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Ontogenetic growth of three cultured species of *Heterocypris* Claus, 1892 (Crustacea: Ostracoda): eggs and valve morphology

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Abstract. Ontogenetic information remains limited for many cypridoid ostracods despite its importance for reliable taxonomy, species identification, and the interpretation of ecological patterns based on size and morphology. This is particularly relevant in paleoecological studies, where, usually, only valves are preserved. We investigated post-embryonic development in three asexual species of *Heterocypris* inhabiting ephemeral aquatic environments: *Heterocypris exodonta* from the Tibetan Plateau, *Heterocypris incongruens* from Mexico, and *Heterocypris salina* from Germany. Based on laboratory cultures maintained under controlled conditions, egg morphology, juvenile valve development, and ontogenetic growth were quantified using measurements of valve length and height. All three species exhibited nine developmental stages, comprising eight juvenile instars and one adult stage. Eggs possess a rough external surface and a distinct internal eggshell structure. Early juvenile stages display a marked polygonal reticulation pattern on their valves that progressively weakens and disappears in adults. Growth ratios, defined as the proportional increase in valve size between successive developmental stages, averaged values close to those expected under geometric growth. However, considerable variation is reported among species and developmental stages. The results provide reference data on ontogenetic development and diagnostic morphological characters, improving the distinction between juvenile stages and both intraspecific and interspecific variation and supporting the interpretation of modern and fossil assemblages.

1 Introduction

Non-marine ostracods are bivalved crustaceans with soft parts enclosed within a carapace composed of a right and a left valve (Boomer et al., 2003). These arthropods grow by a specific number of molts, and ontogeny comprises the egg, juvenile instars, and adult stages, which, together, ensure completion of the life cycle and population persistence (Meisch, 2000; Dumont et al., 2002; Pereira et al., 2017).

Ostracod eggs are surrounded by a multilayered eggshell formed during oogenesis. Classical histological studies indicate that this structure comprises at least two principal layers: an inner layer produced by the oocyte and an outer layer secreted by the ovarian epithelium during oogenesis (Woltereck, 1898; Weygoldt, 1960; Hartmann, 1968;

Roessler, 1982). Furthermore, Kesling (1951) described the eggshell as a double-walled structure composed of chitin impregnated with calcium carbonate, with the inner wall separated from the outer wall and connected to it by fine chitinous processes, indicating a complex eggshell organization before hatching. In addition, SEM studies have documented external egg morphology, particularly in *Heterocypris incongruens*, including surface ornamentation (Dumont et al., 2002; Özuluğ and Suludere, 2012), which may contribute to protection against adverse environmental conditions such as drying and extreme temperatures (Smith et al., 2015).

Resting (dormant) eggs play a key ecological role in non-marine ostracods by enabling populations to persist under fluctuating or adverse environmental conditions (Rossi et al., 2012). This leads to the formation of egg banks, defined as the accumulation of viable dormant eggs in the sediment that hatch when conditions become favorable (De Stasio, 2007; Hairston and Fox, 2009; Rosa et al., 2020, 2023). Egg banks act as temporal reservoirs that enhance resilience in temporary aquatic systems and provide insights into the evolutionary history and adaptive capabilities of these microcrustaceans (Bellin et al., 2020; Wang et al., 2025).

Ontogeny in non-marine ostracods is fundamental to taxonomy and classification as developmental changes in valve morphology are key for species identification and interpreting phylogenetic relationships in modern and fossil assemblages (De Deckker and Martens, 2013). Most podocopid ostracods, particularly within the Cyprididae, exhibit nine post-embryonic instars, including eight juvenile stages and one adult stage (Smith and Martens, 2000). However, in some *Heterocypris* species, such as *H. bogotensis* Roessler, 1982, *H. bosniaca* Petkowski et al. (2000) (Aguilar-Alberola and Mesquita-Joanes, 2013), and *H. incongruens* (Rossi et al., 2015), an additional early developmental stage (A-9), corresponding to a prenauplier stage, has been reported, resulting in a total of 10 instars. Nonetheless, juvenile morphology remains poorly documented despite its importance for avoiding taxonomic misidentifications. Valve characteristics often allow straightforward identification of adult ostracods. In most cases, identification of juveniles at the species level is considerably more challenging, even when soft-part morphology is considered (Holmes, 2001; Lajblová et al., 2014). In parallel, the size, shape, ornamentation, and scar pattern of valves are primarily used for identification. However, ecophenotypic variation, observable morphological differences within a population resulting from environmental influences, should be considered as well (Holmes, 2001). Mainly, water temperature, conductivity, and food availability strongly influence ostracod ontogeny, driving spatial and temporal variations in size and shape across populations, including seasonal and interannual fluctuations (Baltanás et al., 2000).

Recognizing modern analog species is crucial for identifying fossil material at the species level and highlights the importance of ostracod cultures for understanding all developmental instars (Boomer et al., 2003). Under natural conditions, first juveniles generally preserve poorly, mainly because of weak calcification and fragile valves (Tinn and Meidla, 2003). Also, this may be further underrepresented in sediments due to cannibalism by adults or late instars (A-1) (Schreiber, 1922, and personal observation). Nevertheless, lake sediment provides valuable records for studying ontogeny through preserved valves, allowing reconstruction of evolutionary processes and environmental changes, as well as distinguishing autochthonous from allochthonous assemblages (Baltanás et al., 2000; Boomer et al., 2003).

To distinguish juveniles from adults, changes reflecting the addition of new appendages and, in sexual species, the onset of sexual maturity can be used as diagnostic criteria (Retrum and Kaesler, 2005). In males, hemipenis morphology is taxonomically important for identification because their shape differs among species. Meanwhile, in asexual populations, lacking males, the examination of the female genital lobes (vaginas or genital openings) holds significant potential for distinguishing morphologically similar species. This is reflected in interspecific variation in the intersection (junction of seminal ducts or oviduct), which corresponds to the chitinous frame of the vaginal opening sensu Kesling (1951) (see Fontana and Ballent, 2005; Karanovic and Lee, 2012; Kong et al., 2014; Matzke-Karasz et al., 2017; Mesquita-Joanes et al., 2020). This distinction can be of great utility when the morphology of valves and anatomical appendages shows limited interspecific variation (Bonilla-Flores et al. 2024, 2025).

Growth in ostracods is often described by Brooks' rule, which suggests that crustaceans' size doubles their volume through each molt, and this increase shows a linear relationship roughly up to the square root of 2, resulting in the coefficient of 1.26 with each molt (Brooks, 1886). However, deviations in non-marine ostracods are common (Hartmann, 1968; Baltanás et al., 2000; Mezquita et al., 2000, 2002; Van Doninck et al., 2003; Danielopol et al., 2008; Zhai et al., 2015; Mao et al., 2021), limiting its taxonomic value (Martens, 1985; Watabe and Kaesler, 2004), although it remains useful for assessing ontogenetic variability and environmental influences in fossil assemblages (McCormack et al., 2019).

Genus *Heterocypris* is one of the most diverse within the Cyprididae family, with 72 species (Meisch et al., 2024), and detailed comparative ontogenetic studies remain scarce. In particular, SEM-based analyses of valve morphology across instars are still limited, even for widely distributed species such as *H. incongruens* and *H. salina*. This limitation contributes to longstanding uncertainty regarding whether their broad geographic distributions reflect truly cosmopolitan species or complexes of cryptic taxa (Yoo et al., 2017). Recent molecular and population genetic studies indicate substantial genetic structuring and the presence of multiple clonal lineages in these taxa (Rossi et al., 2003, 2006) and, in some cases, suggest the existence of cryptic species complexes (Bonilla-Flores et al., 2025; Kilikowska et al., 2024), but ontogenetic data remain scarce. This underscores the need for integrative approaches combining ontogenetic and SEM-based morphological analyses. To address this gap, this study examines three species from different regions: *H. exodonta* from Nam Co, Tibetan Plateau (TP) (Bonilla-Flores et al., 2025); *H. salina* from Braunschweig, Germany; and *H. incongruens* from San Nicolás Tetelco, Mexico City. These species were selected based on their morphological similarity, occurrence in ephemeral environments, and suitability for laboratory culture. The objectives of this study are to (1) describe valve ontogeny, (2) characterize egg morphology, and (3) evaluate growth ratios to identify potential differences in developmental patterns.

2 Study area

The individuals of *H. exodonta* were collected in the study area located at 4728 m above sea level (m a.s.l.) from a temporary pond near Nam Co, a lake on the southern Tibetan Plateau (Table 1). Such temporary pools and lagoons formed around the lake because of wave action and the uneven terrain along the shoreline (Echeverría-Galindo, personal observation). This endorheic lake is highly exposed to solar radiation, the Indian Summer Monsoon, and the Westerlies (Zhu et al., 2008). Winters on the south-central Tibetan Plateau are dry, with precipitation mainly occurring between May and September (Anslan et al., 2020). Additionally, individuals of *H. incongruens* were collected from a flowerpot in San Nicolás Tetelco, Milpa Alta, southern Mexico City. The pots were watered with tap water and, particularly between June and August, with rainwater. The rainy season extends from June to September, while the dry season occurs during the rest of the year (Bouvier et al., 1993). Furthermore, specimens of *H. salina* were collected at the Botanical Garden in Braunschweig, Germany, an urban green area characterized by semi-controlled environmental conditions. The study site consisted of flowerpots exposed to natural climatic conditions, primarily irrigated with tap water. During the summer months, corresponding to the local rainy season (June–September), irrigation was supplemented by rainfall (Table 1). All ostracods were collected using a spatula and a hand net with a mesh size of 125 µm.

3 Materials and methods

3.1 Laboratory work

The cultures of *H. exodonta, H. incongruens*, and *H. salina* were maintained under the same laboratory temperature (18–23 °C) and a photoperiod of 14:10 h of light to ensure comparability. Under these conditions, the species showed a relatively short cycle between hatching and the final moult of approximately 2 to 3 months. Temperature may influence growth rates and developmental timing; therefore, these values should be considered to be specific to the experimental conditions. The three species were fed spinach, and the cultures were maintained in Vilsa naturelle water (VILSA-BRUNNEN Otto Rodekohr GmbH); its parameters are given in Table 2.

A multiwell cell culture dish (Nunc™) with six compartments, each containing 10 mL of water, was used. When the juveniles were separated, each was fed with small pieces of spinach, and the water was renewed every 3 d, reducing the effects of bacteria or fungi growth.

The purpose of keeping living ostracods was to produce viable cultures that would reproduce multiple times to obtain enough valves for subsequent analysis. It was observed that, during initial trials, juvenile A-8, separated from the group, did not survive despite being provided with spinach as a substrate for feeding. Individually, they did not survive beyond 7–9 d after hatching. However, when multiple juveniles (10–15) were introduced into the same compartment, it was observed that the ostracods had the possibility of surviving for more days (25–30) and reaching adulthood. This is likely due to their tendency to aggregate when water conditions and access to food are favorable (personal observation). Additionally, solitary juveniles showed a higher susceptibility to fungal contamination than individuals maintained in aggregation. Nevertheless, the survival of some solitary specimens was observed after transfer to a clean container and thorough cleaning of the multiwell plates.

Maintaining healthy cultures was also essential for evaluating egg resistance to desiccation. To this end, eggs of *H. exodonta* were exposed to dry conditions for 120 h. Eggs were then rehydrated with distilled water, and morphological changes were monitored during rehydration.

The valves were separated using a size-0 brush from the culture dish and were stored in micropaleontological slides. Subsequently, they were photographed by scanning electron microscopy (SEM, ZEISS EVO Ls 25) at the Institute for Chemical and Thermal Process Engineering, Faculty of Mechanical Engineering, TU Braunschweig. The biological material is temporarily stored at the Institute of Geosystems and Bioindication, TU Braunschweig.

3.2 Statistical analysis

The length and height of individual valves and carapaces of adults and juveniles were measured using a Leica M125 compound microscope. Data were analyzed and plotted in R Studio using the ggplot2 package (R Core Team, 2022).

The mean growth ratio was calculated using valve measurements from juvenile and adult instars. The ratio relates the average increments in length and height from one instar to the following growth stage. To estimate the average growth ratio, individual ratios were summed and divided by the number of growth phases. Values close to those expected under geometric growth (~ 1.260) were considered to be consistent with Brooks' rule, although deviations are expected due to ontogenetic differences among instars, interspecific and intraspecific variation, and environmental influences such as

Table 1. Sampling sites of *Heterocypris* species.

Species	Sample reference number	Locality	Date	Latitude	Longitude	Altitude [m a.s.l.]	Habitat	Water depth [m]
H. exodonta	NC-0919	Nam Co, TP	13.09.2019	30.7900	90.9600	4728	Pond	0.2
H. incongruens	HI-M19	Mexico City, Mexico	24.03.2021	19.2068	−98.9707	2271	Flower pot	0.1
H. salina	HS-G19	Braunschweig, Germany	26.03.2021	52.2705	10.5325	73	Flower pot	0.2

Table 2. Physico-chemical water characteristics of the water used for ostracod cultures.

Parameter	Value
Electrical conductivity [$\mu S\,cm^{-1}$]	170.1
pH	7.7
Cations [$mg\,L^{-1}$]	
Calcium	47.0
Sodium	16.4
Magnesium	3.6
Potassium	2.0
Anions [$mg\,L^{-1}$]	
Hydrogen carbonate	175.0
Chloride	12.0
Sulfate	10.0
Fluoride	0.2

temperature (Brooks, 1886; Watabe and Kaesler, 2004). The systematic position of suprageneric taxa follows Meisch et al. (2024).

4 Results

4.1 Systematic list

Class Ostracoda Latreille, 1802

Subclass Podocopa Sars, 1866

Order Podocopida Sars, 1866

Suborder Cypridocopina Baird, 1845

Superfamily Cypridoidea Baird, 1845

Subfamily Cyprinotinae Bronstein, 1947

Genus *Heterocypris* Claus, 1892

Heterocypris exodonta **Bonilla-Flores** and **Karanovic** in Bonilla-Flores et al. (2025)

Material. Females from a pond near the Nam Co Monitoring and Research Station for Multisphere Interactions (NAMORS), Institute of Tibetan Plateau Research, Chinese Academy of Sciences.

Reproduction. Asexual.

Ontogeny. Egg, eight juvenile instars, and one adult stage (Fig. 1).

Egg characteristics. The eggs display an orange to yellowish coloration (Fig. 2A). The outer surface appears to be rough and is frequently covered by adhering particles such as sediment, diatoms, or plant fragments (Fig. 2B). These particles are likely attached by adhesive secretions during oviposition and do not constitute structural components of the eggshell (Wohlgemuth, 1914). The eggshell itself consists of two layers. The outer surface may appear to be porous or irregular, which could result from post-depositional changes rather than representing the primary structure. After stage-A-8 hatching, the eggshell shows an irregular rupture rather than a predefined opening (Fig. 2E), indicating that no distinct operculum is present (Hartmann, 1968). When dried (120 h), the eggs tended to collapse (Fig. 2F), but after rehydration they turned orange after 20 min and regained their original diameter after 60 min. The average diameter of the eggs is 129 µm.

Adult valves. The coloration is yellowish (see Fig. 1). The external surface has numerous normal pores, each with a single seta. With a lateral view, a small hump is noticeable on the dorsal side. The RV features a broad inner lamella with distinct tubercles on both the anterior and posterior margins. The left valve (LV) is taller and longer, overlapping the right valve (RV).

Juvenile valves. External surface with normal pores; reticulation on the surface of the valves is observable in the early instars, from A-8 to A-2 (Figs. 3 and 4), and gradually dissipates in later instars. Stage A-8 is characterized by a rounded shape, which then develops into a more triangular form in the subsequent molting instars (A-7 to A-2) (Fig. 3). Stage A-8 shows RVs with 206 µm in length and 139 µm in height, on average, and LVs measuring 210 µm in length and 143 µm in height. In contrast, adults display RVs averaging 1131 µm in length and 629 µm in height, and LVs have an average length of 1144 µm and a height of 637 µm (Table 2). The mean growth ratio (length / height) for the RV is 1.237 and 1.214, and for the LV it is 1.236 and 1.205, respectively (Table 2).

Habitat. Ephemeral pond located at the Nam Co Monitoring and Research Station for Multisphere Interactions (NAMORS). Altitude: 4728 m a.s.l. (Anslan et al., 2020).

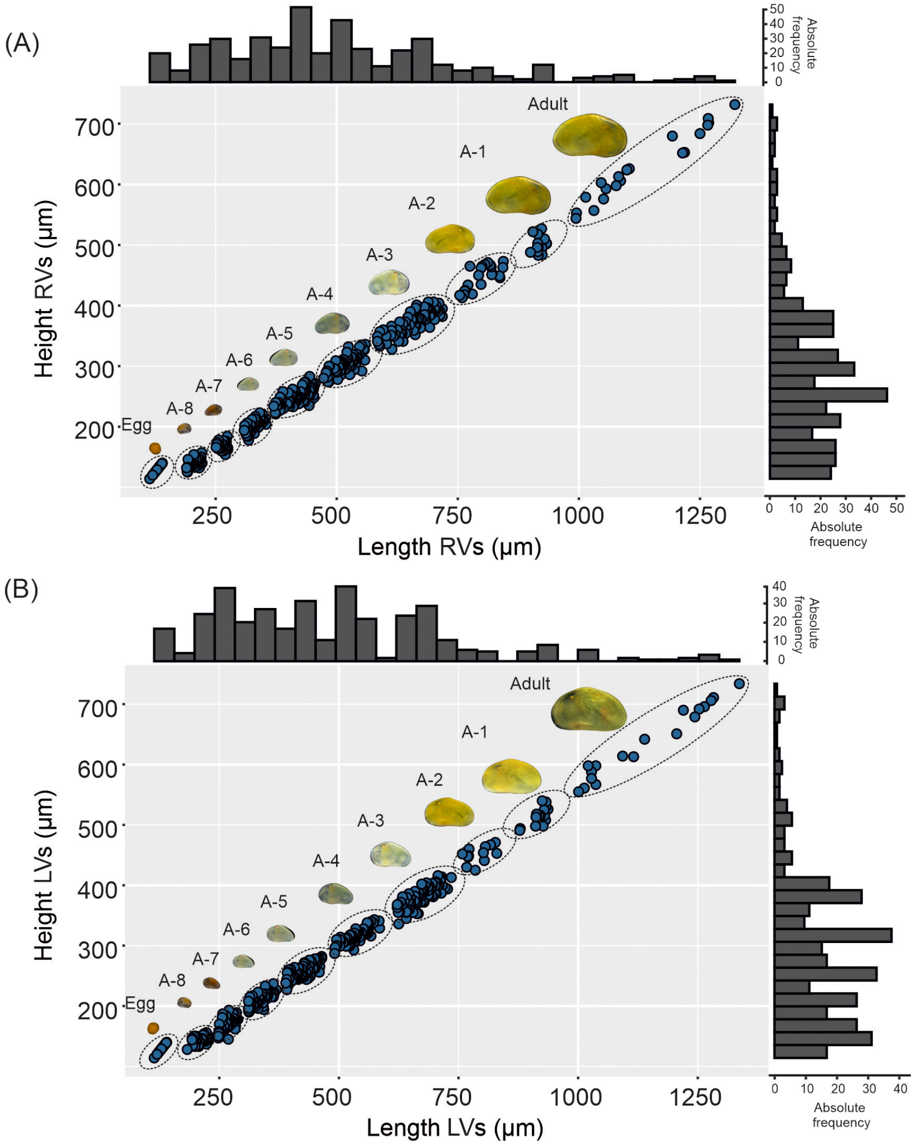

Figure 1. Valve ontogeny of *Heterocypris exodonta*; from Nam Co, Tibetan Plateau (NC-0919). The scatterplot shows the measures of the length and height of **(A)** right valves (RVs), $n = 424$, and **(B)** left valves (LVs), $n = 430$. The histograms display the abundances of eggs, juvenile instars, and adult stage.

Heterocypris incongruens (Ramdohr, 1808)

Material. Females from San Nicolás Tetelco, Mexico City, Mexico.

Reproduction. Predominantly asexual, although sexual populations, including males, have been recorded from multiple localities, particularly in Europe and North Africa (Meisch, 2000; Rossi et al., 2007), as well as in Türkiye (Yavuzatmaca and Külköylüoğlu, 2019).

Ontogeny. Egg, eight juvenile instars, and one adult stage (Fig. 5). Although a ninth juvenile instar (A-9) has been reported for this species (Rossi et al., 2015), it was not observed in the present study.

Egg characteristics. The eggs display an orange-colored (Fig. 6A) outer eggshell layer showing a porous appearance (Fig. 6B–D). On the surface of the egg, remnants of environmental materials, such as sediment or plant remains, are observed (Fig. 6C), providing an additional protective layer for the embryo due to adhesive properties. The inner eggshell layer envelops the embryo (Fig. 6E). Furthermore, the external surface of the eggshell shows small protuberances (Fig. 6F).

Adult valves. The color of the valves is uniformly yellowish; however, some of the larger females were whitish in color (Fig. 5). The surface has normal pores and bumps, and the valves are elongated. The RV is smaller than the LV,

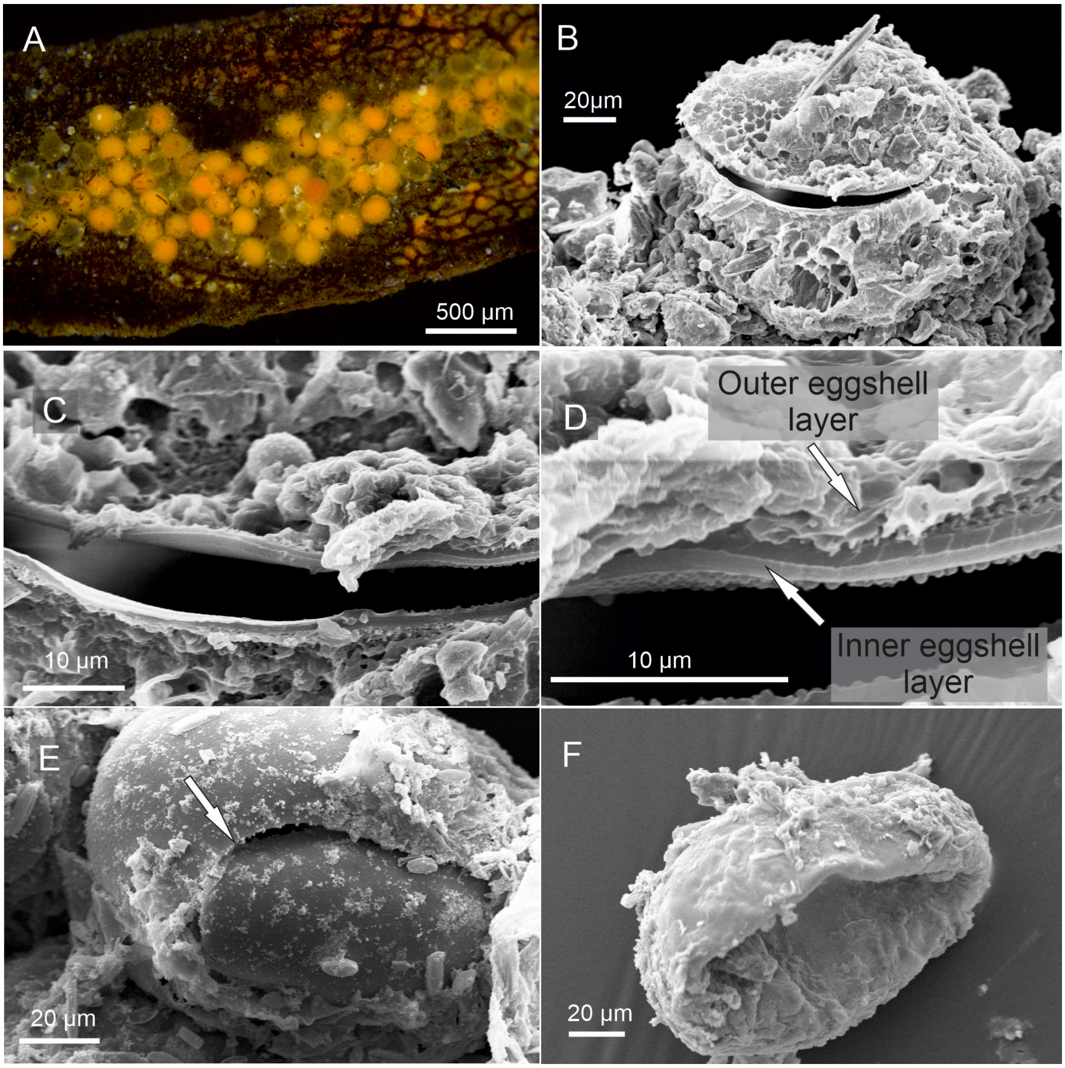

Figure 2. *Heterocypris exodonta*. **(A)** Egg cluster adhered to a plant fragment; **(B)** egg showing adhered particles on the surface and an opening with exposed eggshell layers; **(C–D)** higher-magnification views of the eggshell at the opening, illustrating the inner and outer eggshell layers; **(E)** irregular opening after hatching; and **(F)** collapsed egg after desiccation.

and the LV overlaps the RV. The highest part is in the middle region of the valves (Fig. 7). RVs with a broad inner lamella, crenulation or tubercles in internal view on anterior and posterior edges (see Bonilla-Flores et al., 2025).

Juvenile valves (Fig. 7). Surface with normal pores with setae. The reticulation is hexagonal and includes irregular polygonal forms in A-8 and is less pronounced in the last juvenile stage (A-2) (Fig. 8). The polygonal pattern is variable, including not only hexagons but also other irregular polygonal shapes. A triangular shape was observed for stage A-8, although this form is observable in subsequent molting instars, becoming more arched dorsally in A-1. On average, A-8 juvenile RVs measure 216 µm in length and 151 µm in height, and LVs measure 220 µm in length and 155 µm in height. Adult RVs measure 1349 µm in length and 790 µm in height, and LVs measure 1360 µm in length and 788 µm in height, on average (Table 3). The mean growth ratio (length and height) for the right valves is 1.257 and 1.231, and for the left valves, it is 1.255 and 1.226, respectively (Table 3).

Habitat. Mainly temporary waterbodies (Fryer, 1997; Meisch, 2000; Vandekerkhove et al., 2012).

Heterocypris salina (Brady, 1868)

Material. Females were collected from the Botanical Garden, Braunschweig, Germany.

Reproduction. Asexual populations from Braunschweig, Germany. Males and females were recorded from Crete, Greece (Petkowski et al., 2000).

Ontogeny. Egg, eight juvenile instars, and one adult stage (Fig. 9).

Egg characteristics. Eggs exhibit two colorations, either yellowish or whitish (Fig. 10A). They are laid in clusters,

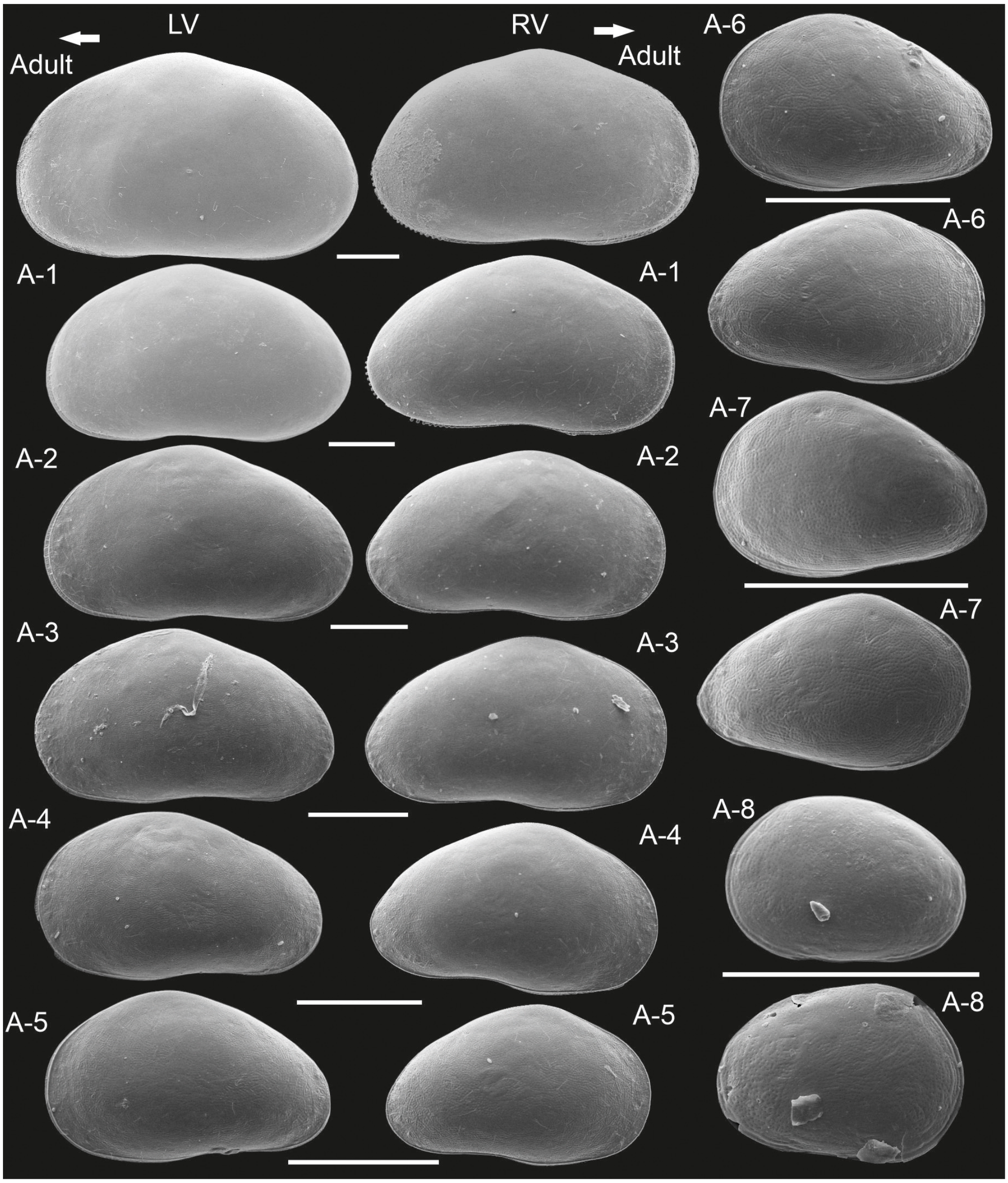

Figure 3. Left (LVs) and right valves (RVs) of adult and juvenile instars (A-1 to A-8) of *Heterocypris exodonta* from Nam Co, Tibetan Plateau (NC-0919). All instars are shown at the largest possible magnification to highlight surface features and valve morphology. The arrow points anteriorly. Scale bars show 200 µm.

adhering to each other through adhesive secretions produced during oviposition (Fig. 10B–D). The eggs are surrounded by an inner eggshell layer and an outer eggshell layer (Fig. 10E–F).

Adult valves. Both valves display a brown coloration pattern, with a pair of lighter perpendicular lines (Fig. 9). The external surface has normal pores with a seta. Compressed and triangular in the dorsal region (Fig. 11). The LV is longer than the RV, overlapping the RV. The external surface of the RV has inconspicuous crenulation along the anterior and posterior margins; detailed high-magnification images of this feature are provided in Bonilla-Flores et al. (2025).

Juvenile valve. External surface with normal pores and setae. The irregular reticulation in surface valves gradually dissipates in the last juveniles (Fig. 12). The shape of stage A-8 is rounded, which then transitions into a more triangular form in the subsequent molting instars to become more arched in A-1; in this stage, small tubercles are also observed along the postero-dorsal margin of the RV (Fig. 11). On average, A-8 juvenile RVs were 181 µm long, and their height was 124 µm, while the LVs were 176 µm long and 125 µm high.

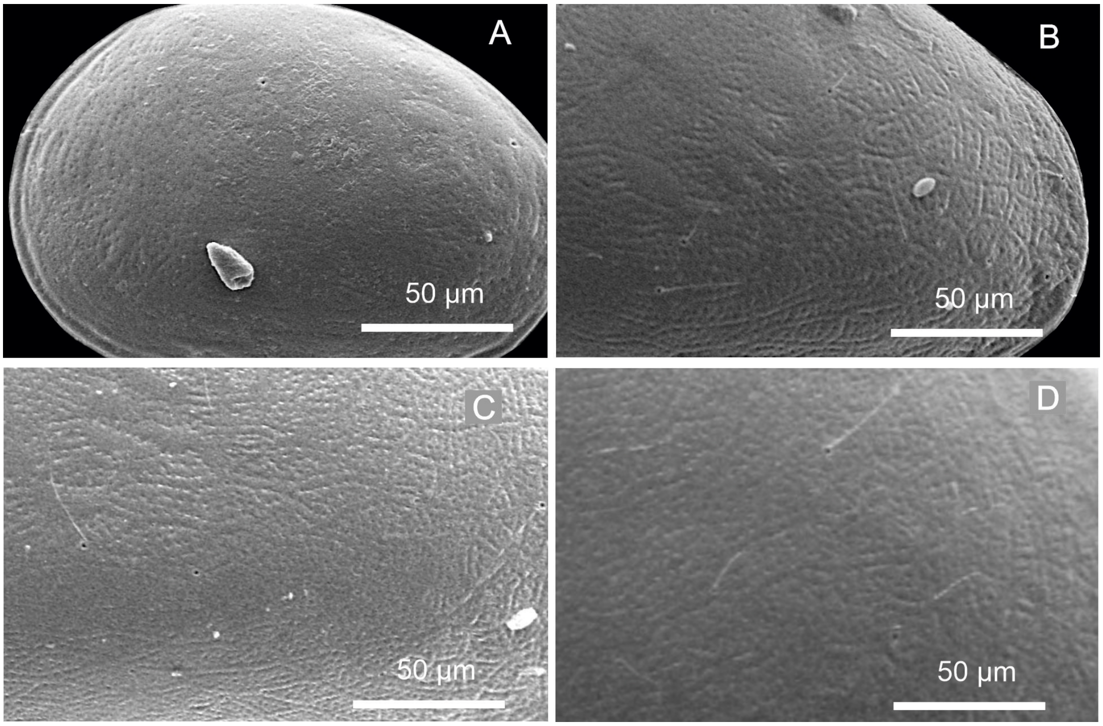

Figure 4. Close-up views of the left valves of juvenile instars of *Heterocypris exodonta* from Nam Co, Tibetan Plateau (NC-0919): **(A)** A-8, **(B)** A-6, **(C)** A-4, and **(D)** A-2, showing the progressive development of the reticulation pattern.

Table 3. Mean values and standard deviations of length and height of right (RVs = 424) and left (LVs = 401) valves and growth ratios between successive instars of *Heterocypris exodonta* collected from Nam Co, Tibetan Plateau; *n* denotes number of valves.

	Size and growth ratios					
Instar and growth phase	RV		*n*	LV		*n*
	Length [µm]	Height [µm]		Length [µm]	Height [µm]	
Adult	1131 ± 106	629 ± 56	20	1144 ± 115	637 ± 58	18
A-1 to adult	1.232	1.255		1.239	1.229	
A-1	918 ± 9	501 ± 13	14	923 ± 81	518 ± 12	14
A-2 to A-1	1.150	1.123		1.150	1.135	
A-2	798 ± 29	446 ± 20	22	802 ± 38	456 ± 19	15
A-3 to A-2	1.212	1.198		1.191	1.193	
A-3	658 ± 37	372 ± 19	75	673 ± 29	382 ± 17	75
A-4 to A-3	1.272	1.227		1.265	1.216	
A-4	517 ± 21	303 ± 13	76	532 ± 21	314 ± 11	76
A-5 to A-4	1.239	1.212		1.266	1.231	
A-5	417 ± 23	250 ± 12	86	420 ± 26	255 ± 15	72
A-6 to A-5	1.263	1.243		1.268	1.220	
A-6	330 ± 12	201 ± 10	47	331 ± 14	209 ± 10	45
A-7 to A-6	1.250	1.250		1.239	1.229	
A-7	264 ± 7	168 ± 6	30	267 ± 12	170 ± 11	52
A-8 to A-7	1.281	1.208		1.271	1.188	
A-8	206 ± 9	139 ± 7	34	210 ± 11	143 ± 6	34
Eggs	129 ± 7		20			
Mean growth ratios	1.237	1.214		1.236	1.205	

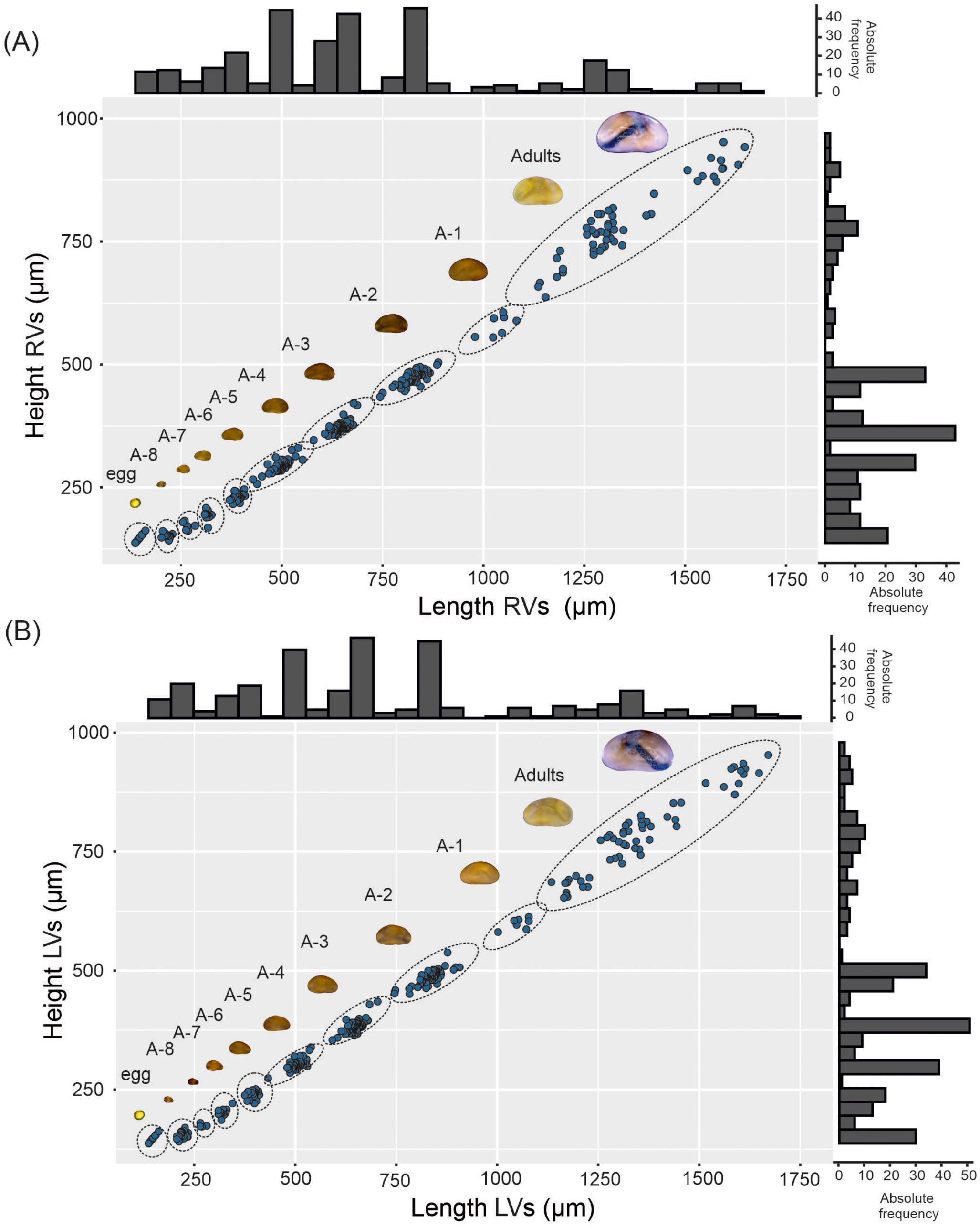

Figure 5. Valve ontogeny of *Heterocypris incongruens* from Mexico City, Mexico (HI-M19). The scatterplot shows the length and height of **(A)** right valves (RVs), $n = 300$, and **(B)** left valves (LVs) $n = 280$. The histograms display the abundances of eggs, juvenile instars, and adult stage.

Adult RVs were 1039 µm long and 622 µm high; LVs were 1045 µm long and 638 µm high (Table 4). The mean growth ratio (length / height) for RVs was 1.246 and 1.211, and for LVs, it was 1.252 and 1.227, respectively (Table 4).

Habitat. Eurytopic species; tolerates high values of water conductivity and organic pollution; found in shallow ponds and temporary pools (Mezquita et al., 1999; Meisch, 2000).

5 Discussion

5.1 Egg morphology and ecological significance

It was observed that the eggs of the three *Heterocypris* species share a remarkably similar overall morphology, including an ellipsoidal shape, comparable size, and a relatively resistant external appearance. These similarities suggest a shared adaptation to ephemeral aquatic environments.

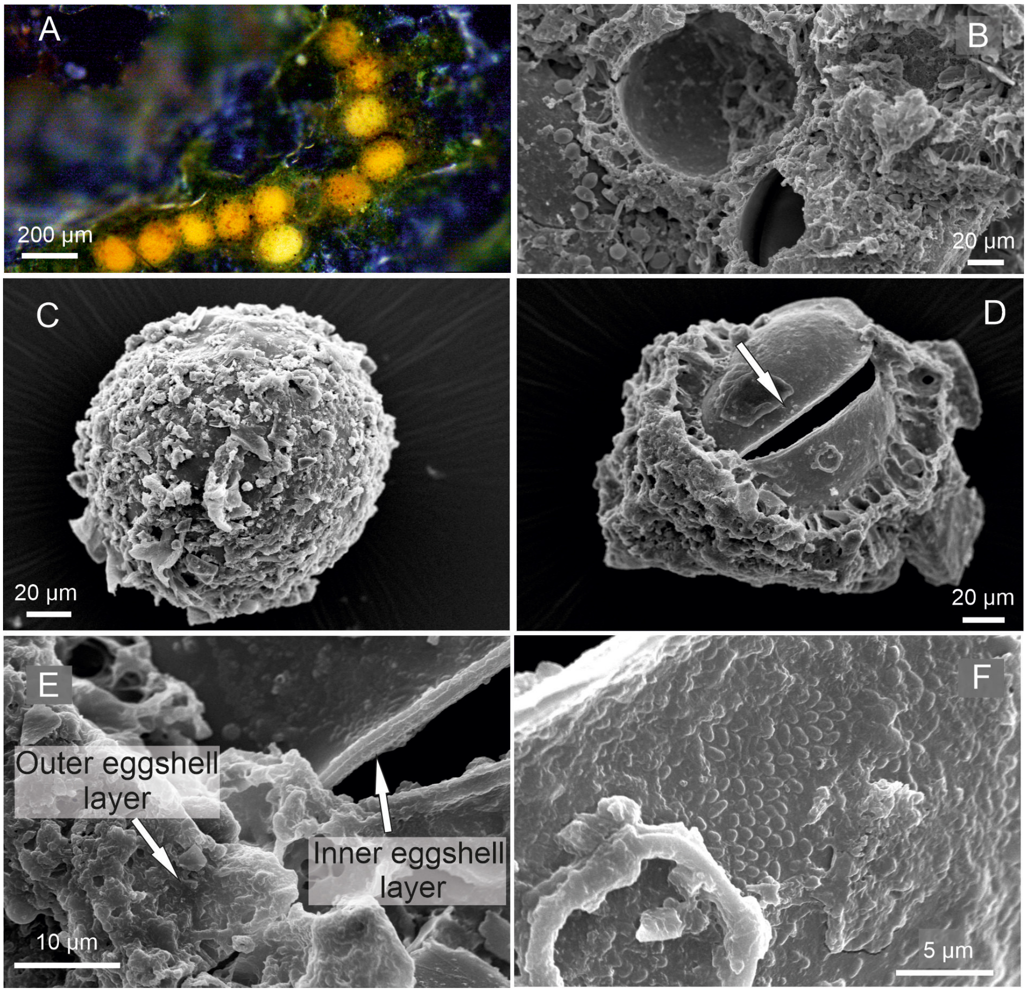

Figure 6. *Heterocypris incongruens.* **(A)** Egg cluster attached to a substrate, **(B)** egg showing an irregular opening after hatching, **(C)** unhatched egg with the outer eggshell layer showing adhered external particles, **(D)** irregular opening indicated by the arrow, **(E)** close-up view of the inner and outer eggshell layers, **(F)** outer surface of inner layer of the eggshell displaying small protuberances.

However, surface features are not uniform across ostracod taxa; for instance, *Chlamydotheca arcuata* (Sars, 1901) exhibits tuberculate ornamentation (Díaz and Lopretto, 2017), indicating that broader comparative studies are required to better understand the diversity and evolutionary significance of egg morphology.

The eggs of *Heterocypris exodonta*, *H. incongruens*, and *H. salina* have two eggshell layers, with the outer layer displaying a rigid-looking roughness, which is vital for protecting the embryo. The structure of ostracod eggs has been described in classical histological studies, which demonstrate that the eggshell is formed during oogenesis and consists of two layers, including an inner layer produced by the oocyte and an outer layer secreted by the ovarian epithelium (Woltereck, 1898; Weygoldt, 1960; Hartmann, 1968). In this context, the interpretation of eggshell morphology based solely on SEM observations should be approached with caution. Features such as a rough or porous surface may not represent primary structural characteristics but instead result from post-depositional processes, including water uptake and expansion of the outer layer (Wohlgemuth, 1914).

Furthermore, early embryological studies have demonstrated that ostracod structures arise through progressive differentiation and proliferation of tissues rather than as static or discrete layers (Müller-Calé, 1913). This dynamic developmental process may lead to misleading structural interpretations when observations are based solely on surface imaging techniques.

Consequently, previously described "layers" based on SEM observations (e.g., Dumont et al., 2002; Özuluğ and Suludere, 2012) for *Heterocypris incongruens* should be reconsidered as they may reflect optical or structural artifacts rather than true biological organization. Similarly, particles or external coatings observed on the egg surface are not intrinsic components of the eggshell but are likely associated with adhesive secretions produced during oviposition.

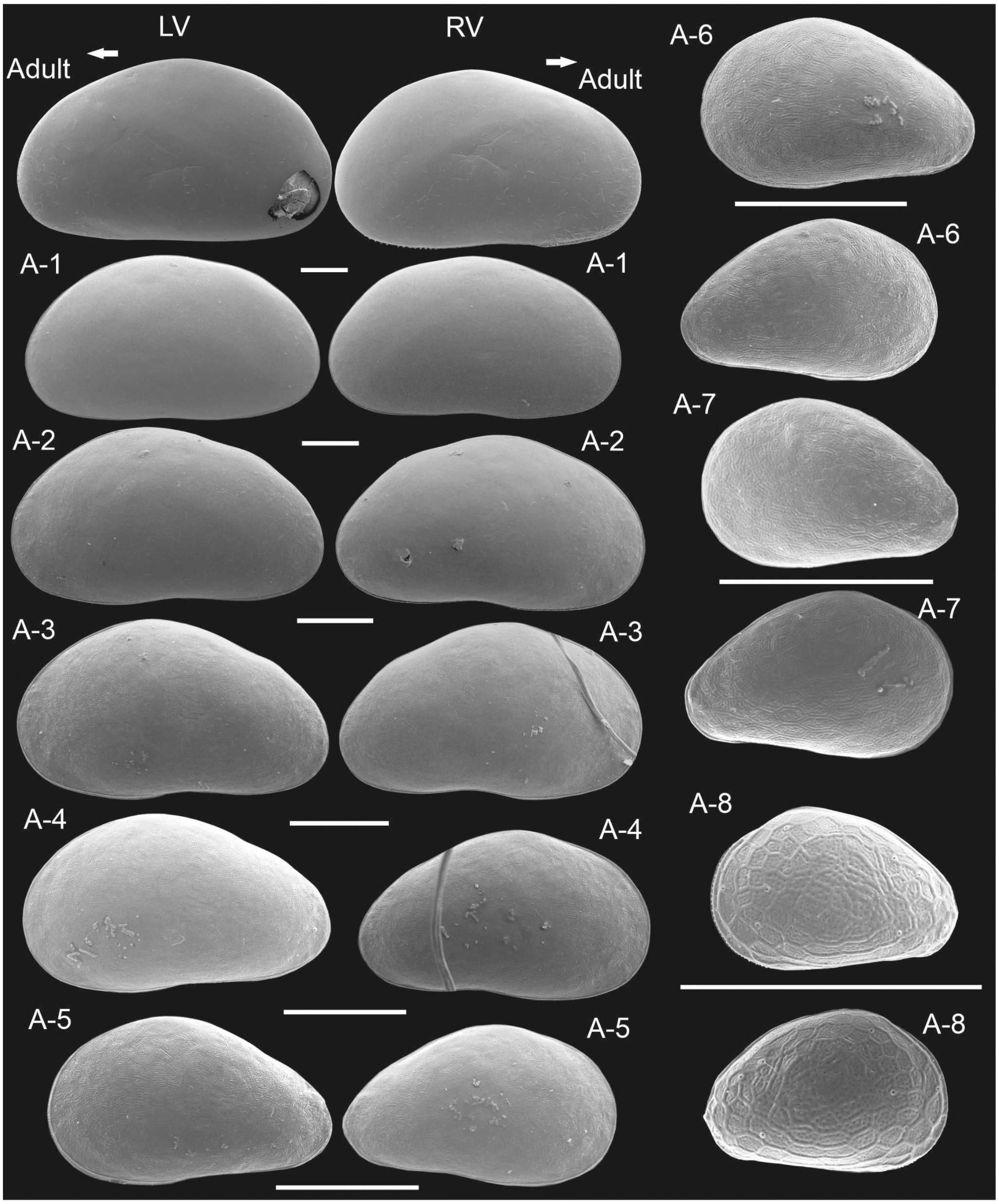

Figure 7. Left (LVs) and right valves (RVs) of adult and juvenile instars (A-1 to A-8) of *Heterocypris incongruens* from Mexico City, Mexico (HI-M19). All instars are shown at the largest possible magnification to enhance visualization of valve morphology and surface features. The arrow points anteriorly. Scale bars show 200 µm.

These findings highlight the importance of integrating classical histological knowledge with modern imaging techniques to avoid misinterpretations of ostracod egg morphology. Despite these limitations, the eggs of *Heterocypris* species are known to exhibit high resistance to environmental stressors, including desiccation and extreme temperatures (−18 to 42 °C), and can remain viable in a dormant state for over 20 years (Angell and Hancock, 1989). Future experimental studies should focus on testing egg resistance under controlled environmental conditions, including temperature extremes and simulated drought, to better understand hatching success, survival, and developmental rates.

In ecological terms, resistant eggs play a key role in the persistence of ostracod populations in temporary and unpredictable environments. However, their production and hatching dynamics are not controlled by temperature alone. Experimental studies on *Heterocypris incongruens* have shown that both temperature and photoperiod influence the proportion of subitaneous versus diapausing eggs, as well as their hatching timing and success (Rossi et al., 1996, 2013). In addition,

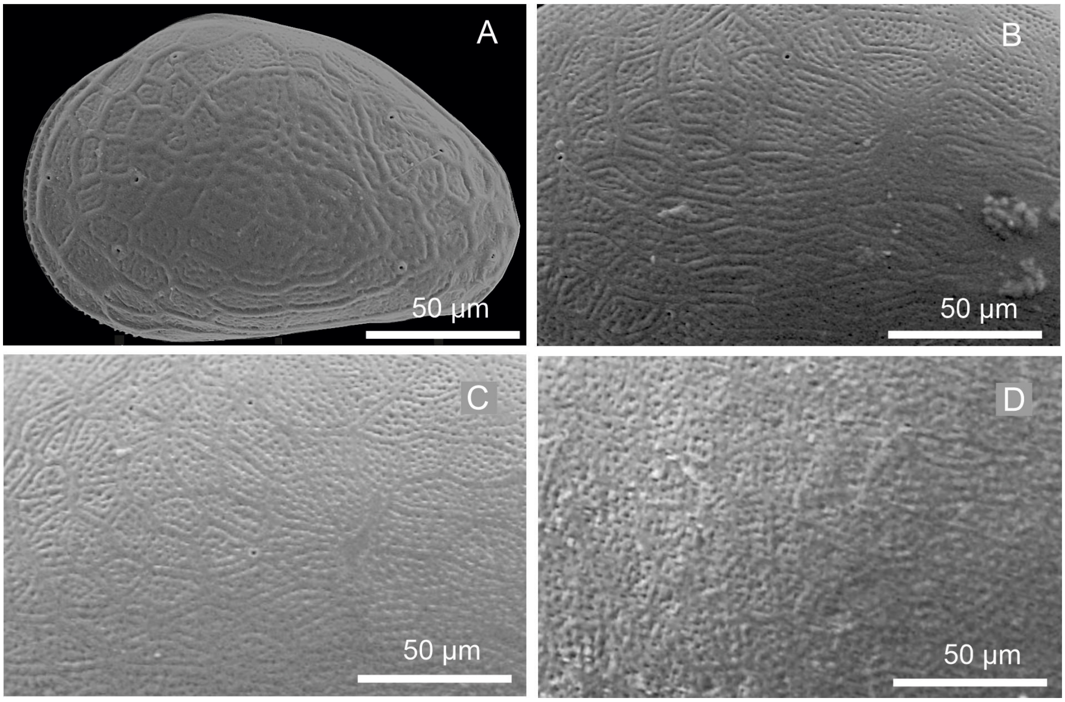

Figure 8. Close-up views of the left valves of juvenile instars: **(A)** A-8, **(B)** A-6, **(C)** A-4, and **(D)** A-2, showing densely reticulated surface pattern.

Table 4. Mean values and standard deviations of length and height of right (RVs = 300) and left (LVs = 280) valves and growth ratios between successive instars of *Heterocypris incongruens* collected from Mexico City; *n* denotes number of valves.

Instar and growth phase	Size and growth ratios					
	RV		*n*	LV		*n*
	Length [µm]	Height [µm]		Length [µm]	Height [µm]	
A	1349 ± 140	790 ± 75	52	1360 ± 146	788 ± 83	57
A-1 to adult	1.302	1.360		1.291	1.315	
A-1	1036 ± 31	580 ± 21	7	1053 ± 26	599 ± 26	7
A-2 to A-1	1.255	1.228		1.262	1.232	
A-2	825 ± 27	472 ± 14	58	834 ± 29	486 ± 15	58
A-3 to A-2	1.287	1.272		1.289	1.272	
A-3	641 ± 17	371 ± 11	69	647 ± 18	382 ± 13	64
A-4 to A-3	1.292	1.266		1.283	1.264	
A-4	496 ± 22	293 ± 12	51	504 ± 14	302 ± 10	45
A-5 to A-4	1.271	1.273		1.269	1.253	
A-5	390 ± 9	230 ± 7	21	397 ± 12	241 ± 11	20
A-6 to A-5	1.242	1.185		1.236	1.193	
A-6	314 ± 4	194 ± 9	13	321 ± 9	202 ± 7	13
A-7 to A-6	1.180	1.134		1.188	1.160	
A-7	266 ± 10	171 ± 8	6	270 ± 7	174 ± 3	4
A-8 to A-7	1.231	1.132		1.227	1.122	
A-8	216 ± 8	151 ± 4	12	220 ± 8	155 ± 6	12
Eggs	147 ± 7		11			
Mean growth ratios	1.257	1.231		1.255	1.226	

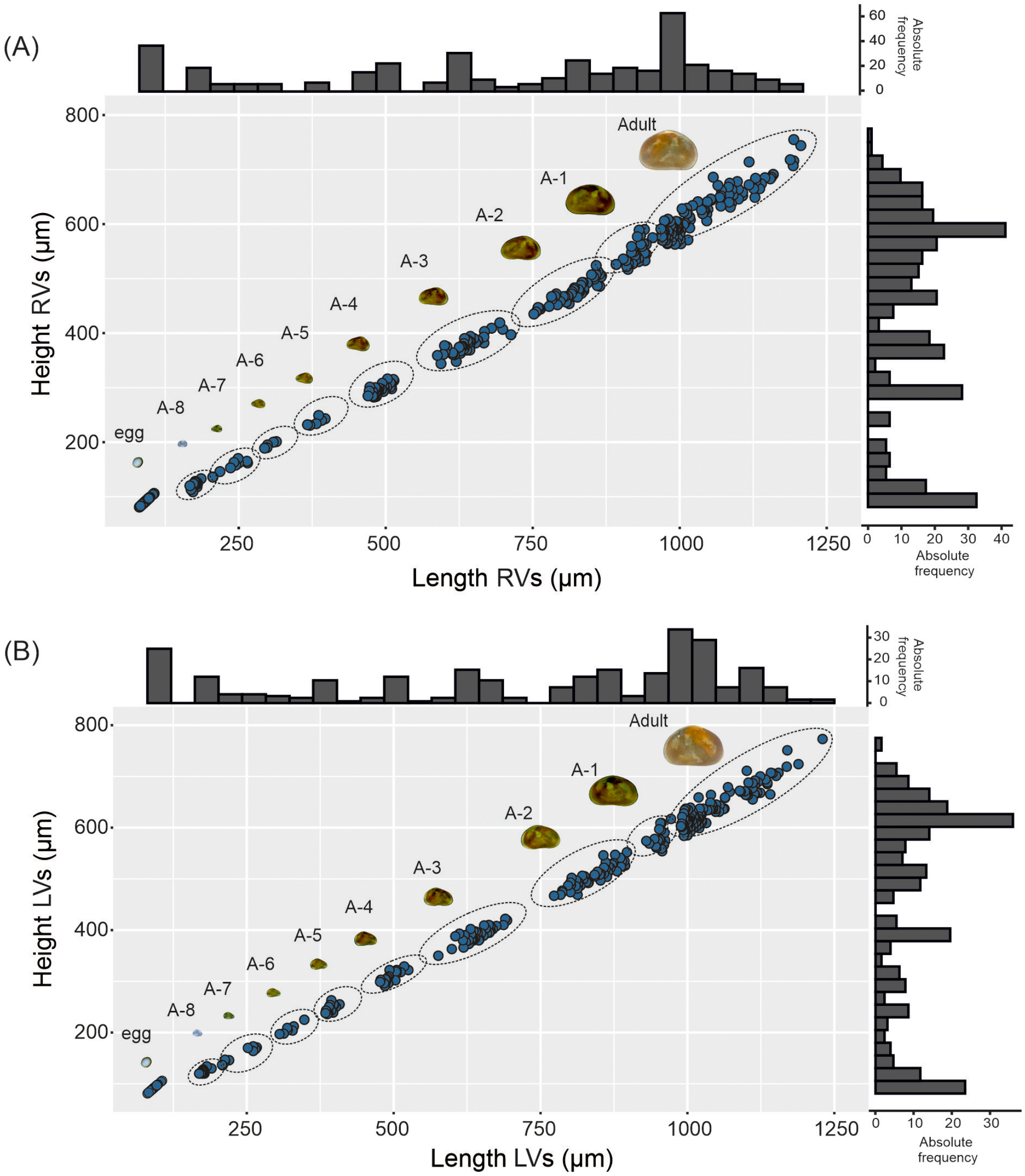

Figure 9. Valve ontogeny of *Heterocypris salina* from Braunschweig, Germany (HS-G19). The scatterplot shows the length and height of **(A)** right valves (RVs), $n = 300$, and **(B)** left valves (LVs), $n = 287$. The histograms display the abundances of eggs, juvenile instars, and the adult stage.

temperature affects growth, development, and fecundity in complex and often non-linear ways rather than acting as a single deterministic factor (Aguilar-Alberola and Mesquita-Joanes, 2014). These environmental cues interact with maternal effects and genotype-specific responses, influencing egg size, structure, and hatching phenology and contributing to variability in life history strategies (Rossi et al., 2013).

In this study, eggs of *H. exodonta*, *H. incongruens,* and *H. salina* exhibited clear interspecific differences in size, with mean values of 129 ± 7 µm ($n = 20$), 147 ± 7 µm ($n = 11$), and 91 ± 6 µm ($n = 31$), respectively. These differences are noteworthy given that all species were maintained under the same laboratory conditions (18–23 °C; 14:10 h light: dark photoperiod), suggesting that egg size variation reflects intrinsic species-specific traits rather than environmental variation alone. However, previous studies have demonstrated that egg size and hatching phenology in *Heterocypris* can be modulated by environmental conditions, particularly photoperiod, as well as maternal effects (Rossi et al., 2013). Therefore, although the controlled conditions in this study allow for direct comparison among species, they may not capture the full extent of phenotypic plasticity observed in natural populations.

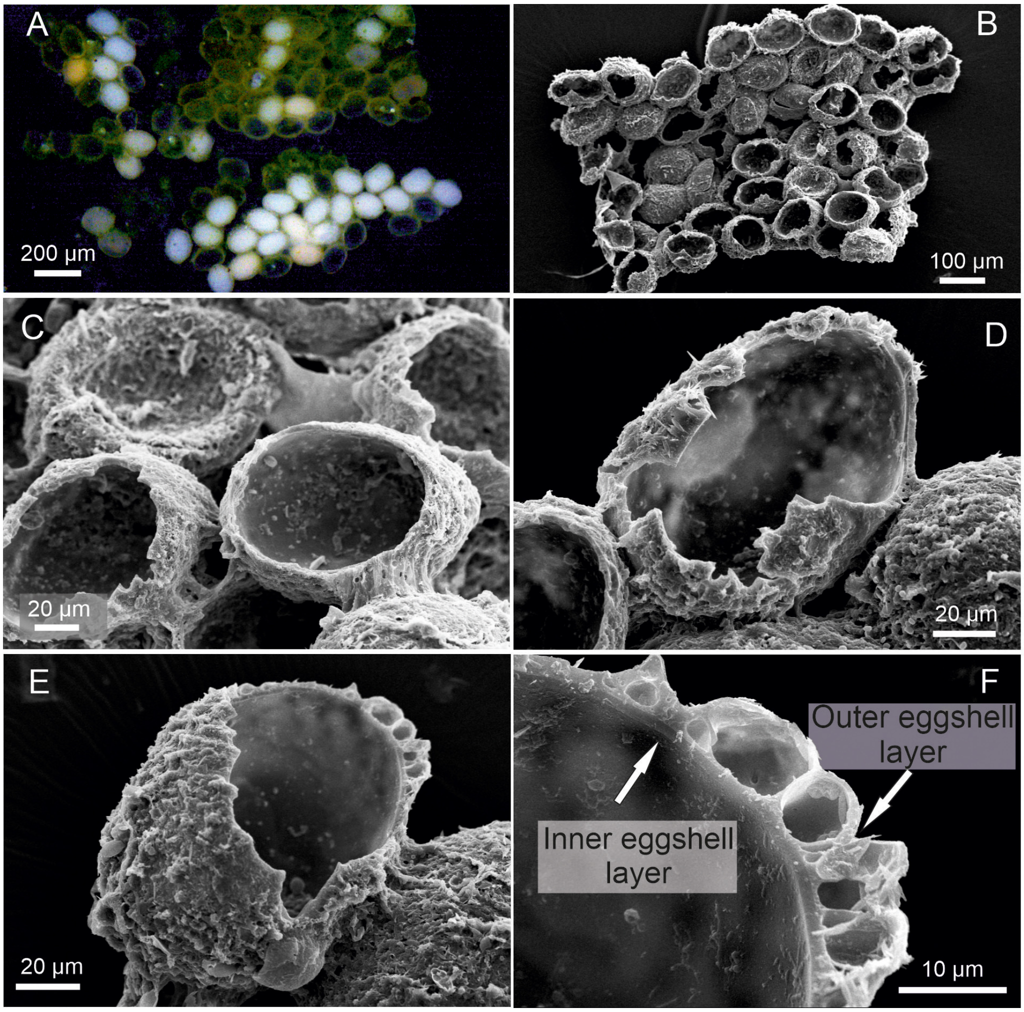

Figure 10. *Heterocypris salina*. **(A–B)** Egg clusters, **(C)** adhesive material between eggs, **(D–E)** eggs showing an irregular opening after hatching with exposed eggshell layers, **(F)** close-up view of the eggshell illustrating the inner and outer eggshell layers.

Furthermore, resting eggs accumulate in sediments, forming egg banks, which act as ecological reservoirs that enhance resilience and facilitate recolonization after disturbance (Rosa et al., 2020). These egg banks contribute to metapopulation dynamics by enabling local extinction and recolonization processes (Rossi and Menozzi, 2012) and may be spatially redistributed by hydrological processes such as flooding (Rosa et al., 2020). In addition, hatching patterns are influenced by species traits and phylogenetic relationships, as well as environmental cues, resulting in staggered emergence and promoting population persistence under fluctuating conditions (Rosa et al., 2021). The persistence of dormant stages, together with broader "ostracod banks", further enhances survival and recolonization capacity in highly variable habitats (Wang et al., 2025).

Finally, it is important to consider that the egg characteristics observed in this study may be influenced by laboratory conditions, including constant temperature and photoperiod regimes. Although these conditions were necessary to ensure comparability among species and to meet the objectives of describing valve ontogeny, characterizing egg morphology and evaluating growth ratios, they may affect egg production, size, structure, and diapause expression, as shown in previous experimental studies (Rossi et al., 1996, 2013). Therefore, the results should be interpreted with caution when extrapolating to natural populations. Despite this limitation, our study provides a comparative framework for understanding interspecific differences in egg morphology and developmental patterns among closely related species from distinct geographic regions. Further studies are needed to evaluate how environmental variability influences egg traits, viability, and hatching success, as well as the physiological and genetic mechanisms underlying these responses (Bellin et al., 2020; Horne and Martens, 1998; Delorme, 2011).

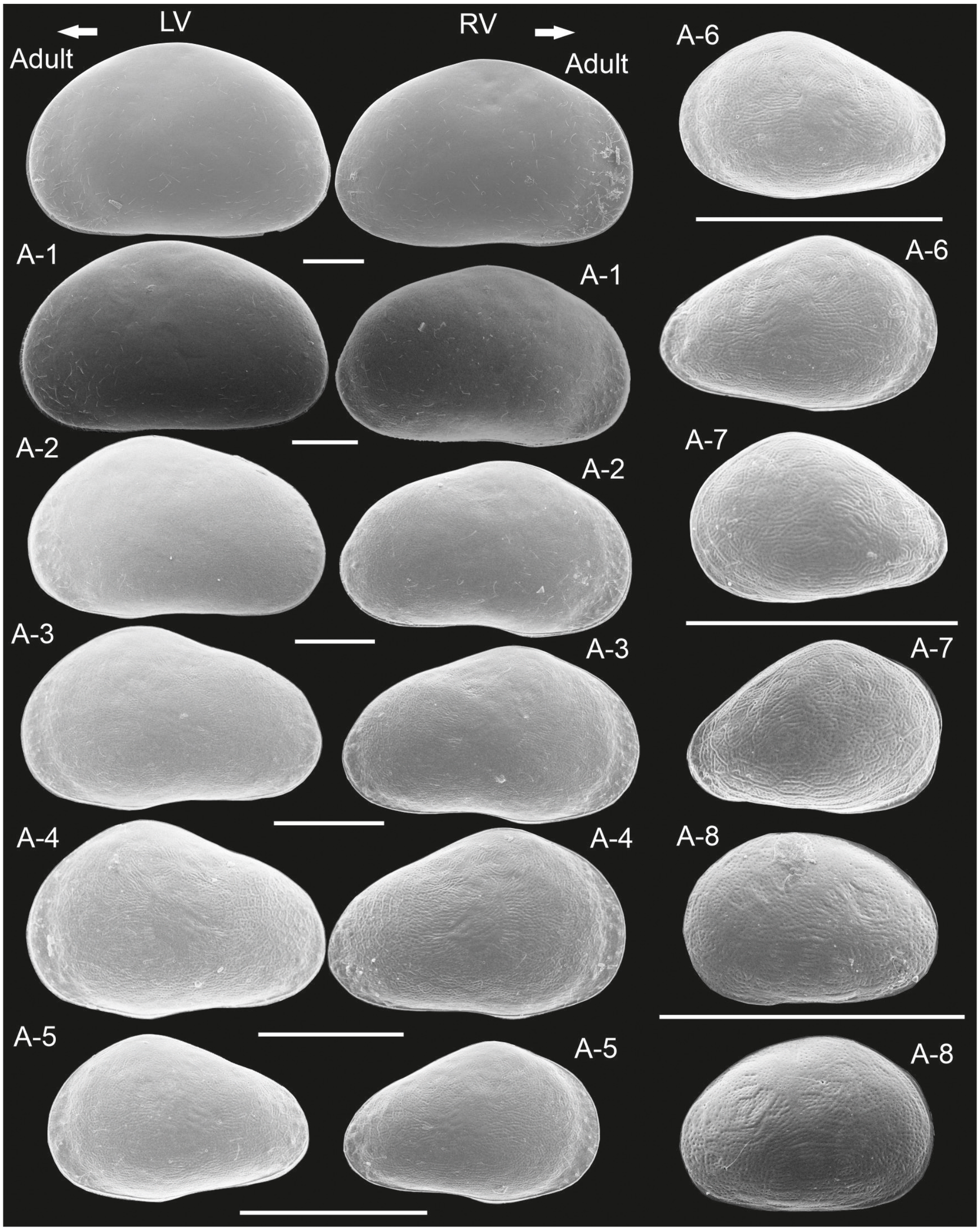

Figure 11. Left (LVs) and right valves (RVs) of adult and juvenile instars (A-1 to A-8) of *Heterocypris salina* from Braunschweig, Germany (HS-G19). All instars are shown at the largest possible magnification to enhance visualization of valve morphology and surface features. The arrow points anteriorly. Scale bars show 200 µm.

5.2 Ontogeny and taxonomic implications

The ontogenies of *H. exodonta*, *H. incongruens*, and *H. salina* (Fig. 13), in terms of the number of molting instars of the valves, are consistent with the general pattern described for podocopid ostracods. All three species exhibit eight juvenile instars followed by a single adult stage, in agreement with previous observations from Marburg, Germany (Schreiber, 1922); Bogotá, Colombia (Roessler, 1983); Büyükçekmece Lake, Istanbul (Kubanç et al., 2007); and temporary pools on the Iberian Peninsula (Aguilar-Alberola and Mezquita, 2008). However, deviations from this standard nine-stage ontogenetic pattern have been reported in some *Heterocypris* species. In particular, the presence of an additional early developmental stage (A-9), interpreted as a prenaupliar stage, has been documented in *H. bogotensis*, *H. bosniaca*, and *H. incongruens* (Roessler, 1983; Aguilar-Alberola and Mesquita-Joanes, 2013; Rossi et al., 2015), in-

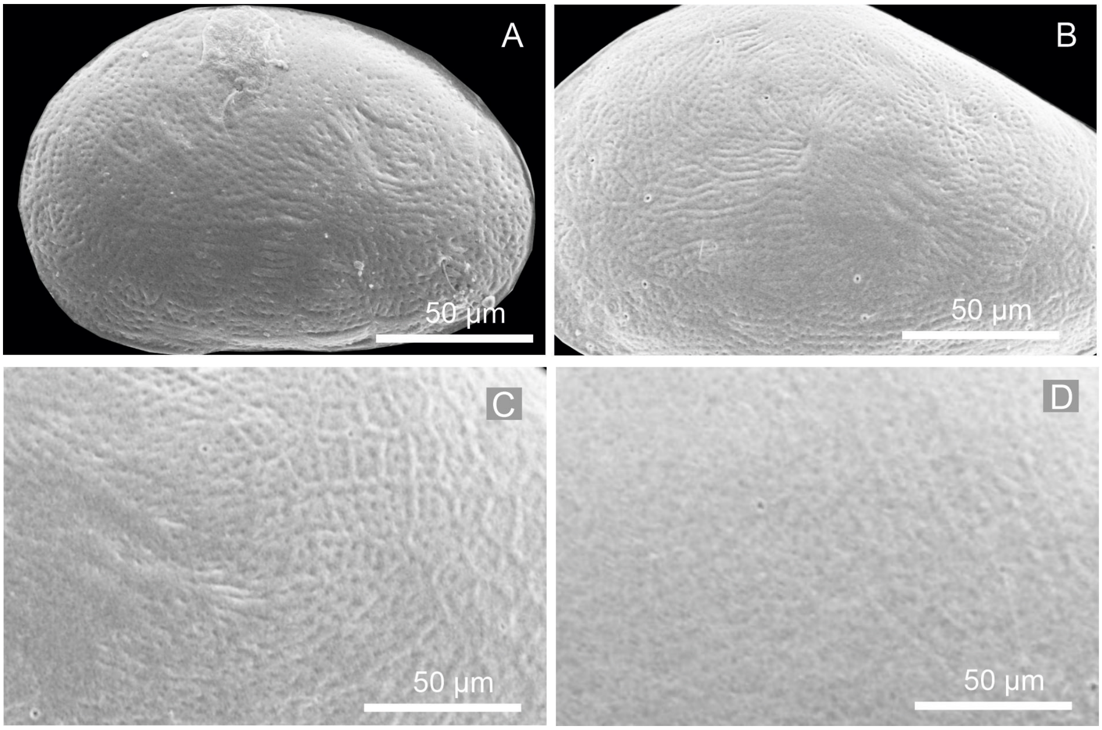

Figure 12. Close-up views of the left valves of juvenile instars: **(A)** A-8, **(B)** A-6, **(C)** A-4, and **(D)** A-2, showing the reticulated surface pattern, which becomes progressively less pronounced and more diffuse in the A-2 instar.

Table 5. Mean values and standard deviations of length and height of right (RVs = 331) and left (LVs = 287) valves and growth ratios between successive instars of *Heterocypris salina* collected from Braunschweig, Germany; *n* denotes number of valves.

	Size and growth ratios					
Instar and growth phase	RV		*n*	LV		*n*
	Length [µm]	Height [µm]		Length [µm]	Height [µm]	
A	1039 ± 64	622 ± 44	110	1045 ± 55	638 ± 37	119
A-1 to adult	1.119	1.130		1.098	1.103	
A-1	928 ± 14	550 ± 21	29	951 ± 9	578 ± 16	17
A-2 to A-1	1.128	1.150		1.132	1.142	
A-2	822 ± 35	478 ± 23	49	840 ± 33	506 ± 22	47
A-3 to A-2	1.290	1.264		1.308	1.297	
A-3	637 ± 27	378 ± 15	43	642 ± 25	390 ± 15	38
A-4 to A-3	1.300	1.272		1.286	1.258	
A-4	490 ± 12	297 ± 9	32	499 ± 22	310 ± 14	20
A-5 to A-4	1.282	1.147		1.269	1.255	
A-5	382 ± 11	238 ± 7	6	393 ± 7	247 ± 8	14
A-6 to A-5	1.264	1.220		1.216	1.193	
A-6	302 ± 8	195 ± 5	5	323 ± 13	207 ± 9	7
A-7 to A-6	1.208	1.211		1.368	1.335	
A-7	250 ± 10	161 ± 5	7	236 ± 26	155 ± 15	10
A-8 to A-7	1.381	1.298		1.340	1.240	
A-8	181 ± 13	124 ± 8	19	176 ± 5	125 ± 8	15
Eggs	91 ± 6		31			
Mean growth ratios	1.246	1.211		1.252	1.227	

dicating that ontogenetic patterns within the genus may be more variable than traditionally assumed. In our study, this additional stage was not observed, which may be related to sampling limitations or to the difficulty of detecting early developmental stages, given the fragility of the exuviation process and the rapid succession of molts.

Ontogenetic analysis remains a fundamental tool in ostracod taxonomy as morphological changes through successive instars provide key characters for species identification and for interpreting phylogenetic relationships (Brooks, 1886; Shaver, 1953; Watabe and Kaesler, 2004; Boomer et al., 2003; Danielopol et al., 2008). In this context, our study extends previous work by providing a complete ontogenetic framework for *H. incongruens* and *H. salina* based on SEM observations. Although Schreiber (1922) presented detailed drawings of *H. incongruens*, high-resolution documentation of surface features across all instars has remained limited until now.

Our SEM images show that valve ornamentation changes throughout development rather than remaining static. Reticulation patterns have also been reported in other non-marine ostracods, including *Cypris pubera* (Yousef et al., 2024), *Chlamydotheca arcuata* (Díaz and Lopretto, 2017), *Eucypris virens* (Smith and Martens, 2000), and *Strandesia bicuspis* (Liberto et al., 2014), and in some candonids such as *Candona xizangensis* (Akita et al., 2016) and *Fabaeformiscandona myllaina* (Smith and Kamiya, 2007). In the three *Heterocypris* species studied here, reticulation follows a clear ontogenetic trend: early instars (A-8 to A-2) display a well-developed and variable polygonal pattern, including hexagonal and irregular forms, which becomes progressively weaker in later stages. This pattern is consistent across *H. exodonta*, *H. incongruens,* and *H. salina*, with minor interspecific differences in terms of its persistence, and is comparable to that observed in juvenile instars of *H. bosniaca* (Aguilar-Alberola and Mezquita, 2008; Aguilar-Alberola and Mesquita-Joanes, 2013). Taken together, these results indicate that valve ornamentation alone is not a reliable taxonomic character, but it becomes informative when evaluated in relation to developmental stage.

Despite being widely distributed and eurytopic species (Meisch, 2000), detailed ontogenetic descriptions for *H. incongruens* and *H. salina* have been scarce, particularly regarding fine surface morphology. Our results, therefore, contribute new data that refine the morphological characterization of these species and improve the identification of juvenile stages, which are often problematic in taxonomic studies.

Overall, our findings highlight the importance of integrating ontogenetic analysis with high-resolution imaging techniques to better understand morphological variability and improve species discrimination. However, given the observed variability in developmental patterns and valve ornamentation, ontogenetic characters should be used cautiously and in combination with other morphological or molecular evidence.

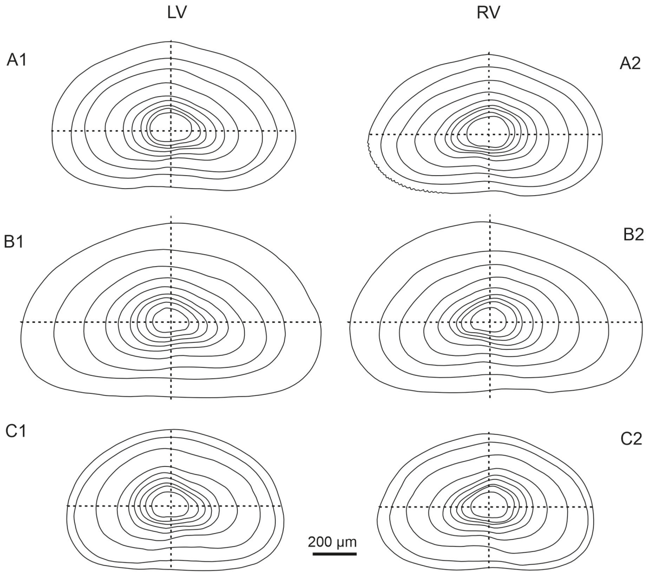

Figure 13. Comparison of external valve outlines across nine ontogenetic instars (from juvenile A-8 to adult). Left (LVs) and right (RVs) valves of **(A1–A2)** *Heterocypris exodonta*, **(B1–B2)** *H. incongruens*, and **(C1–C2)** *H. salina*.

The frequent occurrence of *H. incongruens* and *H. salina* (Meisch, 2000) has long been recognized. However, despite their prevalence, detailed comparative analyses of these species are missing. The contour diagrams presented herein represent a significant step forward in elucidating the ontogenetic development of these ostracod species, providing valuable insights into their morphological similarities and differences. By comparing the outlines of the valves (Fig. 13), differences in shapes among species become apparent, particularly in early instars. In A-8, *H. exodonta* and *H. salina* display a more rounded outline, whereas *H. incongruens* tends to exhibit a more triangular shape. Although early instars may be more flexible and potentially prone to deformation, no clear evidence of preparation artifacts was observed in our material. Therefore, the observed differences are more likely to reflect interspecific variation rather than deformation during SEM processing. However, this interpretation should still be approached with caution as previous observations by Schreiber (1922) show a more rounded outline for the right valve of *H. incongruens*, suggesting that some degree of variability may occur. In addition, the presence of cryptic species within *H. incongruens* may contribute to subtle differences in early ontogenetic stages. Apart from this consideration, the outlines of later instars are more consistent and closely resemble the triangular or conical shapes observed in this study. Furthermore, the A-5 to A-3 juvenile valves of *H. salina* feature a notably rounded dorsal region, unlike the straighter profiles of *H. exodonta* and *H. in-*

congruens. The contour diagrams presented here will serve as crucial benchmarks for future studies, providing a comprehensive understanding of the ontogenetic development of these ostracods and facilitating comparisons with related species.

Our dataset shows that growth ratios in the three species studied converge on average values close to those predicted by Brooks' rule for crustaceans (~ 1.26). However, rather than indicating a strictly predictable pattern, the data reveal considerable variability both among species and across developmental stages. This variability is also evident in the literature, where growth factors span a relatively wide range (Table 6), suggesting that geometric growth represents a general tendency rather than a fixed rule. Although subtle, the observed differences in growth ratios among the studied species do not provide sufficiently consistent information to serve as a reliable diagnostic character for distinguishing closely related taxa with minimal morphological differences. Therefore, confirming the taxonomic utility of growth ratios remains challenging and requires additional data from closely related species, as well as broader geographic and environmental sampling (Brooks, 1886; Watabe and Kaesler, 2004). Furthermore, as noted by Kesling (1952), growth factors should be interpreted with caution as increases in valve size may lead to variations in growth values, and adult stages may differ functionally and morphologically from juvenile instars. In particular, the larger increment observed during the final molt has been attributed to the development of reproductive structures at maturity, which may contribute to deviations from earlier ontogenetic growth patterns (Kesling and Crafts, 1962).

The life cycle study of *Darwinula stevensoni* living in a temporary pond in Belgium from spring to summer revealed a valve length growth ratio of 1.236. The authors suggest that temperature was identified as an important factor influencing developmental timing as higher temperatures were associated with faster development and earlier reproductive maturity (Van Doninck et al., 2003). Similar effects of temperature on growth, including size and growth ratios, have been documented in *Heterocypris*, where thermal conditions influence developmental rates and life history traits (Aguilar-Alberola and Mesquita-Joanes, 2014). In our study, the three *Heterocypris* species were cultured at 18–23 °C and exhibited relatively short life cycles (approximately 35–60 d). However, these observations should be interpreted with caution as temperature interacts with other factors such as photoperiod and intrinsic biological variability, which may also influence growth and development. Likewise, in personal observations, life cycles are slower during winter, with few molts from juveniles to adults in this season. It appears to be the case that juveniles (A-3 to A-1) enter a dormant phase in which they do not molt until they receive an environmental stimulus that signals for them to molt again; in this case, it could be the temperature acquired from sunlight. The observation was made only when the ostracods were near the window where the sunlight was strongest.

Ostracods are opportunistic feeders, and food quality can influence growth and molting (Schmit et al., 2007). In our laboratory observations, increased bacterial growth under winter light conditions may indicate stable culture conditions and frequent molting, suggesting that microbial availability may support these processes, although this relationship requires further investigation. Additionally, it is important to test for competition and potential cannibalism within the population under food-limited conditions as individuals may resort to cannibalism when alternative food sources are scarce (Herman and Heip, 1982).

For species with wide distributions, ontogenetic processes should be described and documented more in the future to fully understand morphological variations in valves and the role of different environmental factors (e.g., ionic concentration and composition, temperature, and dissolved oxygen) (Song et al., 2023) and their effects on development.

Shaver (1953) suggested using growth factors for the taxonomic characterization of supra-specific groups, underscoring the indispensability of ontogenetic studies for resolving questions related to ancestry between taxa. By identifying the similarities and differences in ontogenetic trajectories of ostracod species, these studies can provide insights into their evolutionary connections (Smith and Kamiya, 2002). However, the number of species for which these growth factors and intra-specific variability are known is still limited.

Limited variation in growth patterns may reflect shared evolutionary relationships; however, such patterns are also influenced by factors such as adult body size and egg size, which can vary across ostracod groups. As suggested by the comparative data in Table 6, differences among major taxa (e.g., Lymnocytheridae, Darwinulidae, Candonidae, and Cyprididae) may therefore be better understood in the context of both phylogenetic relationships and size-related constraints.

However, it is important to note that the degree of variation alone may not always indicate genetic connections as environmental factors can also influence growth patterns (Oakley et al., 2012; Vences et al., 2024). Finally, exploring ontogeny unveils how specific morphological traits have evolved. This information is essential for elucidating the evolutionary history of these microcrustaceans, contributing to a comprehensive assessment of their significance in aquatic ecosystems and biodiversity (Horne et al., 2004).

Our contributions include SEM photographs detailing valve surface morphology and ontogenetic changes in three *Heterocypris* species. Beyond their taxonomic value, our results provide insight into growth patterns in ostracods. As organisms with determinate growth, ostracods reach a fixed adult size after the final moult, and growth ratios are influenced by multiple factors, including the relationship between egg size and final body size. Comparative data (Table 6) show that growth factors vary across taxa, with larger species gen-

Table 6. Growth factors for non-marine ostracod species from this study are compared with data from the literature. Quotation marks (“) indicate repetition of the species name and reference. The symbol (?) indicates that information is uncertain or not available.

Superfamily	Family	Species	Site	Growth factor	Data source
Cytheroidea	Limnocytheridae	*Cytherissa lacustris*	Mondsee, Austria	1.27	Danielopol et al. (2008)
“	“	“	Iseo, Italy	1.26	“
Cytheroidea	Limnocytheridae	*Limnocythere inopinata*	Lake Daihai, China	1.22	Zhai et al. (2015)
“	“	“	Lake Dali, China	1.23	“
“	“	“	Lake Hulun, China	1.21	“
“	“	“	Lake Jiang-Co, Tibetan Plateau	1.16	Wang et al. (2021)
Cytheroidea	Limnocytheridae	*Elpidium bromeliarum*	São Paulo, Brazil (bromeliads)	1.23–1.27	Pereira et al. (2017)
Cypridoidea	Darwinulidae	*Darwinula stevensoni*	Temporary pond, Belgium	1.23	Van Doninck et al. (2003)
“	“	“	Lake Pääjärvi, Finland	1.21–1.235	Ranta (1979)
Cypridoidea	Cytherideidae	*Cyprideis torosa*	Dievengat, Belgium	1.268	Herman and Heip (1982)
“	“	“	Santa Pola, Spain	1.266	Mezquita et al. (2000)
Cypridoidea	Candonidae	*Candona candida*	Lake Hańcza, Poland	1.26	Danielopol et al. (2008)
“	“	“	Mondsee, Austria	1.23	“
“	“	“	Iseo, Italy	1.27	“
Cypridoidea	Candonidae	*Candona neglecta*	Lake Hańcza, Poland	1.26	“
“	“	“	Mondsee, Austria	1.26	“
Cypridoidea	Candonidae	*Candona cf. neglecta*	Iseo, Italy	1.27	“
Cypridoidea	Candonidae	*Candona whitei*	Tertiary	1.202	Anderson (1964)
Cypridoidea	Candonidae	*Fabaeformiscandona gyirongensis*	Lake Dalongchi, China	1.25–1.36	Mao et al. (2021)
Cypridoidea	Cyprididae	*Cypridopsis vidua*	Illinois?, USA	1.20	Kesling (1951)
“	“	“	Recent	1.229	Anderson (1964)
Cypridoidea	Cyprididae	*Cyclocypris ovum*	Recent	1.175	Anderson (1964)
“	“	“	Lake La Cruz, Spain	1.15	Mezquita et al. (2002)
Cypridoidea	Cyprididae	*Dolerocypris fasciata*	Recent	1.349	Anderson (1964)
Cypridoidea	Cyprididae	*Chlamydotheca arcuata*	Buenos Aires Province, Argentina	∼ 1.39	Díaz and Lopretto (2017)
Cypridoidea	Cyprididae	*Cypris pubera*	Saudi Arabia	1.26–1.27	Yousef et al. (2024)
Cypridoidea	Cyprididae	*Eucypris virens*	Spain, Italy, UK	1.293	Baltanás et al. (2000)
Cypridoidea	Cyprididae	*E. aragonica*	Saladar, Pez, Rebollón, Zaragoza, Spain	1.24	Marín (1984)
Cypridoidea	Cyprididae	*Tonnacypris stewarti*	Lake Nam Co, Tibetan Plateau	RV = 1.22; LV = 1.23	Bonilla-Flores et al. (2024)
Cypridoidea	Cyprididae	*Heterocypris bosniaca*	Temporary rock pools, Spain	1.26	Aguilar-Alberola and Mesquita-Joanes (2013)
		H. exodonta	Pond near Nam Co, Tibetan Plateau	RV = 1.237; LV = 1.236	This study
“	“	*H. incongruens*	Pond, Mexico City, Mexico	RV = 1.257; LV = 1.255	This study
“	“	“	?	1.259	Anderson (1964)
“	“	*H. salina*	Braunschweig, Germany	RV = 1.246; LV = 1.252	This study
“	“	“	El Salobral, Zaragoza, Spain	1.28	Marín (1984)
Overall mean				1.247	
Mean per species				1.249	

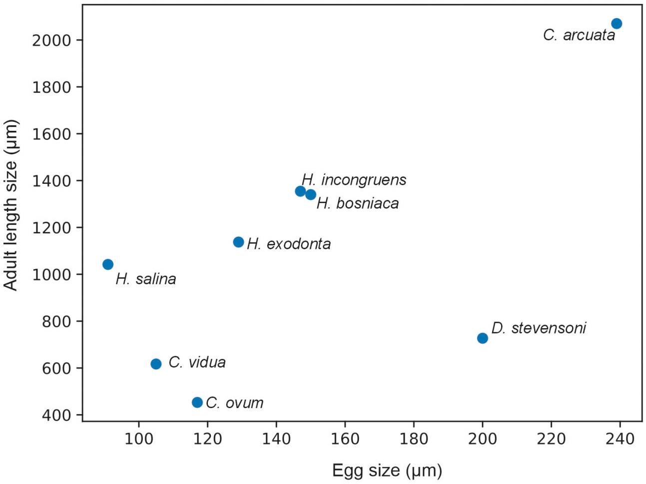

Figure 14. Relationship between egg size (µm) and adult valve length (µm) in selected non-marine ostracod species based on data from this study and the literature. Adult size corresponds to the mean valve length (RV and LV when available). The plot shows a general positive tendency between egg and adult size, although the relationship is not strictly proportional across taxa and should be interpreted with caution.

erally tending to exhibit higher values. For example, high growth factors occur in large-bodied species such as *Eucypris virens* (1.293) and *Dolerocypris fasciata* (1.349), whereas smaller taxa such as *Cyclocypris ovum* show lower values (1.15–1.175). In our dataset, *H. incongruens*, the largest species studied, also shows the highest mean growth ratio (RV = 1.257; LV = 1.255). A comparison between egg size and adult size (Fig. 14) shows a general positive tendency but not a strict proportional relationship, indicating that additional factors beyond initial size influence growth. In ostracods, available evidence suggests that reproductive traits such as egg number or egg volume may be related to female size, although these patterns are not always consistent. For example, a positive relationship between egg number and carapace length has been reported, whereas egg volume may be independent of female size in some species (Yousef and Alahmadi, 2025). Therefore, the relationship between egg size and maternal size remains insufficiently resolved in ostracods, particularly across species, and should be interpreted with caution. Growth ratios are also not constant throughout ontogeny. In our data, higher values occur in intermediate instars, followed by lower values in the final molts. Similar patterns have been reported previously (Liberto et al., 2014; Aguilar-Alberola and Mezquita, 2008), indicating that growth in ostracods is stage-dependent rather than uniform. Overall, growth ratios reflect a combination of developmental constraints, body size scaling, and ontogenetic variation and should not be considered to be fixed species-specific constants.

This applies to both ecological and paleoecological studies. The use of different juvenile instars and adult stages in future paleoecological interpretations is crucial for integrative knowledge of ostracods, increasing their use as paleobioindicators. This approach allows for an appreciation of population structure, coupled with knowledge of relative abundances, facilitating comprehension of ostracod taphonomy (Whatley, 1988; Boomer et al., 2003). Consequently, discerning ontogeny assists in deciphering spatial and temporal alterations in sedimentation rates and transport magnitude, regulated by wind-induced hydrodynamics (Zhai et al., 2013; Zhai et al., 2015). Additionally, it is important to consider the fact that changes in population structure can be related to sediment type and aquatic ecosystem depth, factors that may influence ostracod distribution (Zhai et al., 2010).

6 Conclusions

Laboratory cultures of *Heterocypris exodonta*, *H. incongruens*, and *H. salina* made the documentation of complete ontogenetic sequences possible, including eight juvenile instars and the adult stage. The egg stage plays a key role in persistence under desiccation in ephemeral habitats. This resilience cannot be attributed solely to the eggshell but rather to the entire egg, which is adapted to enter dormancy. The eggshell, composed of an inner and an outer layer, likely contributes to protection against mechanical damage and environmental stress. Particular attention should be paid to the external egg surface as its morphology is not uniform across species and may represent a potentially informative taxonomic character in some taxa.

Ontogenetic changes in valve reticulation show a progressive reduction in ornamentation towards adulthood, highlighting the risk of misinterpretation when isolated valves are analyzed. The limited variation in growth factors further indicates that size ratios alone are not reliable diagnostic characters for closely related taxa. Importantly, laboratory cultures proved to be indispensable for capturing all developmental stages, which are rarely represented in full under natural conditions. By providing a complete ontogenetic framework, this study helps to distinguish ontogenetic variation from true interspecific differences, thereby improving taxonomic resolution and the interpretation of modern and fossil assemblages.

Data availability. The morphometric measurements supporting this study are provided as a Supplement in Excel format associated with the article. Alternatively, the data are available in the doctoral thesis of Mauricio Bonilla Flores, published online by Technische Universität Braunschweig and accessible at https://doi.org/10.24355/dbbs.084-202409201141-0 (Bonilla Flores, 2025).

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