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Description and developmental biology of *Plectus zelli* n. sp. (Nematoda : Araeolaimida)

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Summary — *Plectus zelli* n. sp. is described and illustrated. It is closely related to *P. opisthocirculus* Andrássy, 1952 and *P. inquirendus* Andrássy, 1958, and also to *P. sambesii* Micoletzky, 1916. It has L = 0.57-0.83 mm, a = 18-26, b = 3.5-5.0, c = 8-13, V = 45-56; a continuous lip region, cephalic setae not reaching the apex of the cephalic region and paired lateral alae. *P. zelli* n. sp. reproduces by parthenogenesis, its embryonation time varies from 18-20 h. The first stage juveniles have paired primordia each of which form one sexual branch. The flexure in the ovary is formed at the time of the fourth and final moulting. The total duration of life cycle from egg to adult is 7 to 9 days at 28 ± 2 °C.

Résumé — ***Description et étude du développement de* Plectus zelli *n. sp. (Nematoda : Araeolaimida)*** — *Plectus zelli* n. sp. est décrit et illustré. Il est proche de *P. opisthocirculus* Andrássy, 1952 et de *P. inquirendus* Andrássy, 1958, et également de *P. sambesii* Micoletzky, 1916. Cette nouvelle espèce est caractérisée par : L = 0,57-0,83 mm; a = 18-26; b = 3,5-5,0; c = 8-13; V = 45-56; région labiale continue avec le reste du corps; soies céphaliques n'atteignant pas l'apex de la région céphalique; ailes latérales paires. *P. zelli* n. sp. se reproduit parthénogénétiquement, la période embryonnaire variant de 18 à 20 h. Le premier stade juvénile comporte des primordiums doubles, chaque partie se développant en une branche génitale. La flexion de l'ovaire se produit lors de la quatrième et dernière mue. La durée totale du cycle d'œuf à adulte est de 7 à 9 jours, à 28 ± 2 °C.

Key-words : Nematodes, *Plectus*, development.

An examination of the effluent slurry from the sewer of the Zoology Department, Aligarh Muslim University, revealed a new species of *Plectus* designated as *P. zelli* n. sp. The species is closely related to *P. sambesii* Micoletzky, 1916, *P. opisthocirculus* Andrássy, 1952 and *P. inquirendus* Andrássy, 1958. This paper deals with the description of *P. zelli* n. sp. together with observations on the embryonic and post-embryonic development.

Samples containing *P. zelli* n. sp. were processed by the sieving and decantation and the modified Baermann's funnel technique. The specimens were cultured in water agar to study embryonic and post-embryonic development. Juvenile stages were stained in 2 % lacto-aceto-orcein (Tahseen *et al.*, 1991) to study the development of the gonad.

Specimens for light microscopy were fixed in TAF and dehydrated by the slow method and mounted in anhydrous glycerine. For SEM, the specimens were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in an acetone series and critical point dried in CO_2. The dried specimens were coated with gold and observed in a Hitachi scanning electron microscope at 15 kV.

*Plectus zelli** n. sp.

= *Plectus* sp. *in* Ahmad *et al.*, 1992

(Figs 1, 2)

DIMENSIONS

Females (*paratypes* n = 20) : L = 0.57-0.83 (0.65 ± 0.06) mm; a = 18-26 (20 ± 2.3); b = 3.5-5.0 (4.3 ± 0.9); c = 8-13 (10.2 ± 2.2); c′ = 3-6 (4 ± 1.5); V = 45-56 (48 ± 3.2); stoma = 19-25 (23 ± 3.7) µm; oesophagus = 135-162 (151 ± 11.4) µm; ABD = 14.0-18.0 (16.3 ± 2.1) µm; tail = 60-79 (68 ± 8.3) µm.

Holotype (female) : L = 0.63 mm; a = 19.9; b = 4.2; c = 10.4; c′ = 3.6; V = 48.6; stoma = 25 µm; oesophagus = 147 µm; ABD = 16.5 µm; tail = 60 µm.

DESCRIPTION

Female : Body medium sized, almost straight to slightly curved upon fixation, tapering towards both extremities, more pronounced posteriorly. Cuticle with

* Named after Dr. H. Zell for his help in identification of the species.

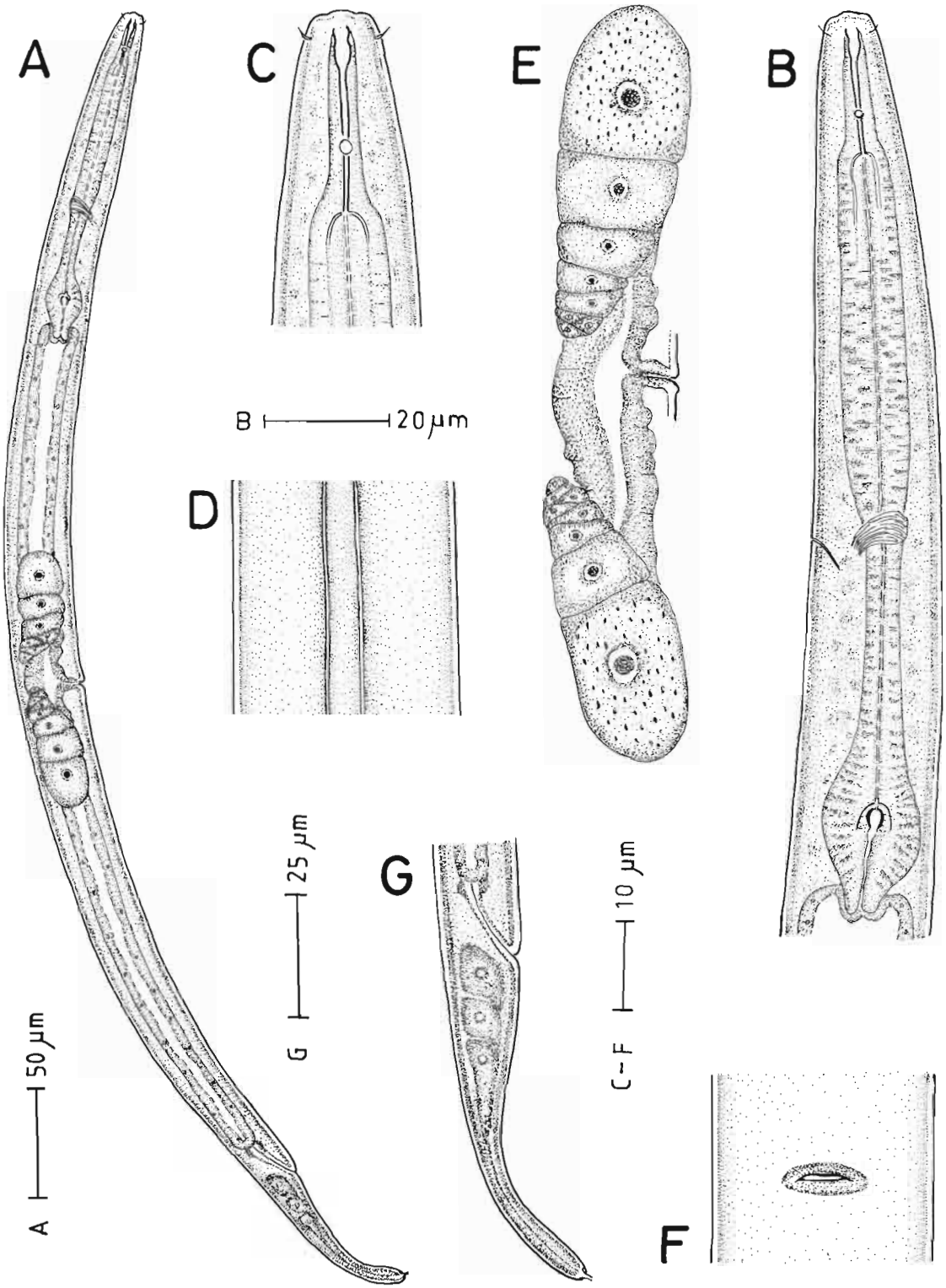

Fig. 1. *Plectus zelli* n. sp. A : Entire female; B : Anterior region; C : Anterior end; D : Lateral alae; E : Reproductive system; F : Vulva; G : Tail.

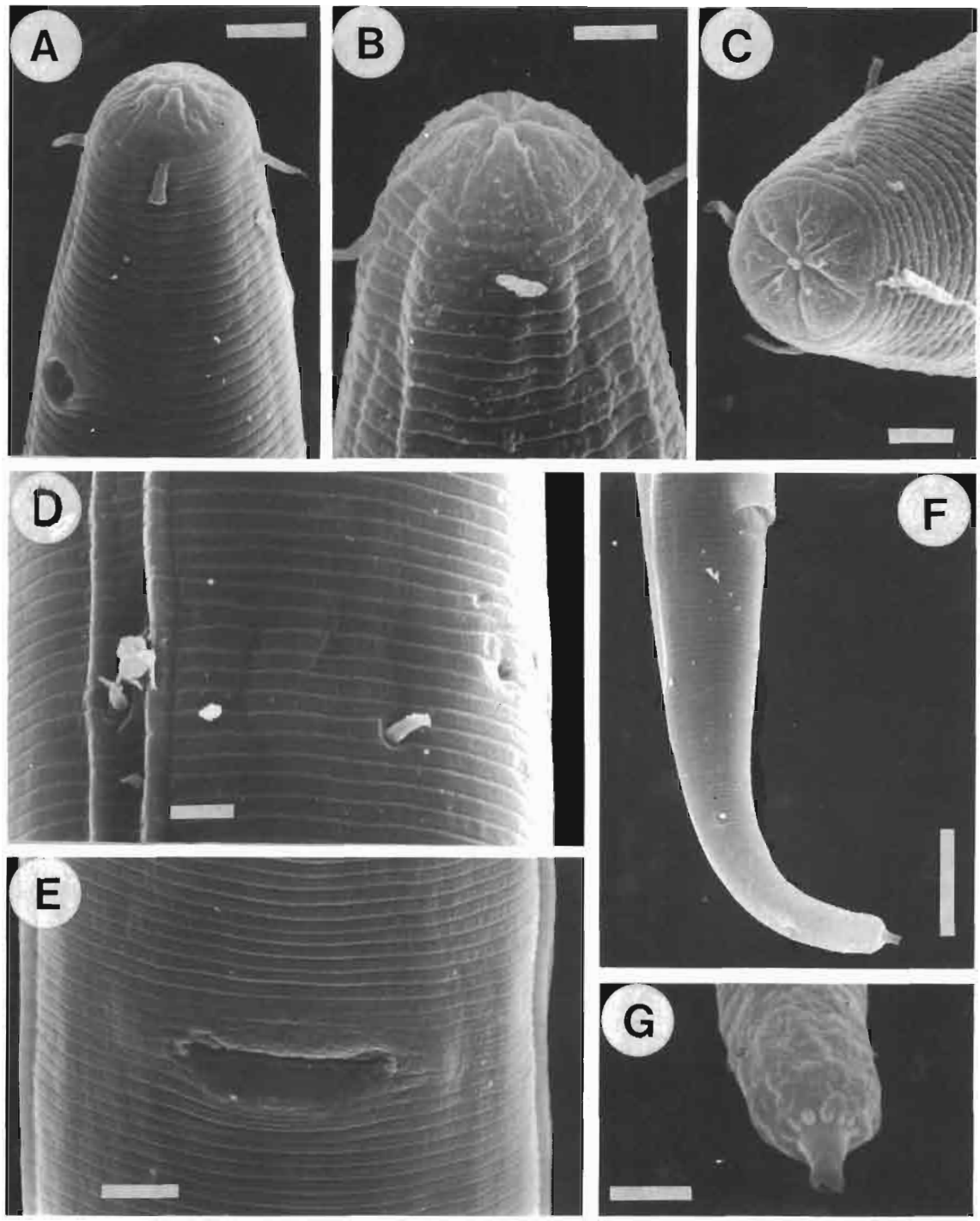

Fig. 2. *Plectus zelli* n. sp. A & B : Anterior end; C : *En face;* D : Excretory pore, lateral alae and cervical papillae; E : Vulva; F : Tail; G : Spinneret and associated papillae *(Bars : A, E : 3 µm; B, C, D, G : 2 µm; F : 10 µm).*

fine transverse striations about 0.5-0.7 µm apart. Lateral alae paired, 2-4 µm apart, starting 25-30 µm from anterior end and ending 1.5-2.0 anal body widths posterior to anus. Transverse striations pass over the lateral alae. Lip region 6-9 µm in diameter, 3.0-4.5 times broader than high, continuous with body contour. Lips six, well demarcated, fused at base and tapering apically. Cephalic setae 2.0-3.5 µm long, located on the third annule from lip base, directed anteriorly in glycerine mounts but irregular in specimens prepared for SEM. Amphidial apertures circular, two or three annules wide, located 13-15 µm from anterior end or 0.5-2.5 µm behind the middle of stoma or fifteen or sixteen annules from lip base. Stoma 19-25 µm long or 2.5-4.0 times the head width or 1/7-1/9 of oesophageal length. Maximum width of stoma 5-6 µm. Nerve ring at 75-100 µm from anterior end or at 55-60 % of oesophageal length. Excretory pore just posterior to nerve ring, 80-100 µm from anterior end or 58-62 % of oesophageal length. Oesophagus 135-162 µm long, basal bulb ovate, 17-21 × 14-16 µm. Posterior extension of basal bulb 6-8 µm long. Intestine granular. Vulva a transverse slit, vulval lips prominent. Vagina slightly anteriorly directed, about 1/3-1/4 of body diameter. Gonad paired, reflexed. Entire reproductive tract 3-4 times body diameter. Rectum about one anal body width long. Tail 60-79 µm or 3-6 anal body diameters long, regularly tapering. Spinneret duct 1-1.5 µm long surrounded by ten minute papillae. Caudal setae three pairs, one subdorsal and two subventral.

Male : Not found.

TYPE HABITAT AND LOCALITY

Sewage slurry from the Department of Zoology, Aligarh Muslim University, Aligarh, India.

TYPE MATERIAL

Holotype : Female on slide *Plectus zelli* n. sp./1 deposited in the nematode collection of the Department of Zoology, Aligarh Muslim University, Aligarh.

Paratypes : Sixteen females on slides *Plectus zelli* n. sp./2-5, deposited in the Department of Zoology, Aligarh Muslim University, Aligarh. Four paratype females deposited at Muséum National d'Histoire Naturelle, Laboratoire des Vers, Paris, France.

DIAGNOSIS AND RELATIONSHIP

Plectus zelli n. sp. is characterised by a medium-sized, finely striated body, continuous lip region, fairly long stoma and a 60-79 µm long tail with three pairs of caudal setae. The species comes closest to *P. opisthocirculus* Andrássy, 1952 in having similar allometric ratios (a, b, c) but can be easily distinguished by a longer body and stoma (L = 0.4-0.6 mm and stoma = 13-16 µm in *P. opisthocirculus*). The new species also resembles *P. inquirendus* Andrássy, 1958 in body length, b value and position of vulva but can be distinguished by the presence of distinct lateral alae and shorter tail (lateral alae indistinct, c = 4.7-6.3, c' = 10-12 in *P. inquirendus*). *P. zelli* n. sp. also shows some resemblance to *P. sambesii* Micoletzky, 1916 in the length of stoma, position of vulva and a and b values but differs from it in having a larger body, longer oesophagus and tail and in the position of nerve ring and excretory pore (L = 505-520 µm, oesophagus = 127-131 µm, tail = 53-73 µm, nerve ring and excretory pore 74-80 µm and 84-88 µm respectively from the anterior end of body in the type specimens of *P. sambesii*). However, the four populations of *P. sambesii* which were described by Micoletzky (1916), De Coninck (1935) and Andrássy (1958*a*, 1985) show significant variations in body measurements particularly in the body size and stoma and tail lengths. Because of the variability in these populations, Meyl (1957) and Andrássy (1958*b*) had possibly confused *P. opisthocirculus* with *P. sambesii*.

Developmental biology

P. zelli n. sp. reproduces parthenogenetically. Intrauterine development was not observed and vaginal prolapse occurred frequently in old females. The eggs were always laid in a single cell condition and measured 30-45 × 48-58 µm (42 × 55). Externally the shell has blunt spines.

EMBRYONIC DEVELOPMENT

The first cleavage, transverse to the longitudinal axis divided the egg unequally into an anterior larger S_1 and a posterior smaller P_1 blastomere (Fig. 3B). The second cleavage followed an oblique plane dividing S_1 into A and B blastomeres (Fig. 3C). The third cleavage furrow was again in an oblique plane and divided P_1 into S_2 and P_2 giving a rhomboid arrangement of the cells (Fig. 3D). Subsequently, A divided into A_1 and A_2 and P_2 gave rise to S_3 and P_3 (Fig. 3E). The blastula was formed 4-7 h after egg laying. Gastrulation occurred 2-3 h later. The first signs of movement were noticed 2-2.5 h after gastrulation and the first stage juvenile was fully formed 16-19 h after egg laying. At this stage the juvenile was 3.5-4 egg folds, showed vigorous movement and exerted pressure on the shell which finally ruptured. The total duration of embryonic development was 18-20 h.

POST-EMBRYONIC DEVELOPMENT (For measurements see Table I)

First stage juvenile : (Fig. 4A, D)

The first stage juveniles had two obliquely placed genital primordia. Each was 3-7 µm long. The anterior one was placed 50-58 % from the anterior end of body. Each primordium had one germinal and two somatic nuclei.

Second stage juvenile (Fig. 4B, E)

The primordia of the second stage juveniles were 5-8 µm in length, the anterior one being placed at

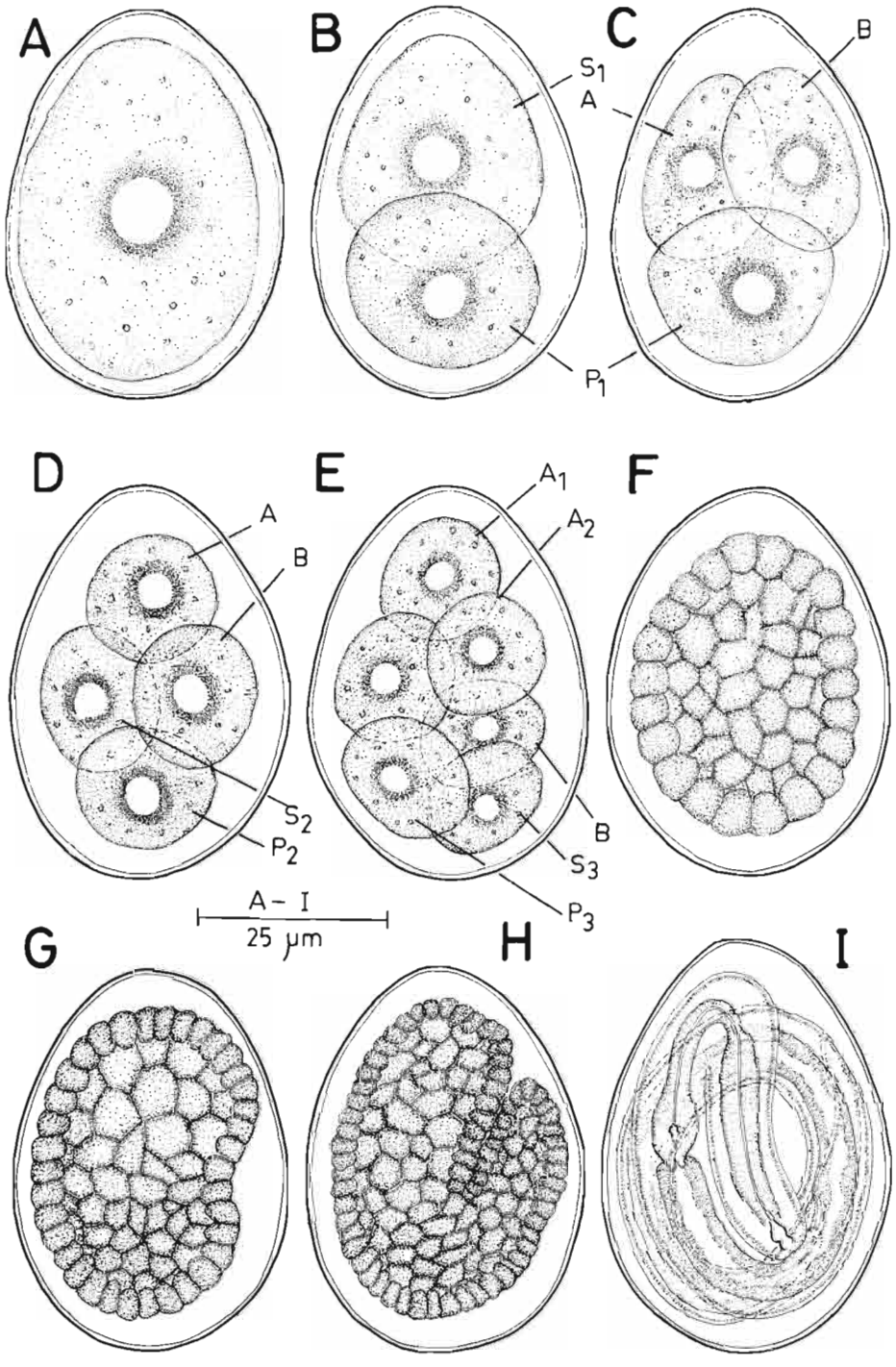

Fig. 3. *Plectus zelli* n. sp. Embryonic development. A : Single cell stage; B : Two cell stage; C : Three cell stage; D : Four cell stage; E : Six cell stage; F : Blastula; G : Lima bean stage; H : Tadpole stage; I : Fully formed juvenile.

Table 1. Dimensions of juvenile stages of *P. zelli* n. sp. (All measurements in µm).

Characters	Juveniles			
	First stage	Second stage	Third stage	Fourth stage
Body length	252.3 ± 25 (220-270)	334.2 ± 50 (280-380)	431.2 ± 42 (390-460)	560.3 ± 67 (470-620)
Body width	13.1 ± 0.9 (12-14)	13.8 ± 1.2 (12-15)	17.7 ± 1.4 (16-19)	21.0 ± 2.1 (18-23)
Stoma	7.6 ± 0.5 (7-8)	9.2 ± 0.9 (8-10)	12.5 ± 1.7 (10-14)	16.3 ± 1.9 (14-18)
Oesophagus	91.4 ± 6.2 (85-97)	110.3 ± 15.9 (90-125)	123.2 ± 9.1 (115-135)	134.8 ± 5.7 (128-138)
Tail	39.3 ± 4.9 (33-43)	48.1 ± 8.6 (39-55)	54.6 ± 6.1 (48-60)	55.3 ± 4.8 (50-60)
ABD	8.6 ± 0.8 (7-9)	9.7 ± 2.1 (8-12)	13.5 ± 1.5 (12-15)	15.2 ± 0.9 (14-16)
a	20.4 ± 2.6 (17-22)	22.2 ± 1.6 (21-24)	24.4 ± 0.9 (23-25)	23.1 ± 0.9 (22-24)
b	2.8 ± 0.4 (2.3-3)	3.1 ± 0.1 (3-3.3)	3.4 ± 0.2 (3.2-3.7)	3.5 ± 0.5 (3-4.1)
c	6.8 ± 0.9 (6-8)	8.1 ± 0.5 (7.5-8.5)	8.3 ± 0.4 (8-9)	7.5 ± 0.7 (7-9)
c'	4.3 ± 0.8 (3-5)	4.4 ± 0.7 (3-5)	4.3 ± 0.2 (4-4.5)	4.5 ± 0.5 (4-5)

48-52 % from the anterior end of body. As a result of growth, the distance between the two primordia was reduced. There were three somatic and one germinal nuclei in each primordium.

Third stage juvenile (Fig. 4C, F)

Further growth of the two primordia resulted in the fusion and the formation of a single structure. The tip of this developing gonad was now 42-48 % from the anterior end of body and its entire length ranged from 16-32 µm. There were six germinal and ten to fourteen somatic nuclei. The anterior and posterior ends of the primordium elongated further and the germinal nuclei migrated into the developing gonoduct. The somatic nuclei were restricted to the central region. For the first time, four to six specialized ventral chord nuclei appeared in this stage.

Fourth stage juvenile (Fig. 3G, H, I)

The developing gonad attained a length of 35-110 µm, the anterior tip was 44-45 % from the anterior end. The five to eight germinal nuclei were generally confined in each sexual branch. The total number of somatic nuclei numbered 13-39 and of these, those destined to form the uterus were somewhat flattened in appearance. The six to twelve specialized ventral chord nuclei became more closely associated to form the vagina which opened to the exterior through a transverse vulval slit in the final moult. The total post-embryonation period was 6-8 days while the total duration of development from egg to adult was 7-9 days at 28 ± 2 °C.

Discussion

SEM characterization of *P. zelli* n. sp. revealed two interesting features. One was the occurrence of lateral cervical papillae between the alae at about the level of excretory pore. This character is not unique to *P. zelli* n. sp. as it has been reported in other species (Mulk & Coomans, 1978) but it is not common to all species either and in *P. minutus* the cervical papillae occur dorsally outside the lateral alae (Maggenti *et al.*, 1990). The second feature, i.e., papillae around the spinneret were first observed by Ahmad *et al.* (1992). These peri-spinneret papillae were also reported in a species of *Tobrilus* but their arrangement was different from that seen in *P. zelli* n. sp. As more information becomes available, the presence or absence of the peri-spinneret papillae and/or their arrangement may provide additional characteristics for species identification.

Like the egg shell surface of *Chromadorita tenuis* as reported by Jensen (1983) and that of *Diploscapter*

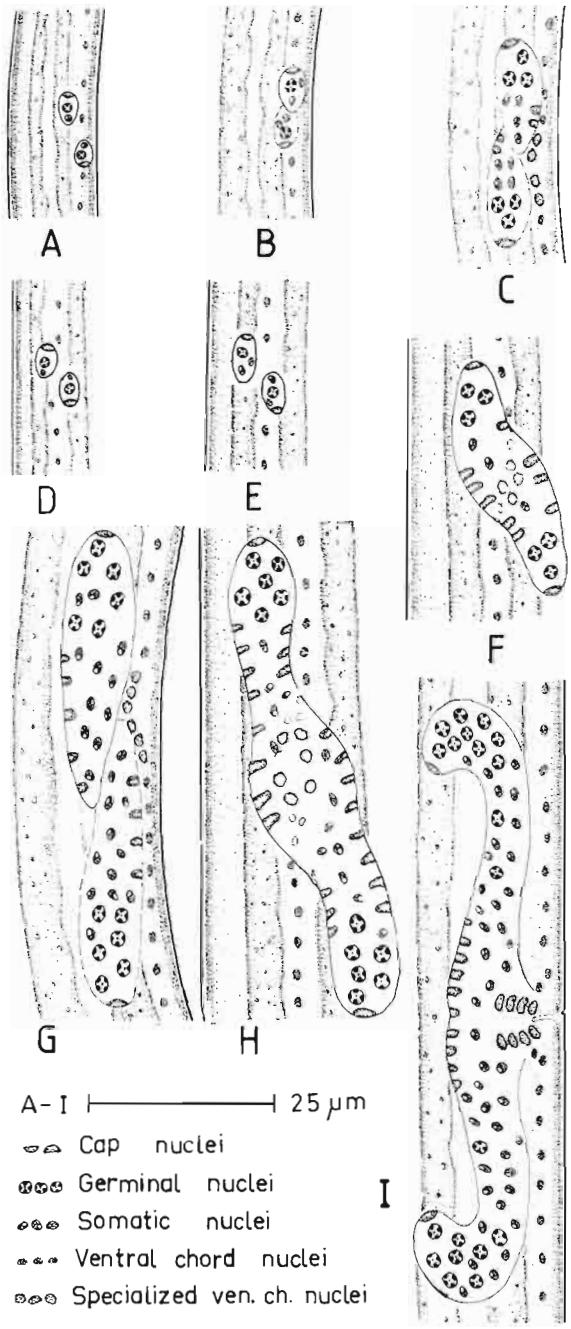

Fig. 4. *Plectus zelli* n. sp. Gonad development. A, D : First stage juvenile; B, E : Second stage juvenile; C, F : Third stage juvenile; G, H : Fourth stage juvenile; I : Late fourth stage (A, B, C, G, I : lateral view; D, E, F, H : ventral view).

orientalis as described by Tahseen *et al.* (1991), the shell surface of *P. zelli* n. sp. is also marked with blunt spines. Such spines have not been reported in other species of *Plectus* and Mulk and Coomans (1978) while giving the dimensions of (probably uterine) eggs did not mention spines in any of the four species. It is not likely that all *Plectus* species will have a similar type of egg shell ornamentation, just as *Prionchulus punctatus* eggs have scaled structures on the shell while those found in *P. muscorum* eggs are echinulate (Arpin *et al.*, 1984).

The observations on the cleavage patterns of *P. zelli* n. sp. show a very striking resemblance to Malakhov's (1983) study on *Hypodontolaimus inaequalis* and to von Ehrenstein and Schierenberg's (1980) and Tahseen and Jairajpuri's (1988) observations on *Caenorhabditis elegans* and *Teratorhabditis andrassyi*, respectively. The first three divisions are all along precisely similar planes resulting in the formation of characteristic rhomboid structure at the four cell stage. Post embryonic development, however, is unlike that of the secernentea primarily because of the fact that the gonads develop from two separate obliquely placed primordia. Lack of information prevents a comparison between araeolaimids but gonad development from paired primordia resembles closely to the mononchs particularly *Miconchus studeri* as described by Khan and Coomans (1980) and *Anatonchus amiciae* studied by Coomans and Lima (1965), the difference being that the connecting strand was not observed in *P. zelli* n. sp.

Acknowledgments

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