HATCHING MECHANISMS AND DEATH OF EUPHAUSIID EMBRYOS DURING HATCHING PROCESS: EVIDENCES FOR EVOLUTIONARY REVERSAL OF THE FREE-LIVING NAUPLIUS?

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ABSTRACT. This essay attempts to summarize and hypothetically reconstruct the original condition for the reproductive strategy and hatching mechanisms of the family Euphausiidae (Order Euphausiacea). Comparison is made of the hatching mechanisms and hatching success rates among five broadcast-spawning (*Euphausia pacifica, Euphausia eximia, Euphausia distinguenda, Thysanoessa spinifera, and Thysanoessa inspinata*) and two sac-spawning euphausiid species (*Nematoscelis difficilis* and *Nyctiphanes simplex*) collected from the Oregon coast, Bahía Magdalena (west coast of Baja California peninsula), and Gulf of California. These along with the discovery of a novel source of embryo mortality during hatching for broadcast-spawning species, and recently published genetic and phylogenetic information of the euphausiids, support the hypothesis that hatching as a free-living nauplius is a reversed character within the Order Euphausiacea in comparison with species belonging to other orders in the Class Crustacea. The hatching of embryos at nauplius stage, with distinct hatching mechanisms, appears repeatedly and intermittently in the Euphausiidae family phylogeny both in euphausiids with broadcast and sac-spawning reproductive strategy. This may represent a condition re-emerging well back in crustacean phylogeny, even though it is not necessarily primitive among the Order Euphausiacea as a whole.

Key words: Euphausiids, hatching mechanisms, hatching success, reproductive estrategy, phylogeny**.**

Mecanismos de eclosión y muerte de embriones de eufáusidos durante el proceso de eclosión ¿Son éstas evidencias que expliquen una reversión evolutiva de nauplios de vida libre?

RESUMEN. En este ensayo se intenta resumir y reconstruir la condición hipotética original de las estrategias de reproducción y mecanismos de eclosión de la Familia Euphausiidae (Orden Euphausiacea). Se hace la comparación de los mecanismos y tasas de éxito de eclosión de cinco especies desovadoras externas (*Euphausia pacifica, Euphausia eximia, Euphausia distinguenda, Thysanoessa spinifera y Thysanoessa inspinata*) y dos especies desovadoras en saco ovígero (*Nematoscelis difficilis* y *Nyctiphanes simplex*) de la costa de Oregon, Bahía Magdalena en la costa oeste de la península de Baja California, y Golfo de California. Esto, junto con el descubrimiento de mortalidad asociada al proceso de eclosión en especies con desove externo y la reciente información publicada sobre genética y filogenética de los eufáusidos, apoyan la hipótesis de que la eclosión de nauplios de vida libre es una característica revertida dentro del Orden Euphausiacea, en comparación con especies de otros órdenes dentro de la clase Crustácea. La eclosión de los embriones en el estadío nauplio, con distintos mecanismos de eclosión, aparece intermitentemente a lo largo de la filogenia de las especies de la Familia Euphausiidae, tanto en especies desovadoras externas como en desovadoras en saco ovígero. Esta eclosión en estadio nauplio aparentemente representa una condición re-emergente de antepasados en la filogenia de crustáceos, y por lo tanto, no es necesariamente una condición primitiva entre las especies de la Orden Euphausiacea.

Palabras clave: Eufáusidos, mecanismos de eclosión, éxito de eclosión, estrategia reproductiva, filogenia.

INTRODUCTION

The crustacean nauplius larva, characterized by a remarkably conserved morphology, has become the focus of studies on life-history evolution that investigate the interplay of animal structure, function, ecology and evolutionary history (Williams, 1994; Dahms, 2000). Several studies claim that the orthonauplius is a primitive (plesiomorphic) character within the Subphylum Crustacea (Lauterbach, 1986; Dahms, 2000). Thus, any larval development without a free-living nauplius has to be a derived (apomorphic) condition. In the Class Malacostraca, only in the Suborder Dendrobranchiata (Decapoda) and seven genera of the Order Euphausiacea (*Euphausia, Meganyctiphanes, Nematoscelis, Nyctiphanes, Stylocheiron, Thysanoessa* and *Thysanopoda*; ~91% of all known 86 valid species) is currently known to hatch as a nauplius, although several sac-spawning euphausiid species usually hatch in older pseudometanauplius (PMN) or metanauplius (MN) larval stages (Gordon, 1955; Brinton, 1966; Ponomareva, 1969; Zilch, 1978; Gómez-Gutiérrez, 2003a,b; Gómez-Gutiérrez & Robinson, 2005). Other malacostracans, like Stomatopoda, Bathynellacea, Anphionidacea, Caridea, Stenopoidea and Reptantia, hatch as a zoea or zoea-like larva; with the exception of the species with direct development (Richter & Scholtz, 2001).

Development with a free-swimming nauplius was one of the main arguments why the Order Decapoda was traditionally considered the nearest relative of euphausiids, both included in the Superorder Eucarida. Gordon (1955), Brinton (1965), Maas & Waloszek (2001) and Casanova *et al.* (2002) also argued that the shared derived characters (synapomorphies) between Decapoda and Euphausiacea are: 1) the carapace attached to 7, not 8, thoracomeres and 2) that in adult stage their thoracic segments are open. Alwes & Scholtz (2004) concluded that the early cleavage and gastrulation pattern of the euphausiid *Meganyctiphanes norvegica* and Dendrobranchiata are maybe homologous features indicating a closely phylogenetic relationship between both groups.

In the other hand, a recent molecular study shows that the suborder Mysida is the closest taxon of Euphausiacea. The Syncaridae are another closely-related, but more basal group and Decapoda are more distantly related to euphausiids (Jarman, 2001; Jarman *et al.*, 2000a). The hypothesis that Euphausiacea and Mysidacea are closely related is not new and has been proposed and discussed in the past. The genus *Thysanopoda* (the first euphausiid species formally described was *Thysanopoda tricuspidata* Milne-Edwards, 1837) was originally placed by Milne-Edwards in his tribe of "Mysiens" and later other authors in late nineteen and early twenty centuries retained the association of these two taxa in the group "Schizopoda" (Calman, 1909). All mysids are sac-spawners forming an ovigerous sac called oosteogite. The nauploid 'eyeless larvae' of mysids have the same appendages as the classic orthonauplius, but lacking the nauplius eye and swimming setae (Wortham-Neal & Price, 2002). Sac-spawning strategy has evolved in four euphausiid genera (*Pseudeuphausia, Nyctiphanes, Nematoscelis* and *Stylocheiron*) including 25 out 86 euphausiid species (Brinton *et al.*, 2000). Several species have distinct hatching mechanisms hatching as nauplii, PMN or MN. This may suggest that sac-spawning reproductive strategy in euphausiids may not be considered strictly and exclusively as an apomorphic feature as mentioned in previous studies (Mauchline & Fisher, 1969; Casanova, 1984, 2004).

Recent arguments based on comparative morphology and embryology suggest that the free-living nauplius of Euphausiacea and Dendrobranchiata has arisen secondarily from an ancestor that developed through an ancestral embryonic "egg nauplius" with hatching at a later larval stage (Shiino, 1958; Strathmann & Eernisse, 1994; Scholtz, 2000). The egg-nauplius is defined as an embryo in which segments and limbs form gradually with the precocious development of the naupliar region (Scholtz, 2000). The Malacostracan egg-nauplius shows a distinct morphology, *i.e.* uniramous mandible, rich yolk and un-differentiated dorsal side due to the yolk content. In contrast, free-living nauplii have biramous mandible, functional limb muscles and little yolk. The

nauplius of *Nematoscelis difficilis* and three broadcast spawning euphausiid species have biramous mandible (Gómez-Gutiérrez, 2003b), but *Nyctiphanes simplex* nauplius has uniramous mandible (Boden, 1951). Thus, whether the Order Euphausiacea has an egg-nauplius or whether hatching as a nauplius stage is a primitive (plesiomorphic) or derived (apomorphic) characteristic are currently in debate and some scientists even believe it is an unsolvable problem to define: which is the most primitive reproductive strategy within the Order Euphausiacea, broadcast or sac-spawning strategy? A comparative study of the embryonic stage at hatching and hatching mechanisms would be of interest in regard to the possible evolutionary reversal of euphausiid development. However, three extensive reviews of hatching process of invertebrate eggs reported no adequate description of hatching for the Order Euphausiacea (Davis, 1968, 1981; Anderson, 1982) showing a gap in knowledge on egg release and brood care in euphausiids that according to Maas & Waloszek (2001) may be an important character in the interpretation of the systematic relationships within the Order Euphausiacea. Until recently it was assumed or generally known within experts studying euphausiid biology and ecology, that all broadcast-spawning euphausiid species hatch as a nauplius, while sac-spawning hatch exclusively in older sta- ges (Boden, 1951; Mauchline & Fisher, 1969; Gopalakrishnan, 1973; Lavaniegos, 1992). However, such distinctions are not so clear since broadcast spawning species may hatch as PMN or MN (Gómez-Gutiérrez, 2002) and sac spawning species may hatch as nauplius (Gómez-Gutiérrez, 2003a,b; Gómez-Gutiérrez & Robinson 2005). Species within each euphausiid genus has exclusively broadcast or sac-spawning spawning reproductive strategy. However, the hatching mechanisms are highly variable among genera and among species of the same genera, supposedly to enhance larval survival. Thus, hatching mechanism may be a characteristic that hypothetically could be tracked throughout euphausiid's phylogeny derived from DNA analysis.

The goal of this essay was to summarize all previous knowledge on euphausiid hatching mechanisms and hatching success of five broadcast and two sac-spawning euphausiid species from the Northeastern Pacific (Gómez-Gutiérrez, 2003a,b; Gómez-Gutiérrez & Robinson, 2005) in the context of previous hypothetical phylogenetic trees of euphausiids deducted from morphological, reproductive strategies (Casanova, 1984; Maas & Waloszek, 2001) and genetic information (Jarman, 2001; Jarman *et al.* 2000a,b) to interpret, in an evolutionary perspective, whether the egg-nauplius could represent ancestral information that has been conserved, a likely condition from which euphausiids diverged by returning to hatching of free-swimming nauplii (Sudhaus & Rehfeld, 1992).

MATERIALS AND METHODS

Euphausiid collection. Euphausiids eggs of *Euphausia pacifica, Thysanoessa spinifera, Thysanoessa inspinata* and *Nematoscelis difficilis* were obtained from gravid females collected in the field at night using a 1 m diameter net with black 333 μm mesh and close cod end (0.75 m long, 0.25 m diameter) during 15 oceanographic cruises (2001-2002) carried out between March and September along the Oregon coast, USA (Gómez-Gutiérrez, 2003a). Females of *Nyctiphanes simplex, Euphausia eximia* and *Euphausia distinguenda* were collected with a similar net in May, July and December, 2004 over the continental shelf and the mouth of Bahía Magdalena, México (24 °N, 112 °N) and along the Gulf of California (November 2005 and July 2007). In all cases, the 10 m to 40 m depth stratum was sampled at drifting towing speed to avoid damage to the live euphausiids. The catches were diluted into 40 L coolers filled with surface seawater from the station sampled.

Euphausiid incubation. During all the oceanographic cruises the incubations were set up at sea within a few minutes after collection in high precision incubator (Fisher) aboard the R/V Wecoma (Oregon State University), R/V New Horizon (Scripps Institution of Oceanography) and in México in a cold room aboard the R/V El Puma (Universidad Nacio-

nal Autónoma de México). We gently removed gravid female euphausiids from the diluted plankton sample with a plastic spoon and placed each female in a 1 L bottle filled with filtered (200 µm) surface seawater. From 2 to 50 gravid females were incubated per station according to availability. Several females were incubated and monitored frequently (< 4 hours) until they spawned, and the embryos were counted and their embryonic development observed every ~10 minutes before gastrula stage and every 1 h to 4 h for older embryos until hatching time at constant temperature 10.5 °C ± 0.5 °C for Oregon specimens and 16 °C ± 1 °C for Baja California and Gulf of California specimens.

Hatching success (HS) and field hatching success (FHS). In broadcast spawning species HS was measured as the percentage of eggs hatched up to the moment all the unhatched eggs began to spoil and the hatching mechanism was monitored using a digital camera (Olympus Camedia 3040, 3.3 x 10^6 pixels resolution). The HS was compared with that of eggs collected from the field > 48 hours and incubated in the laboratory at 10.5 °C ± 0.5 °C (*E. pacifica*), here defined as field hatching success (FHS). In sac-spawning euphausiids hatching success was observed during several days observing the embryos inside the ovigerous sac until the females completely release their embryos of the ovigerous sac.

At the end of an experiment, both females and embryos of the incubations were poured through a sieve (120 ìm) and preserved with 5% formalin buffered with sodium borate for Oregon samples and 96% ethanol for samples collected in México. Preserved females were measured from the posterior part of the eye to the tip of the telson (total length, mm). Brood size was the number of eggs produced per female per spawning event (eggs brood^{-1}) during the 48 h of incubation.

Interpretation of the hatching mechanisms and death of embryos during hatching from a phylogenetic perspective. The hatching mechanisms and average hatching success were compared by a parsimonious inductive process with the phylogenetic trees proposed in several previous publications. I used 13 morphological characters and reproductive strategy (Casanova, 1984), cladistic analysis of adult features and ontogenetic characters (Mass & Waloszek, 2001) and a phylogenetic tree based on the average divergence time using slowly evolving nuclear DNA sequences (partial 28S rDNA) (Jarman, 2001) to attempt to reconstruct a conceptual model of the relative chronological appearance of broadcast spawning and sac-spawning reproductive strategy in the family Euphausiidae. I also attempted to investigate whether free nauplius larvae represent a plesiomorphic feature within the Euphausiidae family as previous studies have suggested (Lauterbach, 1986; Dahms, 2000). The Jarman's (2001) genetic phylogenetic tree was originally shown unrooted, but an interpretation was made to reconstruct the phylogenetic tree for the genera of the family Euphausiidae from the average diverged times for close genera estimated by slow evolving nuclear DNA sequences from Jarman (2001). To reconstruct the Euphausiidae phylogenetic tree, divergence times were obtained mostly from the empty-circles nodes shown in Jarman's Figure 4 and cluster of genera were made from the closest branches in the unrooted tree from Jarman's Figure 3 originally made with cluster analysis.

RESULTS

Hatching mechanism and hatching success of broadcast-spawning species

The emergence of a larva from its egg is a critical period in the life cycle in crustaceans and for broadcast-spawning euphausiid embryos it may involve a relatively low average hatching success, showing large female-to-female variability ranging from ~0% to 100%. Embryos of broadcast spawning species can die during hatching process. When the embryos are ready to hatch (twitching stage), the nauplius pushes against the chorion with the posterior part of the abdomen producing a protuberance. The pressure breaks the chorion and the nauplius pushes itself backward with all its appendages to slide out from the chorion. But sometimes the hole where the nauplius ought to pass through is so small that the

larva ruptures. The naupliar body is mainly filled with yolk-containing endoderm cells because the gut is not formed yet and much yolk is released when the larva ruptures during hatching. This newly discovered source of mortality was frequently observed during incubations under laboratory conditions for eggs of *E. pacifica* (Fig. 1a-d), *T. inspinata* (Fig. 1e-h) and *T. spinifera* in Oregon. Rupture of the lar- va depends on the size of the chorion hole. If an animal can pass most of its body through the small aperture, the nauplius shows a characteristic figure-eight shape with a narrow "waist". The breaking point can be in the anterior (Fig. 1 a-d) or posterior part of the embryo body (Fig. 1 e-h). *Euphausia pacifica* embryos, from females spawned under laboratory conditions, had low hatching success (high mortality) due to failure to break a large enough hole

in the chorion during hatching; rates ranged between 0.5% and 5% (n=9 broods). In contrast, *T. inspinata* (1% to 98%, n=3 broods) and *T. spinifera* (0.2% and 25%, n=5 broods) showed sometimes relatively higher hatching mortality rates (Table 1). The fact that some of the eggs from the same brood died during hatching while others hatched normally suggests different survival probabilities for sibling embryos, based on their individual ability to rupture the chorion. It is uncertain whether laboratory conditions promote the death during hatching. However, embryos that died during hatching process, indicated by tissue fragments attached to the chorion, were occasionally observed from preserved zooplankton samples collected in the field, indicating that mortality during hatching process also happens in the sea, although in very low proportion (<0.1%)
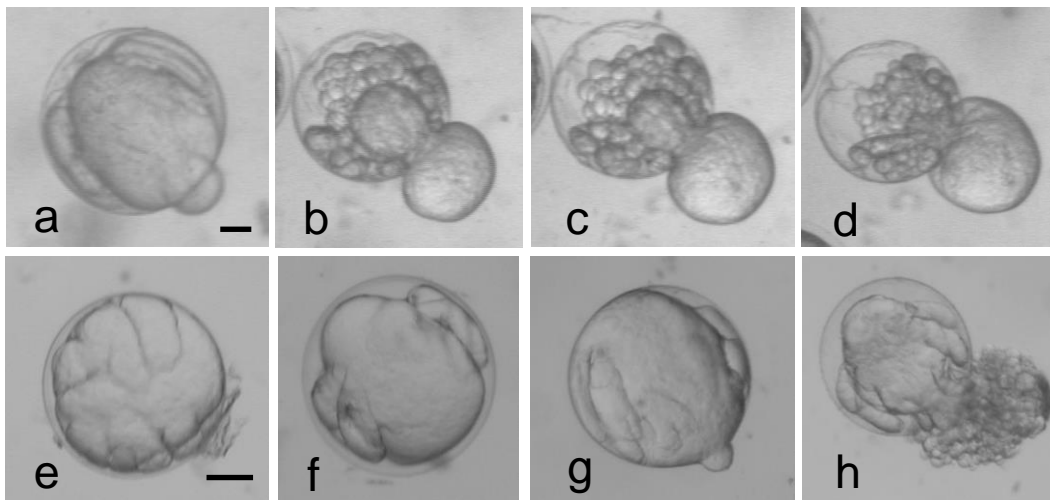

Figure 1. Sequence of the death of the embryos during hatching of *Euphausia pacifica* (a-d) and *Thysanoessa inspinata* (e-h). (a) Twitching nauplius (528 μm diameter) trying to hatch backward and the aperture of the chorion is not big enough to pass through it. (b-d) The pressure on the embryo is high and the nauplius membrane breaks in the anterior part of the nauplius body. This sequence of pictures took <5 seconds. (e) Egg in early limb-bud stage (367 μm) ~18 h after spawning. (f) The egg is in late limb-bud stage 25 h after spawning. (g) The twitching nauplius is hatching backward and the aperture of the chorion is also too small to pass through it. (h) The breaking point here is the posterior part of the body and most of the nauplius body remains inside the chorion. The scale bars are 100 μm.

Figura 1. Secuencia de la muerte de embriones durante la eclosión de *Euphausia pacifica* (a-d) y *Thysanoessa inspinata* (e-h). (a) Nauplio dentro del corión (528 μm diámetro) intentando eclosionar hacia atrás en el cual la abertura del corion no es suficientemente grande para que el embrión pase por el. (b-d) La presión del embrión es alta y la membrana del embrión se rompe de la parte posterior del nauplio. Esta secuencia de fotos duró <5 segundos. (e) Embrión en estadio de apéndices en desarrollo (367 μm) ~18 h después del desove. (f) Embrión en estadio de apéndices ya desarrollados 25 h después del desove. (g) Nauplio dentro del corión esta eclosionando hacia atrás pero la abertura también es demasiado pequeña para pasar a través de ella. (h) El punto de rompimiento del embrión es en la porción posterior del cuerpo y la mayor parte del nauplio se queda dentro del corión. La escala mide 100 μm.

Table 1. Brood size (egg fem^{-1}) and number and percentage of dead embryos during hatching (inverse of hatching success) from females that spawned under laboratory conditions from Oregon (Gómez-Gutiérrez, 2002, 2003a,b) and west coast of Baja California peninsula and Gulf of California (Gómez-Gutiérrez & Robinson, 2005) and this study. Numbers shown between parentheses are averages.

Tabla 1. Tamaño de puesta (embriones hem^{-1}) y número y porcentaje de embriones muertos durante la eclosión (inverso de éxito de eclosión) de hembras que desovaron bajo condiciones de laboratorio en Oregon (Gómez-Gutiérrez, 2002, 2003a,b) y costa oeste de la península de Baja California y Golfo de California (Gómez-Gutiérrez & Robinson, 2005) y este estudio. Los números entre paréntesis son promedios.

Euphausiid species	Date	Latitude, Longitude	Brood size per female	No. of eggs dead during hatching	Percentage (%)
Broadcast spawning species					
Euphausia pacifica (n=9)	Jul 18, 2001	44.39° N, 124.39° W	190	1	0.53
	Aug 5, 2001	44.39° N, 124.39° W	92	1	1.09
	Aug 5, 2001	44.39° N, 124.39° W	151	1	0.66
	Sep 10, 2001	44.00° N, 124.36° W	144	2	1.39
	Apr 10, 2001	44.00° N, 125.00° W	80	1	1.25
	Jun 12, 2002	43.52° N, 124.30° W	170	7	4.12
	Jun 12, 2002	43.52° N, 124.30° W	201	10	4.97
	Jul 23, 2002	44.39° N, 124.39° W	75	2	2.66
	Jul 23, 2002	44.39° N, 124.39° W	251	1	0.40
Thysanoessa spinifera (n=5)	Jun 12, 2002	43.52° N, 124.30° W	477	1	0.21
	Aug 6, 2002	41.54° N, 124.24° W	139	7	5.03
	Aug 6, 2002	41.54° N, 124.24° W	421	14	3.32
	Aug 6, 2002	41.54° N, 124.24° W	71	3	4.22
	Aug 6, 2002	41.54° N, 124.24° W	16	4	25.00
Thysanoessa inspinata (n=3)	Jun 13, 2002	42.30° N, 125.30° W	68	67	98.58
	Jun 13, 2002	42.30° N, 125.30° W	84	2	2.38
	Jun 13, 2002	42.30° N, 125.30° W	86	1	1.16
Euphausia eximia (n=1)	Dec 15, 2004	24.21° N, 112.09° W	46	18	39.13
Euphausia distinguenda (n=1)	Jul 26, 2007	28.36° N, 112.59° W	22	12	54.54
Sac spawning species					
Nematoscelis difficilis (n=7)	Aug 3-17, 2002	44.54-43.59° N, 124.37-125.39° W	34-306	<306	0-100(27.9)
Nyctiphanes simplex (n=55)	Mar, Jul, Dec, 2004	24.20-25.40° N, 111.8-113.00° W	7-105	0	0
Nyctiphanes simplex (n=3)	Nov, 2005; Jan, 2007	24.20-25.40° N, 111.8-113.00° W	35-61	35-61(46)	100

probably due to the difficulty to collect and preserve embryos exactly during the hatching process. *E. eximia* in Bahía Magdalena and Gulf of California was frequently observed hatching backward, but was also observed hatching forward, most times thought it was not possible to measure brood size and hatching success. The only gravid female specimen of *Euphausia distinguenda* that spawned in the laboratory hatched as nauplius using backward hatching mechanism (Table 2).

Quantification of the mortality rates, via field hatching success (FHS), was estimated several times at monospecific spawning events of *E. pacifica* with high densities of eggs in early limb-bud (eLB) and late limb-bud (lLB) stages (Fig. 1) (probably ~20 h - 25 h

Table 2. Experiments of field hatching success (FHS) of *E. pacifica* embryos collected in Oregon (44° 65' N, 124° 18' W, July 2002, and 44° 65' N, 124° 65' W, August 2002) and incubated under laboratory conditions. Most eggs were collected and incubated in early (eLB) and late limb-bud stage (lLB).

Tabla 2. Experimentos de tasas de eclosión de campo de embriones de *E. pacifica* recolectados en Oregon (44° 65' N, 124° 18' O, Julio 2002, y 44° 65' N, 124° 65' O, Agosto 2002) e incubados bajo condiciones de laboratorio. La mayor parte de los huevos fueron recolectados e incubados en estadio temprano de apéndices (eLB) y estadio tardío de apéndices (lLB).

Date	Initial number of embryos (N)	Abundance per egg and nauplius after 48 h of laboratory incubation				
		eLB-TW stages	Embryos hatching	Embryos dead during hatching	Hatched nauplius	FHS (%)
July, 2002	78	38	5	3	32	41.0
	90	51	2	2	35	37.8
	68	19	1	1	47	69.1
	105	33	3	0	69	65.7
	75	26	1	3	45	60.0
	86	23	2	1	60	69.8
	184	54	0	8	122	66.3
	283	112	8	2	161	56.9
	501	220	10	4	267	53.3
	68	32	1	2	33	48.5
	51	38	1	0	12	23.5
Total	1589	40.6%	2.14%	1.64%	55.56%	53.8
August, 2002	65	17	0	5	43	66.2
	118	13	0	0	105	89.0
	82	18	0	2	62	75.6
	106	19	0	0	87	82.1
	49	11	0	0	38	77.6
	72	10	0	1	61	84.7
	76	23	2	0	51	67.1
	91	29	4	0	58	63.7
	100	15	0	4	81	81.0
	41	11	0	0	30	73.2
Total	800	20.8%	0.8%	1.5%	76.9%	76.7

after spawning) collected near shore during July 2002 (44° 65' N and 124 °18' W, 8286.3 eggs m^{-3}) and August 2002 (44° 65' N and 124° 65' W, 2455.2 eggs m^{-3}). At each experiment 10 groups with more than 40 eggs per group were incubated > 48 h (Table 2). Eggs spawned at the sea and incubated in the laboratory hatched by the more frequent backward hatching mechanism to yield healthy nauplii (23.5% and 89.0 % hatching success) with an average mortality of embryos during hatching of 1.5% at both experiments (Table 2).

Hatching mechanism and hatching success of sac-spawning species

The death of eggs during hatching was never observed for eggs of the sac-spawning *N. difficilis* when the embryo used the push-off mechanism as PMN or MN (n=11 broods),

which apparently use a more efficient hatching mechanism (Gómez-Gutiérrez unpubl. data), but death during hatching was observed when the embryo hatched earlier as N2 (Gómez-Gutiérrez, 2003). I interpret the death of the nauplius 1 during hatching in broadcast-spawning species as an inherent disadvantage associated with the backward hatching shared with the less common accelerated hatching in sac-spawning species. In sac-spawning spe- cies when the female dies for any other cause than predation during early embryonic development the whole brood size can not survive. However, when a female dies (excluding predation) during late embryonic development close to hatching time, embryos may survive without the oxygenation provided by the female when it frequently moves up and down its ovigerous sac with the seventh pair of thoracic legs. A summary and description of all hatching mechanisms is shown in Table 3.

DISCUSSION

Currently five distinct hatching mechanisms are known in euphausiid embryos, observed so far in ten euphausiid species around the world (Table 3). Different species share the same hatching mechanisms indepen- dently of their distribution range (*i.e.* temperate euphausiids *Euphausia pacifica* versus tropical euphausiids *Euphausia eximia* and *Euphausia distinguenda*) and the same species collected in two or more distinct regions seem to have similar hatching mechanisms (*Nematoscelis difficilis*). This indicates the hatching mechanism is an inherent feature of the species rather than a regional induced behavior. Gómez-Gutiérrez (2002) reported three different hatching mechanisms: backward, forward and flipping for three broadcast spawning species, *E. pacifica, T. inspinata* and *T. spinifera*. Backward hatching as nauplius 1 (N1) was the most common hatching mechanism of broadcast spawning species and was associated with relatively higher brood size and HS. The present study shows that the tropical euphausiids *E. eximia* and *E. distinguenda* also hatch preferentially as nauplius, but it also has been observed hatching as metanauplius (Table 3). Broadcast euphausiid embryos infrequently hatch in later developmental stages using two

unusual hatching mechanisms associated with relatively low HS: forward and flipping hatching mechanisms (Gómez-Gutiérrez, 2002). The push-off hatching mechanism of the sac-spawning species *N. difficilis*, has been so far observed exclusively in this species (Gómez-Gutiérrez, 2003a,b) (Table 3). Alternatively, the *N. difficilis* embryo can hatch prematurely as N2 using the forward hatching mechanism observed in broadcast spawning species. *Nyctiphanes simplex* embryos hatch as nauplius with the expansion hatching mechanism (so far observed only in this species), they further develop into pseudometanauplii and metanauplii inside the ovigerous sac. The nauplius breaks the thin and fragile chorion by increasing the volume of the body because the embryos have a quite small perivitelline space. *Nyctiphanes simplex* larvae escape from the ovigerous sac late in the MN stage, usually 5 days after spawning, just a few hours before molting into calyptopis 1. This delayed release extends protection by the female, likely decreasing the risk of predation or early cannibalism (Gómez-Gutiérrez & Robinson, 2005). These five different hatching mechanisms observed in euphausiids may have an evolutionary significance associated with their population increase of fitness and larval recruitment rates, partially explaining why in temperate and polar environments numerically dominant euphausiid species have broadcast spawning reproductive strategy, while in tropical and subtropical environments numerically dominant euphausiid species frequently have sac-spawning reproductive strategy. The only possible way to know which of these hatching strategies appeared first in the evolution of the euphausiids is to infer it from the comparison of such hatching mechanisms observed among species and genera with the phylogenetic trees proposed in previous studies using morphological and reproductive strategies (Casanova, 1984; Maas & Waloszek, 2001), based on partial 28S rDNA sequences (Jarman, 2001), or COI (Zane & Patarnello, 2000; Bucklin *et al*., 2007).

A comparison of hatching mechanisms between broadcast (seven species) and sac-spawning (three species) reproductive strategies (Table 3) and the observation relati-

Table 3. Hatching mechanisms of the broadcast-spawning (B) or the sac-spawning (S) euphausiid species observed under laboratory conditions from mature females around the world. The euphausiid species in which the hatching mechanism has been inferred from a brief description of this process, drawings and/or photographs, are indicated with asterisks (Modified from Gómez-Gutiérrez & Robisnon, 2005).

Tabla 3. Mecanismos de eclosión de especies de eufáusidos desovadoras externas (B) o desovadoras en saco ovígero (S) observados bajo condiciones de laboratorio de hembras grávidas recolectadas en el campo en el mundo. Las especies de eufáusidos en las cuales han sido inferidos los mecanismos de eclosión a partir de una breve descripción, fotografías o diagramas son indicadas con asterisco (Modificado de Gómez-Gutiérrez & Robinson, 2005.)

Hatching mechanism	Euphausiid species	Region	References
Backward: The nauplius 1 (N1) pushes against the chorion with the posterior part of the abdomen producing a protuberance. The pressure breaks the chorion, and the N1 pushes itself backward with the first and second antennae and mandible to slide out from the chorion.	*Euphausia superba* (B)* *Euphausia pacifica* (B) *Euphausia eximia* (B) *Euphausia distinguenda* (B) *Thysanoessa spinifera* (B) *Thysanoessa inspinata* (B) *Nematoscelis difficilis* (S)	Antarctic Sea Oregon, USA Bahía Magdalena and Gulf of California Bahía Magdalena and Gulf of California Oregon, USA Oregon, USA Oregon, USA and Gulf of California	Ross & Quetin (1982)*; George (1984)*; Gómez-Gutiérrez (2002, 2003a,b); This study
Forward: The nauplius 2 (N2) and metanauplius (MN) break the chorion with the first and second antennae, hatching forward	*Thysanoessa inermis* (B)* *Stylocheiron carinatum* (S)* *Euphausia pacifica* (B) *Thysanoessa spinifera* (B) *Thysanoessa inspinata* (B) *Euphausia eximia* (B)	Barent Sea Indian Ocean Oregon, USA Oregon, USA Oregon, USA Bahía Magdalena and Gulf of California	Zelikman (1961)*; Ponomareva (1969)*; Gómez-Gutiérrez (2002); Gómez-Gutiérrez & Robinson, 2005; This study
Flipping: The calyptopis (C1) slit the chorion using their telson spines extending and flipping the abdomen outside the egg	*Euphausia pacifica* (B)	Oregon, USA	Gómez-Gutiérrez (2002)
Push-off: The pseudometanauplius (PMN) or MN embryos extend and contract their first and second antennae in a swimming movement, breaking the chorion in almost equal halves joined by one small section in the anterior part of the chorion. The PMN or MN hatch and escapes from the ovigerous sac almost simultaneously	*Nematoscelis difficilis* (S)	Oregon, USA and Gulf of California	Gómez-Gutiérrez (2003a,b) ; This study
Expansion: The nauplius hatch breaking the thin chorion with the body growth, but is the MN stage that escapes from the ovigerous sac about 2 days after hatching	*Nyctiphanes simplex* (S)	Bahía Magdalena and Gulf of California	Gómez-Gutiérrez & Robinson, (2005); This study

vely high mortality rates during hatching process in broadcast spawning euphausiids compared with Jarman's (2001) phylogenetic tree supports the hypothesis that hatching at the N1 (orthonaupliar) stage is a derived (apomorphic) character in the Order Euphausiacea. Thus, the ability of broadcast-spawning species to hatch as N2, MN and C1 represents an intermediate feature between broadcast and sac-spawning reproductive strategies, in other words an evolving atavism of an ancestral egg-nauplius condition (Fig. 2a). Thus, the early hatching of the N2 (forward hatching) observed in the sac-spawning species *N. difficilis* or the *N. simplex* eclosion as naupli with the expansion hatching mechanisms may be distinct steps toward an earlier hatching schedule typical of broadcast spawning species. The general picture emerging from the comparison of hatching mechanisms and hatching success of both euphausiid reproductive strategies appears to support the hypothesis that a free-living nauplius larva is a reversal feature for the Order Euphausiacea and that the push-off hatching mechanism in sac-spawning euphausiids represents a primitive (plesiomorphic) hatching mechanism mode similar to mysids (Fig. 2a). It is interesting that in the euphausiid phylogeny species with sac-spawning reproductive strategy apparently evolved independently among genera since several mechanisms of hatching have been observed in the sac-spawning species *Stylocheiron carinatum* (nauplii, forward), *N. difficilis* (nauplii, backward, PMN or MN, push off) and *N. simplex* (nauplii, expansion) (Table 3) which appears repeatedly and intermittently in the Euphausiidae phylogeny, both in euphausiids with broadcast and sac-spawning reproductive strategy. This agrees with Strathman & Eernisse (1994) in that the most parsimonious interpretation is atavism ('resurrection') of a free nauplius from an egg-nauplius at least once for Euphausiacea and Dendrobranchiata. Because this interpretation agrees with one of the most robust genetic phylogenetic trees currently known (Jarman, 2001) (Fig. 2c), this may be the most parsimonious explanation for evolution of the euphausiid reproductive strategies. However, this perspective of Euphausiidae evolution is quite unconventional since in the twenty century most scientists proposed

that broadcast spawning reproductive strategy is a plesiomorphic reproductive strategy and sac-spawning a apomorphic reproductive in the euphausiid phylogeny based on some convincing evidence to support this view.

The Order Euphausiacea comprises two families: the monophyletic Bentheuphausiidae (*Bentheuphausia amblyops,* G.O. Sars 1885) and the Euphausiidae with 85 valid species (Brinton *et al.*, 2000). The first proposed diagram of phylogenetic relationships of the Order Euphausiacea was based on the exami- nation of 13 generic characters dealing with morphology and reproduction (Fig. 2b) (Casanova, 1984). In this hypothetical tree the monospecific genus *Bentheuphausia* seems to be the most archaic (Casanova, 2004), having the endopod of the first male pleopod unmodified as a petasma, no photophores, three segmented maxilla and three endopodal podomeres in the maxillula (Brinton, 1967; Maas & Waloszek, 2001). *Bentheuphausia amblyops* is presumably a broadcast-spawning species because no ovigerous female has ever been collected during extensive deep sampling collections around the world, but waits for experimental corroboration (Casanova, 1984; Brinton *et al.*, 2000). This is a convincing argument that euphausiids first evolved from a crustacean with broadcast spawning strategy, but genetic analysis so far does not support this view. The Order Euphausiacea lack a fossil record as adult and embryos, thus extinct euphausiid species are absent, for example during the Cretaceous extinction 65 Mya and therefore not included in any of the current euphausiid phylogenetic trees (Jarman, 2001). Thus, it is not possible to know if an ancestral euphausiid with sac-spawning reproductive strategy is currently extinct or evolved in some of the current species with distinct reproductive and hatching mechanism strategy. Casanova (1984) claimed that from the *Thysanopoda*, which represents the first really euphausiacean step, four radiations can be distinguished. This radiation may be explained if sympatric speciation of euphausiids may have occurred simultaneously (in geological time scales) in several regions around the world with changes of the ocean temperature modifying the distribution of individual species according to their physio-
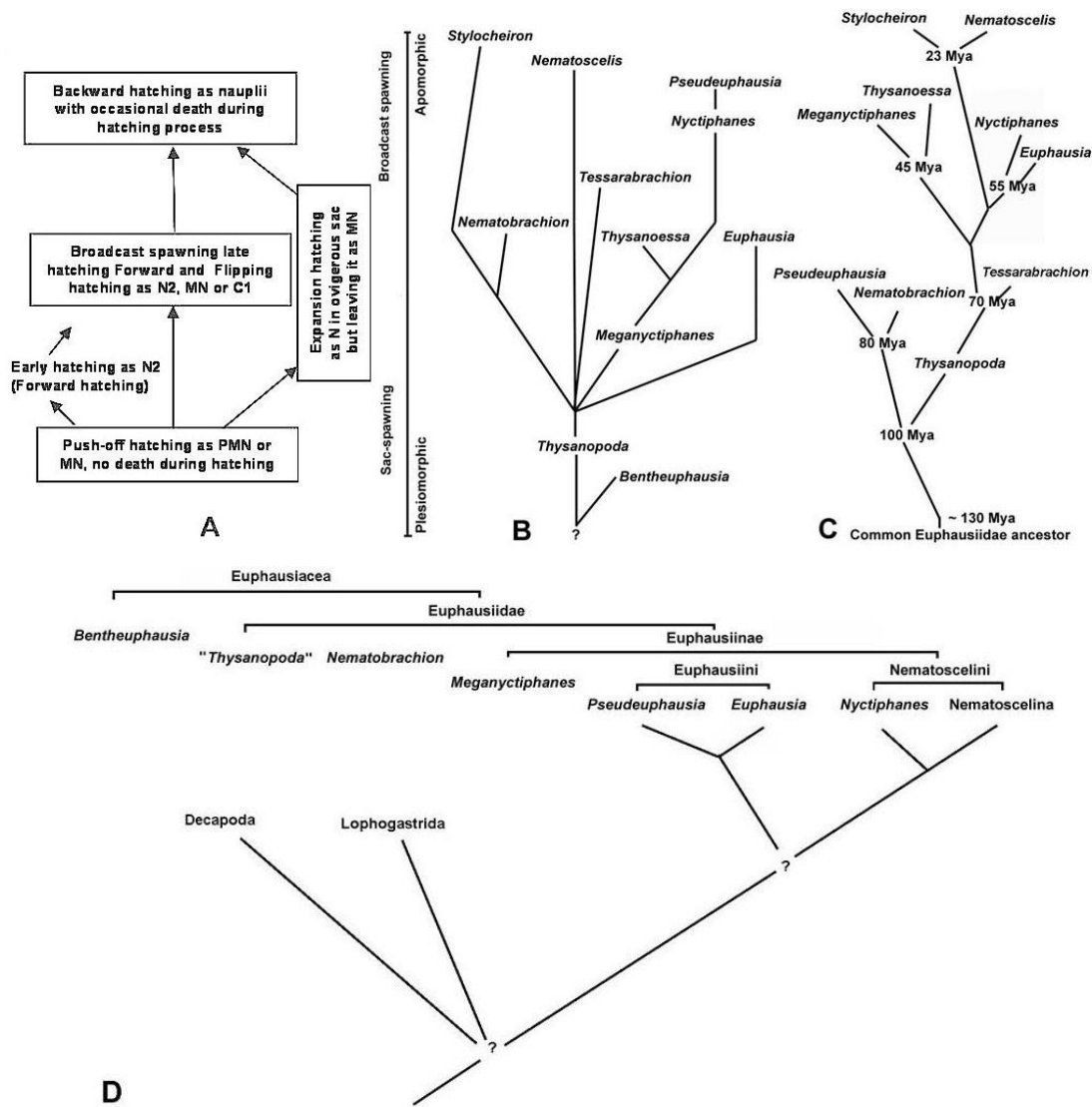
A

Backward hatching as nauplii with occasional death during hatching process

Broadcast spawning late hatching Forward and Flipping hatching as N2, MN or C1

Expansion hatching as N in ovigerous sac but leaving it as MN

Early hatching as N2 (Forward hatching)

Push-off hatching as PMN or MN, no death during hatching

Broadcast spawning / Apomorphic / Sac-spawning / Plesiomorphic

B

Stylocheiron
Nematoscelis
Pseudeuphausia
Nyctiphanes
Tessarabrachion
Nematobrachion
Thysanoessa
Euphausia
Meganyctiphanes
Thysanopoda
Bentheuphausia
?

C

Stylocheiron
Nematoscelis
23 Mya
Thysanoessa
Meganyctiphanes
Nyctiphanes
Euphausia
45 Mya
55 Mya
Pseudeuphausia
Nematobrachion
Tessarabrachion
70 Mya
80 Mya
Thysanopoda
100 Mya
~ 130 Mya
Common Euphausiidae ancestor

D

Euphausiacea
Euphausiidae
Bentheuphausia
Euphausiinae
"Thysanopoda" Nematobrachion
Meganyctiphanes
Euphausiini
Nematoscelini
Pseudeuphausia Euphausia
Nyctiphanes Nematoscelina
?
Decapoda
Lophogastrida
?

Figure 2. (a) Conceptual model of evolution of hatching mechanism of broadcast spawning and sac-spawning strategies in the family Euphausiidae. Late hatching mechanism (forward and flipping) in broadcast spawning species is interpreted here as an atavistism and early hatching schedule (forward and expansion hatching mechanism) in sac-spawning species as an intermediate hatching mechanism between both reproductive strategies. (b) Phylogenetic relationships and evolutionary characters of the Order Euphausiacea based on 13 generic characters dealing with morphology and reproduction (Casanova, 1984; redrawn from his Figure 3). (c) Diagram of the phylogenetic tree for the genera of the family Euphausiidae showing average diverged times for close genera estimated by slow evolving nuclear DNA sequences (modified and interpreted from Jarman, 2001, from his Figure 4). (d) Re-drawn and simplified of the Order Euphausiacea phylogenetic tree where the subfamily Euphausiinae was proposed (Maas & Waloszek, 2001).

Figura 2. (a) Modelo conceptual de la evolución de los mecanismos de evolución de las estrategias de reproducción de desove externo o en saco ovígero en la familia Euphausiidae. Los mecanismos de eclosión tardíos (hacia adelante y por movimientos del abdomen) en especies desovadoras externas se interpreta como un atavismo y la eclosión temprana (mecanismos hacia delante y de expansión) en especies con desove en saco ovígero como un mecanismo de eclosión intermedio entre ambas estrategias de reproducción. (b) Relaciones filogenéticas y caracteres evolutivos del Orden Euphausiacea fundamentado en 13 características genéricas concernientes a la morfología y reproducción (Casanova, 1984; redibujado de su Figura 3). (c) Diagrama del árbol filogenético de los géneros de la familia Euphausiidae mostrando los tiempos de divergencia promedio para géneros cercanos, estimados de secuencias de reducida tasa de mutación de ADN nuclear (modificado e interpretado de Jarman, 2001, Figura 4). (d) Árbol filogenético redibujado y simplificado del Orden Euphausiacea, donde fue propuesta la subfamilia Euphausiinae (Maas & Waloszek, 2001).

logical tolerances (Brinton, 1962; Johnson & Brinton, 1963). Casanova (1984) suggested that the broadcast-spawning is a primitive reproductive strategy in the Order Euphausiacea. According to Casanova (1984, 2004) the evolutive trend in euphausiids is the reduction of pairs of thoracopods in the two sex genders, the elongation of the second and third pair of periopods and the acquisition of bilobed eyes and ovigerous sac. According to Brinton (1965) and Maas & Waloszek (2001) who have a similar perspective as Casanova, the sac-spawning genera *Nematoscelis* and *Stylocheiron* share advanced morphological characteristics like elongation of one or two pairs of thoracopods, a bilobed eye and neotenic external morphology as adults, indicating that these are apomorphic characteristics in comparison with species of other genera within the Order Euphausiacea. However, the molecular phylogeny for all the euphausiid genera of the family Euphausiidae, shows a different perspective (Jarman, 2001). Jarman's unrooted tree based on partial 28S rDNA sequences, which states that no euphausiid is taken to be more ancient than any other, is different from Casanova's phylogenetic tree, excepting the close relationship between the broadcast-spawning genera *Thysanoessa* and *Meganyctiphanes* and that *Tessarabrachion* diverges from any other euphausiid genera clustered in other phylogenetic groups (Fig. 2c).   The main differences were that *Pseudeuphausia* (a sac-spawner) was considered a primitive euphausiid genus which possibly diverged about 100 Mya (Cretaceous) from the common ancestor of the family Euphausiidae (~130 Mya) and that *Nyctiphanes* (sac-spawner) and *Euphausia* (broadcast spawner) were closely related (here is shown that both hatch primarily as nauplii), even though they have different external morphology and reproductive strategy. Another novel aspect of Jarman's perspective is that *Pseudeuphausia latifrons* (sac-spawner) and *Nematobrachion boopis* (broadcast spawner) are closest relatives, while Casanova considered *Pseudeuphausia* closely related with *Nyctiphanes* (both sac-spawning genera). Jarman *et al.* (2000a) supported the idea that if the sac and broadcast spawning genera are distributed throughout the Order Euphausiacea, it is more likely that sac-spawning is primitive and has been lost several times, explaining why euphausiids are most closely related to Mysids. Gómez-Gutiérrez (2003) suggested, from the hatching description of *Stylocheiron carinatum* (Ponomareva, 1969), that this species hatches as N2 using forward hatching mechanism like sometimes *N. difficilis*, *Nematoscelis* and *Stylocheiron* do, which seem to be recent genera that diverged about 23 Mya (Jarman, 2001). This suggests that the hatching mechanism of both genera might not be necessarily shared with other sac-spawning species like *Pseudeuphausia* (so far not observed), or *Nyctiphanes*. However, it is required to observe the hatching mechanism of all the sac spawning genera and species in future research. According with Jarman's phylogenetic tree apparently broadcast spawning is an advanced reproductive strategy because nauplii can swim and escape more efficiently than when hatching in older developmental stages. No euphausiid species have direct embryonic development, considered an apomorphic feature in other crustacean groups (Richter & Scholtz, 2001). However, because this study included only three sac-spawning species it cannot be extrapolated to other species or genera around the world. Such observations must be done in future studies. Relatively less comprehensive genetic studies (fewer genera and species) showed a relatively close match to Casanova's morphological and molecular phylogenies (mitochondrial large-subunit ribosomal partial 28S rDNA and cytochrome oxidase subunit I, COI DNA sequences) (Jarman *et al.*, 2000b; Zane & Patarnello, 2000; Buckin *et al.*, 2007). This makes evident the difficulty to compare and reach phylogenetic conclusions from information with unrepresented species.

Another recent euphausiid cladistic phylogenetic study based solely on morphology and development of euphausiid appendages emphasized the problem of egg release (Fig. 2d) (Maas & Waloszek, 2001). According to those authors, assuming that free release of eggs is a primitive feature, their phylogenetic tree implied that brood care could have developed four times independently within the Euphausiacea. This perspective arose because

the mechanisms of holding the eggs and the number of limbs involved differ among taxa (genera). Alternatively, Maas & Waloszek (2001) suggested that if egg carriage is the primitive stage within Euphausiacea, then loss of this habit should have occurred at least six times independently within Euphausiacea. However, they were inconclusive about what was more likely to have occurred throughout the evolution of the Order Euphausiacea. Also there is a complete lack of information on hatching mode and confirmation of reproductive strategy (broadcast or sac-spawning) for three euphausiid genera *Bentheuphausia, Nematobrachion* and *Tessarabrachion* (Gómez-Gutiérrez, 2002). Maas & Waloszek (2001) proposed a phylogenetic tree with a new subfamily (Euphausiinae), excluding the genera *Thysanopoda* and *Nematobrachion*, including *Meganyctiphanes norvegica* and two other subgroups named Euphausiini and Nematoscelini. *Pseudeuphausia* and *Euphausia* interpreted as sister genera (Euphausinii) and the Nematoscelini which included the genus *Nyctiphanes* closely related with another subgroup named Nematoscelina where they included *Nematoscelis*, *Thysanoessa*, *Tessarabrachion* and *Stylocheiron* provided more phylogenetic relationships than other previously proposed phylogenetic trees (Fig. 2d). However, they did not quote Casanova and Jarman's publications and therefore probably they were unaware of such studies showing relatively unsupported clustering of species. Recently Bucklin *et al.* (2007) made a genetic comparison of ~650 bp region of mitochondrial cytocrome oxidase I (mtCOI) among 40 euphausiid species (10 genera) around the world with the groups of euphausiids derived using morphological features (Brinton *et al.*, 2000). He showed a close inter species match suggesting that this is a useful method to resolve relationships among closely related species to ensure identification, recognition of cryptic species and evaluation of taxonomically meaningful geographic variation. However, because they did not make comparisons among genera they did not provide an insight into evolutionary relationships of euphausiids.

Although the present study proposes that hatching in a nauplius stage in the Order Eup-

hausiacea is a derived (apomorphic) characteristic in their evolutionary phylogeny (Fig. 2a), there is a cost to early hatching, imposed by mortality and certainly high mortality by predation in free-swimming larvae that must be compensated with high fecundity rates typical of broadcast spawning species (Nicol, 1995; Gómez-Gutiérrez *et al.*, 2006). Most euphausiids are highly gregarius animals forming dense swarms that attract multiple predators, if spawning occurs. At least mortality due predators on females after spawning is independent from the eggs, situation that does not happen with sac-spawning species. In broadcast spawning species, mortality during hatching process seems to be relatively small (<2%) in nature coupled with the ability to avoid plankto- nic predators as soon as the nauplius hatch (probably almost negligible in a population perspective), or to avoid deeper layers devoid of food when the eggs are sinking after spawning. Because both reproductive strategies allow survival of the species, reflecting the different ecological strategies, hatching mode perhaps does not reflect an evolutionary advantage for most recent genera explaining why appear irregularly within the Euphausia- cea phylogeny. Even if hatching as nauplius is plesiomorphic within Euphausiacea, it might be an apomorphy for Euphausiacea in relation to other Malacostraceans (Sudhaus & Rehfeld, 1992). This study, like all of the studies published so far on morphology, development and genetic phylogenetic trees, shows partial information of euphausiid biology and phylogeny. Thus, the interpretation provided in this work must be taken with caution. The observations of hatching mechanisms and hatching success reported and summarized here (Table 3) compared chronologically with the phylogeny of euphausiids deducted from DNA analysis, support the evolutionary trend that the free-nauplius has reverted several times within the family Euphausiidae and sac-spawning may be necessarily considered exclusively as an apomorphic condition since several distinctive hatching mechanisms occur in *Nematoscelis, Stylocheiron* and *Nyctiphanes* perhaps reflecting different time adaptations throughout the euphausiid´s phylogeny. Evidently the reproductive and hatching mechanisms of at least one or two species of each

euphausiid genus should be investigated in order to draw more coherent and sound conclusions. This conceptual model predicts that future studies will find that both broadcast and sac-spawning species genera share in different relative proportion distinct hatching embryonic stages according with its time of divergence throughout the Euphausiacea´s phylogeny.

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