



OVARIAN EGG MORPHOLOGY IN CHALCIDOID WASPS (HYMENOPTERA: CHALCIDOIDEA) PARASITIZING GALL WASPS (HYMENOPTERA: CYNIPIDAE)

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ABSTRACT

We provide morphological egg data of 26 species of 5 chalcidoid families associated with cynipid galls (Hymenoptera: Cynipidae) from western Palaearctic, including the first egg data for the family Ormyridae. Adult chalcidoid species were reared from galls, and eggs obtained from dissected female ovaries were examined using scanning electron microscopy (SEM). The shape of the eggs varies from oval to elongate and tapered at both ends. Eggs of Eurytomidae as well as some Eulophidae, Eupelmidae and Pteromalidae are equipped with a peduncle at the anterior end. We found a positive correlation between long eggs and long ovipositors and confirmed the expectation that eggs of endoparasitoids are generally shorter and narrower than eggs of ectoparasitoids. We were able to locate the sperm entrance or micropyle at the anterior pole of eggs of several species. It is situated at the anterior end of the egg and at the end of the peduncle when present. In addition, the eggshells of the endoparasitoid *Sycophila biguttata* (Swederus, 1795) (Hymenoptera: Eurytomidae) and the ectoparasitoid *Cecidostiba fungosa* (Geoffroy, 1785) (Hymenoptera: Pteromalidae), are for the first time described.

Key words: chalcidoid egg ultrastructure; micropyle; eggshell; mode of parasitism; immature stages.

RESUMEN

Morfología del huevo ovárico en calcidoideos (Hymenoptera: Chalcidoidea) parasitoides de avispa de las agallas (Hymenoptera: Cynipidae)

En el presente trabajo se aportan datos morfológicos del huevo de 26 especies del Paleártico occidental pertenecientes a 5 familias de Chalcidoidea asociadas con agallas de cinípidos (Hymenoptera: Cynipidae), incluyendo los primeros datos del huevo de especies de Ormyridae. Los ejemplares adultos de las especies estudiadas fueron obtenidos por emergencia de agallas en laboratorio, los ovarios de las hembras diseccionados para obtener los huevos, que fueron finalmente estudiados utilizando técnicas de microscopía electrónica de barrido. La forma de los huevos estudiados varía de ovalada a alargada y ahusada en ambos extremos. Los huevos de Eurytomidae, así como algunos de Eulophidae, Eupelmidae y Pteromalidae están provistos de un pedúnculo en el extremo anterior. Se encontró una correlación positiva entre aquellos huevos elongados y la presencia de oviposidores largos en las hembras, confirmándose también la hipótesis esperada de que los huevos de especies endoparasitoides son generalmente más cortos y estrechos que los de los ectoparasitoides. Por otro lado los estudios de ultraestructura en los huevos de varias especies han permitido la localización del punto de entrada de esperma o micropilo. Este se encuentra situado bien en el extremo anterior del huevo o bien en el extremo del pedúnculo cuando está presente. Además, por primera vez se estudia y se describe la ultraestructura de la cáscara del huevo de la especie endoparasitoide *Sycophila biguttata* (Swederus, 1795) (Hymenoptera: Eurytomidae) y del ectoparasitoide *Cecidostiba fungosa* (Geoffroy, 1785) (Hymenoptera: Pteromalidae).

Palabras clave: ultraestructura del huevo de chalcidoidea; micropilo; cáscara de huevo; modo de parasitismo; estados inmaduros.

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Introduction

The most diverse group of parasitic wasps is probably the superfamily Chalcidoidea (Hymenoptera) with its 22000 described species and an immense variety of life modes (Noyes, 1978, 1990a; Gordh *et al.*, 1979; Gibson, 1993). Most chalcidoids are entomophagous and attack 339 arthropod families (Clausen, 1940; Noyes, 2002). The great majority are egg and larval parasitoids, while some groups are, at least in part, phytophagous gall inhabitants on as much 444 plant families (Noyes, 2002) or even true gall inducers (La Salle, 2005).

The gall wasps or cynipids (Hymenoptera: Cynipidae) is a large group of Hymenoptera representing one of the more speciose radiations of gall-inducing insects with more than 1400 species described (Nieves-Aldrey, 2001). The classification of the family has been recently revised into twelve tribes which are strongly supported as monophyletic (Ronquist *et al.*, 2015), nine of which are represented in the western Palaearctic. In addition of their intrinsic biological interest, plant galls induced by Cynipidae host large communities of insects which are composed mainly of other cynipid inquiline species and parasitoids which attack all the gall inhabitants. These micro communities support intricate food webs which have been focus of many ecological studies (Askew, 1961; Schönrogge *et al.*, 1995, 1996; Bailey *et al.*, 2009).

The parasitoids inhabitant cynipid galls in western Palaearctic belong mainly to six chalcidoid families: Euytomidae, Torymidae, Ormyridae, Pteromalidae, Eupelmidae and Eulophidae. This parasitoid fauna has been catalogued in recent years (Askew *et al.*, 2006, 2013).

Egg and eggshell can be useful for identification of chalcidoid species (Askew, 1961). For instance Claridge & Askew (1960) showed clear differences in the egg between species of *Eurytoma rosae* Nees, 1834 species-group. Extensive morphological variability in egg structure has been observed between and within families of Chalcidoidea. The egg is short and oval or slender and elongate (Parker, 1924a; Clausen, 1940; Iwata, 1962). A summary of external features of the chalcidoid egg from literature is given in Table 1.

The eggshell ultrastructure has been described for a handful of chalcidoids including *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) (King *et al.*, 1968; Richards, 1969) and *Eurytoma amygdali* Enderlein, 1907 (Hymenoptera: Eurytomidae)

(Mouzaki & Margaritis, 1994; Zarani & Margaritis, 1994). It typically consists of a thin vitelline membrane, adjacent and often attached by interlocking ridges to the oocyte (King *et al.*, 1968) and an outer chorion. The chorion is divided into an inner electron-translucent endochorion and an outer electron-dense exochorion (King *et al.*, 1968; Richards, 1969; Mouzaki & Margaritis, 1994). The endochorion has a uniform smooth structure, whereas the exochorion may have sublayers of granular and columnar structure (Mouzaki & Margaritis, 1994). Spines originating from the endochorion and extending through the exochorion and onto the egg surface were found in *Catolaccus* (Hymenoptera: Pteromalidae) (King *et al.*, 1968). The structure and elasticity of the eggshell facilitate the stretching of the egg through the narrow and long ovipositor. Thus the chorion appears to be reduced in structure, i.e. fewer layers, compared with insects with different oviposition requirements (i.e. diameter of the ovipositor relative to egg width) like the reduviid *Rhodnius prolixus* Stahl that has seven layers in its chorion (Beament, 1946; King *et al.*, 1968; Richards, 1969). The endochorion has been shown to contain peroxidase in *Eurytoma amygdali* (Hymenoptera: Eurytomidae) to provide elasticity (Mouzaki & Margaritis, 1994; Zarani & Margaritis, 1994). The anterior micropylar region of the egg of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) is marked by an opening or by several grooves (King, 1962) on the external surface of the chorion. In *Eurytoma amygdali* (Hymenoptera: Eurytomidae), it is situated at the end of a short posterior filament, the micropylar appendage in the posterior end of the egg (Zarani & Margaritis, 1994). The structure of the eggshell often reflects mode of parasitism. Endoparasitic species often have a thin hydronic eggshell that can take up nutrients from the surrounding host tissue (Margaritis, 1985).

The aim of the study was to provide new data on the egg morphology of chalcidoids by analysing species associated with cynipid galls. Descriptive data on immature stages of species inhabitant cynipid galls are potentially important for studies such as phylogenetic analyses, the ecology of chalcidoid communities, as well as studies of food webs. In recent years some works have been published on comparative morphology of terminal-instar larvae of species of several chalcidoid families associated to gall wasps in Europe (Nieves-Aldrey *et al.*, 2008; Gómez *et al.*, 2008, 2011, 2013; Gómez & Nieves-Aldrey, 2012). However there

Table 1.— Chalcidoid egg data summarized from literature.
 Tabla 1.— Datos de los huevos de Chalcidoidea tomados de la bibliografía.

Family	Egg shape	Ovarian or post-oviposition egg observed	Egg surface	Anterior peduncle	Posterior process	References
Agonidae	dumb-bell shaped (ovarian), elongate (post-ovarian)	Ovarian & post-oviposition	smooth	present	absent	Grandi (1929)
Aphelinidae	elongate, curved, wider at anterior end	Ovarian & post-oviposition	smooth, translucent	present or absent	absent	Compere & Smith (1932), Cendaña (1937), Parker (1924a)
Chalcididae	elongate, curved and wider at anterior end	Post-oviposition	smooth, some sculpture around micropyle	present or absent	absent	Parker (1924b), Dowden (1935)
Encyrtidae	dumb-bell shaped (encyrtiform), or elongate	Ovarian & post-oviposition	may be partly sculptured	present	absent	Ciancy (1946), Hagen (1964), Noyes (1990b)
Eucharitidae	Oval or curved dorsally and flattened ventrally	Ovarian & post-oviposition	smooth	present	absent	Parker (1924a), Heraty & Darling (1984)
Eulophidae	elongate, curved, broader at anterior end	Ovarian & post-oviposition	smooth	present or absent	absent	Parker (1924a), Cameron (1939), Ciancy (1946)
Eupelmidae	Elongate	Ovarian & post-oviposition	smooth	present	present	Phillips & Poos (1921), Ciancy (1946)
Eurytomidae	Elongate	Ovarian & post-oviposition	smooth, some with spines or lattice-structure	present	present	Ciaridge & Askew (1960), Arthur, (1961), Fisher (1965)
Leucospidae	elongate, curved, wider at anterior end	Ovarian & post-oviposition	tubercles, sharply pointed at anterior end, rounded at posterior end	present	absent	Fabré (1886), Parker (1924a)
Mymaridae	elongate	Ovarian & post-oviposition	heavily sculptured	present	absent	Clausen (1940), Jackson (1961)
Perilampidae	elongate, slightly flattened ventrally, tapering at anterior end	Ovarian & post-oviposition	heavily sculptured	Absent	absent	Heraty & Darling (1984), Smith (1917), Parker (1924a)
Pteromalidae	elongate, tapering towards both ends	Ovarian & post-oviposition	smooth or covered with papillae	Most often absent, but present in some	absent	Parker (1924a), Cameron (1939), Ciancy (1946)
Tanaostigmatidae	may be dumb-bell shaped	Ovarian & post-oviposition	unknown	present	absent	La Salle & LeBeck (1983)
Torymidae	elongate, tapering toward both ends	Ovarian & post-oviposition	smooth or covered with spines	Most often absent, but present in some	absent	Parker (1924a), Muesebeck (1931), Askew (1961), Askew (1966), Phillips & Poos (1921)
Trichogrammatidae	ellipsoid or elongate, strongly tapering at posterior end	Ovarian & post-oviposition	smooth or partly heavily sculptured	present or absent	absent	Silvestri (1916), Bakkendorf (1934), Parker (1924a)
Signiphoridae	banana-shaped	Post-oviposition	unknown	present	absent	Rosen <i>et al.</i> (1992)

are not similar comprehensive studies on egg morphology of the same or related species. We give here scanning electron micrographs showing the eggs of 3 species of Eulophidae, 1 Eupelmidae, 8 Eurytomidae, 3 Ormyridae, 4 Pteromalidae and 7 Torymidae as well as transmission electron micrographs showing the eggshell ultrastructure of 1 Eurytomidae (*Sycophila biguttata*) and 1 Pteromalidae (*Cecidostiba fungosa*). Data on egg shape variation, as well as aspects of egg and eggshell properties and oviposition habits are also investigated. Furthermore the ultrastructure of the eggshells of these species is described for the first time. Finally the micropyle and eggshell surface structure are shown for a number of the species.

Material and methods

SELECTED TAXA

We studied the eggs of 26 species of Chalcidoidea collected in Spain and Sweden between 2000 and 2007 (Table 2). The females were reared from cynipid galls on Asteraceae, Fagaceae, Lamiaceae, Papaveraceae, Rosaceae and Sapindaceae induced by species of the tribes Aylacini, Aulacideini, Diastrophini, Diplolepidini, Pediastpidini and Cynipini (Table 2). Taxonomy of cynipids follows Nieves-Aldrey (2001) and Ronquist *et al.* (2015).

REARING AND DISSECTION

Adult females were reared from galls stored in rearing cages or extracted after dissection of galls. Eggs were dissected from the ovaries of the females and counted. It is often difficult to count the number of eggs in the ovaries as the egg may be covered with ovarian sheets and be in varying degree of maturity, thus the egg count given in Table 4 for each species is approximate. Potential difficulties by using only ovarian eggs for descriptions may be that in some species the eggs tend to transform dramatically after oviposition, and sometimes ovarian tissue may conceal the surface structure of each individual egg (HV, personal observation). In the present study only ovarian eggs have been observed with the exception of the very few cases when we happened to find deposited eggs inside galls (see Table 2, Fig. 11). The most mature eggs in the ovaries were selected for the study. We leave the study of post-oviposition eggs for later studies.

PREPARATIONS FOR MORPHOLOGICAL STUDIES

Prior to scanning electron microscopy the adult females were preserved in absolute alcohol and ovarian eggs were dissected and put directly on SEM stubs and into the scanning electron microscope (Zeiss Supra35VP and FEI Quanta 200) on low vacuum without critical point drying or gold coating.

For transmission electron microscopy, the reared females were fixed in PAGF (Stefanini *et al.*, 1967) for 1-2 months. Eggs were dissected and fixed in osmium-tetroxide and dehydrated through an ethanol series, finishing with acetone. The specimens were then infiltrated with 1:1 solution of acetone and epoxy resin (Epon 812) over night and then in pure resin at 60 degrees Celsius for 48 hours before sectioning (60 nm thin sections on copper grids), triple-dyeing in lead citrate- uranyl acetate-lead citrate, and examination in an LEO 912 AB transmission electron microscope.

TERMINOLOGY AND MEASUREMENTS

General terminology used in egg and eggshell descriptions follows King (1962) and Margaritis (1985).

The length of the egg body is measured from the posterior tip to the transition between the egg body and the peduncle (Table 3). The width measurement is taken at the maximum width of the egg body. Only external measurements of the ovipositor were taken. The length of the ovipositor was measured from the side. The ovipositor width was measured from the ventral side at the narrowest point at the apical tip and at the broadest point at the basal articulation (Table 3).

To examine how the egg shape varies within the Chalcidoidea, the different egg shapes were drawn onto a recent phylogeny (Fig. 12) (Heraty *et al.*, 2013). Redrawings from previous authors' illustrations were included (Silvestri, 1916; Parker, 1924a; Grandi, 1929; Dowden, 1935; Cendaña, 1937; Clausen, 1940; Jackson, 1961; La Salle & LeBeck 1983; Noyes, 1990a; Rosen *et al.*, 1992).

Results

Egg and ovipositor measurements are given in Table 3 and egg shape characters, approximate egg counts as well as environment at egg deposition site are given in Table 4.

Pedunculate eggs were observed in 5 families (Figs. 1-3, 5-6; Table 4) and non-pedunculate egg in Eulophidae (Fig. 1), Ormyridae (Fig. 4), Pteromalidae (Fig. 5) and Torymidae (Fig. 6).

For species in our material (Fig. 11), the peduncle does not appear to be attached to the host tissue, but aiding the egg in passing through the ovipositor. The diameter of the eggs with peduncle (mean: 60.85 μm) is narrower than the eggs without peduncle (mean: 62.15 μm) (Table 3). The eggs with a peduncle have a shorter egg body (mean: 205.66 μm) than the ones without a peduncle (mean: 353.69 μm) (Table 3). The non-pedunculate egg may be short and pear-shaped (Eulophidae), oval/subrectangular (*Mesopolobus mediterraneus* and *Torymus rubi*), but most often elongate and rather long egg body.

Table 2.— Taxonomy, hosts and material used in the present study. A = asexual generation, S = sexual generation, – = no alternate generation. The eggshell ultrastructure was examined for the species marked * and post-oviposition egg were observed for species marked #.

Tabla 2.— Taxonomía hospedadores y materiales utilizados en el presente estudio. A = generación asexual, S = generación sexual, – = sin generación alternante. La ultraestructura de la cascara del huevo fue examinada para las especies marcadas * y el huevo después de la puesta fue observado para las especies con el símbolo #.

Studied Chalcidoidea species	Family	Gall wasp inducer host (generation)	Host plant	Chalcidoid life mode	Collecting sites
<i>Aprostocetus epicharmus</i>	Eulophidae	<i>Aylax minor</i>	<i>Papaver dubium</i> .	endoparasitoid	Spain, Madrid, Rivas-Vaciamadrid. 20/VI/99; VI/99 J. L. Nieves leg.
<i>Aulogymnus skianeuros</i>	Eulophidae	<i>Biorhiza pallida</i> (S)	<i>Quercus faginea</i>	ectoparasitoid	Spain, Toledo, Robledo del Mazo 11/III/06 J.L. Nieves leg.
<i>Dichotomus acerinus</i>	Eulophidae	<i>Pediaspis aceris</i> (S)	<i>Acer opalus</i>	inquiline	Spain, Tarragona, Colldejou. 14/VIII/03; 1-7/III/04. J. L. Nieves leg
<i>Eupelmus microzonus</i>	Eupelmidae	<i>Isocolus lichtensteini</i> (-)	<i>Centaurea melitensis</i>	ectoparasitoid	Spain, Madrid, Dehesa de Arganda. 12/II/04; 15-21/III/04. J. L. Nieves leg.
<i>Eurytoma brunniventris</i> #	Eurytomidae	<i>Trigonaspis synaspis</i> (A)	<i>Quercus pyrenaica</i>	ectoparasitoid	Spain, Madrid, Miraflores 3/IX/05. J. L. Nieves leg.
<i>Eurytoma infracta</i>	Eurytomidae	<i>Nealax verbenacus</i> (-)	<i>Salvia verbenaca</i>	ectoparasitoid	Spain, Madrid, Dehesa de Arganda 6/VI/99; VIII/99. J. L. Nieves leg.
<i>Eurytoma rosae</i> #	Eurytomidae	<i>Diplolepis rosae</i> (-)	<i>Rosa</i> sp.	ectoparasitoid	Spain, Madrid, Nuevo Baztán 23/04/05. J. L. Nieves leg.
<i>Eurytoma strigifrons</i>	Eurytomidae	<i>Isocolus lichtensteini</i> (-)	<i>Centaurea melitensis</i>	ectoparasitoid	Spain, Madrid, Dehesa de Arganda. 12/II/04; 15-21/III/04. J. L. Nieves leg.
<i>Sycophila biguttata</i> *	Eurytomidae	<i>Biorhiza pallida</i> (S)	<i>Quercus faginea</i>	endoparasitoid	Sweden, Uppland, Stockholm, Ekshagen, 16/VII/07, H. Vårdal, leg.
<i>Sycophila binotata</i>	Eurytomidae	<i>Plagiotrochus quercusilicis</i> (A)	<i>Quercus ilex</i>	endoparasitoid	Spain, Granada, Vivero Egmasa 17/II/06; IV/06. J. L. Nieves leg.
<i>Sycophila mayri</i>	Eurytomidae	<i>Phanacis centaureae</i>	<i>Centaurea</i> sp.	endoparasitoid	Spain, Madrid, Dehesa de Arganda. 21/IV/05. J. L. Nieves leg
<i>Sycophila submutica</i>	Eurytomidae	<i>Isocolus lichtensteini</i> (-)	<i>Centaurea melitensis</i>	endoparasitoid	Spain, Madrid, Dehesa de Arganda. 12/II/04; 23-29/II/04. J. L. Nieves leg.
<i>Ormyrus nitidulus</i>	Ormyridae	<i>Andricus hispanicus</i> (A)	<i>Quercus canariensis</i>	ectoparasitoid	Spain, Madrid, Algatocín. 19/VIII/02. J. L. Nieves leg.
<i>Ormyrus papaveris</i>	Ormyridae	<i>Aylax minor</i> (-)	<i>Papaver rhoeas</i>	ectoparasitoid	Spain, Madrid, Rivas-Vaciamadrid. 20/VI/99; VI/99 J. L. Nieves leg.
<i>Ormyrus wachtli</i>	Ormyridae	<i>Nealax verbenacus</i> (-)	<i>Salvia verbenaca</i>	ectoparasitoid	Spain, Madrid, Dehesa de Arganda. 1/VI/03; VI/03 J. L. Nieves leg.
<i>Cecidostiba fungosa</i> *	Pteromalidae	<i>Biorhiza pallida</i> (S)	<i>Quercus robur</i>	ectoparasitoid?	Spain, Madrid, Chapinería.15/V/00. J. L. Nieves leg.
<i>Mesopolobus mediterraneus</i>	Pteromalidae	<i>Biorhiza pallida</i> (S)	<i>Quercus faginea</i>	ectoparasitoid?	Spain, Madrid, Chapinería.15/V/00. J. L. Nieves leg.
<i>Pteromalus bedeguaris</i> #	Pteromalidae	<i>Diplolepis rosae</i> (-)	<i>Rosa</i> spp.	ectoparasitoid?	Spain, Madrid, Borox. 7/III/03. J. L. Nieves leg.
<i>Stinoplus lapsanae</i>	Pteromalidae	<i>Timaspis lamsanae</i> (-)	<i>Lampsana communis</i>	ectoparasitoid?	Spain, Madrid, El Escorial, 06/XII/84, J.L. Nieves leg.
<i>Adontomerus impolitus</i>	Torymidae	<i>Aulacidea tragopogonis</i> (-)	<i>Tragopogon</i> spp	ectoparasitoid	Spain, Madrid, El Campillo-Rivas. 6/III/05; V/05. J. L. Nieves leg.
<i>Chalcimerus borceai</i>	Torymidae	<i>Barbotinia oraniensis</i>	<i>Papaver rhoeas</i>	ectoparasitoid	Spain, Madrid, El Campillo-Rivas. 22/II/04; 18/IV/04. J. L. Nieves leg.
<i>Glyphomerus stigma</i>	Torymidae	<i>Diplolepis mayri</i>	<i>Rosa</i> sp.	ectoparasitoid	Spain, Ourense, Rubiá, 16/VI/03, J.L. Nieves leg
<i>Megastigmus aculeatus</i>	Torymidae	-	<i>Rosa</i> sp.	seed eater	Spain, Segovia, Siguero 1/V/07. J. L. Nieves leg.
<i>Torymus bedeguaris</i> *	Torymidae	<i>Diplolepis rosae</i>	<i>Rosa</i> spp.	ectoparasitoid	Sweden, Öland, 3/V/2007. H. Vårdal leg.
<i>Torymus nobilis</i>	Torymidae	<i>Biorhiza pallida</i> (A)	<i>Quercus pyrenaica</i>	gregarious ectoparasitoid	Spain, Madrid, Miraflores. 10/XII/02; VI,03. J.L. Nieves leg.
<i>Torymus rubi</i>	Torymidae	<i>Diastrophus rubi</i> (-)	<i>Rubus</i> spp.	ectoparasitoid	Spain, Asturias, Ajuján-Oviedo. 7/IX/05; X/05. L. Parra leg. 2♀♀

Table 3.— Egg and ovipositor measurements. Ranges are given for the species for which less than 4 specimens were measured, whereas mean and standard deviations are given when $n \geq 4$. The ovipositor width was measured at apical point and at base. All measurements are given in μm .

Tabla 3.— Medidas del huevo y del ovipositor. Se dan los intervalos para los cuales se midieron menos de 4 ejemplares, mientras que la media y la desviación estándar se dan cuando $n \geq 4$. La anchura del ovipositor se midió desde el extremo apical al basal. Todas las medidas se dan en μm .

Studied Chalcidoidea species (families)	Egg body length	Peduncle length	Egg body width	Posterior process length	Ovipositor external length	Ovipositor external width
<i>Aprostocetus epicharmus</i>	135-175 (n=2)	no peduncle	30-45 (n=2)	no process	770 (n=1)	10-15 (n=1)
<i>Aulogymnus skianeuros</i>	150-160 (n=3)	140-160 (n=3)	48-65 (n=3)	no process	1275 (n=1)	15-35 (n=1)
<i>Dichotomus acerinus</i>	113.2±6.8 (n=5)	no peduncle	42±5.7 (n=5)	no process	775 (n=1)	20-35 (n=1)
<i>Eupelmus microzonus</i>	268.8±10.3 (n=4)	323.8±19.7 (n=4)	77.5±2.9 (n=4)	30-35 (n=2)	2125 (n=1)	20-60 (n=1)
<i>Eurytoma bruniventris</i>	200-220 (n=2)	290-310 (n=2)	60-70 (n=2)	35 (n=1)	1075-1750 (n=2)	20-60 (n=2)
<i>Eurytoma infacta</i>	250-320 (n=2)	380 (n=1)	60-70 (n=2)	20-35 (n=2)	1200-1375 (n=2)	10-40 (n=2)
<i>Eurytoma rosae</i>	360 (n=1)	300 (n=1)	90 (n=1)	no process	2275 (n=1)	20-70 (n=1)
<i>Eurytoma strigifrons</i>	254±11.4 (n=5)	435±44.7 (n=5)	82±7.6 (n=5)	50±3.5 (n=5)	2475 (n=1)	20-60 (n=1)
<i>Sycophila biguttata</i>	115±5 (n=5)	always broken (1 at least 200)	57±4.5 (n=5)	20 (n=1)	2325-2625 (n=2)	10-60 (n=2)
<i>Sycophila binotata</i>	148.8±8.5 (n=4)	always incomplete (1 at least 550)	46.3±4.8 (n=4)	22.5±2.9 (n=4)	1875 (n=1)	15-40 (n=1)
<i>Sycophila mayri</i>	150 (n=2)	always incomplete (1 at least 600)	30-45 (n=2)	not visible or flat to egg	1975 (n=1)	10-35 (n=1)
<i>Sycophila submutica</i>	95-130 (n=3)	always incomplete (1 at least 260)	35-55 (n=3)	20-30 (n=3)	ovipositor broken at base, missing	at ovipositor base: 50
<i>Ormyrus nitidulus</i>	760-810 (n=3)	no peduncle	80-120 (n=3)	no process	5100-5250 (n=2)	20-50 (n=2)
<i>Ormyrus papaveris</i>	384±83.2 (n=5)	no peduncle	70±15.8 (n=5)	no process	2075-3125 (n=2)	15-60 (n=2)
<i>Ormyrus wachlii</i>	333±32.1 (n=5)	no peduncle	80±7.1 (n=5)	no process	1350 (n=1)	15-50 (n=1)
<i>Cecidostiba fungosa</i>	250 (n=1)	no peduncle	50 (n=1)	no process	2025 (n=1)	20-45 (n=1)
<i>Mesopolobus mediterraneus</i>	85 (n=1)	no peduncle	50 (n=1)	no process	1250 (n=1)	15-35 (n=1)
<i>Pteromalus bedeguaris</i>	390-520 (n=3)	no peduncle	40-90 (n=3)	no process	2200 (n=1)	15-70 (n=1)
<i>Stinopulus lapsanae</i>	290 (n=1)	no peduncle	60 (n=1)	no process	1550 (n=1)	10-30 (n=1)
<i>Adontomerus impolitus</i>	405-420 (n=2)	no peduncle	60 (n=2)	no process	2450 (n=1)	15-40 (n=1)
<i>Chalcimerus borceai</i>	460 (n=1)	no peduncle	65 (n=1)	no process	3425 (n=1)	15-90 (n=1)
<i>Glyphomerus stigma</i>	660 (n=1)	no peduncle	75 (n=1)	np process	1425-5100 (n=3)	15-120 (n=3)
<i>Megastigmus aculeatus</i>	125-140 (n=2)	480-680 (n=2)	45-60 (n=2)	20 (n=2)	4100-5225 (n=2)	15-70 (n=2)
<i>Torymus nobilis</i>	497.5±37.8 (n=4)	no peduncle	76.3±10.3 (n=4)	no process	4400 (n=1)	20-80 (n=1)
<i>Torymus rubi</i>	53-70 (n=2)	no peduncle	32-40 (n=2)	no process	3825 (n=1)	15-80 (n=1)

Table 4.— Egg characters, counts and oviposition environment. Short peduncle length = less or equal to 340 µm, Long peduncle length = longer than 340 µm. Egg body length: Short: 250 µm or less, Long: longer than 250 µm. Ovipositor length: Short: 2162 µm or shorter, Long: longer than 2162 µm.

Tabla 4.— Caracteres del huevo, conteos y datos ambientales del ovipositor. Longitud del pedúnculo corto=menor o igual a 340 µm, longitud del pedúnculo largo = más largo que 340 µm. Longitud del cuerpo del huevo: Corto: 250 µm o menos, Largo: más largo que 250 µm. Longitud del ovipositor: Corto: 2162 µm o más corto, Largo: más largo que 2162 µm.

Species	Egg shape	Egg surface	Anterior peduncle	Posterior process	Egg count (approx)	Ovipositor	Oviposition substrate/ environment
<i>Aprostocetus epicharmus</i>	Pear, short	smooth	Absent	absent	-	short	inside larva, fluid
<i>Aulogymnus skianeuros</i>	Oval, short	smooth	Present, short	absent	40-50 eggs	short	on larva in gall, medium dry
<i>Dichatomus acerinus</i>	Pear, short	smooth	Absent	absent	50	short	Inquiline, dry gall
<i>Eupelmus microzonus</i>	Oval, long	smooth	Present, short	present	10	short	on larva in gall, dry
<i>Eurytoma brunneiventris</i>	Elongate/Oval, short	papillate	Present, short	present	4-5	short	on larva in gall, dry
<i>Eurytoma infacta</i>	Oval, long	papillate	Present, long	present	4-10	short	on larva in gall, dry
<i>Eurytoma rosae</i>	Oval long	papillate	Present, short	present	10-12	long	on larva in gall, dry
<i>Eurytoma strigifrons</i>	Oval, long	smooth	Present, long	present	20	long	on larva in gall, dry
<i>Sycophila biguttata</i>	Oval, short	smooth	Present, short	present	6-8	long	inside larva, fluid
<i>Sycophila binotata</i>	Oval, short	smooth	Present, long	present	-	short	inside larva, fluid
<i>Sycophila mayri</i>	Oval, short	smooth	Present, long	present	15-16	short	inside larva, fluid
<i>Sycophila submutica</i>	Oval, short	smooth	Present, short	present	60	not measured	inside larva, fluid
<i>Ormyrus nitidulus</i>	Elongate, long	smooth	Absent	absent	-	long	on larva in gall, dry
<i>Ormyrus papaveris</i>	Elongate, long	irregular ridges	Absent	absent	15	long	on larva in gall, dry
<i>Ormyrus wachtli</i>	Elongate, long	smooth	Absent	absent	12-14	short	on larva in gall, dry
<i>Cecidostiba fungosa</i>	Elongate, short	smooth	Absent	absent	19-20	short	on larva in gall, medium dry
<i>Mesopolobus mediterraneus</i>	Oval/Sub- rectangular, short	smooth	Absent	absent	-	short	on larva in gall, dry
<i>Pteromalus bedeguaris</i>	Elongate, long	spiny	Absent	absent	6	long	on larva in gall, dry
<i>Stinoplus lapsanae</i>	Elongate, long	smooth	Absent	absent	20	short	on larva in gall, dry
<i>Adontomerus impolitus</i>	Elongate, long	papillate	Absent	absent	8	long	on larva in gall, dry
<i>Chalcimerus borecai</i>	Elongate, long	smooth	Absent	absent	6	long	on larva in gall, dry
<i>Glyphomerus stigma</i>	Elongate, long	partly spiny	Absent	absent	-	long	on larva in gall, dry
<i>Megastigmus aculeatus</i>	Oval, short	smooth	Present, long	absent	5-9	long	Seed eater, dry
<i>Torymus bedeguaris</i>	Elongate, n.m	smooth	Absent	absent	50-70	long	on larva in gall, dry
<i>Torymus nobilis</i>	Elongate, long	smooth	Absent	absent	10-12	long	on larva in gall, dry
<i>Torymus rubi</i>	Oval/Sub-rectangular, short	smooth	Absent	absent	-	long	on larva in gall, dry

The ovipositor length is positively correlated with egg body length. In 75 % of the species, a short egg body is associated with short ovipositor and long egg body with a long ovipositor (Table 4). There are 3 exceptions in which the egg body is short and the ovipositor is long (*Sycophila biguttata*, *Megastigmus aculeata* and *Torymus rubi*) and 3 species in which the egg body is long and the ovipositor is short (*Eupelmus microzonus*, *Eurytoma infracta*, *Ormyrus wachtlei*) and this may in some cases be attributed to the fact that either the ovipositor or the egg body length was in the vicinity of the limit between what we defined as short and long or perhaps, in a few cases, the eggs may not have been fully mature when examined.

Egg body width is always wider than external ovipositor width measurements (Table 3) meaning that the egg shell must be flexible to facilitate the oviposition through the egg canal.

EULOPHIDAE

The eggs of *Aprostocetus epicharmus* (Walker, 1839) (Tetrastichinae) and *Dichatomus acerinus* (Eulophinae) are elongate and somewhat wider in the anterior end (Fig. 1A) than at the posterior end. The specimens of *Dichatomus acerinus* contained around 50 eggs of approximately the same size (Table 4), The surface is

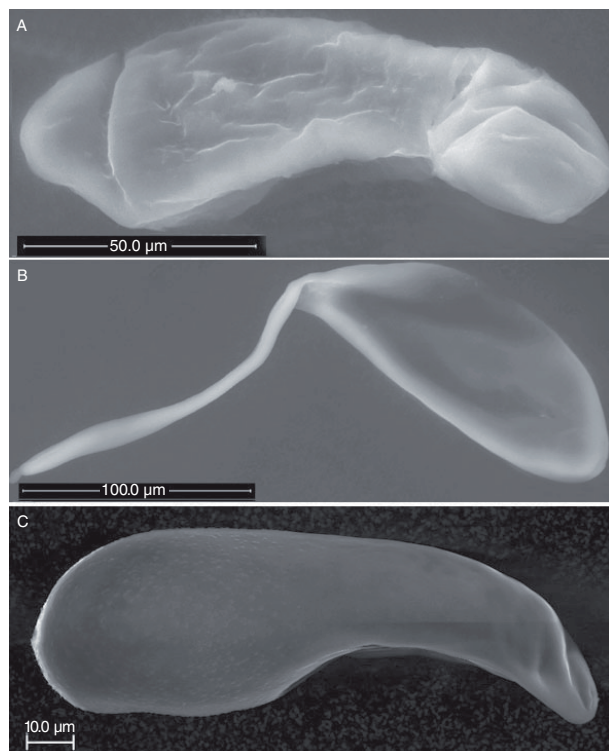


Fig. 1.— Eggs of Eulophidae A) *Aprostocetus epicharmus* B) *Aulogymnus skianeuros* and C) *Dichatomus acerinus*. The anterior end of the egg is to the left.

Fig. 1.— Huevos de Eulophidae A) *Aprostocetus epicharmus* B) *Aulogymnus skianeuros* y C) *Dichatomus acerinus*. La parte anterior del huevo es la izquierda.

mostly smooth, but has tiny papillae in the anterior half in *Dichatomus acerinus* (Figs. 1C, 7A).

The eggs of *Aulogymnus skianeuros* and *Aprostocetus epicharmus* have a smooth surface, peduncles equal in length to the egg body (Fig. 9A).

EUPELMIDAE

The egg of *Eupelmus microzonus* Förster, 1860 is elongate with a peduncle that is slightly longer than the egg body at the anterior end and a short process at the posterior end (Fig. 2). The micropyle is clearly visible at the end of the peduncle (Fig. 9B). The surface of the egg is entirely smooth.

EURYTOMIDAE

Four species each of the genera *Eurytoma* and *Sycophila* represent Eurytomidae in the present study (Table 2). *Eurytoma* eggs have an elongate egg body with a peduncle at one end and a short process at the other end (Figs. 3A-D). The latter is more pronounced in *E. brunniventris* Ratzeburg, 1852 (Fig. 3A) and *E. strigifrons* Thomson, 1876 (Fig. 3D) than in *E. infracta* Mayr, 1904 (Fig. 3B) and *E. rosae* (Fig. 3C). The peduncle is generally between 1 and 1 1/2 times as long as the egg body. Sometimes the eggs are of unequal size and immature eggs can be seen inside the females.

The micropyle was observed at the end of the peduncle in *E. brunniventris* and *E. strigifrons* (Fig. 9C), but not in the other two species. Rounded papillae (* in Fig. 7ABC) and/or small depressions (white arrow Figs. 7B, 8C) (and sometimes both intermixed (Fig. 7B) can be seen on the egg body surface of *E. rosae* (Fig. 7B) and *E. infracta* (Fig. 3B) and *E. brunniventris* (Fig. 8C), whereas the *E. strigifrons* (Fig. 3D) egg surface is entirely smooth.

Observations were done of post-oviposition eggs of *E. brunniventris* in the gall of *Cynips quercus* (agamic generation). What can be seen as small rounded structures in the depressions (white arrow in Fig. 8C) in the ovarian egg, apparently develop into hooks/spines (Fig. 8D) in the post-oviposition egg and the egg expands to at least double the size of the ovarian egg after being deposited in the gall.

The eggs of *Sycophila biguttata* (Swederus, 1795), *S. binotata* (Fonscolombe, 1832), *S. mayri* (Erdős,

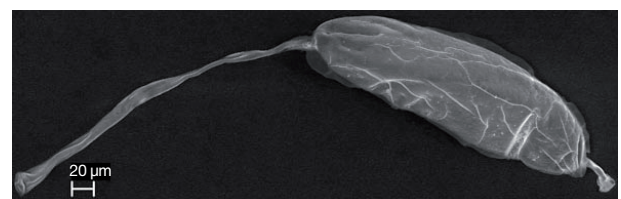


Fig. 2.— Egg of the *Eupelmus microzonus* (Eupelmidae).

Fig. 2.— Huevo de *Eupelmus microzonus* (Eupelmidae).

1959) and *S. submutica* (Thomson, 1876) (Fig. 3E-H) are very similar to each other and are oval and tapered at both ends.

They carry a long slender peduncle at one end and a shorter process at the other end. It is difficult to measure the length of the peduncle as it is almost impossible to disentangle from the rest of the peduncles in the egg mass without breaking and it appears to be very

flexible and is easily stretched out, but it appear to be several times as long as the egg body. The micropyle was seen at the end of the peduncle of *S. submutica*. The surface of the egg body is entirely without structure and appears flaky and soft.

Sections were made of the eggs of *S. biguttata* and examined in the transmission electron microscope (Fig. 10B). The eggshell consists of an inner vitelline membrane (VM) forming a firm layer around the oocyte, and the outer chorion. The vitelline membrane is the thinnest layer of the eggshell (about 0.03 µm in thickness) and is electron dense and uniform in structure. The chorion is separated in the inner endochorion (EN) and the outer exochorion (EX). The endochorion is further divided into an electron dense inner part and a more electron translucent outer part. It is slightly narrower (about 0.070 µm in thickness) than the exochorion (about 0.076 µm in thickness), which appears uniform in structure.

ORMYRIDAE

The eggs of *Ormyrus* (Fig. 4) are highly elongate and slightly broader in the anterior end than in the posterior end. Neither of the 3 examined species have peduncles. The micropyle was seen as quite a large 2 or 3-lobed opening at the anterior end of the eggs of *O. wachtli* Mayr, 1904 (Fig. 4C) and *O. papaveris* (Perris, 1840) (Fig. 4B). The surface appears more or less smooth, but in *O. papaveris* several of the examined species have eggs with irregular ridges on the surface, which appears as a grid pattern (marked by r in Fig. 7D).

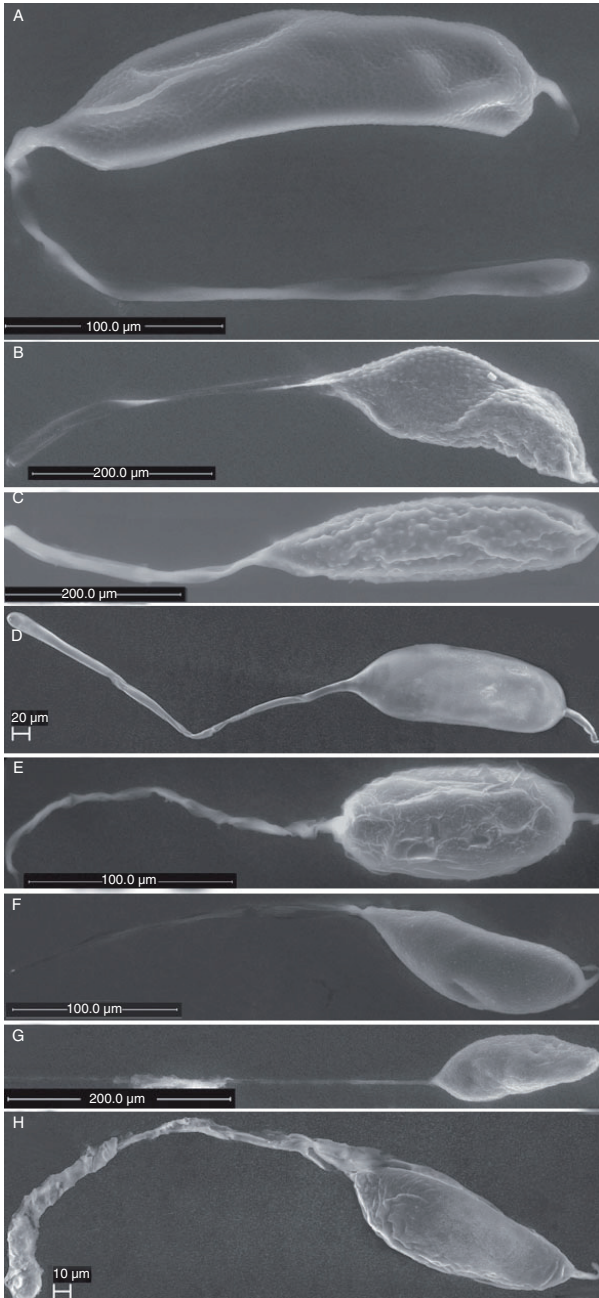


Fig. 3.— Eggs of Eurytomidae A) *Eurytoma brunniventris*, B) *Eurytoma infracta* C) *Eurytoma rosae* D) *Eurytoma strigifrons*, E) *Sycophila biguttata* F) *Sycophila binotata* G) *Sycophila mayri* and H) *Sycophila submutica*.

Fig. 3.— Huevos de Eurytomidae A) *Eurytoma brunniventris*, B) *Eurytoma infracta* C) *Eurytoma rosae* D) *Eurytoma strigifrons*, E) *Sycophila biguttata* F) *Sycophila binotata* G) *Sycophila mayri* y H) *Sycophila submutica*.

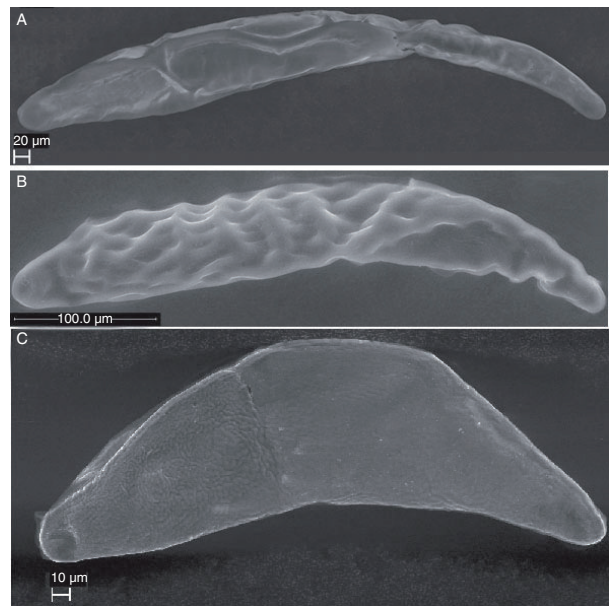


Fig. 4.— Eggs of Ormyridae A) *Ormyrus nitidulus*, B) *Ormyrus papaveris* and C) *Ormyrus wachtli*.

Fig. 4.— Huevos de Ormyridae A) *Ormyrus nitidulus*, B) *Ormyrus papaveris* y C) *Ormyrus wachtli*.

PTEROMALIDAE

The examined eggs of Pteromalidae are elongate and tapered at both ends as in *Pteromalus bedeguaris* (Linnaeus, 1758) (Fig. 5C) or somewhat broader at the anterior end as in *Stinoplus lapsanae* Graham, 1969 (Fig. 5D) and *Cecidostiba fungosa* (Geoffroy, 1785) (Fig. 5A). None of the studied species have pedunculated eggs.

The micropyle was not observed in any of the pteromalid eggs. The surface of the egg is smooth in all the species except *Pteromalus bedeguaris*, in which it is covered in small spine-like structures (marked by s in Fig. 7E).

The eggshell of *Cecidostiba fungosa* was examined using TEM (Fig. 10A). It consists of an inner electron dense vitelline membrane (about 0.3 μm in thickness),

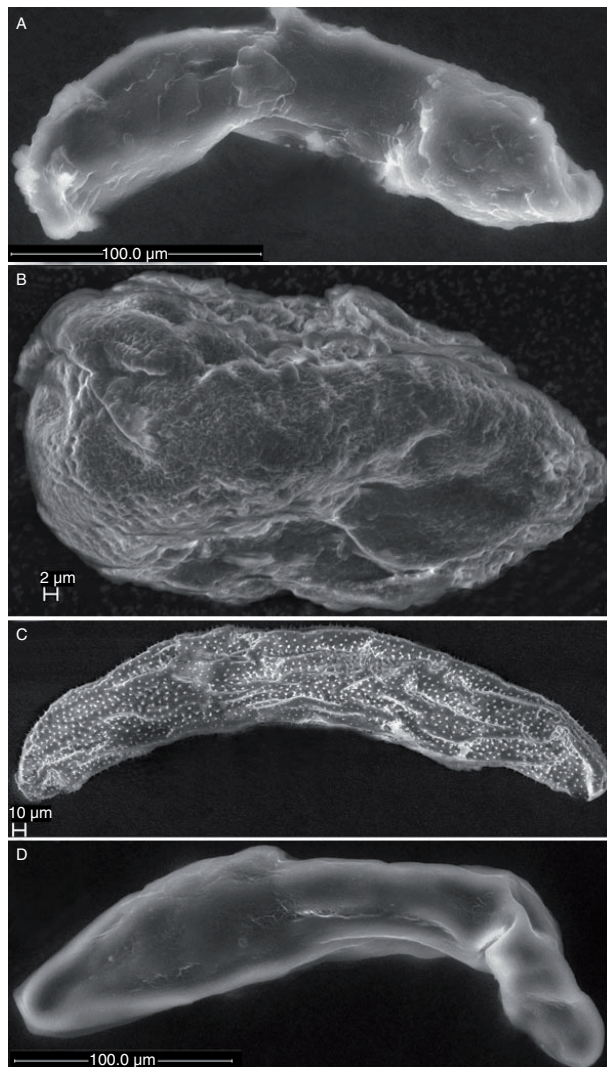


Fig. 5.— Eggs of Pteromalidae A) *Cecidostiba fungosa*, B) *Mesopolobus mediterraneus*, C) *Pteromalus bedeguaris* and D) *Stinoplus lapsanae*.

Fig. 5.— Huevos de Pteromalidae A) *Cecidostiba fungosa*, B) *Mesopolobus mediterraneus*, C) *Pteromalus bedeguaris* y D) *Stinoplus lapsanae*.

a middle endochorion (about 2 μm in thickness) and an outer relatively thin electron dense exochorion (about 0.4 μm in thickness). The exochorion may contain vesicle-like structures (marked by * on Fig. 10A) as well as crystalline structures (black arrows on Fig. 10A), whereas the endochorion is uniform and apparently gelatinous in structure. The innermost layer, the vitelline membrane is electron dense and appears to be anchored by extensions (white arrows on Fig. 10A) into the oocyte.

TORYMIDAE

The shape of the examined torymid eggs varies from elongate and slightly broader at the anterior end in *Adontomerus impolitus* (Askew & Nieves-Aldrey, 1988)

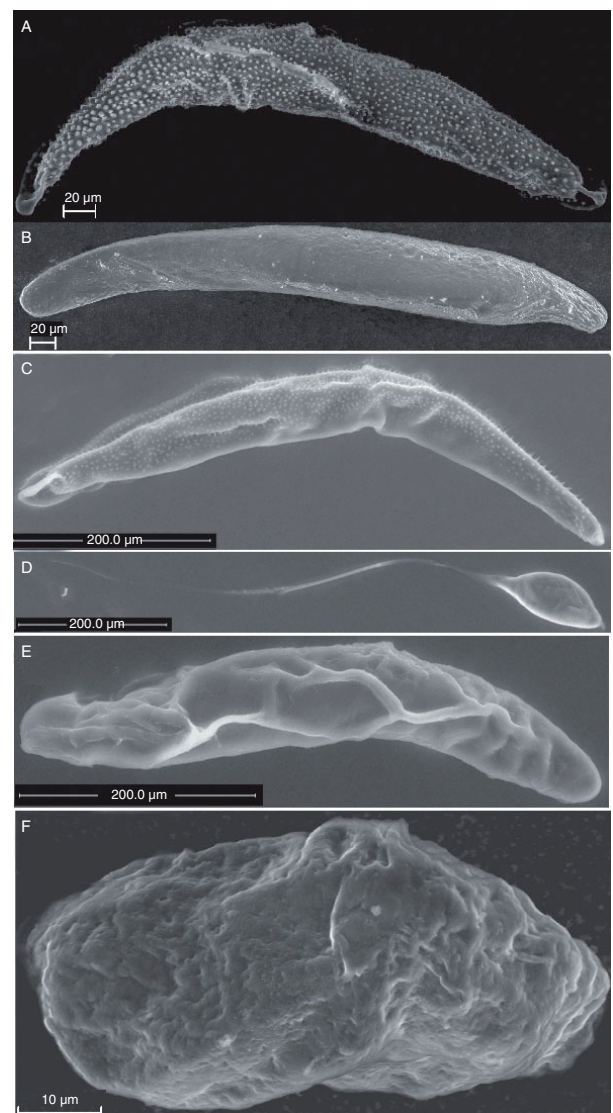


Fig. 6.— Eggs of Torymidae. A) *Adontomerus impolitus* B) *Chalcimerus borceai*, C) *Glyphomerus stigma*, D) *Megastigmus aculeatus*, E) *Torymus nobilis* and F) *Torymus rubi*.

Fig. 6.— Huevos de Torymidae. A) *Adontomerus impolitus* B) *Chalcimerus borceai*, C) *Glyphomerus stigma*, D) *Megastigmus aculeatus*, E) *Torymus nobilis* y F) *Torymus rubi*.

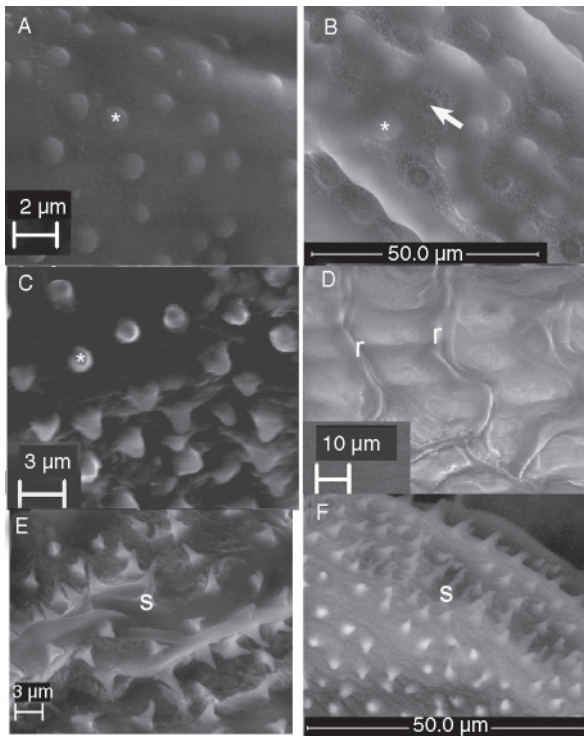


Fig. 7.— Surface of the egg A) partly covered by flat papillae (*) *Dichatomus acerinus* (Eulophidae), B) entirely covered by alternating papillae (*) and depressions (arrow) *Eurytoma rosae* (Eurytomidae), C) elevated rounded papillae (*) *Adontomerus impolitus* (Torymidae), D) irregular ridges (r) *Ormyrus papaveris* or E) sharply pointed spines (s) *Pteromalus bedeguaris* (Pteromalidae) and F) *Glyphomerus stigma* (Torymidae).

Fig. 7.— Superficie del huevo A) en parte cubierta por papilas planas *Dichatomus acerinus* (Eulophidae), B) cubierta enteramente por papilas y depresiones alternando *Eurytoma rosae* (Eurytomidae), C) papilas elevadas redondeadas *Adontomerus impolitus* (Torymidae), D) crestas irregulares *Ormyrus papaveris* or E) espinas puntiagudas *Pteromalus bedeguaris* (Pteromalidae) y F) *Glyphomerus stigma* (Torymidae).

(Fig. 6A), *Chalcimerus borceai* Steffan & Andriescu, 1962 (Fig. 6B), *Glyphomerus stigma* (Fabricius, 1793) (Fig. 6C) and *Torymus nobilis* Boheman, 1834 (Fig. 6E) to short and subrectangular as in *Torymus rubi* (Schrank, 1781) (Fig. 6F) or oval with a long peduncle at the posterior end and a short process at the posterior end as in *Megastigmus aculeatus* (Fig. 6D).

The micropyle was not observed for any of these species. The egg surface is smooth in all the examined torymid species except in *Adontomerus impolitus* (Fig. 7C) in which the entire surface is covered in papilla-like structure and in *Glyphomerus stigma* (marked by * in Fig. 7F) in which at least parts of the egg are covered with small spines.

Discussion

EXTERNAL EGG FEATURES

Several functions have been suggested and shown for the peduncle of eggs of parasitic wasps. Fulton (1933) described the oviposition procedure for

Pteromalus (as *Habrocytus*) *cerealellae* and explained how the egg body, which was 0,14-0,16 mm in diameter distributed its content along the whole length of the peduncle when the egg body enters the egg canal which has a diameter of only 0,04 mm. In other groups, such as *Exenterus* (Hymenoptera: Ichneumonidae), the peduncle varies from a simple stalk with a knob-like anchor to a highly specialized double stalk. The former simply attaches the eggs to the host larva's integument, whereas the latter is firmly inserted into the host larva's cuticle, which makes these eggs much less sensitive to desiccation than the former (Mason, 1967). In the eggs of the eucharitid *Golumiella longipetiolata*, the typical eucharitid peduncle is absent and a posterior anchor attach the egg to the leaf petiole where the eggs are deposited vertically onto the surface (Heraty *et al.*, 2004). At the apical end a pseudostalk covered with secretion, which is believed to attract ants that act as host for the larvae of this species.

Respiration is a documented function of the peduncle in some groups of wasps. In several encyrtid species the egg has been shown to leave a portion of its peduncle on the outside of the host larva, so the encyrtid larva has access to oxygen when it hatches (Maple, 1947). Similarly, for *Eurytoma amygdali* (Hymenoptera: Eurytomidae) a respiratory function was suggested for the tip of the peduncle as this part of the egg apparently remained on the outside of the almond fruit after oviposition (Mouzaki & Margaritis, 1994).

Pedunculate eggs are found in most chalcid families (Fig. 12) in species ovipositing both inside host larvae in a fluid environment as well as in the drier environment on the host larvae inside galls on different plants (Table 4), so pedunculate egg are apparently not restricted to certain oviposition habits.

The sperm entrance or micropyle is usually at the anterior end of the eggs for most parasitic wasps including Chalcidoidea (Rotherham, 1973) or at the tip of the peduncle when present (Maple, 1947; Wishart & Monteith, 1954).

In eggs of at least some species of Eurytomidae that carry a long peduncle at the anterior end as well as a shorter process at the posterior pole, the micropyle has been reported at the end of the egg that carries the short posterior process (Claridge & Askew, 1960; Mouzaki & Margaritis, 1994; Zarani & Margaritis, 1994). We found, however, an opening at the tip of the long anterior peduncle of both *Eurytoma bruniventris* and *Eurytoma strigifrons* (Hymenoptera: Eurytomidae) and assume that this structure is equivalent with the micropyle of the other chalcidoidoid eggs with peduncle.

EGG LOAD

The approximate egg number varies considerably (Table 4). The size of the egg load may be indicative of whether the species is pro-ovigenic

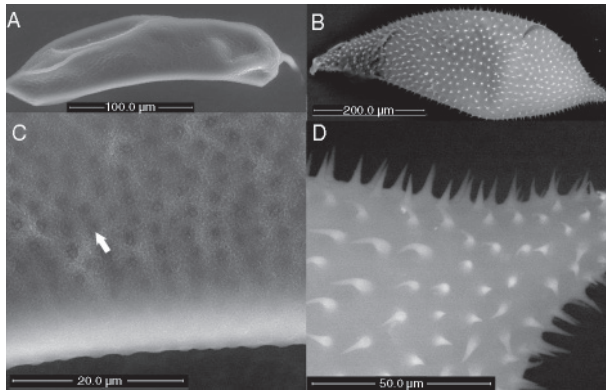


Fig. 8.— Change in the shape and size of the egg of the *Eurytoma brunneviventris* (Eurytomidae) A) ovarian egg and B) post oviposition egg, C) egg surface structure of ovarian egg and D) spiny egg surface of post-oviposition egg.

Fig. 8.— Cambio en el tamaño y forma del huevo de *Eurytoma brunneviventris* (Eurytomidae) A) huevo ovárico y B) huevo después de la puesta, C) estructura de la superficie del huevo ovárico y D) superficie espinosa del huevo después de la puesta.

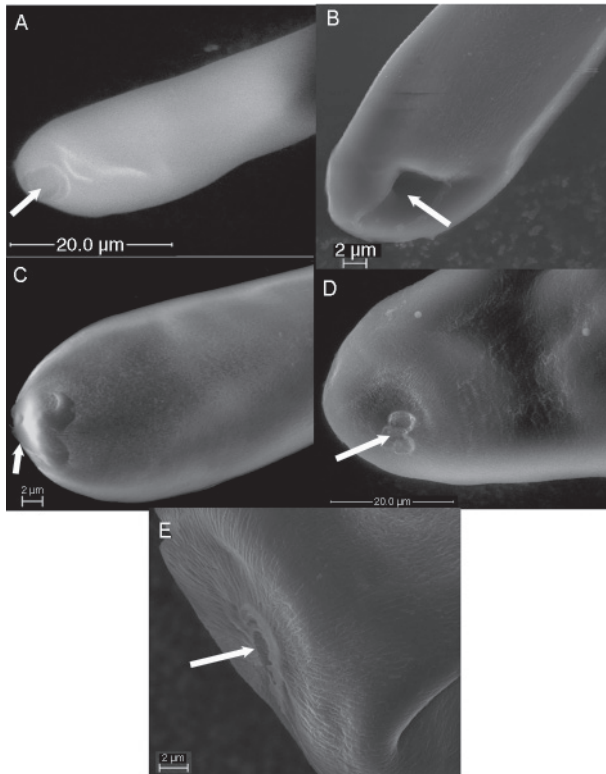


Fig. 9.— Micropyle (arrows) at anterior end of egg of A) *Aulogymnus skianeuros* (Eulophidae) B) *Eupelmus microzonus* (Eupelmidae), C) *Eurytoma strigifrons* (Eurytomidae), D) *Ormyrus papaveris* and E) *Ormyris wachtli* (Ormyridae).

Fig. 9.— Micropilo (flechas) y extremo anterior del huevo de A) *Aulogymnus skianeuros* (Eulophidae) B) *Eupelmus microzonus* (Eupelmidae), C) *Eurytoma strigifrons* (Eurytomidae), D) *Ormyrus papaveris* y E) *Ormyris wachtli* (Ormyridae).

(female emerging with a full complement of mature eggs) or synovigenic (maturing eggs continuously). Compared with many other koinobiont parasitoid

wasps and oak gall wasps that are considered proovigenic and carry hundreds or more than a thousand ovarian eggs (Quicke, 1997; Vårdal *et al.*, 2003), our result show a generally relatively low egg count, which may be an indication of synovigeny in some species (Table 4).

EGG SURFACE

Papillae or hook-like structures, spines and ridges are on the surface of eggs of some ectoparasitoid species (Fig. 7, Table 4). These structures may help to attach the egg to the host larva or the substrate where it is deposited, (Claridge & Askew, 1960; Arthur, 1961; Quicke, 1997). No surface structures were observed in eggs of endoparasitoids like *Aprostocetus* (Eulophidae) and *Sycophila* (Eurytomidae). All the studied endoparasitoids have a smooth chorion (Table 4), and this appears to be the most common state for eggs that are deposited inside another insect (Parker, 1924a; Hagen, 1964).

The surface structure of the mymarid egg is smooth, and this is also the case for the eggs of other outgroup taxa like Scelionidae and Diapriidae (Clausen, 1940) (Fig. 12; Tables 1, 4). This appears to be the most common state for egg surface throughout the superfamily. Surface structures like spines and papillae possibly evolved independently in Eurytomidae, Leucospidae, Torymidae and Pteromalidae and in Ormyridae. The eggshell of some Perilampidae, Trichogrammatidae, Eurytomidae, Encyrtidae and Ormyridae are heavily sculptured with irregular ridges (Table 1, Fig. 12). All the species, for which these surface structures are observed, are ectoparasitoids.

The surface sculpture of the ovarian and oviposited egg is different in *Eurytoma brunneviventris*. In the ovarian egg, the surface is covered by depressions (Fig. 8C). In the depressions small projections with blunt tips can be seen in the ovarian egg, that apparently extend into spines or hooks in the post-oviposition egg (Fig. 8D). Claridge & Askew (1960) also observed that the spines were not present in the ovarian egg of *Eurytoma brunneviventris* (Hymenoptera: Eurytomidae). Development of spines after oviposition appears to be a good strategy as they would probably delay the passage of the egg through the narrow egg canal, yet several species have ovarian eggs with well-developed spines (Fig. 7E-F). King *et al.* (1968) observed that the spines of *Catolaccus* sp. (Pteromalidae) are an integral part of the flexible translucent layer (endochorion) and protrude through the exochorion. Presumably the flexibility of the endochorion will allow the spines to fold when passing through the egg canal.

The egg surface in *Ormyrus papaveris* has irregular ridges (Fig. 2D). In several insect orders similar patterns are believed to be caused by imprints of the

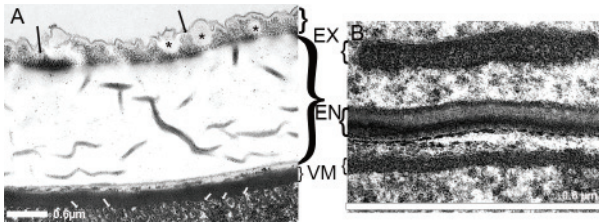


Fig. 10.— Section of the eggshell of A) the ectoparasitoid *Cecidostiba fungosa* (Pteromalidae), (the stars (*) mark vesicle-like structures and black arrows mark crystalline structures in the exochorion, the white arrows mark the extension that anchor the vitelline membrane into the oocyte) and of B) the endoparasitoid *Sycophila biguttata* (Eurytomidae) VM= vitelline membrane, EN= endochorion, EX=exochorion.

Fig. 10.— Sección de la cáscara del huevo de A) el ectoparasitoide *Cecidostiba fungosa* (Pteromalidae), (el asterisco*) indica estructuras en forma de vesícula, la flecha negra apunta a estructuras cristalinas en el exocorion y la flecha blanca marca la extensión que ancla la membrana vitelina en el oocito) y de B) el endoparasitoide *Sycophila biguttata* (Eurytomidae) VM= membrana vitelina, EN= endocorion, EX=exocorion.

follicle cells that have participated in eggshell formation (Hinton, 1981).

THE EGG SHELL

Endoparasitoids often have hydropic eggshells, which have the ability to take up substances through the chorion (Quicke, 1997). To facilitate this action as well as the swelling of the egg after nutrition-uptake, the eggshell is often highly convoluted and flexible and thinner than the eggshell of anhydropic eggs, which contains all the yolk necessary for the embryo development (Quicke, 1997). A thicker impermeable anhydropic eggshell as well as enough yolk to nurture the embryo during the entire egg stage is expected for species exposing their eggs to desiccation (Quicke, 1997). Depending of the environment of the host larva, this may be the case for several ectoparasitic species, which may thus be expected to have thicker eggshells than endoparasitoids into which the eggs are normally deposited directly into the body tissue of the host. The egg of the ectoparasitic *Cecidostiba fungosa* has a thick chorion (2.5 μm) in which the endochorion is about 4 times as thick as the exochorion (Fig. 10A). In the endoparasitic *Sycophila biguttata* (Fig. 10B) the endochorion is as thick as the exochorion and 15 times thicker than in *Cecidostiba fungosa* (0.15 μm). In the examined material it is clear that the eggs of the endoparasitoids are narrower (mean: 44.1 μm) and shorter (mean: 136.5 μm) than the eggs of the ectoparasitoids (width mean: 65.2 μm , length mean: 345.25 μm) (Tables 2, 3) and one reason for this is probably that in our material the female ectoparasitoids in most cases are much larger than the female endoparasitoids.

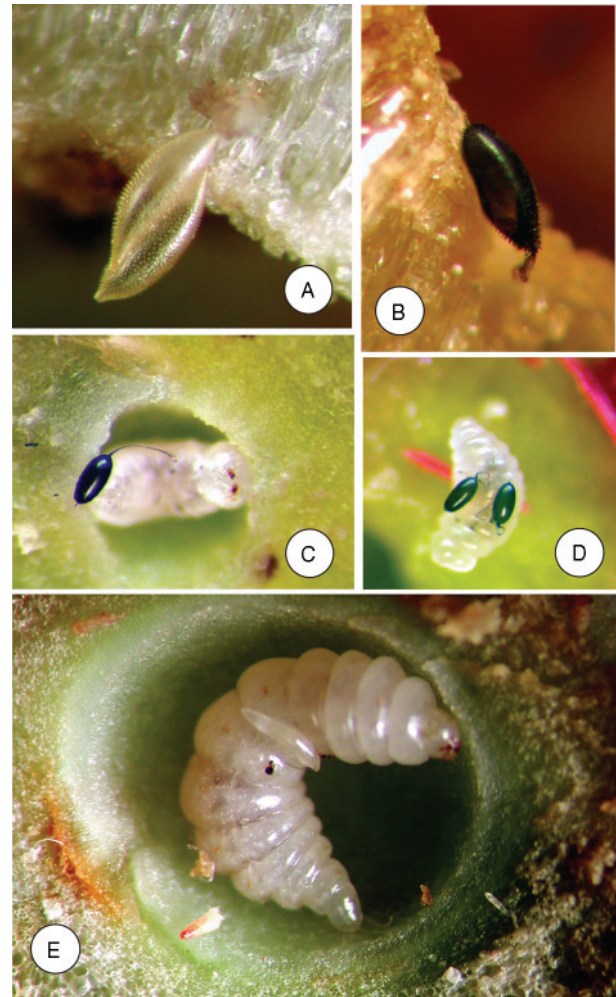


Fig. 11.— Color plate of eggs deposited inside cynipid galls A-B) *Eurytoma bruniventris* (Eurytomidae) egg inside gall of *Cynips quercus*, C-D) *Eurytoma rosae* (Eurytomidae) eggs on larva of the inquiline *Periclistus brandtii* in a *Diplolepis mayri* gall, E) egg of *Pteromalus bedeguaris* (Pteromalidae) on *Diplolepis mayri* larva.

Fig. 11.— Lámina en color de huevos depositados en el interior de agallas de cinípidos A-B) *Eurytoma bruniventris* (Eurytomidae) huevo dentro de la agalla de *Cynips quercus*, C-D) *Eurytoma rosae* (Eurytomidae) huevos sobre larvas del inquilino *Periclistus brandtii* en una agalla de *Diplolepis mayri*, E) huevo de *Pteromalus bedeguaris* (Pteromalidae) sobre una larva de *Diplolepis mayri*.

Conclusions

The chalcidoid egg is extremely variable in size, shape and surface structure. Certain families like polyphyletic Pteromalidae and monophyletic Torymidae exhibit all of the egg types, whereas only one egg type with relatively little variation is observed in certain families like Eurytomidae, Ormyridae, Perilampidae and Eucharitidae. In general, eggs of endoparasitoids have unsculptured eggshell, whereas ectoparasitoid often have egg surface structure, which might be involved in the attachment of the egg on the cuticle of the host.

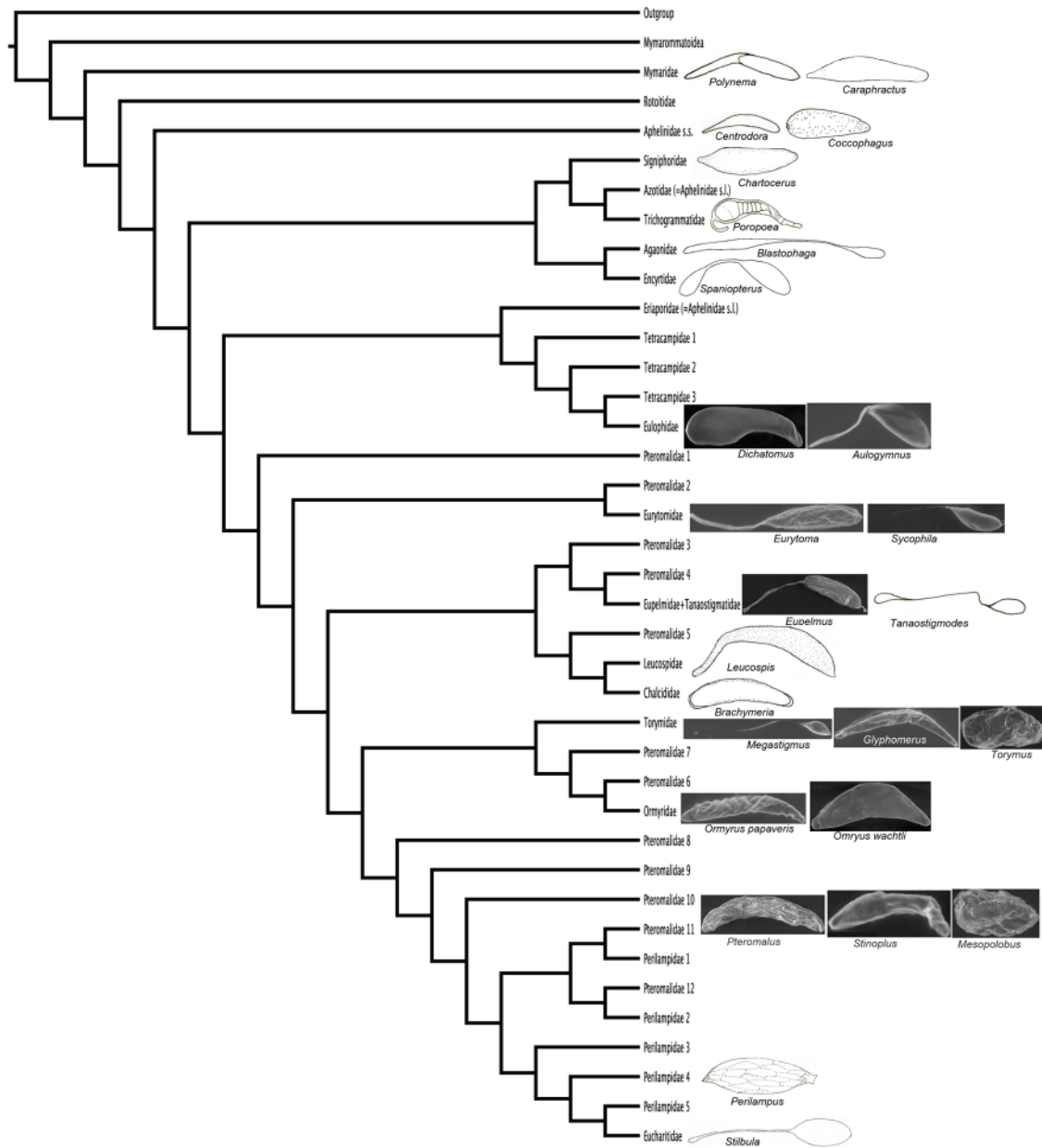


Fig. 12.— Phylogenetic tree modified from the combined implied weight tree in Heraty *et al.* (2013) with egg drawings or SEM micrographs representing egg shapes in families with described eggs. Redrawings were made for representatives of families not examined here (Silvestri, 1916; Parker, 1924a; Grandi, 1929; Dowden, 1935; Cendaña, 1937; Clausen, 1940; Jackson, 1961; La Salle & LeBeck, 1983; Noyes, 1990a; Rosen *et al.*, 1992).

Fig. 12.— Árbol filogenético dibujado a partir del árbol combinado de pesaje implícito en Heraty *et al.* (2013) con dibujos del huevo o SEM microfotografías representando formas de huevos de familias con huevos descritos. Se redibujaron los huevos de representantes de familias que no fueron examinadas en este trabajo (Silvestri, 1916; Parker, 1924a; Grandi, 1929; Dowden, 1935; Cendaña, 1937; Clausen, 1940; Jackson, 1961; La Salle & LeBeck, 1983; Noyes, 1990a; Rosen *et al.*, 1992).

The perception that the endoparasitoid eggshell (endochorion) is thin, whereas the ectoparasitoid eggshell (endochorion) is several times thicker is supported by our results.

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