



## Embryology of the spider crabs *Leurocyclus tuberculatus* (H. Milne-Edwards & Lucas 1842) and *Libinia spinosa* (H. Milne-Edwards 1834) (Brachyura, Majoidea)

XIMENA GONZÁLEZ-PISANI<sup>1,4</sup>, FERNANDO GASPAR DELLATORRE<sup>1</sup> & LAURA LÓPEZ-GRECO<sup>2,3</sup>

<sup>1</sup>National Patagonian Center (CENPAT-CONICET), Puerto Madryn, Chubut, Argentina.

E-mail: [xgpisani@gmail.com](mailto:xgpisani@gmail.com); [dellatorre@cenpat.edu.ar](mailto:dellatorre@cenpat.edu.ar)

<sup>2</sup>Biology of Reproduction and Growth in Crustaceans, Department of Biodiversity and Experimental Biology, FCEyN, University of Buenos Aires, Ciudad Universitaria, C1428EGA Buenos Aires, Argentina

<sup>3</sup>IBBEA, CONICET-UBA, Buenos Aires, Argentina. [laura@bg.fcen.uba.ar](mailto:laura@bg.fcen.uba.ar)

<sup>4</sup>Corresponding author

### Abstract

The embryonic development of the spider crabs *Leurocyclus tuberculatus* and *Libinia spinosa* was divided into five periods based on the differentiation of: I) cleavage, II) embryonic primordium, III) optic lobes, IV) optic lobes pigmented and V) chromatophores presence. Different traits such as spines, setae and telson morphology distinguish the two species from period III until hatching. Egg volume was greater in *Leurocyclus tuberculatus* than in *Libinia spinosa*. The duration of each period was different during development. Whereas in *Leurocyclus tuberculatus* period II (morphogenesis) is the longest, in *Libinia spinosa* the period IV is the longest. Complete embryonic development at 14°C lasted  $36.7 \pm 3.1$  days in *Leurocyclus tuberculatus* and  $57.4 \pm 4.4$  days in *Libinia spinosa*.

**Key words:** Embryonic development, *Leurocyclus*, *Libinia*, Majoidea

### Introduction

Brachyuran crabs show diverse strategies of embryonic development, mainly related to variations in egg size and incubation period (Anderson 1982; Hines 1982; Hartnoll & Gould 1988). Similar sized species can have egg masses composed of few large eggs or many small ones (Hines 1982). Variations in egg and brood size along environmental clines can also occur on a single species but to a minor extent (Diez & Lovrich 2010). These variations can have profound effects on postembryonic life, the egg size is linked to a number of fundamental and adaptive traits such as duration of larval development, larval shape, duration of the facultative feeding period, size at metamorphosis, juvenile growth and survival, resistance to starvation and fertilization success (Moran & McAlister 2009).

Eubranchyuran crabs incubate their eggs attached to the pleopods from spawning to hatching (Guinot 1979). The incubation period varies between species and is mainly affected by temperature (Wear 1974; Garcia-Guerrero *et al.* 2003; Bas *et al.* 2007). Salinity, oxygen availability, and pollution may also affect embryonic development rate and hatching size (Botsford 1991; Bas & Spivak 2000; Giménez & Anger 2001; Fernandez *et al.* 2003). These environmentally induced variations in traits in an early life phase (the embryonic phase) are carried over to the next life phase (larva, juvenile or even adult), having potential effect on population dynamics (Giménez 2006).

Different methods have been used to describe the embryonic development in crustaceans. Some of them are based on visual examination of the embryos while others analyze morphological and anatomical changes and the measurements of embryos. The first is considered an appropriate approach to define the early periods, while the second is considered better to define later ones (Stevens 2006).

Studies on the early life stages of Majoidea often include descriptions of the larval stages (Boschi & Scelzo 1968; Barcardit & Vera 1983; Taishaku & Konishi 2001; Penha-Lopes *et al.* 2006). Instead, the embryonic

development was studied in only four species in Majoidea, three belonging to the family Oregoniidae (Ng *et al.* 2008): *Chionoecetes opilio* Fabricius 1788 (Mallet *et al.* 1993; Moriyasu & Lanteigne 1998; Comeau *et al.* 1999; Webb *et al.* 2007), *Chionoecetes bairdi* Rathbun 1924 (Swiney 2008) and *Hyas araneus* Linnaeus 1758 (Petersen 1995; Petersen & Anger 1997), and one to Epialtidae *Leucippa pentagona* Milne-Edwards 1833 (Varisco & Vinuesa 2011).

Nearly 250 species of majoids have been reported along both coasts of America, eleven of which exhibit an amphioceanic distribution, eight are present in coastal Northern-Patagonia and 4 were observed in the Nuevo Gulf (Argentina): *Leurocyclus tuberculatus*, *Libinia spinosa*, *Leucippa pentagona* and *Rochinia gracilipes* Milne-Edwards 1875 (Spivak 1997; Boschi 2000).

*Leurocyclus tuberculatus* Milne-Edwards & Lucas 1842 and *Libinia spinosa* Milne-Edwards 1834 coexist in northern Patagonian gulfs (41°-43°S 64°-65°W) (González-Pisani 2011). *L. tuberculatus* (Inachoididae, Majoidea) (Ng *et al.* 2008) is distributed along the coasts of southern South America, from Rio de Janeiro (Brazil) to Chile (Boschi 2000). *L. spinosa* (Epialtidae, Majoidea) (Ng *et al.* 2008) is present along both coasts of America, the Pacific coast in Baja California, Galápagos Islands, Hawaii, Perú and northern Chile, and on the shores of the Atlantic Ocean from Nova Scotia to southern Argentina (Boschi 2000). The annual breeding cycle of both species in the northern Patagonian gulfs (41°-43°S 64°-65°W) starts in July and continues until May. During this period, females of a broad size range produce 1–3 broods (Barón *et al.* 2009; González-Pisani 2011).

Embryonic development has not been studied in *L. tuberculatus* and *L. spinosa*, although for both species many aspects have been analyzed such as larval morphology (Boschi & Scelzo 1968; Santana & Marques 2009), distribution (Spivak 1997; Boschi 2000), external morphology (Boschi *et al.* 1992; Braga *et al.* 2002), sexual maturity (Almeida *et al.* 2007; Barón *et al.* 2009; González-Pisani 2011), reproductive anatomy (Sal Moyano *et al.* 2010; González-Pisani *et al.* 2012), breeding seasonality and fecundity (González-Pisani 2011). The aim of this study was to describe the major embryonic features and the chronology of the development of *L. tuberculatus* and *L. spinosa* using morphological characteristics and embryonic measurements. This study can be a baseline for future ecological or fishing studies on Majoidea.

## Material and methods

Ovigerous females of *Leurocyclus tuberculatus* and *Libinia spinosa* were captured with baited traps in Bahía Cracker, Golfo Nuevo (northern Patagonia, Argentina) (42°56'S, 64°21'W) at depths between 5 and 45 m during September 2009. Living specimens were transported in seawater to the laboratory. Twenty similar sized females of each species (45 to 55 mm carapace width) were individually identified using small colored plastic tapes attached to the meropodite of the fifth right pereopod. Groups of five females were placed in 50 l plastic tanks with filtered seawater, constant aeration and a 12:12 (light:dark) photoperiod. Temperature was maintained at  $14 \pm 2^\circ\text{C}$ , resembling the average temperature in the Golfo Nuevo (Dellatorre *et al.* 2012) and salinity was kept constant at 33 psu. Fresh fish was daily offered as food. Females were checked daily until hatching was complete and a new clutch extruded. From this new egg batch and every 48 hours until hatching, a small sample of at least 15 eggs was taken from each of 15 brooding females for both species. The embryos were obtained using a fine-tipped forceps from the pleopods of each female (González-Pisani *et al.* 2006).

Eggs were placed in excavated slides with seawater, and embryonic morphology, growth, color, and yolk-tissue proportion were observed in lateral and frontal views. The coverings of the embryo were removed with fine-tipped forceps and the embryonic appendages and structures observed. Examination of living embryos was done using a Zeiss light microscope equipped with differential contrast interference optics and a LSM JEOL-6460 LV scanning electron microscope (SEM). Illustrations were done with a camera lucida.

Maximum egg diameters in at least 30 eggs per period were measured to the nearest 0.01 mm with a micrometer objective across the dorso-ventral, antero-posterior and lateral axes. Egg volume (V) was calculated as:  $V = (4/3) \times \pi \times r_1 \times r_2 \times r_3$ , where  $r_1$ ,  $r_2$  and  $r_3$  represent the half of each diameter measured (García-Guerrero & Hendrickx 2004).

Embryonic development was divided in periods according to the criteria adapted for crustacean eggs by Sandeman & Sandeman (1991). García-Guerrero & Hendrickx (2004) argue that the use of the terms “stage” or “phase” to describe the embryonic development is somewhat controversial, as both terms are allusive to the

existence of “steps” while the embryonic development is (contrary to larval development) a continuum. According to these arguments we used the term “period” to describe the time between different significant events during development. The extent of each period (in days) was averaged from data of the 15 specimens regularly observed during the experiment. The total duration of embryonic development (DT) was estimated from a control group of five females of each species, which were not stressed with sampling-related manipulation.

Egg dimensions and period duration were compared between periods with a Kruskal-Wallis non-parametric test (K-W test,  $\alpha = 0.05$ ) (Sokal & Rohlf 1995).

## Results

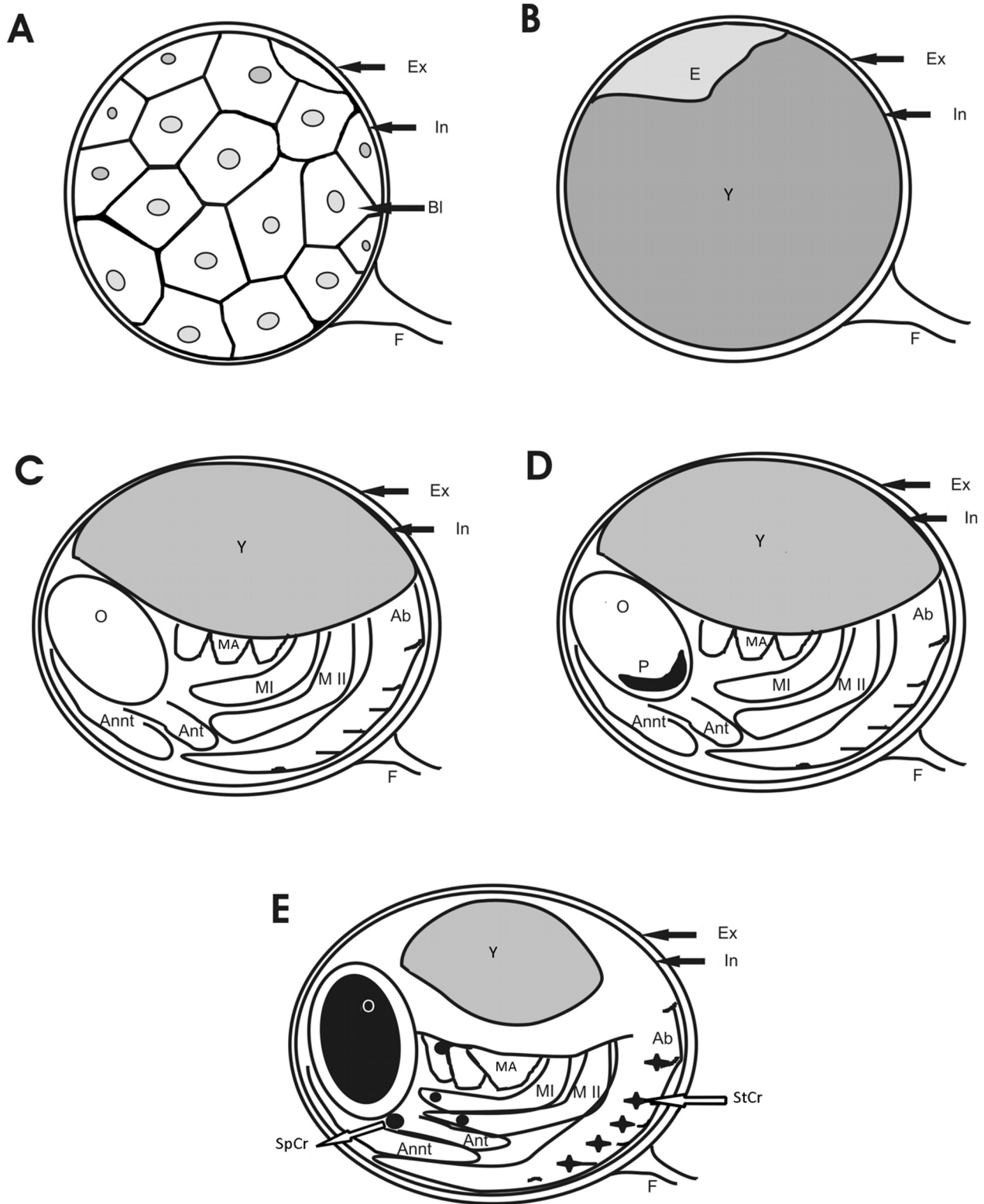
The recently spawned eggs of *Leurocyclus tuberculosus* and *Libinia spinosa* are adhered to the setae and distributed along each pleopod. The chorion is formed by two adherent envelopes. The outer covering, adhesive during extrusion, produces the funiculus that attaches the eggs to the pleopod setae (Fig. 1).

The embryonic development of both species can be divided into five periods based on the differentiation of segmentation (period I, Fig. 1A), the appearance of embryonic primordium (II, Fig. 1B), complete formation of the optic lobes (III, Fig. 1C), appearance of pigmentation in the optic lobes (III, Fig. 1D), development of chromatophores in the mouth parts and abdominal region (IV) and ommatidia completely are developed (V, Fig. 1E). The embryonic developmental periods of *Leurocyclus tuberculosus* and *Libinia spinosa* are described as follows (Table 1.)

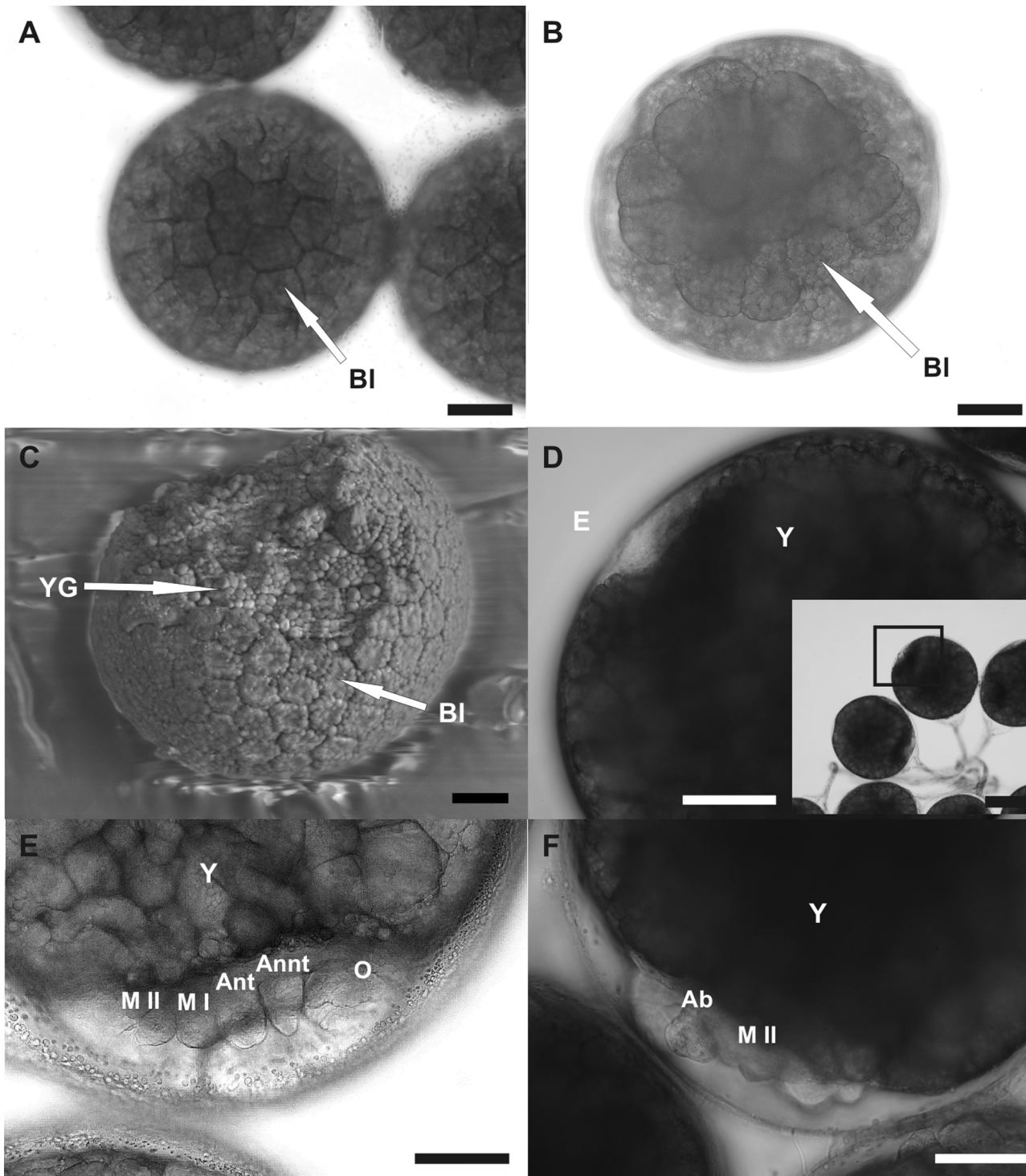
**TABLE 1.** Most relevant morphological traits of each period of embryonic development.

	Common features	Species-specific features	
	<i>L. tuberculosus</i> and <i>L. spinosa</i>	<i>L. tuberculosus</i>	<i>L. spinosa</i>
Period I	Cleavage. Morula.		
Period II	Appearance of the embryonic primordium, the antennules, the antennae and the maxillipeds I and II.		
Period III	The outlines of the optic lobe can be clearly discerned. Appearance of the mandibles, maxilules, maxilla and bilobulated telson.	Telson lobes pointed with five terminal processes on each lobe	Telson lobes rounded with eight terminal processes on each lobe.
Period IV	Appearance of dark pigmentation in the optic lobe.	The four aesthetascs of the antennules are two long and two short and have a terminal setae. The exopodite of the antennae present one subterminal setae.	The four aesthetascs of the antennules are the same large and have two terminal setae. The exopodite of the antennae without setae
Period V	Appearance of chromatophores. Lateral abdominal spines in the 3°, 4° and 5° segment.	Spherical chromatophores located at the bases of the antennae and the oral appendages. Star-shaped chromatophores around the digestive tube. Lateral abdominal spines hook-shaped. The telson presents a median notch in its distal margin.	Spherical chromatophores located at the bases of the antennae, mouth appendages and in the terminal part of the maxillipeds. Lateral abdominal spines straight-shaped. The telson without median notch.

*Period I.* This period begins in both species immediately after egg extrusion and fertilization, when the cleavage occurs and blastomeres are easily distinguishable (Fig. 2A, 2B). Egg color is orange-red, determined only by yolk color (Fig. 2A, 2B). The superficial cleavage leads quickly to a morula (Fig. 2A, 2B). No further structures are distinguishable within the egg, which contains homogeneously distributed yolk granules (Fig. 2C).



**FIGURE 1.** Schematic representation of the different periods of embryonic development of *Leurocyclus tuberculosus* and *Libinia spinosa*. A, Period I, segmentation. B, Period II, presence of the embryonic primordium. C, Period III, the optic lobe can be clearly discerned. D, Period IV, pigmentation of the optic lobe. E, Period V, appearance of chromatophores. Ab, abdomen; Annt, antennula; Ant, antenna; Bl, blastomere; E, embryonic primordium; Ex, external coverings; F, funiculus; In, internal coverings; MI, maxilliped I; MII, maxilliped II; MA, mouth appendages; O, optic lobe; P, pigmentation of the optic lobe; SpCr, spherical chromatophores; StCr, star-chromatophores; Y, yolk.



**FIGURE 2.** Embryonic periods I and II. Eggs in period I in A, *Leurocyclus tuberculatus* (scale bar: 100  $\mu$ m) and B, *Libinia spinosa* (scale bar: 100  $\mu$ m). C, *L. tuberculatus* egg with SEM observed yolk granules (scale bar: 100  $\mu$ m). D, *L. tuberculatus* egg at the beginning of period II observed the embryonic primordium (scale bar: 200  $\mu$ m). Detail: *L. tuberculatus* eggs in period II (scale bar: 200  $\mu$ m). E, *L. spinosa* egg in period II lateral view (scale bar: 50  $\mu$ m). F, *L. tuberculatus* egg in period II posterior view (scale bar: 50  $\mu$ m). Ab, abdomen; Annt, antennula; Ant, antenna; BI, blastomere; E, embryonic primordium; MI, maxilliped I; MII, maxilliped II; O, optic lobe; Y, yolk; YG, yolk granules.

*Period II.* The embryonic primordium is recognized as a small transparent zone at the animal pole of the zygote in both species. It represents the ventral region of the embryo (Fig. 2D) and grows deeper along the antero-posterior axis. The embryonic primordium is formed by a cephalo-thoracic-abdominal plate bearing four pairs of flattened and uniramous buds, primordia of antennules, antennae and the first two maxillipeds (Fig. 2E, 2F). Optic

primordia can also be distinguished by having a diffuse contour (Fig. 2E). The posterior region of the thoracic-abdominal plate shows a short and wide projection (Fig. 2F).

*Period III.* In both species this period begins when the outlines of the optic lobes can be clearly discerned as flat, smooth, unpigmented structures dorso-laterally projected in front of the antennae. The antennae and the maxillipeds are biramous (Fig. 3A, 3B, 3C). The yolk is orange and the embryo is clearly translucent (Fig. 3A, 3B). The antennules and the antennae are placed parallel and grow toward the posterior region (Fig. 3C). The antennules are uniramous, tubular, with a long middle seta (Fig. 3D). Biramous antennae are the most developed appendages during this period. The endopodite is short and wide in both species, and the exopodite is longer with setae forming a row in the distal zone (Fig. 3D). The mandible, maxillule and maxilla are distinguishable between the antennae and the first maxillipeds during this period. The abdomen grows towards the head (Fig. 3B) and its segmentation becomes distinguishable. In *L. tuberculosus* the telson lobes are pointed and bear five terminal processes on each lobe (Fig. 3E). Instead, in *L. spinosa* the telson lobes are rounded bearing eight terminal processes on each lobe (Fig. 3F).

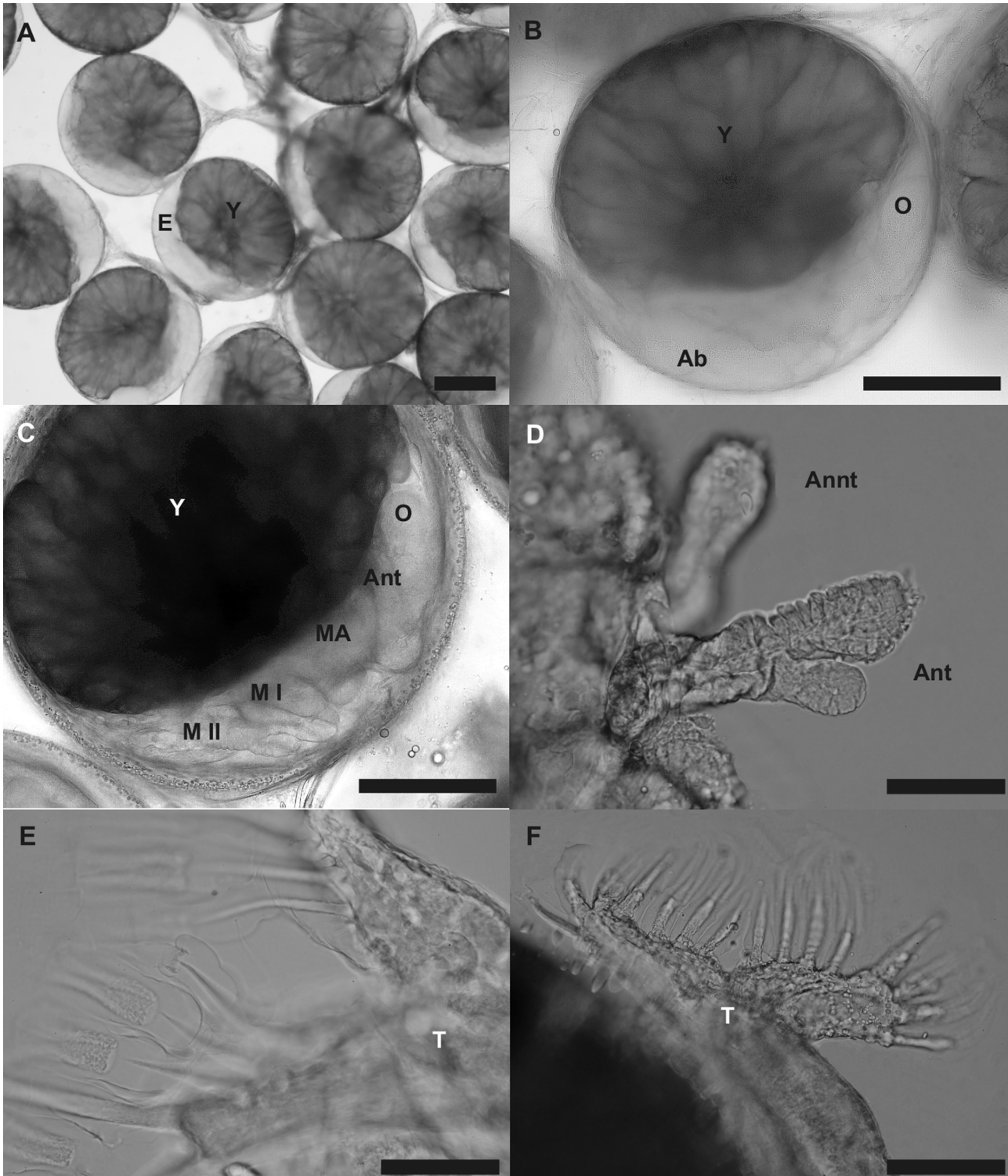
*Period IV.* This period begins in both species with the appearance of a curved line of dark pigmentation in the optic lobes (Fig. 4A, 4B, 4C, 4D). The antennules of both species are uniramous and conic-shaped (Fig. 4E, 4F). In *L. tuberculosus*, however, they have two long and two short aesthetascs and a short terminal setae (Fig. 4E), whereas in *L. spinosa* they have four aesthetascs with the same length and two short terminal setae (Fig. 4F).

The antennae of both species are biramous. In *L. tuberculosus* the antennal endopodite is short, unsegmented and without setae and the exopodite is longer with one large terminal spine, one subterminal short setae and one basal spine with the same length of the exopodite (Fig. 5A). The antennae of *L. spinosa* is similar but without the setae in the exopodite. The mouth appendages are wider and overlap each other in both species. The mandible is overlapped by the maxilla. The mandible has recognizable molar processes, incisor processes in the outer distal edge and palps absent (Fig. 5B). The first and second maxillipeds of both species are biramous (Fig. 5C, 5D). The segmented endopodite has lateral short setae and long terminal plumose setae. The unsegmented exopodite has long terminal plumose setae (Fig. 5C, 5D).

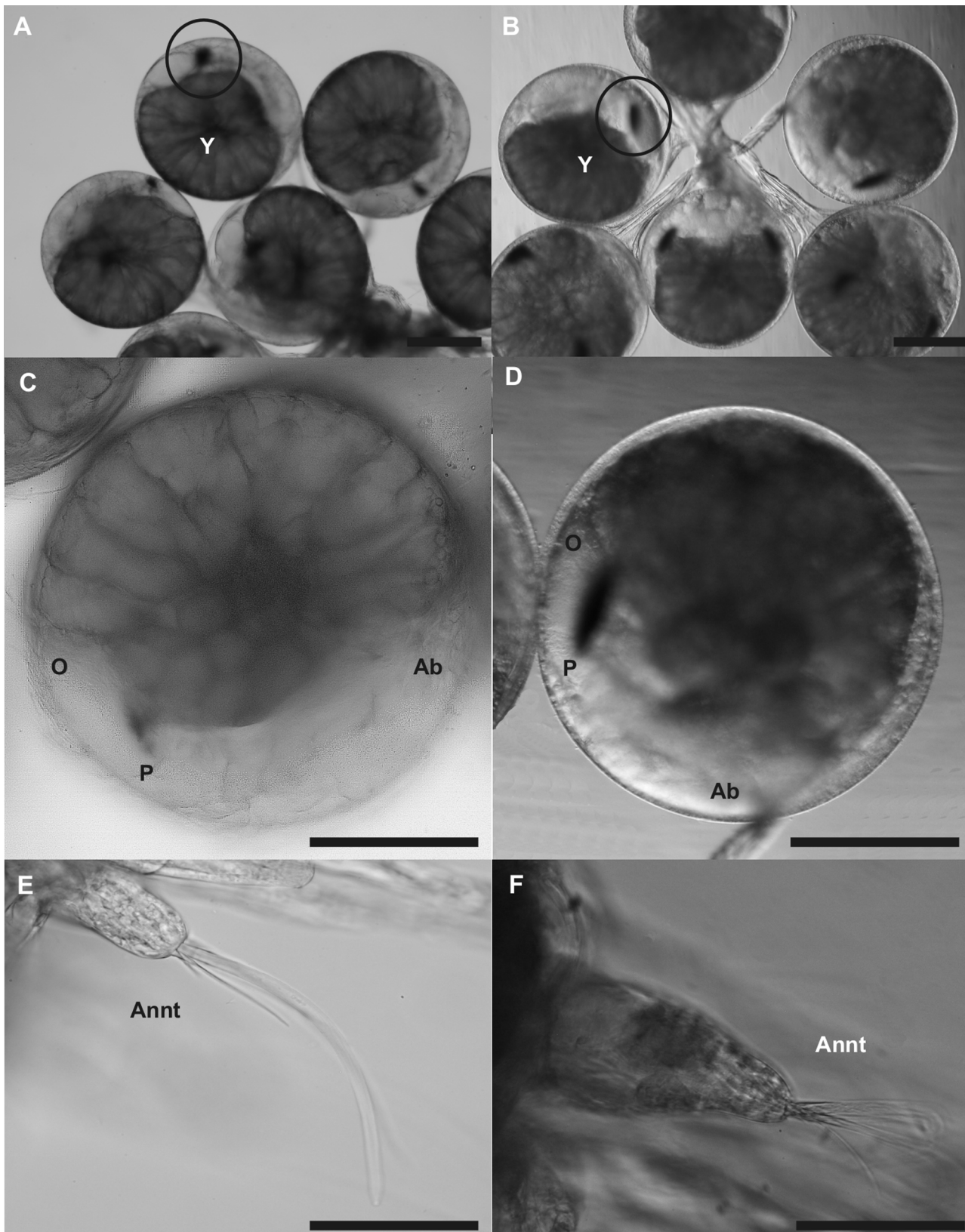
*Period V.* This period is defined in both species by the presence of chromatophores in the embryo (Fig. 6). The position of chromatophores differs between the species. In *L. tuberculosus* rounded chromatophores are located in the base of the antennae and the mouth appendages (Fig. 6A). Star-shaped chromatophores are also located around the digestive tube in the abdomen (Fig. 6B). In *L. spinosa* the chromatophores are located in the base of the antennae and mouth appendages, and in the distal part of the maxillipeds (Fig. 6C, 6D). Lateral spines in the 3<sup>o</sup>, 4<sup>o</sup> and 5<sup>o</sup> abdominal segments are recognizable in both species. Spines are hook-shaped in *L. tuberculosus* (Fig. 6E) and straight-shaped in *L. spinosa* (Fig. 6F). The telson of *L. tuberculosus* has a median notch, six internal processes with the same length. Furcal arms four times longer than the internal processes and a pair of lateral furcal spines similar in length to the internal processes (Fig. 7A). In *L. spinosa*, however, the median notch is absent, and lateral furcal spines are approximately 1/4 the length of the internal process (Fig. 7B). The optic lobes have about 80% oval shaped dark pigmentation (Fig. 6A, 6D). In both species the eyes are sessile and the ommatidia can be observed as hexagonal units in the ocular surface (Fig. 7C, 7D).

Heart beating and sporadic movement of the appendages and the abdomen can be observed, becoming more frequent at the time of hatching. The embryo is completely formed and ready to hatch. The yolk is restricted to the dorsal-medial region of the cephalothorax (Fig. 6A, 6D). At the end of this period the embryos are easily separated from the pleopods and their external envelopes lost their rigidity. Hatching was induced by gently touching the envelope with a dissecting needle. The newly hatched first zoeal stage shows the appendages described above for each species. The third maxilliped and pereopod buds are not recognizable and the dorsal spines are still folded (Fig. 7E, 7F). For both species, a considerable depletion in the yolk amount is observed through the development.

Egg shape changes from spherical to ellipsoidal during development in both species. In *Leurocyclus tuberculosus* eggs were approximately spherical until the end of period II, the three diameters are  $0,43 \pm 0,01$  mm (K-W test;  $H_0$ : no difference between the antero-posterior, the dorso-ventral and the lateral diameter,  $p > 0,05$ ). From the beginning of period III until the end of period V the antero-posterior diameter ( $0,57 \pm 0,01$  mm) becomes longer than the dorso-ventral ( $0,54 \pm 0,01$  mm) and lateral diameters ( $0,53 \pm 0,01$  mm) (K-W test;  $p < 0,05$ ). Consequently, egg volume increases significantly between period I ( $0,35 \pm 0,02$  mm<sup>3</sup>) and period V ( $0,71 \pm 0,03$  mm<sup>3</sup>) (K-W test;  $p < 0,05$ ). The increases rate is not constant through development, however, it is statistically significant between the periods II and IV (K-W test;  $p < 0,05$ ).

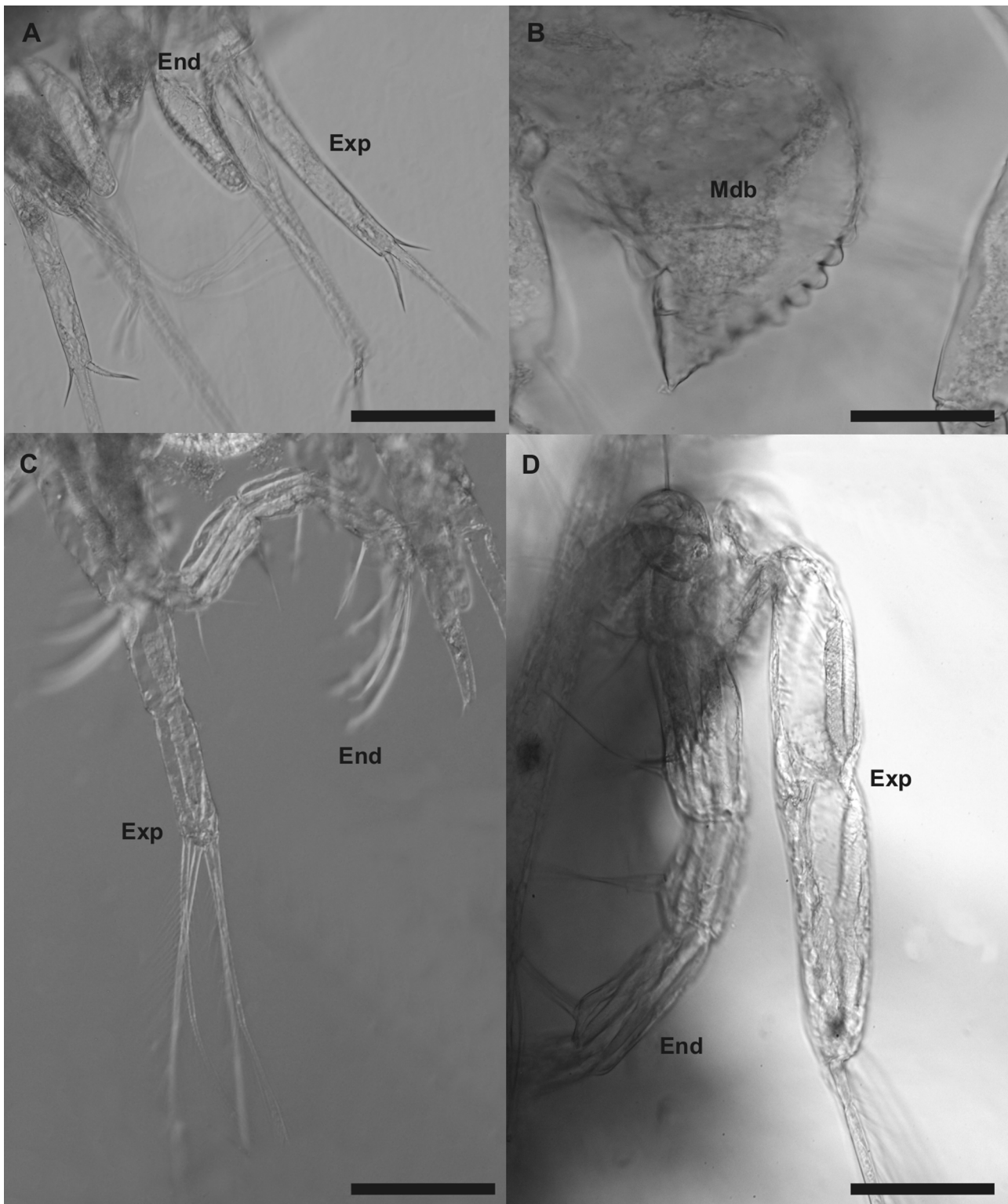


**FIGURE 3.** Embryonic period III in: A, *Leurocyclus tuberculosus*: it can observed the synchronization in the development (scale bar: 200  $\mu$ m), B, *L. tuberculosus*: the outlines of the optic lobe can be clearly discerned (scale bar: 200  $\mu$ m) and C, *Libinia spinosa*: the outlines of the optic lobe and the appendage can be clearly discerned (scale bar: 150  $\mu$ m). D, Detail of *L. spinosa*: antennula and antenna (scale bar: 50  $\mu$ m). E, Detail of *L. tuberculosus*: telson (scale bar: 50  $\mu$ m). F, Detail of *L. spinosa*: telson (scale bar: 50  $\mu$ m). Ab, abdomen; Annt, antennula; Ant, antenna; E, embryo; MI, maxilliped I; MII, maxilliped II; MA, mouth appendages; O, optic lobe; T, telson; Y, yolk.

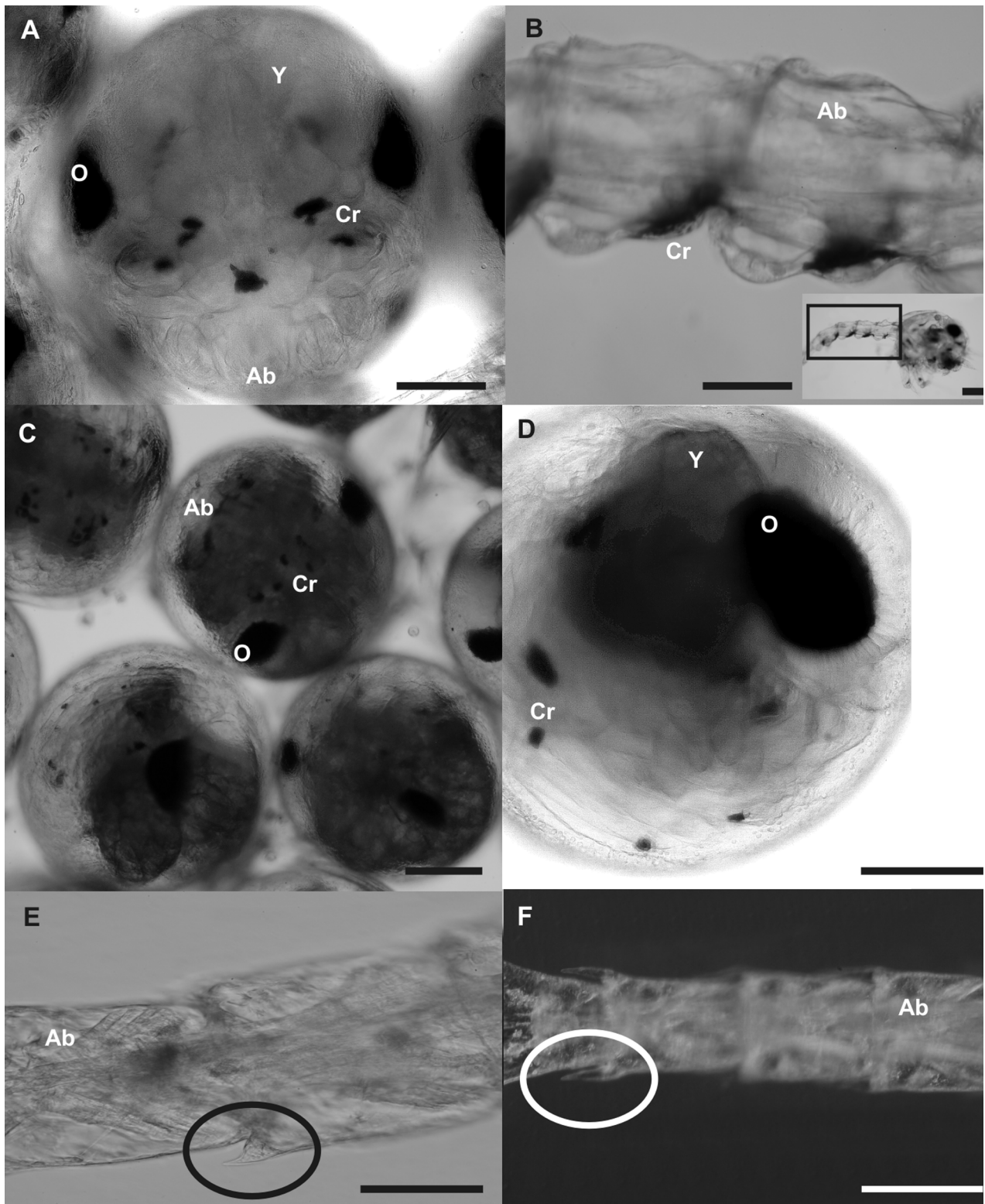


**FIGURE 4.** Embryonic period IV. Eggs with dark pigmentation in the optic lobe (circle) in A, *Leurocyclus tuberculosus* (scale bar: 200  $\mu$ m) and B, *Libinia spinosa* (scale bar: 200  $\mu$ m). Detail of egg with dark pigmentation in the area posterior to the optic lobe in C, *L. tuberculosus* (scale bar: 200  $\mu$ m) and in D, *L. spinosa* (scale bar: 200  $\mu$ m). Detail of the antennula in E, *L. tuberculosus* (scale bar 100  $\mu$ m) and in F, *L. spinosa* (scale bar: 100  $\mu$ m). Ab, abdomen; Annt, antennula; O, optic lobe; P, pigmentation of the optic lobe; Y, yolk.

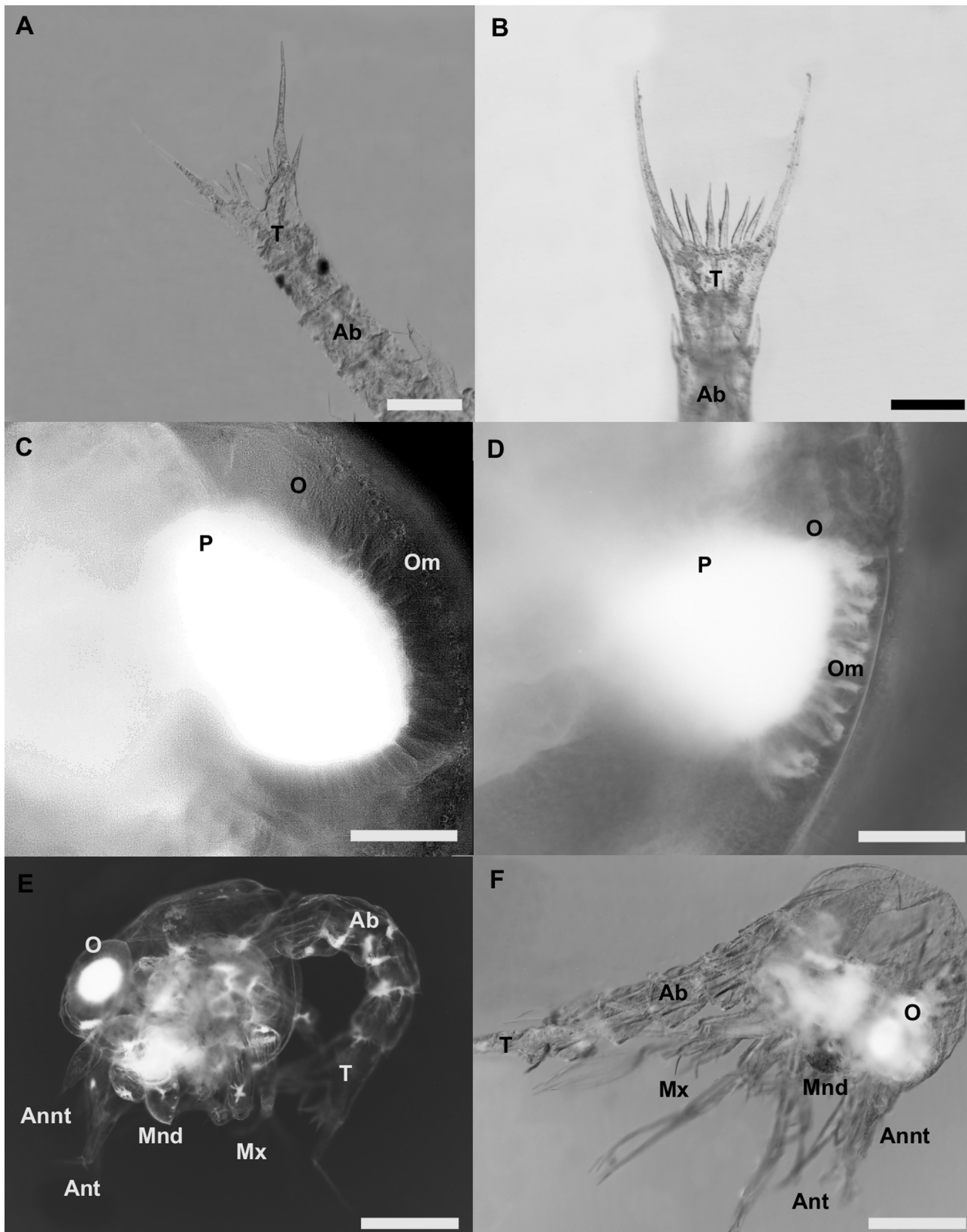




**FIGURE 5.** Period IV of development in embryos of *Leurocyclus tuberculatus* and *Libinia spinosa*. A, Detail of the antenna the *L. tuberculatus* (scale bar: 100  $\mu\text{m}$ ). B, Detail of mandible of *L. spinosa* (scale bar: 50  $\mu\text{m}$ ). Detail of the maxillipeds the C, *L. tuberculatus* (scale bar: 100  $\mu\text{m}$ ) and the D, *L. spinosa* (scale bar: 100  $\mu\text{m}$ ). End, endopodite; Exp, exopodite; Mdb, mandible.



**FIGURE 6.** Embryonic Period V. A, *Leurocyclus tuberculatus* egg with chromatophores (scale bar: 100  $\mu$ m). B, Detail of the embryonic abdomen in *L. tuberculatus* with chromatophores (scale bar: 200  $\mu$ m). C, *Libinia spinosa* eggs, synchronization in the development can be observed (scale bar: 100  $\mu$ m). D, *L. spinosa* egg with chromatophores (scale bar: 100  $\mu$ m). Detail of the lateral spine in the abdomen of E, *L. tuberculatus* (scale bar: 200  $\mu$ m) and F, *L. spinosa* (scale bar: 200  $\mu$ m). Ab, abdomen; Cr, chromatophores; O, optic lobe; Y, yolk.



**FIGURE 7.** Embryonic period V and zoea I in *Leurocyclus tuberculatus* and *Libinia spinosa*. A, Detail of the telson in *L. tuberculatus* (scale bar: 100 µm). B, Detail of the telson in *L. spinosa* (scale bar: 100 µm). C, Detail of the optic lobe in *L. spinosa* (scale bar: 50 µm). D, Detail of the optic lobe in *L. tuberculatus* (scale bar: 50 µm). E, Zoea I of *L. tuberculatus* (scale bar: 200 µm). F, Zoea I of *L. spinosa* (scale bar: 200 µm). Ab, abdomen; Annt, antennula; Ant, antenna; Mnd, mandibule; Mx, maxillipeds; O, optic lobe; Om, omatidias; P, pigmentation of the optic lobe; T, telson.

In *Libinia spinosa* the eggs are larger than in *L. tuberculatus* (K-W test;  $p < 0.05$ ), and spherical only during period I, in which the three diameters are  $0,56 \pm 0,01$  mm. During period II the antero-posterior diameter ( $0,64 \pm 0,01$  mm) is longer than the dorso-ventral and lateral diameters (both  $0,61 \pm 0,01$  mm) (K-W test;  $p < 0.05$ ). This difference persists until hatching. The egg volume increases significantly between the period I ( $0,77 \pm 0,003$  mm<sup>3</sup>) and the period V ( $1,010 \pm 0,003$  mm<sup>3</sup>) (K-W test;  $p < 0.05$ ).

The complete embryonic development lasts an average of 36.7 days (range = 32-44; ) in *L. tuberculatus* and 57.4 days (range = 50-64) in *L. spinosa*. This difference is statistically significant (K-W test;  $p < 0.05$ ). In both species the length of the embryonic development is not significantly different (K-W test;  $p > 0.05$ ) between manipulated females and the control group.

## Discussion

This is the first description of the embryonic development for a species belonging family Inachoididae and Epialtidae and it may be useful for field studies testing reproductive hypothesis at a population level.

Different periods of embryonic development of both species were recognized by the appearance of specific embryonic structures. They are easily recognized without microscope or with a simple magnifying instrument.

During period III all the zoeal appendages are distinguished, at least as primordia, in the embryo and these structures differ between species. Eye formation during periods III and IV in both species clearly followed the pattern described by Cronin & Jinks (2001) for the ontogeny of crustacean vision organs. Sharpening of the distal parts of the maxillipeds during the period V and the presence of four thick terminal setae which appears early during period III may facilitate the chorion rupture before hatching (Dupré 2003; González-Pisani *et al.* 2009).

Pleopod and pereopod buds are not observed in *L. tuberculatus* and *L. spinosa* embryos while these primordia have been observed during embryonic development in *Chionoecetes opilio* (Moriyasu & Lanteigne 1998) and *Hyas araneus* (Petersen & Anger 1997). In these species, pereopod and pleopod buds are visible after the ocular pigmentation (Moriyasu & Lanteigne 1998).

Different criteria have been used to define different periods (or stages) of embryonic development. The morphological change of a specific structure has also been proposed as a classification criterion (Tavonatti & Dupré 1998). These authors define different embryonic periods based on antennal morphology of *Jasus frontalis* Milne Edwards 1837. Our results indicate that this may be the first reported case on which telson morphology and the number and the arrangement of the terminal spines may be used to discriminate between periods of embryonic development. In period I the telson is absent, in the period II the telson is a flattened bilobed primordium at the end of the abdomen; in the period III, the telson bears distal processes (10 in *L. tuberculatus* and 16 in *L. spinosa*). This shape is maintained until period V, when the final shape bearing six distal processes and a spine in each furcal arm is acquired. Therefore, although the telson is not present in all the periods, it might indicate the degree of progress of embryonic development.

An index of optic lobe pigmentation has been useful in species in which ocular pigmentation occurs over more than 70% of the total incubation period (Perkins 1972). This index is not useful for Majoidea species since the pigmentation appears after more than 50% of development time has elapsed (Petersen 1995; Moriyasu & Lanteigne 1998; Swiney 2008; present study). The egg color has also been used as an auxiliary tool in different classification criteria (Moriyasu & Lanteigne 1998). It is useful to determine the period of development under direct observation in species in which eggs change from clear colors to darker colors throughout the development and it has been used in many species of crabs (Ito 1963; Mallet *et al.* 1993; Moriyasu & Lanteigne 1998) including the Majoidea *Chionoecetes opilio* (Moriyasu & Lanteigne 1998). Egg color remains relatively constant (orange) throughout development in *L. tuberculatus* and *L. spinosa*, therefore these criteria may not be useful for the species studied in this work.

The remarkable change of egg shape from spherical to ellipsoidal during the development in both species has been frequently observed in the Anomura (mostly porcellanid species) *Petrolisthes armatus* Gibbes 1850, *P. robsonae* Glassell 1945, *P. laevigatus* Chace 1962, *Pachycheles chubutensis* Boschi 1963 and *Munida gregaria* Fabricius 1793 (Lardies & Wehrmann 1996; García-Guerrero & Hendrickx 2006a; González-Pisani *et al.* 2009; Dellatorre & González-Pisani 2011). In contrast, Brachyura had been reported to have eggs almost spherical shape with minor variations in shape during development (Nagao *et al.* 1999; Pinheiro & Hattori 2003; García-Guerrero

& Hendrickx 2004; García-Guerrero & Hendrickx 2006b). The ellipsoidal shape has been interpreted as a consequence of the appendages growth in the antero-posterior direction (Lardies & Wehrtmann 1996; Hernández & Palma 2003; García-Guerrero & Hendrickx 2006a, b). The increase in the antero-posterior diameter reported for both species in this study from period III onwards, may be a consequence of the simultaneous growth of the cephalo-thoracic appendages.

The increase in egg volume throughout development has been considered as a consequence of increased water absorption rates in several species of Natantia, Anomura and Brachyura (Wear 1974; Valdes *et al.* 1991; Petersen & Anger 1997; Hernández & Palma 2003; Yávar & Dupré 2005). It has also been hypothesized that an increased water absorption rate at the end of the development may facilitate chorionic break by increasing the internal pressure in the egg (Davis 1964; De Vries *et al.* 1991).

Taishaku & Konishi (2001) hypothesized that the presence of remaining yolk in the final period of embryonic development may indicate partial lecithotrophy during the early stages of larval development. This may lead to one or two larval stages that does not need food, as observed in the Majoidea *Goniopugettia sagamiensis* Gordon 1931 (Taishaku & Konishi 2001). Yolk consumption is relatively high in both species studied similar to that observed in other Majoidea (Petersen & Anger 1997; Moriyasu & Lanteigne 1998). This implies, however, a difference with most crustacean embryos, in which yolk consumption during embryonic development is often between 40% and 60% (Petersen & Anger 1997). It may be interpreted as evidence of a short or virtually absent lecithotrophic period in the larval stages of the studied species.

The results show variations in the embryonic development between studied species. The embryonic morphology differentiates the species from the third period of embryonic development until hatching. The periods of the embryonic development of *Leurocyclus tuberculatus* and *Libinia spinosa* described in this study may be easily recognized by naked eye or with a simple magnifying instrument in the field according to the Table 1. It provides a useful tool for ecological studies to estimate the time elapsed since spawning, and the time remaining before hatching in live eggs.

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