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Two new species of dicyemid mesozoans (Dicyemida: Dicyemidae) from *Octopus maya* Voss & Solis-Ramirez (Octopodidae) off Yucatan, Mexico

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Abstract Two new dicyemid species are described from the endemic cephalopod *Octopus maya* Voss & Solis-Ramirez collected off Yucatan, Mexico. The renal sacs of 40 juvenile and adult octopuses from four localities were examined. *Dicyema hochbergi* n. sp. is a medium-sized species that reaches 2,245 µm in length. The vermiform stages consist of 18–24 peripheral cells, a conical calotte and the extension of the axial cell between the base and middle of the metapolar cells. Infusoriform embryos consist of 39 cells with urn cell containing one germinal cell, two nuclei and solid refringent bodies. *Dicyema mexcayae* n. sp. is a relatively small species that reaches 1,114 µm in length. The vermiform stages are constituted by 14–16 peripheral cells, an elongate calotte and the axial cell extending forward to the middle of the metapolar cells. The infusoriform embryos consist of 37 cells, two solid refringent bodies and urn cells with two nuclei each. The present study represents the first description of a dicyemid species from *O. maya* and increases the number of described species from Mexican waters to 11.

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Introduction

Dicyemid mesozoans are endosymbionts that live attached inside the renal appendages of cephalopods (Furuya et al., 2004b). The body of dicyemids consists of eight to 40 cells without differentiated organs or body cavities. The body is organised in a two-layer structure consisting of a single, central axial cell surrounded by ciliated peripheral cells (Furuya et al., 2004b). The life-cycle of dicyemids consists of two morphologically distinct stages: (i) the vermiform stages, in which the dicyemid exists as a vermiform embryo formed asexually from an agamete, and as a final form, the nematogen or rhombogen; and (ii) the infusoriform embryo, which develops from a fertilised egg produced around the hermaphroditic gonad called the infusorigen. The latter is formed from an agamete (Hochberg, 1990; Furuya et al., 2004b).

On the one hand, such a simple mode of organisation could be the result of the endoparasitic mode of life (Nouvel, 1947; Stunkard, 1954, 1972; Ginetsinskaya, 1988). On the other hand, dicyemids have been considered as Protozoa by some authors (Cavalier-Smith, 1993). However, molecular phylogenetic studies have classified them as lophotrochozoans (Kobayashi et al., 1999; Suzuki et al., 2010). Therefore, in order to fully understand the dicyemids, additional research effort is needed to clarify their complete life-cycle, taxonomy, phylogeny and systematics (Hochberg, 1990; Catalano, 2012).

To date 122 dicyemid species have been described from a range of diverse cephalopod hosts worldwide (Hochberg, 1990; Catalano 2012, 2013; Catalano & Furuya, 2013). The first records of dicyemid species from Mexican waters were those of McConnaughey (1949a, b), describing seven dicyemid species from three species of cephalopod in the Mexican Pacific Ocean, namely *Octopus bimaculatus* Verrill, *O. bimaculoides* Pickford & McConnaughey and *O. rubescens* Berry. An additional species was later reported from *Amphictopus burryi* (Voss) in the Gulf of Mexico (Furuya et al., 2002). Recently, *Dicyema guaycurense* Castellanos-Martínez, Gómez, Hochberg, Gestal & Furuya, 2011 has been reported from *O. hubbsorum* Berry in the Gulf of California (off La Paz, Mexico) and the Pacific Ocean off Mexico (Castellanos-Martínez et al., 2011). Additional Mexican localities and cephalopod host species from both deep and shallow waters require critical examination to disclose the diversity of dicyemid fauna and their potential use as natural tags to aid in identifying cephalopods (Pascual & Hochberg, 1996; Overstreet & Hochberg, 2009).

In the present study, two new dicyemid species are described from *Octopus maya* Voss & Solis-Ramirez, collected off the Yucatan Peninsula. This octopus species is the most important cephalopod in Mexico because of its endemic distribution and importance for both fisheries and aquaculture. This is the first description of a dicyemid from *O. maya*.

Materials and methods

In total, 40 juvenile and mature specimens of *O. maya* were collected from four sites during the fishery seasons of 2013 and 2014 (Table 1): off Celestun (20°52′N, 90°27′W), Sisal (21°11′N, 90°04′W), Progreso (21°20′N, 89°37′W) and Rio Lagartos (21°38′N, 88°4′W). Octopuses were caught by local fishermen using an artisanal method called *jimba*. For each octopus, dorsal mantle length and wet body weight were measured to the nearest 0.5 cm and 0.1 g, respectively (Table 1). Host sex was identified by direct observation of the reproductive organs by dissection and maturity (juvenile or mature) was assessed following Avila-Poveda et al. (2016): males and females were considered sexually mature at 242 and 335 g of wet body weight, respectively (Table 1).

Renal appendages were removed from each host and cut into small pieces. Infection with dicyemids was immediately determined by examining fresh renal tissue under a compound light microscope at low magnification (10×). Comparison of the pattern of infection between right and left renal appendages, as well as between male and female specimens is not presented since no differences were found. Fifteen smears per host were made on glass coverslips (22 × 22 mm), fixed in Bouin's fluid for 24 h and then stored in 70% ethanol. The smears were then stained in Harris's hematoxylin and counterstained in Eosin 1% alcoholic solution before being mounted in Entellan (Merck, Damstadt, Germany). Dicyemids were observed, measured and drawn using a light microscope (Olympus BH-2) at magnifications up to 2000×, with the aid of an ocular micrometer and a drawing tube.

All measurements are in micrometres and are given as the range followed by the mean ± standard deviation in parentheses. The terminology for cell nomenclature used in the description of infusoriform larvae is based on Nouvel (1948), Short & Damian (1966), Furuya et al. (1992, 1997) and Furuya (1999). Syntypes of the two dicyemids are deposited in the Colección de Invertebrados of the Universidad Nacional Autónoma de Mexico (CI-IBUNAM 0001–0004), in the collection of the Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH 458713–458716), in the Colección Helmintológica del CINVESTAV-Merida (CHCM 524, 524.2, 524.3, 524.4) and in the author's collection. The symbiotype is deposited in the CHCM.

Family Dicyemidae van Beneden, 1882
Genus *Dicyema* von Kölliker, 1849

Table 1 Dicyemid species recorded in *Octopus maya* from off Yucatan, Mexico

Site	Host no.	DML[a] (mm)	BW[b] (g)	Sex	Maturity	Species
Celestun (20°52'N, 90°27'W)						
	OMY17	86	279	♀♀	J[c]	Uninfected
	OMY18	95	324	♀♀	J	Uninfected
	OMY19	127	541	♂♂	M[d]	*D. hochbergi*
	OMY20	100	317	♂♂	M	*D. hochbergi*
	OMY22	91	295	♀♀	J	Uninfected
	OMY23	87	380	♀♀	M	*D. hochbergi*
Sisal (21°11'N, 90°04'W)						
	OMY24	142	744	♂♂	M	*D. hochbergi* + *D.mexcayae*
	OMY25	125	594	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY26	134	845	♀♀	M	*D. hochbergi* + *D. mexcayae*
	OMY27	128	749	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY28	125	511	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY29	113	463	♀♀	M	*D. hochbergi* + *D. mexcayae*
	OMY30	135	690	♀♀	M	*D. hochbergi* + *D. mexcayae*
	OMY31	120	705	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY32	100	347	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY33	108	540	♀♀	M	Uninfected
	OMY34	118	452	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY36	140	1048	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY37	125	539	♂♂	M	*D. hochbergi* + *D. mexcayae*
Progreso (21°20'N, 89°37'W)						
	OMY01	116	403	♀♀	M	*D. hochbergi* + *D. mexcayae*
	OMY02	123	570	♀♀	M	*D. hochbergi* + *D. mexcayae*
	OMY03	111	372	♂♂	M	Uninfected
	OMY04	138	586	♀♀	M	*D. hochbergi* + *D. mexcayae*
	OMY05	116	330	♀♀	J	*D. hochbergi* + *D. mexcayae*
	OMY06	104	369	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY07	118	467	♀♀	M	Uninfected
	OMY08	126	502	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY09	92	247	♀♀	J	Uninfected
	OMY10	111	357	♀♀	M	Uninfected
	OMY13	95	405	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY14	104	366	♂♂	M	*D. mexcayae*
	OMY15	122	547	♀♀	M	*D. mexcayae*
Rio Lagartos (21°38'N, 88°4'W)						
	OMY57	145	1230	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY58	140	1216	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY59	160	1586	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY60	135	1187	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY61	190	3367	♂♂	M	*D. hochbergi* + *D. mexcayae*
	OMY65	200	2400	♀♀	M	*D. hochbergi* + *D. mexcayae*
	OMY66	140	2513	♂♂	M	*D. hochbergi* + *D. mexcayae*

Table 1 continued

Site	Host no.	DML[a] (mm)	BW[b] (g)	Sex	Maturity	Species
	OMY67	120	640	♂♂	M	*D. hochbergi*+ *D. mexcayae*

[a] Dorsal mantle length; [b]Wet body weight; [c]Juvenile; [d]Mature

Dicyema hochbergi **n. sp.**

Type-host: Red octopus, *Octopus maya* Voss & Solis-Ramirez (Mollusca: Cephalopoda: Octopodidae); male, mantle length 145 mm, wet body weight 1,230 g; (author's collection, SCM-57).
Type-locality: Gulf of Mexico, Mexico, off Yucatan, Rio Lagartos, 21°38′N, 88°4′W, depth 8 m. Collected by *jimba*.
Additional localities: Mexico, Yucatan, Sisal, 21°11′N, 90°04′W; Progreso, 21°20′N, 89°37′W; Celestun, 20°52′N, 90°27’W.
Site in host: Renal appendages within the renal sacs.
Prevalence: 75% (8/8 in Rio Lagartos, 7/13 in Progreso, 12/13 in Sisal, 3/6 in Celestun).
Type-material: Allocation of specimens on slides is as follows: 3 syntype slides deposited in the Colección de Invertebrados of the Universidad Nacional Autónoma de Mexico (CI-IBUNAM 0002, CI-IBUNAM 0003, CI-IBUNAM 0004), 3 in the collection of the Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH 458713, 458715, 458716) and 3 slides in the Colección Helmintológica del CINVESTAV-Merida (CHCM 524, CHCM524.2, CHCM524.4).
Collector and date: Sergio Herrera Peraza, Isidro Puerto Esquivel, Gregory Arjona, Francisco Puc; November-December, 2013, 2014.
Etymology: The species is named for F.G. Hochberg (Curator Emeritus, Invertebrate Zoology, Santa Barbara Museum of Natural History) in honor of his lifetime contribution to teaching and research of cephalopods and their parasites.

Description (Figs. 1–2)

Diagnosis: Medium-sized dicyemid; body length 168–2,245 (748 ± 352). Calotte conical in shape, blunt, rounded; propolar and metapolar cells opposite to each other. Vermiform stages with 18–24 peripheral cells: 4 propolar cells, 4 metapolar cells and 10–16 trunk cells. Infusoriform embryos consist of 39 cells; refringent bodies solid and 2 nuclei in each urn cell.

Stem nematogen [Based on 1 specimen; Fig. 1H.] A single stem nematogen was found in the hosts from off Celestun. Similar to nematogens in shape and body proportions. Body length 454, width 53; with three axial cells (Fig. 1H). Peripheral cell number 22 (4 propolars, 4 metapolars, 2 parapolars, 10 diapolars, 2 uropolars). Agametes (3–11) and vermiform embryos (5–10) found in each axial cell.

Nematogens [Based on 228 specimens; see Table 2, Figs. 1A, B, 2A, C.] Body length 168–2,245 (644 ± 409), trunk width 12–72 (29 ± 10), uniform, slightly wider in the region of parapolar cells (Fig. 1A), peripheral cells number 18–24 (Table 2): 4 propolars, 4 metapolars, 2 parapolars, 6–12 diapolars and 2 uropolars (Fig. 2A). Calotte conical, rounded anteriorly (Fig. 1B); cilia on calotte 4 long, oriented anteriorly. Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively (Fig. 2C). Verruciform cells absent. Axial cell cylindrical, rounded anteriorly; extending forward to the middle of metapolar cells (Fig. 2C). Cytoplasm of propolar and metapolar cells more darkly stained by hematoxylin than that of trunk cells. About 43 vermiform embryos present in an axial cell of a large individual.

Vermiform embryos [Based on 12 specimens, Figs. 1C; 2D–E.] Length of full-grown vermiform embryos 50–80 (68 ± 10), width 8–14 (11 ± 2) (Fig. 1C). Peripheral cells number 18–24 (Table 2); trunk cells arranged in opposed pairs (Fig. 2D). Anterior end of calotte rounded. Axial cell rounded anteriorly, extending forward to overlap with approximately a third (occasionally half way) along the metapolar cell length, and nucleus located in the center of the axial cell (Fig. 2E). Axial cell of full-grown embryos with 1 or 2 agametes; 1 agamete located on each side of the axial cell nucleus, rarely both agametes located in the posterior end (Fig. 1C).

Rhombogens [Based on 43 specimens, Figs. 1D, 2B, F.] Body similar in length to nematogens, length 316–1,960 (859 ± 352), width 20–80 (39 ± 12)

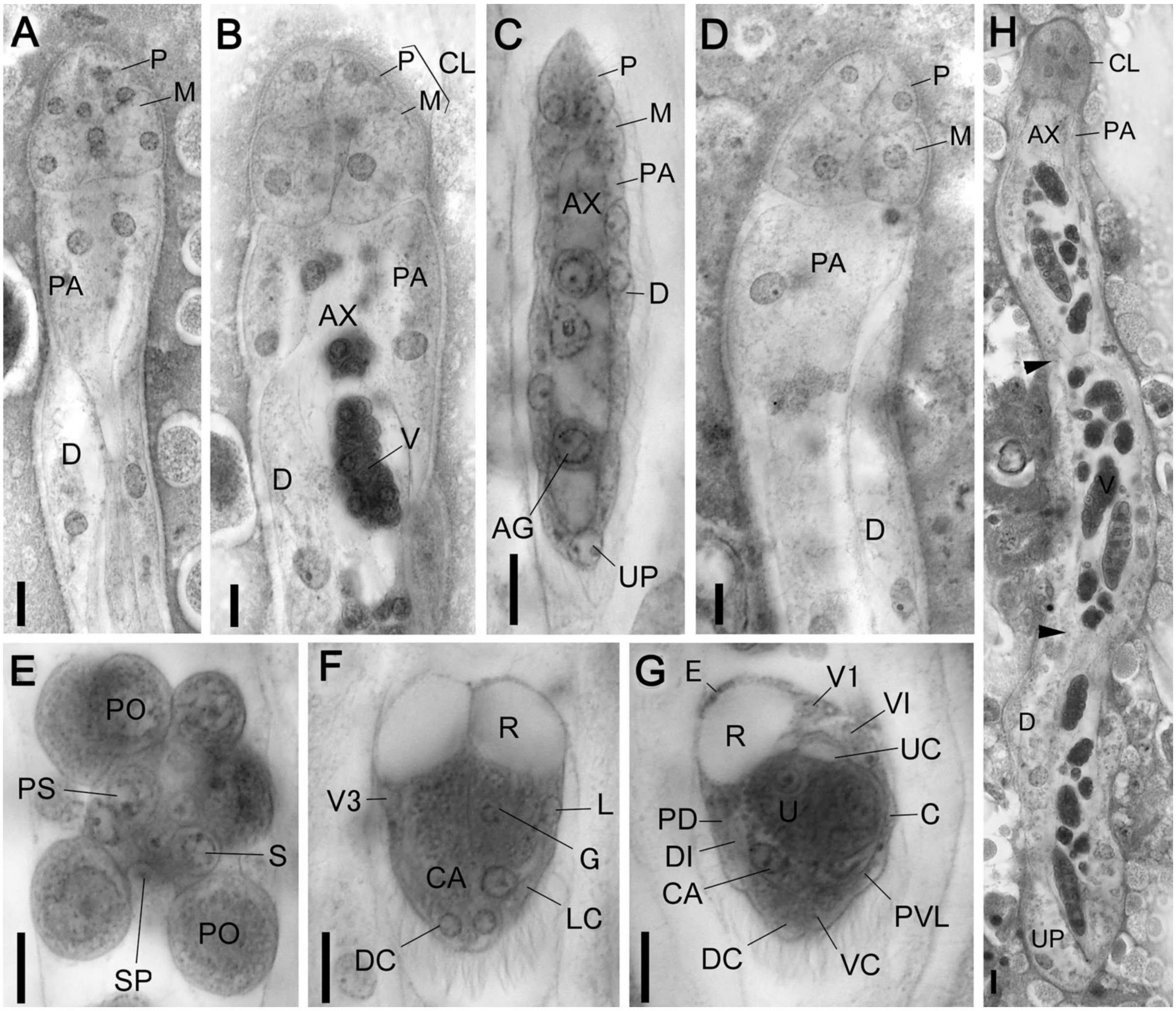

Fig. 1 Light micrograph of *Dicyema hochbergi* n. sp. from *O. maya*. A, B, Nematogens, anterior region; C, Vermiform embryo within axial cell; D, Rhombogen, anterior region; E, Infusorigen; F–G, Infusoriform embryos; F, Horizontal section; G, Sagittal section; H, Stem nematogen with 3 axial cells. *Arrowheads* indicate the borders between axial cells. *Scale-bars*: 10 µm. *Abbreviations*: AG, agamete (axoblast); AX, axial cell; CA, capsule cell; CL, calotte; D, diapolar cell; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PO, primary oocyte; PS, primary spermatocyte; PVL, posteroventral lateral cell; R, refringent body; S, spermatogonium; SP, sperm; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V3, third ventral cell

(Fig. 2B). Peripheral cells number 18, 19 or 22 (Table 2). Calotte conical, rounded anteriorly (Fig. 1D). Axial cell shape and anterior extent similar to those of nematogens (Fig. 2F). From 1 to 4 infusorigens present in the axial cell of large individuals.

Infusorigens [Based on 2 specimens, Fig. 1E.] Mature infusorigens medium-sized, composed of 1–5 external cells (oogonia, primary oocytes) and 1–8 internal cells (spermatogonia, primary and secondary spermatocytes and spermatozoa) (Fig. 1E). Mean diameter of fertilized eggs 13; that of spermatozoa 6. Axial cell round or ovoid, 10–30 in diameter.

Infusoriform embryos [Based on 55 specimens, Figs. 1F, G, 2G–I).] Ovoid, blunt, pointed posteriorly (Fig. 1F). Fully-grown embryos 20–50 (32 ± 6) in

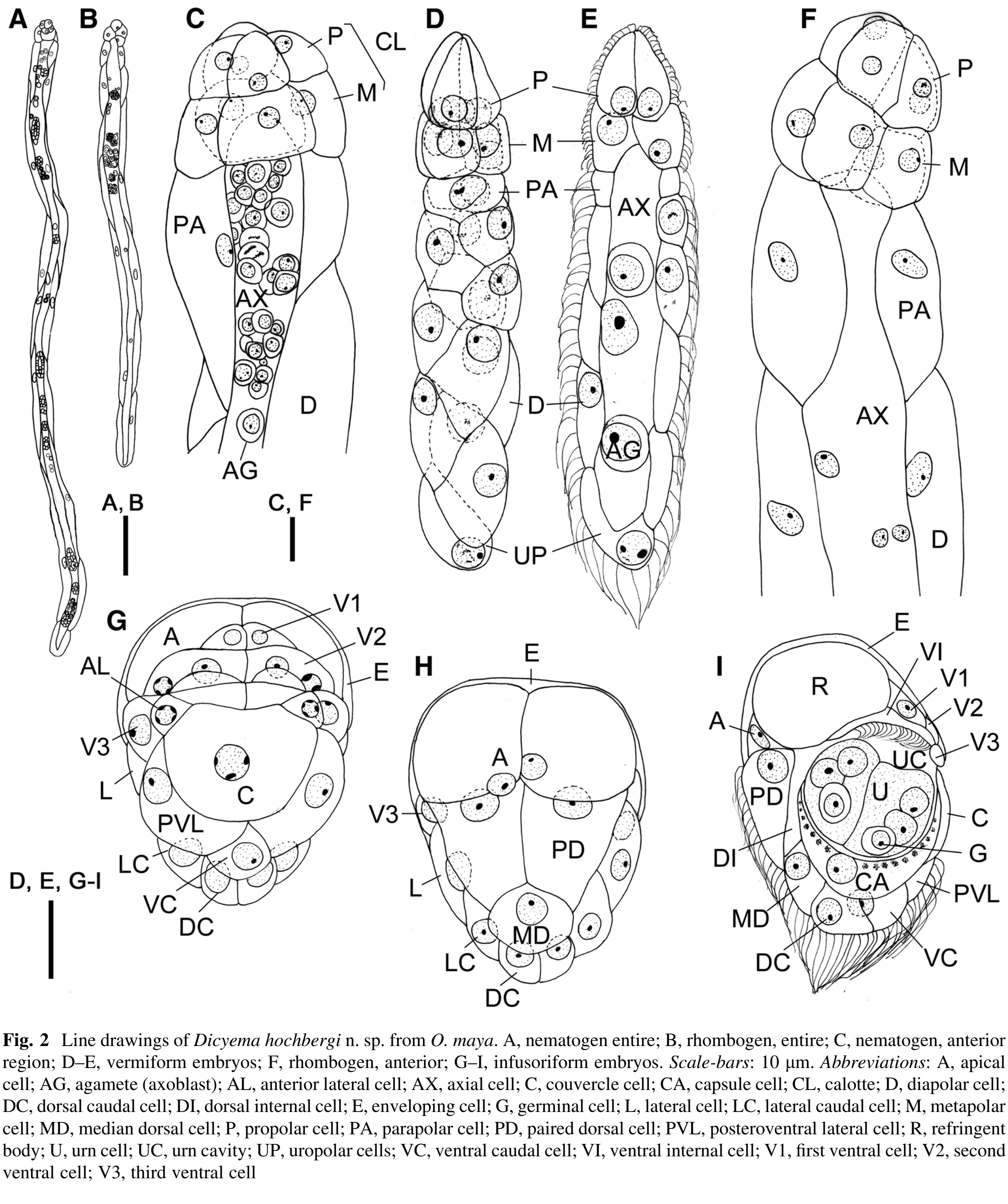

Fig. 2 Line drawings of *Dicyema hochbergi* n. sp. from *O. maya*. A, nematogen entire; B, rhombogen, entire; C, nematogen, anterior region; D–E, vermiform embryos; F, rhombogen, anterior; G–I, infusoriform embryos. *Scale-bars*: 10 µm. *Abbreviations*: A, apical cell; AG, agamete (axoblast); AL, anterior lateral cell; AX, axial cell; C, couvercle cell; CA, capsule cell; CL, calotte; D, diapolar cell; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PVL, posteroventral lateral cell; R, refringent body; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell; V3, third ventral cell

length (excluding cilia); length: width: height ratio 1: 0.82: 0.76. Cilia at posterior end 7 long. Refringent bodies present, solid, occupying anterior 30% of embryo length when viewed laterally (Fig. 1G). Cilia projecting from ventral internal cells into the urn cavity. Fully-grown infusoriform embryos (based on 50 specimens) consisting of 39 cells: 35 somatic and 4 germinal (Figs. 2G–I). Somatic cells of several types:

Table 2 Number of peripheral cells in *Dicyema mexcayae* n. sp. and *Dicyema hochbergi* n. sp.

Number of cells	Number of individuals		
	Vermiform embryo	Nematogens	Rhombogens
Dicyema mexcayae n. sp.			
14	27	42	29
15	0	3	1
16	56	8	0
Dicyema hochbergi n. sp.			
18	2	6	3
19	12	2	1
20	14	64	0
21	12	42	0
22	18	15	1
23	2	1	0
24	1	1	0

external cells covering a large part of anterior and lateral surfaces of the embryo (2 enveloping cells). External cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal and 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, 2 postero-ventral lateral cells) (Fig. 2G), external cells with refringent bodies (2 apical cells) (Figs. 1F, 2H), external cells without cilia (2 first ventral cells, 2 second ventral cells, 2 third ventral cells, 2 anterior lateral cells, 1 couvercle cell) (Figs. 2G, I), internal cells with cilia (2 ventral internal cells), and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells) (Fig. 1G). Each urn cell containing 1 germinal cell plus 2 nuclei (Fig. 2I). All somatic nuclei typically become pycnotic as infusoriform embryos mature.

Remarks

Dicyema hochbergi n. sp. was found together with *Dicyema mexcayae* n. sp. in *O. maya*. However, *D. hochbergi* is distinguishable from *D. mexcayae* by the maximum body length of the adult stages (2,245 *vs* 1,114 µm) and the vermiform embryos (80 *vs* 147 µm), the maximum number of agametes in the vermiform embryo (2 *vs* 11), the number of peripheral cells of vermiform stages (18–24 *vs* 14–16) and the total number of cells in infusoriform embryos (39 *vs* 37).

Most dicyemid species are composed of a constant number of calotte and trunk peripheral cells in vermiform stages (Furuya et al., 2007). In *D. hochbergi* n. sp., intraspecific variation was found in the number of trunk peripheral cells (18–24). Similar variation in peripheral cells is found in several other dicyemid species (Furuya et al., 2007): *Dicyema briarei* Short, 1961; *Dicyema octopusi* Kalavati, Narasimhamurti & Suseela, 1984; *Dicyema dolichocephalum* Furuya, 1999, *Dicyema sphaerocephalum* Furuya, 2005; and *Dicyema balanocephalum* Furuya, 2006. However, *D. hochbergi* differs from *D. sphaerocephalum* and *D. balanocephalum* in the cell number of infusoriform embryos (39 *vs* 37) (Furuya et al., 2004a); and from *D. dolichocephalum* in the calotte shape (conical *vs* long, dome-shaped) (Furuya, 1999). The axial cell of *D. hochbergi* n. sp. extends forward to overlap with about half the length of the metapolar cells whereas in *D. octopusi* it does not extend beyond the base of the metapolar cells (Kalavati et al., 1984). The maximum number and range of peripheral cells in *D. hochbergi* are the same as those for *D. briarei* (Short, 1961). However, *D. hochbergi* n. sp. is distinguishable from *D. briarei* in the anterior extent of the axial cell in vermiform embryos (extending to the middle of the metapolar cells *vs* to the base of propolar cells) and in the maximum body length of adult vermiform stages (2,245 *vs* 961 µm).

***Dicyema mexcayae* n. sp.**

Type-host: Red octopus, *Octopus maya* Voss & Solis-Ramirez (Mollusca: Cephalopoda: Octopodidae); female; mantle length 123 mm; wet body weight 570 g; (author's collection, SCM-2).
Type-locality: Gulf of Mexico, Mexico, off Yucatan, Progreso, 21°20′N, 89°37′W, 8 m deep. Collected by *jimba*.
Additional localities: Mexico, Yucatan, Sisal, 21°11′N, 90°04′W; Rio Lagartos, 21°38′N, 88°4′W.
Site in host: Renal appendages, within the renal sacs.
Prevalence: 73% (8/8 in Rio Lagartos, 9/13 in Progreso, 12/13 in Sisal, 0/6 in Celestun).
Type-material: Two syntype slides deposited in Colección de Invertebrados of the Universidad Nacional Autónoma de Mexico (CI-IBUNAM:0001, CI-IBUNAM:0004), two deposited in the Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH 458714, 458716) and four slides deposited in the Colección Helmintológica del CINVESTAV-Merida (CHCM524–524.4).
Collector and date: Isidro Puerto Esquivel, Sergio Herrera Peraza, Gregory Arjona, Francisco Puc; November-December, 2013, 2014.
Etymology: The specific name is derived from the word *mexcay*, the ancient Mayan name for octopus.

Description (Figs. 3–4)

Diagnosis: Small-sized dicyemid, body length 70–1,114 (322 ± 219). Calotte elongate; propolar and metapolar cells opposite each other with granules occasionally seen in propolar and trunk cells of adults. Vermiform stages with 14–16 peripheral cells: 4 propolars, 4 metapolars, 2 parapolars, 4–6 trunk cells. Infusoriform embryos with 37 cells, refringent bodies solid and urn cell with 2 nuclei each.

Nematogens [Based on 56 specimens; see Table 2, Figs. 3A, B, 4A, C.] Body length 70–1,114 (326 ± 214), width 35–44 (40 ± 5), widest at parapolar cells (Figs. 3A–B). Peripheral cell number 14–16 (Table 2): 4 propolars, 4 metapolars, 2 parapolars, 2–4 diapolar cells, 2 uropolars (Fig. 4A). Granules occasionally observed in propolar and diapolar cells (Fig. 3A). Calotte elongate, rounded anteriorly (Fig. 4B). Mean length of metapolar cells almost twice that of propolar cells (24 *vs* 15 respectively) (Fig. 4C). Cilia on calotte 5 long, oriented anteriorly. Size and nuclei of propolar cells smaller than those of metapolar cells (Figs. 4B–C). Cytoplasm of propolar and metapolar cells more darkly stained than that of other peripheral cells. Verruciform cells absent. Axial cell cylindrical, rounded anteriorly, extends forward to the metapolar cells (Figs. 3B, 4C) and occasionally, can be observed at the base of the calotte. About 14 vermiform embryos present in axial cells of large individuals.

Vermiform embryos [Based on 19 specimens, Figs. 3C, 4D–E.] Length of full-grown vermiform embryos 40–147 (70 ± 29), width 8–18 (12 ± 3) (Fig. 3C). Peripheral cell number 16, occasionally 14 (Table 2): 4 propolars, 4 metapolars, 2 parapolars, 2–4 diapolar cells, and 2 uropolars (Fig. 4D). Trunk cells arranged in opposed pairs. Anterior end of the calotte acutely pointed (Fig. 4E). Axial cell rounded anteriorly and extends forward to overlap with one third the length of metapolar cells (Fig. 4E). Nucleus usually located in centre of cell, occasionally in the anterior portion of the axial cell (Figs. 3C, 4E). Anterior abortive axial cell absent. Axial cell of full-grown embryos with 3–11 (mode 4) agametes usually located at the posterior end (Figs. 3C, 4E).

Rhombogens [Based on 17 specimens, Figs. 3D, 4F–H.] Body similar in length to nematogens, 80 –702 (295 ± 164), width 35–49 (42 ± 7) (Fig. 3D). Peripheral cell number 16–18 (Table 2). Granules occasionally seen in propolar, parapolar and diapolar cells. Calotte elongate (Fig. 4F); metapolar cells twice the size of propolar cells, as in nematogens (Figs. 4G–H). Shape and anterior extent of axial cell similar to those of nematogens (Figs. 3D, 4G). Usually a single infusorigen present in the axial cell of each parent. About 3 infusoriform embryos present in axial cells of large individuals (Figs. 3D, 4H).

Infusorigens [Based on 4 specimens, Fig. 4I.] Mature infusorigen small. Axial cell of infusorigens round to ovoid, 10 in diameter, composed of 2–4 external cells (oogonia and primary oocytes) and 2–3 internal cells (spermatogonia, primary and secondary spermatocytes) (Fig. 4I). Mean diameter of fertilized eggs 10, and that of spermatozoa 5 in diameter.

Infusoriform embryos [Based on 50 specimens, Figs. 3E–F, 4J–L.] Ovoid, blunt, pointed posteriorly

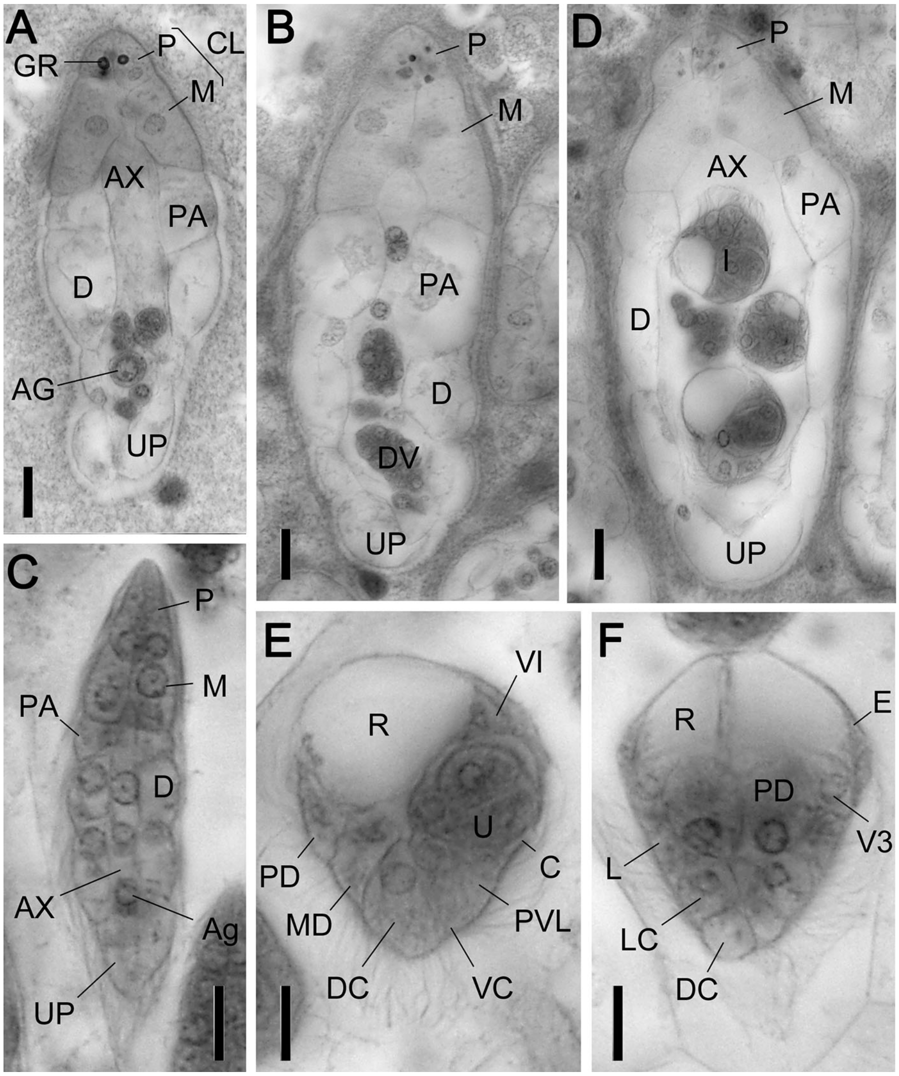

Fig. 3 Light micrograph of *Dicyema mexcayae* n. sp. from *O. maya*. A–B, nematogen, entire; C, vermiform embryo within axial cell; D, rhombogen, entire; E–F, infusoriform embryos. *Scale-bars*: 10 µm. *Abbreviations*: AG, agamete (axoblast); AX, axial cell; C, couvercle cell; CL, calotte; D, diapolar cell; DC, dorsal caudal cell; DV, developing vermiform embryo; E, enveloping cell; GR, granules; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PVL, posteroventral lateral cell; R, refringent body; U, urn cell; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V3, third ventral cell

(Fig. 3E). In full-grown embryos (based on 50 specimens), 20–30 (24 ± 5) in length (excluding cilia); length: width: height ratio 1: 0.82: 0.77. Cilia at the posterior end 7 long. Refringent bodies present, solid, occupying anterior 40% of embryo length when viewed laterally (Figs. 3E–F). Cilia projecting from ventral internal cells into the urn. Full–grown infusoriform embryos (based on 50 specimens) consisting of 37 cells: 33 somatic and 4 germinal cells (Fig. 4L). Somatic cells of several types: external cells covering a large part of the anterior and lateral surfaces of the embryo (2 enveloping cells) (Fig. 4L). External cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, 2 postero-ventral lateral cells) (Figs. 4J–K). External cells with refringent bodies (2 apical cells) (Figs. 3F, 4J–K). External cells without cilia (2 first ventral cells,

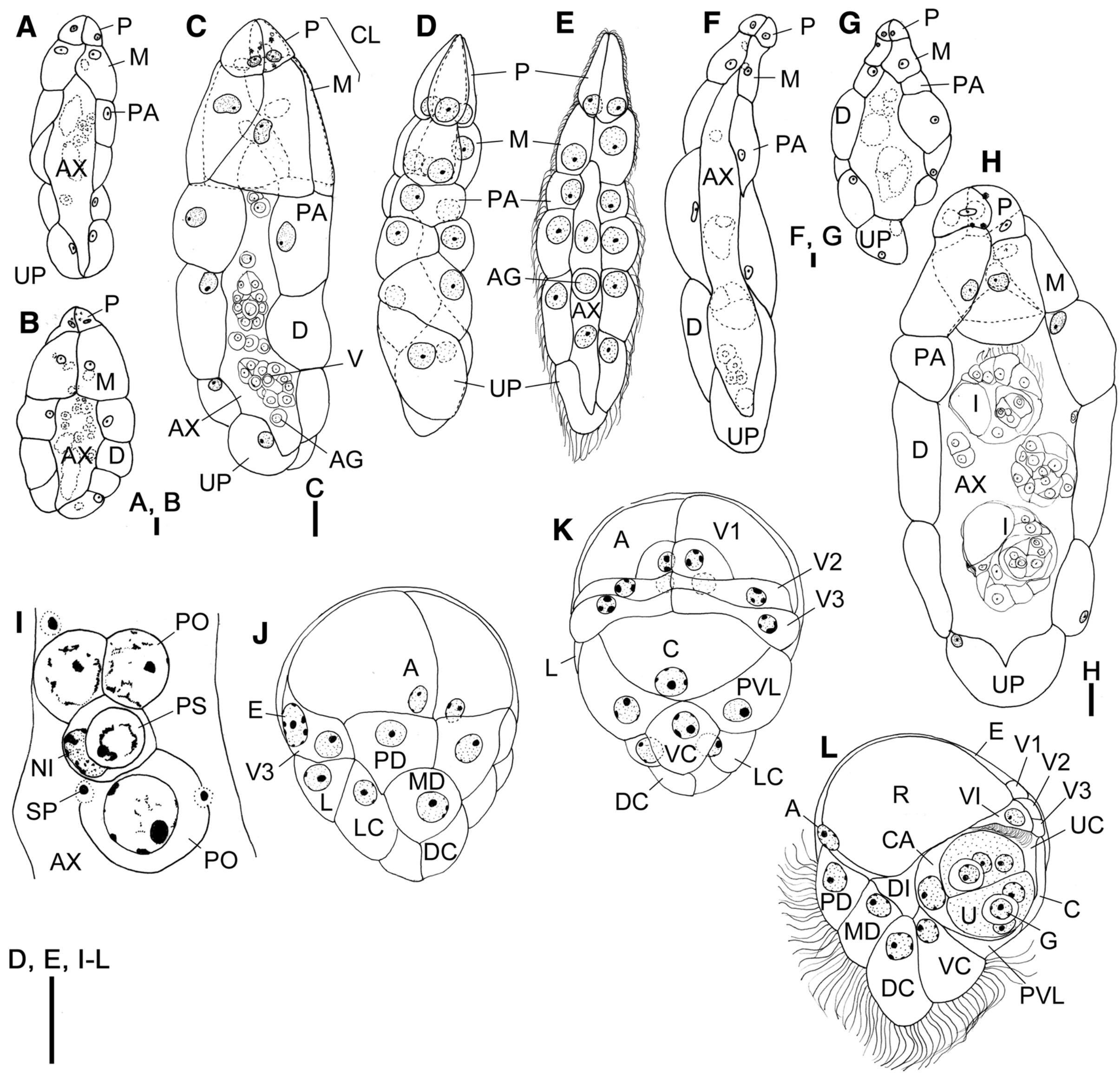

Fig. 4 Line drawings of *Dicyema mexcayae* n. sp. from *O. maya*. A–C, nematogens, entire; D–E, vermiform embryos; F–H, rhombogens, entire; I infusorigen; J–L, infusoriform embryos. *Scale-bars*: 10 µm. *Abbreviations*: A, apical cell; AG, agamete (axoblast); AX, axial cell; C, couvercle cell; CA, capsule cell; CL, calotte; D, diapolar cell; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; NI, nucleus of infusorigen; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PO, primary oocyte; PS, primary spermatocyte; PVL, posteroventral lateral cell; R, refringent body; SP, sperm; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell; V3, third ventral cell

2 second ventral cells, 2 third ventral cells, 1 couvercle cell) (Fig. 4K). Internal cells with cilia (2 ventral internal cells) and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells) (Figs. 3E, 4L). Each urn cell contains 1 germinal cell plus 2 nuclei (Fig. 4L). All somatic nuclei typically become pycnotic as infusoriform embryos mature.

Remarks

Dicyema mexcayae n. sp. is characterised by small, adult vermiform stages and a conical calotte, composed of small, granular propolar cells. This structure is similar in the number of peripheral cells to four other species described from the Gulf of Mexico:

Dicyema apalachiensis Short, 1962, *Dicyema hypercephalum* Short, 1962, *Dicyema bilobum* Couch & Short, 1964 and *Dicyema shorti* Furuya, Damian & Hochberg, 2002. However, *D. mexcayae* n. sp. can be easily distinguished by its calotte composed of small propolar and relatively elongated metapolar cells. In contrast, propolar and metapolar cells are similar in length in the other four species (Short, 1962; Couch & Short, 1964; Furuya, et al., 2002).

Dicyemid species recorded worldwide and similar to *D. mexcayae* n. sp. in the number of peripheral cells and calotte shape are as follows: *D. typus* van Beneden, 1876, *D. acuticephalum* Nouvel, 1947, *D. caudatum* Bogolepova-Dobrokhotova, 1960, *D. oligomerum* Bogolepova-Dobrokhotova, 1960, *D. typoides* Short, 1964, *D. knoxi* Short, 1971 and *D. akashiense* Furuya, 2006. In particular, the number of cells in the infusoriform embryos of *D. mexcayae* n. sp. is similar to that described in those of *D. acuticephalum* and *D. akashiense* (Nouvel, 1947; Furuya, 2006). However, *D. mexcayae* differs from both species in the cell number and composition at this stage. The infusoriform embryos of *D. acuticephalum* and *D. akashiense* have apical internal cells. In contrast, *D. knoxi* has postcapsular instead of third ventral cells (Short, 1971; Furuya et al., 2004a; Furuya, 2006). The infusoriform embryos of *D. mexcayae* n. sp. consist of 37 cells and are thus easily distinguished from *D. typus* and *D. typoides* that are both composed of 35 cells (Furuya et al., 2004a). Finally, *D. mexcayae* n. sp. is easily distinguishable from *D. caudatum* and *D. oligomerum* because these species have a calotte composed of propolar and metapolar cells of similar length. In addition, the axial cell of the vermiform stages of these two species extend up to the propolar cells (Bogolepova-Dobrokhotova, 1960), which is not observed in *D. mexcayae* n. sp.

Discussion

Nine species of dicyemids have been reported from Mexican waters (McConnaughey, 1949a, b; Furuya et al., 2002; Castellanos-Martinez et al., 2011), of which eight were described from the Pacific Ocean off Mexico. *Dicyema shorti* was described from the southern Gulf of Mexico (off Veracruz, Mexico) based on a single host specimen of *A. burryi* (see Furuya et al., 2002). In contrast, seven dicyemid species are known to occur off Florida, USA, in the northern Gulf of Mexico (Short, 1961, 1962; Couch & Short, 1964; Short & Damian, 1966; Furuya et al., 2002; Overstreet & Hochberg, 2009). Until now, the dicyemid fauna of the Mexican territorial waters of the Gulf of Mexico has been poorly studied. Therefore, the present descriptions provide the basis for further research on the dicyemid fauna of this region, which is home to 71 cephalopod species (Salcedo-Vargas, 1991) and potentially harbours additional undescribed dicyemid species.

Specimens of *Octopus maya* were collected off Yucatan, Mexico. This octopus has an ocellus located on the interbrachial membrane between the second and third arms of each side. It lays large eggs (17 mm in length) and is distributed from Belize to Veracruz (Voss & Solis-Ramírez, 1966; Norman et al., 2014). In *O. maya*, *D. hochbergi* n. sp. was the single species found in three octopuses from Celestun, *D. mexcayae* n. sp. was the only species recorded in two octopuses from Progreso; whereas, both dyciemid species co-occurred in 27 octopuses from off Progreso, Sisal and Rio Lagartos (Table 1). The infected hosts were all mature whereas the uninfected ones were mainly juvenile (Table 1). In addition, the lowest prevalence of infection was recorded in Celestun and it might be due to the small body size of the hosts, since a direct relationship between host size and occurrence of dicyemids has been reported in some cephalopod species, such as *O. vulgaris* Cuvier, *Sepioteuthis lessoniana* Lesson and *Sepia papuensis* Hoyle (see Furuya et al., 1992, 2004b; Furuya & Tsuneki, 2005; Catalano, 2013). Dicyemids were not detected or were scarce in smaller individuals in the latter cephalopod species, although the reason for this is not clear. In Yucatan, Velázquez-Abunader et al. (2013) suggested that the collection site Celestun is a recruitment zone for *O. maya*, while the largest octopuses are usually fished off Rio Lagartos (Salas et al., 2009). In this case, the pattern of dicyemid infection in *O. maya* conforms to that for *O. vulgaris*, *S. lessoniana* and *S. papuensis*.

Individuals of vermiform stages specifically inhabit the renal sac and their calotte is critical in adapting to this microhabitat (Furuya et al., 2003). They insert the calotte into the renal tubules (Ridley, 1968; Furuya et al., 1997) or attach it to flatter surfaces of the renal appendages (Furuya et al., 2003; Furuya, 2005, 2006). The calotte in *D. hochbergi* n. sp. and *D. mexcayae* n.

sp. conforms to the conical shape characteristic of the insertion type. These two species insert their calotte into the renal appendage tubules and coexist in the same renal microhabitat. Generally, when dicyemid species co-occur, their calotte shapes differ (Furuya et al., 2003). In contrast, species of dicyemids that possess similar calotte shapes are very rarely found together in a single host individual. When this occurs, one of the species is usually dominant and the most adaptive species for the microhabitat likely becomes the dominant, niche-occupying species (Furuya et al., 2003). For example, in Japanese *O. vulgaris*, two dicyemid species with similar calotte types have been identified, i.e. *D. acuticephalum* and *D. misakiense*, Nouvel & Nakao, 1938, yet these two species never co-occurred in over 150 specimens of the host octopus examined (Furuya et al., 2003). Similarly, the Atlantic pygmy octopus, *O. joubini* Robson harbors two dicyemid species *D. apalachiensis* and *D. hypercephalum* with very similar calotte shapes yet they were never found together in the same host individual (Furuya et al., 2003). In the present study, as far as could be ascertained from the specimens of *D. hochbergi* n. sp. and *D. mexcayae* n. sp. observed on the slides, they coexisted in similar numbers. Therefore, the dominant species (*D. hochbergi* or *D. mexcayae*) could not be determined. The body length of *D. mexcayae* is less than half that of *D. hochbergi*. Perhaps this distinct body size difference somehow enables these two species to coexist in the renal sacs of *O. maya*.

The genus *Dicyema,* to which the two new species belong, is the most common genus recorded in shallow-water cephalopods and the only genus reported for all species in the Gulf of Mexico. A total of nine species from the Gulf of Mexico were previously recorded infecting *O. briareus*, *A. burryi*, *O. joubini* and *O. vulgaris*, which are all shallow-water octopus species (McConnaughey & Kritzler, 1952; Short, 1961, 1962; Couch & Short, 1964; Short & Damian, 1966; Overstreet & Hochberg, 2009). Of the 16 dicyemids described from off the Pacific coast of the USA and Mexico, ten belong to the genus *Dicyemennea* Whitman, 1883 (see McConnaughey 1941, 1949a, 1957, 1959). This may be due to differences in the habitats occupied by the host cephalopods. The octopuses examined off the Atlantic coast of the USA and in the Gulf of Mexico are common shallow-water species that live at depths ranging from 0 to 200 m, whereas cephalopod hosts off the Pacific coast are found up to 500 m. Most described species of *Dicyema* appear to be restricted to shallow-water cephalopod hosts, whereas species of *Dicyemennea* live in hosts with a much wider depth range (Furuya et al., 2002). Additional host cephalopod genera and species remain to be examined, as many other undescribed species of dicyemids likely occur in the Gulf of Mexico.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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