteristically low water temperatures led to the outbreak is discussed.

KEY WORDS: Monogenea · Capsalidae · *Neobenedenia melleni* · Sea cage aquaculture · *Lates calcarifer* · Ectoparasitic disease · Australia

The capsalid monogenean *Neobenedenia melleni* (MacCallum, 1927) Yamaguti 1963 is an infamous pathogen of fish for several reasons. *N. melleni* is recognised as a lethal pathogen of captive marine teleosts, whether held in aquaria (e.g., MacCallum 1927, Jahn & Kuhn 1932, Thoney & Hargis 1991) or in sea cages for aquaculture (e.g., Kaneko et al. 1988, Mueller et al. 1992). Furthermore, unlike most monogenean parasites, *N. melleni* is notorious for its lack of host-specificity. Whittington & Horton (1996) noted records from more than 100 captive and wild teleost species in more than 30 families from 5 orders. Bullard et al. (2000) list 27 teleost species from 18 genera in 14 families from 3 orders as wild hosts of *N. melleni*. The shape and size of the body and haptoral sclerites and the shape of the testes are important taxonomic characters that vary considerably in *N. melleni* (see Whittington & Horton 1996), which has led to significant problems in defining the taxon. Taxonomic difficulties remain because Ogawa et al. (1995) recognised *N. girellae* as a species distinct from *N. melleni* (see also Ogawa & Yokoyama 1998, Koesharyani et al. 1999) whereas Whittington & Horton (1996) synonymise *N. girellae* with *N. melleni*. The definition of *N. melleni* is the subject of ongoing debate. We follow the decision of Whittington & Horton (1996). Throughout this paper, *N. melleni* is defined sensu Whittington & Horton (1996).

The above characteristics make *Neobenedenia melleni* a monogenean species of considerable interest. Knowledge of its global distribution is particularly significant because of its pathogenicity. *N. melleni* was described from numerous fish species in the New York Aquarium (MacCallum 1927) and its true origin (i.e., the fish species on which it was introduced) has long been debated, but remains unknown (Whittington & Horton 1996 and references therein). There are published records of *N. melleni* from the Caribbean Sea, the West Atlantic Ocean, the east and mid-Pacific Ocean, and the Red Sea (Whittington & Horton 1996). Ogawa et al. (1995) reported *N. girellae* from 14 species (6 families; 3 orders) of cultured marine fishes in Japan and provided strong evidence for continuous introduction of the pathogen on the unregulated importation of amberjack fry (*Seriola dumerili* Risso) from Hong Kong and Hainan, China. Koesharyani et al. (1999) reported *N. girellae* (likely to be *N. melleni* sensu Whittington & Horton [1996], but no voucher material was deposited) from serranids in a research station in Bali. The list of wild hosts for *N. melleni* by Bullard et al. (2000) confirms the Caribbean area as a focus for this monogenean species, adding Puerto Rico,

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Grand Cayman Island and the Gulf of Mexico to the growing list of localities.

Restricted surveys of wild marine teleosts by Whittington & Horton (1996) failed to find *Neobenedenia melleni* at the northern and southern tips of the Great Barrier Reef in Australia, but only small sample sizes of a limited range of host species were examined. Whittington & Horton (1996) commented that it was important to determine whether *Neobenedenia* spp., and *N. melleni* in particular, occurred in Australian waters because of the potential threat posed to fin-fish mariculture as shown in other countries. In August 2000, personnel at a marine barramundi farm in Queensland contacted IDW about a 'fluke problem' on cultivated stocks of *Lates calcarifer* (Bloch). Here, we report *N. melleni* for the first time from Australia.

Materials and methods. The monogenean problem was first reported to IDW on 16 August 2000. Culture conditions for the barramundi on which the outbreak was reported were as follows. Specimens of *Lates calcarifer* up to 150 mm in total length were maintained in 2 different kinds of polyethylene mesh cages: up to 50 'nursery' cages (1 m^2, 3 m deep; mesh size 12 mm) or 6 'grow-out' cages (10 × 5 m, 3 m deep; mesh size 20 mm). Larger fish were maintained in 2 cages 10 × 10 m, 3 m deep, or in 5 cages 26 × 10 m, 3 m deep, each with a mesh size of 25 mm. All cages were located in the Hinchinbrook Channel, between Hinchinbrook Island and mainland Australia (18° 29′ 00″ S, 146° 16′ 00″ E). Additional predator nets were deployed with mesh sizes of 50 or 100 mm. Hinchinbrook Channel varies in depth according to tide, but water depth is approximately 4.5 m on an average tide across the farm lease. Stocking density varied, but was estimated at 25 kg of fish m^{-3}.

Four specimens of *Lates calcarifer* (total length range 128 to 174 mm) were air freighted from Cairns to Brisbane in oxygenated seawater. Before examination, each fish was killed by pithing and dorsal chordotomy, placed in a Petri dish and immersed in seawater filtered through 2 sheets of Whatman No. 1 filter paper. Each fish was examined for parasites using a stereodissecting microscope with incident illumination. Live monogeneans were transferred to small Petri dishes containing filtered seawater where many specimens laid eggs. Numerous adult specimens were flattened beneath slight coverslip pressure and preserved in 10% buffered neutral formalin. Other specimens were killed instantly in near-boiling seawater and preserved unflattened in 10% buffered neutral formalin. Some flattened and unflattened material was left unstained, but other specimens were stained using Semichon's aceto-carmine or Mayer's acid haemalum (Humason 1979). Specimens were dehydrated in a graded ethanol series, cleared in cedar wood oil and mounted in Canada balsam beneath a coverslip on glass slides. Some unstained individuals were mounted directly in Hoyer's medium (Pritchard & Kruse 1982) to permit close examination of heavily flattened specimens to ensure that haptoral sclerites lay as flat as possible to reveal their profile (as recommended by Whittington & Horton 1996). All specimens were measured using a computerised digitising system similar to that described by Roff & Hopcroft (1986). All measurements are quoted in micrometres as the range followed by the mean in parentheses. Terminology follows Whittington & Horton (1996) and discussed further by Whittington et al. (2001).

Results. All 4 barramundi specimens examined were infected with capsalid monogeneans. Live worms were recovered from the fins, 'skin' (= body flanks), head and eyes. Infection intensities ranged from 12 to over 400 specimens on the most heavily infected barramundi (total length 145 mm). No capsalids were found on the gills or gill arches.

Monogeneans from each barramundi examined matched the redescription of *Neobenedenia melleni* by Whittington & Horton (1996). Shapes of haptoral sclerites (Fig. 1), eggs and measurements of capsalids removed from *L. calcarifer* (Table 1) are similar to those provided by Whittington & Horton (1996) for *N. melleni*. It should be noted, however, that the low host specificity of *N. melleni* and likely associated 'host-induced morphological variation' (Whittington & Horton 1996), a phenomenon that remains unquantified, appears to result in broad size ranges for this taxon. We conclude that the outbreak on these cultivated barramundi in sea cages in Queensland was *N. melleni*.

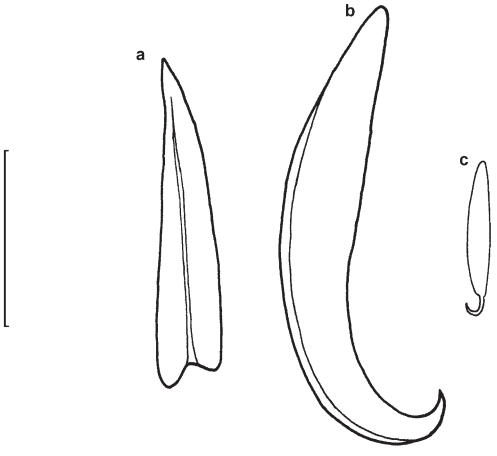

Fig. 1. Haptoral sclerites from the monogenean *Neobenedenia melleni* sensu Whittington & Horton (1996) collected from cultivated *Lates calcarifer* in northern Queensland, Australia. (a) Accessory sclerite; (b) anterior hamulus; (c) posterior hamulus. Drawn from fresh specimens prepared in Hoyer's medium. Scale bar = 125 µm

Table 1. Measurements of specimens of *Neobenedenia melleni* sensu Whittington & Horton (1996) from cultivated *Lates calcarifer* in northern Queensland, Australia. Haptoral sclerites were measured from 10 heavily flattened, mounted specimens (vouchers in the Queensland Museum [QM]: G218291–300); soft body parts were measured from 10 unflattened, mounted specimens (vouchers in QM:G218281–90)

Parameter measured (µm)	Range (mean)
Total length	2230–4190 (2890)
Maximum width	997–1560 (1290)
Haptor length	667–903 (809)
Haptor width	663–916 (793)
Accessory sclerite length	131–190 (165)
Anterior hamulus length	198–390 (252)
Posterior hamulus length	91–163 (130)
Anterior attachment organ diameter	191–374 (312)
Pharynx length	191–374 (312)
Pharynx length	275–335 (293)

There was evidence of loss of scales, some epidermal damage, lifting and loosened scales, and epidermal lesions on the 2 most heavily infected *Lates calcarifer* specimens. The remaining 2 specimens had fewer than 25 worms each and appeared relatively healthy with few external signs of pathology. Estimates of the total barramundi losses resulting from infection by *N. melleni* were 200 000 fish (approximately 50 tonnes) with a value of about AUS $500 000 (= US$277 000).

Twenty voucher specimens of *Neobenedenia melleni* collected in this study are deposited in The Queensland Museum, PO Box 3300, South Brisbane, Queensland 4101, Australia (Accession G218281–300).

Discussion. This is the first confirmed report of *Neobenedenia melleni* from Australia, but sources at the farm where the outbreak arose and local professionals associated with the aquaculture industry nearby suggest that *N. melleni* infections have occurred there before, although not on the scale observed in August 2000. Previous outbreaks of *N. melleni* may have been misdiagnosed as *Streptococcus* infection presumably because wounds caused by the monogeneans may have become secondarily infected by bacteria.

The present report of *Neobenedenia melleni* from the east coast of Australia extends the Pacific Ocean distribution of this pathogen (Whittington & Horton 1996). Our report is also a new confirmed host record, although Leong (1997) listed 2 capsalid species (*Benedenia* sp. I and *Neobenedenia* sp. II) from cultivated *Lates calcarifer* in southeast Asia. Further studies of these capsalid species from various cultivated marine fin-fishes in southeast Asia are required because host distribution data (Whittington et al. 2001) suggest that *Neobenedenia* sp. II may be *N. melleni*. If this is the case, then *N. melleni* is widespread throughout Pacific waters.

There are parallels between the sudden outbreaks of *Neobenedenia melleni* on cultured *Oreochromis mossambicus* (Trewavas) (tilapia) in Hawaii (Kaneko et al. 1988), on cultured amberjack in Japan (Ogawa et al. 1995) and on barramundi cultured in Hinchinbrook Channel in Australia (present study). Kaneko et al. (1988) noted that the natural host of *N. melleni* in Hawaiian waters had not been identified despite an extensive survey of Hawaiian marine fishes for monogeneans conducted previously by Yamaguti (1968). Kaneko et al. (1988) therefore suggested that *N. melleni* may have been introduced into Hawaii after Yamaguti's (1968) survey. Ogawa et al. (1995) provided strong evidence for continuous introduction of *N. melleni* (identified as *N. girellae*) into Japan by the unregulated import of amberjack fry, *Seriola dumerili*, from Hong Kong and Hainan, China. The same 2 scenarios are possible for the outbreak that we report in Australia: either the pathogen occurs naturally on wild specimens of 1 or more marine teleost species near the farm where the outbreak occurred or the capsalid was introduced on imported fish. The latter possibility is unlikely because Australia has strict regulations on the importation of all organisms from overseas and authorities maintain that there is no chance that any marine fish species was imported and stocked locally. The most likely origin of *N. melleni* in Australia, therefore, is a wild population of 1 or, perhaps, several fish species. The current report adds to the mystery about the natural source of *N. melleni*, which has baffled parasitologists since its description from the New York Aquarium.

If *Neobenedenia melleni* has been present in eastern Australian waters on a wild teleost host (or hosts), why did it suddenly transfer to cultivated *Lates calcarifer* and reach epidemic proportions so quickly? We propose that a 3 wk period of low (19°C) seawater temperature in late July to early August 2000, unusual for this region, precipitated the outbreak. A low, or a sudden drop in, temperature is known to stress barramundi (Rimmer 1995, Anderson 1996). Disease, especially among barramundi fingerlings, is more common in winter (Rimmer 1995) and low temperatures slow the production of antibodies and phagocytes and reduce the capacity for barramundi to heal wounds (Anderson 1996). We argue that natural infections of *N. melleni* probably occur on 1 or more wild fish species around the sea cages where the barramundi are cultivated. At seawater temperatures above 20°C, the barramundi can likely combat *N. melleni* infections because their immune system is working optimally. However, in late July and early August 2000, when the seawater temperature fell to 19°C, the immune systems of the cultivated *L. calcarifer* were likely compromised. This, combined with the high fish density in

the sea cages, resulted in the *N. melleni* epidemic. This scenario may also explain why the outbreak by *N. melleni* on barramundi subsided so rapidly. During September 2000, the seawater temperature rose above 20°C and the effectiveness of the immune system of most remaining barramundi likely recovered sufficiently to fight off infections. However, previous unconfirmed outbreaks of *N. melleni* on *L. calcarifer* that were likely misdiagnosed as *Streptococcus* infections at this farm occurred when seawater temperatures were not excessively low. This ubiquitous capsalid is probably able to infect weaker fish that are compromised because of poor nutrition, sickness, reduced efficiency or natural variation of the immune system irrespective of seawater temperature, which makes it a further threat in mariculture.

Glazebrook & Campbell (1987) noted that the range of disease agents identified in Australian barramundi overlapped considerably with those reported from other countries. We suggest that *Neobenedenia melleni* may also be a pathogen to barramundi and other fin-fish cultured in southeast Asia. Mariculture is in its infancy in Australia, which, combined with a lack of diagnostic knowledge, may be why no outbreaks of *N. melleni* have been reported until now. However, *N. melleni* has a broad geographic range and infects a multitude of wild and cultured fish species. Taking into account these 2 key factors alone, we predict that mariculture of not only barramundi, but also other fin-fish species in Australia, is under threat of infection by *N. melleni*. We encourage the reporting of any disease agents in mariculture to appropriate authorities and experts so that pathogens are identified correctly, suitable records are maintained and voucher specimens are deposited in recognised, curated museum collections. These actions will provide an invaluable foundation of data for the future study of marine pathogens of cultivated species.

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