



Title	HOST SWITCHING IN DICYEMIDS
Author(s)	中島, 大暁
Citation	大阪大学, 2026, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/105654
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Doctoral Dissertation

HOST SWITCHING IN DICYEMIDS

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Jan. 2026

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概要

二胚動物門ニハイチュウ (*Dicyemida*) は、底生の頭足類軟体動物、特にタコ類およびコウイカ類の腎嚢内に生息する内部共生生物として、最も一般的かつ最も特徴的な存在である。

通常、単一の宿主個体内には 2 種または 3 種のニハイチュウ類が共存しており、ほとんどのニハイチュウ類は高度な宿主特異性を示す。

一般に、宿主特異的な寄生生物は、分散や新規宿主への定着を妨げる生態学的障壁によって、利用可能な宿主種の範囲が制限されるため、反復的な共進化 (co-speciation) の結果として、宿主と寄生者の系統関係は一致すると予想されることが多い。

しかし実際には、宿主と寄生者の系統が一致しない事例が頻繁に認められており、これは宿主転換、絶滅、その他のマクロ進化的過程によって説明される。

本研究では、ニハイチュウ類の進化史およびそれらと頭足類宿主との関係を、宿主と寄生者の系統を比較することによって検討した。

日本近海に分布する底生頭足類 20 種、すなわち 十腕形上目 Decapodiformes に属する 8 種および 八腕形上目 Octopodiformes に属する 12 種からニハイチュウ類を採集した。

ミトコンドリア cytochrome c oxidase subunit 1 (*COI*) 遺伝子の全長配列を用いて、*Dicyemidae* 科に属する 4 属を代表する 37 種のニハイチュウ類について分子系統樹を再構築した。

COI アミノ酸配列解析に基づいて推定された系統樹は一貫して、ニハイチュウ類が 3 つの主要クレードに分かれることを示した。

さらに、最も種数が多く一般的に認識されている 2 属である *Dicyema* および *Dicyemeneea* が、それぞれ単系統群を形成しないことが明らかとなった。

これらの結果は、形態形質に基づく現行の分類体系が、これら 2 属の進化的関係を適切に反映していないことを示している。

加えて、系統パターンは、ニハイチュウ類の分化および種形成が単一の宿主種内で頻繁に生じ、同一宿主内に近縁種が集積することを示唆している。

さらに、本研究で再構築された系統関係は、Octopodiformes と Decapodiformes という 2 つの主要な頭足類系統群間、あるいはそれぞれの系統群内において、複数回の宿主転換が生じた可能性を示している。

これらの知見を総合すると、ニハイチュウ類の多様化は、厳密な宿主—寄生者共進化のみによって進行したのではなく、宿主内種分化と宿主転換が組み合わさった過程によって進行してきたと考えられる。

本研究は、多数のニハイチュウ類種および属を対象とした分子系統解析に基づき、頭足類宿主との関係を含めたニハイチュウ類の多様化過程を評価した初めての包括的研究である。

ABSTRACT

Dicyemids (phylum Dicyemida) are the most common and most characteristic endosymbionts inhabiting the renal sacs of benthic cephalopod molluscs, particularly octopuses and cuttlefishes. Typically, two or three dicyemid species coexist within a single host individual, and most dicyemids exhibit a high degree of host specificity. In general, host-specific parasites are restricted to a limited range of host species by ecological barriers that constrain dispersal and successful establishment in novel hosts; as a result, the phylogenies of hosts and their parasites are often expected to be congruent due to repeated co-speciation events. However, empirical studies frequently demonstrate incongruence between host and parasite phylogenies, which can be attributed to processes such as host switching, extinction, and other macroevolutionary events.

In this study, the evolutionary history of dicyemids and their associations with cephalopod hosts were examined by explicitly comparing parasite and host phylogenies. Dicyemid species were collected from a total of 20 benthic cephalopod species, including 8 decapodiform species and 12 octopodiform species, sampled from Japanese waters. Using complete mitochondrial cytochrome *c* oxidase subunit 1 (COI) amino acid sequences, a molecular phylogeny was reconstructed for 37 dicyemid species representing four genera within the family Dicyemidae.

Phylogenetic trees inferred from COI sequence analyses consistently indicated that dicyemid species are divided into three major clades. Furthermore, the analyses revealed that the two most species-rich and commonly recognized genera, *Dicyema* and *Dicyemene*, do not form monophyletic groups. These results demonstrate that the current morphology-based classification does not adequately reflect the evolutionary relationships within these genera. The phylogenetic patterns further suggest that divergence and speciation of dicyemid species frequently occur within a single host species, resulting in clusters of closely related species inhabiting the same host.

In addition, the reconstructed phylogeny provides evidence for multiple host-switching events, which may have occurred either between the two major cephalopod lineages, Octopodiformes and Decapodiformes, or within each of these host groups. Taken together, these findings indicate that the diversification of dicyemids has proceeded through a combination of intra-host speciation and host switching, rather than through strict host–parasite co-speciation alone. This study represents the first comprehensive evaluation of dicyemid diversification in relation to cephalopod hosts based on a large number of dicyemid species and genera using molecular phylogenetic data.

INTRODUCTION

In parasite evolution, the relationship with the host is fundamental and cannot be considered independently. This is particularly true for host-specific parasites, whose evolutionary trajectories are constrained by ecological barriers such as host habitat, host life history, and parasite dispersal and transmission pathways. Under such constraints, parasites are often expected to speciate in parallel with their hosts through repeated co-speciation, resulting in congruent host and parasite phylogenies (Hafner et al., 1994; Page et al., 1998). However, strict congruence between host and parasite phylogenies is not universally observed (Huelsenbeck et al., 2003), and alternative macroevolutionary processes—including host switching, duplication, lineage sorting, and failure of parasite speciation—have been proposed to account for such patterns (Brooks and McLennan, 1991; Page, 1996, 2003; Hoberg et al., 1997).

The dicyemids (phylum Dicyemida) provide a distinctive system in which to consider these issues, primarily because of the extreme restriction of their infection site. Dicyemid mesozoans are the most common and characteristic endosymbionts inhabiting the renal sacs of benthic cephalopod molluscs, namely octopuses and cuttlefishes. In cephalopods, the renal organs consist of the renal complex (renal and pancreatic appendages) and the branchial heart complex (branchial heart and pericardial appendage). Prior to excretion, urine accumulates in the renal sac, a fluid-filled lumen that constitutes a highly specialized and enclosed environment supporting a diversity of endosymbionts (Hochberg, 1982; Furuya et al., 2004). Dicyemids are confined to this organ and are not known to inhabit other tissues or host groups.

Dicyemids possess a remarkably simple body organization, consisting of a single central cylindrical axial cell surrounded by a single layer of 8–30 ciliated peripheral cells. They lack body cavities and differentiated organs, and their organization does not correspond to the typical diploblastic or triploblastic body plans of metazoans. On the basis of this simplicity, Van Beneden (1876) introduced the term “Mesozoa” to denote their presumed intermediate status between Protozoa and Metazoa, and dicyemids were long regarded as primitively simple multicellular organisms (Hyman, 1940, 1959; Lapan and Morowitz, 1974). Subsequent zoological and molecular studies, however, have suggested that this simplicity is more plausibly interpreted as secondary reduction associated with parasitism rather than primitiveness (Nouvel, 1947; Stunkard, 1954; Ginetsinskaya, 1988). Molecular phylogenetic analyses now place dicyemids within the Lophotrochozoa, indicating that they are highly derived rather than basal metazoans (Katayama et al., 1995; Kobayashi et al., 1999; Aruga et al., 2007; Suzuki et al., 2010; Lu et al., 2017, 2019).

To date, 149 dicyemid species have been described from cephalopod hosts distributed across a wide geographical range, including the Okhotsk Sea, Sea of Japan, the western and eastern North Pacific, South Pacific, North Indian Ocean, Mediterranean Sea, North Atlantic, Gulf of Mexico, and Antarctic

Ocean (Nouvel, 1947; McConnaughey, 1951; Hochberg, 1990; Furuya, 1999, 2016, 2018; Furuya and Tsuneki, 2003; Catalano, 2013; Castellanos-Martínez et al., 2016; Furuya and Moritaki, 2022). Despite this broad distribution, dicyemids exhibit a high degree of host specificity: typically two or three dicyemid species coexist within a single host individual, often within the same renal sac, and fewer than 10% of known species have been reported from more than one host species (Furuya, 1999, 2018; Furuya and Moritaki, 2022).

The renal sac as a discrete anatomical niche suitable for dicyemid colonization is characteristic of cephalopods and is not found in other molluscan groups. Consequently, the association between dicyemids and cephalopods represents a highly specific correspondence between parasite lineages and a particular host organ, rather than a diffuse association with host species as a whole. Within this restricted and uniform environment, the coexistence of phylogenetically distinct dicyemids raises questions about the processes underlying their diversification. Although the present study does not formally assess host–parasite phylogenetic congruence, the occurrence of distantly related dicyemids associated with the same host species provides an empirical basis for discussing evolutionary scenarios beyond simple parallel divergence.

Experimental observations have suggested that dicyemids may have a direct life cycle without intermediate hosts; however, the empirical evidence supporting this view remains limited, and the routes of transmission among cephalopod hosts are still poorly understood. Patterns of host association and parasite diversity may therefore offer indirect insights into the constraints acting on dicyemid transmission, even if infection pathways are not explicitly resolved.

Interpretation of these host–parasite associations is facilitated by the relatively well-resolved molecular phylogeny of cephalopods, which is established at least at the genus level (Sanchez et al., 2018). Taken together, the extreme restriction of the infection site, the confinement of suitable host organs to cephalopods, and the availability of a robust host phylogeny make the dicyemids–cephalopods system particularly suitable for examining patterns of parasite diversification within a highly constrained anatomical niche.

In the present study, the co-evolutionary history of dicyemids and their host cephalopods was evaluated by comparing their phylogenies. The results indicate that both co-speciation and host switching occur in dicyemid species.

The family Dicyemidae currently comprises the genera *Dicyema*, *Dicyemennea*, *Dicyemodeca*, *Dodecadicyema*, and *Pseudicyema*. *Dodecadicyema* is a rare genus found only once in Indian waters.

In this study, the remaining 4 genera were included in the phylogenetic analysis. These genera are classified based on differences in the number and arrangement of cells in the calotte (Fig. 1). Furuya et al. (2001) reconstructed the phylogenetic relationships among genera based on developmental patterns and suggested that developmental differences among the genera are reflected in morphological differences. However, whether morphological classification accurately reflects

phylogenetic relationships remains to be determined and was addressed in this study.

This work included a large number of dicyemid species, with data on some Australian species collected from data banks, and is expected to make a significant contribution to the phylogeny of this animal taxon.

MATERIALS AND METHODS

Sample collection

Samples from 20 cephalopod species located in Japanese waters were examined for dicyemids between 2002 and 2021 (Table I). Host specimens were identified based on their morphological characters (Sasaki, 1929; Okutani et al., 1987) and comparison of nucleotide sequences of mitochondrial cytochrome *c* oxidase subunit 1 (*COI*). Small pieces of the renal organ with attached dicyemids were removed and smeared on microscope slides to identify the dicyemid species. The smears were fixed promptly in Bouin's fluid for 24 hr and then stored in 70% ethanol. Most were later stained in Ehrlich's hematoxylin and counterstained with eosin. After staining, the samples were dehydrated through an ascending ethanol series to xylene and mounted in Entellan New (Merck, Darmstadt, Germany). Dicyemids were observed with an optical microscope (BH-2, Olympus, Tokyo, Japan).

Contaminating host tissues were carefully removed from the collected dicyemids under a stereomicroscope, and dicyemids were washed several times with artificial seawater. At least 50 individual specimens per species were collected for each DNA extraction, which was conducted with a DNeasy Blood and Tissue kit (QIAGEN, Nordrhein- Westfalen, Germany) following the manufacturer's instructions or with the HOT-Shot method (Truett et al., 2000). Based on morphological characters, 8 genera were recognized and placed in 3 families: Conocycemidae, Dicyemidae, and Kantharellae (Hochberg, 1990; Furuya, 2016). In the present study, 37 dicyemid species from the 4 genera (*Dicyema*, *Dicyemennea*, *Dicyemodoca*, and *Pseudicyema*) were collected and their collection dates recorded (Table I).

Amplification and sequencing of a target gene *COI*

Whole *COI* sequences were amplified by PCR using 3 primer sets (COI_9F: 5'-ATYATYGCWGTYCCBACWGG-3'; COI_6R: 5'-CCRAAGAAYCARAANARRTG-3'; COI_1-1-4F: 5'-TTRYTRATTCKTATCATTG-3'; COI_1-1-4R: 5'-ACAAGRATVCCRTAHACRAGWGA-3'; COI_1-1-5F: 5'-TTYCCNCGDWTDAATGC-3'; and COI_1-1-5R: 5'-CCWGADGTWCC-NCCRAT-3'), which were designed to amplify dicyemid *COI* based on the report by Watanabe et al. (1999). PCR products were treated with Freeze 'N Squeeze™ DNA Gel Extraction Spin Columns (BioRad, Hercules, California) to remove excess primers and dNTPs. Some samples were directly sequenced with ABI BigDye® Terminator v. 3.1 in an ABI 3730XL Sequencer (Thermo Fisher, Waltham, Massachusetts) using the same primers. The other amplified DNA fragment was cloned in p-GEM-T Vector (Promega, Madison, Wisconsin). Plasmid DNA from transformant colonies was

purified with a QIAprep® Miniprep kit (QIAGEN). Both strands of plasmid DNA were fully sequenced with the T7 primer upstream and the SP6 primer downstream of the insert site using the dideoxy chain-termination method with ABI BigDye Terminator v. 1.1 or 3.1 (Sanger et al., 1977). Dicyemid mitochondrial genes are separate circular DNA molecules (minicircles) (Watanabe et al., 1999). For each species, 1,288–2,284 bp were determined from the whole *COI* minicircle molecule containing the *COI* gene and an associated non-coding region. The sequences were deposited in the DNA Data Bank of Japan database; accession numbers are listed in Table I.

Data processing for phylogenetic analysis

The ORFs (open reading frames) were identified and translated into amino acid sequences using the transeq command in EMBOSS:6.5.0.0 (Rice et al., 2000; Goujon et al., 2010) and the invertebrate mitochondrial code (translation table 5). The amino acid sequence was searched for the COI domain with hmmscan of Hmmer 3.1 (<http://hmmer.org>), and a part of the sequence was extracted. Multiple alignments of extracted COI sequences were designed using MAFFT (Strategy, L-INS-I, Katoh et al., 2005) with the homologous sequences of 49 dicyemids and 1 sequence of the gastrotrich *Diuronotus aspetos* (accession number LC575089) as the outgroup. Sequences with exact matches were removed to avoid node density artifacts (Webster et al., 2003; Venditti et al., 2006), and gaps and low-reliability regions were removed by the gappyout method of trimAl v. 1.2 (Capella-Gutierrez et al., 2009).

Phylogenetic analysis

The maximum likelihood method was used with Aminosan (Tanabe, 2011) to select the mtZoa + F Gamma model as the optimal model for molecular evolution. Phylogenetic trees were inferred with this model using the maximum likelihood method with RAxML 8.2.9 (Stamatakis, 2014). The phylogenetic trees were tested for reliability with a bootstrap test (1,000 trials) in RAxML and displayed with bootstrap values in FigTree v. 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

We mapped the host cephalopod (Octopodiformes or Decapodiformes) to the phylogenetic tree of dicyemids and inferred which ancestral host in each clade was the Octopodiformes or Decapodiformes host. The most recent common ancestor (MRCA) for host usage was inferred by the maximum likelihood method using Mesquite version 3.7 (Maddison and Maddison, 2021). For each operational taxonomic unit, 3 states were set for host usage: free-living invertebrates, Octopodiformes, or Decapodiformes. I set whether the host was Octopodiformes or Decapodiformes as the character states. *Diuronotus aspetos* (Gastrotricha) was selected as the outgroup, but this species is not a cephalopod parasite; thus, the character state was set as free-living invertebrate. Ancestral character states were estimated by maximum likelihood. I selected the Likelihood Ancestral State for the ancestral state reconstruction method in the program and used the Stored Probability Model for the probability

character model. In the case of a clade formed by co-speciation of dicyemids and cephalopods, either Octopodiformes or Decapodiformes, the MRCA probability indicates a value of 100%. If a host switch has occurred, the MRCA probability will never show a value of 100%, and if many host switches have occurred, the value is small and it is unclear whether the Octopodiformes or Decapodiformes was the MRCA.

Genus name abbreviations

To achieve brevity but avoid confusion, the following genus name abbreviations are employed here: A., *Amphioctopus*; C., *Callistoctopus*; D., *Dicyema*; Dn., *Dicyemene*; Dd., *Dicyemodonta*; O., *Octopus*; Op., *Opisthoteuthis*; P., *Pseudicyema*; S., *Sepia*; Sl., *Sepiella*; and St., *Sepioteuthis*.

RESULTS

Molecular phylogenetic relationships among dicyemid species

Phylogenetic trees based on the analyses of *COI* genes consistently suggest that dicyemid species are separated into 3 major clades (Fig. 2). The relationships among clades cannot be determined due to the low bootstrap values.

The first clade (clade A) includes 16 species of 3 genera: *Dicyema clavatum*, *Dicyema coffinense*, *Dicyema erythrum*, *Dicyema furuyi*, *Dicyema koinonum*, *Dicyema orientale*, *Dicyema oxycephalum*, *Dicyema papuceum*, *Dicyema multimegalum*, *Dicyema rhadinum*, *Dicyema sepiellae*, *Dicyema* sp., *Dicyema vincentense*, *Dicyemennea minabense*, *Pseudicyema nakaoi*, and *Pseudicyema* sp. Ten dicyemid species in this clade belong to *Dicyema* from decapodiform cuttlefishes, the exceptions being *D. clavatum* from *Callistoctopus minor*, *D. erythrum* from *Amphioctopus fangsiao*, and *D. orientale* from the squid *Sepioteuthis lessoniana*. This cluster contains 6 *Dicyema* species from Australian cuttlefishes: *D. coffinense*, *D. koinonum*, and *D. multimegalum* from *Sepia apama*; *D. furuyi* and *D. papuceum* from *Sepia papuensis*; and *D. vincentense* from *Sepia novaehollandiae*. *Pseudicyema nakaoi* from *Sepia esculenta* and *Pseudicyema* sp. from *Sepia lycidas* are clustered and form a sister relationship with *D. erythrum* from *A. fangsiao*. *Dicyemennea minabense* from *S. esculenta* is included in a cluster of *Dicyema* with *D. sepiellae* from *Sepiella japonica* and *Dicyema* sp. from *Euprymna morsei*.

The second clade (clade B) consists of 8 species of *Dicyema* found in the shallow water octopuses: *Dicyema acuticephalum*, *D. japonicum*, and *D. misakiense* from *Octopus sinensis*; *D. akashiense*, *D. awajiense*, *D. colurum*, and *D. helocephalum* from *A. fangsiao*; and *D. sphyrocephalum* from *Callistoctopus minor*. In this clade, each dicyemid species is closely related to the other dicyemids within its host species: *O. sinensis* or *A. fangsiao*.

The third clade (clade C) is composed of 3 subclades. One subclade is composed of 2 species of different genera: *Dicyema koshidai* from *St. lessoniana* and *Dicyemennea leptcephalum* from *Octopus longispadiceus*. A second subclade includes 3 genera from octopod species and decapodiform sepiid species: *Dicyema codonocephalum* from *O. sasakii*; *Dicyemennea floscephalum* from *Octopus berrima*; *Dicyema leiocephalum* from *Amphioctopus areolatus*; *Dicyema lycidoeceum* from *S. lycidas*; *Dicyema tosaense* from *Amphioctopus kagoshimensis*; *Dicyemennea desmocephalum* from *Octopus tenuicirrus*; *Dicyemennea gyrinodes*, *Dicyemennea ophioides*, and *Dicyemennea trochocephalum* from *Octopus hongkongensis*; *Dicyemennea spencerense* from *S. novaehollandiae*; *Dicyemennea umbraculum* from *Opisthoteuthis depressa*; *Dicyemennea* sp. A from *Metasepia tullbergi*; and *Dicyemodoca* sp. from *Octopus conispadiceus*. A third subclade was composed only of species of *Dicyemennea* from octopods and decapodiform sepiolids: *Dicyemennea brevicephaloides* from *Rossia*

pacifica, *Dicyemeneea mcconnaugheyi* and *Dicyemeneea megalosomum* from *O. longispadiceus*, *Dicyemeneea nouveli* from *Enteroctopus dofleini*, *Dicyemeneea rossiae* from *R. pacifica*, and *Dicyema* sp. B from *O. conispadiceus*.

Of the 4 dicyemid genera examined, the 2 most common genera, *Dicyema* and *Dicyemeneea*, are not monophyletic and so were never observed as a single clade. The 2 *Pseudicyema* species are included in clade A, and the *Dicyemodoca* species are included in clade C.

Intra-host speciation and host switching

In general, 2 or 3 (rarely 4) dicyemid species are found in a single cephalopod species: 4 (*D. akashiense*, *D. awajiense*, *D. colurum*, and *D. helocephalum*) in *A. fangsiao*, 3 (*D. acuticephalum*, *D. japonicum*, and *D. misakiense*) in *O. sinensis*, 2 (*Dn. brevicephaloides* and *Dn. rossiae*) in *Rossia pacifica*, *Dn. mcconnaugheyi* and *Dn. megalosomum* in *O. longispadiceus*, and *Dn. trochocephalum* and *Dn. gyrinodes* in *O. hongkongensis*. If the dicyemids had co-speciated with their host cephalopod species, the phylogenetic trees would show congruence, but congruence is incomplete. Clade B consists of Octopodiformes-specific dicyemid species, whereas clade A consists mostly of Decapodiformes-specific dicyemid species, except for *D. clavatum* and *D. erythrum* with their octopod hosts *C. minor* and *A. fangsiao*, respectively (Fig. 2). Clade C includes species from both major cephalopod groups (Fig. 2). Closely related species, such as *D. leiiocephalum* and *D. tosaense*, have been described from *A. kagoshimensis* and *A. areolatus*, respectively.

Possible host-switching events occur both within and between the Octopodiformes and Decapodiformes (Fig. 3). In clade A, the MRCA value is very high (95%), suggesting that the dicyemid species in this clade speciated within the Decapodiformes and subsequently *D. clavatum* and *D. erythrum* switched to hosts in the Octopodiformes. In clade B, the MRCA value is similar (95%), suggesting that the MRCA parasitized an octopodiform host and co-speciation occurred in *A. fangsiao* and *O. sinensis*. In clade C, the MRCA value is not high (67%) possibly due to a switch from 2 decapodiform hosts. However, the MRCA was apparently in an octopodiform host.

There are 2 instances where 2 genetically distant dicyemid species are found in a single cephalopod host species: *St. lessoniana* and *S. lycidas*. *Sepioteuthis lessoniana* has 2 genetically distant dicyemid species: *D. koshidai* and *D. orientale*. The phylogenetic tree indicates that *D. koshidai* moved from a host in the Octopodiformes, and *D. orientale* moved from a sepiid. *Sepia lycidas* also harbors 2 species, *D. lycidoecum* and *Pseudicyema* sp., which are genetically distant dicyemid species.

The phylogenetic tree (Fig. 2) shows that closely related dicyemid species can be associated with different host genera, such as *D. sepiellae* in *Sl. japonica*, *Dicyema* sp. in *Euprymna morsei*, and *Dn. minabense* in *S. esculenta*.

DISCUSSION

Phylogenetic relationships among dicyemid species from Japanese hosts

I used whole mitochondrial COI amino acid sequences to reconstruct a phylogeny of 37 dicyemid species including 4 genera (*Dicyema*, *Dicyemennea*, *Dicyemodeca*, and *Pseudicyema*) representing the family Dicyemidae. Japanese dicyemid species are separated into 3 major clades (Fig. 2). However, the topology of the clades is not conclusive given the low bootstrap support. The divergence of each clade may have occurred in a short period of time from their common ancestor. Relationships within the clades suggest that they are approximately in parallel with relationships among the 4 genera. The largest number of dicyemid species is included in the genus *Dicyema* (about half the total number of dicyemid species) followed by *Dicyemennea* with about 40% of the species. *Dicyemodeca* and *Pseudicyema* each account for 5% of species. Therefore, species in the phylogenetic tree primarily reflect relationships among the 2 most common genera, *Dicyema* and *Dicyemennea*. The tree (Fig. 2) suggests that the currently recognized genera are each paraphyletic, which is inconsistent with the morphological classification. The genera are separated morphologically based on the cell constitution and arrangement of calottes (Nouvel, 1947; McConnaughey, 1949; Hochberg, 1990). The cells of the propolar tier in *Pseudicyema* alternate with those of the metapolar tier, whereas the orientation of cells of the propolar and metapolar tiers are opposite in *Dicyema*. *Dicyema* and *Pseudicyema* species have 4 metapolar cells, *Dicyemennea* species have 5, and *Dicyemodeca* species have 6. Considering that the body of dicyemids is composed of very few cells of fixed number depending on the species, this character could be considered sufficient to distinguish the genera. Even if the difference in cell number were significant, these differences in traits are still too subtle to distinguish the genera with confidence.

Dicyemodeca, including the synonym *Pleodicyema*, is a small genus, and just 5 species have been described, which are found only in the bathyal or mid-depth cephalopods *Bathypolypus arcticus* (Prosch, 1849) (see Nouvel, 1961), *E. dofleini* (see Bogolepova, 1957; McConnaughey, 1957; Furuya, 1999), and *O. tenuicirrus* (see Furuya, 2018). Three species, *Dicyemodeca deca*, *Dd. dogieli*, and *Dd. anthinocephalum*, have been described from *E. dofleini* distributed in the eastern North Pacific Ocean (Washington and Oregon), the Okhotsk Sea (Russia), and the western North Pacific Ocean (Japan), respectively. *Dicyemodeca kukii* was described from *O. tenuicirrus* in the western North Pacific Ocean (Japan) (Furuya, 2018). Most *Dicyemodeca* species are rare, but *Dicyemodeca* sp. is the predominant dicyemid found in *O. conispadiceus* (Sasaki, 1917). *Dicyemodeca* sp. is included in a mixed clade of *Dicyema* and *Dicyemennea*, in which *Dicyemodeca* sp. forms a sister taxon with the *Dicyemennea* cluster. Therefore, *Dicyemodeca* sp. is more closely related to species of *Dicyemennea* than to species of *Dicyema*. This small cluster is composed of the dicyemid species of *O. conispadiceus*, *O. hongkongensis* (Hoyle, 1885), and *O. tenuicirrus*. *Octopus conispadiceus* and *O. hongkongensis* are

“long-ligula octopuses” of the family Enteroctopodidae (Gleadall, 1993; Strugnell et al., 2014). *Octopus tenuicirrus* shares such characters and so belongs to the Enteroctopodidae. Genus *Dicyemodeca* in the Pacific Ocean might have its origin in species of the Enteroctopodidae.

Genus *Pseudicyema* was established by Nouvel (1933) to include a single species, *Dicyema truncatum* Whitman, 1883, which is distinguishable from other species of *Dicyema* principally by the orientation of cells in the calotte. *Pseudicyema* is recorded from shallow-water cephalopod species in both Europe and Japan (Whitman, 1883; Nouvel, 1947; Furuya, 1999, 2009), mainly in *Sepia* species and *Rossia macrosoma* (Chiaie, 1830) (Nouvel, 1947). In *Pseudicyema*, the cells in the propolar tier alternate with cells in the metapolar tier, whereas the tiers typically are opposite each other in *Dicyema*, to which the terms plagiotropal (alternate or bilaterally symmetrical) and orthotropal (opposite or radially symmetrical) have been applied (McConnaughey, 1949). McConnaughey (1949) and Hochberg (1990) identified *Pseudicyema* as a junior synonym of *Dicyema*. In the phylogenetic tree, the *Pseudicyema* cluster of species appears in the branch within the *Dicyema* clade. There is only 1 difference between *Dicyema* and *Pseudicyema*: the orientation of the propolar and metapolar cells in the vermiform stages. The characteristic orientation patterns are easily observed shortly after the vermiform embryos begin to develop in both genera (Furuya et al., 2001). In *D. truncatum* and *P. nakaioi* the cells of the propolar tier consistently alternate with those of the metapolar tier, whereas they are always opposite in *D. acuticephalum* and *D. japonicum* (Furuya et al., 1994, 2001). It is considered most likely that *Pseudicyema* arose within the *Dicyema* species.

The current classification of dicyemids is based on the cell constitution and arrangement of calottes. In vermiform stages of dicyemids, the number of somatic (peripheral) cells is species specific and thus represents significant features to be used in the identification and classification of dicyemids. Developmental studies show no intraspecific variations in calotte peripheral cells (Furuya et al., 1994, 2003), although some variations have been found in trunk peripheral cells (Furuya et al., 2007). Therefore, it is appropriate to use calotte characteristics as the basis for distinguishing among species. Even in the presence of strong selective pressures, mutations in the number of cell divisions of 1 or 2 cells could occasionally occur in the calottes of any dicyemid lineages, and variations in the number of calotte cells are known in each clade, e.g., *Dn. minabense* in the *Dicyema* clade (clade A), in which the calotte cell number probably increased.

Relationships between Australian species and Japanese species

The phylogenetic tree indicates that Australian dicyemid species are found within each clade of Japanese dicyemids, which suggests that there are no significant genetic differences between the Japanese and Australian species, although Australian waters are geographically distant from Japanese waters. Except for the dicyemid species *D. coffinense* and *D. multimegalum* from *S. apama*, the other three Australian species (*D. furuyi*, *D. floscephalum*, and *D. spencerense*) are more closely related to

Japanese species than to other Australian species. These relationships may reflect similarities between the parasites' hosts. Australian species with octopodiform hosts are close to Japanese species that also have an octopodiform host, and Australian species with a decapodiform host are close to Japanese species that also have a decapodiform host. Certainly, the dicyemids are phylogenetically divided by the host group, Octopodiformes or Decapodiformes, excluding those species that have experienced host switching. The ancestral dicyemid presumably lived in the common ancestor of the Octopodiformes and the Decapodiformes and co-specified, more or less, after the Octopodiformes and Decapodiformes separated.

Intra-host speciation and possible co-speciation

In all clades, 2–4 closely related dicyemid species cluster in a single host species, as in *A. fangsiao*, *O. hongkongensis*, *O. longispadiceus*, *O. sinensis*, and *R. pacifica*. The presence of such monophyletic dicyemid species groups suggests that the current species were generated as a result of intra-host speciation from their common ancestor. Because such intra-host speciation occurs in any lineage, it presumably represents the usual way of speciation for dicyemid species.

In coexisting dicyemid species, their calotte shapes are distinctly different, although they are closely related (Furuya et al., 2003). Calotte shapes in dicyemid species from different host species more closely resemble each other than those of dicyemids observed within the same host species, apparently as a result of adaptation to different structural features of host renal tissues, and this helps to facilitate niche separation of coexisting species (Furuya et al., 2003). Whenever intra-host speciation occurs, only species with a calotte shape distinctly different from others could survive in a different niche and thereby avoid competition.

Co-speciation events seem to occur among several closely related dicyemid species. For example, the two closely related dicyemid species *D. leiocephalum* and *D. tosaense* use congeneric host species *A. areolatus* and *A. kagoshimensis*, respectively. The 4 *Dicyemenea* species *Dn. mcconnaugheyi*, *Dn. megalosomum*, *Dn. nouveli*, and *Dicyemenea* sp. form a single congeneric cluster and are closely related. However, their host species (*E. dofleini*, *O. conispadiceus*, and *O. longispadiceus*) are not congeners. Although current taxonomic confusion obscures the fact, both the morphological evidence (Gleadall, 1993) and the molecular phylogeny (Kaneko et al., 2011; Strugnell et al., 2014) suggest that *O. conispadiceus* and *O. longispadiceus* are closely related to *E. dofleini*. Therefore, an ancestor of these *Dicyemenea* species is considered to have co-specified with the ancestor of the 3 octopod species.

Host switching at high frequency

The resulting phylogenies did not indicate a congruent relationship between dicyemids and

cephalopods. In general, host–parasite coevolution can occur in the presence of a tight interaction between hosts and parasites. The prevalence of dicyemids depends upon the cephalopod species and the host growth stage (Nouvel, 1937; Hochberg, 1990; Furuya et al., 1992a, 2003; Furuya, 1999, 2018). Dicyemids are often observed heavily infesting cephalopod renal organs. In contrast to the high prevalence of these parasites in adult cephalopods, juveniles and small cephalopods have a low number of dicyemids (Furuya et al., 1992a). There are also some benthic cephalopod species in which dicyemids have not been detected; e.g., no dicyemids have been found in *Amphioctopus aegina*, *Octopus cyanea*, and *Callistoctopus ornatus* distributed on coral reefs in the subtropical seas of Okinawa and Hawaii (Furuya et al., 2003; F. G. Hochberg, pers. comm.). Although the reason for this absence is not clear, some octopus species do not need dicyemids. Cephalopods and dicyemids do not necessarily have a tight relationship; therefore, complete coevolution is not likely to be established between dicyemids and cephalopods.

Host switching appears to have occurred in this system. The obvious examples are *D. clavatum* and *D. erythrum* found in *C. minor* and *A. fangsiao*, respectively. Based on reconstruction of the ancestral state using the maximum likelihood method, the MRCA is estimated to have been present originally in a cuttlefish host. Therefore, *D. clavatum* and *D. erythrum* probably infested the ancestor of their present octopus hosts via a host switch from the cuttlefish species *S. esculenta*, *S. lycidas*, or one of their common ancestors. *Amphioctopus fangsiao* and *C. minor* share a common habitat with cuttlefish species, which may have enabled the dicyemid species to be transmitted between these cephalopod groups in their habitat.

The coexistence of genetically distant species of dicyemids inhabiting a single host species also should be considered. The big-fin reef squid, *St. lessoniana*, has 2 dicyemid species, *D. koshidai* and *D. orientale* (Nouvel and Nakao, 1938; Furuya and Tsuneki, 2005). Compared with other dicyemid species, the prevalence of these 2 species is not high, 14.2 and 48.7%, respectively (Furuya and Tsuneki, 2005). Pelagic cephalopod species rarely harbor dicyemids, as indicated by reports from *Loligo* sp. in the Indian Ocean (Kalavati and Narasimhamurti, 1980) and from the oceanic squid *Todarodes pacificus* Steenstrup, 1880 in Toyama Bay (Furuya and Tsuneki, 2003). Bayard H. McConnaughey found dicyemids in *Loligo opalescens* off California but did not describe the species (F. G. Hochberg, pers. comm.). Most cephalopod hosts harboring dicyemids are indeed benthic species. It appears that the behavior of dicyemid larvae reduces the chance of contact with pelagic cephalopod species. Dicyemids produce a dispersal stage, the infusoriform larva, which is not planktonic. In the anterior region of the larva, a pair of unique cells, the apical cells, each contain a refringent body composed of a hydrated magnesium salt of inositol hexaphosphate (Lapan, 1975). Its high specific gravity imparts a negative buoyancy to the dispersal larvae, suggesting that the role of the refringent bodies is to help the larvae remain near the sea bottom, where they can encounter another host (McConnaughey, 1951; Lapan, 1975).

Pelagic squid presumably were not original hosts for dicyemids, and it seems that dicyemids may have difficulty in entering such a predominantly pelagic host, resulting in exclusion. *Sepioteuthis lessoniana* forms a complex of three closely related species or subspecies, known by the vernacular Japanese names akaika, kuaika, and shiroika (Izuka et al., 1994, 1996), but dicyemids have been detected only in shiroika. This finding suggests that dicyemids are not closely associated with the *St. lessoniana* complex and have not diverged in tandem with their hosts during the split into this species complex. Although it is not clear why only the shiroika of *St. lessoniana* is associated with dicyemid species, the dicyemids have presumably switched from a different host species. Based on DNA sequence similarities (Fig. 2), *D. koshidai* probably switched from an octopodiform host species, and *D. orientale* probably came from a cuttlefish.

Sepia lycidas harbors two genetically distant dicyemid species, *D. lycidoeceum* and *Pseudicyema* sp., which are in different phylogenetic clusters, clades A and C, respectively. *Pseudicyema* sp. is a rare species found only in hosts from the Pacific Ocean off Miyazaki Prefecture, Kyushu, Japan. Although the characteristics of this species have not been described, the phylogenetic tree (Fig. 2) suggests that *Pseudicyema* sp. is very close to *P. nakaoui*, which is found predominantly in *S. esculenta*. The host species *S. esculenta* and *S. lycidas* are assigned to the Acanthosepion species complex (characterized by the morphology of the sepion or cuttlebone; Khromov et al., 1998), and their affinity is supported by their molecular phylogeny (Yoshida et al., 2006) (Fig. 2). Although *S. esculenta* and *S. lycidas* are certainly related species, probably they have not arisen recently from a common ancestor (Yoshida et al., 2006). Therefore, it is unlikely that *Pseudicyema* sp. has cospeciated in tandem with host speciation, so *P. nakaoui* probably has not switched hosts from *S. esculenta* to *S. lycidas*. However, it seems likely that *D. lycidoeceum* has switched from an octopod host species in clade C.

Some closely related dicyemid species, e.g., *D. sepiellae*, *Dicyema* sp., and *Dn. minabense*, have host species in different genera, such as *Sl. japonica*, *Euprymna morsei*, and *S. esculenta*. These host species share the same habitat and may encounter each other, so a host switch has probably occurred among the sympatric host species. Host switching may have occurred among species in Japanese waters regardless of the large genetic distance between the Octopodiformes and Decapodiformes.

Dicyemid species may be able to switch among cephalopod species sharing the same habitat, with transfer occurring at a relatively high frequency. However, the availability of a suitable niche space for the newcomer may be a factor affecting the success of a host transfer, because presumably there is competition for the niche with the prior inhabitants. Therefore, perhaps only a few dicyemids can survive in a new host, and most new arrivals are unable to compete with the incumbent dicyemid species and are eventually excluded.

The success rate of dicyemids infecting new hosts can be estimated by considering the dicyemid life cycle. The dicyemid community may develop from a small number of individuals (1 or a few) at the initiation of infestation of the renal sac because the success of infesting a new host is low for

dispersal larvae. Dicyemids are occasionally found in only one of the two renal sacs in a host octopus. Two different dicyemids species are occasionally detected, one each in the right and left renal sacs of the same host (Furuya et al., 1992b; Furuya, 2006, 2018). This finding suggests that only a small number of propagules may infest an individual host. For switching hosts, such a low infestation rate could be an advantage because of reduced competition and may be the most significant factor in making a host switch possible. In this context, the probability of a host switch is hypothetically higher in the Octopodiformes than in the Decapodiformes because of the presence of 2 renal sacs in the Octopodiformes but only a single renal sac in the Decapodiformes.

This study is the first to investigate a large number of dicyemid species and genera based on whole COI sequences. The results were used to identify dicyemid species and separate the major clades, therefore elucidating the relationships between families, such as Conocyemidae and Dicyemidae. However, the relationships among the clades cannot be determined, suggesting the limitations of using the *COI* gene for whole phylogenetic reconstruction of dicyemid species. Another gene sequence appropriate for the analysis, such as ITS (nuclear rRNA internal transcribed spacer), would be needed.

Host Switching, Transmission Constraints, and Host Specificity

If host switching has occurred at a high frequency in dicyemids, as suggested by phylogenetic patterns that are difficult to explain otherwise, this implies that their apparently high host specificity is more likely constrained by transmission routes than by strict physiological incompatibility with alternative hosts. If differences in urinary composition, renal tissue architecture, or host immune responses among potential host species strongly prevented establishment, such frequent host-switching events would be unlikely to occur. Rather, it is plausible that the physiological conditions within the renal sacs of different cephalopod hosts are broadly permissive for dicyemid establishment once transmission has been achieved.

Support for this interpretation can be found in other host-specific parasite systems. For example, avian feather lice exhibit high host specificity under natural conditions, yet transplantation experiments have demonstrated that they can survive and reproduce on non-native bird hosts. These results indicate that host specificity in feather lice is largely determined by ecological barriers to transmission rather than by intrinsic physiological incompatibility with alternative hosts (Clayton et al., 2004). Although feather lice are ectoparasites and differ ecologically from endosymbiotic parasites such as dicyemids, this example illustrates how strong host specificity can arise from constraints on transmission rather than from post-infection failure.

In dicyemids, several factors may impose such transmission constraints, including the presumably limited dispersal ability of infusoriform larvae and differences in the reproductive timing of cephalopod hosts. Once even a single parasite individual successfully infects a new host species, however, subsequent establishment may be facilitated by the reproductive biology of dicyemids.

Within the renal sac, parasites can proliferate asexually and generate large numbers of infusoriform larvae through self-fertilization, making reinfection within the same host species relatively easy. Under these conditions, limitations on transmission are likely to play a major role in shaping the observed patterns of host specificity in dicyemids.

CONCLUDING REMARKS

This study investigated the evolutionary history of dicyemids distributed in coastal waters of Japan using molecular phylogenetic analyses. The results indicate that the diversification of dicyemids has been shaped by a combination of processes, including speciation occurring within a single host species and host switching among cephalopod hosts. The associations between dicyemids and their cephalopod hosts cannot be adequately explained by a simple model of strict co-divergence, but instead suggest a more complex and dynamic evolutionary history.

These findings demonstrate that even parasitic animals with extremely simplified body organization, such as dicyemids, can exhibit highly dynamic evolutionary trajectories. Accordingly, this study provides important insights into the mechanisms underlying parasite evolution and diversification, and contributes to a broader understanding of host–parasite evolutionary relationships.

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FIGURES

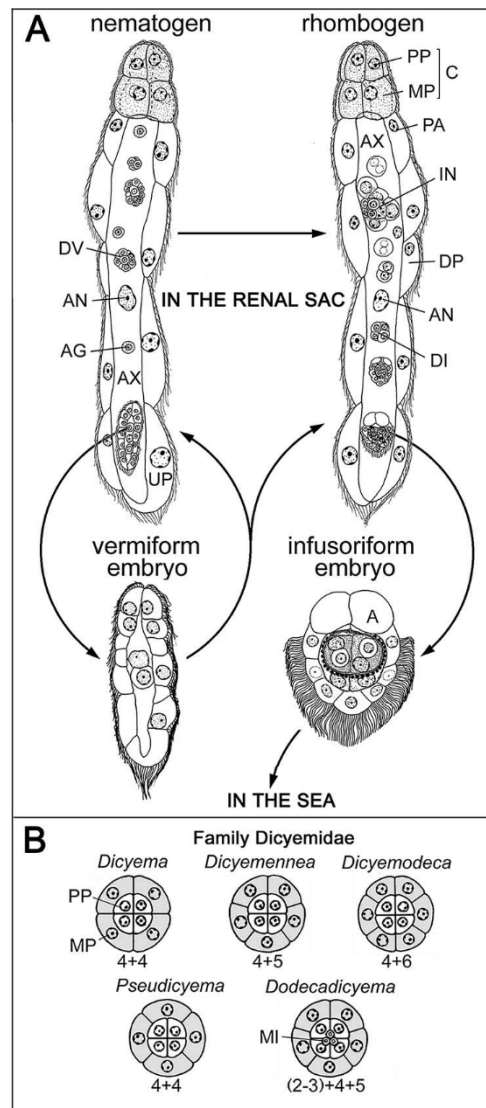


Figure 1. Morphology, life cycle, and classification of genera in dicyemids. (A) The life cycle consists of 2 phases of different body organization (modified from Furuya and Tsuneki, 2003): (1) the vermiform stage, in which a vermiform embryo formed asexually from an agamete (axoblast) develops into the nematogen or rhombogen; and (2) the infusoriform embryo, which develops from a fertilized egg produced around a hermaphroditic gonad called an infusorigen. In the vermiform stage, a large cylindrical axial cell is surrounded by peripheral cells. Four to 10 anterior peripheral cells (propolars and metapolars) form a calotte. The other peripheral cells are called diapolars. Two posterior diapolars are somewhat specialized as uropolars, often containing many granules. How the infusoriform larva develops into the vermiform stage in the new host has not been determined. (B) Enface view of the calottes of 5 genera in the family Dicyemidae (modified from Hochberg, 1990). Genera are classified by the difference in the number of cells in the propolar and metapolar tiers.

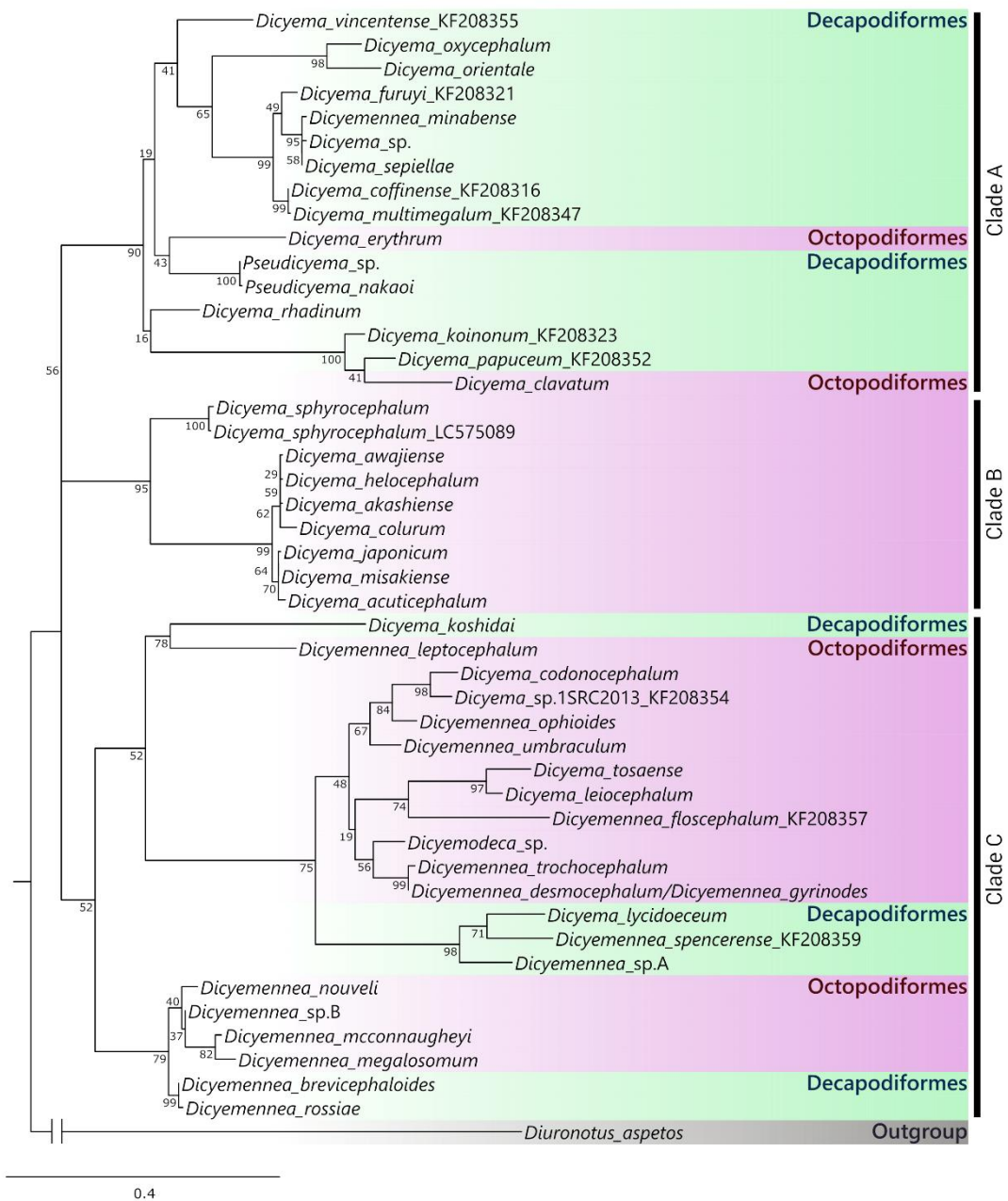


Figure 2. Phylogenetic tree obtained from analyses of mitochondrial COI amino acid sequences. A–C indicate 3 clades; see text for descriptions. Numbers on nodes indicate bootstrap support values .50% (1,000 replicates). Australian species have accession numbers beginning with KF. Bar represents number of substitutions per site.

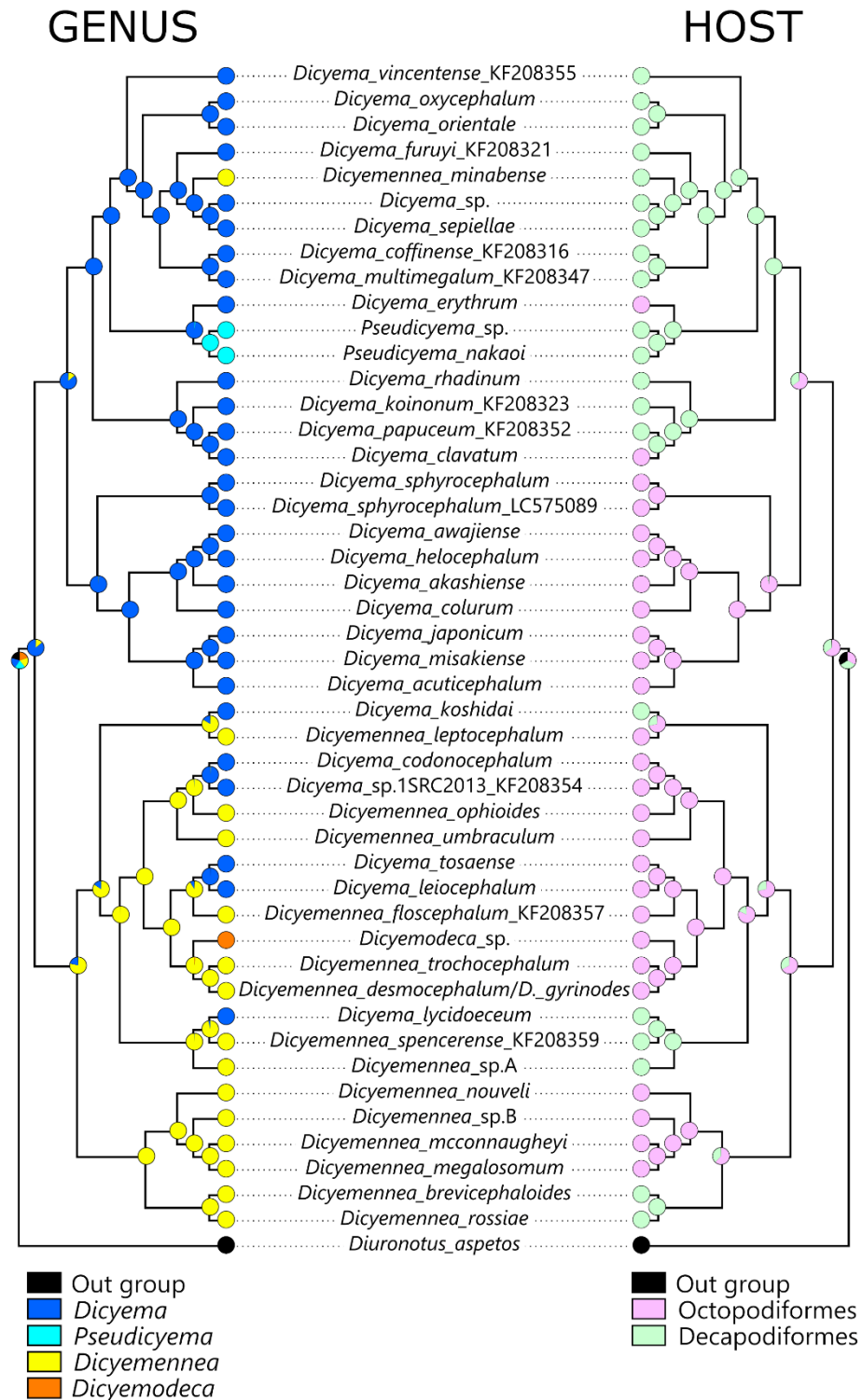


Figure 3. Estimated most recent common ancestor based on the ancestral state reconstruction inferred by maximum likelihood. Australian species have accession numbers beginning with KF.

TABLE

Table I. Dicyemid species and cephalopod hosts.

Dicyemid species	Cephalopod host species	Locality	Collection date†	Accession number‡
<i>Dicyema acuticephalum</i>	<i>Octopus sinensis</i>	Osaka Bay, Osaka, Japan	30-Sep-04	LC770219
<i>Dicyema akashiense</i>	<i>Amphioctopus fangsiao</i>	Harima Sea, Akashi, Japan	17-Dec-04	LC770220
<i>Dicyema awajiense</i>	<i>Amphioctopus fangsiao</i>	Harima Sea, Akashi, Japan	9-Mar-05	LC770221
<i>Dicyema clavatum</i>	<i>Callistoctopus minor</i>	Harima Sea, Akashi, Japan	23-Apr-02	LC571907
<i>Dicyema codonocephalum</i>	<i>Octopus sasakii</i>	Tosa Bay, Irino, Japan	27-Apr-06	LC770222
<i>Dicyema colurum</i>	<i>Amphioctopus fangsiao</i>	Harima Sea, Akashi, Japan	17-Dec-04	LC770223
<i>Dicyema erythrum</i>	<i>Amphioctopus fangsiao</i>	Harima Sea, Akashi, Japan	17-Dec-04	LC770224
<i>Dicyema helocephalum</i>	<i>Amphioctopus fangsiao</i>	Harima Sea, Akashi, Japan	9-Mar-05	LC770225
<i>Dicyema japonicum</i>	<i>Octopus sinensis</i>	Osaka Bay, Osaka, Japan	30-Sep-04	LC770226
<i>Dicyema koshidai</i>	<i>Sepioteuthis lessoniana</i>	Wakasa Bay, Obama, Japan	14-Nov-05	LC770227
<i>Dicyema leioccephalum</i>	<i>Amphioctopus areolatus</i>	Uwa Sea, Yawatahama, Japan	6-Jul-06	LC770228
<i>Dicyema lycidoceum</i>	<i>Sepia lycidas</i>	Osaka Bay, Osaka, Japan	21-Apr-05	LC770229
<i>Dicyema</i> sp.	<i>Euprymna morsei</i>	Harima Sea, Akashi, Japan	31-Jan-06	LC770230
<i>Dicyema misakiense</i>	<i>Octopus sinensis</i>	Osaka Bay, Osaka, Japan	30-Sep-04	LC770231
<i>Dicyema orientale</i>	<i>Sepia lessoniana</i>	Wakasa Bay, Obama, Japan	14-Nov-05	LC770232
<i>Dicyema oxycephalum</i>	<i>Sepia longipes</i>	Suruga Bay, Izu, Japan	24-Apr-08	LC770233
<i>Dicyema rhadinum</i>	<i>Sepia esculenta</i>	Tosa Bay, Irino, Japan	27-Apr-06	LC770234
<i>Dicyema sepiellae</i>	<i>Sepiella japonica</i>	Seto Inland Sea, Ushimado, Japan	12-May-05	LC770235
<i>Dicyema sphyrocephalum</i>	<i>Callistoctopus minor</i>	Harima Sea, Akashi, Japan	23-Apr-02	LC571908
<i>Dicyema tosaense</i>	<i>Amphioctopus kagoshimensis</i>	Osaka Bay, Osaka, Japan	25-Jan-07	LC770236
<i>Dicyemennea brevicephaloides</i>	<i>Rossia pacifica</i>	Sea of Japan, Karo, Japan	24-Sep-06	LC770237
<i>Dicyemennea desmocephalum</i>	<i>Octopus tenuicirrus</i>	Kumano Sea, Owase, Japan	27-Feb-17	LC770238
<i>Dicyemennea gyrinodes</i>	<i>Octopus hongkongensis</i>	Kumano Sea, Owase, Japan	20-Dec-05	LC770239
<i>Dicyemennea</i> sp. A	<i>Metasepia tullbergi</i>	Tosa Bay, Irino, Japan	12-Oct-06	LC770240
<i>Dicyemennea leptoccephalum</i>	<i>Octopus longispadiceus</i>	Sea of Japan, Tottori, Japan	24-Sep-06	LC770241
<i>Dicyemennea mconnaugheyi</i>	<i>Octopus longispadiceus</i>	Sea of Japan, Karo, Japan	1-Nov-14	LC770242
<i>Dicyemennea megalosomum</i>	<i>Octopus longispadiceus</i>	Sea of Japan, Karo, Japan	1-Nov-14	LC770243
<i>Dicyemennea minabense</i>	<i>Sepia esculenta</i>	Kii Channel, Minabe, Japan	26-Oct-05	LC770244
<i>Dicyemennea noveli</i>	<i>Enteroctopus dofleini</i>	Sea of Japan, Tottori, Japan	25-Sep-06	LC770245
<i>Dicyemennea ophioides</i>	<i>Octopus hongkongensis</i>	Kumano Sea, Owase, Japan	20-Dec-05	LC770246
<i>Dicyemennea trochocephalum</i>	<i>Octopus hongkongensis</i>	Kumano Sea, Owase, Japan	20-Dec-05	LC770247
<i>Dicyemennea umbraculum</i>	<i>Opisthotectis depressa</i>	Kumano Sea, Owase, Japan	20-Nov-17	LC770248
<i>Dicyemennea rossiae</i>	<i>Rossia pacifica</i>	Sea of Japan, Karo, Japan	24-Sep-06	LC770249
<i>Pseudicyema nakaoi</i>	<i>Sepia esculenta</i>	Tosa Bay, Irino, Japan	27-Apr-06	LC770250
<i>Pseudicyema</i> sp.	<i>Sepia lycidas</i>	Osaka Bay, Osaka, Japan	21-May-15	LC770251
<i>Dicyemennea</i> sp. B	<i>Octopus conispadiceus</i>	Pacific Ocean, Shiranuka, Japan	21-Jan-21	LC770252
<i>Dicyemodeca</i> sp.	<i>Octopus conispadiceus</i>	Pacific Ocean, Shiranuka, Japan	21-Jan-21	LC770253
<i>Dicyema furuyi</i>	<i>Sepia papuensis</i>	Shark Bay, Western Australia, Australia		KF208321
<i>Dicyema koinonum</i>	<i>Sepia apama</i>	Spencer Gulf, South Australia, Australia		KF208323
<i>Dicyema multimegalum</i>	<i>Sepia apama</i>	Tasman Sea, Cronulla, New South Wales, Australia		KF208347
<i>Dicyema papuceum</i>	<i>Sepia papuensis</i>	Shark Bay, Western Australia, Australia		KF208352
<i>Dicyema</i> sp. 1SRC2013	<i>Octopus kaurana</i>	Spencer Gulf, South Australia, Australia		KF208354
<i>Dicyema vincentense</i>	<i>Sepia novaehollandiae</i>	Gulf St. Vincent, South Australia, Australia		KF208355
<i>Dicyemennea floscephalum</i>	<i>Octopus berrima</i>	Spencer Gulf, South Australia, Australia		KF208357
<i>Dicyemennea spencerense</i>	<i>Sepia novaehollandiae</i>	Spencer Gulf, South Australia, Australia		KF208359
<i>Dicyema sphyrocephalum</i>	<i>Callistoctopus minor</i>	Yellow Sea, Shinan, South Korea		LC575089
<i>Dicyema coffinense</i>	<i>Sepia apama</i>	Coffin Bay, South Australia, Australia		KF208316

† Collection dates for species examined in the present study.

‡ Accession numbers for the DNA Data Bank of Japan (LC) and GenBank (KF).