

PHYLUM DICYEMIDA IN AUSTRALIAN WATERS: FIRST RECORD AND DISTRIBUTION ACROSS DIVERSE CEPHALOPOD HOSTS

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ABSTRACT: Dicyemid mesozoans are marine organisms that live exclusively in the excretory organs of cephalopod hosts. This study constitutes the first record of the Phylum Dicyemida from Australian waters and provides information on the degree of infections within and between shallow-water host species, and on the distribution of dicyemids across latitude. A total of 38 cephalopod species from 8 families were collected throughout southern, eastern and western Australia, and investigated for the presence of dicyemids. Individuals from 24 cephalopod species were found to be infected, generating new host records of dicyemids for 23 species, 5 genera (*Euprymna*, *Grimpella*, *Hapalochlaena*, *Sepiadarium*, *Sepioloidea*) and one family (Sepiariidae). This investigation represents the first detailed cross-latitudinal survey of dicyemids. Findings of dicyemids in three tropical octopus species (*Octopus alpheus*, *O. dierythraeus* and *O. ornatus*) and multiple findings of uninfected adult benthic cephalopods in temperate regions allow us to re-evaluate and modify concepts of latitudinal trends proposed in previous studies on this group of parasites. Implications of our observations on the largely unknown dicyemid life cycle are discussed.

INTRODUCTION

Dicyemid mesozoans are a highly specialised phylum of marine organisms found exclusively in the excretory organs of octopuses, squids and cuttlefishes (coleoid cephalopods). Dicyemids spend the majority of their life cycle attached to the renal appendages of their hosts, deriving their nutrition from their host's urine. Dicyemids are microscopic worm-like (vermiform) organisms that typically occur in densities of thousands per cm³. They consist of a fixed number of cells and grow from an early stage by cell enlargement. The adult vermiform stages typically are made up of 14–40 ciliated peripheral cells (depending on the species) that surround a single elongate axial cell. The anterior end is modified into a head-like calotte covered with short thigmotactic cilia that facilitate attachment to the renal tissue of the host. The vermiform body hangs free in the renal coelom and is constantly bathed in the urine of the host.

The known stages in dicyemid life cycle as they occur in the cephalopod host have been documented by numerous researchers (for review see Hochberg, 1990).

The phylogenetic position of this phylum is still subject to considerable controversy. Recently the dicyemids were shown to be most closely related to higher, triploblastic metazoans (Kobayashi *et al.* 1999). Thus the simple body organisation of dicyemids most likely is the product of degeneration attributable to parasitism (see discussion in Katayama *et al.* 1995).

Dicyemids often are treated as parasites, however, the precise nature of the relationship between the dicyemid and the host has not been resolved. The general contention in the literature is that dicyemids are commensal, having little effect, positive or negative, on the host (Hochberg 1990). There are, however, a few findings that suggest otherwise. Lapan (1975) proposed that

dicyemids facilitate host excretion of ammonia and, therefore, are engaged in a symbiotic relationship beneficial to the host. In contrast, the presence of dicyemids in some cases causes the renal appendages to have an eroded or pitted appearance (Hochberg unpubl. data) and electron-microscopy has revealed that dicyemids may damage the delicate brush border of the host's renal appendages (Ridley 1968). The effect of this erosion on the function of the renal appendages is not known but it has been proposed that it may have a pathogenic impact (Ridley 1968).

The majority of investigators working on the phylum have concentrated on cephalopod hosts obtained from coastal waters in the Northern Hemisphere (see Hochberg 1990). Areas of prime focus have included the Bering Sea (Russia), the North Pacific Ocean (Japan, Russia, USA south to Mexico), the North Atlantic Ocean (Canada south to USA, Bermuda, Europe, Mauritania), the Gulf of Mexico (USA), the Mediterranean Sea (Europe), and the Bay of Bengal in the Indian Ocean (India). In comparison there have been relatively few published works on dicyemids from the Southern Hemisphere (see Hochberg 1990). In this region the focus has been in the South Atlantic Ocean (Argentina, the Falkland Islands, South Africa), the South Pacific Ocean (New Zealand), and the Southern Ocean (Antarctica). This overview of the dicyemid infection rates of Australian cephalopods constitutes the first formal report of dicyemids from Australian waters.

In previous reviews Hochberg (1982, 1983, 1989, 1990) summarised these widespread reports and proposed several theories that attempted to explain the distribution of dicyemids across taxa and latitude. Based on information existing at the time Hochberg (1990) postulated that 'dicyemids parasitize only benthic or epibenthic cephalopods'. In temperate and polar waters 'adult, benthic cephalopods generally are 100% infected' while in the tropics and off oceanic islands 'no cephalopods have been reported to be infected' (Hochberg, 1990). In subtropical regions 'the infection rates are variable and range from 10 to 20%' (Hochberg, 1990).

Australia contains one of the most diverse cephalopod faunas in the world (Lu and Phillips

1985, Lu and Dunning 1997, Norman and Reid 2000). Dicyemids are formally reported from Australian waters for the first time. Ongoing studies on the cephalopod fauna of Australia's south coast (Lu and Tait 1983, Stranks 1988 1990, Lu and Stranks 1992, Stranks and Norman 1993) and east coast (Norman 1991, 1992, 1993a, 1993b, 1993c, 2001, Norman and Finn 2001) have provided a solid taxonomic foundation for cephalopod-related investigations.

Coastal surveys undertaken by the authors in recent years have provided the opportunity to investigate the presence and composition of dicyemids in Australian cephalopods. As sites surveyed spanned across 19 degrees of latitude from 19°S to 38°S, it also was possible to investigate latitudinal trends. Dicyemids proved to be abundant in Australian waters.

MATERIALS AND METHODS

A total of 223 submature and mature cephalopods, representing 38 species in eight families, were collected from coastal sites between Magnetic Island, Queensland in the north (19°08'S, 146°50'E) and Cockburn Sound, Western Australia (32°12'S, 115°42'E) in the west across a latitudinal range from north to south of 19°S to 38°S (Figure 1). The majority of cephalopods sampled were collected from sub-tidal habitats, using SCUBA, during both day and night. Additional collection techniques also were employed, including intertidal reef walks, beach seines, benthic trawls, and the use of octopus pots and squid jigs. Trawling was conducted in Moreton Bay, Queensland during July 1997 in conjunction with the Queensland Department of Primary Industries. Freshly dead specimens of rarer fauna, including *Argonauta nodosa* and *Architeuthis sanctipauli*, were obtained by the authors and incorporated into this study. Taxonomic authorities for all taxa examined are listed in Table 1.

All living cephalopods were anaesthetised prior to dissection by placing the animals in a solution of 5% ethanol in seawater until they did not respond to external stimulus (*i.e.*, they failed to retract a pinched arm tip). Data sheets were prepared for each species of host examined. Sex, stage of

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Table 1. Australian cephalopods examined for dicyemid parasites. * new host family; ** new host genus and species; *** new host species; ● dicyemids present; ○ dicyemids not present.

Cephalopod Host Species	Dicyemids Detected	No. Examined	No. Infected	Collection Localities (°S Localities)	
Family SEPIIDAE					
<i>Sepia apama</i> Gray, 1849***	●	11	11	33°00'to34°30'	
<i>Sepia hedleyi</i> Berry, 1918***	●	1	1	36°25'	
<i>Sepia mestus</i> Gray, 1849***	●	2	2	35°05'	
<i>Sepia novaehollandiae</i> Hoyle, 1909***	●	1	1	35°06'	
<i>Sepia plangon</i> Gray, 1849***	●	7	7	27°05'to32°43'	
<i>Sepia smithi</i> Hoyle, 1885***	●	1	1	27°07'	
Family IDIOSEPIIDAE					
<i>Idiosepius notooides</i> Berry, 1921	○	3	0	38°22'	
<i>Idiosepius pygmaeus</i> Steenstrup, 1881	○	2	0	27°06'	
Family SEPIADARIIDAE*					
<i>Sepiadarium austrinum</i> Berry, 1921**	●	19	17	33°00'to38°10'	
<i>Sepiadarium</i> sp.1 (see Norman, 2000)	○	4	0	34°30'to35°06'	
<i>Sepioloidea lineolata</i> (Guoy and Gaimard, 1832)**●	●	15	12	23°30'to35°06'	
Family SEPIOLIDAE					
<i>Euprymna tasmanica</i> (Pfeffer, 1887)**	●	18	14	27°05'to38°21'	
Family LOLIGINIDAE					
<i>Uroteuthis</i> sp.	○	5	0	27°07'to27°14'	
<i>Sepioteuthis australis</i> Quoy and Gaimard, 1832	○	11	0	32°30'to38°10'	
<i>Sepioteuthis lessoniana</i> Ferrusac in Lesson, 1830	○	1	0	23°30'	
Family ARCHITEUTHIDAE					
<i>Architeuthis sanctipauli</i> (Velain, 1877)	○	1	0	38°21'	
Family OCTOPODIDAE					
<i>Octopus alpheus</i> Norman, 1993***	●	2	2	23°30'	
<i>Octopus aspilosomatis</i> Norman, 1993	○	2	0	23°30'	
<i>Octopus australis</i> Hoyle, 1885***	●	2	2	32°42'	
<i>Octopus berrima</i> Stranks and Norman, 1993***	●	16	16	33°15'to38°23'	
<i>Octopus bunurong</i> Strank, 1990***	●	5	3	38°10'to38°19'	
<i>Octopus cyanea</i> Gray, 1849	○	6	0	19°08'to23°30'	
<i>Octopus dierythraeus</i> Norman, 1993***	●	6	6	19°08'to27°14'	
<i>Octopus</i> cf. <i>kagoshimensis</i> (see Norman, 2000)	○	7	0	23°30'to27°05'	
<i>Octopus kaurna</i> Stranks, 1990***	●	16	13	34°04'to38°10'	
<i>Octopus maorum</i> Hutton, 1880	●	3	2	33°51'to38°28'	
<i>Octopus ornatus</i> Gould, 1852***	●	6	2	23°30'	
<i>Octopus pallidus</i> Hoyle, 1885***	●	5	3	37°59'to38°07'	
<i>Octopus tetricus</i> Gould, 1852	○	11	0	32°42'to36°25'	
<i>Octopus</i> cf. <i>tetricus</i> (see Norman, 2000)***	●	3	2	33°51'to34°00'	
<i>Octopus warringa</i> Strank, 1990	○	1	0	38°19'	
<i>Octopus</i> sp.4 (see Norman, 2000)	○	1	0	23°30'	
<i>Octopus</i> sp.7 (see Norman, 2000)***	●	1	1	38°10'	
<i>Hapalochlaena fasciata</i> (Hoyle, 1886)**	●	2	2	27°14'	
<i>Hapalochlaena maculosa</i> (Hoyle, 1883)**	●	21	15	33°00'to38°28'	
<i>Hapalochlaena</i> sp.3 (see Norman, 2000)***	●	2	2	32°08'	
<i>Grimpella thaumastocheir</i> Robson, 1928**	●	1	1	34°30'	
Family ARGONAUTIDAE					
<i>Argonauta nodosus</i> [Lightfoot, 1786]	○	2	0	38°16'	
TOTALS		38 species 24infected	223	138	19°08'to38°28'

maturity, wet weight and standard length measurements were recorded for all cephalopods examined. Only mature and submature animals were examined.

The renal appendages of cephalopods lie on the ventral surface of the viscera within the mantle cavity. The viscera were exposed by a longitudinal incision along the ventral mid-line of the mantle. The renal appendages of cephalopods are usually cream to brown in colour and are immersed in coelomic fluid within renal sacs surrounded in turn by a single renal membrane. Renal appendages were removed through a small incision made in the membrane covering the renal coelom. The appendages were cut into small pieces and immersed in a small volume of coelomic fluid collected using a pipette inserted directly into the renal cavity. In later samples, the above procedure was performed separately (with equipment cleaned and sterilised in absolute ethanol) for each renal appendage and each host to avoid cross contamination of preparations.

The reno-pancreatic appendages and branchial heart appendages have been reported as additional sites of dicyemid infection (Hochberg 1990). These sites were not examined in this study.

An indication of the presence of dicyemid infections was immediately determined by examining freshly dissected and macerated renal tissue at low magnification using a compound microscope. This initial check determined whether a host was infected. In cases where dicyemids were observed a preliminary assessment of the degree of infection and the stage(s) infecting the host could be obtained. In some instances an infection could not be detected by this preliminary examination due to low densities of dicyemids. As a consequence replicate smears were fixed, stained and examined before individuals were recognised as uninfected. Preserved smears were retained as vouchers of this study and also to provide specimens for ongoing taxonomic studies.

Renal smears were prepared using the following methodology. A small piece of renal appendage was held with fine forceps and brushed lightly in one direction over the surface of a square glass coverslip (22 x 22 mm) in a series of parallel lines. To avoid desiccation, the coverslip was

dropped immediately smear-side-down into a Petri dish of Bouin's fluid. Each piece of renal appendage was used to make one coverslip smear. Up to 30 coverslip smears were made per host depending on the volume of renal tissue available. All remaining pieces of renal appendage were immediately fixed in 5% formaldehyde in seawater for future reference and histological examination. Coverslip smears were floated in Bouin's fluid for 10-24 hr and then transferred to screw top vials containing 70% ethanol for storage prior to staining. Coverslips were separated by pieces of gauze or cheesecloth. Detailed labels were placed in the vials with each series of smears.

Following trials of a range of different staining techniques, the method chosen as providing the best result across a range of dicyemid species was extended immersion of smears (30 hr) in Harris' Hematoxylin (diluted 1 in 20 parts). Once stained, cover slip smears were mounted using DPX mountant (BDH Lab Supplies), smear side down, on micro slides. The slides were air-dried in an oven (maintained at 47°C) for 48 hr, the excess mounting medium trimmed off and labels applied. Smears were scanned using a Leica compound microscope and the presence or absence of dicyemids was recorded. Where an infection was not obvious, great care was taken to examine all slides of that series plus tissue material fixed in formalin, at a wide range of magnifications. If dicyemids were not detected the host was designated as 'uninfected'. Renal smears are lodged in the Santa Barbara Museum of Natural History, California, USA. Host vouchers and symbiotypes are archived with the Museum of Victoria, Melbourne, Australia.

RESULTS

First record of the Phylum Dicyemida in Australian waters: Cephalopods from a total of 38 species and eight families were collected off the coasts of southern, eastern, and western Australia between the latitudes of 19°08'S (Magnetic Island, Queensland) and 38°28'S (Flinders, Victoria) (Table 1). Individuals from 24 of the 38 species were infected with dicyemids, representing the first documented record of this phylum from Australian

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waters. Of the 24 species with positive infections, 23 species represent new host records. Dicyemids previously have been described from *Octopus maorum* from New Zealand (Short and Hochberg 1969, Short 1971). This octopus also occurs in Australia and was examined in this study. Dicyemids were recorded for the first time from five genera (*Sepiadarium*, *Sepioloidea*, *Euprymna*, *Hapalochlaena*, *Grimpella*) and one family (Sepiadariidae).

Multiple species of the dicyemid genera *Dicyema* and *Dicyemenea* were encountered (see Plate 1 for examples). Species descriptions are currently being completed for new species. Initial examination of the predominant *Dicyema* species infecting the southern blue-ringed octopus *Hapalochlaena maculosa* found the species to be new (see Finn 1997).

A number of undescribed cephalopod species (see Norman 2000) were encountered by the authors in the course of this investigation (Table 1). Taxonomic descriptions presently are being produced for members of the families Sepiadariidae (*Sepiadarium* sp. 1) and Octopodidae (*Octopus* cf. *kagoshimensis*, *O.* sp. 4, *O.* sp. 7, *Hapalochlaena* sp. 3).

Infection rates by host taxonomic group: The presence/absence of dicyemids within individual cephalopods varied between families. Dicyemids were not recorded in any members of the squid families Idiosepiidae, Loliginidae, Architeuthidae and the pelagic octopod family Argonautidae. In contrast, all specimens of all species of cuttlefishes (family Sepiidae) were infected. The remaining families examined (Sepiadariidae, Sepiolidae, and Octopodidae) possessed variable rates of infection as indicated below.

Individuals of eight species examined were 100% infected: Octopodidae - *Octopus alpheus* (2/2), *O. australis* (2/2), *O. berrima* (16/16), *O. dierythraeus* (6/6), *O.* sp. 7 (1/1), *Hapalochlaena fasciata* (2/2), *H.* sp. 3 (2/2), *Grimpella thaumastocheir* (1/1).

In ten species a variable number of individuals (less than 100%) were infected: Sepiadariidae - *Sepiadarium austrinum* (17/19) *Sepioloidea lineolata* (12/15); Sepiolidae - *Euprymna tasmanica* (14/18); Octopodidae - *Octopus bunurong* (3/5),

O. kaurna (13/16), *O. maorum* (2/3), *O. ornatus* (2/6), *O. pallidus* (3/5), *O. cf. tetricus* (2/3), *Hapalochlaena maculosa* (15/21).

In seven species all individuals examined were uninfected: Sepiadariidae - *Sepiadarium* sp. 1 (0/4); Octopodidae - *Octopus aspilosomatis* (0/2), *O. cyanea* (0/6), *O. cf. kagoshimensis* (0/7), *O. tetricus* (0/11), *O. warringa* (0/1), *O.* sp. 4 (0/1).

Infection rate by collection locality: When comparing rates of infection to collection locality, no correlations were observed. At many survey sites, multiple species of cephalopods co-existed and were sampled. The percentage of individuals found to be infected varied between co-existing species. Three examples discussed below (two from temperate waters and one from tropical waters) clearly demonstrate this phenomenon.

In temperate waters of South Australia, under Edithburgh Jetty in the Gulf Saint Vincent (35°06'S), seven species of cephalopods from the families Sepiadariidae, Sepiolidae and Octopodidae co-exist. Of the individuals examined from this locality, five species were 100% infected: Sepiadariidae: *Sepiadarium austrinum* (1/1); Sepiolidae: *Euprymna tasmanica* (2/2); Octopodidae: *Octopus berrima* (1/1), *O. kaurna* (2/2), *Hapalochlaena maculosa* (5/5). Individuals of one species were variably (less than 100%) infected: Sepiadariidae: *Sepioloidea lineolata* (4/5). Two individuals of one species were uninfected: Sepiadariidae: *Sepiadarium* sp. 1 (0/2).

In the temperate waters of Victoria, in both Port Phillip Bay and Western Port Bay (37°59'S to 38°28'S), 10 species of cephalopods representing the families Sepiadariidae, Sepiolidae and Octopodidae were found to co-exist. All individuals belonging to five species were infected: Sepiadariidae: *Sepiadarium austrinum* (16/16); Sepiolidae: *Euprymna tasmanica* (6/6); Octopodidae: *Octopus berrima* (15/15), *O. maorum* (2/2), *O.* sp. 7 (1/1). Individuals of four species were variably infected (less than 100%): Octopodidae: *Octopus bunurong* (3/5), *O. kaurna* (9/12), *O. pallidus* (3/5), *Hapalochlaena maculosa* (8/14). A single animal was uninfected: Octopodidae: *Octopus warringa* (0/1).

In the tropical waters of southern Queensland surrounding One Tree Island (23°30'S) a total of

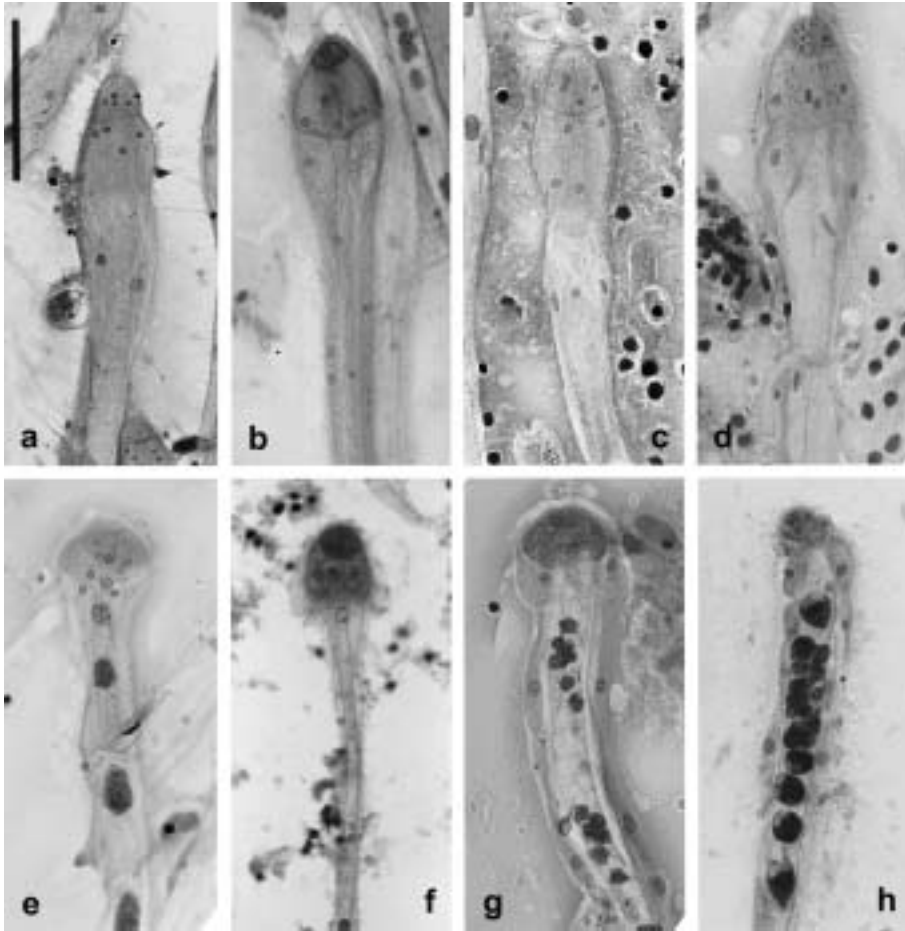


Plate 1. a-h. Selection of dicyemid fauna of co-inhabitant cephalopod hosts. **a.** *Dicyema* sp. from *Hapalochlaena maculosa*. **b.** *Dicyemenea* sp. from *Octopus berrima*. **c.** *Dicyema* sp. from *Octopus bunurong*. **d.** *Dicyema* sp. from *Octopus kurna*. **e.** *Dicyema* sp. from *Octopus maorum*. **f.** *Dicyemenea* sp. from *Octopus pallidus*. **g.** *Dicyema* sp. from *Euprymna tasmanica*. **h.** *Dicyemenea* sp. from *Sepiadarium austrinum*. Scale bar (a-h) = 100m

seven species of cephalopods from the families Sepiadariidae and Octopodidae were found to co-exist. All individuals belonging to one species were infected: Octopodidae: *Octopus alpheus* (2/2). Individuals of two species were variably (less than 100%) infected: Sepiadariidae: *Sepioloidea lineolata* (1/3); Octopodidae: *Octopus ornatus* (2/6). All individuals of four species were uninfected: Octopodidae: *Octopus aspilosomatis* (0/2), *O. cyanea* (0/3), *O. cf. kagoshimensis* (0/1), *O. sp. 4* (0/5).

Variation between individuals of the same species: In a number of instances variations in infections were detected between individuals of the same species. For species where dicyemids were detected in less than 100% of individuals the incidence of infection was not correlated with location, sex or maturity of hosts, nor date or time of sampling. Multiple individuals of *Octopus bunurong*, *O. kurna*, *O. ornatus*, *O. pallidus* and *Hapalochlaena maculosa* of the same sex and

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maturity, were collected from the same site, at the same time, and were found to vary in infection. In one case, four individuals of *O. pallidus* (two mature males and two mature females) of similar size, maturity and condition were collected from Portarlington Pier, Victoria (5 March 1997). Dense infections of dicyemids were detected in both renal appendages of two individuals (one male and one female), while rigorous examination of all renal material obtained from the other two individuals (one male and one female) failed to detect a single dicyemid.

Dicyemids were not detected in the single specimen of *Sepioteuthis lessoniana* sampled in this study from Queensland waters. Individuals of this species have been reported to contain dicyemids in Japanese waters (Nouvel and Nakao 1938, Nouvel 1947, Furuya 1999). A recent study, however, suggested that more than one species of loliginid squid is represented by this species name (Segawa *et al.* 1993). The genus *Sepioteuthis* requires extensive taxonomic revision.

Variation within individuals: Variations in infections also were detected on an individual level in octopuses that were approximately the same size and supposed age. In two cases (*Octopus kaurna* and *O. bunurong*), two individuals were found in which dicyemids were present in only one and not both renal coeloms. In octopuses, in contrast to other families of cephalopods, two separate renal coeloms are present. The appendages in one coelom were densely infected with dicyemids while rigorous examination of the appendages in the adjacent coelom failed to reveal a single dicyemid. Individual examination of renal appendages in each coelom also found variability in the life cycle stages of dicyemids in adjacent appendages. Typically, in most octopuses examined, the life cycle stage of the dicyemids within both renal appendages corresponds to the maturity of the hosts. In smaller, immature host individuals dicyemids of the nematogen stage are present whereas larger, mature host individuals harbour dicyemids of the rhombogen stage. In sub-mature hosts a combination of both stages is present. However, in one individual of *Hapalochlaena maculosa* nematogens were only found in the left renal coelom while only rhombogens were present in the right

coelom. Similar variations in infections between the renal appendages have been found in Japanese octopus (Furuya *et al.* 1992).

In summary, variations in infections occurred between different families, between species of the same family, between individuals of the same species, and in the case of three octopus between the renal coeloms of the same individual. Occurrence of varying infections between renal coeloms may be more widespread than suggested by the few examples detected in this investigation because independent treatment of the renal appendages from each renal coelom was a late modification to sampling procedures.

Dicyemids occur across latitude: Dicyemids were present in cephalopods across the entire latitudinal range of this investigation, from the tropical waters at 19°08'S (Magnetic Island, Queensland) through subtropical waters to the temperate waters at 38°28'S (Flinders, Victoria) (Table 1, Figure 1).

To allow comparison of our data with latitudinal theories postulated by Hochberg (1990), Australia's coastal waters were divided into tropical, subtropical and temperate regions (see Figure 1). The Tropic of Capricorn (23°30'S) is taken as the boundary between tropical and subtropical regions in both eastern and western Australia. Following Wilson and Allen (1987), Cape Howe (37°31'S) marks the boundary between subtropical and temperate regions in eastern Australia, while Cape Leeuwin (34°22'S) marks the subtropical/temperate boundary in the west. The waters between Cape Howe and Cape Leeuwin, including the waters of the Great Australian Bight and the South Australian gulfs are considered temperate.

Two small squid species, *Euprymna tasmanica* (family Sepiolidae) and *Sepioloidea lineolata* (family Sepiadariidae) occur across more than one biogeographic region considered in this study (Norman and Reid 2000). Individuals of *E. tasmanica* collected from temperate waters off Victoria and South Australia including the waters of Spencer Gulf and the Gulf Saint Vincent (32°30'S to 38°21'S) were found to be infected with dicyemids while individuals collected in the subtropical waters of eastern Australia (27°05'S

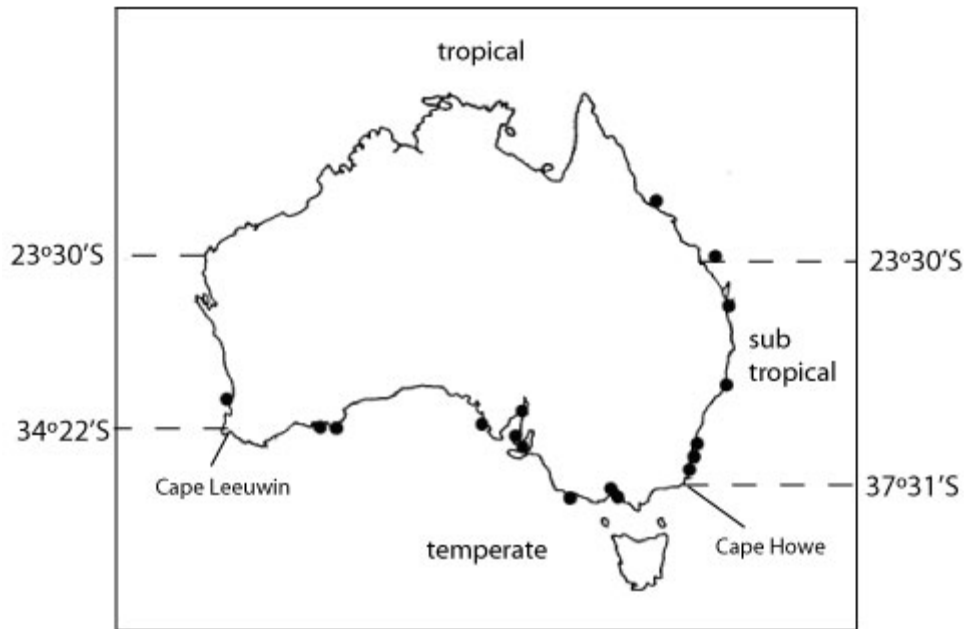


Figure 1. Collection sites of cephalopods examined for dicyemid parasites.

to 36°25'S) were found to be uninfected. Individuals of *S. lineolata*, in contrast, were infected across an extensive latitudinal range including the temperate waters off South Australia - 35°06'S (4/5), subtropical waters of New South Wales - 35°05'S (6/6), 32°43'S (1/1), and the tropical waters off southern Queensland - 23°30'S (1/3).

The majority of cephalopods examined in this study belonged to the family Octopodidae. Multiple species of octopuses were sampled from all latitudes investigated and dicyemids were found to infect species across the entire latitudinal range (19°08'S to 38°28'S). The percentage of individuals infected for each species did not correlate with latitude. Species were found to be 100% infected (*i.e.*, dicyemids present in all individuals examined) and completely uninfected (*i.e.*, dicyemids absent in 100% of the individuals examined) at both the tropical and temperate extremities of this investigation.

At the species level, the incidence of Octopodidae species in which dicyemids were found decreased across the three latitudinal zones examined, progressing south to north – temperate,

subtropical, tropical. Conversely, the percentage of species that were completely uninfected within each zone increased with decreasing latitude (Figure 2). This phenomenon is most evident when comparing the infection rates of Octopodidae species from Port Phillip Bay/Western Port Bay, Victoria (37°59'S to 38°28'S) at the southern extremity of this study, and One Tree Island, Queensland (23°30'S) near the northern extremity. Of the eight octopodids collected in Port Phillip Bay and Western Port Bay, seven species (87.5%) possessed dicyemids (three species were 100% infected: *Octopus berrima* (15/15), *O. maorum* (2/2), *O. sp. 7* (1/1); four species were variably infected: *O. bunurong* (3/5), *O. kaurna* (9/12), *O. pallidus* (3/5), *Hapalochlaena maculosa* (8/14)). A single animal was uninfected (*O. warringa* (0/1)). Of the six octopodids collected from One Tree Island, only two species (33.3%) possessed dicyemids (one species was 100% infected: *Octopus alpheus* (2/2); one species was variably infected: *O. ornatus* (2/6)). All individuals of four species were uninfected (*Octopus aspilosomatis* (0/2), *O. cyanea* (0/3), *O. cf. kagoshimensis* (0/1), *O. sp. 4*

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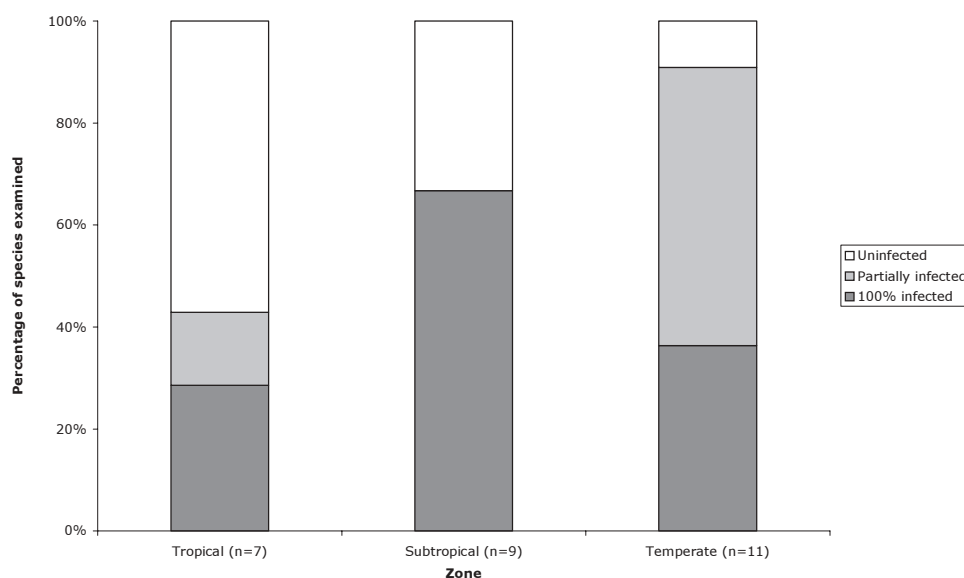


Figure 2. Percentage of octopus species infected across the three biogeographic regions sampled in this study.

(0/5)). Thus 12.5% of species in the Port Phillip/Western Port region were uninfected compared with 66.7% of species sampled from One Tree Island.

Octopus tetricus occurs along the east coast of Australia. It can be separated from an as yet unnamed sibling species, *O. cf. tetricus* (see Norman, 2000) that occupies an equivalent latitudinal range on the west coast. In our examination of the 11 individuals of the genuine *O. tetricus* examined throughout its warm temperate range (32°42'S to 36°25'S) no specimens were found to contain dicyemids whereas dicyemids were present in 2 of the 3 specimens of *O. cf. tetricus* sampled from roughly equivalent latitudes on the west coast (33°51'S to 34°00'S).

DISCUSSION

Coastal cephalopod surveys by the authors allowed the investigation of 38 cephalopod species, from 8 families, across 19 degrees of latitude, for the presence of dicyemids. This broad scale sampling provided the unique opportunity to examine trends in dicyemid infection. As a consequence of the broad scale of this sampling

the number of individuals examined for each species was often low – this was especially the case for rarer fauna. Recent research has indicated that in many cases a larger number of individual cephalopods (at least 10) needs to be sampled to detect infection in some variably infected species (Furuya pers. comm.). The absence of infection in some species, as found by this study, should therefore be treated as tentative until further replication is undertaken.

Trends across host taxa: The high diversity of cephalopod species inhabiting the coastal waters of south-eastern Australia provided the opportunity to gain insights into the distribution of dicyemids across a large number of host taxa. Different families of cephalopods varied in their degree of susceptibility to dicyemids. Dicyemids were present in cephalopods in the families Sepiidae, Sepiolidae, Sepiadariidae and Octopodidae. In contrast all individuals of the squid families Loliginidae, Idiosepiidae and Architeuthidae, and the pelagic octopod family Argonautidae were uninfected. Dicyemids previously have been reported from the families Sepiidae, Sepiolidae, Loliginidae and Octopodidae (for review, see Hochberg 1990). Our study provides the first record of dicyemids from

the family Sepiadariidae. Negative reports from the family Argonautidae are consistent with the literature (Hochberg 1982), while the families Idiosepiidae and Architeuthidae previously had not been examined for dicyemids.

Latitudinal trends: In reviewing the existing literature, Hochberg (1982, 1983, 1989, 1990), proposed several theories that attempted to explain the distribution of dicyemids across taxa and latitude. Based on information existing at the time Hochberg (1990) postulated that 'dicyemids parasitize only benthic or epibenthic cephalopods'. In temperate and polar waters 'adult, benthic cephalopods generally are 100% infected' while in the tropics and off oceanic islands 'no cephalopods have been reported to be infected' (Hochberg, 1990). In subtropical regions Hochberg (1990) stated that 'the infection rates are variable and range from 10 to 20%'.

The results of this study clearly counter Hochberg's earlier hypotheses. As a percentage of individual octopus examined 33.3% of tropical octopus were found to be infected (as opposed to 0% as proposed by Hochberg, 1990). Subtropical octopus were infected 31.6% of the time (as opposed to 10–20% proposed by Hochberg, 1990) while temperate octopus were found to be infected only 78.1% of the time (compared to 100% proposed by Hochberg, 1990) (see Figure 3). Recent studies in polar regions also support reassessment of this early hypothesis. Results of cephalopods examined for dicyemids in the Southern Ocean off Antarctica showed some species to be uninfected, others to have variable rates of infection, and still other species to be 100% infected (Hochberg unpubl. data).

The high number and scope of investigations conducted in Northern Hemisphere temperate regions in the past and limited reports from tropical regions and oceanic islands led to the earlier conclusion that the phylum Dicyemida was restricted to polar, temperate and subtropical waters (Hochberg 1990). This earlier proposal was largely based on findings of uninfected cephalopods collected in the central Pacific Ocean off Hawaii, Fiji and the Marshall Islands (McConnaughey 1949, Koshida *et al.* 1986, Hochberg unpubl. data), and in the Gulf of Mexico off the Virgin Islands

(Hochberg and Couch 1971). Dicyemids clearly do occur in tropical latitudes as reported in the earlier literature on the group: southeastern Atlantic Ocean, West Africa, Mauritania, Port Etienne (~21°N; Nouvel 1934), and the Bay of Bengal, India, Kalingapatnam to Madras (~13° to 18°N; Kalavati *et al.* 1978, 1984, Kalavati and Narasimhamurti 1980). More recently, dicyemids have been reported from cephalopods inhabiting upwelling areas in the Caribbean Sea off La Guaira-Macuto, Venezuela (~11°N: Penchaszadeh *et al.* 1996). Heavy infections of dicyemids are also known to be present in *Sepia latimanus* from Guam (G. Pauly pers. comm.). In our study dicyemids were detected in three tropical octopuses, namely *Octopus alpheus*, *O. dierythraeus* and *O. ornatus*. Two attributes of infections in tropical hosts may account for the scarcity of positive records of dicyemids in tropical cephalopods. Firstly, tropical animals often have lower densities of dicyemids within the renal appendages than typically observed in temperate hosts. As a consequence the presence of dicyemids was not initially detected in the examination of fresh material of several species in the field. It was only detected later in the laboratory when stained preparations were examined. Previous studies of tropical cephalopods may have discounted infections on the basis of such fresh examinations. Secondly, in the case of two tropical octopus species, *Octopus ornatus* and *O. dierythraeus*, the dicyemids were extremely tiny species probably related to the rare genus *Conocyema*. These dicyemids were firmly attached to the renal appendages of the host so that they were not dislodged by conventional smearing techniques. Their detection was the result of observations of finely macerated, fresh renal tissue and not fresh smears or fixed and stained preparations.

In summary, the susceptibility of potential cephalopod hosts to dicyemids, as a percentage of species examined, was found to decrease with decreasing latitude (Figure 2): 10 of 11 species (90.9%) examined in the temperate zone were found to possess dicyemids, while six of the nine species (66.6%) were infected in the subtropical zone and only three of seven species (42.8%) were infected in the tropical zone.

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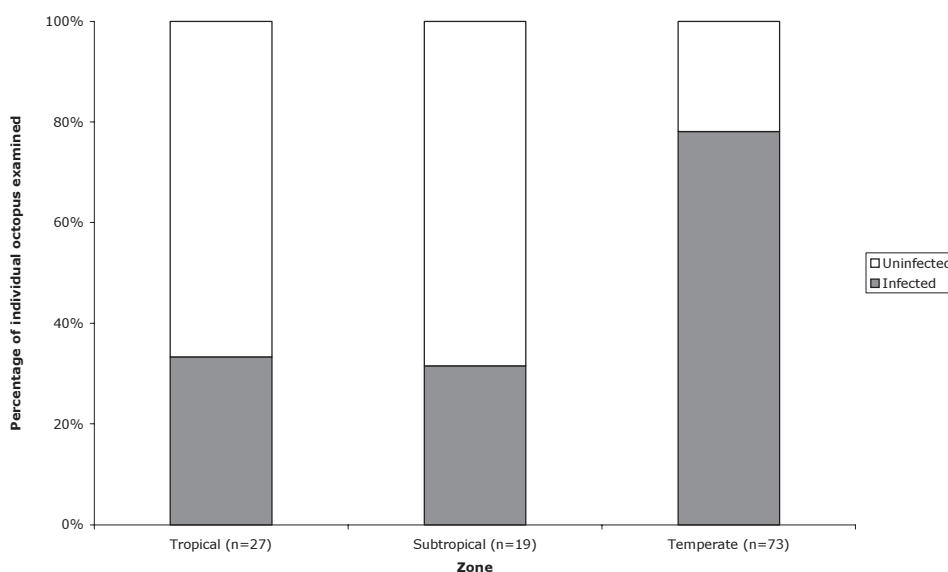


Figure 3. Percentage of individual octopus infected across the three biogeographic regions sampled in this study.

Cues for dicyemid maturation: Observations made during this investigation may provide some insight into the little-known life cycle of dicyemids and the unresolved pathway of infection. In several of the octopuses examined, individual hosts were found to have different densities or stages of dicyemids in the two separate renal coeloms. The majority of previous studies typically treated both renal appendages as a single entity. Dicyemids in the separate renal coeloms of individual octopus hosts were found to be at different stages of maturity. In several individual hosts, dicyemids were present in only one of the two renal coeloms.

Dicyemids move through three worm-like (vermiform) stages within the host cephalopod (see Hochberg 1990). The earliest known stage within the host is the stem nematogen. It is replaced by nematogens that reproduce asexually to increase the number of vermiform individuals in the coelom. In response to an unknown trigger nematogens are either directly transformed into or begin to produce rhombogens, a form within which the sexual phase of the life cycle occurs. Following fertilization and development a dispersal phase (infusoriform) is produced that leaves the host.

The factor, or factors, that trigger the transition from nematogen to rhombogen production has long been speculated. As the transition in stages has been seen to occur in the majority of dicyemids in an individual host at approximately the same time, it is generally accepted that the change in phase is induced by a factor that affects the dicyemid population as a whole rather than the simple maturation of each individual dicyemid (McConnaughey 1951). McConnaughey reported dicyemid infections to differ in reproductive stage between individual hosts of the same species, size and maturity that had been sampled at the same time. According to McConnaughey asynchrony indicates that life cycle stages are not dictated by external seasonal influences.

Instead, the stage of the dicyemid life cycle more often appears to correlate with the maturity of the cephalopod host. Juvenile to immature hosts generally harbour mixed populations of stem nematogens and nematogens, while more mature hosts usually are found to possess infections dominated by rhombogens (Hochberg 1983, 1990). Apparent synchrony with sexual maturation of the host has lead some authors to speculate that the

hormonal flux associated with host maturation may act as trigger to life cycle changes (see summary in Hochberg 1983). A change in phase also can be correlated with density of dicyemids, usually occurring when the renal appendages are heavily populated with nematogens (Lapan and Morowitz 1972). Lapan and Morowitz (1972) proposed that high population density may trigger the shift in dicyemid life cycle stage, mediated via chemical or mechanical cues from the dicyemid rather than the host.

In our study the stage of dicyemid infections was found to differ between adjacent renal coeloms within single octopus hosts. In the most marked example, an individual of *Hapalochlaena maculosa* had only nematogens within the left renal coelom, while the right coelom was populated entirely with rhombogens. If the transition from the nematogen to the rhombogen stage was the result of host hormonal flux it would be expected that both coeloms would be equally affected, and therefore harbour the same stage in the life cycle. Our findings indicate that the infection within each coelom develops independently with isolated developmental cues and also supports the suggestion that the transition from nematogen to rhombogen is triggered from elements within the dicyemid population rather than the host individual.

Insights into the infection mode of dicyemids: The mode of entry of an infecting agent into the host and the initiation of infection also remains unknown. Lapan and Morowitz (1972) proposed that germinal cells from the urn of the infusiform could directly infect the circulatory system of the host and from there penetrate into the kidneys. Our findings of dicyemid infections in only a single renal appendage of single individuals (*Octopus bunurong* and *O. kaurna*) however suggest independent infection of each renal coelom. This phenomenon is consistent with motile infecting agents entering the kidney via a passage restricted

to each coelom (*i.e.* renal pore) and not via the blood, a medium common to both renal coeloms.

The proposal of independent infection via the renal papillae could also explain dicyemids at different stages of maturity in each renal coelom of a single host. In the *Hapalochlaena maculosa* individual discussed above, the right renal appendages could have been infected earlier than the left and, therefore, at the time of sampling the dicyemids were at a later stage in the life cycle. In addition, different degrees and stages of infection within adjacent renal appendages confirm that the renal appendages are isolated in the octopods and the passage of dicyemids between appendages does not occur.

This investigation has raised more questions than answers. There is still much to be learned of the taxonomy, life cycles, and distributions of members of this unusual phylum.

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