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THE USE OF INTEGUMENTAL PORE SIGNATURE IN THE CHARACTERISATION OF SPECIES OF THE GENUS *THERMOCYCLOPS* KIEFER, 1927 : THE CASE OF *THERMOCYCLOPS EMINI* (MRÁZEK, 1895) (CRUSTACEA : COPEPODA : CYCLOPOIDA)

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Abstract. The possibility to use the integumental pore signature as a tool in the identification of cyclopoid copepods is explored. A full classical description of female specimens of *Thermocyclops emini* (Mrázek, 1895) is supplemented by mapping of the integumental perforations. These are bilaterally symmetrical, and most occupy constant geometrical positions. On the metasome and urosome the pattern changes from one segment to another, and differs in ventral and dorsal position (on the urosome). The total number of perforations varies between 193 and 202.

Key words : Copepods, *Thermocyclops emini* (Mrázek, 1895), integumental pore pattern, characterisation.

INTRODUCTION

Following the investigations of FLEMINGER (1973), the number and position of integumental organs became used as taxonomic tools to classify calanoid copepods. Many species of copepods are now known to possess integumental perforations; these pores, with their underlying soft tissue, form integumental organs that are usually arranged in a discrete pattern (KOOMEN, 1992). The perforations can be made visible after a preparation of the cuticle that includes digestion of all tissues and staining. Investigating the calanoid *Paramisophria platysoma* Susumu & Mitsuzumi, 1990 by Scanning Electron Microscopy (S.E.M), SUSUMU & MITSUZUMI (1990) found that the integumental organs of the female cephalothorax were nearly symmetrical in distribution.

In their habitats, copepods migrate, feed, breed and perform different socio-behavioural activities (MAUCHLINE, 1977) for which these eye-less animals must rely on chemical and vibrational communication. Integumental organs are probably essential to locate and correctly identify a potential mate or predator without visual aids (FLEMINGER, 1973).

Such monitoring and interpretation suggests a variety of organ types, such as sensory receptors and glands (MAUCHLINE, 1977), with a possibly species-specific distribution across the body surface. Indeed, it is now known that each species of the genus Eucalanus Dana, 1853 has a distinct pore signature (FLEMINGER, 1973; MAUCHLINE, 1977). MALT (1983) successfully used integumental pore patterns to separate two Poecilostomatoida of the genus *Oncaea* Philippi, 1843: *O. ornata* Giesbrecht, 1902 and *O. englishi* Heron, 1977, which otherwise have a similar morphology.

Unfortunately information on the taxonomic value of the pore signature is still limited, and little is known of cyclopoids. Therefore, we aimed in the present study to provide a basis for investigations on the taxonomic value of the pore pattern of a variety of cyclopoid species and genera.

We studied the pore signature of the genus *Thermocyclops* Kiefer, 1927. As a test case, *Thermocyclops emini* (Mrázek, 1895) is here redescribed using classical morphological characters, including mouth parts and thoracopods, but its pore signature is also mapped. Mapping was carried out on the dorsum and sides of the rostrum and cephalosome; the dorsum of the metasome; the dorsum, ventrum and sides of the urosome. Perforations occur across the whole length of an animal and occupy bilaterally symmetrical positions. They are either simple, double or in groups of three. The preparation for routine work made it impossible to differentiate the integumental organs between sensilla (hair, cone, peg, pit) and gland openings. Thus, this work is limited to mapping the number and position of these organs.

MATERIAL AND METHODS

Dr. L. Mwebaza Ndawula collected material in 1990 from Lake Victoria. Samples were preserved in 4% formalin. Only adult females were used as no male was present in the studied material. Specimens were identified on the evidence of their elongated shape, antennules, long furcal rami, and dorsal setae of furcal rami, and sorted from the sample. For routine morphometrical analysis, unstained specimens were used. After dissection under a dissecting microscope, parts were mounted in glycerine on a covered slide. For the digestion of tissues, staining and washing procedure, the method of FLEMINGER (1973) was used: specimens were first heated at 80-100°C for four hours in KOH 10%. The remaining exoskeletons were first kept for some minutes in distilled water and in 70% alcohol for washing. Clean animals were then stained in Chlorazol black in lactophenol. Parts were studied separately except for the urosome that was studied intact. Dissections were done using tungsten needles electrolytically sharpened in KOH solution. Perforations were analysed and drawn under immersion oil using a camera lucida on a Medilux-12 microscope. For each part investigated (rostrum, cephalosome, each metasomal somite and urosome), a minimum of five specimens was used. Scanning Electron Microscopy (S.E.M) micrographs of critical point dried, gold-coated specimens were taken to complete optical images. Total body length excluded the furcal setae and was measured from the anterior basis of the rostrum to the posterior edge of the caudal rami. The width of the cephalosome was measured at its widest part.

The terminology used in VAN DE VELDE (1984), HUYS & BOXSHALL (1991) was adopted. All measurements are in µm and abbreviations are as follows: T. L: Total Length, L: Length, W: Width, Spi: Internal spine, Spe: External spine, Si: Internal seta, Smi: Median internal seta, Sme: Median external seta, Se: External seta, Sd: Dorsal seta, Sl: Lateral seta, SD: Standard Deviation.

RESULTS

Redescription of females

Measurements are given in Table 1. The animals are elongate with a length range of 828-928 µm, mean 878.8 µm (n=10). DUSSART'S (1982) and EINSLE'S (1970), material from the same Lake measured 1.000-1.100 µm and 900-946 µm, respectively. In his original material, Mrázek (1895) cited 990 µm. Cephalosome and genital somite longer than broad; ratios 1.28 and 1.43, respectively. On Enp_3P_4, Spi 2.43 times as long as Spe, and segment 1.15 times as long as Spi, Furca 3.52 times as long as broad, Smi 1.47 times as long as Sme, Sd 1.14 as long as Si, Sd 4.80 as long as Sl, Sd 3.03 as long as Se, Si 2.65 as long as Se and Si 1.9 as long as Furca.

Antennule (Fig. 2: N): Long and reaching the 4th metasomal somite, 17 segmented, with a row of minute spinules on first segment, 10th and 13th segments devoid of setae; segments 16th and 17th with a relatively well developed hyaline lamella (Fig. 6: B, C, D, E).

Antenna: Spine pattern on frontal and caudal sides of basipodite as in Fig. 5: H, I. Second endopodite with four setae (Fig. 5: I).

Labrum: Ventrally with eleven sharp teeth, long hair dorsally surrounding two strong median teeth; lateral edges rounded (Fig. 5: C, D).

Mandible: Pars molaris with series of sharp teeth, mandibular palp provided with three unequal setae: a small and naked seta plus two long and feathered setae (Fig. 5: A).

Maxillule (Fig. 5: G).

Maxilla (Fig. 5: B): Inner side precoxa with two strong and feathered setae. Coxa with a median seta, provided with strong spines. Outgrowth of coxa with two unequal setae, the strongest feathered, with spinules at the end. A stout claw-like seta bearing a strong seta prolongates the maxilla. Stout seta provided with variable strong spines. Endopodite one-segmented with two spinous setae and three strong feathered setae with spinules at the end.

Maxilliped: Basal segment (fusion of precoxa and coxa) provided with three feathered setae, frontal side glabrous (Fig. 5: E), caudal side with spinules (Fig. 5: F). Basis of maxilliped with two feathered setae, frontal side with two groups of minute spinules (Fig. 5: E); caudal side with long, strong spines (Fig. 5: F). Endopodite two-segmented. Caudal side of proximal segment with a group of spines, segment bearing a long feathered seta. Distal segment with three unequal setae, the longest two feathered.

Thoracopods P_1-P_4: Spine formula: 2-3-3-3, armature of the segments (Figs 3: B; 4: B) outgrowths of the connecting lamellas naked.

P_1: Inner distal margin of basipodite with a spine, inner part of basipodite with setules (Fig. 3: A, D).

TABLE 1

Measurements of the females of Thermocyclops emini *(Mrázek, 1895) from Lake Victoria*

		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Mean*	*SD*
Total length	T. l	915.00	928.00	853.00	881.00	859.00	896.00	878.00	900.00	850.00	828.00	878.80	29.92
Cephalosome	L	321.00	337.00	325.00	325.00	328.00	343.00	343.00	328.00	318.00	315.00	328.30	9.31
	W	259.00	243.00	243.00	262.00	256.00	275.00	275.00	262.00	253.00	243.00	257.10	11.43
	L/W	1.24	1.39	1.34	1.24	1.28	1.25	1.25	1.25	1.26	1.30	1.28	0.05
Thorax	L	243.00	237.00	243.00	240.00	231.00	240.00	234.00	237.00	228.00	209.00	234.20	9.60
Genital Somite	L	128.00	128.00	109.00	131.00	115.00	115.00	128.00	140.00	115.00	112.00	122.10	9.64
	W	84.00	87.00	84.00	84.00	84.00	87.00	81.00	87.00	87.00	87.00	85.20	1.99
	L/W	1.52	1.47	1.30	1.56	1.37	1.32	1.58	1.61	1.32	1.29	1.43	0.12
Enp3P4	L	60.00	62.00	59.00	59.00	58.00	64.00	54.00	64.00	63.00	58.00	60.10	3.01
	W	17.00	17	17	18	15	18	18	17	17	18	17.20	0.87
	L/W	3.53	3.65	3.47	3.28	3.87	3.56	3.00	3.76	3.71	3.22	3.49	0.25
	Spi	53.00	52.00	53.00	50.00	53.00	51.00	48.00	54.00	53.00	52.00	51.90	1.70
	Spe	25.00	20.00	21.00	20.00	21.00	23.00	20.00	21.00	20.00	24.00	21.50	1.75
	Spi/Spe	2.12	2.60	2.52	2.50	2.52	2.22	2.40	2.57	2.65	2.17	2.43	0.18
Abdomen	L	275.00	275.00	281.00	256.00	228.00	250.00	259.00	265.00	231.00	228.00	254.80	19.11
Furca	L	75.00	75.00	71.00	68.00	78.00	78.00	71.00	71.00	75.00	78.00	74.00	3.38
	W	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	0.00
	L/W	3.57	3.57	3.38	3.24	3.71	3.71	3.38	3.38	3.57	3.71	3.52	0.16
Furcal setae	Si	137.00	146.00	134.00	153.00	146.00	140.00	140.00	156.00	150.00	150.00	145.20	6.87
	Smi	281.00	300.00	293.00	290.00	291.00	287.00	290.00	287.00	271.00	296.00	288.60	7.66
	Sme	181.00	190.00	203.00	203.00	203.00	193.00	203.00	203.00	196.00	187.00	196.20	7.72
	Se	53.00	56.00	59.00	53.00	56.00	59.00	50.00	56.00	50.00	56.00	54.80	3.06
	Sd	162.00	156.00	171.00	171.00	156.00	171.00	165.00	171.00	159.00	178.00	166.00	7.14
	Sl	31.00	37.00	34.00	37.00	37.00	37.00	31.00	34.00	31.00	37.00	34.60	2.62
	Smi/Sme	1.55	1.58	1.44	1.43	1.43	1.49	1.43	1.41	1.38	1.58	1.47	0.07
	Sd/Si	1.18	1.07	1.28	1.12	1.07	1.22	1.18	1.10	1.06	1.19	1.14	0.07
	Sd/Sl	5.23	4.22	5.03	4.62	4.22	4.62	5.32	5.03	5.13	4.81	4.80	0.38
	Sd/Se	3.06	2.79	2.90	3.23	2.79	2.90	3.30	3.05	3.18	3.18	3.03	0.18
	Si/Se	2.58	2.61	2.27	2.89	2.61	2.37	2.80	2.79	3.00	2.68	2.65	0.21

P_4: Inner margin of the basipodite and both sides of the outgrowths of connecting lamella glabrous; distribution of setules on sides of coxopodite as in Fig. 4: C, G. Enp_3, 3.49 times as long as broad, Spi 2.43 times as long as Spe and 1.15 times shorter than the segment.

P_5: Setae on terminal segment almost equal (Fig. 4: E).

P_6: Three setae on thoracopod: two strong, dwarfed, naked setae next to a relatively long, feathered seta (Fig. 6: G). A variable number of openings on distal area of the implantation of P6, as described in *Mesocyclops leukarti* (Claus, 1857) by Van de Velde (1984).

Lateral margins of last metasomal somite glabrous (Figs1: I, J, K, L; 5: H).

Genital somite and receptaculum seminis: Somite 1.43 times as long as broad.

Lateral arms of the receptaculum almost straight, as described in DUMONT *et al.* (1981) and Einsle (1970). Copulatory pore and pore canal in the middle of receptaculum; a large «gland opening» (Figs 1: J, K; 6: A, G, H) at proximal edge of integument, near distal edge of last metasomal somite. P6 implanted laterally on genital somite (Fig. 6: G).

Last (anal) somite: Ventrally, distal edge with a row composed of a variable number of spines (Fig. 1: K; 6: A), naked dorsally (Fig. 1: I, 6: F).

Integumental pore pattern

Rostrum and cephalosome

Rostrum (Fig. 1: A, C, D.): Eight perforations present. One in the middle, another at the limit of the rostrum and cephalosome. The other six surround two sieve plates. In five investigated specimens, there was no variation in number or position of perforations.

Cephalosome: Maximum 91 pores were counted. Seventy-seven are in dorsal position (Figs 1: B, Ca, E; 2: A, B), 14 in lateral position (Fig. 1: C; a). Their distribution delimits three zones (Fig. 1: B, C, E; I, II, III). The first includes a maximum of 37 perforations, arranged symmetrically. Thirty-six of them are geometrically disposed around a single mediocentral one. Half of fifteen specimens analysed had double perforations at some positions as highlighted in Fig. 1: B & E (arrow in Fig. 2: Photograph A), they were simple in the other half (Fig. 1: B, E).

The second area has 26 perforations. Twenty two, symmetrically disposed, surround four mediocentral perforations.

The third zone is composed of 14 perforations and lacks median pores.

Metasome

First somite: Integument with nine pores, geometrically disposed (Fig. 1: F).

Second somite: The integument has between 24 and 26 perforations. Two of them are exactly on the longitudinal axis. A position in front of the posterior perforation can hold either a symmetrical perforation or not, as in Figs 1: G; 2: C. On the same somite median pores occur, either single or double (highlight in Fig. 1: G & M, arrow in Fig. 2: C). Seven out of ten checked animals presented double perforations at this position.

Third somite: Integument with 21 pores (Fig. 1 : H ; 2 : D). The number and positions were constant in five investigated specimens.

Fourth somite: Figs 1 : I, L ; 2 : E. The perforation number varied from four to eight.

Urosome

Genital somite (*First somite*) (Figs 1 : I, J, K ; 2 : F) : Dorsally with 10 perforations, three on the anterior middle of the somite, six in a transverse row near the posterior perforation and one on the longitudinal axis, close to the distal edge. Ventral side with four perforations.

Second somite (Figs I, J, K ; 2 : G) : Seven perforations are present dorsally ; six on the sides and one on the longitudinal axis. In ventral position, only two perforations are seen.

Third somite (Figs 1 : I, J ; 2 : G) : One perforation only on dorsum, ventral side without any pores.

Fourth (anal) somite (Figs 1 : 1 : I, J, K ; 2 : G) : six pores present dorsally, bilaterally symmetrical around the longitudinal axis. In ventral position, two pores near the distal edge of the somite.

Furcal ramus

With six perforations : two on the dorsum and four on the ventrum (Fig. 1 : I, J, K).

CONCLUSION

To date, only morphology and morphometrical analysis have been used to identify species within *Thermocyclops* Kiefer, 1927. The distribution of the integumental perforations, sites of the integumental organs, herein used to redescribe *Thermocyclops emini* (Mrázek, 1895), revealed limited variation in total pore number (from 193 to 202). Most positions were fixed. Further work will seek to clarify whether this conclusion can be generalised to other populations, and whether the pore signature of *Thermocyclops emini* (Mrázek, 1895) is unequivocally distinct from that of its congeners.

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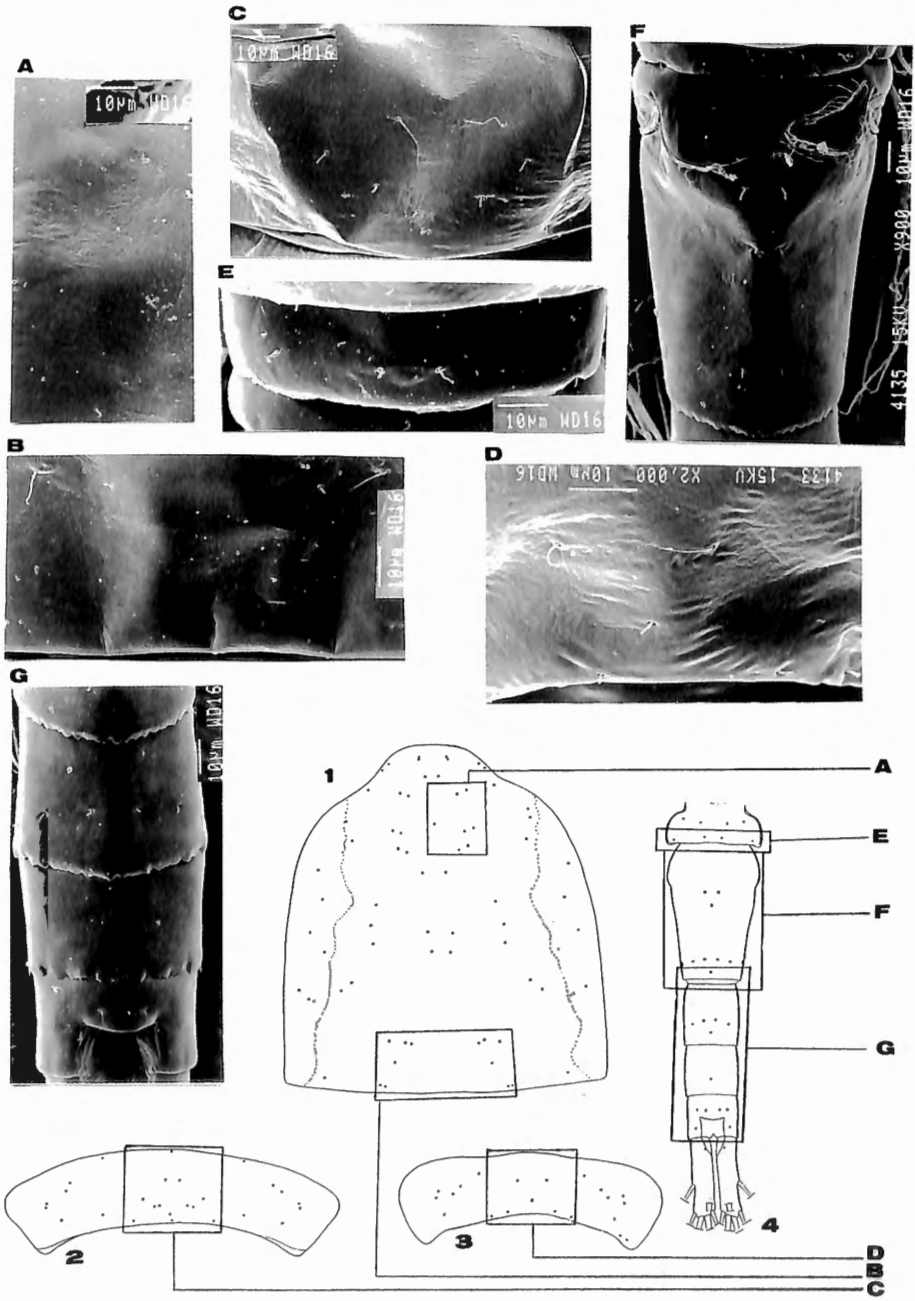

Fig. 1 . – *Computer-edited S.E.M micrographs.* – 1. : Cephalosome : Photos A and B ; 2. : Second metasomal somite : Photo C ; 3. : Third metasomal somite : Photo D ; 4. : Urosome and last metasomal somite : Photos E, F, G.

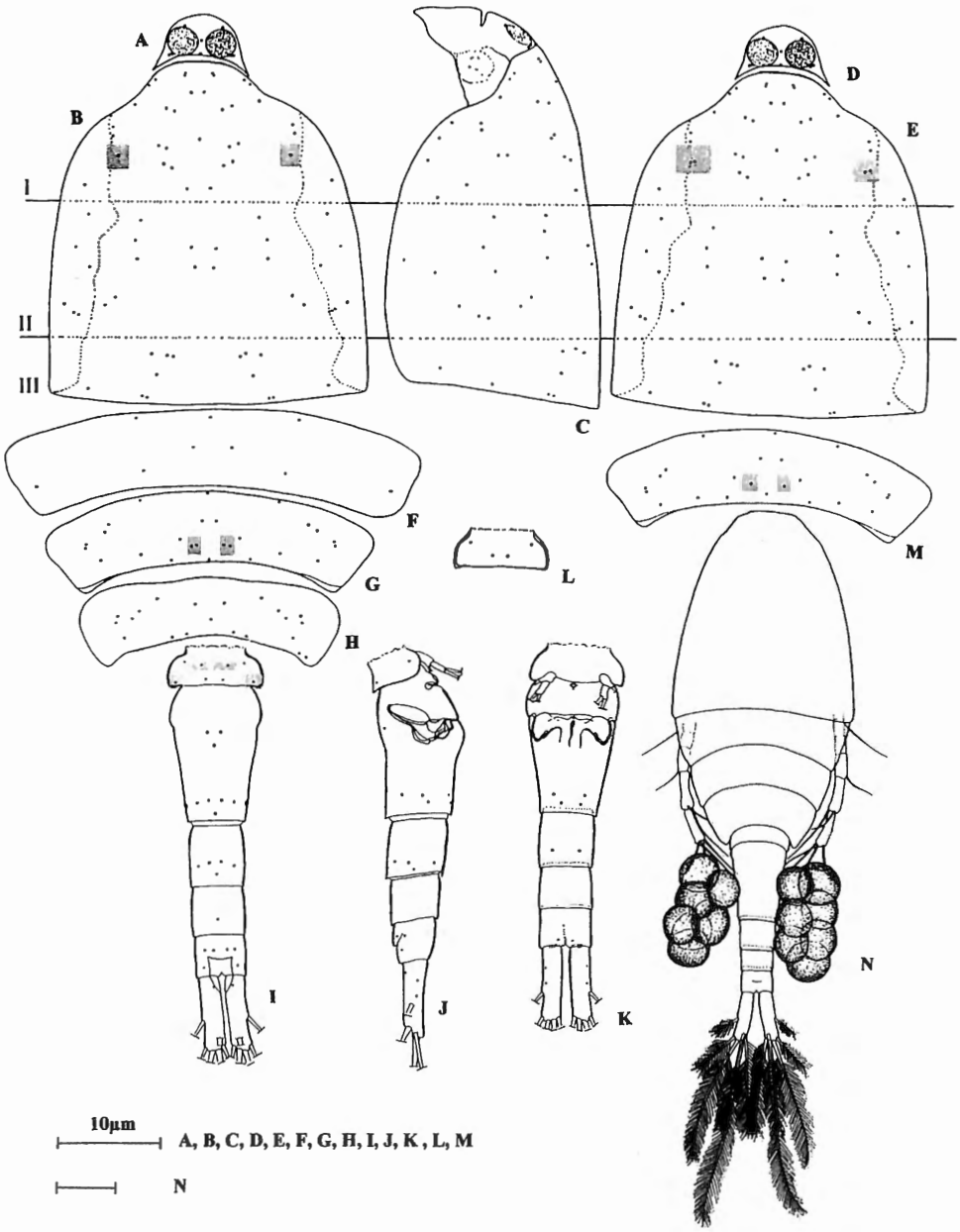

Fig. 2. – *Integumental perforations and habitus.* – A, D : Rostrum ; B, E : Cephalosome (dorsal) ; C : Cephalosome (lateral) F : First thoracic segment ; G, M : Second thoracic segment ; H : Third thoracic segment ; I : Urosome (dorsal) with last thoracic segment ; J : Urosome (lateral) ; K : Urosome (ventral) ; L : Last thoracic segment ; N : Habitus. Scale bars = 10 µm.

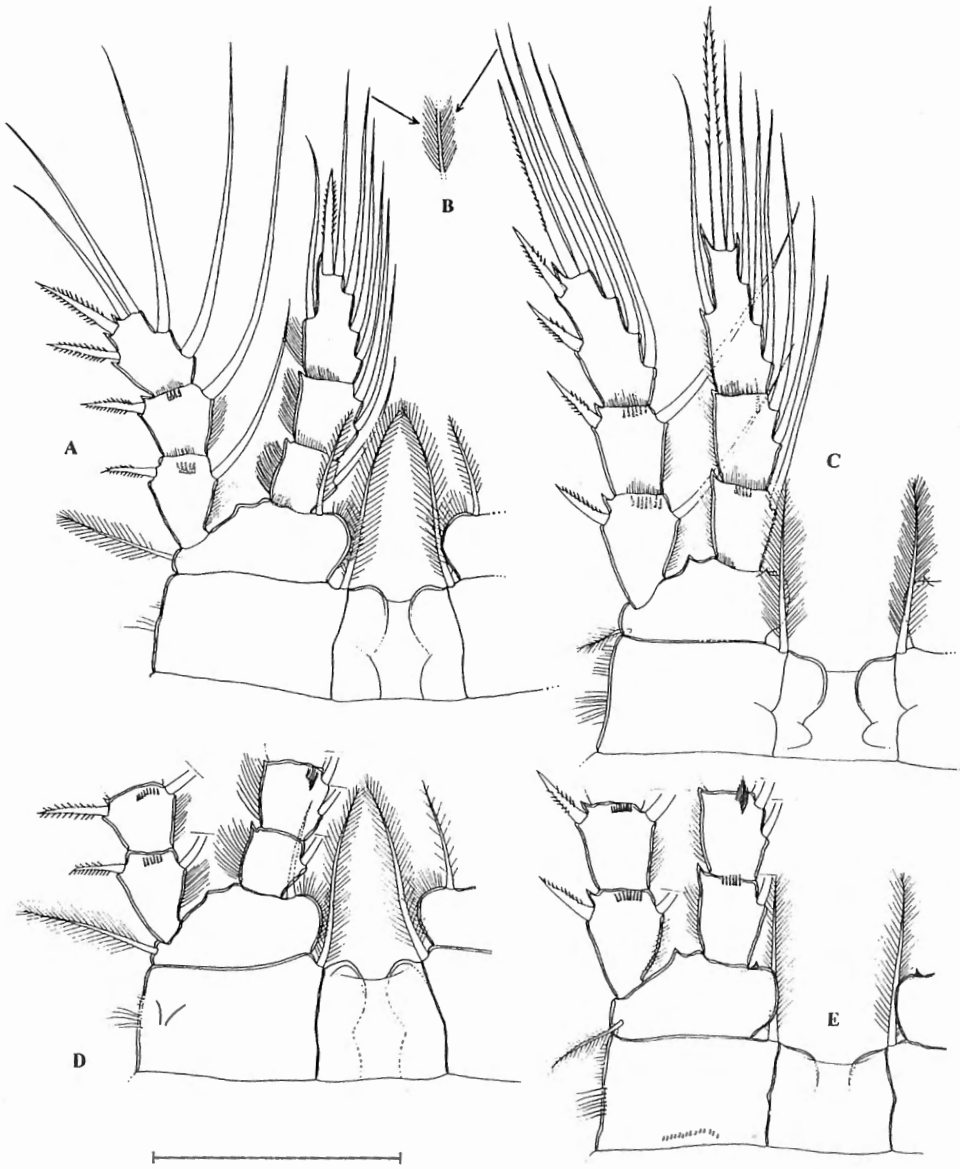

Fig. 3. – *Thoracopods.* – A, C : P_1-P_2 frontal side ; D, E : P_1-P_2 caudal side ; B : Ornamentation of setae of endo. and exopodites. Scale bars = 10 µm.

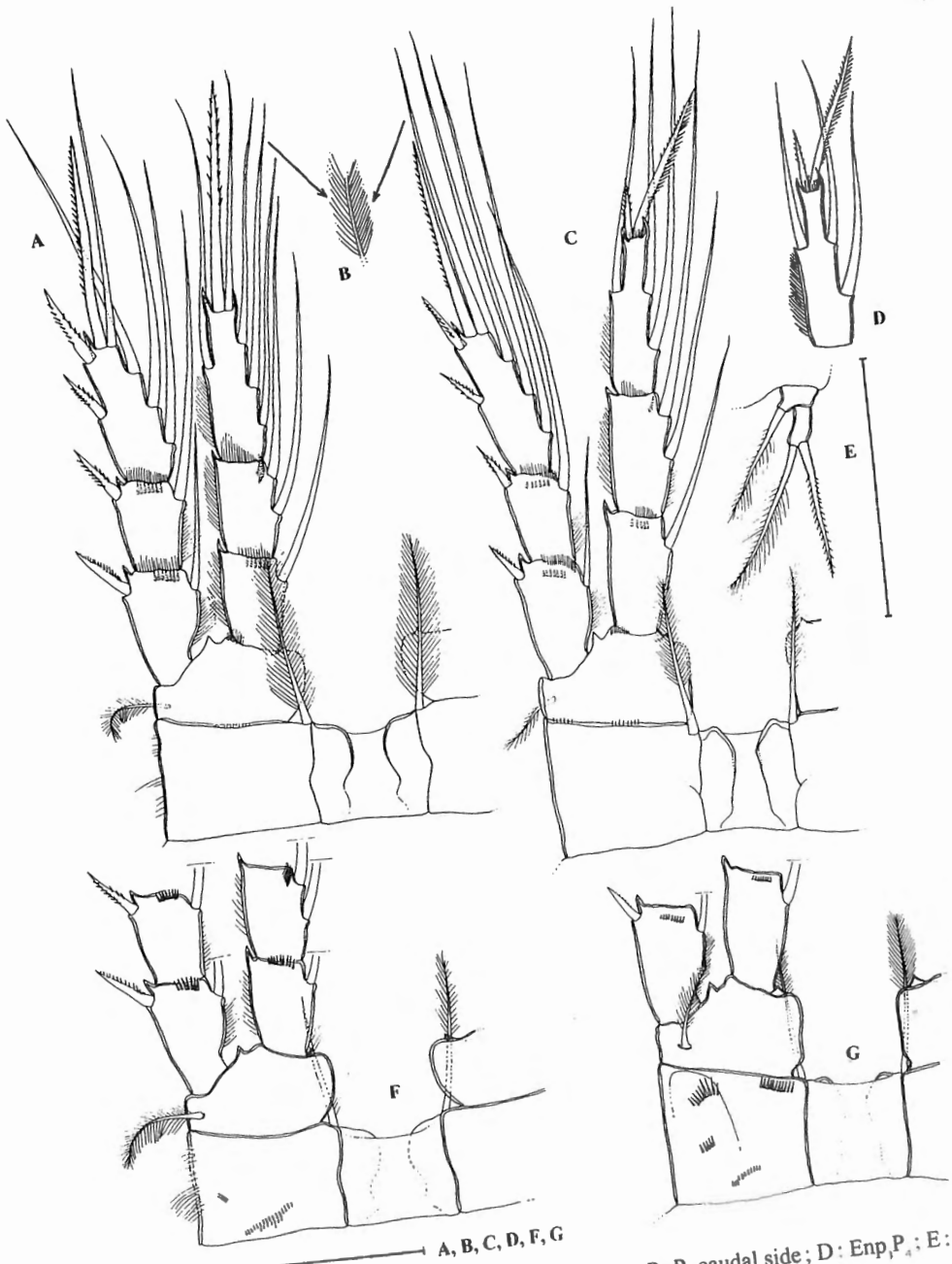

Fig. 4. – *Thoracopods.* – A, C : P_3-P_4 frontal side ; F, G : P_3-P_4 caudal side ; D : Enp,P_4 ; E : P5 ; B. – Ornamentation of setae of endo. and exopodites. Scale bars = 10 µm.

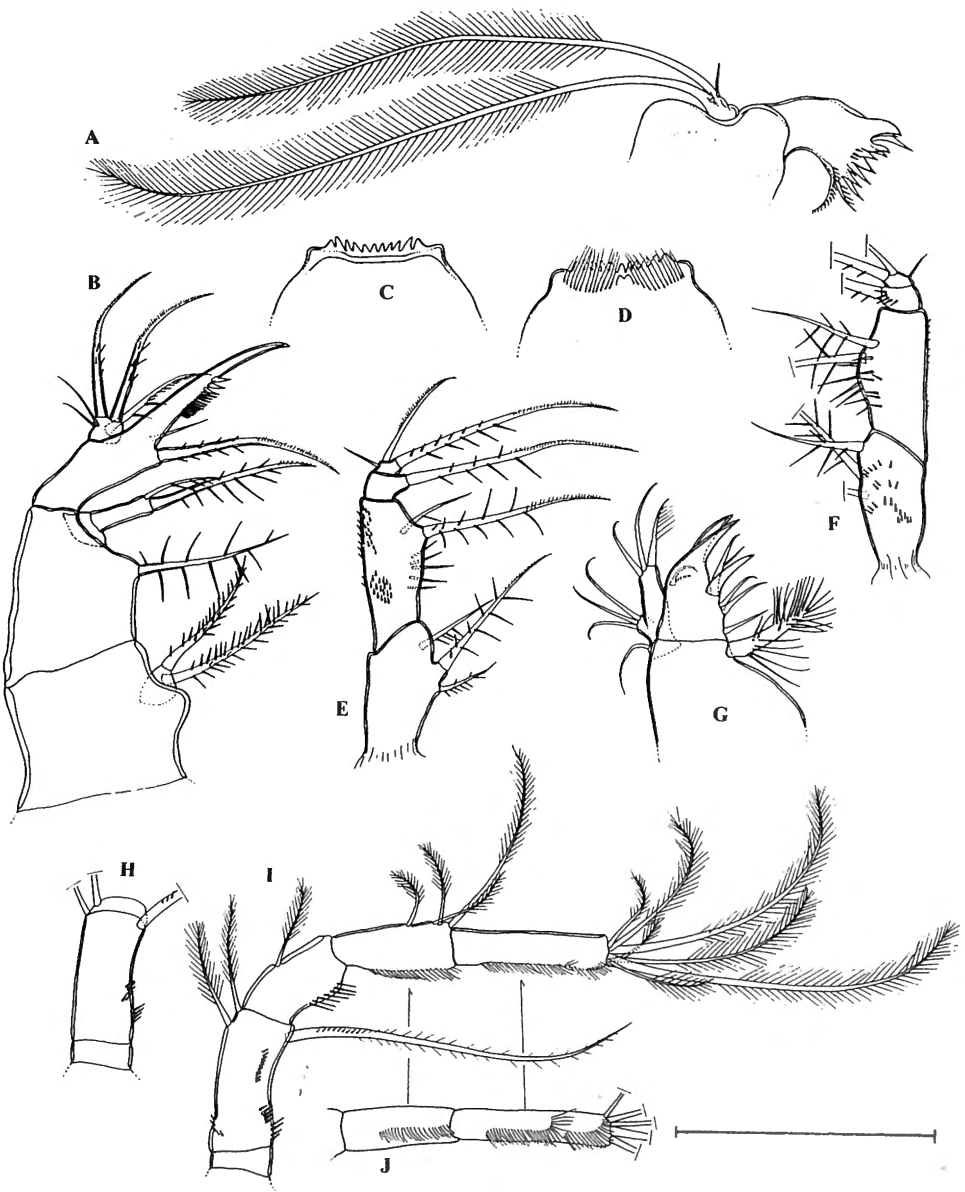

Fig. 5. – *Mouth parts and antenna.* – A : Mandible ; B : Maxilla ; C, D : Labrum (C : ventral, D : dorsal) ; E, F : Maxilliped ; G : Maxillule ; H : Basipodite A_2 (frontal side) ; I : Antenna (caudal side) ; J : $Enp_{2,3}$ Antenna (postero-ventral). Scale bars = 10 µm.

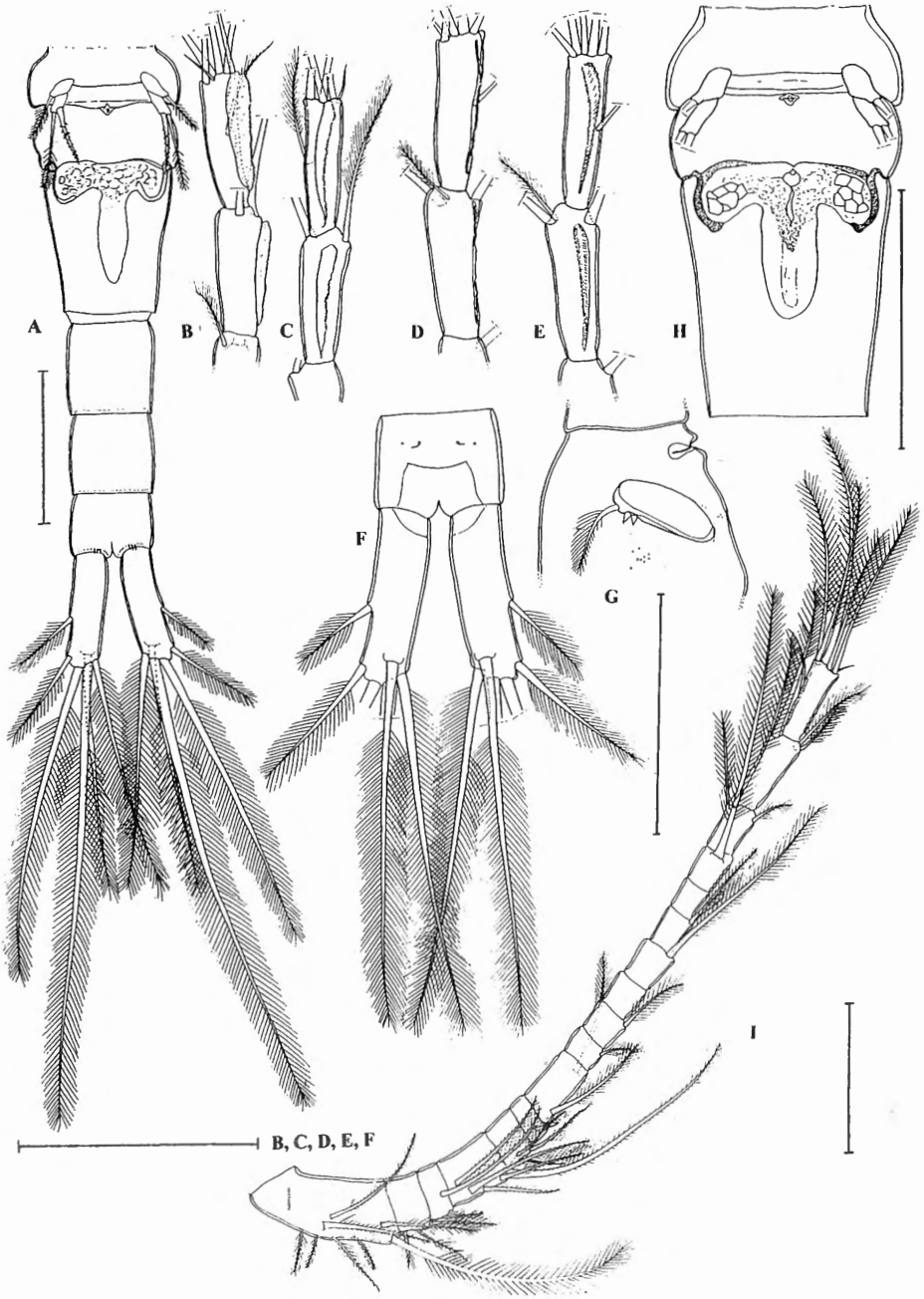

Fig. 6. – *Urosome, furca and antennule.* – A : Urosome and furca (ventral), B, C, D, E : Hyline lamella ; F : Anal segment and furca (dorsal side), G : Antennule. Scale bars = 10 µm.

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