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A Hidden Larva in Nemerteans of the Order Hoplonemertini

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Nemerteans are characterized by a wide diversity of developmental types. The representatives of order Heteronemertini have a pilidium or pelagic larva [1–3]. A hidden ciliate larva developing in the egg coats has been described in a representative of Heteronemertini [4]. With some changes, this larva repeats the development and metamorphosis of a pilidium. The representatives of the order Paleonemertini also have a pelagic larva, which is different in its structure from the pilidium of Heteronemertini [3, 5, 6]. This larva has special larval organs (eyes and apical cilia). Some authors suggest the presence of larval ectoderm in the larvae of Paleonemertini [6]. In some representatives of the order Hoplonemertini, as well as in Bdellonemertini, young nemerteans hatch from eggs [7–9]. This gave grounds to consider the representatives of the order Hoplonemertini to have direct development [3]. Alternatively, planktonic larvae have been found in other Hoplonemertini [2]. This suggests that, actually, a hidden larva is present in the representatives of this order in which direct development was found.

To test this suggestion, we investigated the embryonic development of *Tetrastemma candidum* Mueller, 1774, a representative of Hoplonemertini. Sexually mature females and males of this species were collected in the littoral zone of Kandalaksha Bay, the White Sea, in August 1996 and August 1997. Females and males were placed into Koch's dishes; they grouped together and excreted a mucous cocoon. In this cocoon, unfertilized eggs were laid (Fig. 1a). The observations on the consecutive developmental stages were conducted in surviving developing embryos. To study the internal structure, the embryos were fixed in Bouin's fluid and mounted as standard histological sections. The sections were stained with Caracci's hematoxylin.

Spheroid eggs of *T. candidum* are rich in yolk. When observed in transmitted light, they were nontransparent; in incident light, they were milky-white. The mean egg diameter was 0.22 mm. The yolk was regularly distributed, and the animal pole was marked by polar cor-

puscles. The egg cleavage was spirally homoquadrantal (Figs. 1d–1i). The interval between consecutive cleavage divisions was approximately 1 h. A specific feature of the cleavage divisions in *T. candidum* was the inversion of the sizes of the first-quartet micro- and macromeres: that is, animal micromeres are significantly greater than vegetative ones (Fig. 1f). The further cleavage, which was monitored until the 64-blastomere stage, occurred according to the pattern of spiral homoquadrantal cleavage (Figs. 1g–1j).

Gastrulation began approximately a day after the beginning of cleavage and showed signs of both polar in-growth and invagination. The blastopore was a round invagination that gradually became narrower and then was completely closed (Figs. 1k and 1l).

Two days after the beginning of cleavage, cilia developed in embryos. Owing to these cilia, the embryo rotated inside the egg coat. The number of ciliate cells increased during development, and their size decreased. The rotation rate reached 40 rpm. The ciliated embryo was being extended; the extension was likely to occur in the future cerebro-caudal direction. On the lateral surface of the embryo, lobes developed (Figs. 1m and 1n). In the majority of embryos, three lobes were found; they surrounded the pit with the central evagination. In other regions of the embryo surface, single small furrows or pits developed irregularly. Unfortunately, we failed to determine whether the lobes corresponded to the position of the future blastopore, or whether their positions were not related to that of the gastrulation pole.

Four days after the beginning of lobe formation, they were gradually smoothed over, and the embryo became ellipsoid. At one end of the ellipsoid, three deep, narrow invaginations (two lateral and one central) were detected (Fig. 1o). As seen from the data on the internal structure of the embryo, the two lateral invaginations corresponded to the germs of the nervous system; the central one, to the stomodeum that leads to the germ of the intestine (Fig. 2a). At this stage, the embryo was a hidden larva with specific larval organs (Fig. 2a). Larval organs included the apical plate located terminally at the cerebral end of the embryo and the larval ectoderm. The latter was represented by large flattened ciliate cells, which covered the entire surface of the

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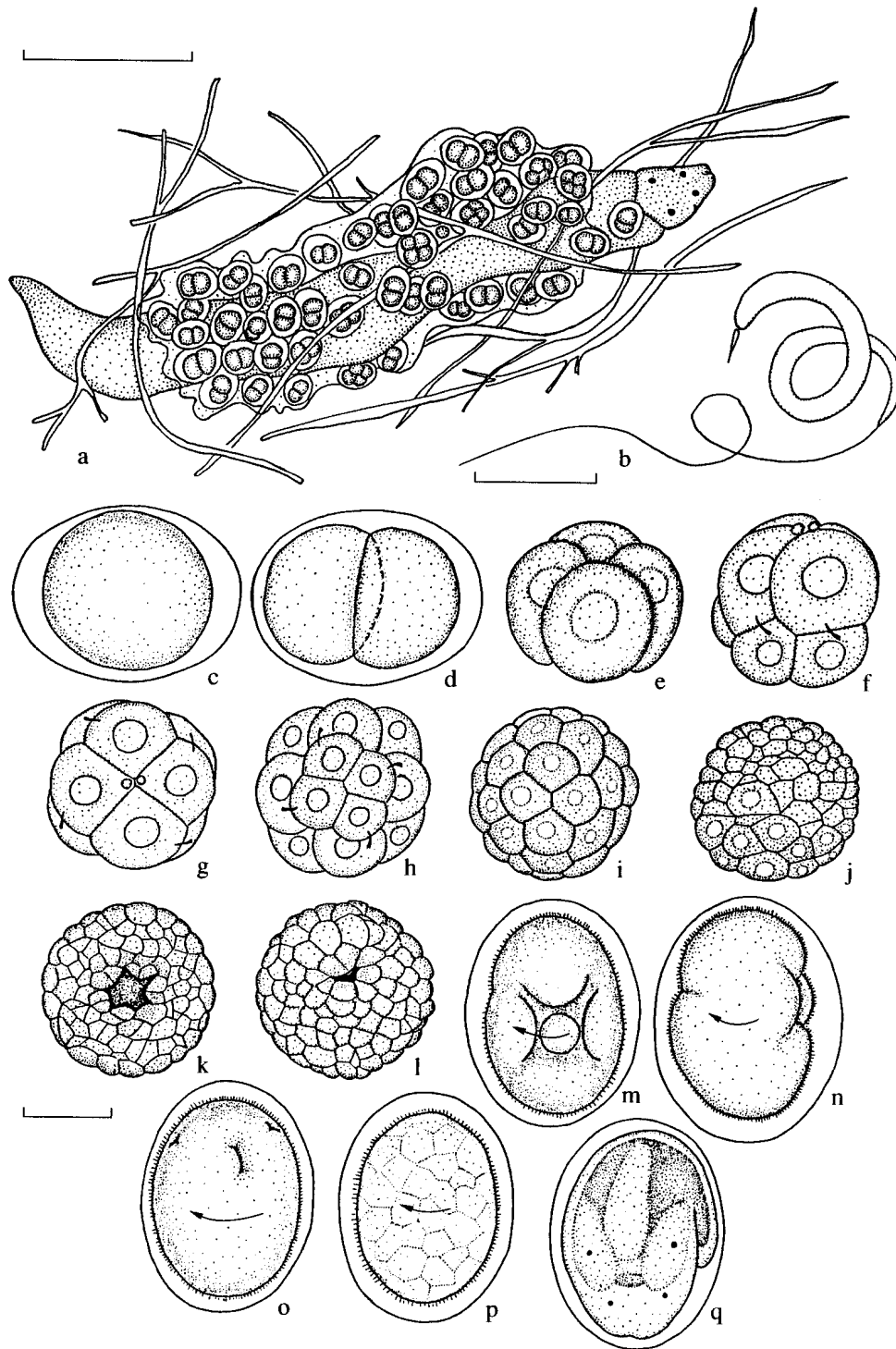
Fig. 1. Embryonic development of *T. candidum*: (a) a female in the mucous clutch; (b) a spermatozoid; (c) an egg; (d) the two-blastomere stage; (e) the four-blastomere stage; (f) and (g) the eight-blastomere stage, viewed from the side and from the animal pole, respectively; (h) the 16-blastomere stage; (i) and (j) the late cleavage stage (about 128 cells), top and side views, respectively; (k) gastrulation; (l) the blastopore closing; (m) and (n) an embryo with lobes; (o) an embryo with invaginations on the animal pole; (p) the large-cell surface in the late embryo; (q) a young nemertean before hatching. Scale: (a) 1 mm; (b) 5 μm; and (c)–(d) 100 μm.

embryo. A thick mass consisting of small cells with small compact nuclei and densely stained cytoplasm was located between the larval ectoderm and the germ of the intestine.

The cells of the apical organ and larval ectoderm were very different from the remaining cells of the embryo in their large vesicle-like nuclei and light cytoplasm (Figs. 2a and 2b). At late developmental stages,
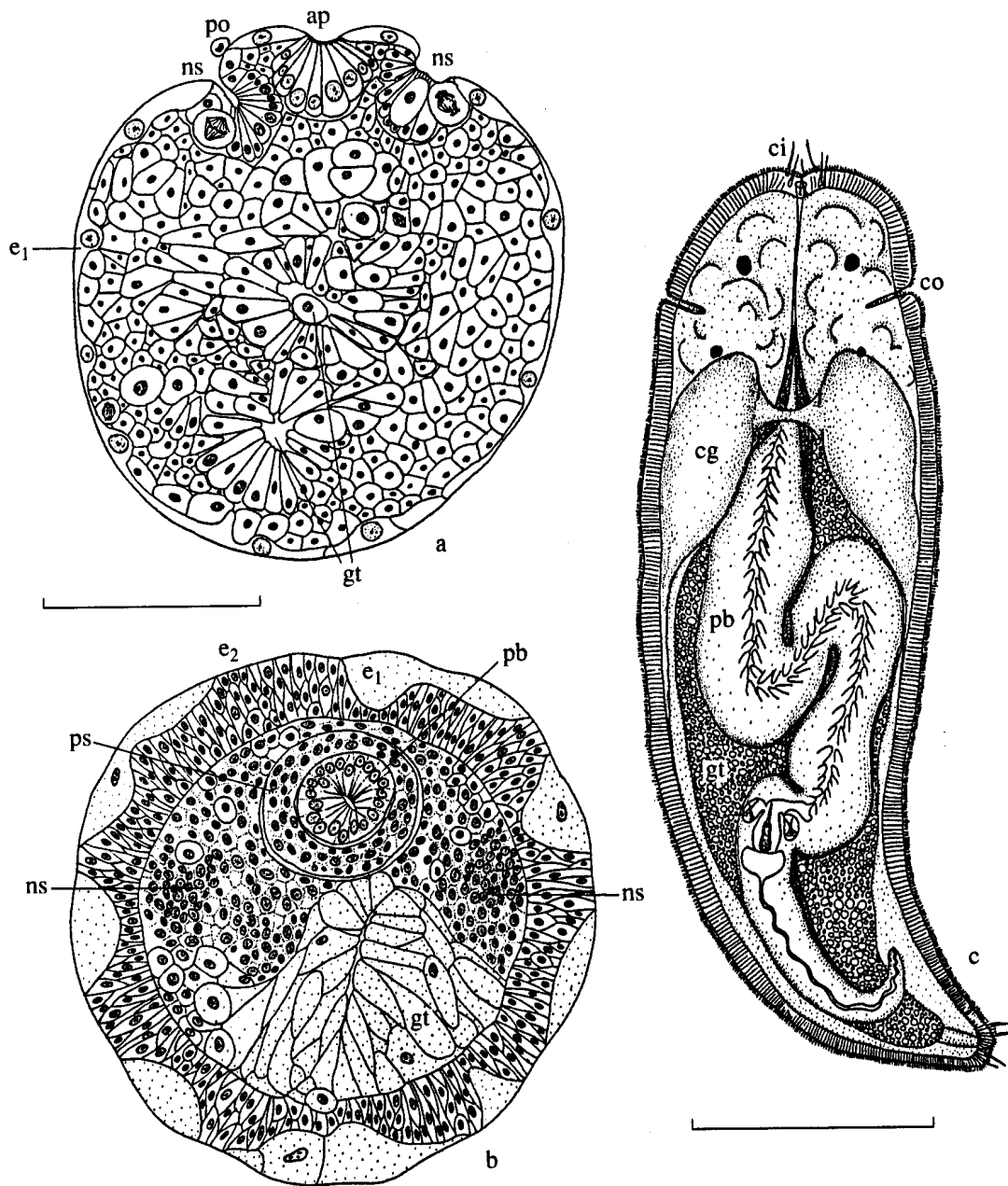
Fig. 2. (a) The frontal section of the embryo with invaginations; (b) the transverse section of the late embryo; (c) a young nemertean (four days after hatching). Scale: (a) and (b) 100 μm; (c) 0.5 μm. Designations: ap, apical organ; cg cerebral ganglia; ci, ciliate cirri; co, cerebral organs; e_1, larval ectoderm; e_2, imaginal ectoderm; gt, intestine; ns, germinal nervous system; pb, proboscis; po, polar corpuscle; and ps, proboscis sheath.

the edges of the larval ectoderm can be easily detected in living embryos (Fig. 1p). The cells of the larval ectoderm were as large as 30–40 μm in diameter. The late embryos with the large larval-ectoderm cells slowed their rotation inside the egg coats down to several revolutions per minute and then stopped rotating altogether. Further development was accompanied by extension of the embryo, which gradually assumed a wormlike shape (Fig. 1q).

Nine or ten days after the beginning of development, the young nemerteans hatched from their eggs. The length of the newly hatched young nemerteans was approximately 2 mm. Their organization is typical of the representatives of genus *Tetrastemma* (Fig. 2c). The differences consisted in a relatively larger size of cerebral ganglia and a greater length of the preneural part of the head. The neck furrow, which is typical of the adult representatives of this genus, was not expressed in the newly hatched young nemerteans.

The development of the nemerteans of genus *Tetrastemma* was studied by Ya.N. Lebedinskii [7], who did not identify the larval ectoderm. However, large superficial cells can be seen in the figures presented in [7]. These cells are likely to correspond to the larval ectoderm. The ciliate larval ectoderm was found in other Hoplonemertini, e.g., in *Prostoma graecense* [10], *Emplectonema gracile* [11], and *Geonemertes australiensis* [9]. The embryonic stages that include the development of some organs, such as the apical plate and the larval ectoderm, should be considered to be hidden larvae. These larvae undergo some form of metamorphosis because neither the apical plate nor the large cells of the larval ectoderm are found in young nemerteans hatching from eggs. However, the mechanism of resorption of these purely larval organs remains unknown. The hidden larva of *T. candidum* and other Hoplonemertini is comparable to Desor's larva described in the development of *Lineus viridis* (Heteronemertini) [4]. However, whereas the development with a hidden larva is an exception in the order Heteronemertini, in which the majority of species have a pilidium, the development with a hidden larva is likely to be a rule in Hoplonemertini.

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