

Research Article

Description of the eudoxid stages of *Lensia havock* and *Lensia leloupi* (Cnidaria: Siphonophora: Calyco-phorae), with a review of all known *Lensia* eudoxid bracts

MARY M. GROSSMANN^{1,2}, ALLEN G. COLLINS³ & DHUGAL J. LINDSAY^{1,4}

¹Yokohama City University, 236-0027 Yokohama, Japan

²Okinawa Institute of Marine Science and Technology (OIST), 904-0495 Onna, Okinawa, Japan

³National Systematics Laboratory, National Museum of Natural History, MRC-153, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012, USA

⁴Japan Agency for Marine–Earth Science and Technology (JAMSTEC), 237-0061 Yokosuka, Japan

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The majority of calyco-phoran siphonophores release cormidia from the end of the polygastric colony to form a free-living sexual unit, called the eudoxid stage. Despite having a very different morphology from that of the polygastric colony, nearly all calyco-phoran species have had their eudoxid stages described, through a combination of morphological and distributional similarities with the polygastric stages. However, in the catch-all genus *Lensia*, less than half of the small, rocket-shaped polygastric stages have had eudoxid stages assigned to them. In this work, DNA barcoding of the mitochondrial 16S gene allowed two new *Lensia* eudoxid stages to be reliably linked with their polygastric stages: *L. havock* and *L. leloupi*. A re-examination of the latter species has indicated that *L. nagabhushanami* is a junior synonym. A review of all known *Lensia* eudoxid bracts is given, with some notes on five as-yet unassigned eudoxid stages.

<http://zoobank.org/urn:lsid:zoobank.org:pub:0C7F3376-B07C-481D-8F2F-367E2B7152CC>

Key words: bract, Diphyidae, DNA barcoding, *Lensia*, mitochondrial 16S, morphology, ridge pattern, sexual eudoxid stage

Introduction

Siphonophores are complex colonial pelagic hydrozoans, most well known for their long gelatinous colonies that can exceed 30 m in length (Mackie *et al.*, 1987; Robison, 2004). Over 60% of known siphonophore species, however, are small rocket-shaped colonies that rarely exceed 20 cm in total length. The majority of these species belong to the calyco-phoran family Diphyidae Quoy & Gaimard, 1827 (Mapstone & Schuchert, 2013). The genus *Lensia* Totton, 1932, a catch-all genus of small diphyid calyco-phoran siphonophores, is one of the most species-rich genera of the Siphonophora. Belonging to the subclass Hydroidolina of the class Hydrozoa, diphyid siphonophores have a complex life cycle, composed of a succession of several different life stages that share few similarities in size or morphology. The largest, and believed to be the

most long-lived, is the polygastric stage, usually comprising two large zooids, an anterior and a posterior nectophore, and a siphosomal stem bearing many cormidia. Cormidia, composed of gonads, a stomach (gastrozoid) and tentacle, and a protective shield (bract), are released from the posterior end of the polygastric stage in many diphyid species, to form free-living sexual units, called the eudoxid stage. In calyco-phoran siphonophores, specific identification is most commonly, and sometimes exclusively, associated with the morphology of the anterior nectophore of the adult asexual colony – the polygastric stage. However, the free eudoxid stage of the majority of diphyid species has been successfully linked to the polygastric stage by association of morphological and distributional similarities between these life stages.

For species of the genus *Lensia*, however, the large number of known species, combined with the often small morphological characteristics that differentiate them, has made the identification of free eudoxid stages difficult and, presently, free eudoxid stages have been described

Correspondence to: M.M. Grossmann. E-mail: grossmann.mary@gmail.com

for less than half of the known *Lensia* species. The specific identification of free eudoxid stages has traditionally been possible only when large numbers of a single type of polygastric stage have been collected in conjunction with large numbers of a single type of eudoxid (e.g. Gamulin, 1966). This method has proved useful for the description of the eudoxid stages of the majority of the species present in the Mediterranean Sea (Totton, 1932; Gamulin, 1966; Carré, 1968; Gamulin & Kršinić, 2000), where the diversity of Siphonophora is relatively low.

In the Atlantic, Indian and Pacific Oceans, and in many tropical seas, siphonophore alpha biodiversity can be exceptional (e.g. Gao *et al.*, 2002; Zhang, 2005; Gibbons *et al.*, 2010), with, for example off southern Japan, the coexistence of more than 60% of the known siphonophore species (Grossmann & Lindsay, 2013). In such areas, although morphological and distributional similarities can be established between eudoxid and polygastric stages, the specific identity of previously undescribed eudoxid stages cannot be established with certainty in the absence of further information.

Following successful applications of DNA barcoding techniques using the mitochondrial 16S gene in linking different hydrozoan life stages under the same species name (Miranda *et al.*, 2010; Moura *et al.*, 2011; Grossmann *et al.*, 2013), we combined morphological and distributional information obtained from intensive sampling in Japanese waters with DNA barcoding techniques in order to link previously undescribed eudoxids to their known polygastric stage. A review of the morphological and diagnostic characters of all known *Lensia* eudoxid bracts was compiled, in an effort to standardize the terminology and diagnostic characters, and facilitate the identification of as-yet unknown eudoxid forms.

Materials and methods

Twenty-nine specimens, representing six species of the genus *Lensia* and three species of the genus *Diphyes* Cuvier, 1817 were collected using plankton nets, preserved in 99.5% ethanol and stored at -20°C after onboard identification to species level (Table 1). Two kinds of free eudoxid stages of unknown parentage, tentatively identified combining morphological and distributional similarities of preserved bracts and anterior nectophores ('Bract C' and 'Eudoxid A' in Grossmann & Lindsay, 2013), were collected, identified onboard, preserved in 99.5% ethanol and stored at -20°C . For the *L. leloupi* specimen KF724464, DNA extraction, amplification and sequencing were performed following the protocol established in Collins *et al.* (2008). For the other specimens, total DNA was extracted using the Qiagen (Valencia, California, USA) DNEasy Blood & Tissue kit, and a 623 bp segment of the mitochondrial 16S gene was amplified and sequenced using 'primer 1' and 'primer 2' from Cunningham & Buss (1993) with the TaKaRa (Otsu, Shiga, Japan) ExTaq and

BigDye kits. Sequencing was performed on an ABI 3130xl sequencer. The 16S rDNA sequence for each sample was established as the consensus of the forward and reverse reads and deposited on GenBank (Table 1). Thirty-two sequences, available on GenBank, were added to the analysis (Table 2), creating a dataset of sequences of 12 species of *Lensia* and three species of *Diphyes*. The sequences were aligned using MAFFT v. 7 (Katoh & Standley, 2013), using the E-INSI strategy (recommended for < 200 sequences with multiple conserved domains and long gaps). A General Time Reversible, gamma distribution and invariant sites (GTR+G+I) evolutionary model was chosen after applying the MrModelTest v.2.3 (Nylander, 2004) test to the alignment. A Bayesian analysis was performed using MrBayes ver. 3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) using the MrModelTest output command block with 1 million generations and a sampling every 1000 generations. The convergence of the data was verified using Tracer v. 1.5 (Rambaut & Drummond, 2003). The consensus tree was visualized using FigTree v. 1.4.0 (Rambaut, 2007). A Maximum Likelihood analysis was performed using MEGA v. 5.05 (Tamura *et al.*, 2011) under a GTR+G+I model with complete gap deletion. Bootstrap support was estimated over 1000 replications.

Formalin-seawater preserved material was collected in a 330- μm -mesh IONESS net (Kitamura *et al.*, 2001), a 330- μm -mesh ORI net and a 330- μm -mesh 1.13-m-diameter fry net around the eastern and southern coasts of Japan between 2006 and 2013. Additional material was collected with a 0.25 m² 100- μm -mesh multiple opening-closing net system (Weikert & John, 1981) in eastern Antarctica during the CEAMARC cruise (Grossmann & Lindsay, 2011) and around the Antarctic Peninsula and Weddell Sea between 1988 and 1992 during several ANTARKTIS expeditions aboard the R/V *Polarstern* (Pagès & Kurbjeweit, 1994; Pagès & Schnack-Schiel, 1996). Samples collected using a 300- μm -mesh MOCNESS net with a 1 m² mouth area (Wiebe *et al.*, 1985) were obtained in February 2000 from the Celebes and Sulu Seas and in July 2012 from the Mediterranean Sea. Sampling was performed near Rosslyn Bay, Australia in March 2009, using a 250- μm -mesh NORPAC net.

Artwork was created from photographs of formalin-preserved specimens using Adobe Illustrator CS 5.1. Siphonophore terminology (Fig. 1) follows Mapstone (2009).

Results

Phylogenetic analyses

Sequences were obtained from 12 *Lensia* species and from the three temperate-water species of the genus *Diphyes*, which were used as outgroups. Each species formed a well-supported, monophyletic clade in the Bayesian analysis (Fig. 2).

Table 1. Characteristics of the samples sequenced for the present work.

Species	Accession No.	Zooiid	Depth (m)	Date	Lat.	Sampling location	
						Long.	Location
<i>Diphyes bojani</i>	KF724481	anterior nectophore	0–70	14-Sep-2011	24°13.9'N	123°47'E	east of Taiwan
<i>Diphyes bojani</i>	KF724482	eudoxid	0–400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
<i>Diphyes bojani</i>	KF724480	eudoxid	0–600	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
<i>Diphyes chamissonis</i>	KF724483	anterior nectophore	0–5	10-Oct-2011	4°2.76'N	100°37.28'E	Malaysia
<i>Diphyes chamissonis</i>	KF724484	anterior nectophore	0–400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
<i>Diphyes dispar</i>	KF724479	eudoxid	0–1439	24-Apr-2012	34°59.15'N	139°21.05'E	Sagami Bay (Japan)
<i>Lensia conoidea</i>	KF724475	anterior nectophore	0–500	24-Mar-2009	35°0.50'N	139°20'E	Sagami Bay (Japan)
<i>Lensia conoidea</i>	KF724477	anterior nectophore	0–400	16-May-2012	37°N	141°29.47'E	off Fukushima (Japan)
<i>Lensia conoidea</i>	KF724478	anterior nectophore	400–550	23-Jun-2012	40°58.16'N	02°03.71'E	Mediterranean Sea
<i>Lensia conoidea</i>	KF724474	bract	0–1000	8-Mar-2012	35°N	139°20'E	Sagami Bay (Japan)
<i>Lensia conoidea</i>	KF724476	posterior nectophore	0–1075	24-Apr-2012	34°59.18'N	139°20'E	Sagami Bay (Japan)
<i>Lensia cordata</i>	KF724458	anterior nectophore	550–600	23-Mar-2006	35°0.50'N	139°20'E	Sagami Bay (Japan)
<i>Lensia cordata</i>	KF724457	anterior nectophore	600–650	24-Mar-2006	34°42'N	139°50'E	off Oshima (Japan)
<i>Lensia havock</i>	KF724456	bract	0–1439	24-Apr-2012	34°59.15'N	139°21.05'E	Sagami Bay (Japan)
<i>Lensia leloupi</i>	KF724466	anterior nectophore	0–1000	8-Mar-2012	35°N	139°20'E	Sagami Bay (Japan)
<i>Lensia leloupi</i>	KF724467	anterior nectophore	0–1000	8-Mar-2012	35°N	139°20'E	Sagami Bay (Japan)
<i>Lensia leloupi</i>	KF724468	anterior nectophore	0–1000	24-Apr-2012	36°27.75'N	141°43.28'E	off Fukushima (Japan)
<i>Lensia leloupi</i>	KF724469	anterior nectophore	0–1000	8-Mar-2012	35°N	139°20'E	Sagami Bay (Japan)
<i>Lensia leloupi</i>	KF724464	anterior nectophore	250–350	24-Apr-2009	35°N	139°20'E	Sagami Bay (Japan)
<i>Lensia leloupi</i>	KF724470	gonophore	0–1439	24-Apr-2012	34°59.15'N	139°21.05'E	Sagami Bay (Japan)
<i>Lensia leloupi</i>	KF724465	gonophore	0–1439	24-Apr-2012	34°59.15'N	139°21.05'E	Sagami Bay (Japan)
<i>Lensia leloupi</i>	KF724471	posterior nectophore	0–1357	24-Apr-2012	34°59.18'N	139°20'E	Sagami Bay (Japan)
<i>Lensia panikkari</i>	KF724472	anterior nectophore	0–600	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
<i>Lensia panikkari</i>	KF724473	anterior nectophore	0–1076	8-Jun-2012	27°50.05'N	127°00'E	Izena Hole (Japan)
<i>Lensia subtiloides</i>	KF724461	anterior nectophore	0–400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
<i>Lensia subtiloides</i>	KF724460	anterior nectophore	0–400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
<i>Lensia subtiloides</i>	KF724462	anterior nectophore	0–400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
<i>Lensia subtiloides</i>	KF724459	anterior nectophore	0–946	7-Jun-2012	27°47.73'N	126°54.11'E	Izena Hole (Japan)
<i>Lensia subtiloides</i>	KF724463	posterior nectophore	0–400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)

Table 2. Calycophoran sequences obtained from GenBank.

Species	GenBank accession No.	Development stage	Location	Reference
<i>Diphyes chamissonis</i>	JQ715939	polygastric	China Seas	He <i>et al.</i> (unpublished)
<i>Diphyes chamissonis</i>	JQ715940	polygastric	China Seas	He <i>et al.</i> (unpublished)
<i>Diphyes chamissonis</i>	JQ715941	polygastric	China Seas	He <i>et al.</i> (unpublished)
<i>Diphyes chamissonis</i>	JQ715942	polygastric	China Seas	He <i>et al.</i> (unpublished)
<i>Diphyes dispar</i>	AY935276	polygastric	north-west Atlantic	Dunn <i>et al.</i> , 2005
<i>Lensia achilles</i>	KC782553	polygastric	Sagami Bay (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia achilles</i>	KC782554	polygastric	Sagami Bay (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia achilles</i>	KC782555	polygastric	eastern Antarctica	Grossmann <i>et al.</i> , 2013
<i>Lensia achilles</i>	KC782556	polygastric	eastern Antarctica	Grossmann <i>et al.</i> , 2013
<i>Lensia achilles</i>	KC782557	polygastric	eastern Antarctica	Grossmann <i>et al.</i> , 2013
<i>Lensia campanella</i>	KC782540	polygastric	off Kagoshima (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia campanella</i>	KC782541	polygastric	off Kagoshima (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia conoidea</i>	AY935318	polygastric	north-eastern Pacific	Dunn <i>et al.</i> , 2005
<i>Lensia cossack</i>	KC782542	eudoxid	off Kagoshima (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia cossack</i>	KC782543	polygastric	Izena Hole (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia cossack</i>	KC782544	polygastric	Izena Hole (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia cossack</i>	KC782545	polygastric	Izena Hole (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia cossack</i>	KC782546	polygastric	Izena Hole (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia cossack</i>	KC782547	eudoxid	Izena Hole (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia cossack</i>	KC782548	eudoxid	off Fukushima (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia cossack</i>	KC782549	eudoxid	off Fukushima (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia exeter</i>	KC782550	polygastric	Izena Hole (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia havock</i>	KC782532	polygastric	eastern Antarctica	Grossmann <i>et al.</i> , 2013
<i>Lensia havock</i>	KC782533	polygastric	eastern Antarctica	Grossmann <i>et al.</i> , 2013
<i>Lensia havock</i>	KC782534	polygastric	off Kamogawa (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia havock</i>	KC782535	polygastric	Sagami Bay (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia havock</i>	KC782536	polygastric	Sagami Bay (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia hostile</i>	KC782551	polygastric	Sagami Bay (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia hostile</i>	KC782552	polygastric	off Oshima (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia multicristata</i>	KC782537	polygastric	eastern Antarctica	Grossmann <i>et al.</i> , 2013
<i>Lensia multicristata</i>	KC782538	polygastric	Sagami Bay (Japan)	Grossmann <i>et al.</i> , 2013
<i>Lensia multicristata</i>	KC782539	polygastric	Sagami Bay (Japan)	Grossmann <i>et al.</i> , 2013

The genus *Lensia* was split into two distinct clades in both the Bayesian and Maximum Likelihood analyses (Fig. 2), the first corresponding to what are commonly called ‘5-ridged *Lensia*’ (excluding species with 5 faint ridges), and the second clade regrouping *Lensia* species with 7 or more ridges, and those with 5 vestigial ridges. In species of the first clade, the anterior nectophore of the polygastric stage is pentagonal in shape, the five lateral facets separated by marked longitudinal ridges, joining in a point at the apex of the nectophore. The posterior nectophores of the polygastric stage have 5 straight, complete ridges, the two lower ones delimiting the hydroecial groove. Although lacking support at many nodes, this clade could be subdivided into 4 groups, corresponding to distinct morphological characteristics of the species contained: *Lensia achilles* and *L. cordata*, in which the lateral ridges of the anterior nectophore curve sharply towards the upper side at ostial level; *L. conoidea*, a species with 5 straight ridges, the upper extending to form a marked posterior tooth at ostial level; *L. leloupi* and *L. panikkari*, species in which the lateral ridges do not extend to ostial level; and *L. subtiloides*, the type species of the genus

Lensia, with 5 straight, complete ridges, and without an upper ostial tooth. The second *Lensia* clade could also be subdivided into 4 groups: *L. havock*, a species with 7 complete ridges on the anterior nectophore; *L. multicristata*, in which only 5 of the 7 ridges of the anterior nectophore join at the apex of the nectophore; *L. exeter* and *L. hostile*, species with more than 7 ridges on their anterior nectophore; and *L. campanella* and *L. cossack*, species with 5 vestigial ridges that do not meet at the apex of the nectophore. Posterior nectophores are known for only two species of this clade. In *Lensia campanella*, the posterior nectophore has only two ridges, delimiting a shallow hydroecium, and extending to less than half the nectophore from the apex (Carré, 1968). The posterior nectophore of *L. multicristata* has 5 marked ridges like the posterior nectophores of 5-ridged *Lensia*, but the lateral ridges are incomplete in their posterior part, and curve sharply towards the upper side of the nectophore (Mapstone, 2009).

The free eudoxid stages of *Lensia cossack* (KC782542, KC782547-KC782549) and *L. conoidea* (KF724474, KF724476) clustered within their respective species

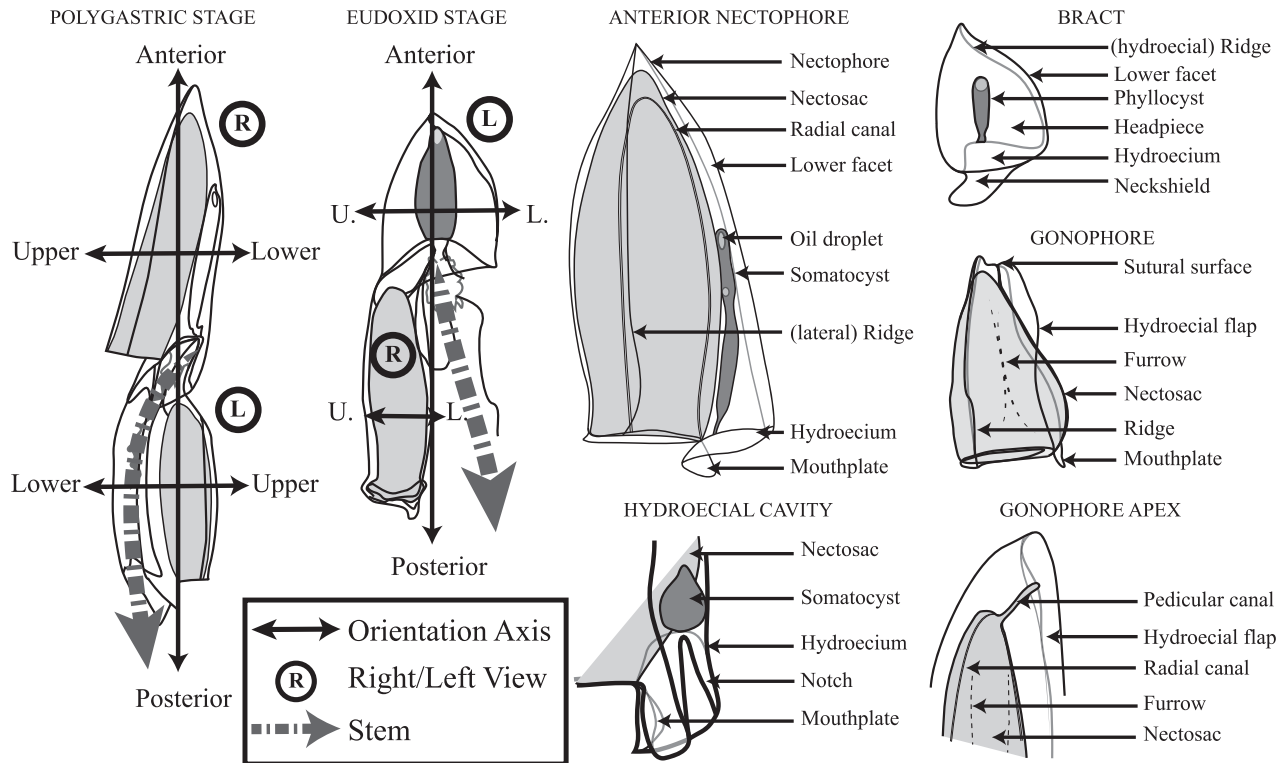


Fig. 1. Orientation and terminology used in the present study. Polygastric and eudoxid stages based on *Chelophyes contorta*, anterior nectophore on *Lensia achilles*, hydroecium inset on *L. havock*, bract on *L. conoidea*, gonophore on *L. leloupi*, and gonophore inset on *L. cossack*. Not to scale.

clusters. The sequenced bract of Eudoxid A *sensu* Grossmann & Lindsay (2013) (KF724456) from Japan clustered within the *L. havock* group, and more specifically within the group of samples collected in Japanese waters, this group under one species name exhibiting high inter-geographic genetic distances. The gonophores associated with Bract C *sensu* Grossmann & Lindsay (2013) (KF724465, KF724470) clustered with *L. leloupi*. A posterior nectophore (KF724471), found to be abundant in upper-mesopelagic IONESS net samples from south-eastern Japan (Grossmann unpubl. data), also clustered within the *L. leloupi* clade.

Taxonomic descriptions

Lensia havock Totton, 1941

(Figs 3–13)

Lensia havock Totton, 1941 (Figs 17–19: 160, tropical Atlantic); Totton, 1965 (Fig. 114: 175); Alvariño, 1981 (Fig. 174.45: 438); Kirkpatrick & Pugh, 1984 (Fig. 38: 95); Alvariño, 1985 (Fig. 7: 80); Daniel, 1985 (Fig. 100: 376); Alvariño *et al.*, 1990 (Fig. 53: 35); Pugh, 1999 (Fig. 3.93: 505); Gao *et al.*, 2002 (Fig. 70: 130 – Illustrations of *L. cordata* (Fig. 71: 131) and *L. havock* inverted);

Mapstone, 2009 (Fig. 49: 192) *Muggiaea havock* Stepanjants, 1967 (Fig. 126: 185, Northern Pacific); Zhang, 2005 (Fig. 54: 65) – see Pugh, 1974 for the affiliation of *L. havock* to the genus *Lensia*. Eudoxid A Grossmann & Lindsay, 2013 (Fig. 8 b: 407, Japanese waters).

Diagnosis. Anterior nectophore with 7 complete ridges, upper laterals curving towards upper surface at ostial level, lower laterals curving towards lower side and extending onto mouthplate; hydroecium extending anterior of ostial level, lower facet with extensive triangular posterior notch; somatocyst club-shaped, with pedicle, 10–20% nectophore height, with orange pigmentation. Posterior nectophore unknown. Bract conical, headpiece without marked ridges; neckshield at least as long as headpiece, with tooth-like projections on baso-lateral corners; hydroecial cavity flattened; phyllocyst spindle-shaped to globular, with orange pigmentation. Gonophore with six ridges, incomplete in posterior part except for the two lower, running onto large rounded mouthplate; hydroecium shallow; articulate surface concave; nectosac wide, with rounded, conical apex.

Material Examined. Two hundred and seventeen anterior nectophores, 307 bracts, 649 gonophores and 152 complete eudoxid stages collected between 250 and

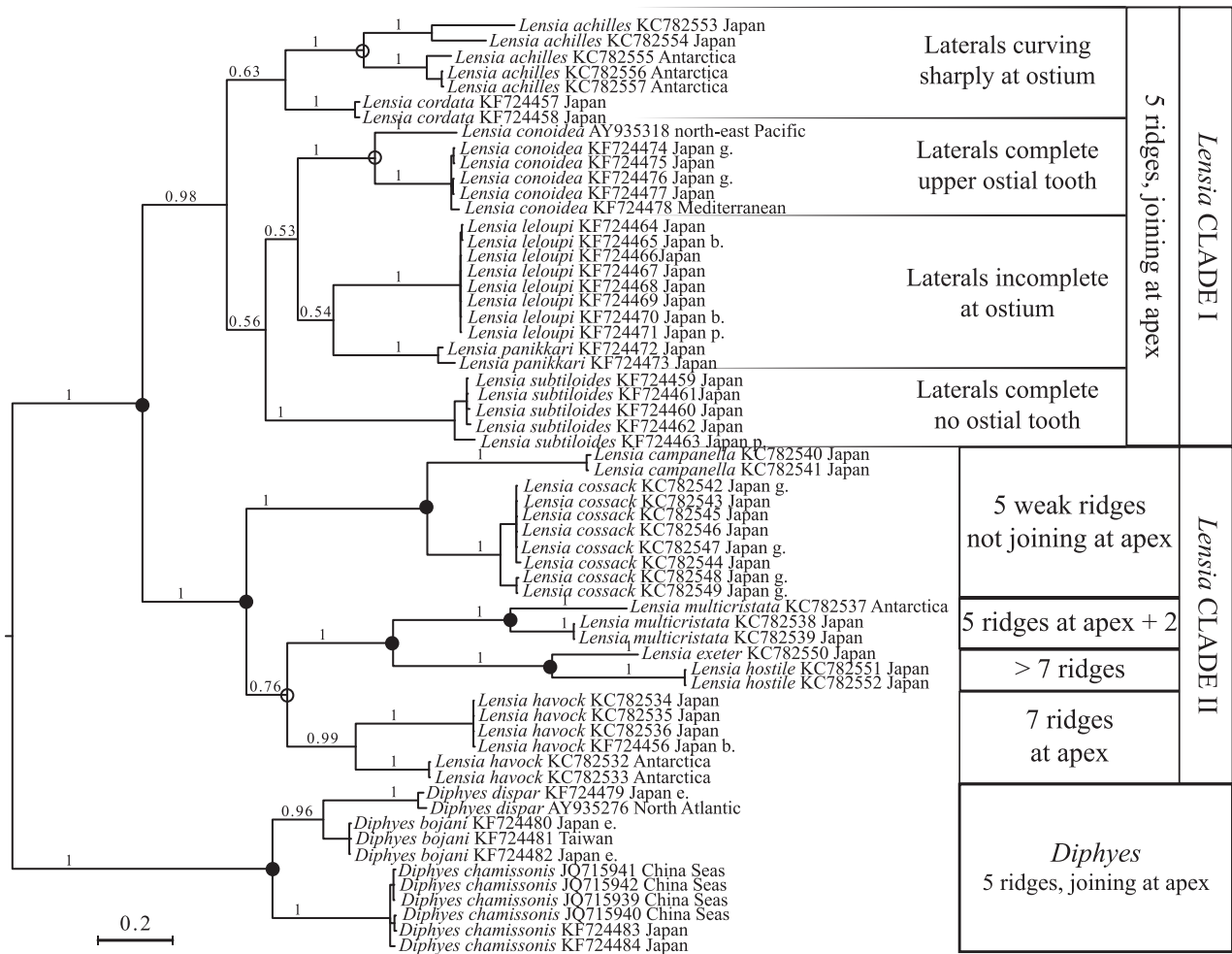


Fig. 2. Bayesian consensus tree based on the mitochondrial 16S gene, with a description of the different *Lensia* sub-groups. Terminal Bayesian posterior probabilities not shown. Maximum Likelihood node support: open circles: bootstrap $\geq 70\%$; closed circles: bootstrap $\geq 95\%$; terminal bootstrap values not shown. Scale represents 0.2 substitutions per site. All anterior nectophores unless mentioned: b.: bract; e.: eudoxid stage; g.: gonophore; p.: posterior nectophore.

1200 m in Sagami Bay (34°59.18'N, 139°20.06'E) during the March 2006 MULTI-SPLASH cruise (Grossmann & Lindsay, 2013); 36 anterior nectophores, 103 bracts, 311 gonophores and 18 complete eudoxid stages collected between 500 and 1600 m in March 2006 east of Oshima Island (34°42'N, 139°50'E); 47 anterior nectophores, one bract, 62 gonophores and 32 complete eudoxid stages collected between 300 and 1000 m south of Kamogawa (34°51.07'N, 140°02.97'E) in March 2006; 3 anterior nectophores, 4 bracts and one gonophore, collected in Sagami Bay (34°59.18'N, 139°20.06'E) in April 2012; one anterior nectophore collected off north-eastern Japan (36°27.75'N, 141°43.28'E) in March 2012; 2 bracts collected north of Okinawa (27°47.73'N, 127°E) in June 2012; 4 anterior nectophores collected between 600 and 800 m on 19 February 2000 in the Celebes Sea (Nishikawa *et al.*, 2007); 2 anterior nectophores collected between 1000 and 2000 m at CEAMARC station UM17

(63°30.52'S, 140°1.81'E) on 31 January 2008, 3 anterior nectophores collected between 1000 and 2000 m and one gonophore collected between 500 and 1000 m at CEAMARC station UM8 (65°30.64'S, 143°1.17'E) on 12 February 2008 (Grossmann & Lindsay, 2011); 7 anterior nectophores, 18 bracts, 2 gonophores and 7 complete eudoxids collected during several ANTARKTIS campaigns of the R/V *Polarstern* to the Antarctic Peninsula and the Weddell Sea between 1988 and 1992 (cf. Pagès & Kurbjeweit, 1994; Pagès & Schnack-Schiel, 1996).

Description

Polygastric Stage. Anterior nectophore (Figs 3–5). Up to 16.5 mm high, with 7 complete ridges, the upper laterals curving towards the anterior side at ostial level, the lower laterals curving to join the outer rim of the mouthplate;

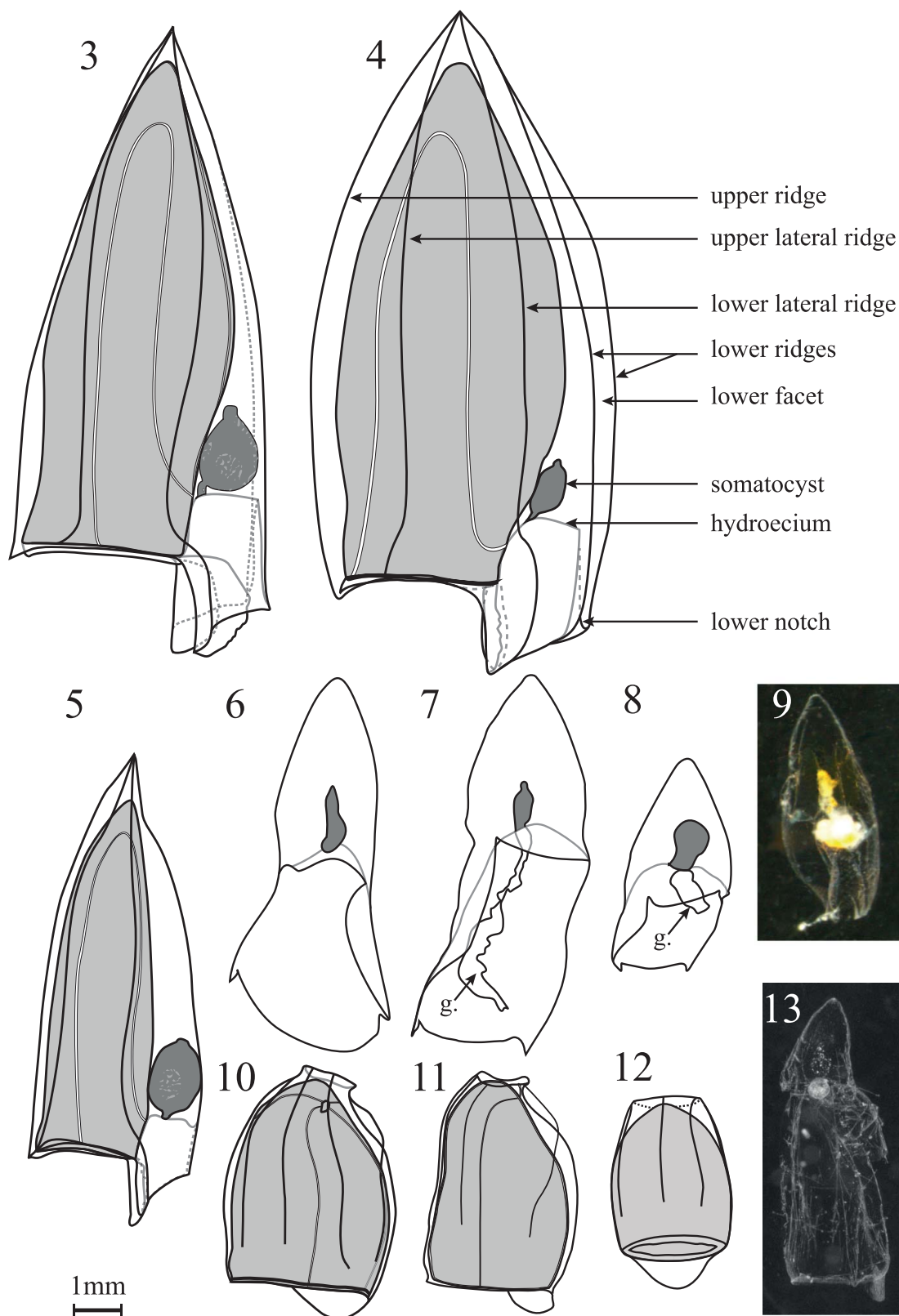


Fig. 3–13. *Lensia havock* Totton, 1941: 3–5: anterior nectophore, right lateral view (3: Japan; 4: eastern Antarctica; 5: Weddell Sea); Figs 6–9: bract, 6 in lower view; 7, 8: right lower-lateral view; 9: upper view (6, 7, 9: Japan, 8: Weddell Sea); 10–12: gonophore, 10: lower-lateral view; 11: right lateral view, 12: upper view (10, 11: Japan, 12: eastern Antarctica); 13: eudoxid stage, bract in left lateral view, gonophore in right lateral view (Weddell Sea). 4 and 9 of living animals. g.: gastrozoid. Scale bar = 1 mm.

nectosac extensive, occupying 88–95% of the nectophore in height, radial canals following a normal diphyine course; hydroecium about 1/10th of the nectophore in height, with a pronounced triangular split on the lower side, extending nearly to the anterior limit of the hydroecium; mouthplate large, with rounded overlapping flaps, slightly serrated on the inner margins; somatocyst large, club-shaped with a short pedicle, 10–20% the nectophore in height, a vivid orange colour in living animals.

Posterior nectophore. Unknown.

Eudoxid Stage. Bract (Figs 6–9). Up to 6.4 mm high, by 1.7 mm wide; conical headpiece without marked ridges; neckshield at least as long as headpiece, wider at posterior margin, with 2 tooth-like projections, one on each basolateral corner; hydroecial cavity flattened; phyllocyst about half the height of the headpiece, spindle-shaped, slightly asymmetrical, containing oil droplets, orange in colour in living animals.

Gonophore (Figs 10–12). Up to 5.7 mm high by 3.2 mm wide; 6 incomplete ridges extending to 1/5 the gonophore length from the ostium except for lower pair, extending onto large rounded mouthplate; hydroecium shallow; articulate surface concave; nectosac as wide as gonophore, with rounded conical apex, radial canals straight, joining on ventral facet at about 1/5th nectosac length from its apex.

Remarks. The large size of this eudoxid, its lower mesopelagic distribution, as well as the bright orange pigmentation of the phyllocyst when alive (Fig. 9), made this a likely eudoxid stage of *Lensia achilles*, *L. cordata* or *L. havock* – all present in the sampling zone (Grossmann & Lindsay, 2013) and having orange-pigmented somatocysts of a similar globular shape to the phyllocyst of ‘Eudoxid A’. However, because a gonophore (Fig. 12) was also collected in the Southern Ocean north-east of the Dumont d’Urville Antarctic base (CEAMARC campaign, station UM8 (65°30’S, 143°E), 500–1000 m, Grossmann unpubl. data) and also found, attached to a bract, in the Weddell Sea (Fig. 13), this eudoxid was unlikely to be associated with a tropical mesopelagic species such as *L. cordata*.

The sequencing of a bract collected in Sagami Bay confirmed this eudoxid stage to be that of *Lensia havock* Totton, 1941 (Fig. 2 – *Lensia havock* KF724456), and it clustered with the polygastric stages collected in Japanese waters, *L. havock* exhibiting significant inter-geographic intra-specific genetic distances. Such large genetic distances may correspond to the presence of cryptic species (Grossmann & Lindsay, 2013). However, morphological characters of the anterior nectophores such as the relative heights of the nectophore, nectosac and hydroecium, and the shape of the lateral ridges showed a great deal of variation within each geographic location (Figs 3–5),

and none of these characters could reliably differentiate between the Antarctic and Japanese nectophores. The eudoxids collected in the Southern Ocean during the ANTARKTIS cruises, and preliminarily identified by the late Francesc Pagès (Figs 8, 13) sufficiently resembled those collected in Japanese waters to be associated with this species. Unfortunately, having been preserved in formalin, no sequences could be obtained from these samples in order to test which of the two thus far known genetic clades of *L. havock* they more closely resembled. The size of the somatocyst of the anterior nectophore, and of the phyllocyst of the eudoxid bract tended to be larger in samples collected in the Southern Ocean (Figs 5, 8). However, somatocysts and phyllocysts function as storage organs, and their size is directly influenced by the quantity of lipid reserves they contain (Figs 4, 5). The large reserves observed in many of the specimens from the Southern Ocean may therefore be necessary to survive the harsh winter conditions when food may be scarce (water temperature of –1.8 to 0°C in Antarctica, 4–6°C in Japanese waters), rather than to an allopatric morphological differentiation. The eudoxid gonophores collected in the Southern Ocean (eastern Antarctica and Weddell Sea) could not be differentiated in size or morphology from the Japanese samples (Figs 10–12).

***Lensia leloupi* Totton, 1954**
(Figs 14–26)

Lensia leloupi Totton, 1954 (Fig. 55 C: 113, equatorial Atlantic); Totton, 1965 (Fig. 97: 162); Daniel, 1974 (Fig. 12 Q: 140 – non Fig. 12 R: *species inquirenda*); Daniel, 1985 (Fig. 58: 231); Margulis & Alekseev, 1985 (Fig. 1 A: 3); Kitamura, 1997 (Fig. 27); Pugh, 1999 (Fig. 3.96: 505); Zhang, 2005 (Fig. 59: 71) – non Alvarino *et al.*, 1990 (Fig. 58: 37: *Lensia subtiloides?*); Gao *et al.*, 2002 (Fig. 61: 120: *Muggiaea delsmanni?*).

Lensia nagabhushanami Daniel, 1971; 1974 (Fig. 12 E: 140, Indian Ocean); 1985 (Fig. 61: 335); Margulis & Alekseev, 1985 (Fig. 1 H: 3).

Bract C Grossmann & Lindsay, 2013 (Fig. 8 d: 407, Japanese waters).

Diagnosis. Anterior nectophore with five ridges joining at apex, the laterals incomplete in their posterior part; hydroecium extending slightly anterior of ostial level; mouthplate medium-sized; posterior border of the lower facet with small central notch; somatocyst elongate, tubular to club-shaped, without a pedicle, 25% the nectophore in height. Posterior nectophore with five complete ridges, the lower running onto short, slightly bilobed mouthplate; articulate surface truncate; hydroecial flaps thick, asymmetrical; nectosac extensive; pedicular canal arising near apex of nectosac. Bract tentatively assigned to this species

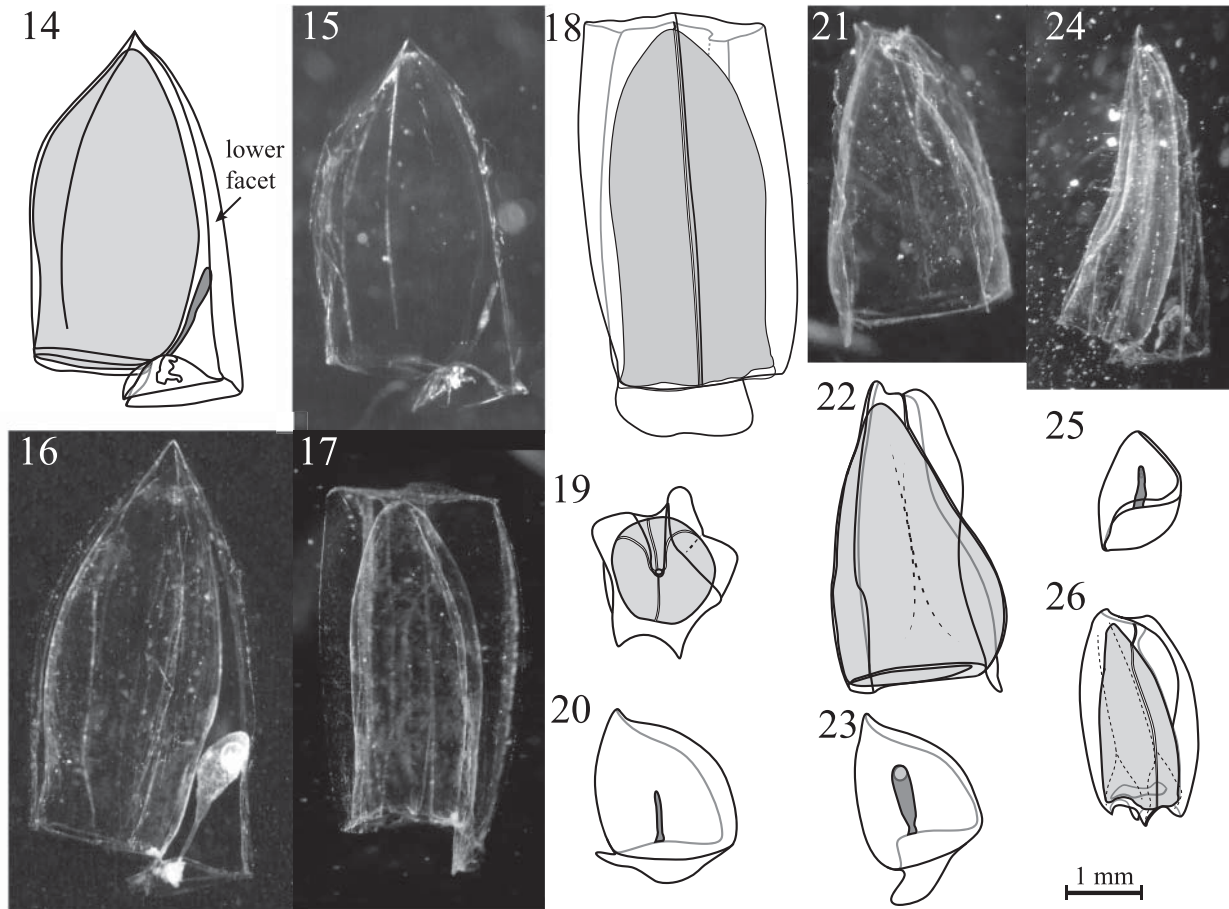


Fig. 14–26. *Lensia leloupi* Totton, 1954: 14–16: anterior nectophore (14, 15: Holotype; 16, from Japanese waters); 17–19: posterior nectophore; 20: eudoxid bract; 21, 22: gonophore; 23: *Lensia conoidea* eudoxid bract; 24–26: *Lensia subtiloides*, 24: anterior nectophore; 25: eudoxid bract; 26: gonophore. Dotted lines indicate furrows. All in right lateral view except 18: upper view, 19: anterior view (hydroecium towards top of page), 21: left lateral view. 14, 15: from the equatorial Atlantic; 16–20, 21–23: from Japanese waters; 24, from the Celebes Sea; 25, 26: from the Mediterranean Sea. Scale bar = 1 mm. (20, 23: from Grossmann & Lindsay, 2013, Fig. 8d: 407).

bluntly conical, with marked bracteal ridges; neckshield 1/3 the height of headpiece; hydroecial cavity flattened; phyllocyst elongate, without terminal swelling, about 50% the headpiece in height. Gonophore conical, with four marked, complete ridges; mouthplate short; hydroecial flaps thin, more developed in anterior part; necotosac the height of the gonophore.

Material Examined. *Lensia leloupi* Holotype (BMNH 1952.11.19.1) and 123 additional anterior nectophores from the Discovery collections (BMNH 1958.4.29.106–1958.4.29.108), collected at Discovery station 277 in the equatorial Atlantic (01°44'S, 08°38'E), between 88 m and the surface on 7 August 1927 (Totton, 1954); 239 anterior nectophores and 143 posterior nectophores collected between 100 and 350 m in Sagami Bay (35°N, 139°20'E) in March 2006 (Grossmann & Lindsay, 2013), 79 anterior

nectophores, 49 posterior nectophores and 46 gonophores collected between 50 and 300 m east of Oshima Island (34°42'N, 139°50'E) in March 2006, 322 anterior nectophores, 43 posterior nectophores and 45 gonophores collected between 100 and 500 m off Kamogawa (34°59.43'N, 140°02.97'E) in March 2006, 2 anterior nectophores from the north-eastern coast of Japan (36°28'N, 141°43'E) in March 2012, 37 anterior nectophores collected between 75 and 300 m in the Celebes Sea on 19 February 2000, 21 anterior nectophores collected between 50 and 175 m on 24 February 2000 in the Sulu Sea, and one very small anterior nectophore from near Rosslyn Bay, Australia (23°10'S, 150°48'E) in July 2011. Additionally, one *Lensia subtiloides* (Lens & van Riemsdijk, 1908) anterior nectophore collected north of Okinawa, Japan (27°47.73'N, 126°54.11'E) in June 2012, 5 anterior nectophores collected near Kagoshima, on the

south-western coast of Japan (34°59.43'N, 140°02.97'E) in November 2011, 21 anterior nectophores collected off north-eastern Japan (37°25'N, 141°30'E) in September 2013, 102 anterior nectophores, collected in the Celebes Sea (2°25.8'N, 122°28.2'E) on 19 February 2000, 304 anterior nectophores and 48 bracts from the Sulu Sea (8°N, 120°E) on 24 February 2000, and one eudoxid and 4 gonophores collected in Malaysia (2°27.64'N, 101°51.06'E) in February 2011 were examined. A total of 1171 bracts tentatively assigned to *Lensia leloupi* were collected in Sagami Bay (35°N, 139°20'E), east of Oshima Island (34°42'N, 139°50'E) and off of Kamogawa (34°59.43'N, 140°02.97'E) in March 2006.

The DNA sequences obtained (Table 1) included 4 anterior nectophores (KF724464, KF724466, KF724467 and KF724469), one posterior nectophore (KF724471), and 2 gonophores (KF724465, KF724471) from Sagami Bay, and one anterior nectophore from north-eastern Japan (KF724468).

Description

Polygastric Stage. Anterior nectophore. Holotype 4.3 mm high by 2.4 mm wide (Figs 14, 15), with 5 longitudinal ridges, joining in a point at the apex of the nectophore, lateral ridges incomplete in their posterior part, the left lateral ridge extending further towards the ostium than the right lateral one; nectosac extensive, occupying 90% of the nectophore in height, musculature causing a central bulge; hydroecium extending slightly above ostial level in its central part, mouthplate 10% the length of the nectophore, with overlapping, rounded flaps; posterior border of the lower facet with small rounded central notch; somatocyst elongate, without pedicle but thinner in its posterior part, 25% the nectophore in height, in contact with the lower facet of the nectophore in its anterior part.

Posterior nectophore (Figs 17–19). Up to 5 mm high by 2.5 mm wide, with 5 marked, complete ridges; mouthplate short, slightly bilobed, 20% the length of the nectophore in length; hydroecial flaps well developed in their anterior part, asymmetrical, the left slightly shorter and with thicker mesogloea, the right slender and pointed; articulate surface truncate; nectosac with blunt conical apex, same height as nectophore, pedicular canal straight, following antero-posterior axis, originating at 5% nectosac height from the apex, radial canals following a normal diphyne course.

Eudoxid Stage. Bract (Fig. 20). Up to 3 mm high, bluntly conical with marked bractal ridges joining at the apex; neckshield 1/3 the height of the headpiece; hydroecial cavity flattened; phyllocyst elongate, without terminal swelling, about 50% of the headpiece in height.

Gonophore (Figs 21, 22). Up to 5 mm high by 2.4 mm at widest point; conical in shape, distal portion up to twice as wide as articulate surface; 4 marked, complete ridges; mouthplate 10% nectophore in length, with squared corners; hydroecial flaps thin, more developed in anterior part; nectosac the height of the nectophore, course of the radial canals unknown.

Remarks. The anterior nectophores of *Lensia leloupi* labelled with Discovery collection numbers 1958.4.29.106–1958.4.29.108 and those collected in Japanese waters (Fig. 16) were generally larger than the holotype, measuring up to 7.5 mm in height, the posterior nectophores thereby being markedly shorter than the anterior ones. The *L. leloupi* anterior nectophore collected off eastern Australia, although measuring only 2.75 mm in height, showed all the morphological characteristics of the larger specimens. Not visible in the holotype specimen, the radial canals of the nectosac followed a typical diphyne course, reaching to 5% the nectosac height from its apex. With increased lipid reserves, the anterior part of the somatocyst may increase in size to come into contact with the nectosac; the posterior part remaining thin. Most *L. leloupi* anterior nectophores examined were of the general shape described by Daniel (1971) for *Lensia nagabushanami* as 'a pointed tip, bulged middle region and a broad ostium'. Many of the more poorly preserved specimens also had large longitudinal furrows on either side of the lateral ridge. Although slightly larger than the specimens from Japanese waters, the specific characteristics of *L. nagabushanami* Daniel, 1971 are not sufficient to differentiate it from *L. leloupi* Totton, 1954, and it is therefore synonymized with the latter.

Apart from the incomplete lateral ridges in *Lensia leloupi*, the main characters differentiating the anterior nectophores of *L. leloupi* from those of *L. subtiloides* (Fig. 24) are the length of the mouthplate and lateral wall of the lower facet, the height of the hydroecium, larger in the former species, and the presence of a rounded notch in the posterior margin of the hydroecium in *L. leloupi*. The somatocyst, while usually taller in *L. leloupi* (25–30% of nectophore length, compared with 15–20% in *L. subtiloides*), exhibited a large amount of variation in size and shape in both species, depending on the number of oil droplets it contained (Figs 15, 16). When preserved in formalin, *L. leloupi* specimens usually displayed a smaller width/height ratio than *L. subtiloides*, but these characters may not apply to live material. However, most of the aforementioned characters showed a great deal of variability when considering specimens from different geographic locations. Additionally, off Japan (Grossmann & Lindsay, 2013; Grossmann unpubl. data) and in the Celebes and Sulu Seas (Lindsay, unpubl. data), *L. leloupi* and *L. subtiloides* were rarely found in the same net samples, showing either vertical or temporal segregation.

Therefore, unless in a position to directly compare the two species, the main diagnostic characteristic remains the incomplete lateral ridge found in *L. leloupi*.

Genetically, these two species each formed distinct clades within the 5-ridged *Lensia* group (Fig. 2). *Lensia panikkari*, another 5-ridged *Lensia* species with lateral ridges incomplete in their posterior portion, clustered close to *L. leloupi*. In *L. panikkari*, the somatocyst extends to more than half the nectophore in height, and remains in contact with the nectosac along its entire surface. Little is known of the three other *Lensia* species with incomplete lateral ridges: *L. lebedevi* Aleskeev, 1985, *L. paritii* Aleskeev, 1985 and *L. tottoni* Daniel & Daniel, 1965, and they are all presently considered *species inquirenda* (Mapstone & Schuchert, 2013). However, all these species appear to have a very shallow hydroecium, at or below ostial level, contrary to that observed in *L. leloupi* and *L. panikkari*.

Very similar in shape to those of *Lensia achilles*, the posterior nectophores of *L. leloupi* could be differentiated by the more conical shape of the apex of the nectosac. The nectophores were also much smaller (up to 5 mm in length), than the 13 mm reported as the maximum size for *L. achilles* posterior nectophores (Mapstone, 2009). However, the *L. achilles* anterior nectophores found in Japanese waters were also smaller than the 18 mm found in Canadian Pacific waters (Mapstone, 2009), with a maximum length of 15.4 mm, and a mean length of only 8 mm. The posterior nectophores of *L. leloupi* were shorter and wider than those of *Lensia conoidea*, and with a much shorter, less bilobed mouthplate. They could be differentiated from the posterior nectophores of *L. fowleri* and *L. subtiloides* by the extent of the nectosac anterior to the insertion point of the pedicular canal. All posterior nectophores observed and sequenced had 5 straight, complete ridges. This is contrary to the characters of a posterior nectophore Daniel (1985) associated with *L. leloupi* and briefly described as being the same height as the anterior nectophore, and having incomplete lateral ridges. The illustration provided (Fig. 58: 231) does not show any lateral ridges, and the height of the posterior nectophore is only about half that of the anterior.

The bracts associated with *Lensia leloupi* were the most common bract of unknown parentage in the upper 200 m of the water column during the day in Sagami Bay in March 2006 (Grossmann & Lindsay, 2013) and their phyllocyst showed some resemblance in shape to the somatocyst of *L. leloupi* anterior nectophores. Most similar in size and shape to the bracts of *Lensia conoidea* (Fig. 23), the bracts of *L. leloupi* could be differentiated by the less pronounced teardrop shape, the flattened hydroecial cavity and the shorter, thinner phyllocyst (Fig. 20). The bracts of *L. subtiloides* (Fig. 25), generally smaller than those of *L. leloupi*, could be differentiated by the shape of the neckshield and hydroecial cavity.

The gonophores of *L. leloupi* (Figs. 21, 22) could be differentiated from other described *Lensia* gonophores by their conical shape, marked ridges and untwisted apex. The hydroecial flaps were less developed than in *L. subtiloides* gonophores (Fig. 26) and, although conical in shape, the apex of the nectosac of *L. leloupi* gonophores was more rounded than in *L. subtiloides*. These characters, however, may be altered during preservation, and a review of all the known *Lensia* eudoxid stage gonophores may be necessary in order to establish species-specific diagnostic characteristics.

Review of known *Lensia* eudoxid stages

Of the 26 valid *Lensia* species recorded in the World Register of Marine Species (Mapstone & Schuchert, 2013), 12 have now had eudoxid stages assigned to them with certainty [*Eudoxia indica* and the eudoxid stage of *Lensia hotspur* listed in Daniel, 1985 are not considered here, as the descriptions and illustrations provided are insufficient to be able to reliably identify them or differentiate them from other *Lensia* eudoxids]: *L. achilles*, *L. asymmetrica*, *L. campanella*, *L. conoidea*, *L. cossack*, *L. fowleri*, *L. havock*, *L. leloupi*, *L. meteori*, *L. multicristata*, *L. subtilis* and *L. subtiloides*. In order to facilitate the further study of these sexual stages, a review of all known *Lensia* bracts is provided, with the focus placed on illustrating samples from Japanese waters. Eudoxid gonophores tend to show much less morphological variability than the eudoxid bracts. The specific characteristics of the former will not be treated herein. Several bracts of unknown or doubtful parentage have also been included, and their possible affiliation with the genus *Lensia* discussed.

Lensia achilles Totton, 1941

Tentative bract described and illustrated by Mapstone (2009, Fig. 46: 183).

Lensia achilles bigelowi Stepanjants, 1970 (Fig. 8: 242).

? *Lensia achilles bigelowi* Stepanjants, 1967 (Fig. 119 B: 179).

In Japanese waters, bract up to 7 mm high; headpiece conical, with 2 marked bracteal ridges extending to the apex of the headpiece on the lower side; right hydroecial flap with large notch; shallow serration of the distal margin of the neckshield; phyllocyst spindle-shaped, orange-coloured and containing oil droplets (Fig. 27).

Although the identity of this bract has yet to be confirmed by genetic methods, similarities in size, morphology, colouration and distribution with the polygastric stage of *Lensia achilles* seem to corroborate Mapstone's identification. However, a study of the mitochondrial 16S gene in *L. achilles* from Japanese and Antarctic waters showed very high

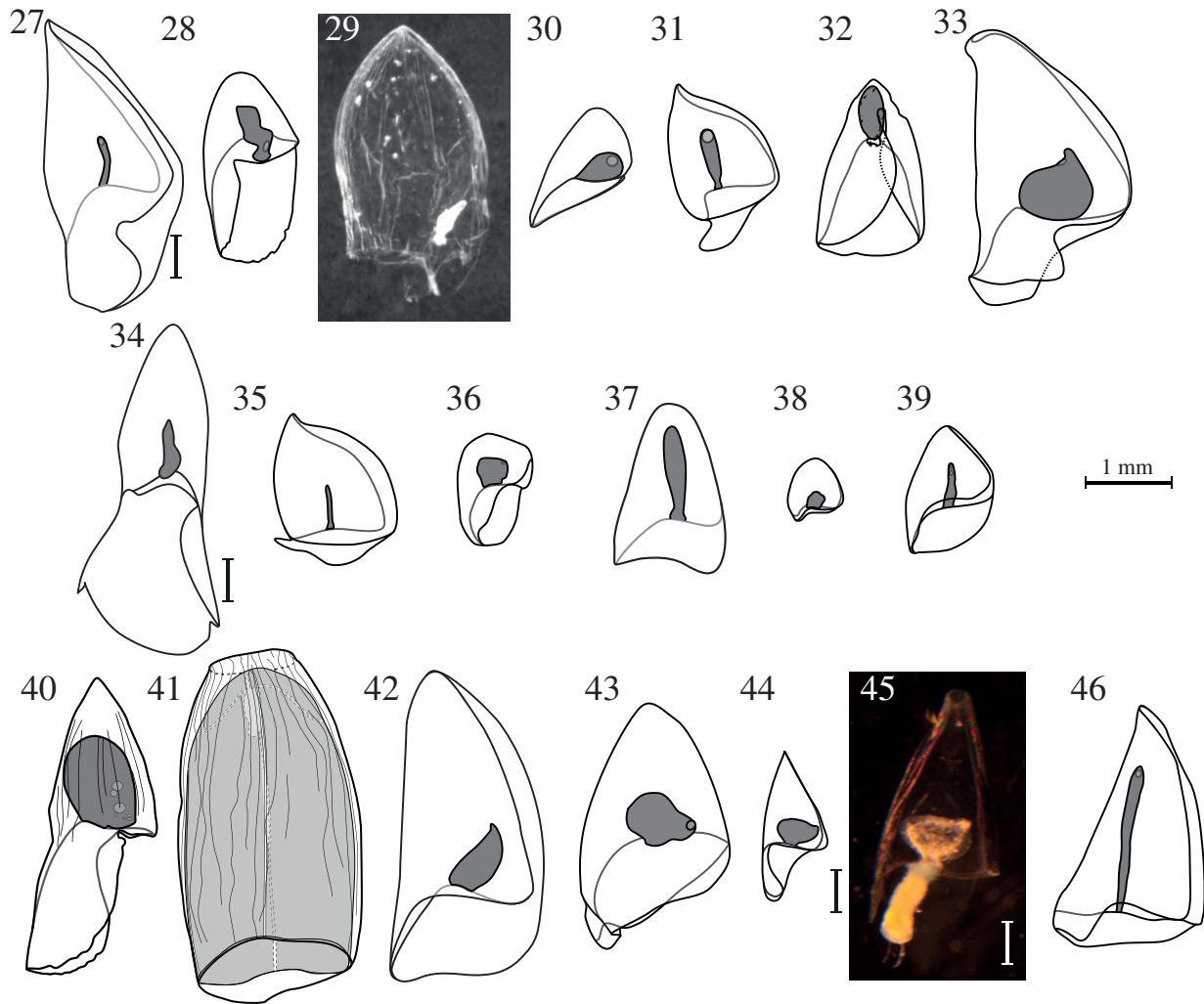


Fig. 27–46. *Lensia* eudoxid bracts. 27: *Lensia achilles*; 28: *L. asymmetrica* bract; 29: *L. asymmetrica* anterior nectophore; 30: *L. campanella*; 31: *L. conoidea*; 32: *L. cossack*; 33: *L. fowleri*; 34: *L. havock*; 35: *L. leloupi*; 36: *L. meteori*; 37: *L. multicristata*; 38: *L. subtilis*; 39: *L. subtiloides*; 40: *L. sp.* (multistriate) bract; 41: *L. sp.* (multistriate) gonophore; 42: *Eudoxia dohmi*; 43: *Eudoxia galathea*; 44, 45: Bract I; 46: *Eudoxia B.* All in right lateral view except 32, 34: lower view, and 42: upper view. All from Japanese waters except: 30, 33, 37–39, 42: from the Mediterranean Sea; 45 from the Weddell Sea. 30: redrawn after Carré, 1968 (Fig. 3: 441); 37: redrawn after Patrili, 1965 (Plate 4, a: 31, as *Eudoxia tenuis*); 42: redrawn after Gamulin, 1966 (Fig. 6: 4); 31, 35, 43 from Grossmann & Lindsay, 2013 (Fig. 8: 407); 32: from Grossmann *et al.*, 2013 (Fig. 1). Scale bars = 1 mm (27, 34, 44, 45 at half-scale).

inter-geographic genetic distances, possibly pointing to a cryptic species complex (Grossmann *et al.*, 2013). It is not known whether the bracts of *L. achilles* from the Southern Ocean resemble those from the northern Pacific.

Lensia asymmetrica Stepanjants, 1970

Eudoxid stage described and illustrated by Pugh and Pagès (1997, Figs 4, 5: 157–158).

Bract (Fig. 28) up to 2.2 mm high; headpiece rounded without bracteal ridges; notch at hydroecial level in right and left hydroecial flaps, less marked on left side; neck-shield more than half the bract in length, with faint serration of the outer distal edges; hydroecial cavity rounded;

phyllocyst asymmetrically bilobed, one lobe extending towards lower surface, in contact with the hydroecial cavity, the second vertical, about half the headpiece in height.

In samples from Japan and the Celebes and Sulu Seas, both the anterior nectophores and the eudoxid bracts of this species were covered in a fine network of longitudinal ridges or reticulation (Fig. 29), similar to that described and illustrated by Stepanjants (1970, Fig. 11: 245). Although we agree with Pugh & Pagès (1997) that the visibility of this network is most likely a preservation artefact, a couple of well-preserved specimens from Japanese waters being devoid of them, it may be interesting to study the underlying structure of the mesogloea in this species,

and its phylogenetic distribution with respect to multistriate *Lensia* species or the diphyid species *Gilia reticulata*.

The presence of serration on the outer distal edges of the bract neckshield, unrecorded by Pugh and Pagès (1997), may also be a function of sampling and preservation methods, as these forms are small and fragile, and can be easily damaged by deep-sea sampling methods.

Lensia campanella (Moser, 1925)

Eudoxid stage described and illustrated by Carré, 1968 (Fig. 3: 441).

Bract about 1 mm high, conical, without marked bracteal ridges; neckshield reduced; globular phyllocyst flattened along the hydroecial cavity on the lower side of the bract (Fig. 30).

This bract has yet to be collected from Japanese waters, but this may be due to its fragility and diminutive size, the anterior nectophores of *L. campanella* being relatively common in the surface waters of Japan and south-east Asia (e.g. Gao *et al.*, 2002; Kitamura *et al.*, 2003; Grossmann & Lindsay, 2013).

Lensia conoidea (Keferstein & Ehlers, 1860)

Eudoxid stage described and illustrated by Sars, 1846 (Plate 7, Fig. 13 – as *Diphyes truncata*).

Bract up to 2 mm high, teardrop shaped, with 2 marked bracteal ridges extending to the apex of the headpiece on the lower side; neckshield asymmetric, undeveloped on right side; hydroecial cavity forming a characteristic right-angle on the upper side of the phyllocyst; phyllocyst club-shaped, over half the headpiece in height (Fig. 31).

Lensia cossack Totton, 1941

Eudoxid stage described by Totton, 1954 (Fig. 62: 119), under the name *Eudoxia macra* (Grossmann *et al.*, 2013, Figs 1–4: 383).

Bract up to 2 mm high, conical, flattened in the upper-lower plane, without marked bracteal ridges; smooth transition between headpiece and neckshield; small notch in distal margin of neckshield; phyllocyst globular, vertical, extending nearly to bract apex (Fig. 32).

Lensia fowleri (Bigelow, 1911)

Eudoxid stage described and illustrated by Gamulin, 1966 (Figs 2, 3: 3).

Eudoxia vasconiensis Patriiti, 1965 (Plate 3: 30).

Bract up to 3 mm high, headpiece conical, with 2 marked bracteal ridges extending to the apex of the headpiece on the lower side; neckshield less than half the headpiece in length, with a central triangular notch in the distal margin; marked notch in right hydroecial flap; phyllocyst

globular, sometimes with a small vertical projection at the apex (Fig. 33).

Lensia havock Totton, 1941
(present work)

Bract up to 6.4 mm high, without marked bracteal ridges; conical headpiece; neckshield at least as long as headpiece, with tooth-like projections on baso-lateral corners; hydroecial cavity flattened; phyllocyst globular, with orange pigmentation (Fig. 34).

Lensia leloupi Totton, 1941
(present work)

Bract up to 3 mm high, bluntly conical, with 2 marked bracteal ridges extending to the apex of the headpiece on the lower side; neckshield 1/3 the height of the headpiece; hydroecial cavity flattened; phyllocyst elongate, without terminal swelling, about half the headpiece in height (Fig. 34).

Lensia meteori (Leloup, 1934)

Eudoxid stage described and illustrated by Gamulin & Kršinić, 2000 (Fig. 47: 86).

Bract 1–1.5 mm high, without marked ridges; neckshield half the bract in height, forming a sac-like hydroecial cavity opening onto the lower side of the bract; phyllocyst globular, occupying up to half the headpiece in height (Fig. 36).

Lensia multicristata (Moser, 1925)

Eudoxia tenuis Patriiti, 1965 (Plate 4, a–f: 31)

Bract about 2 mm high, headpiece conical, the bracteal ridges extending only halfway to the apex of the headpiece; neckshield less than half the bract in height, with small distal notch; phyllocyst club-shaped, nearly reaching the apex of the bract (Fig. 37).

Synonymized with *Lensia multicristata* by Gamulin & Kršinić (2000), the specific identity of *E. tenuis* has yet to be assessed genetically, although the shape of the phyllocyst and the lack of marked bracteal ridges extending to the apex of the bract make this a possible eudoxid stage of *L. multicristata*. However, this eudoxid has not so far been recorded from Japanese waters, despite the large abundance of *L. multicristata* polygastric stages between 100 and 1000 m off the southern (Kitamura, 1997, 2000, 2009; Grossmann & Lindsay, 2013) and along the north-eastern coast (Grossmann, unpubl. data) of Japan. Additionally, genetic studies of the mitochondrial 16S (Grossmann *et al.*, 2013) and CO1 (Ortman *et al.*, 2010) genes uncovered the presence of several genetically distinct groups within *L. multicristata*, which may reflect the presence of cryptic species. It is also interesting to note that

while the large majority of *L. multicristata* anterior nectophores collected off south-eastern Japan resembled, in ridge pattern and somatocyst shape, the '*Lensia multicristoides*'-type described by Zhang & Lin (1988, Fig. 1: 116), about 1% resembled the Mediterranean samples illustrated by Bigelow & Sears (1937) (Figs 40–42: 57), and about 0.5% resembled that illustrated by Moser (1925) from the equatorial Atlantic Ocean (Plate. III, Fig. 9), with a somatocyst of similar shape to that illustrated by Bigelow and Sears (1937, Figs 40–43: 57–58), but of the same height as that of the '*L. multicristoides*'-type. The shape and size of the phyllocyst of the eudoxid may therefore vary depending on the morphology of the polygastric stage from which it was produced.

Lensia subtilis (Chun, 1886)

Eudoxid stage first illustrated by Totton, 1954 (Fig. 59: 116).

?*Ersaea elongata* Will, 1844 (Plate II, Fig. 30).

Bract small, less than 1 mm high, headpiece rounded, without marked bractal ridges; reduced neckshield; phyllocyst globular, without the marked pedicle characteristic of the somatocyst of the anterior nectophore (Fig. 38).

Lensia subtiloides (Lens & van Riemsdijk, 1908)

Eudoxid stage first illustrated by Totton, 1932 (Fig. 33: 366).

Bract up to 1.5 mm high, headpiece conical, with 2 marked bractal ridges extending to the apex of the headpiece on the lower side; neckshield asymmetrical, undeveloped on right side; phyllocyst elongate, without terminal swelling (Fig. 39).

Eudoxid stage bracts of unknown polygastric stage

Lensia sp. (multistriate)

Bract (Fig. 40) up to 3.5 mm high, headpiece conical, without bractal ridges; neckshield at least as long as the headpiece, with marked serration of the outer edges of the distal border; faint pattern of longitudinal ridges in the distal part of the headpiece; hydroecial cavity flattened; phyllocyst very large, occupying more than half the headpiece in height, globular, slightly asymmetrical, filled with large oil droplets. In preserved specimens, a contraction can often be observed at the base of the headpiece.

Gonophore up to 3.6 mm long by 2.1 mm wide (Fig. 41), the outer surface covered in longitudinal ridges, more or less pronounced, incomplete in their distal part; articulate surface concave; hydroecial flaps small, only on

proximal part of gonophore; mouthplate short, rectangular; nectosac large and filling gonophore, with a rounded apex.

A total of 11 gonophores and 6 bracts of this large eudoxid stage were collected between 500 and 700 m off south-eastern Japan. The size of the phyllocyst, and serration of the posterior margin of the bract's neckshield most closely resemble the size of the somatocyst and serration of the inner edges of the mouthplate found in the anterior nectophores of *Lensia zenkevitchi* Margulis, 1970. Anterior nectophores of *L. lelouveteau* and *L. zenkevitchi*, as well as those of *L. ajax sensu* Margulis & Alekseev, 1985 were present in the net samples containing the present eudoxid stages. The gonophores, although having a large number of longitudinal ridges over their upper and lateral sides, showed no evidence of a transversal ridge, a defining characteristic in *L. exeter* and *L. lelouveteau* anterior nectophores.

A re-examination of a eudoxid stage described by Alvarino & Wojtan (1984) under the specific name *Lensia lelouveteau* by Pugh and Pagès (1995) showed the bract to have both apical and distal extensions to the phyllocyst, a characteristic of the mesopelagic species *Dimophyes arctica*.

Eudoxia dohrni Gamulin, 1966

(Fig. 6: 4)

Bract up to 5.5 mm high; headpiece tall and bluntly conical, with 2 marked bractal ridges extending to the apex of the headpiece on the lower side; neckshield asymmetrical, undeveloped on right side, without a distal notch; somatocyst globular, with pointed apex (Fig. 42).

This bract has not been reported from Japanese waters, and it may eventually prove to be a morphological variation of *L. fowleri* (Fig. 33).

Eudoxia galathea Moser, 1925

(Plate 4, Figs 9–11)

Bract up to 2.9 mm high, without bractal ridges; headpiece conical; neckshield usually half the headpiece height in length, posterior margin bilobed due to wide central notch; hydroecium deep, evenly rounded; phyllocyst globular, asymmetrical, without pedicle, varying greatly in size depending on the number of oil droplets contained (Fig. 43).

Bract I Grossmann, 2013 (Fig. 40: 220)

?*Lensia* sp. e: Pagès & Kurbjeweit, 1994.

?*Eudoxia* X: Pagès & Schnack-Schiel, 1996 (non *Eudoxia* X Pagès & Pugh, 2002).

Bract up to 4 mm high; headpiece conical, with two marked bractal ridges extending to the apex of the

headpiece on the lower side; neckshield up to half the headpiece in length, large notch near the junction with the headpiece on right side, well-marked central distal notch; hydroecial cavity flattened; phyllocyst short and globular, with central projection on lower side, orange-coloured and containing oil droplets.

Collected primarily around 500 m in Japanese waters (Fig. 44), the orange pigmentation and globular shape of the phyllocyst, as well as the particular sheen of the bract mesogloea (resembling that often found in preserved *Cheilophyes contorta* nectophores, for example) made this form a likely eudoxid stage of the little-known mesopelagic species *Lensia cordata* Totton, 1965. However, several bracts, appearing to be of the same species, were also collected during ANTARKTIS cruises to the Weddell Sea in Antarctica (ANT IX/2 station 067: 66.5°S, 27°W; ANT X/3 station 373: 68°S, 7.5°W) by the German R/V *Polarstern* (Pagès & Kurbjeweit, 1994, as '*Lensia* sp. e.'; Pagès & Schnack-Schiel, 1996, as '*Eudoxia* X') (Fig. 45). The maximum water temperature at the latter sampling points being lower than 1 °C, it seems unlikely this eudoxid stage could be that of *L. cordata*, a primarily tropical species found in the tropical Indian and eastern Pacific Oceans, but also sometimes found in the more temperate waters of Sagami and Suruga Bays, off southern Japan (Grossmann & Lindsay, 2013; Lindsay, personal observation). Further sampling and genetic analyses will be necessary to determine whether the Japanese and Southern Ocean eudoxid bracts truly belong to the same species, and to allow these eudoxids to be linked to their polygastric stage.

Eudoxid B Grossmann & Lindsay, 2013 (Fig. 8: 407)

Bract up to 3.19 mm high; headpiece conical, with 2 marked bracteal ridges extending to the apex of the headpiece on the lower side; neckshield greatly reduced; hydroecium shallow; phyllocyst long and thin, without pedicel, occupying 80% of the headpiece in height (Fig. 46).

Discussion

The present study, where we analysed DNA sequences of 12 of the 26 valid *Lensia* species (Mapstone & Schuchert, 2013), verified the validity of all the studied species by virtue of the clear genetic distinctness displayed at species level (Fig. 2). Furthermore, two clades that correspond with some distinct morphological characteristics could be identified within the genus. While, traditionally, *Lensia* species have been identified by the number of ridges present on the anterior nectophore of the polygastric stage, the present study showed that species with 5 ridges on the anterior nectophore did not form a monophyletic group with respect to the 16S rDNA analysis, but were found in each of the two genetically defined *Lensia* clades.

However, the shape, extent and markedness of the lateral ridges appeared to be of some diagnostic importance. Within the first *Lensia* clade, *L. achilles* and *L. cordata* are both characterized by having anterior nectophores with 5 complete ridges, the laterals curving towards the upper side at ostial level, and have a brightly coloured orange somatocyst. As seen in Grossmann *et al.* (2013), *L. achilles* may represent a cryptic species complex and additional sampling of both *L. achilles* and *L. cordata* from different geographic areas may increase the support at this node. The sub-group composed of *L. leloupi* and *L. panikkari* can be differentiated from the other groups by the presence of 5 straight ridges on the anterior nectophore of the polygastric stage, all complete in their apical part, but the laterals ending some distance anterior of ostial level. The lack of support at the deeper nodes within this first *Lensia* clade may be due to the lack of representative 5-ridged *Lensia* species, such as *L. challengeri* or *L. hotspur*, the latter being a sister-taxon to *L. conoidea* in a study of the mitochondrial COI gene (Ortman *et al.*, 2010).

Lensia species with 5 vestigial ridges on their anterior nectophores (*L. campanella* and *L. cossack*) were found to be more closely related to the 7-ridged and multi-ridged *Lensia* species than they were to the other 5-ridged *Lensia* species. Although these two species share few common morphological characteristics with 7-ridged and multi-ridged species when comparing the size and morphology of the anterior nectophores, the eudoxid stage bracts of species of the second *Lensia* clade all shared a common morphological trait: the lack of marked bracteal ridges reaching the apex of the bract on the lower side. Indeed, a distinct lower facet cannot be distinguished in the bracts of these species, as opposed to that clearly seen in the bracts of *Lensia achilles*, *L. conoidea* or *L. subtiloides* (Figs 27, 31, 39). Although the present study focuses primarily on the morphology of the eudoxid bracts, a similar observation may be made for eudoxid gonophores: on the gonophores of *L. campanella* (Carré, 1968), *L. cossack* (Grossmann *et al.*, 2013), *L. havock* (Figs 10–12), or gonophores associated with multistriate *Lensia* (Fig. 41), for example, the upper and lateral ridges, when present, do not extend to ostial level, contrary to observations on the gonophores of other 5-ridged *Lensia* such as *L. leloupi* or *L. subtiloides* (Figs 22, 26), where all four ridges of the gonophores are complete in both their proximal and distal parts. Additionally, the 5 vestigial ridges present on the anterior nectophores of *L. campanella*, although complete in their posterior part, do not join at the apex of the nectophore: the left lateral and lower ridges do not, at any point, come into contact with the upper, right lateral and lower ridges. This character is unique amongst 5-ridged *Lensia*, with the possible exception of the closely related *L. cossack*, in which the ridge pattern has yet to be properly studied.

The two 7-ridged *Lensia* species studied here, *L. havock* and *L. multicristata* did not form a monophyletic clade, *L. multicristata* being closer to the multi-ridged *Lensia* species than it was to *L. havock* (Fig. 2). However, although both species are characterized by the presence of 7 ridges on their anterior nectophore, the morphology of these ridges differs greatly between these two species. Indeed, in *L. havock*, all 7 ridges are complete and join at the apex of the nectophore. In *L. multicristata*, only 5 of the ridges join at the apex, the upper-laterals being incomplete in their posterior part, and the lower-lateral ridges reaching neither the apex nor the ostium of the nectophore. Both upper and lower lateral ridges tend to curve sharply in their most posterior part, but the direction and degree of curvature of the ridges is extremely variable, even amongst individuals from the same net sample, and does not seem to be directly linked to the size and shape of the nectophore or somatocyst.

Although none of the three non-ridged *Lensia* species, *L. asymmetrica*, *L. meteori* and *L. subtilis* were successfully sequenced, if the morphology of the bract and gonophore of the eudoxid stage is of diagnostic importance, it would seem likely that these non-ridged species are more closely related to *L. campanella*, *L. cossack*, the 7-ridged and multi-ridged *Lensia* species than to the other 5-ridged *Lensia*. Indeed, in non-ridged *Lensia* species, the eudoxid bracts are devoid of marked hydroecial ridges (Figs 28, 30, 36, 38), and the gonophores have no ridges besides those delimiting the hydroecial cavity. Additionally, nearly all *L. asymmetrica* anterior nectophores collected in Japanese waters were covered in a network of poorly defined ridges (Fig. 29), a character that may point to genetic similarities with the multi-ridged *Lensia*.

The effect the morphology of siphonophore nectophores and other zooids may have on swimming behaviour or predation is not yet well understood. The presence of a multitude of ridges (e.g. *L. lelouvetau*, *L. quadriculata*), or of underlying reticulation such as found in *L. asymmetrica*, the only meso- and bathypelagic non-ridged *Lensia*, may also play an important role in counteracting increased viscosity at higher pressures, as these characters appear only in species living preferentially below 500 m, and may therefore be an analogous, functional convergence rather than an indication of genetic relatedness of these species. The presence of marked ridges, especially near the apex of the colony, would most likely affect the physics of propulsion of the colony through the water, and therefore affect their metabolic rates and predation avoidance mechanisms.

The somatocyst of the anterior nectophore and phyllocyst of the bract are used as storage organs for lipid reserves that will help the colony survive times of low food availability. The size of these organs will therefore vary considerably depending on the physiological condition of the animal at the time of sampling. The large

number of animals observed during the present study allowed us to confirm that although differences in lipid content greatly influence the width and roundness of the storage organs (Figs 3–5, 14, 15), characters such as the total height of the organ, its position (e.g. somatocyst of *Lensia fowleri*), or the present of a marked pedicle, show only minimal intra-specific variations, and may therefore be safely used as diagnostic characteristics.

The mitochondrial 16S gene proved useful for the identification of calycophoran eudoxid stages to species level, both within the genus *Lensia* (Grossmann *et al.*, 2013; this study), and within the genus *Diphyes*. However, this gene was not robust enough to resolve all of the deeper nodes, which would allow for a more clear understanding of the phylogenetic relationships between the considered species. A combination of genes, both nuclear and mitochondrial, may be necessary to achieve this goal. The genetically defined *Lensia* sub-groups were found to correspond to distinct morphological traits of both the anterior nectophores of the polygastric stages and of the eudoxid stages. It is hoped the continued use of a combination of morphological, distributional and genetic information for the study of small diphyid siphonophores may finally allow for the description of the eudoxid stages of the remaining *Lensia* species. Indeed, *Lensia* is the only calycophoran genus for which, in over half the described species, the sexual stage is still unknown. Increased knowledge of the sexual ‘medusa’ stages of the Siphonophora should prove invaluable to the study and modelling of marine communities and ecosystems. Indeed, despite their size and pelagic lifestyle, the polygastric stage of diphyid siphonophores corresponds to what is called the polyp stage in other Hydrozoa species. Therefore, the presence of asexual polygastric stages of siphonophores in an environment does not necessarily equate to the presence of a reproducing siphonophore standing stock, should the conditions be unfavourable for the development, release and maturation of sexual eudoxid stages.

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