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Description and developmental biology of the predatory diplogastrid *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962 (Nematoda: Rhabditida)

S. AHLAWAT, Q. TAHSEEN[1]

Nematode Research Laboratory, Department of Zoology, Aligarh Muslim University, Aligarh-202002, India,
E-mail: *qtahseen@gmail.com*

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Summary

Acrostichus nudicapitatus (Steiner, 1914) Massey, 1962 is redescribed and illustrated along with observations on its developmental biology. Most morphometrics of the present population agree well with those of *A. nudicapitatus* (Steiner, 1914) Massey, 1962. The largely oviparous females of *A. nudicapitatus* lay eggs in single-celled or two-celled stage, 1.5 – 2 h after fertilization. In cultured females, the uterine tract was observed to accommodate occasionally as many as 4 – 6 eggs. The eggs are smooth-shelled, oval in shape measuring 45 – 48 x 23 – 26 µm in dimension. The pole of entry of sperm marks the posterior end of the developing embryo. The embryonation time has been recorded to be 20 – 25 h at 25 ± 2 °C. The first moult occurs inside the egg and the juvenile hatches as second stage juvenile. The gonad development follows the trends found in most rhabditids, however, three prime cells of the 12 vulval precursor cells have been observed to be involved in vulva formation.

Keywords: *Acrostichus nudicapitatus*; description; taxonomy; developmental biology

Introduction

Diplogastrid nematodes are widely found in phoretic, necromenic, and parasitic associations with insects (Sudhaus & Fürst von Lieven, 2003). Their occurrence and distribution may largely be undocumented due to their niche diversity and adaptability to a variety of conditions. Being predominantly predators, the diplogastrids are characterized by extensive modifications (Fürst von Lieven & Sudhaus, 2000) in feeding apparatus including teeth, cutting plates and denticles. *Acrostichus* Rahm, 1928, the genus of Diplogastridae Micoletzky, 1922, has been raised on fairly robust and conspicuous characters (Sudhaus & Fürst von Lieven 2003). The species list of the genus was revised by Sudhaus and Fürst von Lieven (2003) who considered 28 valid species. Till now, three more species have been added by Kanzaki *et al.* (2009, 2010a, 2010b). It is a fact that due to insufficient or poor descriptions and illustrations, certain species cannot be clearly distinguished or differentiated. However, few high weight characters that have the diagnostic value, can be aptly used in species differentiation e.g., the morphology of stoma (with special reference to the dorsal tooth), female gonad, spicules, gubernaculum and male genital papillae. Besides, the developmental biology and the type of insect associates may further provide important supporting evidence for species characterization.

Barring few sporadic taxonomic reports by Khera (1965, 1970), Suryawanshi (1971), Tahseen *et al.* (1992), Hussain *et al*. (2004), Ahmad *et al*. (2004, 2005), Mahamood *et al*. (2006, 2007), Khan *et al*. (2008), Mahamood and Ahmad (2009), Ahmad *et al*. (2009), Singh *et al.* (2014) and Mahamood (2014), the diplogastrids of India have largely remained neglected.

The present paper deals with the description of a diplogastrid predator, *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962 with added features and minor variations. The information on its embryogenesis and gonad development has also been supplemented to view the species holistically.

Materials and Methods

The soil samples were processed using Cobb's (1918) sieving and decantation and modified Baermann's funnel (1917) techniques. The nematodes were extracted and fixed in hot formalin-glycerol fixative, dehydrated by the slow evaporation method (Seinhorst, 1959) and mounted in anhydrous glycerin. Permanent mounts were prepared using the paraffin wax-ring method (de Maesneer & d'Herde, 1963). The measurements were taken with an ocular micrometer. LM photographs were taken with a Jenoptik ProgRes digital camera mounted on an Olympus BX-51 DIC microscope.
The nematodes were grown on standard NGM (Nematode Growth Medium) at 25 °C and seeded with *Escherichia coli* as the food source. Stock cultures were maintained from single gravid female. Agar strips 0.4 mm thick were prepared as described by Sulston *et al.* (1983). Freshly-laid eggs (at single-celled stage) of *A. nudicapitatus* were transferred from agar plates and mounted on agar strip that was placed on cavity slide covered with a cover slip. The

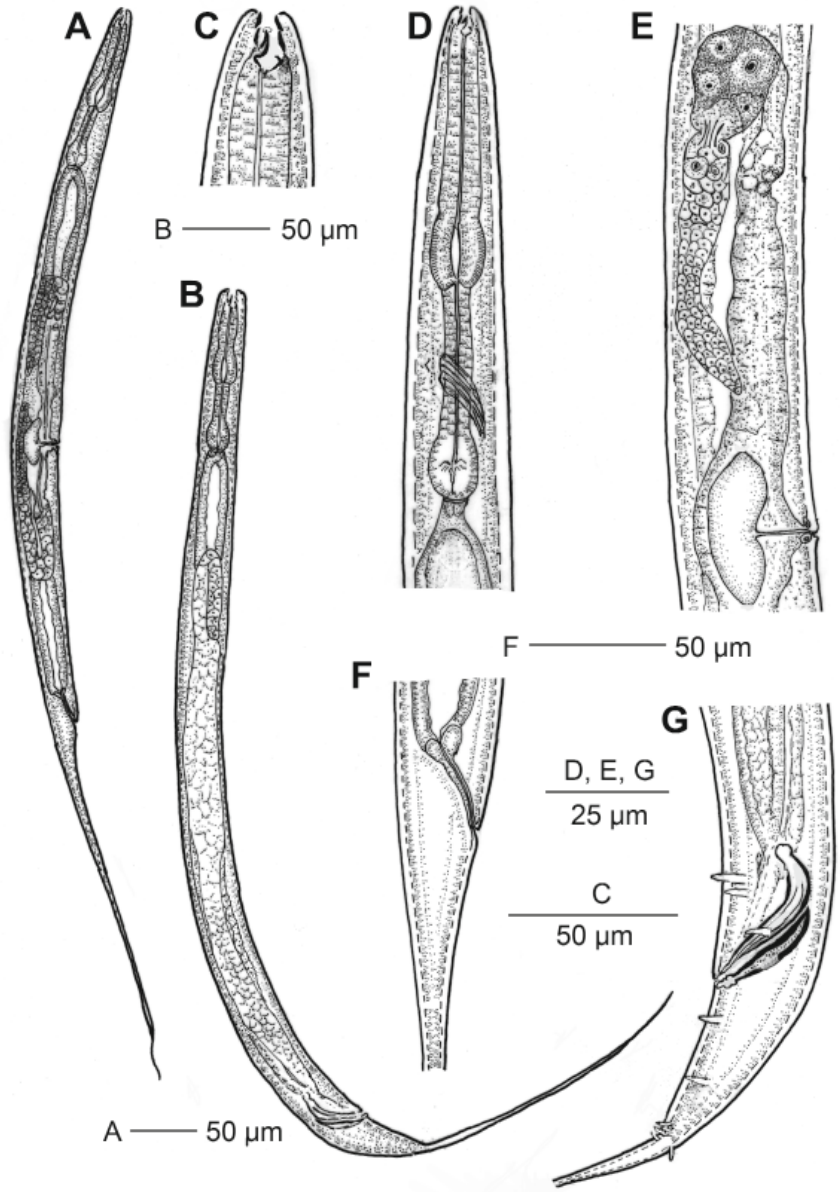

Fig. 1. *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962: A – Entire female, B – Entire male, C – Female anterior end, D – Female pharyngeal region, E – Female anterior genital branch, F – Female posterior region, G – Male posterior region

eggs were observed under microscope for embryogenesis and cleavage patterns. Gonad development was studied by picking the juveniles from the corresponding cultures and placing them in 1.5% lacto-aceto-orcein stain (Tahseen *et al.,* 1992) for 2 – 3 h. The primordium and related structures were studied in the juvenile stages.

Systematics

Acrostichus nudicapitatus (Steiner, 1914) Massey, 1962
(Fig. 1 and 2)

Measurements
See Table 1.

Description
Female. Body slender, medium to large-sized; straight or slightly curved after fixation, tapering at both extremities. Cuticle with fine transverse striations and longitudinal ridges; lateral fields not demarcated. Lip region flattened to slightly truncate, continuous or demarcated by a slight depression from adjoining body. Lips amalgamated; labial sensilla slightly raised. Amphidial apertures elliptical, situated about 4 – 6 µm from anterior end of stoma. Cheilostom cuticularised, smaller than gymnostom, converging anteriorly with six faintly visible adradial plates. Stegostom anisotopic, anisomorphic. Dorsal metastegostomal wall with an anteriorly directed, upright to slightly arcuate tooth, each sub-ventral wall provided with a small tooth. Pharyngeal corpus muscular, 40 – 45 µm long; metacorpus ovoid, strongly developed with thickened lumen; isthmus distinctly differentiated from metacorpus, 15 – 19 µm long. Basal bulb small, glandular in nature, continuous with isthmus, 16 – 18 µm x 12 µm in dimension. Nerve ring encircling isthmus at 73 – 75 % of pharyngeal length from anterior end. Hemizonid usually conspicuous in basal bulb region. Secretory-excretory pore placed posterior to hemizonid or at 78 – 85 % of pharyngeal length from anterior end. Body at pharyngeal end *ca* 2.9 – 3.4 times labial diam. wide. Cardia 6 – 7 µm long. Intestine thin-walled, often with a bacterial pouch present in anterior part. Rectum *ca* 1.2 – 1.5 times anal body diam. long, with inconspicuous rectal glands. Reproductive system didelphic, amphidelphic; ovaries reflexed, usually reaching the level of vulva but occasionally crossing each other; anterior ovary on right side and posterior on left side of in-

Table 1. Morphometrics of present population of *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962

Characters	Female (n=10)	Male (n=8)
Body length	700.0 ± 1.0 (699 – 701)	579.3 ± 10.0 (567 – 591)
Body diameter	28.2 ± 2.1 (26 – 31)	23.0 ± 0.7 (22 – 24)
a	23.3 ± 0.7 (22.6 – 24.1)	25.2 ± 0.6 (24.4 – 24.7)
b	6.7 ± 0.1 (6.6 – 6.8)	6.1 ± 0.1 (5.9 – 6.3)
c	3.0 ± 0.2 (2.7 – 3.3)	3.6 ± 0.1 (3.4 – 3.8)
c’	14.9 ± 0.9 (14.0 – 15.8)	8.5 ± 0.7 (8.1 – 9.0)
V/T	40.1 ± 1.8 (38.3 – 42.0)	47.9 ± 1.3 (46.2 – 49.9)
G1	27.6 ± 3.0 (24.6 – 30.6)	–
G2	29.1 ± 2.5 (26.6 – 31.6)	–
Lip region height	2.2 ± 0.3 (2 – 3)	2.2 ± 0.4 (2 – 3)
Lip region diameter	7.0 ± 0.9 (7 – 8)	6.1 ± 0.4 (6 – 7)
Stoma length	8.5 ± 0.2 (8 – 9)	7.4 ± 0.9 (7 – 9)
Stoma diameter	3.5 ± 0.5 (3 – 4)	3.2 ± 0.4 (2 – 3)
Pharynx length	100.5 ± 1.6 (98 – 102)	93.7 ± 1.9 (91 – 96)
Nerve ring – anterior end	74.0 ± 1.0 (73 – 75)	70.5 ± 0.8 (70 – 72)
Secretory-excretory pore – anterior end	91.7 ± 2.6 (89 – 95)	86.0 ± 1.1(85 – 87)
Rectum length	22.1± 1.5 (20 – 24)	27.1 ± 1.7 (25 – 29)
Anal body diameter	16.0 ± 1.2 (15 – 18)	18.7 ± 1.3 (17 – 20)
Tail length	230.6 ±16.9 (213 – 253)	160.2 ± 7.2 (148 – 167)
Spicule length	–	30.5 ± 1.1 (29 – 32)
Gubernaculum length	–	24.7 ± 0.4 (24 – 25)

Measurements in µm and in the form: mean ± standard deviation (range)

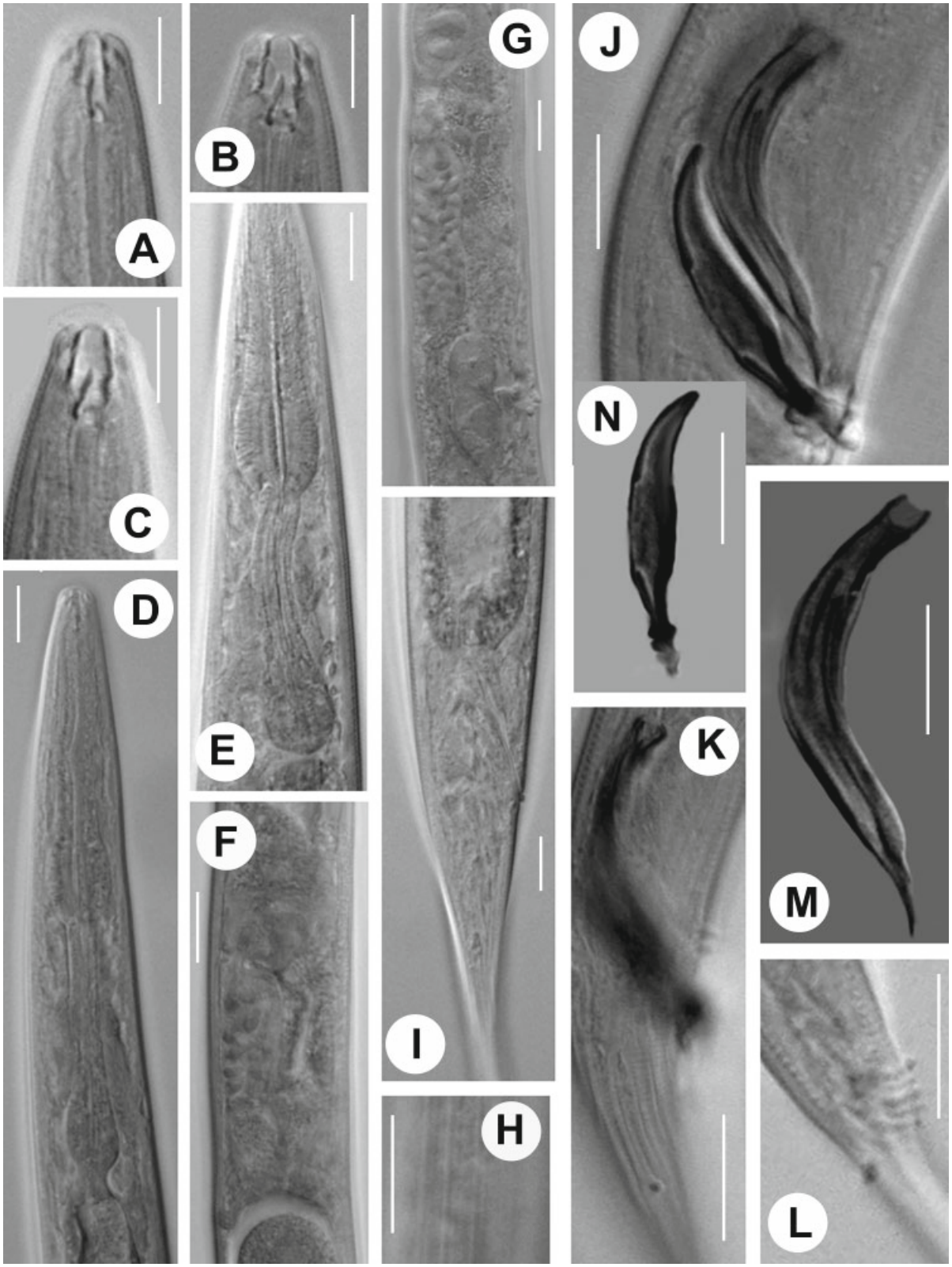

Fig. 2. *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962: A – C: Anterior end, D, E: Female pharyngeal region, F, G: Female anterior genital branch, H: Cuticular markings, I: Female posterior region, J – L: Male cloacal region, M: Spicule, N: Gubernaculum (Scale bar: 10 µm)

testine. Posterior genital branch usually larger as compared to anterior genital branch. Oocytes arranged in double row in proliferation zone of each ovary; proximal part of ovary separated from rest of ovary by a sphincter. Oviduct a narrow tube leading to wider, ovoid spermatheca. Uteri containing 1 – 2 eggs of 45 – 48 µm x 23 – 26 µm in dimension occasionally in early stage of segmentation. Older females contained as many as 4-6 intra-uterine eggs. Each uterus with a bilobed or kidney-shaped dorsal pouch connected to vagina, occasionally containing sperms. Vagina 10 – 12 µm, cuticularised, with thick lumen occupying about 1/3rd – 1/4th of corresponding body diam.; epiptygma present. Vulval lips slightly protruding. Distance between vulva-anus 0.6 – 0.8 times tail length. Tail long filiform with whip-like terminus.

Male. Similar to female in general appearance but shorter in length and strongly curved in posterior region. Testis single, laterally reflexed, reflexed part 45 – 52 µm long. Vas deferens continuous with a tapering ejaculatory duct joining with rectum to form cloaca. Spicules strongly-built, ridged, arcuate in proximal half or bent at 40 % of length proximally, 1.5 – 1.8 times anal body diam. long with rounded capitula, a distinct neck with small ventral protrusion/ blister in its ventral wall; dorsal wall of spicule without a spur or appendix and distal part deeply attenuated. Gubernaculum stout, heavily-built, 70 – 85 % of spicule length, proximally arcuate with a tapering end; dorsal wall partially without sclerotization; distal end with two pairs of short spines (in ventral view) that appear obtuse tubercles in lateral view. Tail demarcated into two parts; a short, conoid anterior part and a long whip-like posterior part. Genital papillae divided into three precolacal and six postcloacal pairs. v1 slightly longer than and close to subventral v2; v3d lateroventral; v4 subventral slightly posterior to cloaca; ad lateral, situated more than one cloacal body diam. posterior to cloaca; v5, v6 and v7 long, tubular, grouped together subventrally; pd posterior most, subdorsal in position. Copulatory muscles representing 5 – 6 pairs of broad bands. Phasmids pore-like, about 1.0 – 1.3 cloacal body diam. posterior to cloacal opening.

Habitat and locality

Samples containing *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962 were collected from soil rich in decaying leaf litter close to a tree base near State Bank of India at Aligarh (Geographical coordinates: 27.88334 N, 78.07475 E.), Uttar Pradesh, India.

Voucher specimens

Ten females and eight males on slide *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962 SA39/ 1-10 deposited in the Nematode Collection, Department of Zoology, Aligarh Muslim University, Uttar Pradesh, India.

Remarks

The present population shows conformity to *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962 in most morphological and morphometric characters barring few minor differences *viz*., dorsal wall of spicules without a fine spur (*vs* a fine spur or appendix present); gubernaculum narrow and tapering proximally (*vs* proximal end relatively thicker); relatively smaller 'c' value (2.7 – 3.3 *vs* 2.9 – 4.3); smaller intra-uterine eggs (45 – 48 x 23 – 26 µm *vs* 50 – 63 x 25 – 36 µm) and smaller spicules (29 – 32 µm *vs* 32 – 40 µm in original population of *A. nudicapitatus apud* Weingartner, 1955). The present population although closely related to *A. ponderosus* Massey, 1962 shows some differences *viz*., greater 'a' value (22.6 – 24.1 *vs* 16.0); smaller 'V' value (38.3 – 42.0 *vs* 48.0) and larger gubernaculum (24 – 25 µm *vs* 22 µm). The two species, *A. nudicapitatus* and *A. ponderosus* show striking similarities with each other in most morphological and morphometric characters including the body length, nature of longitudinal lines, shape of lip region, nature of labial and cephalic sensilla, position of amphids; shape and size of stoma, uterine chamber and spicules and structure of gubernaculum (in having two pairs of distal conical processes or tubercles). *A. nudicapitatus* was described with fairly good number of specimens by Steiner (1914) while *A. ponderosus* was described by Massey (1962) on the basis of one male and one female. Based on their original descriptions, both can be differentiated by 'a' (20.8 – 28.0 *vs* 16.0) and 'b' (5.0 – 5.5 *vs* 7.0) values in females and by L (0.55 – 0.56 mm *vs* 0.50 mm) and 'c' (2.8 – 3.2 *vs* 5.0) values in males, respectively. The imprecisely drawn stoma (Fig. 8: Steiner, 1914) of *A. nudicapitatus* in original publication does not offer enough for comparison. Further, in *A. ponderosus*, the disparity in the number of precloacal genital papillae in males [two precloacal pairs (Fig. 1C *apud* Massey, 1962; Fig. 1D *apud* Massey, 1966) *vs* three precloacal pairs (Fig. 50F *apud* Massey (1974)] and the conspicuously broad and arcuate dorsal tooth (Kanzaki *et al*. (2009) Fig. 4C, D) in apparently flattened original specimens (Fig. 4A, B, F) vouch for a thorough study of the two representatives of the species which shows no subsequent reports. Gagarin (2002) reported two populations of *A. nudicapitatus* from sewage and cow manure with a wide range of values (a= 16 – 22, V= 36.2 – 44.3, spicule length= 32 – 39 µm and gubernaculum length= 22 – 31 µm) which nearly overlapped with the values of the single male and female specimens of *A. ponderosus*. On the basis of overlapping morphometrics and similar morphological features, Gagarin (2002), synonymized *A. ponderosus* with *A. nudicapitatus* considering the latter as senior synonym, however, he endorsed Andrássy's (1984, 2005) views to consider them both under the genus *Diplogasteritus* Paramonov, 1952. The observations of Mahamood (2014) further indicate wide ranges in body length (552 – 722 µm), 'a' value (20.2 – 25.2), spicule (28 – 35 µm) and gubernaculum (22 – 25 µm) lengths of *A. nudicapitatus*. The unusual range of 'b' value (15.1 – 6.3) in the Table 1 (page no. 149) of above publication, however, appears to be due to oversight (hence corrected in Table 2 of present publication) while the statement about presence of circular amphids, also seems to be erroneous for diplogastrids. Keeping in view the conspicuous overlaps in morphometrics as well as morphological

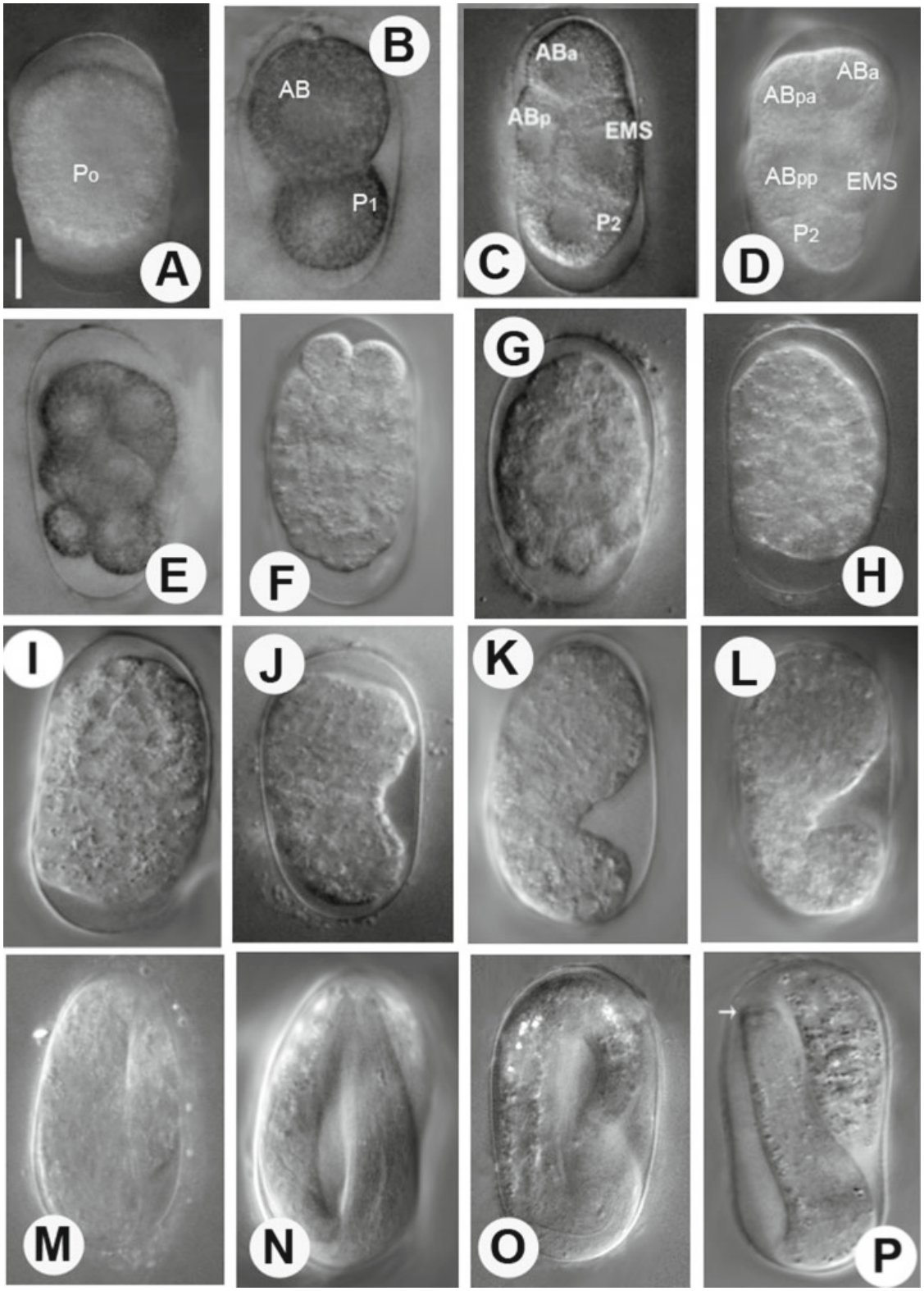

Fig. 3. Embryonic stages in *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962: A – Single-celled stage, B – Two-celled stage, C – Four-celled stage, D – Five-celled stage, E – Eight-celled stage, F – Morula stage, G – Early blastula stage, H – Late blastula stage, I – Gastrula stage, J – Lima bean stage, K – Comma stage, L – Tadpole stage, M – Plum stage, N – Loop stage, O,P – Pretzel stage (Scale bar: 10 μm)

characteristics and in view of inadequate number of original specimens, the status of *A. ponderosus* with respect to *A. nudicapitatus* needs to be ascertained. Perhaps a molecular characterization and a holistic comparison may help when a population of the former species is reported in future.

Developmental biology of *Acrostichus nudicapitatus*

The extracted natural population of *A. nudicapitatus* showed the male: female sex ratio to be 1:1. Later single gravid female culture also showed equal proportion of males and females.

Embryogenesis (Fig. 3, 4): The females were oviparous and the eggs were laid in single-celled or two-celled stage, 90 – 160 min after fertilization. The uterine tract was observed to accommodate rarely as many as 4 – 6 eggs. The eggs were smooth-shelled, oval in shape measuring 45 – 48 x 23 – 26 µm in dimension. The pole of entry of sperm marked the posterior end of the developing embryo. Soon after the sperm's entry, the cytoplasmic streaming led to formation of false cleavage furrow. During the event, the sperm pronucleus migrated to the centre of egg where it finally fused with the egg pronucleus. The first cleavage furrow was formed perpendicular to the longitudinal embryonic axis after 10 – 15 min of pronuclear fusion. The resulting blastomeres were unequal with anterior one (AB) slightly larger than the posterior (P_1). At the commencement of the second cleavage division the cytoplasm of cell AB showed streaming. Thus, the second oblique cleavage after 10 – 15 min gave rise to cells ABa (anterior) and ABp (posterior) blas-

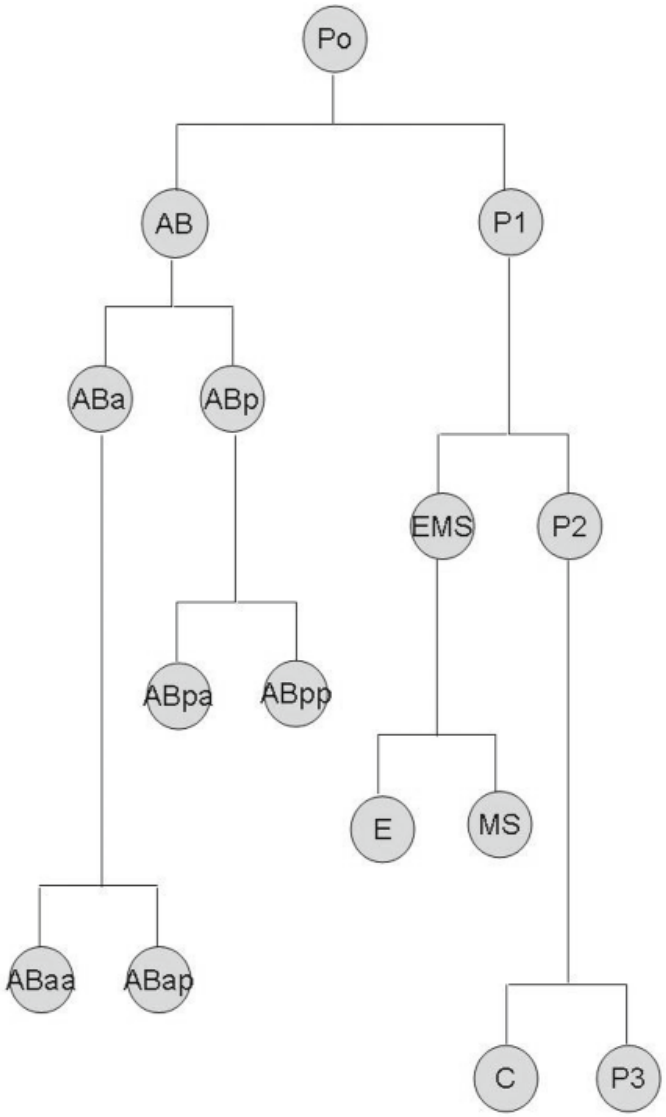

Fig. 4. Embryonic lineage in *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962 up to seven cleavage division

Table 2. Morphometrics of *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962 as provided by different investigators over the time

Character	Steiner (1914) (from mud of ditch)	Weingartner (1955) (from compost)	Gagarin (2002) (from sewage)	Gagarin (2002) (from cow manure)	Andrassy (2005) (from dung)	Mahamood (2014) (from sewage)	Present population (from leaf litter)
			FEMALE				
Body Length	579 – 699	540 – 1260	741 – 938	610 – 736	700 – 1050	552 – 722	699 – 701
Body width	22 – 28	~26 – 45	38 – 42	~36 – 38	~35 – 40	23 – 34	26 – 31
a	20.8 – 28.0	20.6 – 27.4	19 – 22	16 – 20	20 – 26	20.2 – 25.2	22.6 – 24.1
b	5.0 – 5.5	5.4 – 8.8	5.4 – 6.4	5.7 – 6.8	5.0 – 7.2	5.1 – 6.3	6.6 – 6.8
c	2.8 – 3.2	2.9 – 4.3	2.9 – 3.4	2.4 – 2.9	2.8 – 4.3	2.7 – 3.3	2.7 – 3.3
c'	–	~12.4	11.5 – 15.4	10.9 – 13.8	11 – 15	11.5 – 16.3	14.0 – 15.8
V	~<50	38.6 – 41.0	39.2 – 44.3	36.2 – 38.8	39 – 41	38.7 – 42.1	38.3 – 42.0
Stoma length	–	~11	8.5 – 11.0	8.5 – 10.0	9 – 11	9 – 10	8 – 9
Pharynx length	112 – 126	~110	126 – 147	102 – 118	~140 – 145	104 – 121	98 – 102
Tail length	187 – 220	~186 – 293	238 – 287	240 – 281	240 – 280	179 – 238	213 – 253
			MALE				
Body length	558 – 565	470 – 920	650 – 780	487 – 575	470 – 800	480 – 611	567 – 591
Body width	22 – 25	~21 – 30	~31 – 36	~25 – 30	~22 – 26	20 – 26	22 – 24
a	22.3 – 26.0	21.5 – 30.6	18 – 25	16 – 22	21 – 31	21.5 – 26.3	24.4 – 24.7
b	4.8 – 5.2	5.9 – 7.6	5.1 – 5.7	5.2 – 6.3	4.8 – 7.0	4.9 – 5.5	5.9 – 6.3
c	2.8 – 3.2	3.3 – 5.6	3.4 – 4.2	2.6 – 3.2	3.0 – 5.6	3.2 – 3.8	3.4 – 3.8
c'	~11.5	~7.1 – 8.2	7.1 – 9.4	7.6 – 11.0	7 – 9	7.6 – 9.4	8.1 – 9.0
Pharynx length	108 – 115	~79 – 120	122 – 137	90 – 109	~98 – 114	87 – 111	91 – 96
Tail length	173 – 198	~142 – 164	168 – 210	161 – 217	170 – 200	126 – 175	148 – 167
Spicule length	~31	~36 – 38	35 – 39	32 – 36	35 – 40	28 – 35	29 – 32
Gubernaculum length	~22	~22 – 25	28 – 31	22 – 28	28 – 30	22 – 25	24 – 25

Measurements are in µm with values (~) estimated from illustration or calculated from other related measurements

tomeres. The blastomere P_1 after 5 – 10 min, also divided obliquely to form EMS and P_2 resulting into four-celled stage (Fig. 3B). Simultaneously within 10 – 15 min ABp transversely divided into ABpa and ABpp blastomeres (Fig. 3C) followed by division of EMS into MS and E (Fig. 3D) after 15 – 20 min. P_2 divided into C and P_3 (Fig. 4) after 15 – 20 min. The eight-celled stage was reached 90 – 110 min after egg laying followed by ten-celled stage after 100 – 120 min. Morula (Fig. 3F) representing a loose mass of cells was formed after 1.5 – 2.5 h of ten-celled stage. Blastula (Fig. 3G, H) reached 2.5 – 3.0 h of morula formation. Gastrulation (Fig. 3I) started after 1.0 – 1.5 h marking the germ layer differentiation. The stage involved the differentiation of ectoderm as an outer layer covering inner layer and an inconspicuous blastocoel. The ingression of endodermal cells led to the differentiation of anterior and posterior regions of the gut. The elongating embryo showed invagination along longitudinal axis followed by a depression slightly away from the centre of egg. The invagination marked the 'Lima bean' stage (Fig. 3J) 50 – 60 min after the initiation of gastrulation and further deepening of the cleft resulted into 'Comma' stage subsequently after 40 – 50 min (Fig. 3K). The embryo then prepared for organogenesis and acquired a worm-like body in 'tadpole' stage (Fig. 3L) after about 40 – 50 min of comma stage, with an anterior shallow depression representing the presumptive stoma. Plum stage (Fig. 3M) followed after about 1.0 – 1.5 h with the embryo showing first sign of movement which became more frequent later. Loop stage (Fig. 3N) was attained subsequently after about 30 – 40 min from plum stage when the embryo acquired almost two egg-folds length and moved continuously inside the shell in the anterior-posterior direction. The vermiform embryo exhibited a broader anterior end with small stomal cavity and a long pointed tail showing its posterior end. The loop stage was followed by pretzel stage after about 3 – 3.5 h, in which the stoma, pharynx, intestine, tail and rectum were differentiated. Continuous movements involving body rotation and turning were observed in the embryo during this stage with the embryo attaining 4.0 – 4.5 egg-fold length. About 3.0 – 3.5 h after the commencement of pretzel stage (Fig. 3O, P), the stoma is differentiated into cheilostom, gymnostom and stegostom while other structures *viz*., pharynx, genital primordium, rectum and tail were fully formed. After the completion of organogenesis, the juvenile underwent a moult within the egg shell (Fig. 3P) before finally

moving out of the shell. As a result of the increased plasticity due to excessive pressure caused by the body movements as well as labial probations of the juvenile, the egg shell became very thin and finally ruptured in the form of a slit. However, the second-stage juvenile hatched out of the egg shell in several attempts 3 – 4 h after completion of organogenesis. The total embryonation time from single-celled stage to hatching was 20 – 25 h at 25 ± 2 °C.

Gonad development (Fig. 5): During post-embryogenesis the gonad development continued from second stage juvenile (J_2) to fourth stage juvenile (J_4). The second stage juvenile showed a primordium similar to the first stage juvenile which remains in the egg shell. The juvenile stages could not be differentiated exclusively on morphometrics due to overlap of values. However, the growth patterns of the genital primordium served good marker to differentiate these stages.

In the egg shell, the first-stage juvenile showed an obliquely-oriented primordium containing two large central germinal (Z_2, Z_3) and two relatively smaller somatic cells (Z_1, Z_4) at poles. The genital primordium was similar in all first stage juveniles with fixed number of primordial cells and was sexually undifferentiated.

The primordium (Fig. 5A) of second stage juvenile (J_2) that hatched out of the egg shell, was located at 42.2 – 44.3 % from anterior end and was similar (Fig. 5B, C) to that of first stage juvenile and identical in all juveniles that were to develop into males or females. However, during the moulting period, the localization of cells and the resulting primordial growth indicated the future sex of juvenile. In second stage juvenile destined to develop into female, by the end of moulting, the cells present in the genital primordium included two distal tip cells (DTCs) helpful in gonad elongation and germ-line patterning to form all future somatic gonad cells; and 4 germinal cells ready to form the future germline of the female gonad. In second stage moulting juveniles destined to develop into males, the two DTCs were positioned at the posterior distal end and the somatic cells showed an anterior placement in the primordium indicating its future direction of elongation.

Third-stage female juveniles of *A. nudicapitatus* possessed a much developed genital primordium, present at 42.1 – 47.2 % of body length from anterior end. The primordium (Fig. 5D, E) showed a bidirectional growth and elongated anteriorly, as well as posteriorly, as a result of cellular proliferation. The genital primordium contained germinal and somatic cells including two DTCs at the anterior and posterior extremes. Some of the germinal cells migrated to the ends of the elongating arms while the somatic cells largely remained in the centre (Fig. 5 E) along with few germinal cells and an anchor cell (AC). The latter was a transient cell that primarily functioned to indicate the site for vulva formation and helped in vulva patterning. The moulting period marked the proliferation of somatic cells in the centre of primordium resulting in its extension. Ventral hypodermal chord cells, differentiated into precursors for the vulva and aggregated on the ventral aspect of primordium at 45 – 55 % of body length from anterior end (Fig. 5E).

The genital primordium of third-stage male juveniles was located at 42.7 – 45.5 % of body length from anterior end. It consisted of germinal and somatic cells including two DTCs at the distal end and a linker cell at proximal end. The primordium (Fig. 5H) showed anterior growth due to proliferation of the somatic cells and eventually developed a flexure (Fig. 5J) during the moulting period. Two dense cellular aggregates were observed each on dorso-lateral side of rectum representing the precursors of the spicules and associated structures (Fig. 5K).

Fourth-stage female juveniles possessed a much elongated genital primordium located at 37.7 – 39.1 % of body length from anterior end. It consisted of two primordial arms directed in anterior and posterior directions (Fig. 5F). The arms represented the germline while the central broader part showed the somatic parts of the future reproductive system. The part between the presumptive oviduct and uteri showed germinal cells that proliferated later to mark spermatogenesis in the hermaphrodite juvenile. The area later served to store the spermatids. The anchor cell (AC) located in the centre of primordium (Fig. 5E) and morphologically distinct from all other somatic cells, played a significant role in vulva induction. The cell also played important role in signaling to other ventral uterine cells and finally generating the connection between the uterus, vagina and the outside environment. Of the 12 VPCs (vulval precursor cells) the central three (Fig. 5F) divided to give rise to up to 16 descendants that actively participated in vulva formation. The rest of the precursor cells disappeared in late stage due to programmed cell death. In the moulting stage, the two extreme ends of the primordium containing germinal cells reversed forming the germ line/ ovaries while the remaining gonoduct formed the somatic gonad. The vulva formation along with the differentiation of the somatic parts of the gonad was completed with the termination of moulting (Fig. 5G).

The fourth-stage male juveniles possessed a genital primordium at 36.1 – 37.4 % of body length from anterior end. The genital primordium showed a reflexed part showing the germline of the future gonad with germinal cells contained in and a considerably longer tube elongating posteriorly. The somatic tube contained proliferating somatic cells with the linker cell placed ahead to join with the rectum to form cloaca during late moulting stage. The spicular precursor cells showed more compaction (Fig. 4K, L) and formed the spicules and gubernaculum in the late fourth stage initially as refractory lines. The genital papillae were the last structures to develop by special hypodermal cells known as papillae precursor cells. The caudal region of male juvenile distinctly differed from that of the female juvenile (Fig. 5M).

Discussion

Generally the species of *Acrostichus* have been reported from a wide range of habitats including fresh and polluted water, more specifically in aquatic mulm or slime flux or sewage; from soil to rotten decaying matter or from moist husk to rotten plants and from dung to frass of beetles. The species *A. nudicapitatus* too, is a widely dis-

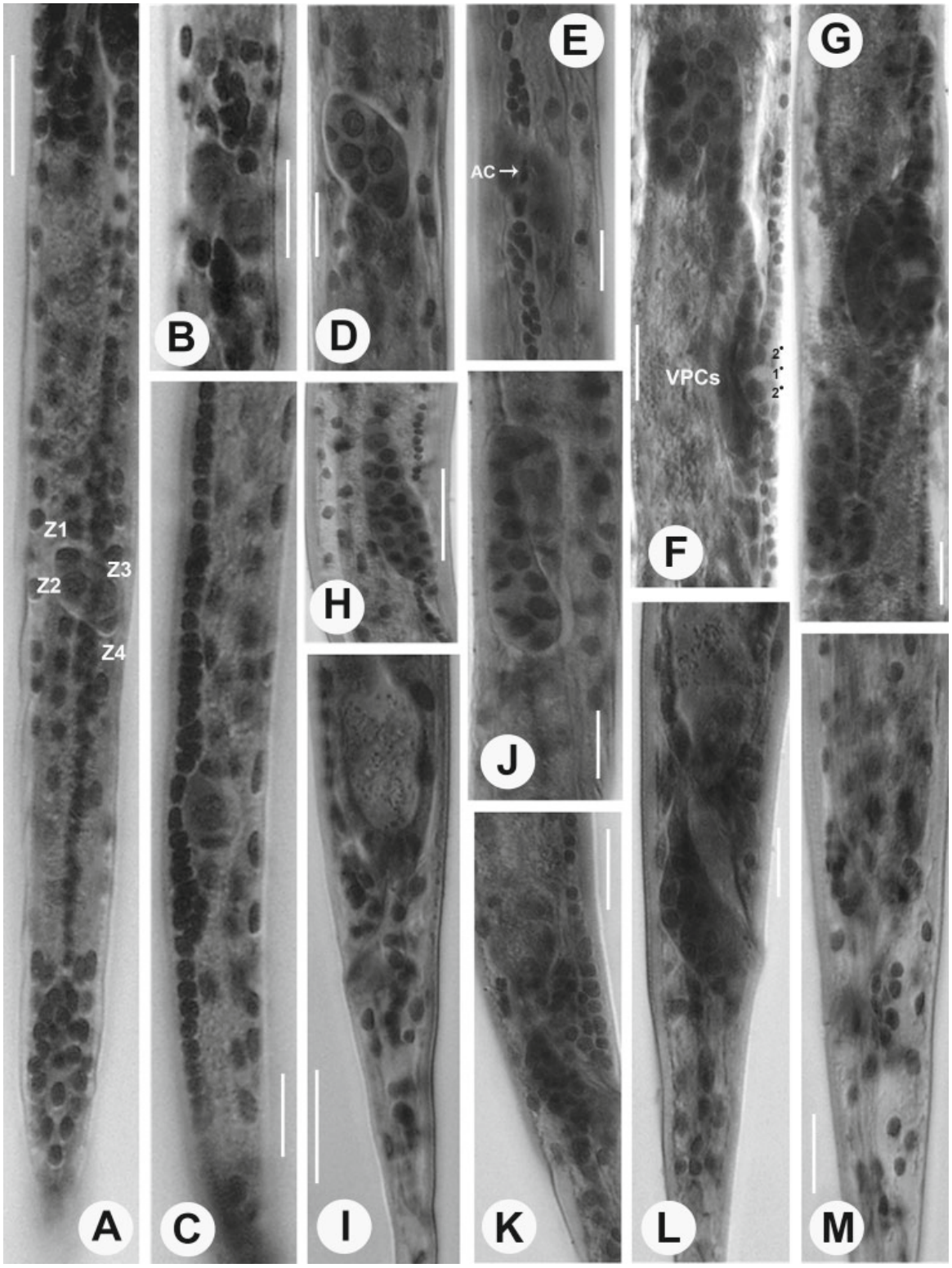

Fig. 5. Gonad development in *Acrostichus nudicapitatus* (Steiner, 1914) Massey, 1962: A – Newly hatched second stage juvenile, B,C – Genital primordiun in second stage juvenile, D, E – Genital primordiun in third stage female juvenile, H – Genital primordiun in third stage male juvenile, F,G – Genital primordiun in fourth stage juvenile, J – Genital primordium in early fourth stage male juvenile, I – Cloacal region in undifferentiated second stage juvenile, K,L – Cloacal region in third and fourth stage male juveniles respectively with spicular primordium, M – Anal region in fourth stage female juvenile (Scale bar: 10 µm)

tributed species reported from polluted water, dung as well as bark beetles. Its closely related species *viz*., *A. arcuatus* Massey, 1962, *A. concolor* Massey, 1962, *A. gubernatus* Massey, 1974 and *A. taedus* Massey, 1962, have also been reported from bark beetles whereas the insects like honey bees and curculionids were reportedly found to harbour *A. occidentalis* (Steiner, 1932) Massey, 1962 and *A. lineatus* (Fuchs, 1915) Massey, 1962 respectively.

A. nudicapitatus is unique in having a slightly arcuate and sharply pointed dorsal tooth compared to related species *A. taedus* and *A. gubernatus* that possess narrow and sharply pointed tooth and broad and blunt tooth, respectively although some intermediate variants have also been found. Due to overall similarity in gonad morphology, the taxonomically reliable characters appear to be the spicules and gubernaculum which are amber colour and strongly cuticularised. *A. nudicapitatus* has the spicules bent at 40% of the total spicule length from its proximal end whereas spicules of related species were slightly arcuate (*A. taedus*) to straight (*A. concolor* and *A. gubernatus*). The proximal end of the gubernaculum of *A. nudicapitatus* is relatively tapering and slightly arcuate compared to the gubernacula of other species which are relatively wider and bluntly pointed proximally.

The embryogenesis and gonad developmental patterns resemble those of other diplogastrids including *Mononchoides fortidens* (Tahseen *et al*., 1992). The embryogenesis time has been found to be greater (20 – 25 h *vs*16 – 20 h) in *A. nudicapitatus*, however, it could well be comparable to *M. fortidens* keeping in view the temperature conditions which were 25 ± 2 ºC in the former while 30 ± 2 ºC in the latter. It is a fact that embryonic variations in addition to morphological and molecular information may serve as phylogenetic markers (Schierenberg, 2001). An important phenomenon observed was the moulting of first-stage juvenile within the shell without undergoing feeding in conformity to tylenchids (Yüksel, 1960; Hirschmann, 1962; Yuen, 1965; Hirschmann and Triantaphyllou, 1967). The phenomenon is contrary to that found in closely related group of rhabditids where the first stage juvenile moults after hatching. The results are in conformity to those of Furst von Lieven (2005). Despite some variations in the sequence of cleavages, the embryonic lineages showed similarity to the rhabditid, *Caenorhabditis elegans* where the germ cell, P_4 was formed very late and the initial divisions occurred along AB line up to 16-celled stage before P4 (germ cell) was finally differentiated (Schierenberg *et al*. 1997). Development of the reproductive system from a single, obliquely-oriented primordium has been a feature similar to other rhabditids (Tahseen *et al*., 1990 and Tahseen & Nisa, 2006) and diplogastrids (Tahseen *et al*., 1992). The pattern and morphological changes in genital primordium to form the gonad revealed similar trends as observed in species having a diovarial female and monorchic male (Tahseen *et al*., 1990, 1991). The vulva formation involving 12 VPCs of which only three taking part in vulva development is a feature also reported in the diplogastrid, *Pristionchus pacificus* where the rest of them were reported to have undergone programmed cell death.

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