THREE NEW SPECIES OF DICYEMA (PHYLUM DICYEMIDA) FROM CEPHALOPODS IN THE WESTERN MEDITERRANEAN

H Furuya, F G Hochberg

VIE ET MILIEU, 1999, 49 (2/3) : 117-128

THREE NEW SPECIES OF *DICYEMA* (PHYLUM DICYEMIDA) FROM CEPHALOPODS IN THE WESTERN MEDITERRANEAN

H. FURUYA, F.G. HOCHBERG*

Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, 2559 Puesta del Sol Road, Santa Barbara 93105-2936 California, USA
Email : fghochberg@sbnature2.org
** Present address : Department of Biology, Graduate School of Science, Osaka University, 1-16 Machikaneyama-cho, Toyonaka, Osaka 560-0043 Japan*

CEPHALOPOD
CUTTLEFISH
OCTOPUS
PARASITE
PHYLUM DICYEMIDA

ABSTRACT. – Three new species of dicyemid mesozoans are described from cuttlefish and octopus hosts (Mollusca : Cephalopoda) collected off Italy and France in the western Mediterranean Sea. *Dicyema whitmani* sp. nov. from *Sepia officinalis* is an elongate, slender dicyemid with body lengths that commonly exceed 7,000 µm. The calotte is disc-shaped and at least twice as wide as the body. The axial cell extends to the base of the propolar cells. Vermiform stages have 28 peripheral cells. Infusoriform embryos have 37 cells. *Dicyema banyulensis* sp. nov. from *Octopus salutii* is a medium-sized dicyemid with body lengths that rarely exceed 1,500 µm. The calotte is relatively small and conical in shape. Vermiform stages have 22 peripheral cells. Infusoriform embryos have 39 cells. *Dicyema benedeni* sp. nov. also from *Octopus salutii* is a medium-sized dicyemid with body lengths typically less than 1,500 µm. The species is characterized by a disc-shaped calotte. Vermiform stages have 18 peripheral cells. Infusoriform embryos have 37 cells. The dicyemid fauna found in *Sepia officinalis* and *Octopus salutii* is briefly discussed.

CEPHALOPODE
SEICHE
POULPE
PARASITE
PHYLUM DICYEMIDA

RÉSUMÉ. – Les auteurs décrivent trois espèces nouvelles de mésozoaires Dicyémides trouvées dans deux Céphalopodes-hôtes (Seiche, Poulpe) récoltés sur les côtes italiennes et françaises de Méditerranée occidentale. *Dicyema whitmani* sp. nov. parasite de *Sepia officinalis* est un Dicyémide longiforme, mince dont la longueur dépasse normalement 7 000 µm. La calotte se présente en forme de disque, le diamètre étant au moins deux fois celui du corps. La cellule axiale s'étend jusqu'à la base des cellules propolaires. Les stades vermiformes ont 28 cellules périphériques, les embryons infusoriformes 37 cellules. *Dicyema banyulensis* sp. nov. qui habite *Octopus salutii* offre une taille moyenne dépassant rarement 1 500 µm. La calotte est relativement petite, conique. Les stades vermiformes ont 22 cellules périphériques, les embryons infusoriformes, 39 cellules. *Dicyema benedeni* sp. nov., également trouvé chez *Octopus salutii*, est un Dicyémide de taille moyenne, normalement inférieur à 1 500 µm. L'espèce est caractérisée par une calotte en forme de disque. Les stades vermiformes ont 18 cellules périphériques, les embryons infusoriformes 37 cellules. Une brève discussion est consacrée à la faune des Dicyémides observée chez *Sepia officinalis* et *Octopus salutii.*

INTRODUCTION

The dicyemids that inhabit the kidneys of cephalopods have been studied in Europe since the mid-19th century by a number of researchers (Krohn 1839, von Kölliker 1849, Wagener 1857, Lankester 1873, Van Beneden 1876, 1882, Whitman 1883, Nouvel 1944, 1947, 1948). Following nearly 150 years of study a total of 14 species of dicyemid mesozoans have been described from 17 cephalopod species in Europe (northeastern Atlantic Ocean, English Channel, North Sea, and Mediterranean Sea). In the Mediterranean Sea the following dicyemids repeatedly have been reported from the shallow-water cuttlefish, *Sepia officinalis* Linnaeus, 1758 : *Dicyemennea gracile* Wagener, 1857 ; *Microcyema vespa* van Beneden, 1882 ; and *Pseudicyema truncatum* Whitman, 1883 (Hochberg 1990 ; Furuya and Hochberg, in prep). Nouvel (1945) reported the presence of *Dicyemennea eledones* Wagener, 1857, along with the apostome ciliate *Chromidina*, in a single small mid-depth octopus, *Octopus salutii* Verany, 1839, examined off Monaco on 23 April 1938.

In this paper 3 new species in the genus *Dicyema* are described from the western Mediterranean in the following cephalopod hosts : *Sepia officinalis* (1 new species); and *Octopus salutii* (2 new species). These are the first new species of dicyemids to be described from Europe in over 35 years.

MATERIALS AND METHODS

Specimens in the collections of the Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH) were examined during the course of this study. Slide preparations and formalin-fixed material of dicyemids were obtained from three principal sources as elucidated below.

Henri Nouvel (HN ; Université Paul-Sabatier de Toulouse, France) worked extensively on the dicyemid and ciliate parasites that are found in the renal sacs of cephalopods throughout the Mediterranean and northeastern Atlantic Ocean (including the English Channel) (Beetschen & Bitsch 1975). Following his death in August, 1974 Nouvel's extensive microslide collection was sent for safe keeping to C. Combes (Laboratoire de Biologie Animale, Université de Perpignan, France). This collection, which documents Nouvel's life-long work on dicyemids and apostome ciliates, was located in 1996 and reexamined. The material of interest in this paper was collected in 1930-1935 when Nouvel worked at the Station Biologique in Roscoff, France and at the Musée Océanographique in the Principality of Monaco. Duplicate microslides of some material referenced in this paper are archived at SBMNH.

John L. Mohr (JLM ; Allan Hancock Foundation, University of Southern California, Los Angeles, California, USA) prepared smears of cephalopod kidney parasites during a sabbatical in Europe in 1957 and early 1958. During this trip he obtained dicyemid material while working at the Marine Biological Laboratory in Plymouth, England and the Stazione Zoologica in Naples, Italy. Mohr's collection of microslides of dicyemids and apostome ciliates was donated to the SBMNH in 1997.

F.G. Hochberg (FGH ; University of California, Santa Barbara, California, USA) worked in Europe in the summer of 1969 while a graduate student. During this trip he collected cephalopods and prepared smears of both dicyemids and apostome ciliates at the Laboratoire Arago in Banyuls-sur-Mer, France, the Stazione Zoologica in Naples, Italy and at the Marine Biological Laboratory in Plymouth, England. Hochberg's collection of microslides of dicyemids and apostome ciliates from this trip are deposited in the collections of the SBMNH.

Additional microslides sent by Nouvel and Mohr to B.H. McConnaughey (University of Oregon, Eugene, Oregon, USA) and by Nouvel to R.B. Short (Florida State University, Tallahassee, Florida, USA) also were examined for this study. The large collections of both McConnaughey and Short are archived at the SBMNH.

In this study prepared slides from 77 *Sepia officinalis* collected at 4 localities were examined. Autopsy numbers were inscribed on all slides examined as indicated in Table III, however, host data sheets were not available for any of the Nouvel material examined nor for about half of the *Sepia* examined by Mohr. A total of 23 freshly dead *Octopus salutii* were examined for presence of dicyemid mesozoans or apostome ciliates (*Chromidina*) by the second author (FGH). 22 host octopus were collected by otter trawl in July 1969 off the coast of Banyuls-sur-Mer, France and an additional individual was obtained at the fish market in Rosas, Spain presumably captured in the immediate vicinity of the Bahía de Rosas. Coverslip smears were prepared from 12 of the freshly dead hosts autopsied and live preparations were examined from the remaining hosts (Table IV). Data sheets were prepared in the field for each octopus host examined which include a FGH autopsy number. All octopus hosts were sexed, the stage of maturity determined, and the dorsal mantle length (ML) measured.

When parasites were detected in the renal coelom of the host cephalopod small pieces of renal appendages with attached dicyemids were removed and smeared on glass coverslips. The smears were fixed immediately in Bouin's fluid for 10-24 hr and then stored in 70 % ethanol alcohol. A diversity of hematoxylins have been used to stain dicyemid preparations. In this study the majority of the coverslips examined were stained in Heidenhain's or Ehrlich's acid hematoxylin and counterstained in eosin. Following staining smears were mounted on glass microslides using Canada Balsam, Permount or Entellan (Merck). Additional pieces of renal tissue from a number of hosts examined in the study by FGH were fixed and preserved in 5 % formalin (F) in seawater (see Table IV).

Observations of dicyemids were made with a Zeiss compound light microscope. Measurements and drawings were made with the aid of an ocular micrometer and drawing tube, respectively. Unless otherwise indicated we examined details of the various stages in the following number of individuals : 20 vermiform stages (i.e., nematogens, vermiform embryos, and rhombogens); 20 infusorigens; 50 infusoriform embryos. Unless otherwise indicated all measurements of dicyemids are in micrometers (μm). Syntypes of all dicyemids described herein are deposited in the Santa Barbara Museum of Natural History (SBMNH).

The terminology for description of cell names in infusoriform embryos is from Nouvel (1948), Short and Damian (1966), Furuya (1999), Furuya and co-workers (1992a, b ; 1997). Abbreviations used in figures and tables : A, apical cell ; AG, agamete (axoblast) ; AL, anterior lateral cell ; AX, axial cell ; AXI, axial cell of infusorigen ; C, couvercle cell ; CA, capsule cell ; 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 ; N, nucleus of axial cell ; NI, nucleus of infusorigen ; O, oogonium ; P, propolar cell ; PA, parapolar cell ; PD, paired dorsal cell ; PO, primary oocyte ; PVL, posteroventral lateral cell ; R, refringent body ; S, spermatogonium ; SP, sperm ; U, urn cell ; UP, uropolar cell ; UC, urn cavity ; VC, ventral caudal cell ; VI, ventral internal cell ; V1, first ventral cell ; V2, second ventral cell ; V3, third ventral cell.

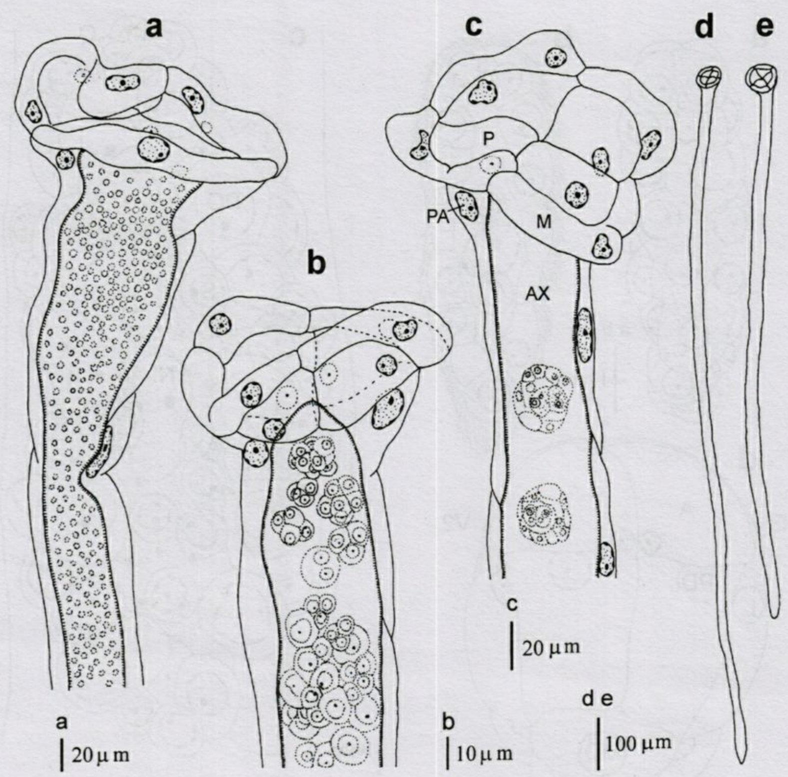

Fig. 1. – *Dicyema whitmani* sp. nov. a-c. Anterior part of vermiform stages. a, Nematogen or transitional individual. b-c, Rhombogens. d-e, Vermiform stages, entire. d, Nematogen. e, Rhombogen.

FAMILY DICYEMIDAE Van Beneden, 1882

GENUS *DICYEMA* VON KÖLLIKER, 1849

***Dicyema whitmani* sp. nov.** (Fig. 1, 2, Tables I, II)

Materials examined

See Table III. Description based on dicyemids present on 15 slide preparations from 1 cuttlefish host.

Description

Diagnosis : Large dicyemids; body lengths typically greater than 7,000 μm. Calotte large; disc-shaped. Vermiform stages (i.e., vermiform embryos, nematogens, and rhombogens) with 28 peripheral cells : 4 propolars; 4 metapolars; 2 parapolars; and 18 trunk cells. Infusoriform embryos with 37 cells; 2 nuclei present in each urn cell.

Nematogens (Fig. 1a, d; n = 20) : Body elongate, slender, nail-shaped, widest in region of metapolar cells; lengths of largest individuals range from 5,000-8,000 μm; trunk width uniform, maximum widths from 50-90 μm. Peripheral cell number 28 : 4 propolars; 4 metapolars; 2 parapolars; 16 diapolars; and 2 uropolars (Table I). Calotte large, flat, disc-shaped; in *enface* view metapolar cells appear to form narrow ring around propolar cells; cilia on calotte about 8 μm long, oriented anteriorly. Cytoplasm of both propolar and metapolar cells more darkly stained by hematoxylin than trunk cells. Propolar cells and nuclei slightly smaller than the metapolars. Cephalic enlargement, composed of calotte and parapolar cells. Verruciform cells absent. Axial cell cylindrical, rounded anteriorly; extends forward to base of propolar cells.

Table I. – Vermiform stages : numbers of peripheral cells in 3 new species of *Dicyema* from European waters (listed alphabetically).

Cell Number	Number of Individuals Examined		
	Vermiform Embryos	Nematogens	Rhombogens
Dicyema banyulensis sp. nov.			
22	15	5	2
Dicyema benedeni sp. nov.			
18	6	1	2
Dicyema whitmani sp. nov.			
26	0	1	1
27	0	0	1
28	5	10	5

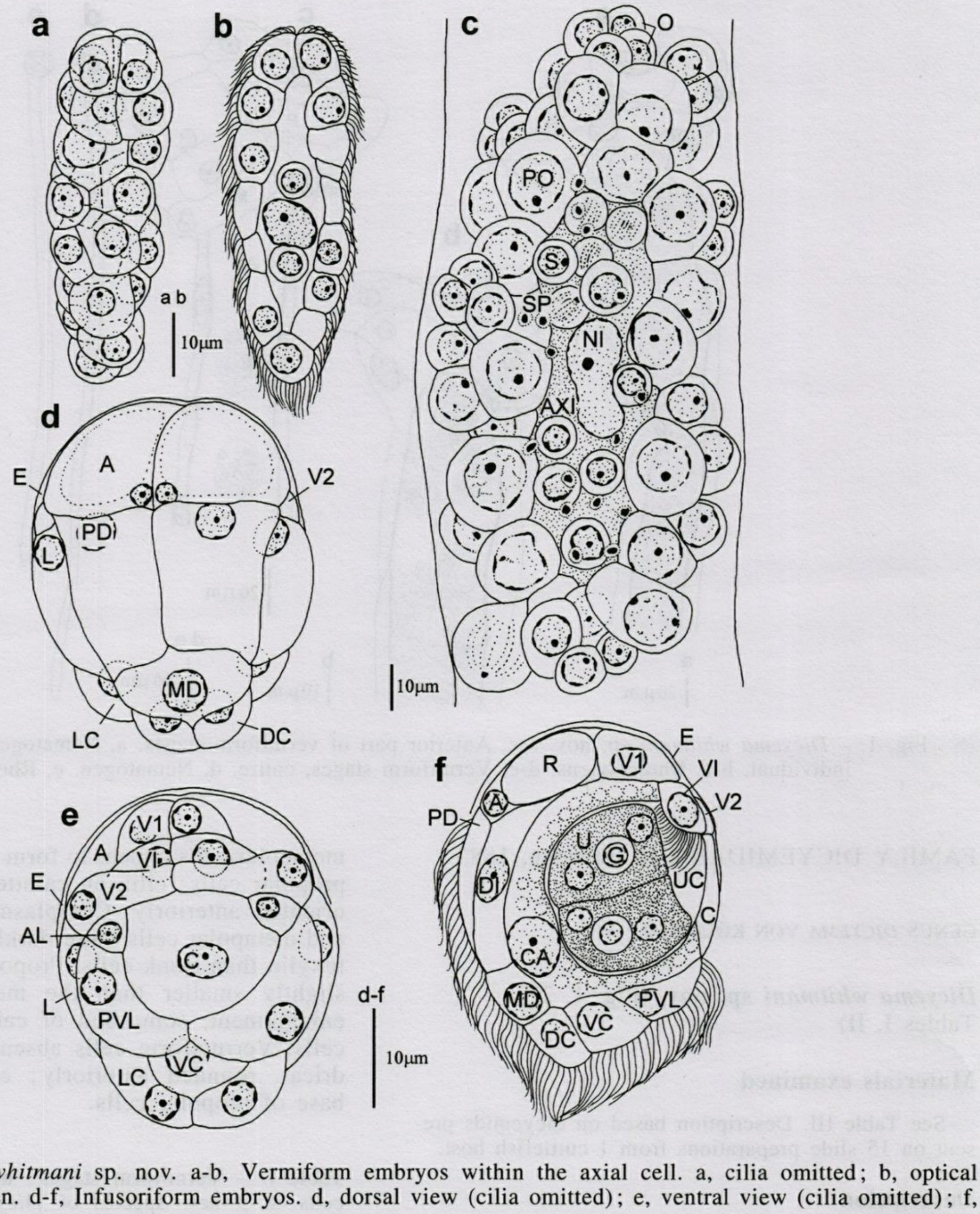

Fig. 2. – *Dicyema whitmani* sp. nov. a-b, Vermiform embryos within the axial cell. a, cilia omitted; b, optical section. c, Infusorigen. d-f, Infusoriform embryos. d, dorsal view (cilia omitted); e, ventral view (cilia omitted); f, sagittal section.

Vermiform embryos (Fig. 2a, b; n = 20): Vermiform embryos small; lengths of largest individuals within axial cell of parent nematogens range from 40-50 μm, widths average 15 μm. Peripheral cell number 28 (Table I); trunk cells arranged in opposed pairs. Anterior end of calotte rounded. Axial cell rounded anteriorly; extends forward to base of propolar cells; nucleus usually located in center of axial cell; nucleus diameter almost twice as large as agamete diameter. Axial cells of full-grown embryos contain 2 agametes; typically 1 on either side of nucleus.

Rhombogens (Fig. 1b, c, e; n = 20): Slightly shorter and stockier than nematogens, otherwise generally similar in shape and body proportions; lengths range from 5,000-7,000 μm; widths from 70-90 μm. Peripheral cell number typically 28, rarely 26 or 27 (Table I). Calotte disc-shaped. Cephalic enlargement, composed of calotte and parapolar cells. Verruciform cells absent. Axial cell similar to nematogens in shape and anterior extent. Variable number of infusorigens, never more than 4, present in axial cell of each parent individual. About 40 infusoriform embryos pre-

Table II. – Infusoriform embryos : types and numbers of cells, and number of nuclei in urn cells in 3 new species of *Dicyema* from the Mediterranean (listed alphabetically).

Species of *Dicyema*	Somatic Cell Number	Infusoriform Embryos: Types and Numbers of Cells and Nuclei																				Urn Cell Nuclei
		External Cells*														Internal Cells*						
		E	V1	V2	V3	AL	C	A	L	PVL	LC	VC	PD	DC	MD	VI	AI	DI	CA	U	G	
banyulensis sp. nov.	39	2	2	2	2	2	1	2	2	2	2	1	2	2	1	2	0	2	2	4	4	2
benedeni sp. nov.	37	2	2	2	2	0	1	2	2	2	2	1	2	2	1	2	0	2	2	4	4	2
whitmani sp. nov.	37	2	2	2	0	2	1	2	2	2	2	1	2	2	1	2	0	2	2	4	4	2

* For abbreviations see Materials and Methods

sent in axial cell of largest individuals. Accessory nuclei occasionally observed in uropolar cells.

Infusorigens (Fig. 2c ; n = 20) : Mature infusorigens large, composed of 50-100 (mode 64) external cells (oogonia and primary oocytes) ; 10-20 (mode 13) internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes) ; and 15-30 (mode 16) spermatozoa. Mean diameter of fertilized eggs, 11.8 µm ; spermatozoa, 2.5 µm.

Infusoriform embryos (Fig. 2d-f ; n = 50) : Full-grown embryos small, lengths (excluding cilia) average 24.4 ± 1.0 µm (mean ± SD) ; length-width-height ratio, 1 : 0.85 : 0.80 ; shape ovoid, bluntly rounded to pointed posteriorly ; cilia at posterior end 7 µm long. Refringent bodies present ; solid ; size about same as single urn cell ; occupy anterior 25 % of embryo length when viewed laterally (Fig. 2f). Cilia project from ventral internal cells into urn cavity (Fig. 2f). Capsule cells contain many large granules on side adjacent to urn. Full-grown infusoriform embryos with 37 cells : 33 somatic plus 4 germinal cells. Somatic cells of several types present (Table IV) : external cells that cover large part of anterior and lateral surfaces of embryo (2 enveloping cells) ; external cell 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 posteroventral lateral cells) ; external cells with refringent bodies (2 apical cells) ; peripheral cells without cilia (2 first ventral cells, 2 second ventral cells, 2 anterior lateral cells, 1 couvercle cell) ; internal cell with cilia (2 ventral internal cells) ; and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells). Each urn cell contains 1 germinal cell plus 2 nuclei ; nuclei shape round (Fig. 2f). Nuclei of anterior lateral cells pycnotic. All somatic nuclei become pycnotic as embryos mature.

Taxonomic Summary

Type Specimens : **Syntypes** on 15 slides of the JLM host series N1 (SBMNH 345290).

Type Host : *Sepia officinalis* Linnaeus, 1758 (Mollusca : Cephalopoda : Sepiidae).

Additional Host(s) : None.

Type Locality : Western Mediterranean Sea, Italy, off Naples, depth of capture unknown.

Collector and Date : John L. Mohr, January 1958.

Distribution : Known only from the type locality.

Site of Infection : Attached to the renal appendages within the renal sacs.

Prevalence : In 1 of 77 cephalopod hosts examined (Table III).

Etymology : The species is named in honor of Charles O. Whitman who studied dicyemid taxonomy at the Stazione Zoologica in Naples, Italy.

Remarks

Dicyema whitmani was found together with *Pseudicyema truncatum* in a single host specimen. This appears to be a very rare species that previously has not been observed in any other species of cephalopod examined in Europe.

Dicyema whitmani is similar to *D. macrocephalum* Van Beneden, 1876 in both calotte shape and size of vermiform stages. The species differs from *D. macrocephalum* in the number of peripheral cells in the vermiform stages (28 vs. 30-31), the number of cells in the infusoriform embryos (37 vs. 39), and the species of cephalopod hosts (*Sepia officinalis* vs. *Sepia elegans, Sepiola rondeleti, Sepiola steenstrupiana*, *Sepietta obscura,* and *Sepietta oweniana*) (Nouvel 1947 ; Hochberg 1990 ; Furuya & Hochberg unpubl. obs.).

Although the vermiform stages of *Dicyema whitmani* and *D. paradoxum* von Kölliker, 1849 have the same number of peripheral cells the two species can be distinguished based on calotte shape (disc-shaped vs. cap-shaped), the number

Table III. – *Sepia officinalis* : dicyemids observed on slide material collected and prepared by Henri Nouvel (1930-35) and J.L. Mohr (1957-58) from several localities in the English Channel and western Mediterranean Sea.

Host Autopsy Number	Dicyemids Observed	Host Autopsy Number	Dicyemids Observed
English Channel: Roscoff, France (Nouvel collection)		248	*P. truncatum, M. vespa*
30	*Pseudicyema truncatum, Microcyema vespa*	249	None
30.BD	*M. vespa*	250	None
30.CA	*M. vespa*	251	None
31	*P. truncatum, M. vespa*	252	*P. truncatum*
31.12	*M. vespa*	253	None
32.2	*P. truncatum, Dicyemennea gracile*	254	*P. truncatum, M. vespa*
32.3	*P. truncatum, D. gracile*	255	*P. truncatum*
33.1	*P. truncatum*	256	*P. truncatum, M. vespa*
33.2	*P. truncatum, D. gracile, M. vespa*	257	*P. truncatum*
33.4	*P. truncatum, D. gracile*	258	None
33.5	*P. truncatum*	259	None
33.6	*P. truncatum*	260	None
33.7	*P. truncatum, D. gracile*	261	*P. truncatum*
34.1	*P. truncatum*	262	*P. truncatum, M. vespa*
35.1	*P. truncatum, D. gracile, M. vespa*	263	*P. truncatum, M. vespa*
		264	*P. truncatum, M. vespa*
English Channel: Plymouth, England (Mohr collection)		265	None
001	*P. truncatum*	266	*P. truncatum*
011	*P. truncatum*	267	*P. truncatum, M. vespa*
013	*P. truncatum*	268	*P. truncatum, M. vespa*
028	*P. truncatum*	291	*P. truncatum, M. vespa*
045	*P. truncatum, M. vespa*	292	None
057	*P. truncatum*	293	None
086	*P. truncatum*	294	None
093	*P. truncatum*	295	*P. truncatum, M. vespa*
094	*P. truncatum, M. vespa*	296	None
106	*P. truncatum*	299	None
109	*P. truncatum*	300	None
114	*P. truncatum*		
115	*P. truncatum*	**Western Mediterranean Sea: Naples, Italy (Mohr collection)**	
116	*P. truncatum*	N1	*P. truncatum, **Dicyema whitmani*** **sp. nov.**
117	*P. truncatum*	N3	*P. truncatum*
179	*P. truncatum, M. vespa*	N4	*P. truncatum*
215	*P. truncatum*		
217	*P. truncatum*	**Western Mediterranean Sea: Monte Carlo, Monaco (Nouvel collection)**	
218	*P. truncatum*	off.1	*P. truncatum, D. gracile*
219	*P. truncatum*	off.4	*P. truncatum, D. gracile*
220	*P. truncatum*	off.5	*P. truncatum, D. gracile*
230	*P. truncatum*	off.6	*P. truncatum, D. gracile*
236	*P. truncatum*	off.7	*P. truncatum, D. gracile*
243	*P. truncatum, M. vespa*	off.8	*P. truncatum, D. gracile*

of cells in the infusoriform embryos (37 vs. 39), and the species of cephalopod hosts (*Sepia officinalis* vs. *Octopus vulgaris* and *O. macropus*) (Nouvel 1947, Hochberg 1990, Furuya & Hochberg unpubl. obs.).

Dicyema banyulensis **sp. nov.** (Fig. 3, 4, Tables I, II)

Material examined

See Table IV. Description based on dicyemids present on a total of 33 slide preparations from 6 octopus hosts plus observations of live animals from 7 hosts.

Description

Diagnosis : Small to medium-sized dicyemids ; body lengths typically less than 1,500 µm. Vermiform stages (i.e., vermiform embryos, nematogens, and rhombogens) with 22 peripheral cells : 4 propolars ; 4 metapolars ; 2 parapolars ; and 12 trunk cells. Calotte relatively small, conical in shape. Infusoriform embryos with 39 cells ; 2 nuclei present in each urn cell.

Nematogens (Fig. 3a, c ; n = 20) : Body elongate, slender, widest in region of parapolar cells ; lengths of largest individuals range from 500-1,500 µm ; trunk width uniform, maximum widths range from 30-100 µm. Peripheral cell number 22 : 4 propolars ; 4 metapolars ; 2 parapolars ; 10 or 11 diapolars ; and 2 or 1 uropolars (Table I). Calotte small ; shape conical ; cilia on calotte short, about 5 µm long, oriented anteriorly. Cytoplasm of both propolar and metapolar cells more darkly stained by hematoxylin than trunk cells. Propolar cells and nuclei slightly smaller than the metapolars. Cephalic enlargement formed by calotte and swollen parapolar cells. Trunk cells arranged in opposed pairs. Verruciform cells absent. Axial cell cylindrical, rounded anteriorly; extends forward to base of propolar cells. About 30 vermiform embryos present in axial cells of largest individuals. Accessory nuclei occasionally observed in trunk peripheral cells.

Vermiform embryos (Fig. 3f, g; n = 20): Full-grown vermiform embryos medium size; lengths range from 65-80 µm, widths from 17-23 µm. Peripheral cell number 22 (Table I); trunk cells arranged in opposed pairs. Anterior end of calotte rounded. Axial cell tapered anteriorly, occasionally pointed; extends forward to base of propolar cells; nucleus typically located in center or in anterior half of axial cell; nucleus diameter equal to agamete diameter. Axial cell of full-grown embryos contain 2-4 agametes; located on both sides of nucleus.

Rhombogens (Fig. 3b, d, e; n = 20): Slightly stockier than nematogens, otherwise generally similar in shape and proportions; lengths range from 500-1,500 µm; widths from 80-100 µm. Peripheral cell number 22 (Table I). Calotte shape conical. Axial cell similar in shape and anterior extent to nematogens. Variable number of infusorigens, never more than 4, present in axial cell of each parent individual. About 50 infusoriform embryos present in axial cells of largest individuals. Accessory nuclei occasionally observed in peripheral cells.

Infusorigens (Fig. 4a; n = 20): Mature infusorigens medium-sized; composed of 10-30 (mode 21) external cells (oogonia and primary oocytes); 4-10 (mode 6) internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes); and 4-20 (mode 8) spermatozoa. Mean diameter of fertilized eggs, 13.6 µm; of spermatozoa, 2.2 µm. Axial cells of infusorigens typically irregular in shape.

Infusoriform embryos (Fig. 4b-d; n = 50): Full-grown embryos medium-sized, lengths (excluding cilia) average 30.0 ± 1.8 µm (mean ± SD); length-width-height ratio, 1 : 0.90 : 0.92; shape ovoid, bluntly rounded to pointed posteriorly. Cilia at posterior end short, 5 µm long. Refringent bodies present; solid; occupy anterior 30 % of embryo length when viewed laterally (Fig. 4b). Cilia project from ventral internal cells into urn cavity (Fig. 4b). Capsule cells relatively large, contain numerous large granules on side adjacent to urn. Full-grown infusoriform embryos consist of 39 cells: 35 somatic plus 4 germinal cells. Somatic cells of several types present (Table II): external cells that cover much of anterior and lateral surfaces of embryo (2 enveloping cells); external cell 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 posteroventral lateral

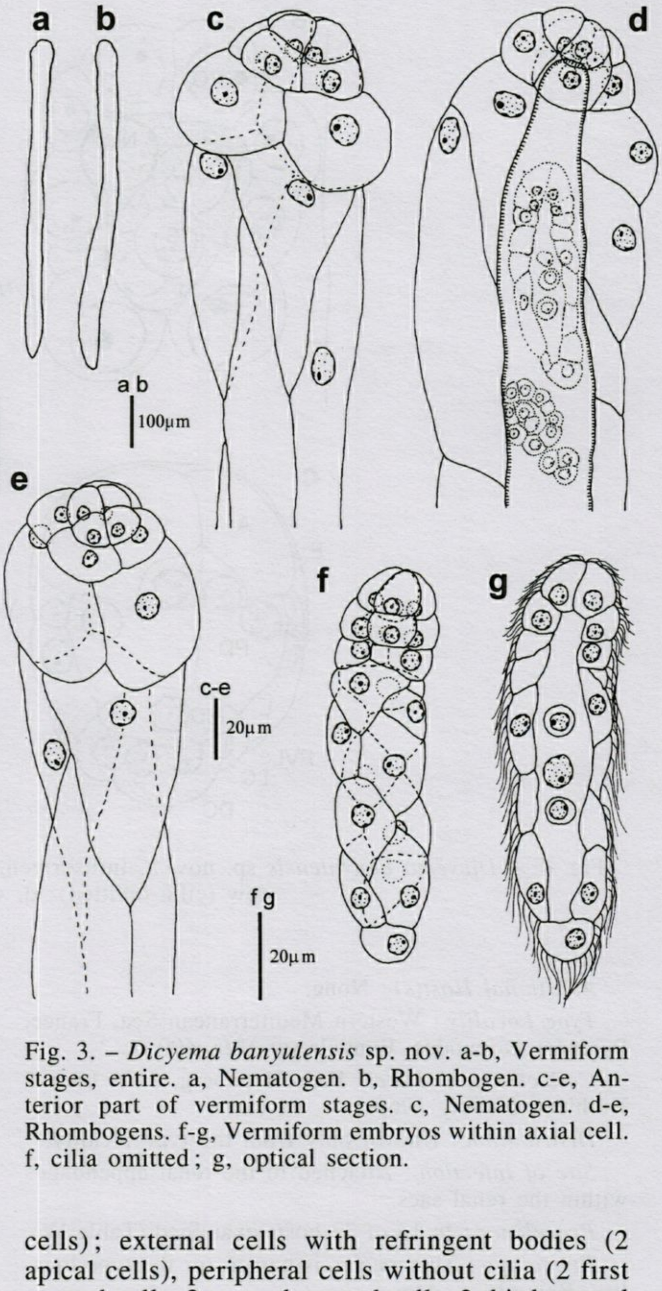

Fig. 3. – *Dicyema banyulensis* sp. nov. a-b, Vermiform stages, entire. a, Nematogen. b, Rhombogen. c-e, Anterior part of vermiform stages. c, Nematogen. d-e, Rhombogens. f-g, Vermiform embryos within axial cell. f, cilia omitted; g, optical section.

cells); external cells with refringent bodies (2 apical cells), peripheral cells without cilia (2 first ventral cells, 2 second ventral cells, 2 third ventral cells, 2 anterior lateral cells, 1 couvercle cell); internal cell with cilia (2 ventral internal cells); and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells). Each urn cell contains 1 germinal cell plus 2 nuclei; nuclei shape round (Fig. 4b). All somatic nuclei become pycnotic as embryos mature.

Taxonomic Summary

Type Specimens: Syntypes on slides in the FGH host series 502 (9 slides, SBMNH 345291); 503 (4 slides, SBMNH 345306); 504 (3 slides, SBMNH 345292; 507 (6 slides, SBMNH 345307).

Type Host: *Octopus salutii* Verany, 1839 (Mollusca : Cephalopoda : Octopodidae).

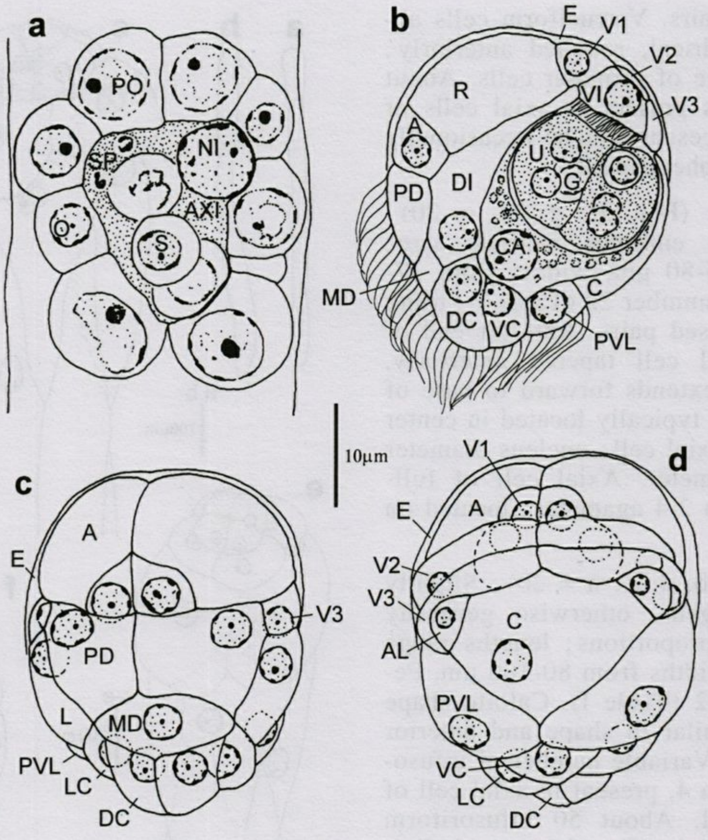

Fig. 4. – *Dicyema banyulensis* sp. nov. a, Infusorigen. b-d, Infusoriform embryos. b, sagittal section; c, dorsal view (cilia omitted); d, ventral view (cilia omitted).

Additional Host(s) : None.

Type Locality : Western Mediterranean Sea, France, Pyrénées-Orientales, Banyuls-sur-Mer, 400 m.

Collector and Date : F.G. Hochberg, R/V Lacaze Duthiers, 23 July 1969.

Distribution : Known only from the type locality.

Site of Infection : Attached to the renal appendages within the renal sacs.

Prevalence : In 13 of 23 hosts examined (Table IV).

Etymology : The species is named for the type locality, Banyuls-sur-Mer.

Remarks

Dicyema banyulensis is similar to *D. caudatum* Bogolepova-Dobrokhotova, 1960 in calotte shape, however, it differs from *D. caudatum* in the number of peripheral cells (22 vs. 16), species of cephalopod host (*Octopus salutii* vs. *Rossia pacifica* Berry, 1911 plus an unidentified octopod), and geographic distribution (western Mediterranean Sea, France vs. Okhotsk Sea, Russia).

Dicyema banyulensis differs from all other species in the genus that have 22 peripheral cells principally on the basis of its small, conical calotte and large, swollen parapolar cells in adult vermiform stages.

***Dicyema benedeni* sp. nov.** (Fig. 5, 6, Tables I, II)

Materials examined

See Table IV. Description based on dicyemids present on a total of 59 slide preparations from 9 octopus hosts plus observations of live animals from 6 hosts.

Description

Diagnosis : Medium-sized dicyemids; body lengths typically less than 1,500 µm. Calotte large, disc-shaped. Vermiform stages (i.e., vermiform embryos, nematogens, and rhombogens) with 18 peripheral cells : 4 propolars; 4 metapolars; 2 parapolars; and 8 trunk cells. Infusoriform embryos with 37 cells; 2 nuclei present in each urn cell.

Nematogens (Fig. 5a, c; n = 20) : Body relatively short, stocky, nail-shaped, widest in region of metapolar or parapolar cells; lengths of largest individuals range from 500-1,500 µm, maximum widths from 80-120 µm. Peripheral cell number 18 : 4 propolars; 4 metapolars; 2 parapolars; 6 diapolars; and 2 uropolars (Table I). Calotte large, disc-shaped; cilia on calotte short, about 5 mm

Table IV. – *Octopus salutii* : dicyemids observed in material collected and prepared by H Nouvel (1938) and FG Hochberg (1969) from several localities in the western Mediterranean Sea.

HOST DATA			PREPS	DICYEMIDS
Autopsy Number	Sex (maturity)	ML (mm)*	No. Slides + Formalin	Species Observed
Monaco: Monte Carlo (Nouvel collection)				
1938-1	Female (juvenile)	[15 g]	2	*Dicyemennea eledones* + *Chromidina***
France: Banyuls-sur-Mer (Hochberg collection)				
477	Female (mature)	85	10 + F	*D. eledones* + *Dicyema benedeni* + *Chromidina***
480	Female (mature)	75	live	*D. eledones* + *D. benedeni*
498	Female (immature)	55	5	*D. benedeni*
502	Male (mature)	70	9 + F	*Dicyema banyulensis*
503	Female (mature)	95	4	*D. eledones* + *D. benedeni* + *D. banyulensis*
504	Male (mature)	75	3 + F	*D. eledones* + *D. banyulensis*
507	Male (mature)	60	6 + F	*D. eledones* + *D. benedeni* + *D. banyulensis*
508	Female (mature)	92	6 + F	*D. eledones* + *D. benedeni* + *D. banyulensis*
509	Male (mature)	70	live	*D. eledones* + *D. benedeni* + *D. banyulensis*
512	Male (mature)	60	2 + F	*D. eledones*
513	Female (mature)	87	9 + F	*D. eledones* + *D. benedeni*
517	Female (mature)	100	5 + F	*D. eledones* + *D. benedeni*
518	Female (mature)	85	live	*D. benedeni* + *D. banyulensis*
519	Female (mature)	125	9 + F	*D. eledones* + *D. benedeni*
531	Male (mature)	80	5 + F	*D. eledones* + *D. benedeni* + *D. banyulensis*
536	Female (brooding)	95	live	*D. benedeni*
540	Female (mature)	100	live	*D. benedeni* + *D. banyulensis*
553	Male (mature)	65	live	*D. banyulensis*
554	Female (mature)	75	live	*D. eledones* + *D. banyulensis*
555	Female (mature)	80	live	*D. banyulensis*
556	Female (mature)	85	live	*D. eledones* + *D. benedeni* + *D. banyulensis*
Spain: Rosas (Hochberg collection)				
494	Female (mature)	115	F	*D. eledones* + *D. benedeni* + *Chromidina***

* = measurements of mantle lengths (ML) taken from freshly dead animals
** = *Chromidina* cf. *elegans* (Ciliophora: Apostomea) also found in the kidneys of the octopus host
F = host kidney tissue preserved in 5% formalin in seawater
live = identifications of dicyemids determined from live material only; preparations not made

long, oriented anteriorly. Cytoplasm of both propolar and metapolar cells more darkly stained by hematoxylin than trunk cells. Propolar cells and nuclei slightly smaller than the metapolars. Cephalic enlargement composed of calotte and parapolar cells. Verruciform cells absent. Axial cell cylindrical, rounded anteriorly; extends forward to base of propolar cells. About 50 vermiform embryos present in axial cells of largest individuals.

Vermiform embryos (Fig. 5e, f; n = 20) : Vermiform embryos small; lengths of largest individuals within axial cell of parent nematogens range from 40-50 µm, widths from 17-23 µm. Peripheral cell number 18 (Table I); trunk cells arranged in opposed pairs. Anterior end of calotte rounded. Axial cell rounded anteriorly; extends forward to base of propolar cells; nucleus usually located in anterior half of axial cell; nucleus diameter equal to agamete diameter. Axial cells of embryos typically contain 2 agametes; both located in posterior half of cell.

Rhombogens (Fig. 5b, d; n = 20) : Slightly stockier than nematogens, otherwise generally similar in shape and body proportions; lengths range from 500-1,500 µm; widths 80-150 µm. Peripheral cell number 18 (Table I). Calotte disc-shaped. Cephalic enlargement composed of calotte and parapolar cells. Verruciform cells absent. Axial cell similar to nematogens in shape and anterior extent. Typically 1-2, sometimes 3-4, infusorigens in axial cell of each parent individual. About 60 infusoriform embryos present in axial cell of largest individuals. Accessory nuclei occasionally observed in uropolar cells.

Infusorigens (Fig. 6a; n = 20) : Mature infusorigens medium-sized; composed of 30-70 (mode 52) external cells (oogonia and primary oocytes); 15-50 (mode 25) internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes); and 20-80 (mode 26) spermatozoa. Mean diameter of fertilized eggs, 12.8 µm; of spermatozoa, 2.2 µm.

Infusoriform embryos (Fig. 6b-d; n = 50) : Full-grown embryos medium-sized, lengths (excluding cilia) average 26.7 ± 1.3 µm (mean ± SD); length-width-height ratio 1 : 0.84 : 0.83; shape ovoid, bluntly rounded to pointed posteriorly; cilia at posterior end short, 5 µm long. Refringent bodies present; solid; size about same as single urn cell; occupy anterior 25 % of embryo length when viewed laterally (Fig. 6b). Cilia

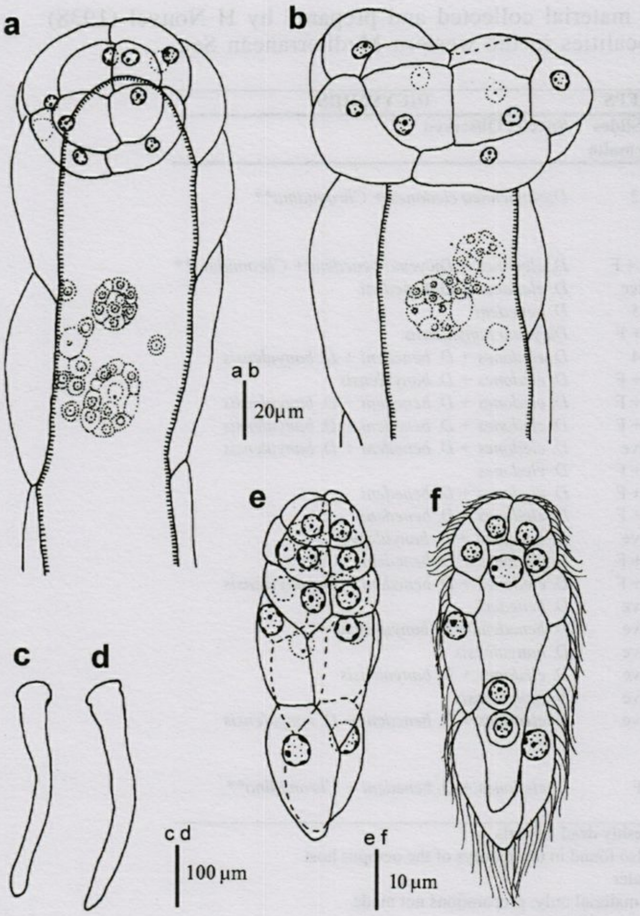

Fig. 5. – *Dicyema benedeni* sp. nov. a-b, Anterior part of vermiform stages. a, Nematogen. b, Rhombogen. c-d, Vermiform stages, entire. c, Nematogen. d, Rhombogen. e-f, Vermiform embryos within axial cell. e, cilia omitted; f, optical section.

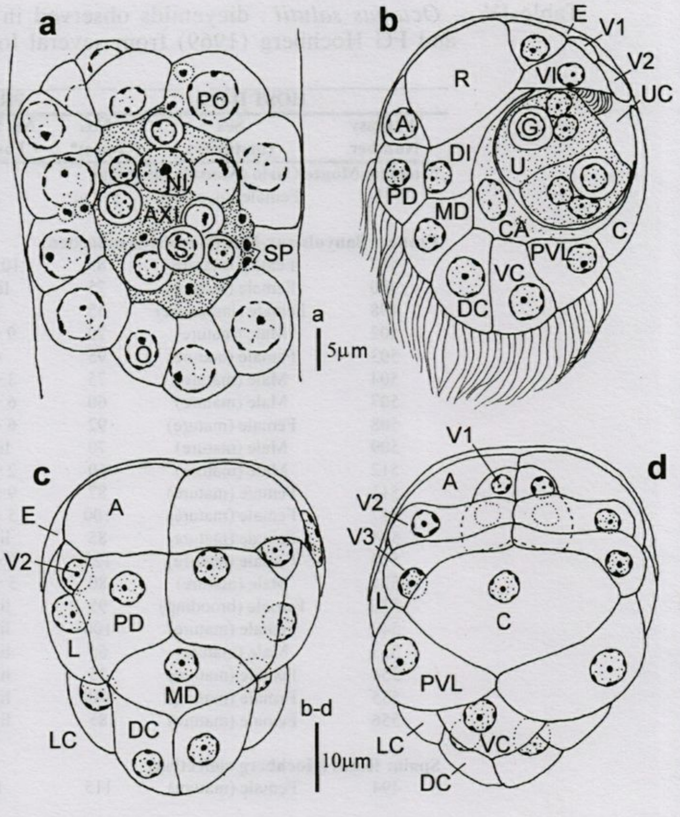

Fig. 6. – *Dicyema benedeni* sp. nov. a, Infusorigen. b-d, Infusoriform embryos. b, sagittal section; c, dorsal view (cilia omitted); d, ventral view (cilia omitted).

project from ventral internal cells into urn cavity (Fig. 6b). Capsule cells contain many large granules on side adjacent to urn. Full-grown infusoriform embryos with 37 cells : 33 somatic plus 4 germinal cells. Somatic cells of several types present (Table II) : external cells that cover large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cell 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 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); peripheral cells without cilia (2 first ventral cells, 2 second ventral cells, 2 third ventral cells, 1 couvercle cell); internal cell with cilia (2 ventral internal cells); and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells). Each urn cell contains 1 germinal cell plus 2 nuclei; nuclei shape round (Fig. 6b). Nuclei of anterior lateral cells pycnotic. All somatic nuclei become pycnotic as embryos mature.

Taxonomic Summary

Type Specimens : Syntypes on slides in the FGH host series 503 (4 slides, SBMNH 345306); 507 (6 slides, SBMNH 345307); 513 (9 slides, SBMNH 345293); and 517 (5 slides, SBMNH 345294).

Type Host : *Octopus salutii* Verany, 1839 (Mollusca : Cephalopoda : Octopodidae).

Additional Host(s) : None.

Type Locality : Western Mediterranean Sea, France, Pyrénées-Orientales, off Banyuls-sur-Mer, 400 m.

Collector and Date : F.G. Hochberg, R/V Lacaze Duthiers, 23 July 1969.

Distribution : Also found in one host collected off Rosas, Spain (Table IV).

Site of Infection : Attached to the renal appendages within the renal sacs.

Prevalence : In 16 of 23 hosts examined (Table IV).

Etymology : The species is named in honor of Van Beneden who studied dicyemids and other primitive multicellular animals in Europe and erected the name "Mesozoa" for this unusual group of cephalopod parasites.

Remarks

Although *Dicyema benedeni* and *D. banyulensis* may be found together in the same host individual the two species can be distinguished easily based on the number of peripheral cells in vermiform stages (18 vs. 22), the shape of the calotte (disc-like vs. conical), and the number of cells in infusoriform embryos (37 vs 39).

Dicyema benedeni is similar to *D. macrocephalum* (Van Beneden, 1876) and *D. whitmani* in calotte shape, and sizes of vermiform stages. *Dicyema benedeni* differs from *D. macrocephalum* in the number of peripheral cells in the vermiform stages (28 vs. 30-31), the number of cells in infusoriform embryos (37 vs. 39), and the species of cephalopod hosts (*Octopus salutii* vs. *Sepia elegans* Blainville, 1827, *Sepiola rondeleti* Leach, 1834, *Sepiola steenstrupiana* Levy, 1912, *Sepietta obscura* Naef, 1916, and *Sepietta oweniana* Orbigny, 1839-1841 in Férussac & Orbigny, 1834-1848) (Nouvel 1947, Hochberg 1990, Furuya & Hochberg unpubl. obs.). *Dicyema benedeni* differs from *D. whitmani* (described above) in the number of peripheral cells in the vermiform stages (18 vs. 28), the lengths of adult vermiform stages (vs. 7,000 µm), and the species of cephalopod hosts (*Octopus salutii* vs. *Sepia officinalis*).

DISCUSSION

Dicyemids from Europe obtained by H. Nouvel, J.L. Mohr and F.G. Hochberg and housed in the collections of the Santa Barbara Museum of Natural History were studied. A total of 4 species of dicyemids were found in 63 of 77 individuals (82 %) of the cuttlefish host, *Sepia officinalis*, namely : *Dicyema whitmani*; *Dicyemennea gracile*; *Microcyema vespa*; and *Pseudicyema truncatum* (see Table III). As shown in Table III dicyemids were not detected on slide preparations from 14 of 77 hosts examined (18 %). Three species of dicyemids, *D. gracile, M. vespa,* and *P. truncatum* were detected together in 2 hosts (3 %). Two species of dicyemids were detected together in 27 hosts (35 %). A single species was found in the remaining 34 hosts (44 %). Four different collection localities are represented, namely : Italy (Naples); Monaco (Monte Carlo); England (Plymouth); and France (Roscoff). *Pseudicyema truncatum* was present at all four localities in the Mediterranean and English Channel. *Microcyema vespa* was found in hosts collected in Monaco, Plymouth, and Roscoff and thus probably is distributed throughout the hosts range (Furuya & Hochberg, unpubl. obs.). *Dicyemennea gracile* has a more disjunct distribution and was only found in Monaco and Roscoff. In contrast to the other species, *D. whitmani* was found in only one cuttlefish host from Naples. This latter species is very rare and may have a very narrow geographic distribution.

Three species of dicyemids have been found in *Octopus salutii* namely : *Dicyema banyulensis*, *Dicyema benedeni*, and *Dicyemennea eledones*. All 3 species of dicyemids were found together in a total of 6 of 23 hosts examined in the Mediterranean (26 %; Table IV). Two species were detected together in another 10 hosts (44 %) and a single species was found in 7 hosts (30 %). The apostome ciliate *Chromidina elegans* Foettinger, 1881 was found together with dicyemids in 3 hosts.

This study increased the number of described dicyemid species for *Sepia officinalis* from 3 to 4. Other host species in which more than 3 dicyemids, have been described include : *Octopus dofleini* Wülker, 1910 (4 species – North Pacific Ocean; Hochberg 1990); *Octopus fangsiao* Orbigny, 1839-41 (5 species – northwestern Pacific Ocean; Furuya 1999); *Octopus rubescens* Berry, 1953 (5 species – northeastern Pacific Ocean; Hochberg 1990); *Octopus vulgaris* Cuvier, 1797 (6 species – northeastern Atlantic Ocean & Mediterranean, Nouvel 1947, Hochberg 1990),

Octopus bimaculoides Pickford & McConnaughey, 1949 (7 species – northeastern Pacific Ocean, Hochberg 1990), *Rossia pacifica* Berry, 1911 (9 species – North Pacific Ocean, Hochberg, 1990). Thus, it appears to be relatively common for a single species of cephalopod to be host to 4 or more species of dicyemids (Hochberg 1990, Furuya 1999).

ACKNOWLEDGMENTS. – The work presented in this paper was undertaken while the senior author (HF) was in residence as a Postdoctoral Research Fellow at the SBMNH. During this time he was supported in part by a grant for Japanese Junior Scientists from the Japan Society for Promotion of Science (JSPS research grant no. 2952). The junior author (FGH) would like to thank Drs. K. Mangold and S.v. Boletzky for their generous hospitality and assistance in obtaining cephalopods for a study of their parasites during the summer of 1969 while visiting the Laboratoire Arago, Banyuls-sur-Mer, France as a graduate student. We extend special thanks to Dr. J.L. Mohr (University of Southern California, retired) who donated his collection of dicyemid and ciliate preparations to the SBMNH in which we discovered one of our new species described above. C. Combes (Université de Perpignan, France) generously allowed us to study slides in H. Nouvel's collection of dicyemids for which he is the current custodian. A preliminary survey of Nouvel's collection was undertaken during a visit by FGH to France in October 1996. The visit was organized and hosted by Dr. S.v. Boletzky (Laboratoire Arago, Banyuls-sur-Mer, France). Sigurd assisted with French translations of several portions of this paper.

REFERENCES

Beetschen JC, Bitsch J 1975. Henri Nouvel (1905-1974). *Bull Soc Hist Nat Toulouse* 111 : 7-16.

Bogolepova-Dobrokhotova II 1960. Dicyemidae of the Far-eastern Seas. II. New species of genus *Dicyema*. *Zool Zhur* 39 : 1293-1302 [in Russian, with English abstract].

Furuya H 1999. Fourteen new species of dicyemid mesozoans from six Japanese cephalopods, with comments on host specificity. *Species Diversity* (in press).

Furuya H, Tsuneki K, Koshida Y 1992a. Two new species of the genus *Dicyema* (Mesozoa) from octopuses of Japan with notes on *D. misakiense* and *D. acuticephalum*. *Zool Sci* 9 : 423-437.

Furuya H, Tsuneki K, Koshida Y 1992b. Development of the infusoriform embryo of *Dicyema japonicum* (Mesozoa : Dicyemidea). *Biol Bull* 183 : 248-157.

Furuya H, Tsuneki K, Koshida Y 1997. Fine structure of a dicyemid mesozoan, *Dicyema acuticephalum*, with special reference to cell junctions. *J Morphol* 231 : 297-305.

Hochberg FG 1990. Diseases caused by protistans and mesozoans. *In* Diseases of Marine Animals, Vol. III. Edited by O Kinne, Biologische Anstalt Helgoland, Hamburg : 47-202.

Kölliker A von 1849. Über *Dicyema paradoxum*, den Schmarotzer der Venenanhänge der Cephalopoden. *Ber. König Zoot Anst Würzburg* 2 : 53-58.

Krohn A 1839. Über das Vorkommen von Entozoen und Krystallablagerungen in den schwammingen Venenanhängen einiger Cephalopoden. *Neue Notiz Geb Natur Heilk* 11 : 213-216.

Lankester ER 1873. The parasite of the renal organ of Cephalopoda. *In* Summary of zoological observations made at Naples in the winter of 1871-1872. *Ann Mag Nat Hist* 11 : 81-97.

Nouvel H 1944. Les Dicyémides des Sepiolidae des côtes françaises. *Bull Inst Océanog Monaco* 869 : 1-12.

Nouvel H 1945. Les Dicyémides de quelques Céphalopodes des côtes françaises avec indication de la présence de Chromidinides. *Bull Inst Océanog Monaco* 887 : 1-8.

Nouvel H 1947. Les Dicyémides. 2e partie : systématique, générations, vermiformes, infusorigène et sexualité. *Arch Biol Paris* 58 : 59-220.

Nouvel H 1948. Les Dicyémides. 2e partie : infusoriforme, tératologie, spécificité, du parasitisme, affinités. *Arch Biol Paris* 59 : 147-223.

Short RB, Damian RT 1966. Morphology of the infusoriform larva of *Dicyema aegira* (Mesozoa : Dicyemidae). *J Parasitol* 52 : 746-751.

Van Beneden É 1876a. Recherches sur les Dicyémides, survivants actuels d'un embranchement des Mésozories. *Bull Acad R Belg Cl Sci* 41 : 1160-1205.

Van Beneden É 1876b. Recherches sur les Dicyémides, survivants actuels d'un embranchement des Mésozories. *Bull Acad R Belg Cl Sci* 42 : 3-111.

Van Beneden É 1882. Contribution à l'histoire des Dicyémides. *Arch Biol Paris* 3 : 195-228.

Wagener GR 1857. Über *Dicyema* Kölliker. *Arch Pathol Anat Physiol* 1857 : 354-364.

Whitman CO 1883. A contribution to the embryology, life history, and classification of the dicyemids. *Mitt Zool Stat Neapel* 4 : 1-89.

Reçu le 15 septembre 1998; received September 15, 1998
Accepté le 22 octobre 1998 : accepted October 22, 1998