

## Postembryonic development of nonmarine ostracod *Chlamydotheca arcuata* (Sars, 1901) (Crustacea: Ostracoda), reared in the laboratory

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**Abstract:** We describe here 8 instars (from A-8 through A-1) of the postembryonic development of the Cyprididae *Chlamydotheca arcuata* (Sars, 1901) from hatching to sexually mature individuals. To study the development of this species, cultures of adult parthenogenetic female specimens were established and maintained in the laboratory. The ostracod specimens were collected from temporary ponds and streams in Buenos Aires Province, Argentina. This report provides a description of the chaetotaxy of each limb along with detailed illustrations. Photographs of eggs and carapaces were taken using scanning electron microscopy. The complete development of *Chlamydotheca arcuata* lasted between 80 and 90 days. In the first instars, the active swimmers are characterized by carapaces that have a greater height in front of the first half of the maximum length and taper toward the rear end to become pointed. The appearance and numbers of the sensory setae on the carapace increase in the latest instars.

**Key words:** Nonmarine ostracods, *Chlamydotheca*, ontogeny, chaetotaxy

### 1. Introduction

The ontogeny of only 5 of the 8 extant superfamilies of the order Podocopida has been documented in detail, including the early and late instars of: Terrestrial Cytheroidea (Horne et al., 2004), Cytheroidea (Smith and Kamiya, 2003, 2005), Bairdioidea (Smith and Kamiya, 2002), Darwinuloidea (Smith and Kamiya, 2008), and Cypridoidea (reviewed by Smith and Martens, 2000; Kubanç et al., 2007).

Most cypridoidean ostracods pass through 8 consecutive larval stages, numbered sequentially from the last to the first instars as A-1 through A-8. The number indicates the number of molts needed to attain adulthood (Smith and Martens, 2000). Therefore, the A-1 instar corresponds to the last juvenile stage before adulthood (Aguilar-Alberola and Mesquita-Joanes, 2013). Nevertheless, Roessler (1982a, 1982b, 1998) found an earlier instar called the “prenauplius” or A-9 in some species of the family Cyprididae, and Aguilar-Alberola and Mesquita-Joanes (2013) and Rossi et al. (2015) described a prenauplius or postembryonic stage A-9 in *Heterocypris bosniaca* and *H. incongruens*, respectively. Usually, the ontogeny of the species of the family Cyprididae is similar, with specific limbs forming during the same instar stages.

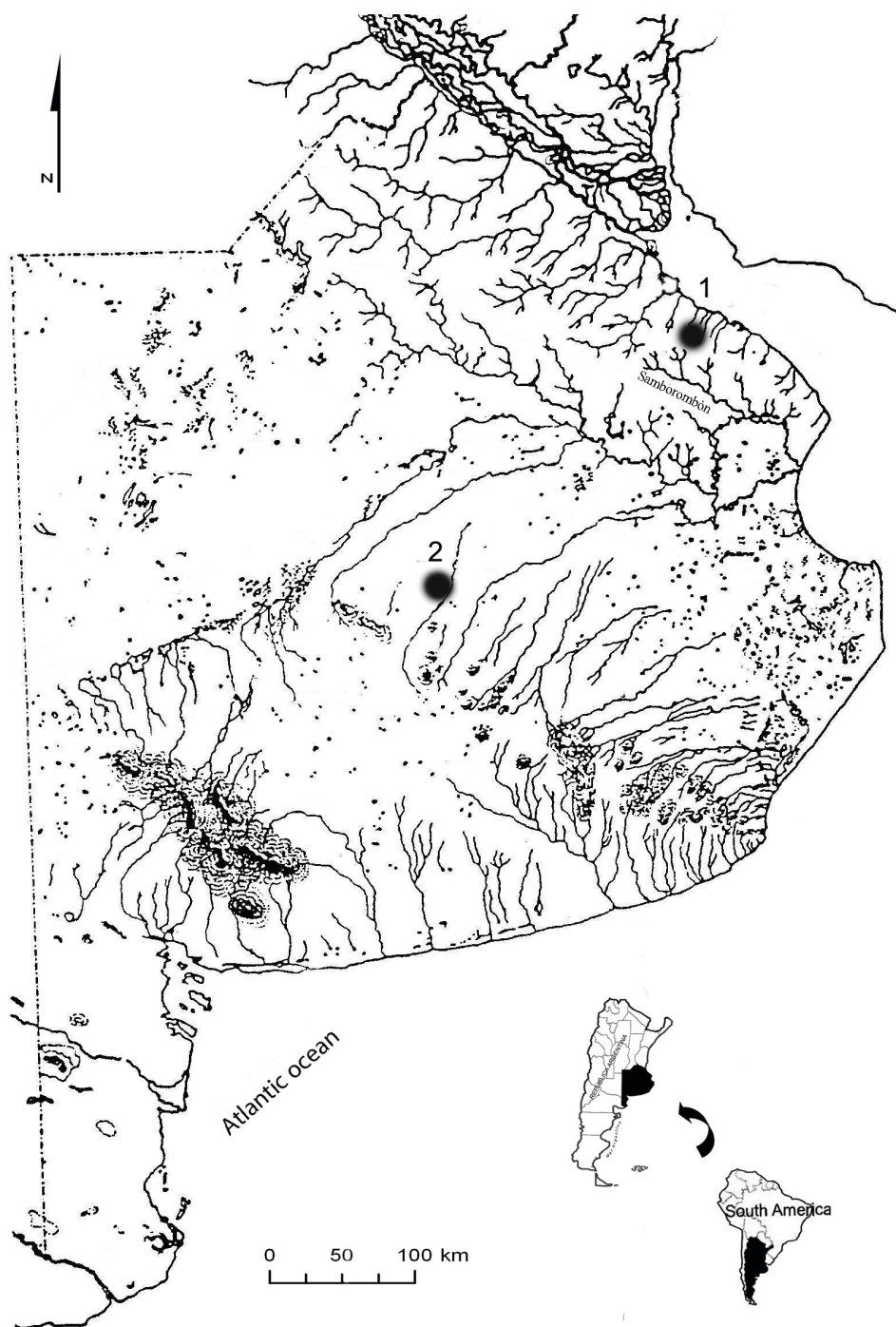
Among the Cyprididae, *Chlamydotheca* is the only genus for which the ontogeny is still poorly known.

In this report, we provide a description of the juvenile instars of *Chlamydotheca arcuata* (Sars, 1901), a species of the genus *Chlamydotheca* that is typically representative of the Neotropical ostracod fauna that inhabits lotic and lentic environments, including temporary and permanent ponds. *Chlamydotheca arcuata* was selected for the present study on ostracod ontogeny considering that this parthenogenetic species is large enough to allow each juvenile instar to be easily seen, handled, and dissected. The adult stage of *C. arcuata* was redescribed in a previous study about the distribution of the genus (Díaz and Lopretto, 2011). This work presents the ontogenical study of the carapace and limbs of the genus *Chlamydotheca* based on this species.

### 2. Materials and methods

Ostracod specimens were collected from temporary ponds in Buenos Aires Province (Argentina) (Figure 1) from October to December of 2007 and 2008. Samplings were performed on the 5th day of each month at Pereyra Iraola Park (34°50'S, 58°13'W) and in the La Matilde stream

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**Figure 1.** Sampling sites in Buenos Aires Province, Argentina. 1. Pereyra Iraola Park. 2. La Matilde stream.

(36°35'S, 59°39'W) on 10 November 2008, where 200 and 50 specimens were collected, respectively.

The material was collected using an enameled white spoon and a fine-meshed (0.25 mm) hand net. The spoon is an effective tool to collect samples because it enables an immediate recognition of the ostracods present in

shallow streams. For the identification of these nonmarine ostracods, adult females were placed in 70% alcohol for permanent storage. After immersion in polyvinyl-lactophenol, the specimens were dissected under a Zeiss standard stereo microscope. Limb morphology was studied under light microscope and line drawings

were made using a camera lucida. Scanning electron microscope (SEM) photographs of the valves were taken using a JEOL JSM 6360 LV scanning electron microscope at the Faculty of Natural Sciences and Museum (FCNyM, La Plata, Argentina).

Cultures were established in the laboratory from specimens collected in the field. The culture medium was prepared in an aquarium (3 L) with filtered and previously boiled water from the ponds where ostracods specimens were collected. The aquarium was equipped with Atman HF 0600 aerators. The culture medium was kept at room temperature (20 to 23 °C) and neutral pH with a photoperiod of 16:8 h of light:darkness. It was periodically supplemented with 2 g of commercial canned water-packed tuna. Each adult female was isolated in a culture plate (5 mL) containing the medium and they were fixed after they laid eggs for taxonomical classification. In order to follow individuals grown in culture chambers, larvae and juveniles were each placed in 24-well culture plates (2 mL), previously disinfected with 10% NaClO in order to prevent microbial activity and rinsed with distilled water. Each single culture plate well was monitored every day and the water in each well was renewed when necessary at the same time depending on the amount of feces and food waste present. The procedure consisted of the daily monitoring of each juvenile to observe survival and molting events. Valves and carapaces were collected to obtain information about the size and molting time of each instar. The egg cases were also studied using a SEM to examine the ornamentation patterns of each specimen. The samples are deposited in the Carcinological Collection of the Museum of La Plata, La Plata, Argentina under the indicated MLP numbers.

The chaetotaxy of the limbs mostly follows Broodbakker and Danielopol (1982). For the second antenna, we used the revised model proposed by Martens (1987), and for the second and third thoracopods we adopted Meisch's nomenclature (2000). Taxonomy classification of Ostracoda follows Horne et al. (2002).

The abbreviations used in the text, tables, and figures are as follows: Cp, carapace; H, height of the valves; L, length of the valves; W, width of the carapace; LV, left valve; RV, right valve; IV, internal view; EV, external view; An, anlagen; An1, antennula; An2, antenna; Md, mandible; Rlo, rake-like organ; Mx, maxillula; Prmx, respiratory plate of Mx; T1, first thoracopod; T2, second thoracopod; T3, third thoracopod; CR, caudal ramus; CRa, attachment of the caudal ramus; Db, dorsal branch; Vb, ventral branch.

Measurements of valves and carapaces are expressed in µm along with the numbers of the individuals, the maximum and minimum values, and the arithmetic means ± SD in parentheses.

### 3. Results

Class Ostracoda Latreille, 1806

Subclass Podocopa G.W. Müller, 1894

Order Podocopida G.O. Sars, 1866

Superfamily Cypridoidea Baird, 1845

Family Cyprididae Baird, 1845

*Chlamydotheca* Saussure, 1858

*Chlamydotheca arcuata* (Sars, 1901) (Figures 2A–2L, 3A–3G, 4A–4F, 5A–5G, 6A–6D, 7A–7G, 8A and 8B, 9A–9I, 10A–10F, 11A–11H)

Along with the adults that were cultured for 2 to 5 days after being isolated, 3 to 5 eggs were found and gathered from the floor of the aquarium; these hatched asynchronously after incubation in culture media. All single eggs were covered with a jelly-like substance; in the absence of this substance, the surface appeared covered with small, regularly spaced tubercles (Figures 2A–2C). Some of the eggs were attached to the internal surface of the valves (Figure 2D).

#### 3.1. Description of first instar (A-8; n = 10)

Cp: L 133–180; H 100–130. Anterior margin of carapace acuminate, posterior margin rounded. Surface of Cp with small puncta (Figure 2E).

An1 (Figure 4A). With 5 segments. Second segment with 1 ventral seta. Third segment with 2 long subequal dorsal setae and 1 short ventroapical seta. Terminal segment with 2 long setae, 1 ventroapical short seta, and 1 aesthetasc ( $y_a$ ).

An2 (Figure 4B). Biramous with a protopod, endopod, and small exopod. Protopod with 1 subapical seta. The exopod is on the apical outer face of the protopod consisting of 1 seta. The endopod has 3 segments. First segment with 1 long subapical natatory and 1 short seta (anlage of aesthetasc Y) and with 1 long ventral seta. Second segment with 1 long  $G_3$  seta and 1 short  $G_1$  claw-like seta. Terminal segment with 2 large  $G_M$ ,  $G_m$  claw-like setae, 1 smooth g-seta, and 1 aesthetasc ( $y_3$ ).

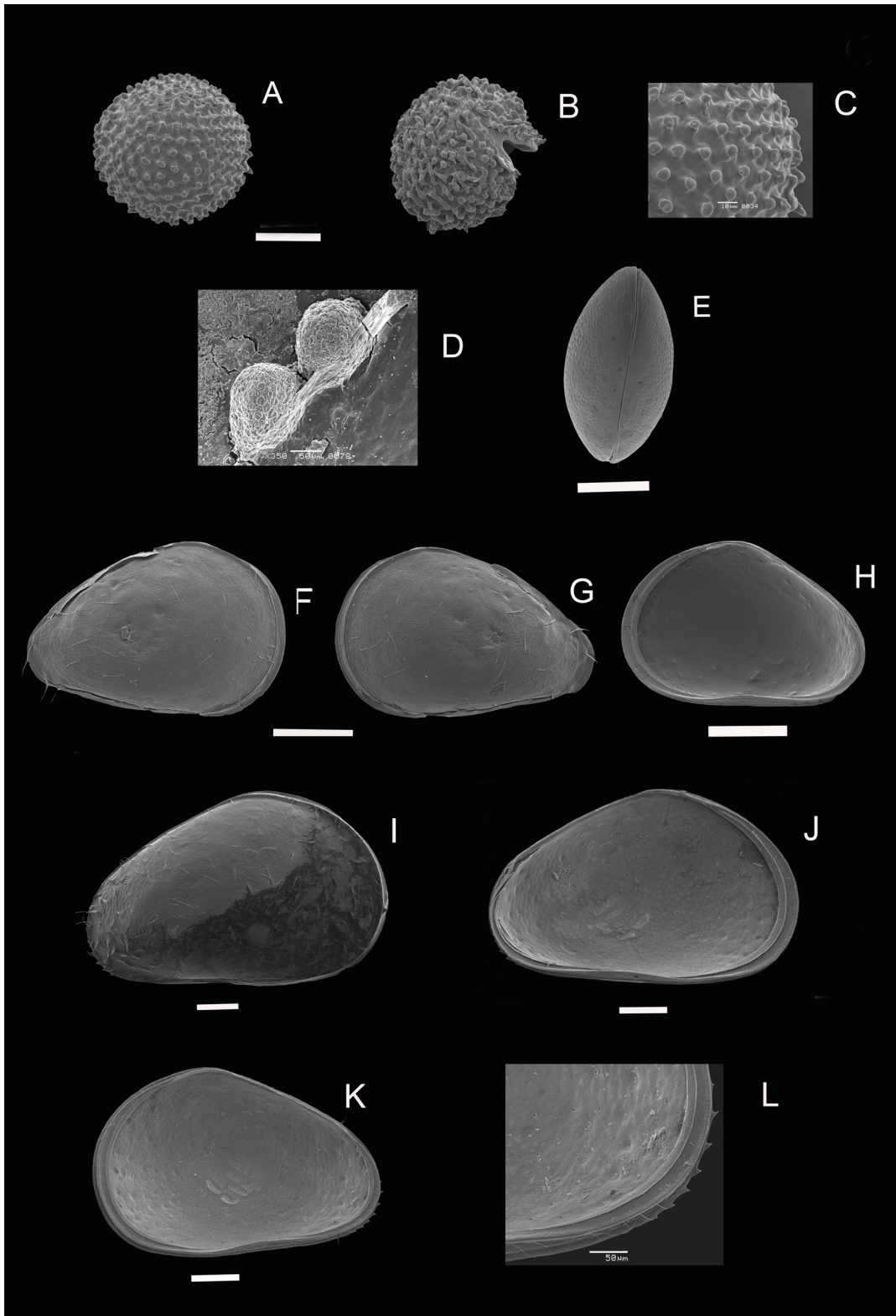
Md (Figure 4C). Points backwards and attached to the body just above and to the side of the mouth region. Consists of 3 segments. Terminal segment with a long curved seta and 2 small setae.

#### 3.2. Description of second instar (A-7; n = 6)

RV: L 216–290; H 161–280; LV: L 227–300; H 230–250. The maximum H in the anterior part of valve. Anterior margin rounded and acuminate at the posterior margin. Valves subequal, the RV overlaps the LV dorsally. Surface rough with numerous long setae in the posterior margin (Figures 2F and 2G).

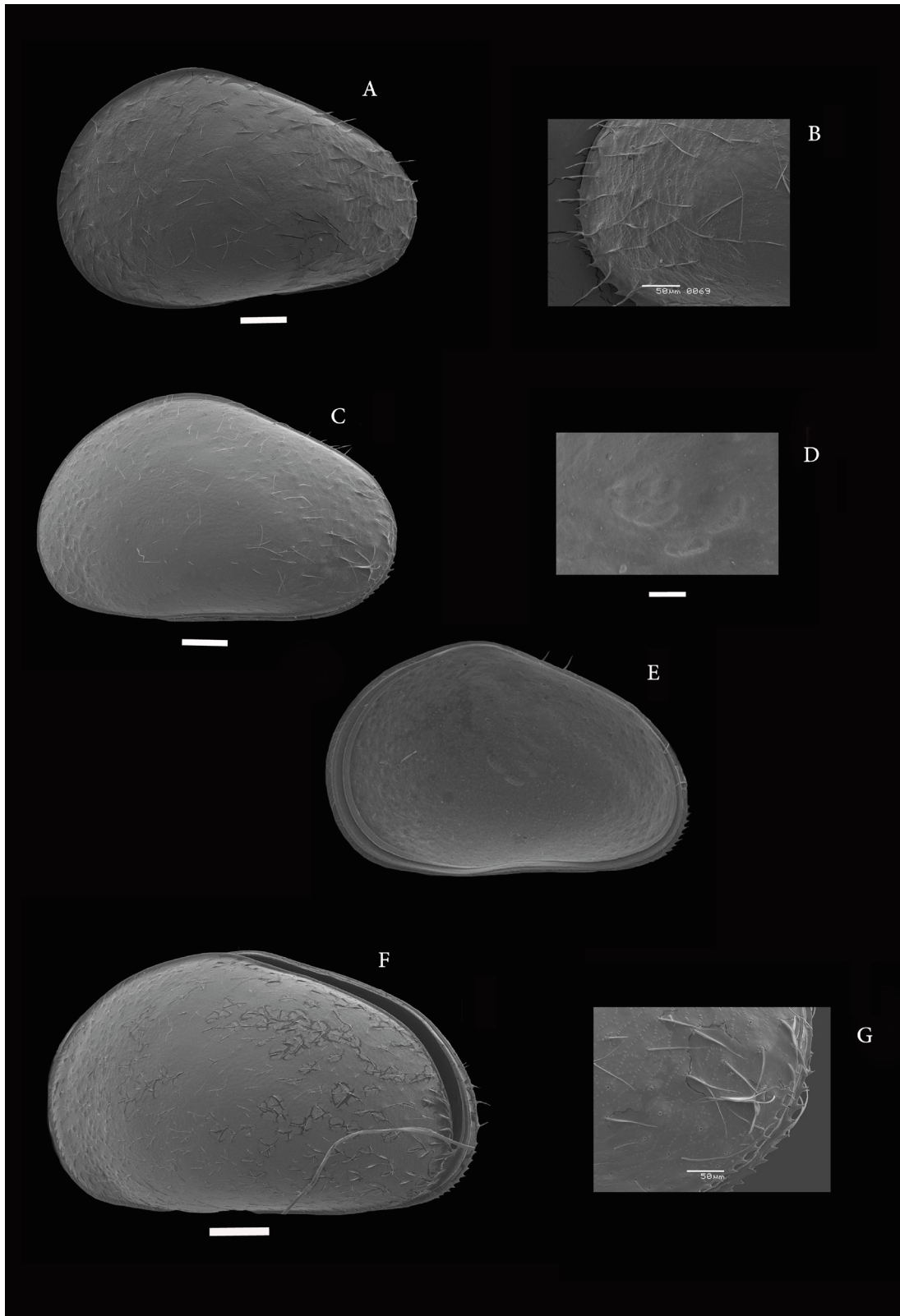
An1. Similar to A-8 instar, with setae proportionally longer than in the previous instar.

An2 (Figure 4D). First segment of protopod with 1 long subapical ventral seta. Second segment same as the previous instar. The exopod consists of 1 long seta and 1

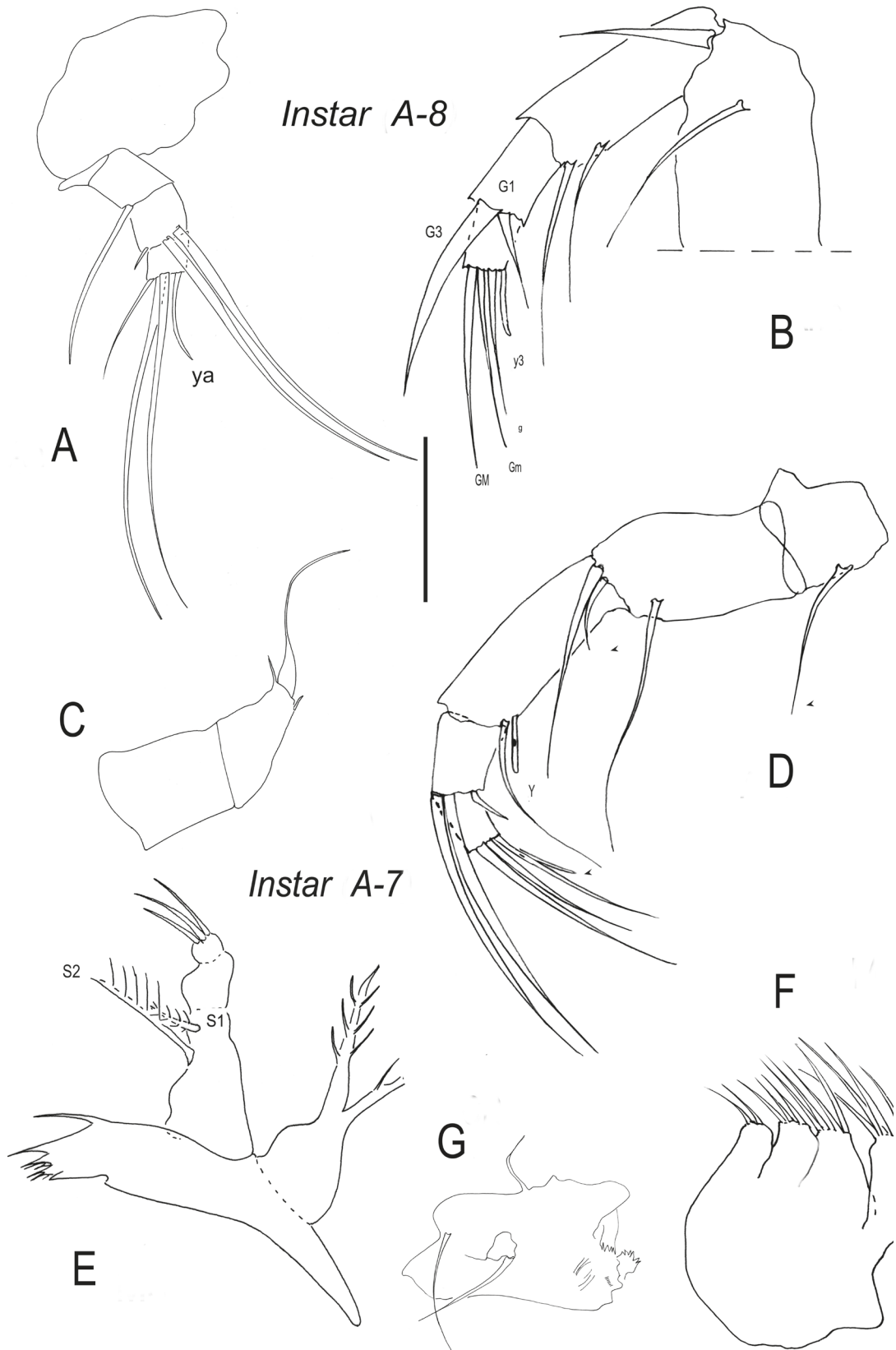


**Figure 2.** *Chlamydotheca arcuata* (Sars, 1901). A–D, SEM photographs of eggs (MLP 26771). A, B, eggs. C, detail of egg. D, eggs attached to the internal surface of carapace. E, instar A-8 (MLP 26771), Cp, DV. F, G, instar A-7 (MLP 26772). F, RV, EV; G, LV, EV. H, instar A-6 (MLP 26773), RV, IV. I–L, instar A-5 (MLP 26774). I, RV, EV; J, LV, IV; K, RV, IV; L, detail of posterior part of RV. Scale bars: 50 µm for A, B, E, F, G; 100 µm for H, I, J, K.





**Figure 3.** *Chlamydotheca arcuata* (Sars, 1901). A, B, instar A-4 (MLP 26775). A, LV, EV; B, detail of posterior part of LV. C–E, instar A-3 (MLP 26776). C, LV, external view; D, detail of muscle scars; E, LV, IV. F, G, instar A-2 (MLP 26777). F, LV, EV; G, detail of posterior part of LV. Scale bars: 100 µm for A, C, 200 µm for E, F.



**Figure 4.** *Chlamydotheca arcuata* (Sars, 1901). A–C, instar A-8 (MLP 26771). A, An1; B, An2; C, Md. D–G, instar A-7 (MLP 26772). D, An2; E, Md; F, Mx; G, CR. Scale bar: 100  $\mu$ m. Arrows indicate first appearance of a feature.

short seta. The aesthetasc (Y) is clearly visible in subapical position. Claws of the second segment of endopod proportionally longer than in the A-8 instar, this segment with the addition of 1 short  $G_2$  ventral seta. Last segment same as the A-8 instar.

Md (Figure 4E). Developed into a feeding appendage consisting of a coxa, a palp, and a respiratory plate (exopod). Coxa pointed dorsally and slightly raked backward. Coxa with 5 teeth, 1 subapical dorsal seta, and intervening setae protruding on either side towards the mouth. First segment of the palp with 2 apical ventral  $S_1$  and  $S_2$  setae. Second segment without setae. Last segment with 3 apical setae. Exopod consists of 2 rays.

Rlo. Appears for first time in the anterior region of the hypostome with 5 or 6 rounded teeth.

Mx (Figure 4F). Appears for first time, consists of a stout palp and 3 endites. First and second endites with 2 and 3 apical setae, respectively. Third endite with 4 apical setae. Palp with 4 subequal apical setae.

Pediform anlage of CR (Figure 4G) consisting of 1 segment with a long terminal curved seta.

### 3.3. Description of third instar (A-6; n = 3)

Cp: L 314–370; H 214. The maximum H displaced behind the middle. Outline subtriangular. Anterior margin rounded, posterior margin more pointed than in previous instars. Dorsal margin straight. Ventral margin concave. Duplicature well developed in the anterior part. Numerous normal pores with small setae (Figure 2H).

An1 (Figure 5A). Similar to A-7 instar. First segment lost. Second segment with an additional long seta. Third segment with long setae and proportionally longer. Fourth segment with an additional long ventral seta.

An2 (Figure 5B). Exopod with 1 short additional seta. Aesthetasc (Y) slightly shifted in position towards the protopod. The first segment of the endopod with 2 additional long natatory setae. Terminal segment remains unchanged, but aesthetasc  $y_3$  is now bifurcated.

Md (Figure 5C). Slightly larger compared to previous instars. Coxa with cusped teeth. Palp with 3 recognizable segments. First segment with 1 additional plumose  $\beta$ -seta, 2 long smooth subapical ventral setae, and 1 dorsoapical seta. Second segment with 2 ventral setae and 1  $\gamma$ -seta with a swollen base. Terminal segment with 1 additional claw-like seta.

Rlo (Figure 5D). With 9 teeth.

Mx (Figure 5E). The palp has 2 segments. First segment with 2 dorsoapical setae. Respiratory plate with 13 rays.

T1 (Figure 5F). Anlage of T1 protrudes from behind the base of Mx, consists of 1 segment.

CR (Figure 5G). Same as in previous A-7 instar.

### 3.4. Description of fourth instar (A-5; n = 2)

RV: L 450–650; H 485–510. LV: L 500–714; H 550. Anterior margin of RV with 1 spine. Posterior margin with

a row of small spines on the ventral side. Ornamentation most strongly developed, consisting of a gentle reticulate pattern and numerous setae. Muscle scars long and strongly marked, situated in the middle part of the valve above the maximum L (Figures 2I–2K). Internal margins of both valves with a row of small spines (Figure 2L).

An1 (Figure 6A). Second segment with an additional short dorsoapical seta.

An2 (Figure 6B). Claws of the second segment of endopod are proportionally longer than in the previous instar. Second segment of endopod with the addition of 1 short ventroapical seta. Terminal segment remainder unchanged.

Md and Rlo. Same as in the A-6 instar.

Mx (Figure 6C). Third endite with the addition of 1 plumose apical seta. One of the apical seta of the first segment of palp appears plumose.

T1 not obtained.

T2 (Figure 6D). Consist of 3 segments. Terminal segment with 1 long curved serrated claw and 2 short setae.

CR. Same as in the previous instar.

### 3.5. Description of fifth instar (A-4; n = 3)

Cp: L 785–800; H 514–600. The maximum H in the middle L. Ventral side more convex than in the A-5 instar but not too high. Surface of carapace with a delicate reticulation in the posterior margin. Normal pores more numerous than in the A-5 instar (Figures 3A and 3B).

An1 (Figure 7A). First segment of endopod with a long dorsal seta.

An2 (Figure 7B). Same as in the A-5 instar.

Md (Figure 7C). Coxa with 2 additional plumose ventroapical setae. Palp with 4 recognizable segments. First segment with 2 plumose  $S_1$  and  $S_2$  setae. Second segment with 3 subequal dorsoapical setae and with 1 plumose seta and 1 smooth seta ventrally. Third segment of palp with 2 unequal smooth setae and 2 subequal setae dorsally inserted.  $\gamma$ -Seta smooth. Last segment with 4 unequal apical setae.

Rlo. Same as in the A-5 instar.

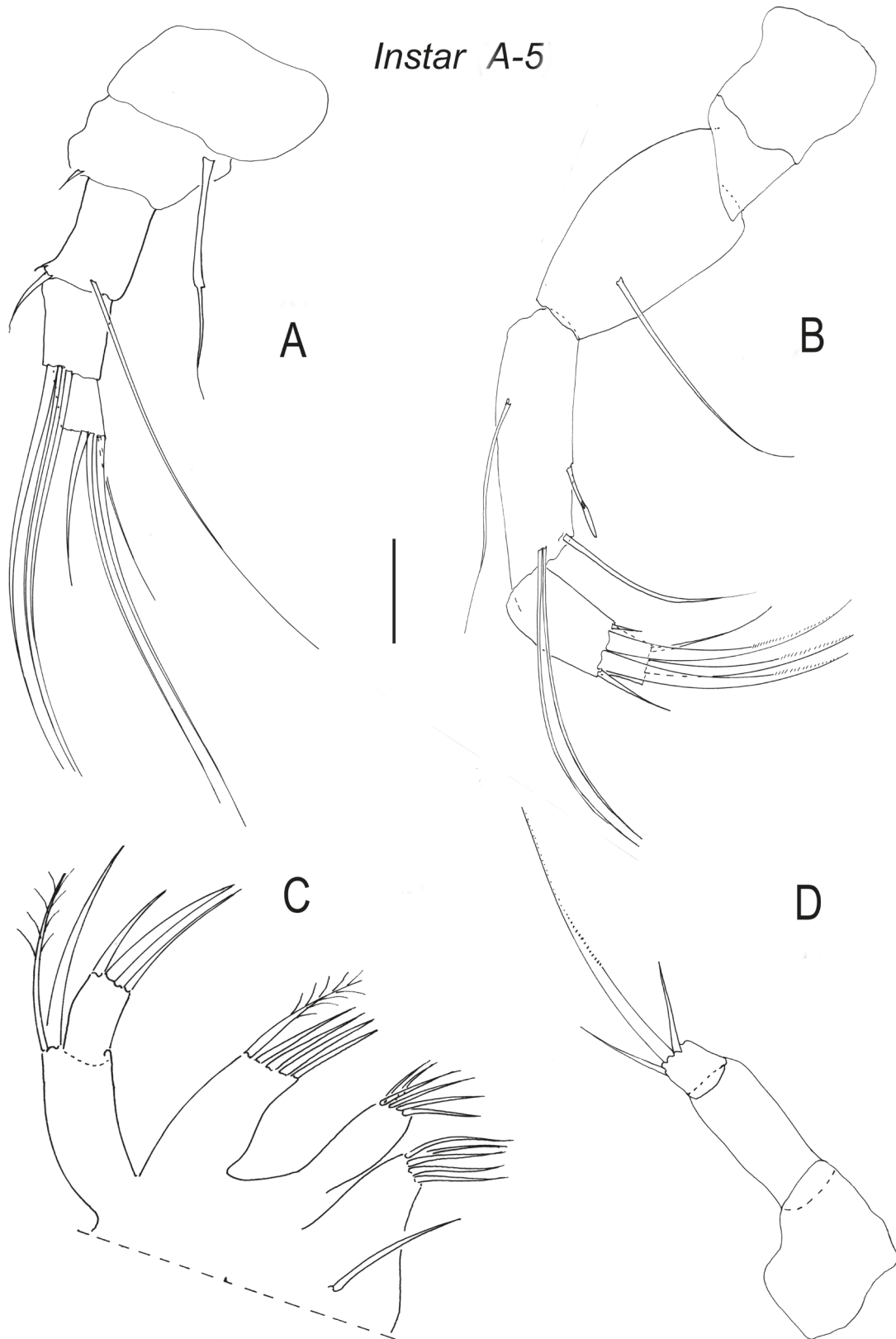
Mx (Figure 7D). Third endite with 4 dorsoapical setae and 2 apical setae now transformed into claw-like Zahnborsten setae.

T1 (Figure 7E). Masticatory process with 4 smooth subapical and 5 smooth apical setae.

T2 (Figure 7F). First segment of endopod with 1 ventral seta and second segment with 1 ventral apical seta. Last segment is unchanged from the A-5 instar.

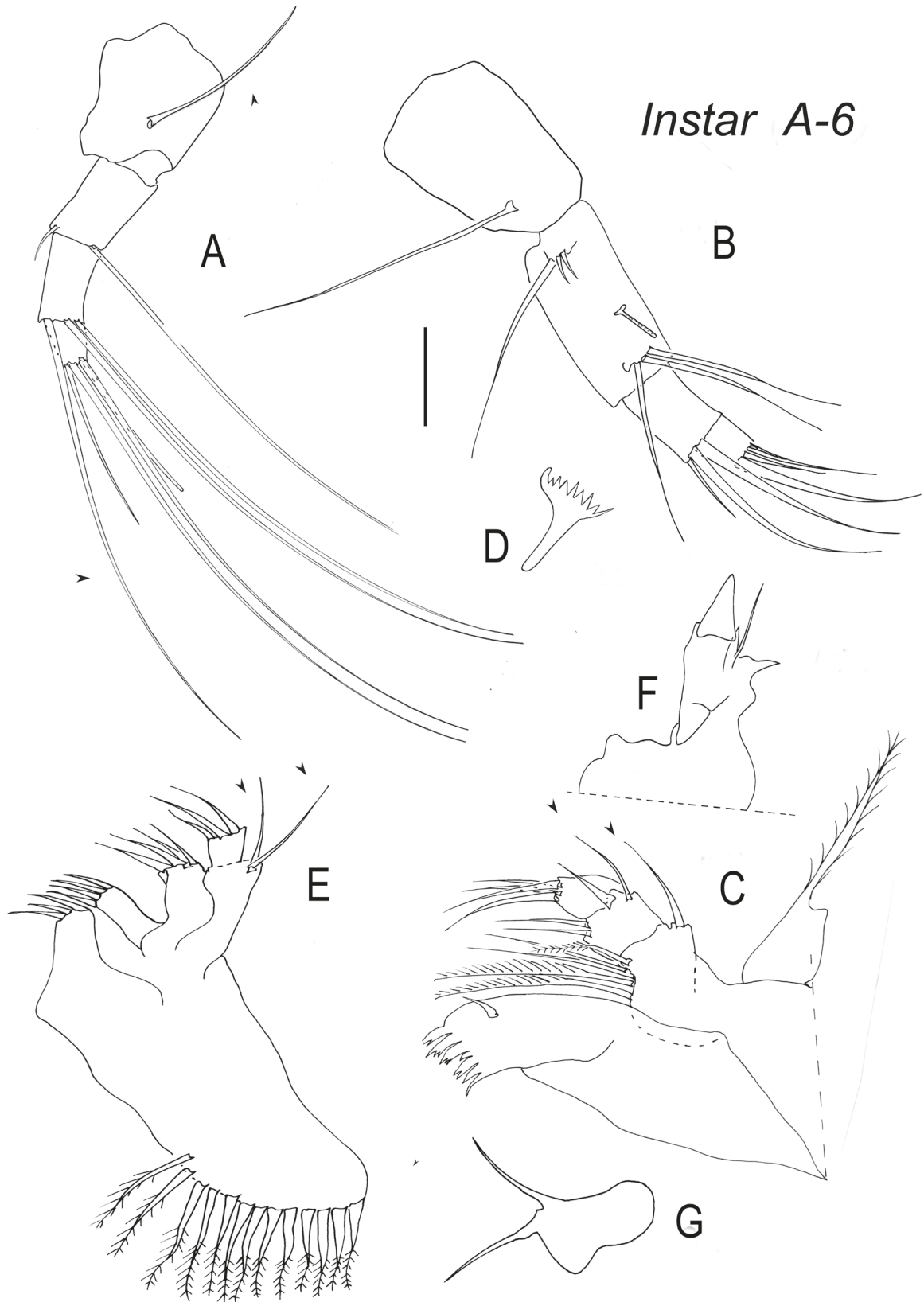
T3 (Figure 7F). Anlage consists of 1 segment.

CR (Figure 7G). With 1 posterior seta (sp) and 2 subequal claws (Ga, Gp). The CR looks relatively lightly sclerotized and hence may not be functional in this instar; this is corroborated by the absence of any supporting attachment of the endoskeleton.

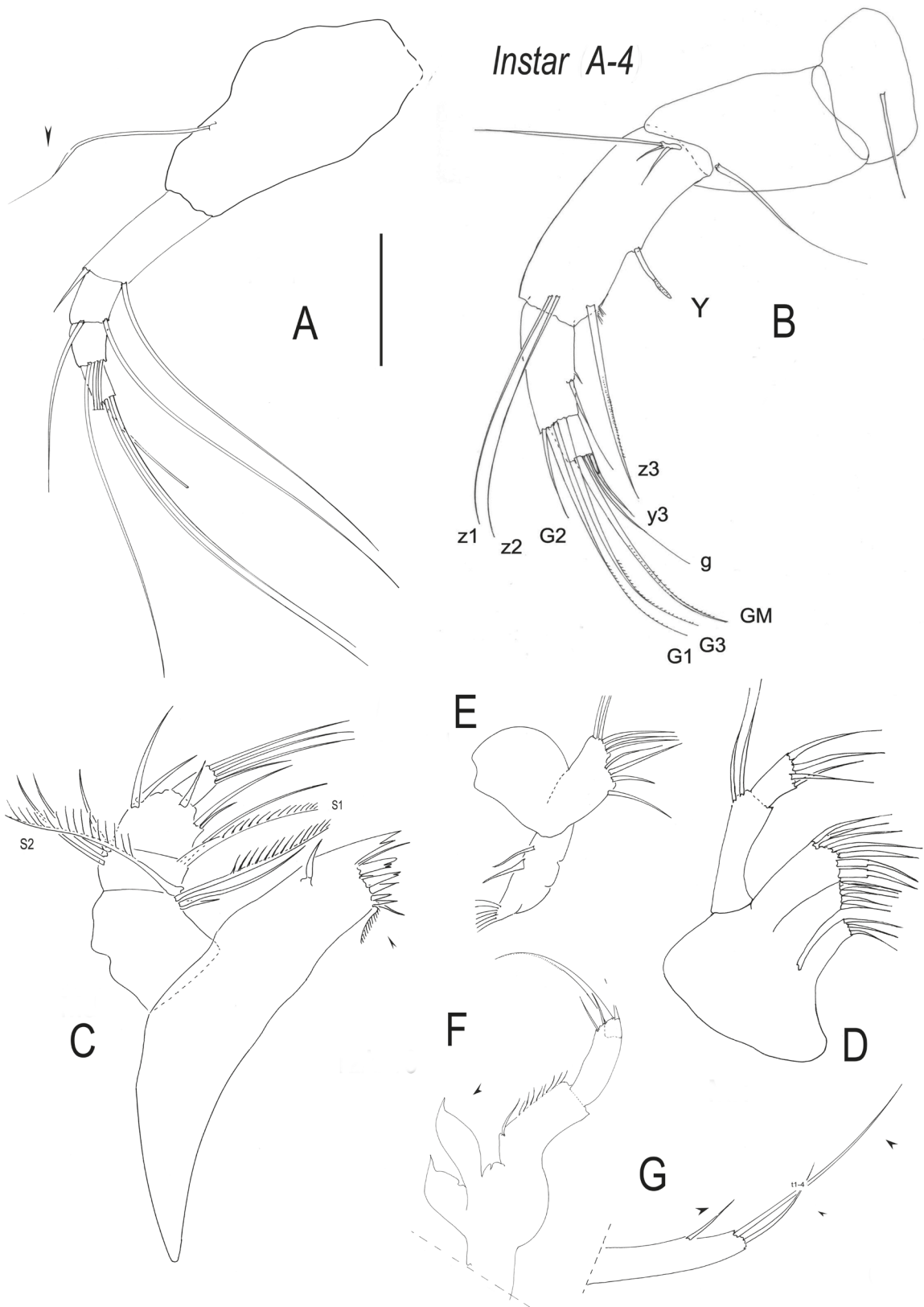


**Figure 5.** *Chlamydotheca arcuata* (Sars, 1901). Instar A-6 (MLP 26773). A, An1; B, An2; C, Md; D, Rlo; E, Mx; F, AnT1; G, CR. Scale bar: 100  $\mu$ m. Arrows indicate first appearance of a feature.





**Figure 6.** *Chlamydotheca arcuata* (Sars, 1901). Instar A-5 (MLP26774). A, An1; B, An2; C, Mx; D, T2. Scale bar: 100  $\mu$ m. Arrows indicate first appearance of a feature.



**Figure 7.** *Chlamydotheca arcuata* (Sars, 1901). Instar A-4 (MLP 26775). A, An1; B, An2; C, Md; D, Mx; E, T1; F, T2A-T3; G, CR. Scale bar: 100  $\mu$ m. Arrows indicate first appearance of a feature.

### 3.6. Description of sixth instar (A-3; n = 4)

Cp: L 900–1003; H 670–770. Surface of carapace less reticulate than in the previous instars with additional pustules in the anterior margin (Figures 3C and 3E). Muscle scars consist of a group of 6, the ventral scars longer than the others (Figures 3D and 3E).

An1 (Figure 8A). With 6 segments that result from the fourth segment splitting into 2 smaller quadrate segments. First segment of endopod large, broad, and subrectangular in shape and supporting 2 plumose subapical setae on its dorsal edge; the ventral seta appears plumose. Second segment of endopod short with ventral seta plumose. Third segment of endopod as an elongated rectangle and with a length approximately twice the width, supporting a long ventral seta and a short dorsal seta apically. Fourth segment of endopod subquadrate bears a long ventral seta and 1 short dorsal seta. Fifth segment of endopod with 2 long ventral setae and 1 short dorsal seta. Last segment as A-4 instar.

An2 (Figure 8B). First segment of protopod with 3 additional short ventral setae. The setae of the second segment of protopod appear plumose. First segment of endopod with 2 additional long setae and 1 short natatory seta (a total of 4+1). Second segment of endopod with 2 additional mediodorsal setae. Ventrally are 4 long t-setae and apically the aesthetasc ( $y_2$ ); claws ( $G_1$ ,  $G_3$ ) become bigger than in the previous instars. Last segment with claw GM, Gm well developed and serrated, seta g, and aesthetasc  $y_3$ .

Md (Figure 9A). Coxa with 6 teeth. Two pectinate setae appear between the first and second teeth. Third segment of palp with 2 smooth ventral setae and 4 smooth subapical dorsal setae. Last segment with 3 claw-like setae and 4 smooth setae. Exopod with 7 rays.

Rlo. Same as in the A-4 instar.

Mx (Figure 9B). Third endite with the addition of 1 plumose subapical seta and 2 bipectinate apical setae. The short apical seta present in previous instars now is subapical and plumose. First segment of palp with 1 additional apical seta and 4 subapical setae. Second segment of palp with 1 additional smooth apical seta. Respiratory plate (Figure 9C) with 28 rays.

T1 (Figure 9D). Protopod with 2 additional plumose setae (d, b). Masticatory process with 5 additional setae. All setae appear plumose. Endopod with 3 setae ( $h_1$ ,  $h_2$ ,  $h_3$ ). Exopod with 5 rays.

T2 (Figure 9E). Second segment of protopod with 1 additional plumose subapical ventral seta. First segment of endopod stout with 2 additional subapical setae. Second segment with 1 ventroapical seta. Third segment with 1 additional subapical ventral seta. The claw of the last segment present in the A-4 instar with a row of small teeth.

T3 (Figures 9F and 9G). Developed into a cleaning limb, consisting of 3 elongated segments. First segment

with 1 plumose subapical ventral seta and 2 subapical plumose setae. Second segment with 1 subapical plumose ventral seta. Third segment capped in a fully developed cleaning pincer organ as seen in adult specimens.

CR (Figure 9H). Proportionally larger than in the A-4 instar and finely denticulate. Posterior (sp) seta and both claws (Ga, Gp) appear plumose. An additional anterior seta (sa).

CRA (Figure 9I). Appears in this instar as a slender and bifurcating attachment.

### 3.7. Description of seventh instar (A-2; n = 2)

Cp: L 1300–1500; H 900–960. Anterior margin of carapace with a fine flange. Posterior margin with a rhomboid pattern of ornamentation, also with many pores and delicate setae (Figure 3F). Posterior margin of both valves with delicate denticles (Figure 3G). Muscle scars in a central position.

An1 (Figure 10A). With 7 segments. Second segment of endopod with 1 additional short ventral seta; third segment with 1 ventroapical seta and 1 short dorsoapical seta; fourth segment with 2 long subequal ventral setae and 2 unequal dorsal setae, the shorter plumose; fifth segment with 2 long ventral and 2 long dorsal setae. Fourth segment of endopod also with 4 long setae and 1 short natatory seta. Last segment is the same as in the A-3 instar.

An2 (Figure 10B). Proportionally longer than in previous instars with 1 additional long natatory seta (5+1), with the chaetotaxy in the rest of the segments remaining unchanged.

Md (Figure 10C). Dorsal setae of coxa appear plumose. Third segment of palp with  $\gamma$ -seta larger than in A-3 instar. One of the claw-like setae of the last segment appears serrated.

Rlo not obtained.

Mx (Figure 10D). First endite with 2 plumose setae at the base. Third endite with 1 additional smooth seta. First segment of palp with 1 medial seta, second segment with an additional smooth seta.

T1 (Figure 10E). Similar to A-3 instar, but 4 of the apical setae of the masticatory process appear serrated. Seta (b) present.

T2 (Figure 10F). Same as in A-3 instar.

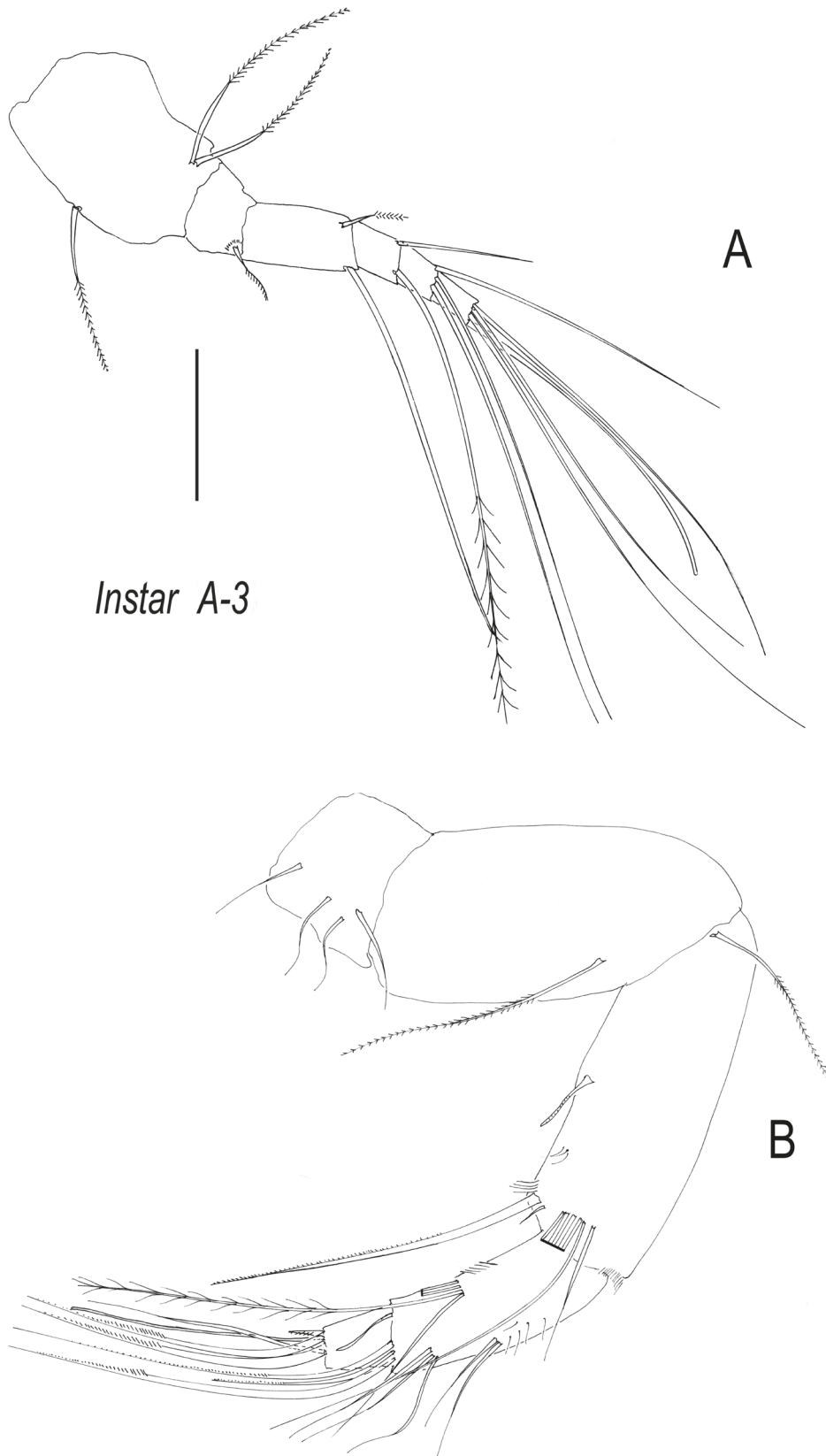
T3 (Figures 11A and 11B), CR (Figure 11C), and CRA (Figure 11D). Same as in A-3 instar.

### 3.8. Description of eighth instar (A-1; n = 2)

LV: L 1650; H 980. No photographs were obtained for valves in this instar because they broke up during the mounting procedure.

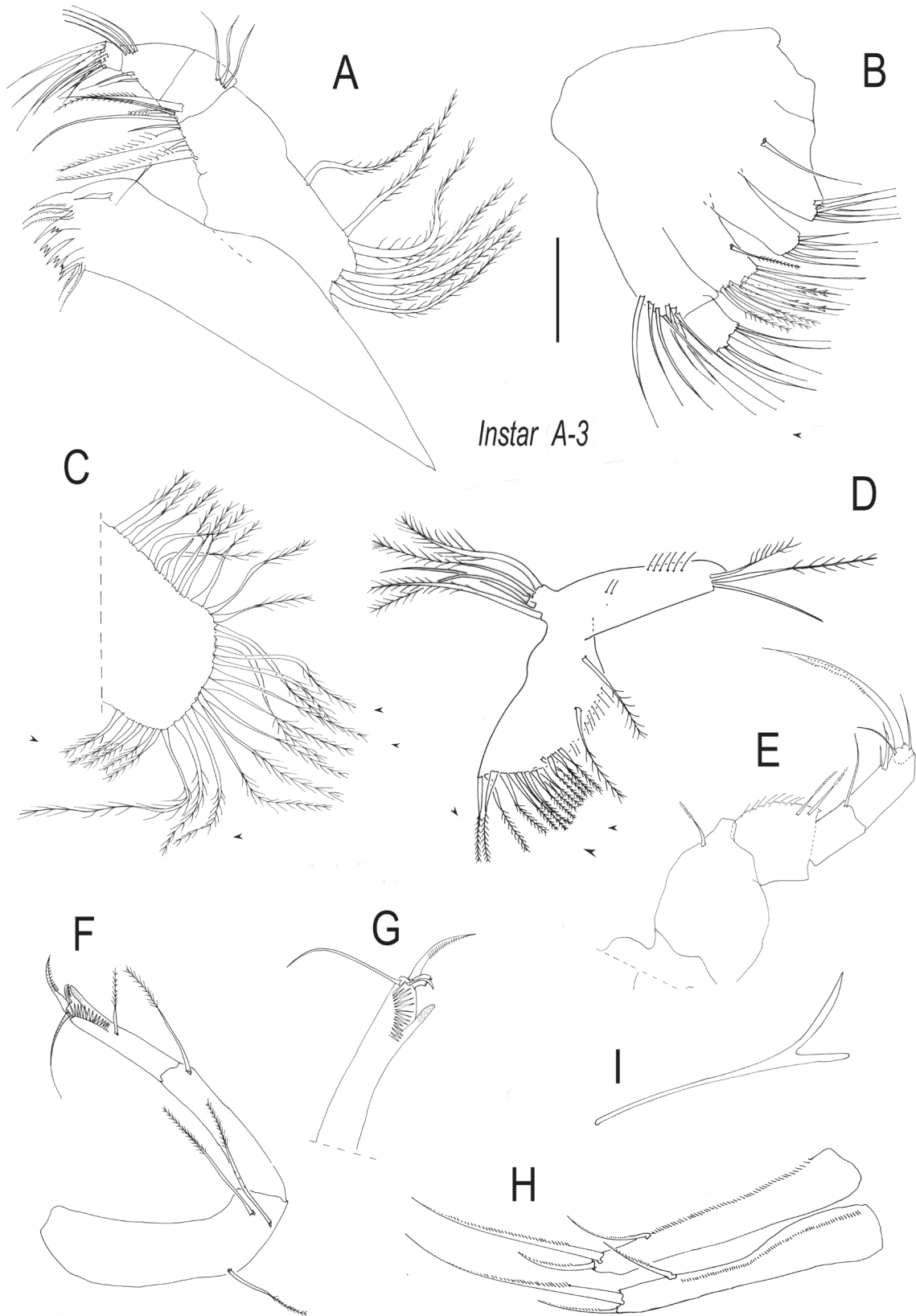
An1 and An2. Same as in A-2 instar.

Md (Figure 11E). Coxa with 7 teeth. Two short setae appear between the second and third tooth. Fifth and 6th teeth bicuspid. Seventh tooth bicuspid with a long seta behind.

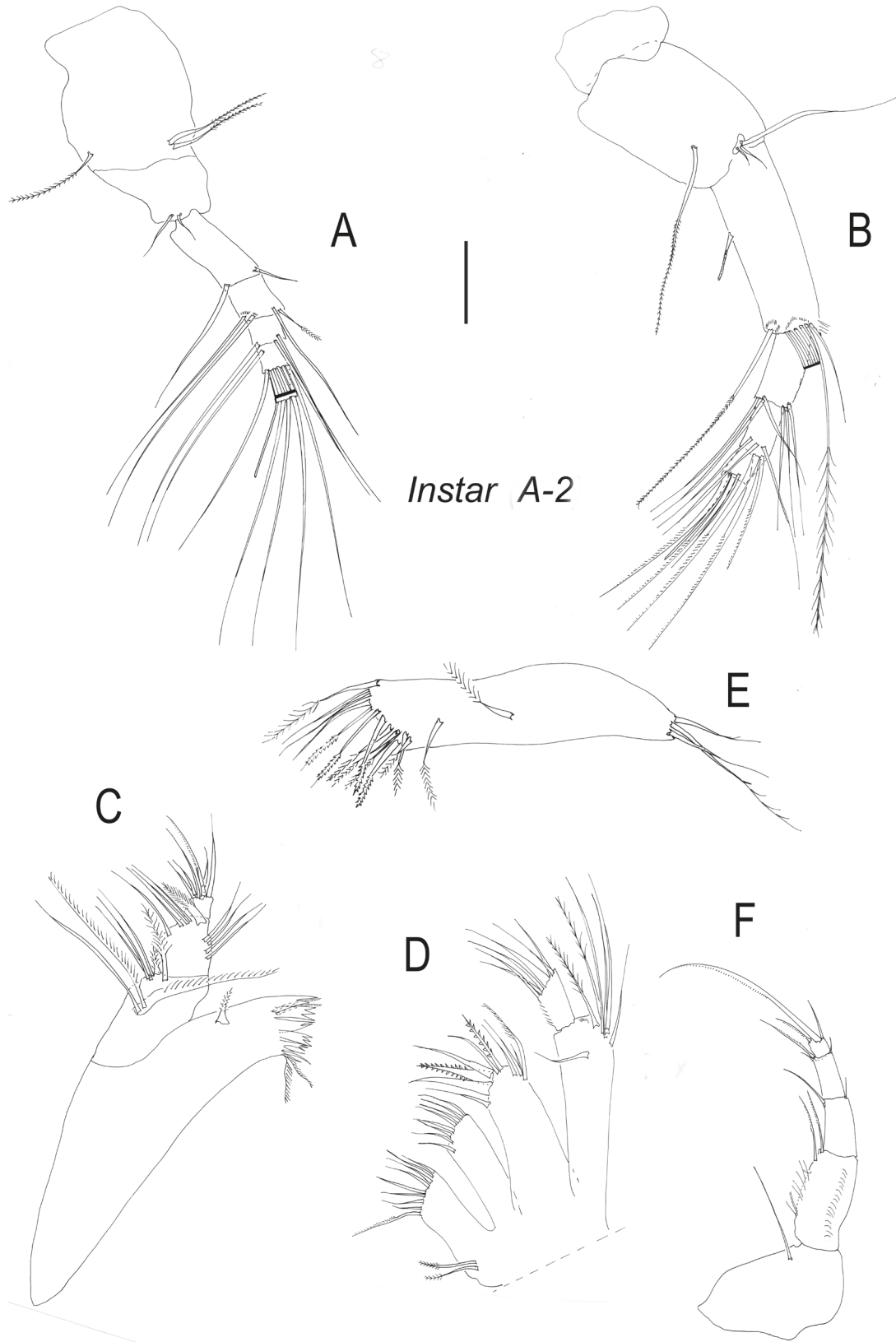


**Figure 8.** *Chlamydotheca arcuata* (Sars, 1901). Instar A-3 (MLP 26776). A, An1; B, An2. Scale bar: 50  $\mu$ m. Arrows indicate first appearance of a feature.

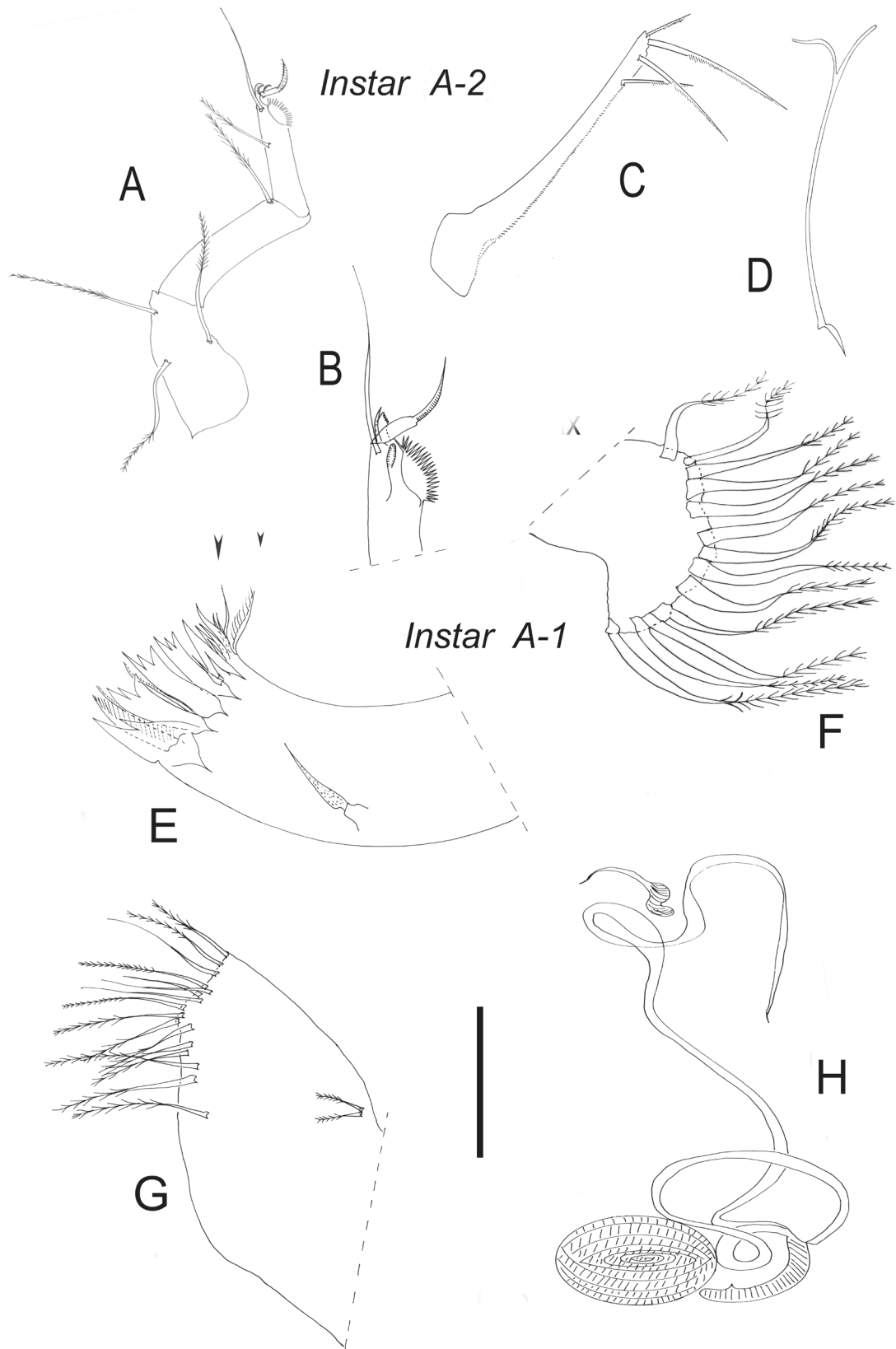




**Figure 9.** *Chlamydotheca arcuata* (Sars, 1901). Instar A-3 (MLP 26776). A, Md; B, Mx; C, Prmx; D, T1; E, T2; F, T3; G, detail of T3; H, CR; I, CRa. Scale bar: 50  $\mu$ m. Arrows indicate first appearance of a feature.



**Figure 10.** *Chlamydotheca arcuata* (Sars, 1901). Instar A-2 (MLP 26777). A, An1; B, An2; C, Md; D, Mx; E, T1; F, T2. Scale bar: 50  $\mu$ m. Arrows indicate first appearance of a feature.



**Figure 11.** *Chlamydotheca arcuata* (Sars, 1901). A–D, instar A-2 (MLP 26777). A, T3; B, detail of T3; C, CR; D, CRa. E–H, instar A-1 (MLP 26778). E, Md; F, Prmx; G, T1; H, Glo. Scale bars: 100  $\mu$ m. Arrows indicate first appearance of a feature.

Prmx (Figure 11F). Same as in previous instar. Respiratory plate with 16 rays.

Rlo. Same as in A-2 instar.

T1 (Figure 11G). With the addition of 2 (a) setae.

T2, T3, CR, and CRa. Unchanged from the previous instar.

Glo (Figure 11H). Genital lobe present with genital operculum rounded, internally connected by tubes.

The order of appearance of appendages of *Chlamydotheca arcuata* from A-8 through A-1 is summarized in Table 1.

### 3.9. Description of adult (A)

Carapace tumid in dorsal view, maximum width just behind the middle, pointed and acuminate anteriorly and rounded posteriorly. Surface of valves smooth with delicate setae. RV (n = 200): L = 1990 ± 150 (1800–1990), H = 1003 ± 0.02 (100–109); LV (n = 200): L = 2150 ± 300 (2000–2200), H = 1190 ± 700 (1020–1190).

The chaetotaxy of limbs is the same as in previous instars, but in the adults the genital organ is completely developed. The vaginal opening is rimmed with chitinized rings and connected to the seminal receptacle by long, coiled canals.

### 3.10. Complete development

The complete development of *C. arcuata* lasted between 80 and 90 days. The nauplius A-8 hatched between 10 and 15 days after laying. The time elapsed between each molt varied with the extent of development (Table 2). A great

number of juveniles died upon reaching the sixth instar (i.e. A-3). The eighth and final molt occurred 20 to 30 days after the females had laid the eggs.

In the A-5 instar, we observed that the second thoracic limb had a definite shape, which indicates that the appendage had become a true walking limb. In the A-3 instar, we noted that the respiratory function was completed with the branchial plate at the eighth instar. When the fifth thoracic limb changed, another thoracic limb appeared along with the well-developed caudal ramus. This transformation is achieved when a newly formed appendage takes over the locomotor function of the preexisting appendage, which loses that function to adopt a different function (e.g., feeding).

### 4. Discussion

The growth of *Chlamydotheca arcuata* is typically discontinuous and it involves a characteristic number of stages. This species, like other podocopids, exhibits a determinate growth with the number of juveniles in each species being fixed (Kesling, 1951) and the development consists of 8 postembryonic stages that occur successively in the process of reaching adulthood.

The juvenile A-8 to A-1 instars and the adult stage of *C. arcuata* follow a growth pattern that is similar to the rest of the Cyprididae (Roessler, 1983; Smith and Tsukagoshi, 2005; Aguilar-Alberola and Mesquita-Joanes, 2008, 2013).

**Table 1.** Summary of the ontogeny of *Chlamydotheca arcuata* (Sars 1901) from A-8 through A-1. X, presence; O, absence, An: Anlagen.

Instar	An1	An2	Md	Rlo	Mx	T1	T2	T3	CR	CRa
A-9	Not recovered									
A-8	X	X	An	O	O	O	O	O	O	O
A-7	X	X	X	X	X	O	O	O	An	O
A-6	X	X	X	X	X	An	O	O	An	O
A-5	X	X	X	X	X	X	X	O	An	O
A-4	X	X	X	X	X	X	X	An	X	O
A-3	X	X	X	X	X	X	X	X	X	X
A-2	X	X	X	X	X	X	X	X	X	X
A-1	X	X	X	X	X	X	X	X	X	X

**Table 2.** Instars A-8 to A-1: time of molt occurrence (in days) and number of individuals observed (n).

Instar	A-8	A-7	A-6	A-5	A-4	A-3	A-2	A-1
Days	4–5	1–2	1–2	1–2	9–10	5–7	15–20	
n	10	6	3	2	3	4	2	2



In *C. arcuata* the appearance of limbs is progressive but occurs nonsequentially, and the most important feature is noticed in the extensive growth of the mandibles and maxillae in A-7 instar and the development of the second thoracic limb in A-5 instar. There are also many differences from the other species of Cyprididae, such as the time of laying and the lack of ornamentation of the eggs as well as the position and appearance of limbs (see Roessler, 1983 for *Heterocypris bogotensis*; Baltanás et al., 2000 and Smith and Martens, 2000 for *Eucypris virens*; Kubanç et al., 2007 for *H. salina*; Aguilar-Alberola and Mesquita-Joanes, 2008, 2013 for *H. bosniaca*).

The phylogenetic appearance and development of the feeding limbs, which contain the rake-like organ, establish the beginning of the transition from a filtering habit to a detritivorous subsistence (Roessler, 1998). According to Roessler (1998), changes also occur in the carapace color of the genus *Chlamydotheca* that passes from a red-orange in the first stages to a pale white in the adult, which suggest the consumption of metabolic reserves. We registered changes in the carapace color that passes from yellow pale to dark green. The emergence of aesthetascs ( $Y, y_2$ ) along with the sensory setae of the carapace in the first instars is also related to this detritivorous mode of feeding.

We also registered great changes in the carapace related to the appearance of many small spines within the margins of the valves. These changes possibly indicate

the remarkable influence of environmental conditions on carapace shape (Baltanás et al., 2000) and also of the temperature range on carapace size (Marín, 1984; Latifa, 1987).

The muscle scars acquired a notable development in the last (A-4, A-3) instars and they were related to the progressive ontogeny of the carapace, which in turn became more calcified than in the previous instars. In the A-3 instar, well-developed tubercles appeared.

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