Int. J. Morphol.,
43(1):194-202, 2025.

Embryonic Development of *Gyraulus albus*

Desarrollo Embrionario de *Gyraulus albus*

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FUERTES-RECUERO, M. & FONTANILLAS-PÉREZ, J. C. Embryonic development of *Gyraulus albus*. *Int. J. Morphol., 43(1)*:194-202, 2025.

SUMMARY: Here we describe the embryonic and larval development of *Gyraulus albus*. The morphogenetic changes along its development were followed by *in vivo*, using recently obtained egg clutches kept at 25 ºC, by taking serial photographs to determine the mean time and the different structures that characterize the embryonic stages. Our results on the detailed characterization of the embryonic development of *Gyraulus albus* could serve as a reference for future studies on parasitology, ecological indicators and taxonomic characteristics, among others.

KEY WORDS: *Gyraulus albus*, Gastropods, Embryonic development, Planorbidae.

INTRODUCTION

Studies of embryonic development in mollusks have been extensive. Most of these studies have focused on the class Gastropoda, as they are a good model for embryological study and monitoring. In particular, fresh water snails of the genus Lymnae, such as *Limnaea stagnalis* (van den Biggelaar, 1971; Taylor, 1977; Verdonk, 1979; Dorresteijn *et al*., 1981; de Jong-Brink *et al*., 1984; Zivkovic *et al*., 1991a,b), but also in other members of the family Planorbidae, such as *Biomphalaria glabrata* (Kawano *et al*., 1992) or the genus *Patella* (van den Biggelaar & Guerrier, 1979).

The *Gyraulus albus* (O. F. Müller, 1774) also known as *Planorbis albus*, commonly known as the white ram's horn snail, is a small species of freshwater snail, an aquatic pulmonate gastropod mollusc in the family Planorbidae. This species is widespread throughout most of Europe and western Asia (Kerney, 1999; Michalik-Kucharz, 2008). The shell is characteristically yellowish or greenish-whitish, transparent, radially and spirally striate, with 3-4 regularly rounded whorls, without a keel, with a concave upper surface with a large umbilicus and an opening slanting towards the upper surface. The average size is usually 1.5-2.5 x 4-7 mm (Kerney, 1999; Sumner, 2006). They are found in stagnant and slow moving waters, lake margins, shallow water on mud and aquatic plants (Dussart, 1979; Kerney, 1999; Zealand & Jeffries, 2009). Ecological studies have been carried out to assess dispersal and the effects of degradation of wetlands where *Gyraulus albus* is found (Niggebrugge *et al*., 2007; Spyra, 2010; Lorencová *et al*., 2021). Understanding the population and dispersal mechanisms is essential for further studies to evaluate reintroduction and enhancement as recovery tools. Other studies focus on their importance as carriers, vectors and intermediate hosts of parasites, such as trematodes and cestodes, that infect humans or other animals, causing significant economic losses to livestock farms and significant health damage to humans (Schwelm *et al*., 2018).

To the best of our knowledge, only a few studies have studied the embryonic and larval development in the Planorbidae family, such as *Biomphalaria glabrata* (Kawano *et al*., 1992), but none of them have described it in *Gyraulus albus*, so its development remains poorly known. The aim of this study is to describe the embryonic and larval development of *Gyraulus albus*, using recently obtained egg clutches kept at 25 ºC, by taking serial photographs to determine the mean time and the different embryonic stages, covering the stages between initial karyogamy and hatching. This study could serve as a reference for future studies on parasitology, ecological indicators, and taxonomic characteristics, among others.

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Received: 2024-08-06 Accepted: 2024-10-02

MATERIAL AND METHOD

Animals. Thirty *Gyraulus albus* were used and kept in three 38 L aquaria. The standard dimensions of the aquaria were 50 cm long and 25 cm high, and they were equipped with 300 L/h external activated carbon filters. They were also equipped with probe thermometers to record maximum and minimum temperatures, 75 W heaters and a 40 W cold light screen controlled by a central control unit. The snails were fed fresh floating vegetables and naturally produced phytoplankton. To prevent any pathologies that might affect reproductive function and subsequent embryonic development, the water and specimens were monitored regularly. An antifungal treatment with malachite green (3ml/100L), an antiparasitic treatment with methylene blue (3 ml/100L) and an antibacterial treatment with oxolinic acid (20 g/l-10 cc/100L) were administered.

Floating egg-layers were placed on the surface of the aquarium and the clutches that were only located on them were collected. These were collected immediately after oviposition. Fifty clutches with an average of 25 eggs each were collected. After the clutches were collected, they were placed in Petri dishes (35 mm by 10 mm), water was added from the aquarium where they were placed, and they were kept in an incubator at 25 °C with an estimated hatching time of 6 days (156 degrees/day).

Determination and observation of larval stages. Of the 50 clutches collected, 10 eggs from each clutch were observed and the mean time and SD of each stage were recorded. To determine the embryonic stages, the development of the early embryonic stages was recorded. A sterile environment was always maintained during handling and recording. A video recording system coupled to a stereoscopic magnifier was used to obtain the recordings, which allowed us to follow the development of the embryo. Serial photographs of the different stages were obtained from the recordings. The evolution of the different embryonic stages in *Gyraulus albus* has an average duration of 6 days (165 hours).

Although there is some asynchrony within clutches, in general the different eggs in the clutch are at the same stage at the same time. Based on recently laid clutches, the starting point for the different stages of embryonic development has been identified as the moment of karyogamy.

The photographs were taken continuously in the early stages due to the rapidity with which the first stages of embryonic development occur in this species. After the fourth segmentation, the photographs were taken at increasing intervals.

RESULTS

Initially, after oviposition, the sperm penetrates the oocyte and binds to the germinal vesicle of the oocyte, initiating karyogamy, which is taken as time zero (Fig 1. 1 and 2). After penetration of the sperm head into the oocyte germinal vesicle, the first cleavage begins at 10.18 min (SD 0.4) (Fig 1. 3) and the presence of the two polar bodies or polyocytes is visualized at 11.12 min (SD 0.19) (Fig 1. 4). At 15.06 min (SD 0.23) the end of the first cleavage occurs, at which time the newly formed blastomeres are connected by a small cytoplasmic area (Fig 1. 5).

At 20.38 min (SD 0.48), the two blastomeres produced in the first cleavage begin to fuse gradually along a broad contact surface (Fig 1.6). At 30.02 min (SD 0.72), an elongated cavity is visible along the equator of the sphere formed by the union of the two blastomeres, called the division cavity. Immediately afterwards, the second polar body emerged, and the second cleavage began. The cavity becomes larger and four rounded blastomeres are formed (Fig 1. 7).

The onset of the second cleavage occurs at 60.22 min (SD 0.85) (Fig 1. 8). This second cleavage is meridional and complete. The blastomeres do not divide synchronously, but the cleavage furrow appears in one of the blastomeres before the other, implying an asymmetric shape (Fig 1. 9 and 10). The onset of the third cleavage occurs at 92.03 min (SD 3.07). It can be seen that the blastomeres are very close to each other and it can be observed that the cleavage cavity begins to appear between them (Fig 1. 11 and 12). At 180.94 min (SD 8.0) the second polar body is released through the cleavage cavity (Fig 1. 13).

The third cleavage took place at 190 minutes. It can be seen that the embryo shows a general compression, with a visible reduction in its diameter (Fig 1. 14 and 15). This cleavage takes place in the sub-equatorial plane of the oocyte, with the formation of the first four blastomeres at 210.96 minutes (SD 8.53) (Fig 1. 16). Subsequently, a gradual increase in the size of the cleavage cavity is observed, indicating the onset of the fourth cleavage. The fourth cleavage occurs at 240.24 min (SD 52.89). It occurs in a sub-equatorial plane, giving rise to the second quartet of micromeres at 15.01 hours (SD 0.88) (Fig 1. 17).

The blastula stage occurred 15 hours after karyogamy (Fig 1. 18). This was followed by the gastrula stage at 26.01 hours (SD 1.30) (Fig 1. 19). The formation of the gastrula stage results from the approach of the vegetative pole towards the animal pole, through a process

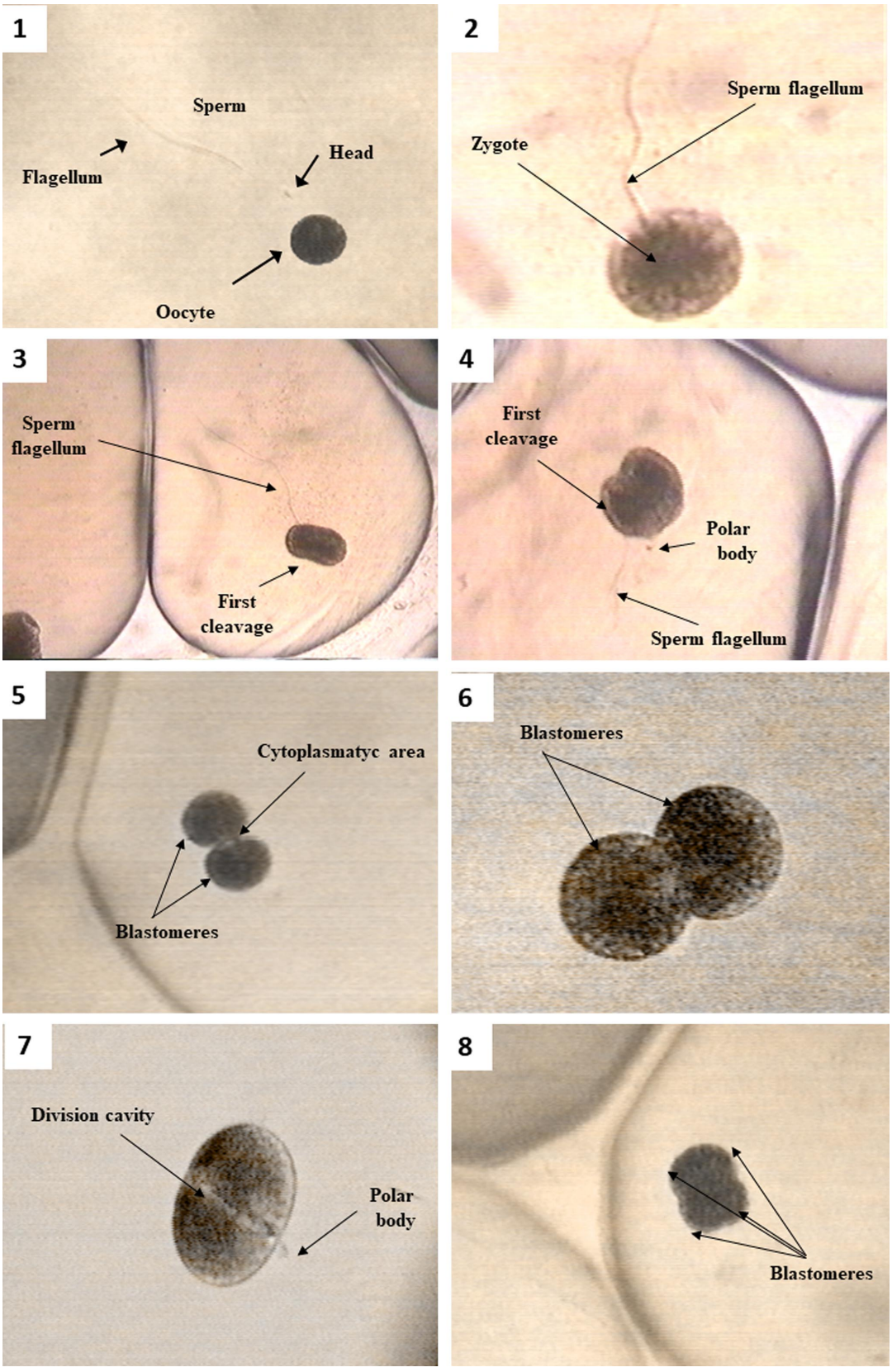

Fig. 1. 1-8. Photographs of the different stages of development of *Gyraulus albus*.

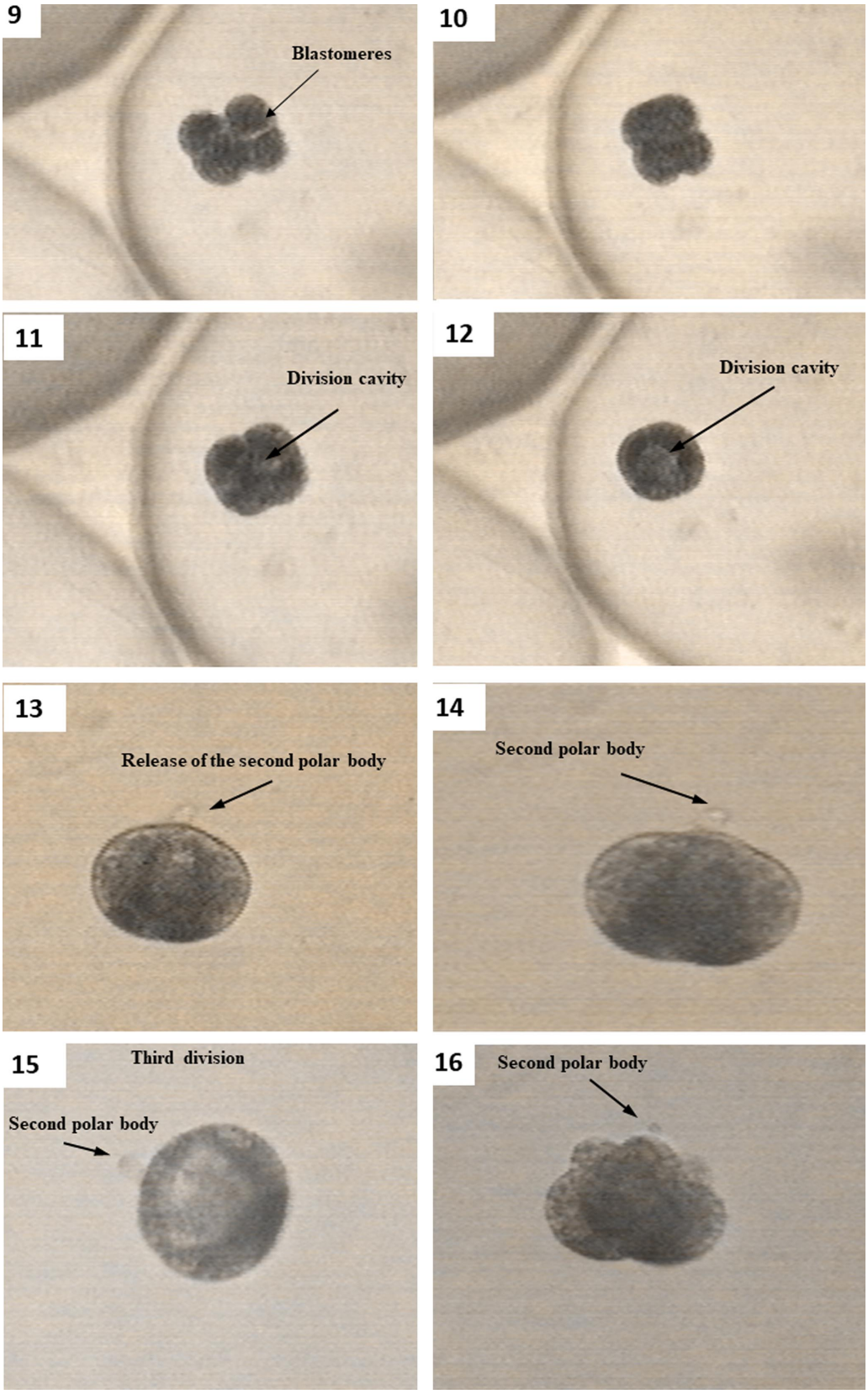

Fig. 1. 9-16. Photographs of the different stages of development of *Gyraulus albus*.

of invagination of this region with the formation of a spherical cavity. The blastomeres in turn begin a gradual reduction in size until the trochophore larva is formed. The early trochophore larva is visualized at 45.01 hours (SD 1.25). The first characteristic larval movements can be seen through the ciliated cells of the prototroch, which divide the larva into the pretrochlear and posttrochlear regions. The pretrochlear region is characterized by the presence of the apical plate, vesicle, and cephalic plate. The posttrochal region consists of the shell gland, stomodeum, and foot (Fig 1. 20).

The late trochophore larva occurs after 65.08 hours (SD 1.39). The larva elongates slightly and takes on a kidney shape. At this stage, the cells responsible for head and foot formation are located in the anterior and dorsal regions of the shell gland. At 96.03 hours (SD 1.28), the early veliger larval stage and the beginning of shell and foot formation can be observed (Fig 1. 21). At 121.9 hours (SD 1.07), further development of the shell and foot can be seen (Fig 1. 22). The ocelli of the individual begin to become visible, and elevation of the tentacle region, mouth, foot and shell can be observed. The apical plate and head remain unchanged while the cephalic plate splits to form tentacles and ocelli (Fig 1. 23).

At 164.08 h (SD 1.31), the beginning of the juvenile stage, a phase of larval development that takes place inside the egg capsule, was observed (Fig 1. 24). At this stage, a strong development of ocelli and tentacles was observed in the pre-trochlear region. In the post-trochlear region, growth and differentiation of the foot was observed (Fig 1. 24). The shell began to coil and cover the entire body (Fig 1. 25). At 165.02 hours (SD 1.31), the juvenile stage of the snail hatched at 25 ºC (Fig 1. 26).

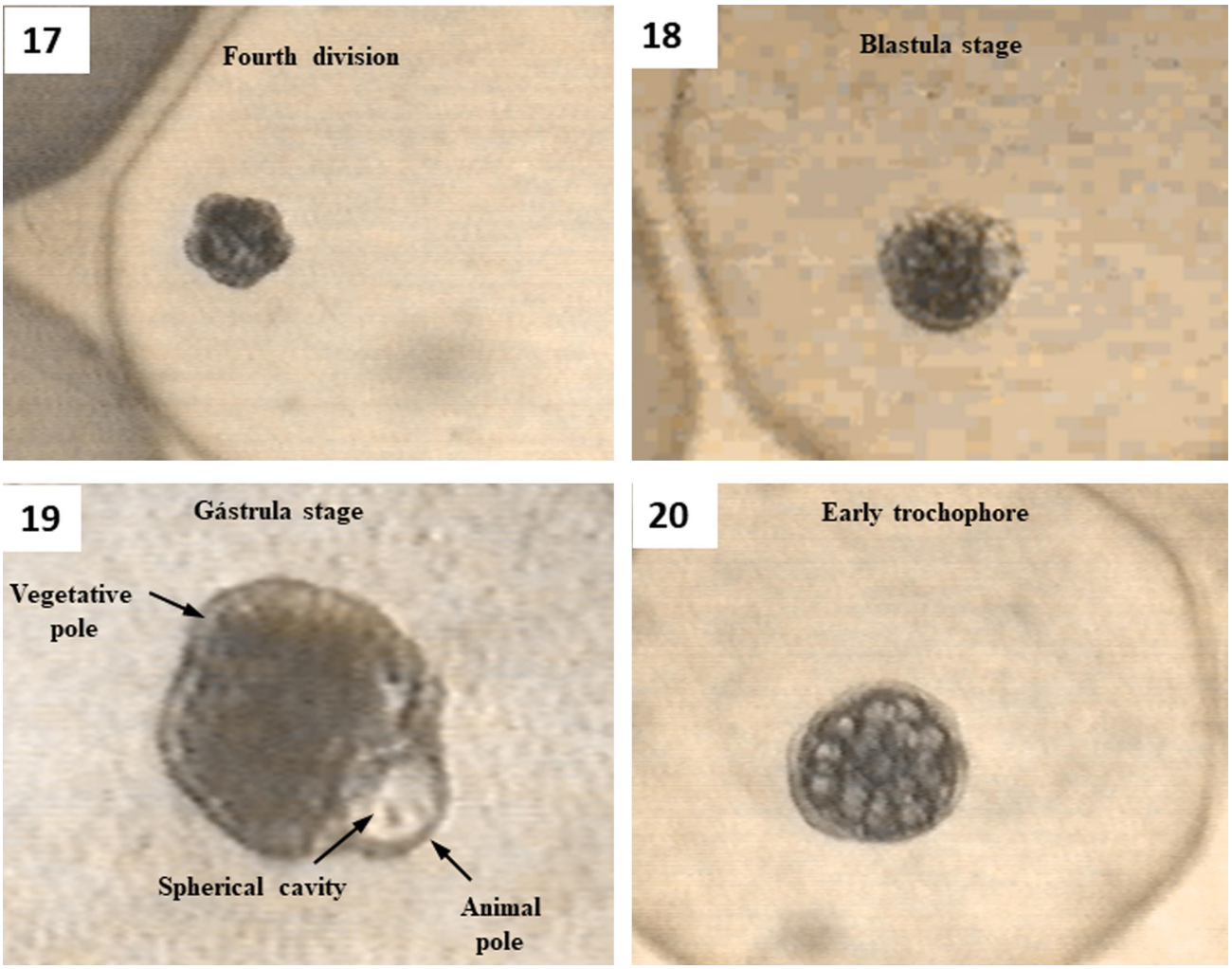

Fig. 1. 17-20. Photographs of the different stages of development of *Gyraulus albus*.

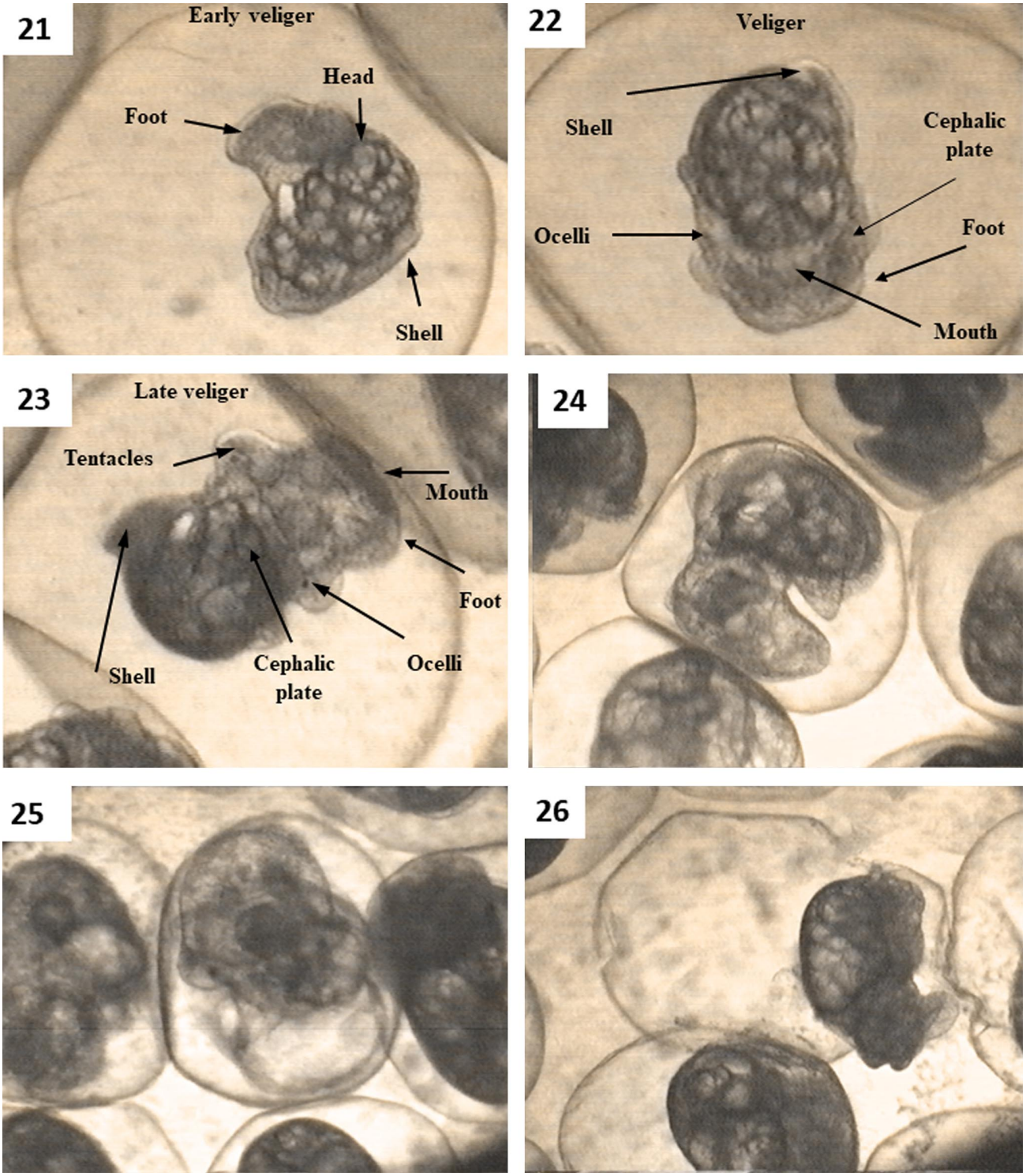

Fig. 1. 21-26. Photographs of the different stages of development of *Gyraulus albus*.

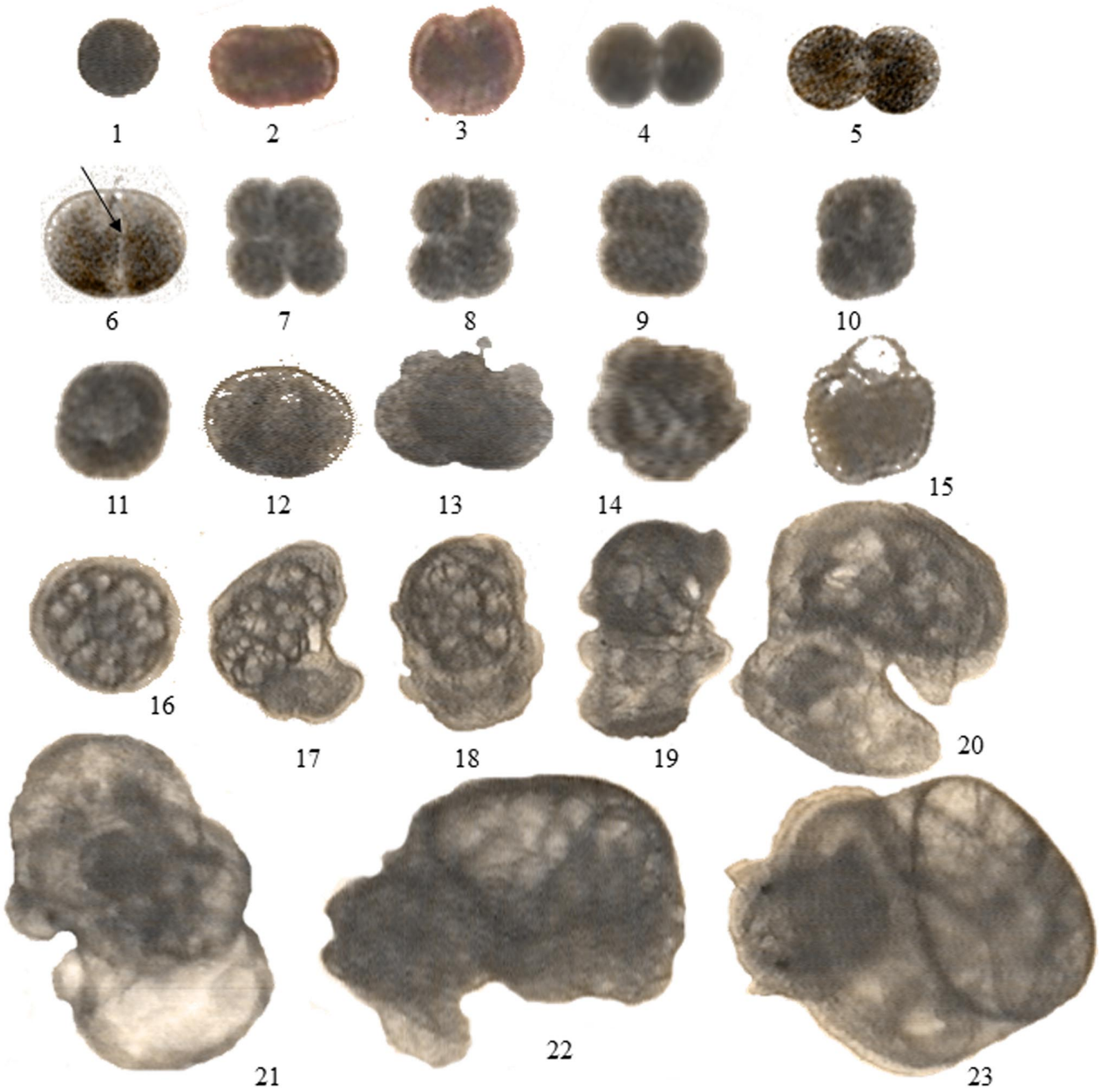

Fig. 2. 1-23. Different stages of development of *Gyraulus albus* at different times.

DISCUSSION

This study describes the embryonic development of *Gyraulus albus* by recording the evolution of the early stages of the embryo at 25 ºC. Due to their semi-transparency and easy maintenance in laboratory conditions, *Gyraulus albus* eggs represent a suitable material for embryological studies, allowing good *in vivo* observation.

The embryonic developmental stages in molluscs have been widely studied. Most studies have used *Limnaea stagnalis* as an embryonic model (Raven, 1963, 1970, 1974; Verdonk, 1979), its initial development during the 185 minutes after the first cell division and the activity of different ions on the embryonic cells have been described (Zivkovic *et al*., 1991a,b). Another study described the morphological changes that take place during the first two divisions in *Limnaea stagnalis* in relation to the cleavage cavity, from the first constriction of the egg to form the first two blastomeres to the appearance of the first four blastomeres

(Taylor, 1977). In addition, other studies have provided a complete description of the gastrulation process in this species, which occurs between 40 and 45 hours after the first cleavage. These studies compare the polarity of two gastrulae (-1b and -1a) with a normal gastrula and describe the stages of gastrulation and the quadrants that form the head and the quartet of micromeres from which they arise (Verdonk, 1979; Willen *et al*., 1983). Other studies have focused on cell-cell interactions in *Limnaea stagnalis* embryos during the interval between the formation of the third and fourth micromere quartets. During this period, the cleavage cavity disappears and most of the animal pole micromeres and vegetative pole macromeres fuse in the center of the embryo. This is followed by a differentiation of the macromeres with respect to their spatial position. The 3D stem cell, which determines the dorsoventral organization of the embryo, differs from the other macromeres with respect to these parameters (van den Biggelaar, 1971; Dorresteijn *et al*., 1981; Arnolds *et al*., 1983). In turn, the configuration of the first quartet of micromeres is very important in determining the differentiation of the macromeres. When two of the first four micromeres' quartets disappear, the remaining two can have a symmetrical or asymmetrical configuration with respect to the animal-vegetative axis, the asymmetrical configuration being more effective than the symmetrical one (Verdonk, 1979; Dorresteijn *et al*., 1981; Willen *et al*., 1983).

In turn, studies on *Bithynia tentaculata* and *Dentalium vulgare* developed the first embryonic mechanisms consisting of the formation of the vegetative pole during the first two divisions. Each of the stem cells divides unequally to form the polar lobe. The development of the first four cells irreversibly establishes the position of the blastomere 'D' and thus the dorsoventral axis (Guerrier *et al*., 1978; van Dam & Verdonk, 1982).

In other gastropods, e.g. *Patella vulgata*, these differences do not appear in the macromere row when the union of animal and vegetative blastomeres is affected by partial dissociation of the embryo. In *Patella vulgata* the partial disappearance of the first quartet is less frequent and there is less differentiation between the macromeres (van den Biggelaar & Guerrier, 1979) compared to *Gyraulus albus*.

Another study on another species of the family Planorbidae (*Biomphalaria glabrata*; (Kawano *et al*., 1992) describes the embryonic development from the first cell division to the birth of the new individual. Our results are similar in terms of embryonic development described in *Biomphalaria glabrata*. However, our findings differed from the observed in terms of time development. In our study, *Gyraulus albus*, the end of the first cell division occurs at 15.06 min (SD 0.23), at which time the newly formed blastomeres are joined by a small cytoplasmic area. In contrast, *Biomphalaria glabrata* approximately 30 minutes, the emission of the first polar body occurs, as a result of the first meiotic division. Approximately 60 minutes later, the second polar body emerges. Both polar bodies remain in the animal pole during the first cleavages. Within 60 minutes after the emission of the second polar body, the fusion of the male and female pronuclei occurs, the egg now being ready for the first cleavage (Kawano *et al*., 1992). The results obtained on the embryonic development of this species are similar to those obtained in our study on *Gyraulus albus*.

With the exception of cephalopods, the cleavage pattern in molluscs is of the spiral type, i.e. the cleavage of the blastomeres is oblique to their axis and is of a determinative type, since the different regions of the egg will give rise to the future organs. The developmental stages described here are similar to those of *Limnaea stagnalis*, with some differences. Cleavage is laeotropic or reverse in *Biomphalaria glabrata* (Camey & Verdonk, 1970) and dexiotropic in *L. stagnalis* (Verdonk, 1979). The differentiation of the type of cleavage occurs according to the orientation of the division spindle. When this is oblique in relation to the axis of the egg in clockwise direction, cleavage is dexiotropic, and when the spindle is oblique but in a counterclockwise direction, cleavage is laeotropic. The first indication of the orientation of the cleavage spindle in *Biomphalaria glabrata* occurs during the third cleavage (Camey & Verdonk, 1970). In the trochophore stage, the shell gland is shifted to the right side in *Biomphalaria glabrata* and to the left side in *L. stagnalis* (Verdonk, 1979). In our study, the shell gland is shifted to the right side as well.

In conclusion, this study showed the embryonic and larval development in *Gyraulus albus*. This detailed characterization of the embryonic development can contribute to better understand the cycle of *Gyraulus albus* and serve as a reference for future studies on parasitology, ecological indicators, and taxonomic characteristic.

FUERTES-RECUERO, M. & FONTANILLAS-PÉREZ, J.C. Desarrollo embrionario de *Gyraulus albus*. *Int. J. Morphol., 43(1)*:194-202, 2025.

RESUMEN: En este trabajo se describe el desarrollo embrionario y larvario de *Gyraulus albus*. Se han seguido *in vivo* los cambios morfogenéticos a lo largo de su desarrollo, utilizando puestas de huevos recientemente obtenidas y conservadas a 25 ºC, tomando fotografías seriadas para determinar el tiempo medio y las diferentes estructuras que caracterizan los estadios embrionarios. Nuestros resultados sobre la caracterización detallada del desarrollo

embrionario de *Gyraulus albus* podrían servir como referencia para futuros estudios sobre parasitología, indicadores ecológicos y características taxonómicas, entre otros.

PALABRAS CLAVE: *Gyraulus albus*; Gasterópodos; Desarrollo embrionario; Planorbidae.

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