Cell Number and Cellular Composition in Infusoriform Larvae of Dicyemid Mesozoans (Phylum Dicyemida)

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ABSTRACT—Cell numbers and cellular composition were examined in infusoriform larvae of 44 species of dicyemid mesozoans belonging to 6 genera; *Conocyema*, *Dicyema*, *Dicyemennea*, *Dicyemodeca*, *Microcyema*, and *Pseudicyema*. In addition, literature on infusoriform larvae of another 20 species was reviewed. Infusoriform larvae consist of a constant cell number which is species-specific. Small interspecific variations are found in total cell numbers, 35, 37, 39, 41 and 42. The most frequent cell number encountered in infusoriform larvae studied is either 37 or 39. Infusoriform larvae with 35 cells are found in three genera. Infusoriform larvae with 37 cells are found in four genera. Infusoriform larvae with 39 cells are found in four genera. Most differences in total cell numbers are due to the absence or presence of particular ventral cells. In all infusoriform larvae, the lateral, dorsal and caudal areas are cell constant, whereas in the apical and ventral areas a distinct and variable configuration of cells are present. In cellular composition, a total of 29 cells (15 cell types) were recognized in all infusoriform larvae examined. Additional cell types are characteristic of a relatively few species. Even in infusoriform larvae with the same total cell numbers, cellular composition varies by species. Thus, there are 7 variations of cellular composition in infusoriform larvae with 37 cells. Differences in larval cell numbers and types do not warrant traditional generic separation of dicyemids.

Key words: cell numbers, cellular constitution, dicyemids, infusoriform larvae, mesozoans

INTRODUCTION

Dicyemid mesozoans are endoparasites that live exclusively in the renal sacs of benthic cephalopod molluscs. Although recent studies have revealed that they might not be truly primitive animals deserving the name of mesozoans (Katayama *et al.*, 1995; Kobayashi *et al.*, 1999), they are still one of the most interesting groups of lower invertebrates. Their body, consisting of the smallest number of cells among multicellular animals (usually 10 to 40), is organized in a very simple fashion. Their life cycle is also unusual. They produce two distinct types of larvae; vermiform larva from an asexual agamete and infusoriform larva from a fertilized egg (Furuya *et al.*, 1992a, 1993, 1994, 1996). High population densities in the cephalopod kidney may trigger or initiate the shift from an asexual mode to a sexual mode of reproduction (Lapan and Morowitz, 1975). Vermiform individuals have a distinct anterior region, termed a calotte, that

attaches to the surfaces or are inserted into tubules, crypts, or folds of the renal appendages of cephalopod hosts (Ridley, 1968; Furuya *et al.*, 1997; Furuya *et al*, 2003a). On the other hand, the infusoriform larvae escape from the host into the sea to search for a new host. However, the life cycle remains unknown nor is it understood how infusoriform larvae develop into vermiform stages in a new host.

The body forms of invertebrate larvae are spectacularly diverse and a large number of larval forms have been given specific names (Young, 2002). Infusoriform larvae of dicyemids are distinct larvae, which consist of larger numbers of cells and are more complicated in body organization than adult forms. All infusoriform larvae are ciliated posteriorly, and most of them possess two refringent bodies anteriorly. These bodies contain highly hydrated magnesium salt of inositol hexaphosphate (Lapan, 1975). In some dicyemid species, infusoriform larvae were described in detail (Nouvel, 1948, 1961; Short and Damian, 1966). Subsequently some interspecific variations in total cell numbers and cellular composition have been found in these larvae (Short, 1971; Furuya *et al.*, 1992b; Furuya, 1999; Furuya and Hoch-

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berg, 1999). In infusoriform larvae both total cell number and cellular composition appear to be species-specific and thus may represent significant features to be used in the identification and classification of dicyemids. However, in more than half of the species described to date details of both cell number and composition are not known.

In this study we examined cellular constitution and total cell numbers in infusoriform larvae of 44 species belonging to 6 genera; *Conocyema*, *Dicyema*, *Dicyemennea*, *Dicyemodeca*, *Microcyema*, and *Pseudicyema*. In addition infusoriform larvae of 20 previously described species served for comparison. These observations revealed that a total cell number of infusoriform larvae shows some variations and most of them are attributable to the presence or absence of particular ventral cells.

MATERIALS AND METHODS

In this study 44 species of dicyemids were examined. Fifty to 100 infusoriform embryos were studied in each species. These are specimens in the authors' collections mainly from northwestern Pacific Ocean, including the sea off Japan, and the collections of the Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH).

Authors' collections were prepared as follows. When dicyemids were detected in the kidney of the host cephalopods, small pieces of renal appendages with attached dicyemids were removed and smeared on glass microslides. The smears were fixed immediately in Bouin's fluid for 24 hr and then stored in 70% ethyl alcohol. The majority of fixed smears were stained in Ehrlich's hematoxylin and counterstained in eosin. Stained smears were mounted using Canada Balsam, Damar, Permount or Entellan (Merck). Slide preparations in SBMNH were obtained principally from five sources as follows: 1) Henri Nouvel (Université Paul-Sabatier, Toulouse, France) studied dicyemids in cephalopod hosts collected throughout the Mediterranean and northeastern Atlantic Ocean (including the English Channel); 2) Bayard H. McConnaughey (University of Oregon, Eugene, Oregon, USA) worked on dicyemids in cephalopods in the northeastern Pacific Ocean; 3) Robert B. Short (Florida State University, Tallahassee, Florida, USA) examined dicyemids in cephalopods collected in the northwestern Atlantic Ocean, the Gulf of Mexico, and in the Southern Ocean off Antarctica; 4) John L. Mohr (University of Southern California, Los Angeles, California, USA) prepared smears of cephalopod kidney parasites in Europe at the Marine Biological Laboratory, Plymouth, England and the Stazione Zoologica, Naples, Italy; and 5) F. G. Hochberg (Santa Barbara Museum of Natural History, Santa Barbara, California, USA) prepared dicyemids from cephalopods captured in the northeastern Pacific Ocean off California and Mexico, the Mediterranean off France and Italy, and the English Channel.

Dicyemids were observed with a light microscope at magnifications up to 2000x. Measurements and drawings were made with the aid of an ocular micrometer and a drawing tube (Olympus U-DA), respectively.

The literature, describing infusoriform larvae of an additional 20 species, also was consulted for comparison (see Table 2). Nouvel (1948), Short and Damian (1966), and Furuya (1999) give the terminology for description of cell names in infusoriform larvae. Abbreviations for the names of the cells that make up the body of infusoriform larvae are provided in Table 1.

Table 1. Cell types and numbers in infusoriform larvae of dicyemid mesozoans. The presence or absence of cilia is noted for each cell type.

Cell Types (abbreviation)	Number	Cilia
SOMATIC CELLS		
External Cells		
Apical (A)*	2	+
Anterior lateral (AL)	2	−
Apical micro (AM)	2	−
Couvercle (C)*	1	−
Dorsal caudal (DC)*	2	+
Enveloping (E)*	2	−
Lateral (L)*	2	+
Lateral caudal (LC)*	2	+
Median dorsal (MD)*	1 or 2	+
Paired dorsal (PD)*	2	+
Posteroventral lateral (PVL)*	2	+
Shield (SH)	2	−
Ventral caudal (VC)*	1	+
Ventral 1 (V1)	2	−
Ventral 2 (V2)	2	−
Ventral 3 (V3)	2	−
Internal Cells		
Anterior internal (AI)	2	−
Capsule (CA)*	2	−
Dorsal internal (DI)	2	−
Postcapsular (PC)	2	−
Urn (U)*	4	−
Ventral internal (VI)*	2	(+)
GERMINAL CELLS (G)*	4 or 8	−

 * Cell types found in infusoriform larvae of all dicyemid species.
(+) Cilia on these cells project internally.

RESULTS

General notes

To date 91 species of dicyemid have been named and described in the world. The largest number of species have been placed in the genus *Dicyema,* followed by *Dicyemennea*. Several other genera are known but they are monotypic or contain only a small number of species: *Conocyema* (1 species); *Microcyema* (1); *Dicyemodeca* (4); *Dodecadicyema* (1); *Kantharella* (1); and *Pseudicyema* (2). Details on the infusoriform larva of *Kantharella antarctica* are not available. The species has been inadequately described and is not treated in this study.

The above genera have been variously allocated to 3 families, namely Dicyemidae, Conocyemidae (=Heterocyemidae) and Kantharellidae.

Infusoriform embryos are formed within the axial cell of

Table 2. Differences in cell types and cell numbers in infusoriform larvae of 64 species.

Species	AI	AL	AM	DI	PC	SH	V1	V2	V3	Total cell number	Cellular Composition Type**
Conocyema polymorpha	−	−	−	+	−	−	+	+	+	37	2
Dicyema acciacatum	−	+	−	+	−	−	+	+	+	39	1
D. acuticephalum	+	−	−	+	−	−	+	+	−	37	6
D. aegira	−	+	−	+	−	−	+	+	+	39	1
D. apollyoni	−	+	−	+	−	−	+	+	+	39	1
D. banyulensis[1]	−	+	−	+	−	−	+	+	+	39	1
D. benedeni[1]	−	−	−	+	−	−	+	+	+	37	2
D. bilobum	−	−	−	+	−	−	+	+	+	37	2
D. briarei	−	+	−	+	−	−	+	+	+	39	1
D. clavatum[2]	−	+	−	+	−	−	+	+	+	39	1
D. colurum[3]	−	+	−	+	−	−	+	+	+	39	1
D. dolichocephalum	−	+	−	+	−	−	+	+	+	39	1
D. erythrum[3]	−	−	−	+	−	−	+	+	+	37	2
D. hadrum	−	+	−	+	−	−	+	+	+	39	1
D. japonicum[2]	−	+	−	+	−	−	+	+	−	37	3
D. knoxi	−	−	−	+	+	−	+	+	−	37	5
D. lycidoeceum[3]	−	−	−	+	−	−	+	+	+	37	2
D. macrocephalum	−	+	−	+	−	−	+	+	+	39	1
D. maorum	−	+	−	+	+	−	+	+	−	37	5
D. misakiense	−	+	−	+	−	−	+	+	−	37	3
D. moshatum	−	+	−	+	−	−	+	+	+	39	1
D. orientale	−	−	−	+	−	−	+	+	+	37	2
D. paradoxum	−	+	−	+	−	−	+	+	+	39	1
D. rhadinum[3]	−	−	−	+	−	−	+	+	+	37	2
D. robsonellae	−	−	−	+	+	−	+	+	−	37	5
D. rondeletielae	−	+	−	+	−	−	+	+	−	37	3
D. schulzianum	−	−	−	+	−	−	+	+	+	37	2
D. sphyrocephalum[3]	−	−	−	+	−	−	+	+	+	37	2
D. sullivani	−	+	−	+	−	−	+	+	+	39	1
D. typoides	−	−	−	+	−	−	+	+	−	35	4
D. typus	−	−	−	+	−	−	+	+	−	35	4
D. whitmani[1]	−	+	−	+	−	−	+	+	−	37	3
D. sp.[4]	−	−	+	+	−	+	−	−	−	35	7
Dicyemennea abelis	−	+	−	+	−	−	+	+	+	39	1
D. abreida	−	+	−	+	−	−	+	+	+	39	1
D. abreviata	−	+	−	+	−	−	+	+	+	39	1
D. adminicula	−	+	−	+	−	−	+	+	−	37	3
D. adscita	−	+	−	+	−	−	+	+	+	39	1
D. antarcticensis	−	+	−	+	−	−	+	+	+	39	1
D. brevicephala	−	+	−	+	−	−	+	+	+	39	1
D. brevicephaloides	−	−	−	+	−	−	+	+	−	35	4
D. californica	−	+	−	+	−	−	+	+	+	39	1
D. canadensis[5]	−	−	−	+	−	+	+	+	−	37	8
D. dorycephalum[6]	−	−	−	+	−	−	+	+	+	37	2
D. eledones	−	−	−	+	−	−	+	+	+	37	2
D. eltanini	−	−	−	+	−	−	+	+	+	37	2
D. gracile	−	−	−	+	−	−	+	+	+	37	2
D. granularis	−	+	−	+	−	−	+	+	+	39	1
D. gyrinodes[3]	−	−	+	+	−	+	+	+	+	41	9
D. kaikouriensis	−	−	−	+	−	−	+	+	+	37	2
D. lameerei	−	−	−	+	−	−	+	+	−	37	3
D. mastigoides[3]	−	−	−	+	−	−	+	+	+	37	2
D. minabense[3]	−	−	−	+	−	−	+	+	+	37	2
D. nouveli	−	+	−	+	−	−	+	+	+	39	1
D. ophioides[3]	−	+	−	+	−	−	+	+	+	39	1
D. parva	−	−	+	+	−	−	+	+	−	37	10
D. rostrata	−	+	−	+	−	−	+	+	+	39	1
D. trochocephalum[3]	−	+	−	+	−	−	+	+	+	37	2
Dicyemodeca anthinocephalum[3]	+	−	+	+	−	+	−	−	−	42#	11
D. deca	−	+	+	−	−	+	−	−	−	35	12
D. delamarei[7]	−	−	−	+	−	−	+	+	+	37	2
Microcyema vespa	−	+	−	+	−	−	+	+	+	39	1
Pseudicyema nakaoi[3]	−	+	−	+	−	−	+	+	+	39	1
P. truncatum	−	+	−	+	−	−	+	+	+	39	1

* Cells common to all dicyemid species are omitted. For abbreviations see Table 1.
** See Table 3.
# This species has 8 germinal cells.
References: 1, Furuya and Hochberg (1999); 2, Furuya *et al.* (1992a); 3, Furuya (1999); 4, Furuya and Hochberg (pers. obs.); 5, Furuya *et al.* (2002); 6, Furuya and Hochberg (2002); 7, Nouvel (1961).

parent rhombogens (Fig. 1a). Upon maturation, and following eclosion from the axial cell of the parent, infusoriform embryos are flushed from the cephalopod host into the sea when urine is voided. As larvae they search for a new host. In our study only mature infusoriform embryos were examined. They are essentially the same as infusoriform larvae in size, cell number and cell composition.

Size of infusoriform larvae

The infusoriform larva is bilaterally symmetrical and usually ovoid or spheric (Fig. 1). Body lengths of infusoriform embryos within axial cells of parent rhombogens range from 22.4–51.0 μm; mean±S.D., 30.78±5.13 μm; median,

30.0 μm (n=64). Infusoriform embryos of *Dicyemennea* are slightly larger than those of *Dicyema* (32.9±5.71 μm vs. 29.1±4.27 μm). The smallest embryos are found in *Dicyema paradoxum*, whereas the largest are observed in *Dicyemennea antarcticensis* (Fig. 2a).

Cell number of infusoriform larvae

Cell numbers in infusoriform larvae are species constant. There are 5 basic variations, namely 35, 37, 39, 41, and 42 (Fig. 2b; Table 2). The 2 most frequent cell numbers encountered are 37 (30 species) or 39 (27 species), which constitute 89% of all dicyemid species examined. Infusoriform larvae with 37 cells are found in 4 genera, *Conocyema*,
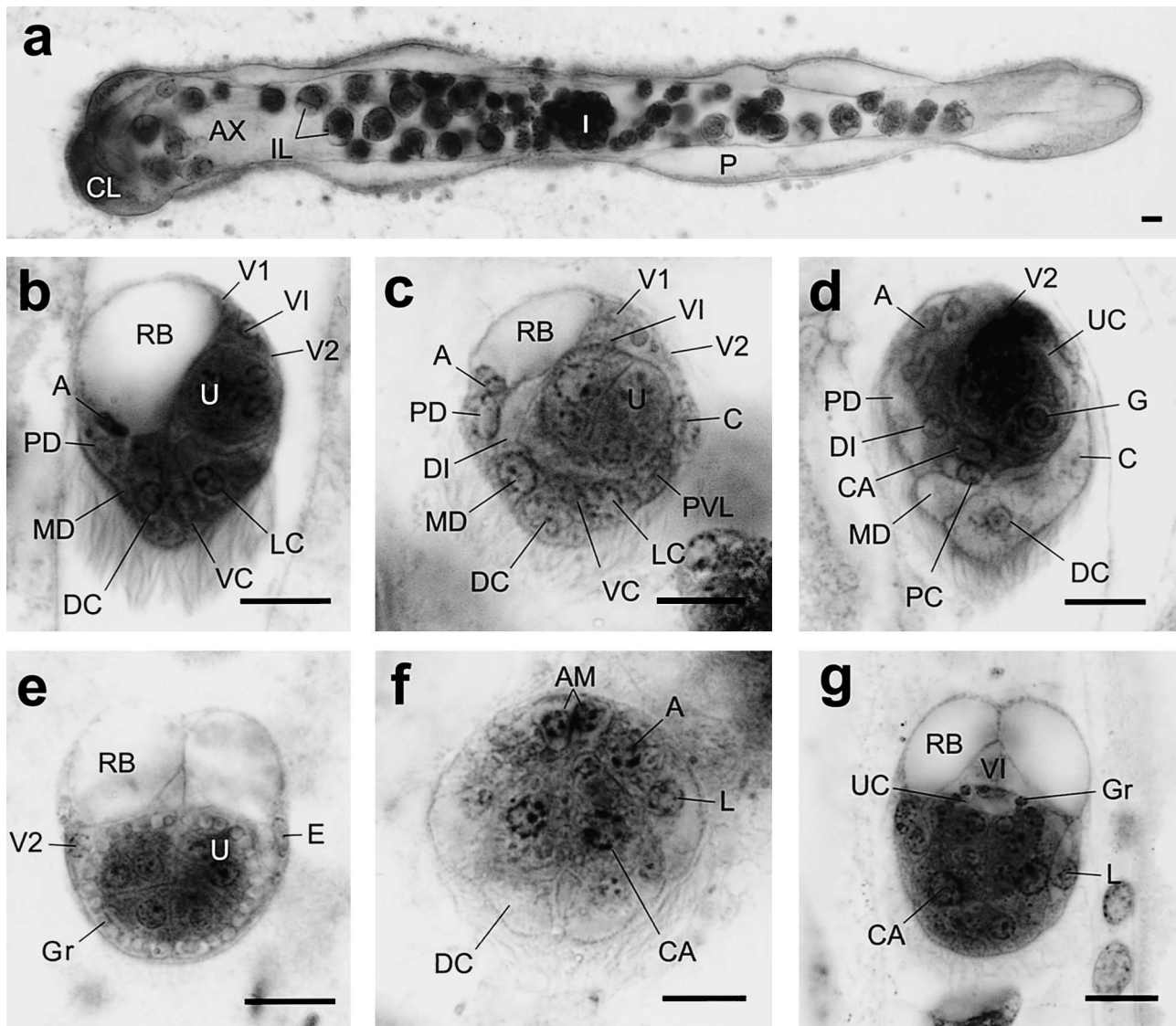

Fig. 1. Light micrographs of a rhombogen stage and a diversity of infusoriform larvae. **a**, entire body of a rhombogen of *Dicyema japonicum*; **b–g**, infusoriform embryos within axial cells of parent rhombogens. **b**, *Dicyema typoides*; **c**, *Dicyema japonicum*; **d**, *Dicyema maorum*; **e**, *Dicyemennea abreida* ; **f**, *Dicyemennea gyrinodes*; **g**, *Dicyema apollyoni*. Scale bars represent 10 μm. Abbreviations: A, apical cell; AM, anterior micro cell; AX, axial cell; C, couvercle cell; CA, capsule cell; CL, calotte; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; Gr, granules; I, infusorigen; IL, infusoriform larvae; L, lateral; LC, lateral caudal cell; MD, median dorsal cell; P, peripheral cell; PC, postcapsular cell; PD, paired dorsal cell; PVL, posteroventral lateral cell; RB, refringent body; U, urn cell; UC, urn cavity; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell. Orientation of optical sections: b–d, sagital; e–g, horizontal.
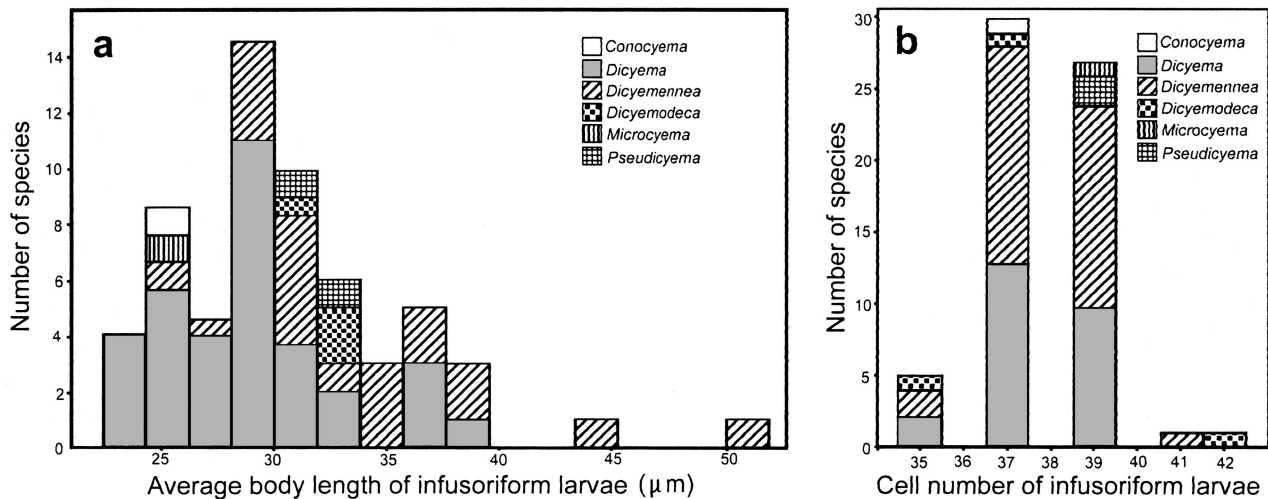
Fig. 2. Distribution of body lengths in μm (a) and cell numbers in infusoriform larvae (b).

Dicyema, *Dicyemennea*, and *Dicyemodeca*. Infusoriform larvae with 39 cells are found in four genera, *Dicyema*, *Dicyemennea*, *Microcyema* and *Pseudicyema*. The next most frequent cell number is 35, which is found in 5 species of 3 genera, *Dicyema*, *Dicyemennea*, and *Dicyemodeca*. The remaining 2 cell numbers, 41 and 42, are rare, and to date have been found only in one species each, namely, *Dicyemennea gyrinodes* and *Dicyemodeca anthinocephalum*. All infusoriform larvae with 35, 37, 39, and 41 cells, are composed of 4 germinal cells, plus 31, 33, 35, or 37 somatic cells, respectively. The infusoriform larva with 42 cells consists of 8 germinal cells plus 34 somatic cells (Furuya, 1999).

Cell types of infusoriform larvae

A total of 23 types of cells (22 somatic cells and one germinal cell) have been identified in infusoriform larvae (Table 1). Fourteen cell types, including a total of 29 cells, are found in all species examined. The presence or absence of cilia on different larval cell types is summarized in Table 1.

External Cell Types Present in All Species Studied

Apical cells (A): a pair of large cells located in the anterior region that each contain a refringent body (Figs. 1, 3–6). Refringent bodies typically are solid. Some species have fluid (mucoid) refringent bodies and in a few species these bodies are entirely absent (Fig. 1d, f). A small patch of short cilia is present in the dorsal region (Fig. 3c).

Couvercle cell (C): a single flattened cell located on the midline of the ventral region (Figs. 1, 3–6). Depending on the species the outline shape varies from a reverse triangle to a heart, diamond, home plate, U- or V-shape.

Dorsal caudal cells (DC): a pair of cells located in the posterior ventral to median dorsal region (Figs. 1, 3–6).

Enveloping cells (E): a pair of large cells that cover the anterior and lateral surfaces of immature embryos (Figs. 3–

6). These cells are unusual in that they are lost in swimming larvae.

Lateral cells (L): a pair of cells located in the medial-lateral region (Figs. 3–6).

Lateral caudal cells (LC): a pair of cells located in the ventro-lateral region (Figs. 3–6).

Median dorsal cell (MD): a single cell typically located in the mid-dorsal region (Figs. 1, 3–6). Depending on the species the outline shape varies from a reverse triangle to a diamond, heart or oval shape. Infusoriform larva of *Dicyemodeca anthinocephalum* is unique in the presence of a pair of median dorsal cells (Fig. 5j).

Paired dorsal cells (PD): a pair of cells located in the dorsal region (Figs. 1, 3–6). Depending on the species the outline shape varies from a trapezoid to a parallelogram, rectangle or square.

Posteroventral lateral cells (PVL): a pair of cells located in the postero-ventral region bordering the couvercle cell anteriorly and the lateral cells laterally (Figs. 1, 3–6).

Ventral caudal cell (VC): a single cell located in the ventral caudal region adjacent to the posteroventral lateral cells on the ventral side and to the dorsal caudal cells on the dorsal side (Figs. 1, 3–6).

Internal Cell Types Present in All Species Studied

Capsule cells (CA): a pair of cup-shaped cells surrounding the urn cells (see below). The cytoplasm typically contains conspicuous granules which vary in both size and number depending on the species (Figs. 1b, 3a, 4–6).

Urn cells (U): four large cells located in the center of the larva (Figs. 1, 3a, 4–6). The cytoplasm is stained dark by hematoxylin. Depending on the species each urn cell has either 1 or 2 nuclei and 1 or rarely 2 germinal cells in the cytoplasm.

Ventral internal cells (VI): a pair of cells located in the ventro-median region, underlying the ventral series of cells (see below). Cilia from these cells project into the urn cavity
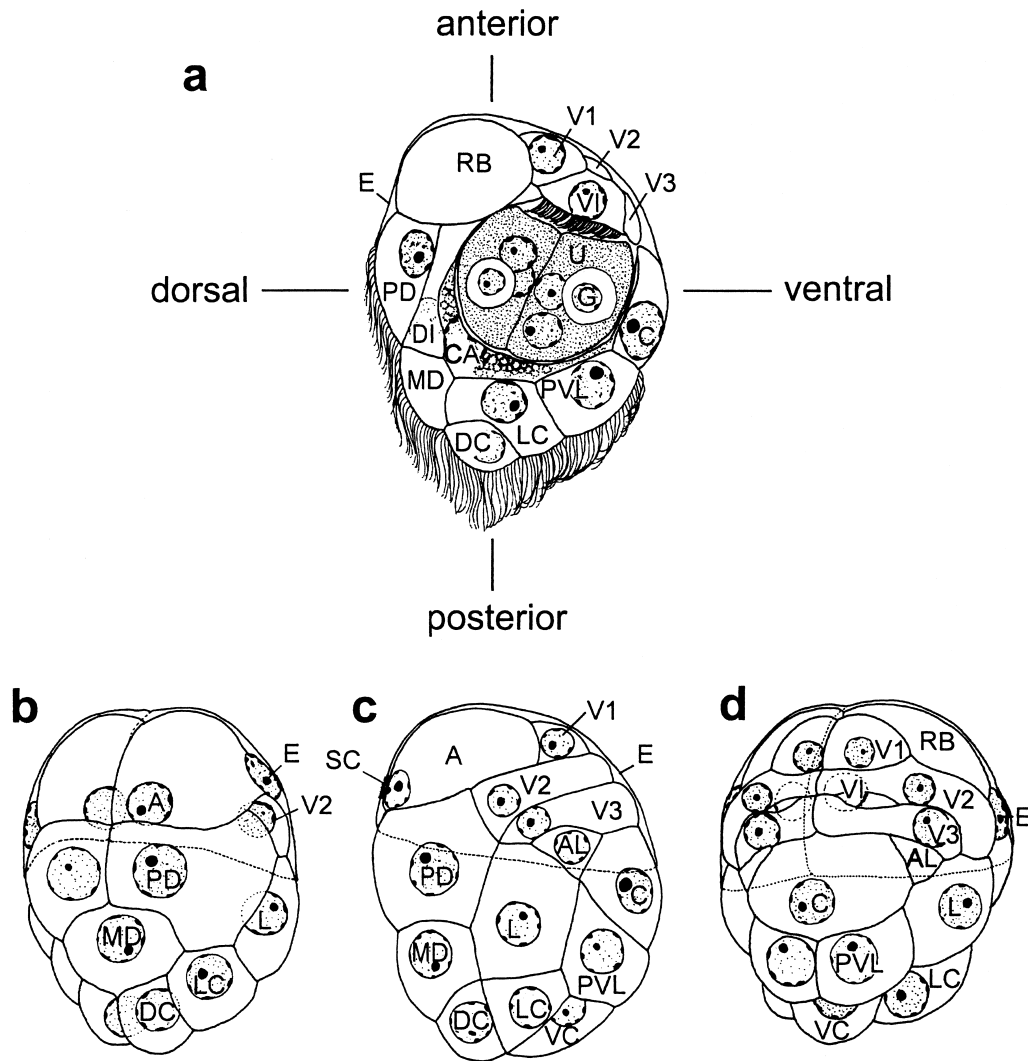
Fig. 3. Generalized schematic drawings of infusoriform larvae with 39 cells. **a**, sagittal section; **b**, dorsal view; **c**, lateral view; **d**, ventral view. RB, refringent body; SC, short cilia. See Table 1 for the other abbreviations.

(Figs. 3a, 4–6). Short (1971) noted that cilia were absent in the ventral internal cells of the infusoriform larvae of *Dicyema knoxi*, *D. maorum*, and *D. robsonellae*. However, when critically reexamined, the ventral internal cells in larvae of these 3 species actually possess very short cilia (Figs. 4o, 6c). Similarly Nouvel (1948) noted that cilia were absent in the ventral internal cells of *Dicyema moschatum*. Larvae of this species also were found to have short cilia in these cells.

Germinal cells (G): a single, and rarely 2, cells located in the cytoplasm of each of the 4 urn cells. Two germinal cells are found in an urn cell of *Dicyemodeca anthinocephalum* (Fig. 5l) and *Dicyema dolichocephalum*.

External Cell Types Common But Not Consistently Present
    Anterior lateral cells (AL): a pair of small cells located in the anterio-lateral to ventro-lateral region (Figs. 3–6). Nuclei of these cells become pycnotic during embryonic development. Anterior lateral cells are not present in one

third of the dicyemid species examined (Table 2).

First ventral cells (V1): a pair of small cells located in the midline on the antero-ventral surface of the apical cells (Figs. 1, 3–6). These cells are not present in 3 species of dicyemids, *Dicyema* sp., *Dicyemodeca anthinocephalum*, and *D. deca* (Table 2).

Second ventral cells (V2): a pair of band-shaped cells located in anteroventral region (Figs. 1, 3–6). These cells, as well as the first ventral cells, are absent in 3 dicyemid species, *D.* sp., *D. anthinocephalum*, and *D. deca* (Table 2).

Third ventral cells (V3): a pair of band-shaped cells located in antero-ventral region (Figs. 3–6). The third ventral cells are absent in one-third of all dicyemid species examined or described (Table 2).

Internal Cell Types Common But Not Consistently Present
    Dorsal internal cells (DI): a pair of flattened cells located between the paired dorsal cells and the capsule cells, anteriorly bordering apical cells and posterioly median dorsal
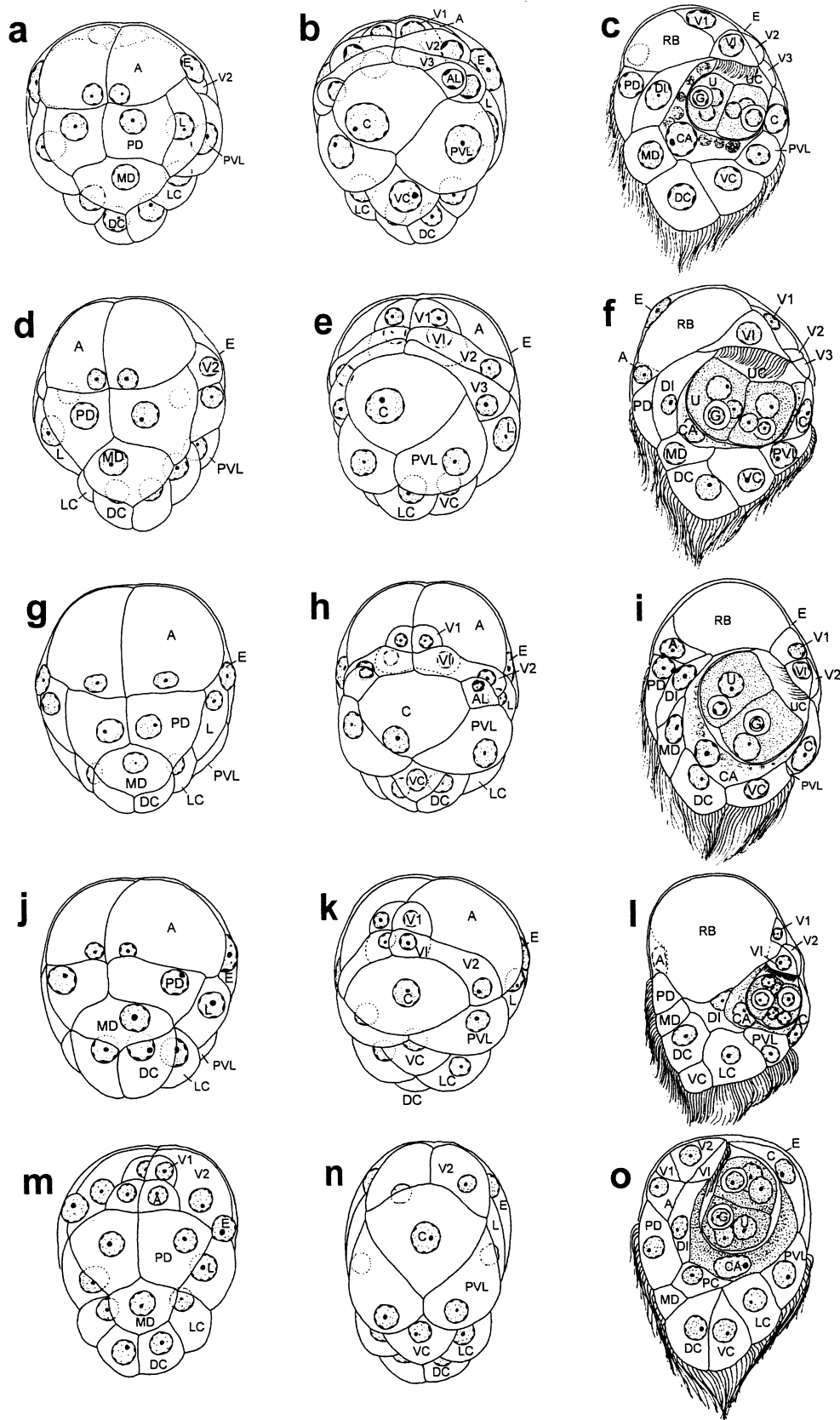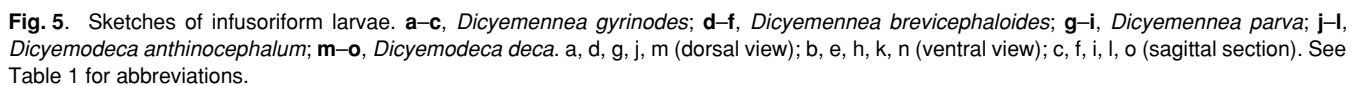
Fig. 4. Sketches of infusoriform larvae. **a–c**, *Microcyema vespa*; **d–f**, *Conocyema polymorpha*; **g–i**, *Dicyemennea adminicula*; **j–l**, *Dicyema typus*; **m–o**, *Dicyema knoxi*. a, d, g, j, m (dorsal view); b, e, h, k, n (ventral view); c, f, i, l, o (sagittal section). See Table 1 for abbreviations.
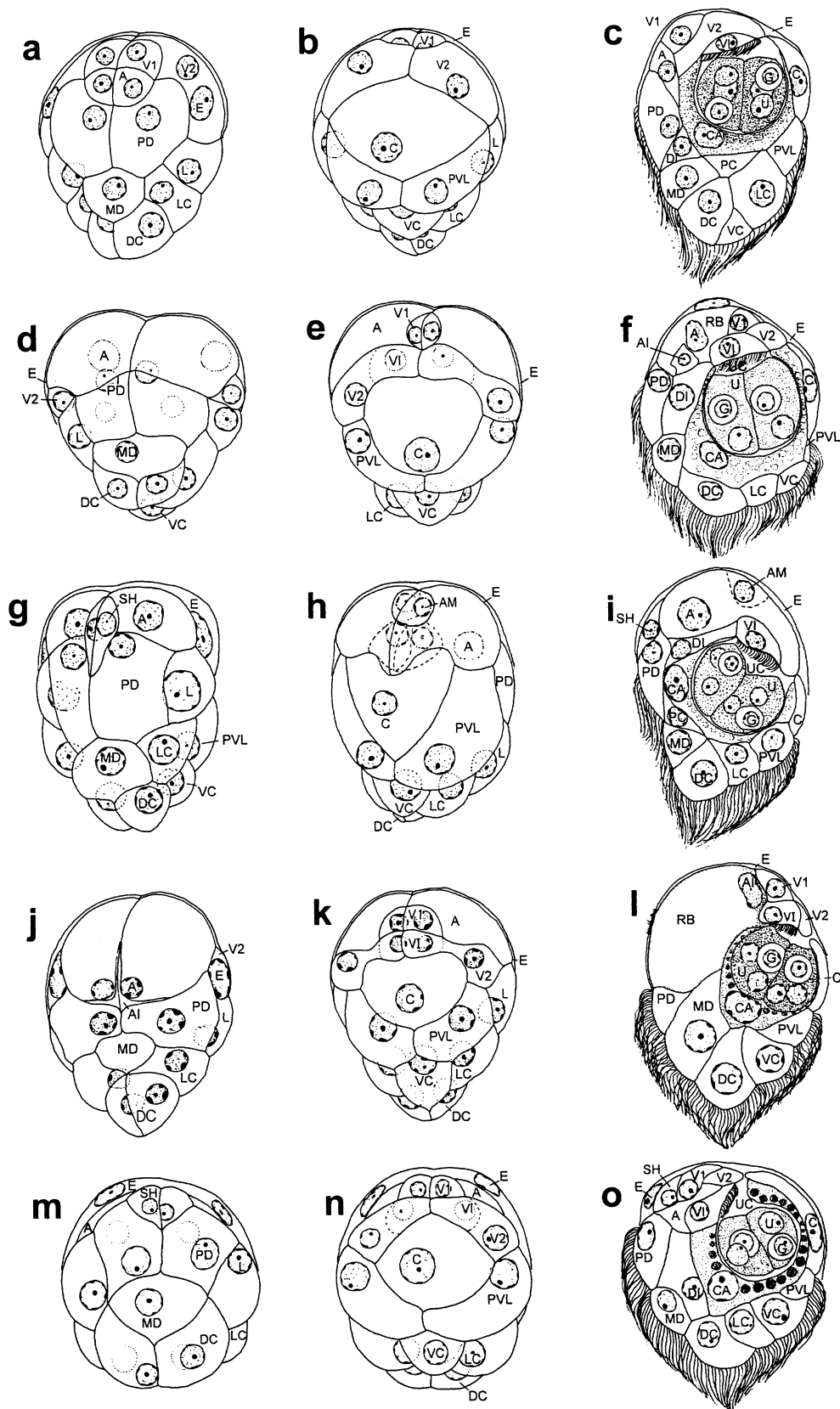
Fig. 5. Sketches of infusoriform larvae. **a–c**, *Dicyemennea gyrinodes*; **d–f**, *Dicyemennea brevicephaloides*; **g–i**, *Dicyemennea parva*; **j–l**, *Dicyemodeca anthinocephalum*; **m–o**, *Dicyemodeca deca*. a, d, g, j, m (dorsal view); b, e, h, k, n (ventral view); c, f, i, l, o (sagittal section). See Table 1 for abbreviations.

Fig. 6. Sketches of infusoriform larvae. **a–c**, *Dicyema maorum*; **d–f**, *Dicyemennea lameerei*; **g–i**, *Dicyema* sp.; **j–l**, *Dicyema acuticephalum*; **m–o**, *Dicyemennea canadensis*. a, d, g, j, m (dorsal view); b, e, h, k, n (ventral view); c, f, i, l, o (sagittal section). See Table 1 for abbreviations.

cells (Figs. 1c, 3a, 4–6). This pair of internal cells is absent only in the larva of *Dicyemodeca deca* (Table 2, Fig. 5o).

Rare and Unusual Cell Types

Apical micro cells (AM): a pair of small cells located externally in the midline between the apical cells and attached to the first ventral cells on the ventral side of the larva (Figs. 1f, 5a, g, j). Apical micro cells have been found only in 5 species of dicyemids, namely *Dicyema* sp., *Dicyemennea gyrinodes*, *Dicyemennea parva*, *Dicyemodeca anthinocephalum*, and *Dicyemodeca deca* (Table 2).

Shield cells (SH): a pair of shield-shaped cells located externally in the midline on the dorsal surface (Figs. 5a, j, m, 6g, m). Shield cells have been found in 4 species of dicyemids, namely *D.* sp., *Dicyemennea canadensis*, *D. gyrinodes*, and *D. anthinocephalum* (Table 2).

Anterior internal cells (AI): a pair of thin cells located internally between the apical cells (Figs. 6j, l). The anterior internal cells are rare and have been found only in *Dicyema acuticephalum* (Table 2).

Postcapsular cells (PC): a pair of cells located internally between the capsule cell and the posterior external cells (dorsal caudal cells, median dorsal cell, lateral caudal cells, ventral caudal cell) (Figs. 1d, 6i). Postcapsular cells have been found only in 3 species of dicymids from New Zealand, namely, *Dicyema knoxi*, *D. maorum*, and *D. robsonellae* (Table. 2).

Cellular constitution of infusoriform larvae

A total of 12 variations in cellular composition were observed in infusoriform larvae of 64 species of dicyemids examined (Table 3). Cellular composition may differ between larvae with the same cell number. In infusoriform larva with 39 cells, however, only a single pattern of cell composition is found. In infusoriform larvae with 35 or 37

cells, most variations are attributable to the presence or absence of cells in the ventral series, such as the anterior lateral cells, and the first, second or the third ventral cells (Table 3). Additional variations are due to the presence of rare or unusual cell types, such as the shield, anterior internal, apical micro, and postcapsular cell pairs.

The 12 types, or patterns, of cell numbers and composition are listed below by order of frequency (see also Table 3). In the cellular composition of infusoriform larvae, cells consistently observed in all species are located on the external surface of lateral, dorsal and caudal areas. In contrast, distinct and unusual cells are located on the external surface of apical and ventral areas (Figs. 4–6).

Type 1: characteristic of 27 species that belong to 4 genera, *Dicyema*, *Dicyemennea*, *Microcyema*, and *Pseudicyema*. Larvae of this type have 39 cells which invariably consist of 29 common cells, plus a pair of anterior lateral cells, dorsal internal cells, and cells in all 3 ventral series.

Type 2: characteristic of 18 species that belong to 4 genera, *Conocyema*, *Dicyema*, *Dicyemennea*, *Dicyemodeca*. Larvae of this type consist of 37 cells , 29 common cells, dorsal internal cells, plus pairs of cells in all 3 of the ventral series.

Type 3: present in 6 species of dicyemids that belong to 2 genera, *Dicyema* and *Dicyemennea*. Larvae of this type also consist of 37 cells but they have 29 common cells, anterior lateral cells, dorsal internal cells, plus pairs of cells in 2 of the ventral series.

Type 4: present in 3 species that belong to 2 genera, *Dicyema* and *Dicyemennea*. Larvae of this type consist of 35 cells, 29 common cells, dorsal internal cells, plus pairs of cells in 2 of the ventral series.

Type 5: present in 3 species of *Dicyema* from New Zealand. This type of larva has 37 cells and is characterized by postcapsular cells.

Table 3.   Summary of infusoriform larval types based on cell number and composition.

Larval Types	No. of Species	Included genera*	Cell Types Present**									Total Cell No.
			AI	AL	AM	DI	PC	SH	V1	V2	V3	
1	27	D, Dn, M, P	−	−	−	+	−	−	+	+	+	39
2	18	C, D, Dn, Dd	−	−	−	+	−	−	+	+	+	37
3	6	D, Dn	−	+	−	+	−	−	+	+	−	37
4	3	D, Dn	−	−	−	+	−	−	+	+	−	35
5	3	D	−	−	−	+	+	−	+	+	−	37
6	1	D	+	−	−	+	−	−	+	+	−	37
7	1	D	−	−	+	+	−	+	−	−	−	35
8	1	Dn	−	−	+	+	−	+	+	+	+	41
9	1	Dn	−	−	−	+	−	+	+	+	−	37
10	1	Dn	−	−	+	+	−	−	+	+	−	37
11	1	Dd	+	−	+	+	−	+	−	−	−	42
12	1	Dd	−	+	+	−	−	+	−	−	−	37

 * C, *Conocyema*; D, *Dicyema*; Dn, *Dicyemennea*; Dd, *Dicyemodeca*, M, *Microcyema*; P, *Pseudicyema*
 ** Cell types common to all dicyemid species are omitted. For abbreviations see Table 1.

Types 6–12: each of these larval types is present in only a single species of dicyemid. They are characterized by rare and unusual cell types, such as shield, anterior internal, or apical micro cells.

DISCUSSION

General morphology

Infusoriform larvae of dicyemids are unusual in that they consist of larger numbers of cells and are more complicated in body organization than adults. The Phylum Orthonectida, until recently treated as mesozoans together with the dicyemids, also have distinct larvae which consistently differ morphologically among species (Caulley and Lavallée, 1908, 1912; Atkins, 1933). The infusoriform larvae of dicyemids also differ both in cell number and composition depending on the species. However, as stated by McConnaughey (1951), morphological variations are rather small among species. Orthonectids live in tissues of a wide diversity of marine invertebrate hosts. They have been recorded in the following phyla: Platyhelminthes, Nemertea, Annelida, Mollusca, Echinodermata, Bryozoa, and Urochordata. Species of dicyemids, on the other hand, are known to live only in the renal organs of benthic cephalopod hosts. Differences in larval morphology are possibly related to differences in host diversity (Furuya, 2002). In addition, orthonectids are associated with a variety of organs and tissues within their hosts, namely, the gonads, parenchyma, and body wall. Differences in location within the host might also contribute to morphological variations in larvae of orthonectids. Currently these two mesozoan groups are treated as distinct and separate phyla and considered not to be closely related based on fine structure and life cycles (Kozloff, 1969; Hochberg, 1983). In their study of 18S rRNA nucleotide sequences Pawlowski *et al.* (1996) also concluded that dicyemids and orthonectids had separate origins.

Infusoriform larvae of dicyemids are free-swimming organisms, whereas adult vermiform stages are restricted to the renal organs of host cephalopods. The vermiform body shape of adult stages might be simplified as a reflection of their specialization in their habitat, the tubular space in the cephalopod renal organs. A similar elongate, vermiform shape also is observed in the apostome ciliate, *Chromidina*, of which habitat is restricted to the renal organs in pelagic or oceanic cephalopods (Hochberg, 1982). In the dicyemids infusoriform larvae thus seem to represent the true level of organization. Nevertheless, the body organization of infusoriform larva is not regarded as achieving the grade of the tissue level (Furuya *et al.*, 1996). A germ layer is absent in the infusoriform embryos, and groups of cells are characterized only as being external or internal in their location.

During the embryogenesis of infusoriform larvae, cells of the vegetal hemisphere appear to be passively incorporated into the interior of the embryo as cells of the animal hemisphere proliferate (Furuya *et al.*, 1992a). Enclosed blastomeres develop into germinal or internal cells. This is similar to epiboly as seen in the stereoblastula stage of some invertebrates (Hyman, 1951; Kume and Dan, 1968). In infusoriform development a cavity appears between the urn cells and the ventral internal cells. The urn cavity does not represent a blastocoel, because this cavity is a slit-like space that is secondarily derived. This cavity possibly represents the vegetal pole region where the external cells fail to cover the interior cell mass completely. Thus it may be comparable to the blastopore of metazoan embryos. The minute blebs are characteristics of the margins of the urn cells which face the anterior open area of the cavity (Matsubara and Dudley, 1976). The inclusion in blebs may be transported from the urn cavity by pinocytosis. The fluid, contained within the urn cavity, appears to be circulated by movement of cilia on the ventral internal cells. Seawater may be drawn in by the current through the slit between the couvercle cell and ventral internal cells. The seawater, thus exchanged, likely provides nutrients and oxygen for both urn cells and germinal cells.

Size of Infusoriform larvae

Infusoriform larvae are very small in size compared to the larval stages of most other marine invertebrates. This is due both to adult body size and their unusual method of reproduction. Dicyemids are parasites or symbionts that live exclusively in the renal organs of cephalopods. The largest known species measure less than 10 mm in length. Larvae develop within the axial cell of vermiform parent adults. Larval size most likely is limited by the size of the parent. Infusoriform size also is correlated with the size (mantle lengths) of the cephalopod host (Furuya *et al.*, 2003b). Although infusoriform size appears to be a function of host size, it is not clear what character is directly correlated with the larval size. One character that may limit larval size is the diameter of the host's renal pore through which the infusoriform larvae escape. Another possible character is the nature and size of the site where the infusoriform first contacts or infects the cephalopod host. However, at present it is not known whether infusoriform larvae reach their cephalopod host directly or via an intermediate host.

Cell numbers of infusoriform larvae

In infusoriform larvae cell numbers are fixed and species-specific. This study reveals a small but distinct differences in cell numbers in infusoriform larvae of different dicyemid species. There are 5 variations in total cell numbers, 35, 37, 39, 41, and 42 for which somatic cell numbers are 31, 33, 35, 37, and 34 respectively. Vermiform larvae also have fixed numbers of somatic cells. However, in comparison to infusoriform larvae, vermiform larvae exhibit a larger range in somatic cell numbers (8-32 cells). In vermiform larvae, variations in somatic cell numbers are attributed to differences in the number of divisions in a particular pair of cells during embryogenesis (Furuya *et al.*, 2001). Recently we examined embryogenesis in three types of infusoriform larvae with 39, 37, and 35 cells, namely, *Dicyema*

apollyoni, D. misakiense and *D. typoides* (Furuya *et al*., unpublished). Differences in cell numbers between different species of infusoriform larvae are attributed to the number of terminal divisions in particular cell lineages as well. In ancestral dicyemids, the two types of larvae probably consisted of a fixed and specific number of somatic cells. In subsequent speciation the number of terminal divisions likely varied in each specific larva. In dicyemids, infusoriform larvae function as the dispersal stage. Thus they may have evolved in response to selective pressures to increase the incidence of new host infections. In contrast, vermiform larvae are restricted to the renal organs, and likely evolved in response to selective pressures to increase population densities within the renal sac. It is also possible that these differences are of little adaptive significance but are neutrally fixed during the process of speciation.

Cellular composition of infusoriform larvae

In infusoriform larvae 12 types or patterns of cellular composition are recognized. In some cases variations in the cellular composition are found even in infusoriform larvae with the same total cell number. Most variations in cell composition are attributed to the presence or absence of external cells in the ventral region of the body, such as the anterior lateral cells and the three ventral series of cells. Additional variations are due to the presence of rare or unusual cell types, such as the shield, anterior internal, apical micro cells, and the internally located postcapsular cells. Except for the postcapsular cells, these cells are externally located in the anterior and ventral regions of the body of the larvae. In contrast, cells commonly found in all types of larvae are restricted to external cells located in the lateral, dorsal and posterior (caudal) regions of the larvae. In developmental patterns and cell lineages of infusoriform larvae, blastomeres situated in the animal hemisphere give rise to ciliated external cells that form the dorsal and posterior part of the larva during early stages of embryogenesis (Furuya *et al*., 1992a; Furuya *et al*., unpublished). Whereas blastomeres situated in the vegetal hemisphere give rise to anterior and ventral cells plus all internal cells of the larva during later stages of embryogenesis. This suggests that, in the larval cell composition, with the exception of the germinal cells, common cells differentiate much earlier than unusual cells and these latter cells cause species-specific differences.

In infusoriform larvae thus far studied, common cells formed in early embryogenesis appear to be homologous in most species because the early development is identical (Furuya *et al*., 1992a; Furuya *et al*., unpublished). Among cells formed in the later embryogenesis, most appear to be homologous on the ground of cell lineage. In the case of infusoriform larvae with 37 cells, differences typically are in the absence of either the anterior lateral or the 3rd ventral cell pairs. These two cell types are distinct in morphology but are located in the same region of the larval body. The anterior lateral and the 3rd ventral cell pairs are possibly formed in the same cell lineage. Unusual cells, such as the

apical micro and shield cells, often replace cells in the ventral series. This suggests that they might be comparable to some cells of ventral series, although it is unknown whether these cells are homologous or not.

Systematic view

As was originally observed by Nouvel (1948, 1961) we found specific differences in larval size and in the shape, number and composition of cells in infusoriform larvae. These species-specific differences are important taxonomic characters that can be used to describe and identify dicyemids. Short (1971) suggested that larval characters may aid in separating genera. However, to date, clear-cut differences in delineating genera of infusoriform larvae have not been found. Genera and families of dicyemids traditionally are separated based on differences in the cell number and arrangement in the calottes of vermiform adults. Larval characters are not necessarily correlated to adult characters. For instance, unusual dicyemids, such as *Conocyema polymorpha* and *Microcyema vespa*, are the most highly differentiated species, of which adults have very irregular bodies (van Beneden, 1882). Traditionally *C. polymorpha* and *M. vespa* have been placed in a separate family, the Conocyemidae. In contrast to the adult vermiform stages, their infusoriform larvae are not unusual. The adult forms of these two taxa likely are adapted to the renal habitat of the host in combination with selective competition with other co-occuring dicyemid species (Furuya *et al*., 2003a). Infusoriform morphology apparently does not support placement of these 2 genera in a separate family.

Based on this study it appears that meristic and morphological features that characterize infusoriform larvae may have evolved independently from the morphology of the vermiform adult stages. Molecular phylogeny may cast some light on the problem of higher classification in dicyemids.

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