Systematics and Biodiversity (2013), **11(3)**: 381–387

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Research Article

The end of an enigmatic taxon: *Eudoxia macra* is the eudoxid stage of *Lensia cossack* (Siphonophora, Cnidaria)

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(Received 15 March 2013; revised 25 June 2013; accepted 12 July 2013)

The greatest challenge in the study of the cnidarian order Siphonophora lies in the identification of all parts of the fragile colonies usually separated during net sampling. Species are usually established based on the morphology of at least one swimming bell of the polygastric stage, and a single genus, *Eudoxia*, has housed the description of free sexual eudoxid stages until these can be reliably linked to a polygastric stage. This genus, extensively used until the early 20th century, has progressively been emptied and, at the present day, contains only one form for which the identification of the polygastric stage has remained completely enigmatic: *Eudoxia macra* Totton, 1954. DNA barcoding techniques using the mitochondrial 16S gene allowed this eudoxid stage to finally be linked with its polygastric stage, *Lensia cossack* Totton, 1941.

http://zoobank.org/urn:lsid:zoobank.org:pub:F253E52B-C14D-4440-9BA5-EB9EAC6B42E0

Key words: DNA barcoding, *Eudoxia macra*, *Lensia cossack*, geographic divergence, mitochondrial 16S, Siphonophora

Introduction

The greatest challenge in the study of the cnidarian order Siphonophora lies in the identification of all parts of the fragile colonies usually separated during net sampling. The identification of different life stages of species under different names is not uncommon in the history of binomial taxonomy. Within the Cnidaria, the Anthomedusae and Athecata orders, and the Leptomedusae and Thecata orders separated the medusoid and polypoid forms, respectively, of species presently regrouped into the Anthoathecata and Leptothecata.

In the order Siphonophora, species are usually established based on the morphology of at least one swimming bell of the polygastric stage, and a single genus, *Eudoxia*, has housed the description of free sexual eudoxid stages until these can be reliably linked to a polygastric stage. No other life stages have been described unless they could be linked to a known polygastric stage (e.g. Russell, 1938; D. Carré, 1972; C. Carré, 1979), except for the small post-larval physonect stage *Mica micula* Margulis, 1982, which may well be conspecific with *Pyrostephos vanhoeffeni* Moser, 1925 (Grossmann *et al*., 2013).

The genus *Eudoxia* (and its precursor *Ersaea*), extensively used until the early twentieth century, has progressively been emptied and, at the present day, contains only one completely enigmatic form: *Eudoxia macra* Totton, 1954. The association of eudoxid and polygastric stages is primarily done through morphological and distributional similarities. Indeed, the phyllocyst of the bract of many eudoxid stages resembles, in shape, the somatocyst of the anterior nectophore of the polygastric stage (Totton, 1965; Mapstone, 2009); and polygastric stages having nectophores with marked ridges tend to have eudoxid stages with ridged bracts (personal observation: e.g. 5-ridged *Lensia*, genera *Diphyes*, *Eudoxoides*, *Muggiaea*, etc.). Although this identification of eudoxid stages has been performed successfully many times in the Mediterranean Sea (e.g. Totton, 1932; Gamulin, 1966; Gamulin & Kršinić, 2000), where the diversity of Siphonophora is relatively low, the extremely high biodiversity of Japanese waters (Fujikura *et al*., 2012) would not normally lend itself to this type of enterprise.

Applying DNA barcoding techniques to the cnidarian order Siphonophora, the position of *Eudoxia macra* within the

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ISSN 1477-2000 print / 1478-0933 online

http://dx.doi.org/10.1080/14772000.2013.825658

family Diphyidae Quoy & Gaimard, 1827 and, more specifically, amongst the non-ridged *Lensia* species is discussed, using both morphological and genetic data from specimens caught in south-east Asian and Antarctic waters.

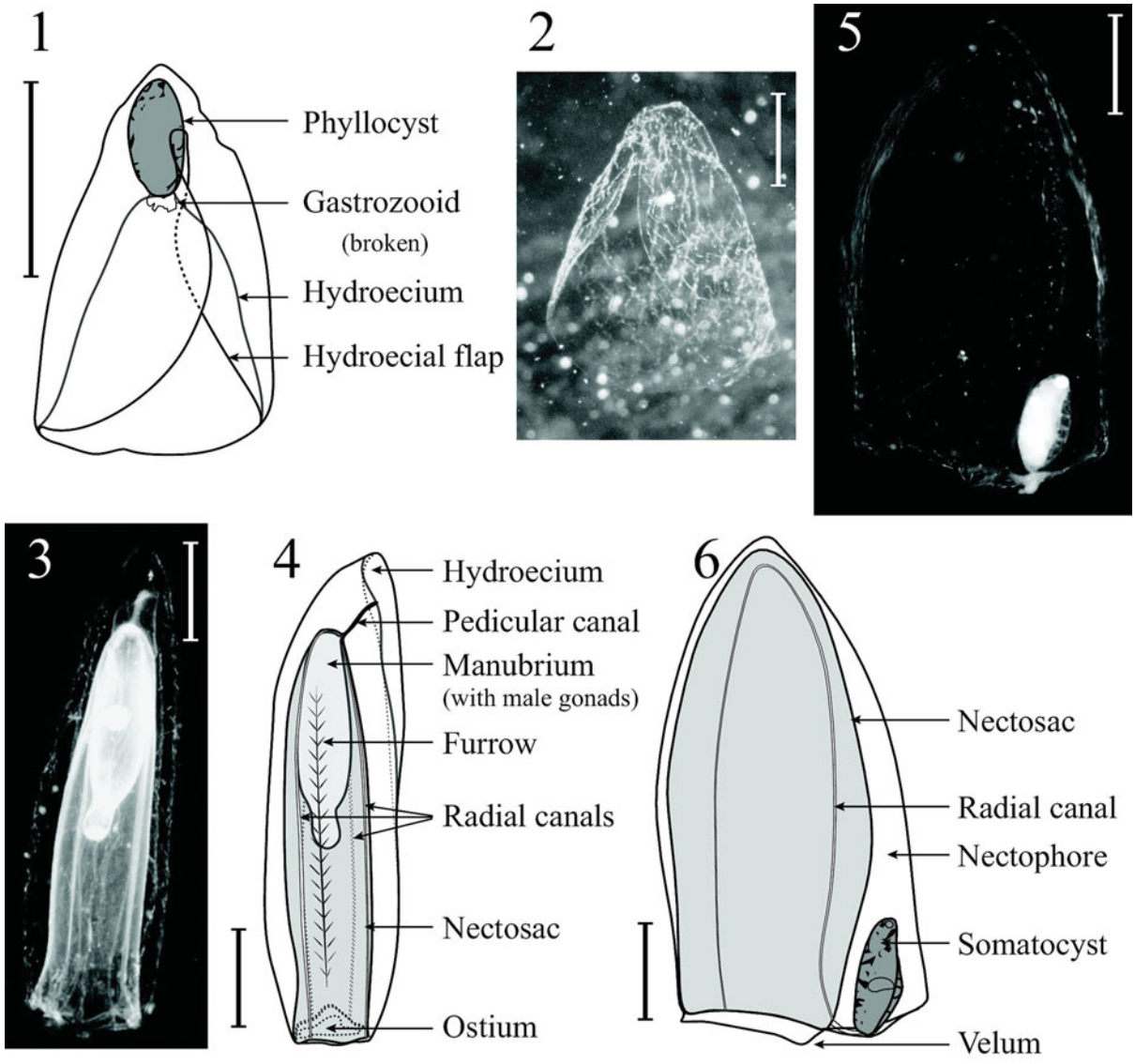
Material and methods

The formalin-seawater preserved material included four complete *Eudoxia macra* eudoxids, nine bracts and 14 gonophores collected in 330 μm mesh IONESS (Kitamura *et al*., 2001) tows in March 2006 off south-eastern Japan; seven gonophores collected with a 1.13 m diameter fry net (330 μm mesh) between October and December 2010 off the north-eastern coast of Japan; eight gonophores collected by MOCNESS (Wiebe *et al*., 1985) from the Sulu Sea in November 2002 (Nishikawa *et al.*, 2007); and two bracts and four gonophores from MOCNESS tows in the Celebes Sea, November 2002 (Nishikawa *et al.*, 2007). Specimens of 23 species of *Lensia*, from the collections of the Japan Agency of Marine-Earth Science and Technology, were also examined.

Twenty-two specimens of seven species of the genus *Lensia*, and four *Eudoxia macra* gonophores were collected using plankton nets and preserved in 99.5% ethanol or frozen at –20°C onboard after identification to species level (Table 1). Frozen specimens were transferred to 99.5% ethanol before DNA extraction. For 15 specimens (access numbers KC78253434, 40–53), total DNA was extracted using the Qiagen (Valencia, California, USA) DNEasy Blood & Tissue kit, and a 623 bp segment of the mitochondrial 16S gene 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. For the other 11 samples, DNA extraction, amplification and sequencing were performed following the protocol established in Collins *et al.* (2008). Sequence alignment was performed manually using the Se-Al v.2.0a11 software (Rambaut, 2002). Intra- and inter-specific genetic variation was calculated as the Kimura 2 parameter (K2P) genetic distance using MEGA version 5.05 (Tamura *et al*., 2011). A

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

Species	GenBank accession No.	Depth (m)	Date	Sampling location		
				Lat.	Long.	Location
Eudoxia macra	KC782542	0-400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
E. macra	KC782547	0-1076	8-Jun-2012	27°50.05'N	127°00'E	Izena Hole (Japan)
E. macra	KC782548	0-400	15-May-2012	36°40'N	141°50'E	off Fukushima (Japan)
E. macra	KC782549	0-400	14-May-2012	37°30'N	142°00'E	off Fukushima (Japan)
Lensia achilles	KC782553	750-800	19-Mar-2006	35°0.25'N	139°20'E	Sagami Bay (Japan)
L. achilles	KC782554	400-600	23-Apr-2009	35°0.25'N	139°20'E	Sagami Bay (Japan)
L. achilles	KC782555	1000-2000	11-Jan-2008	65°30.06'S	143°0.05'E	eastern Antarctica
L. achilles	KC782556	1000-2000	31-Jan-2008	64°0.62'S	140°0.76'E	eastern Antarctica
L. achilles	KC782557	1000-2000	11-Jan-2008	65°30.06'S	143°0.05'E	eastern Antarctica
Lensia campanella	KC782540	0-400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
L. campanella	KC782541	0-400	18-Nov-2011	31°N	132°E	off Kagoshima (Japan)
Lensia cossack	KC782543	0-1076	8-Jun-2012	27°50.05'N	127°00'E	Izena Hole (Japan)
L. cossack	KC782544	0-946	7-Jun-2012	27°47.73'N	126°54.11'E	Izena Hole (Japan)
L. cossack	KC782545	0-946	7-Jun-2012	27°47.73'N	126°54.11'E	Izena Hole (Japan)
L. cossack	KC782546	0-946	7-Jun-2012	27°47.73'N	126°54.11'E	Izena Hole (Japan)
Lensia exeter	KC782550	767-1100	24 Oct. 2010	27°47.73'N	126°54.11'E	Izena Hole (Japan)
Lensia havock	KC782532	200-500	29-Jan-2008	62°0.45'S	139°58.80'E	eastern Antarctica
L. havock	KC782533	500-1000	12-Feb-2008	65°30.64'S	143°1.17'E	eastern Antarctica
L. havock	KC782534	600-650	24-Mar-2006	34°59.43'N	140°15.54'E	off Kamogawa (Japan)
L. havock	KC782535	700-900	24-Apr-2009	35°0.25'N	139°20'E	Sagami Bay (Japan)
L. havock	KC782536	300-600	24-Apr-2009	35°0.25'N	139°20'E	Sagami Bay (Japan)
Lensia hostile	KC782551	200-300	19-Mar-2006	35°0.25'N	139°20'E	Sagami Bay (Japan)
L. hostile	KC782552	800-850	27-Mar-2006	34°42'N	139°50'E	off Oshima (Japan)
Lensia multicristata	KC782537	0-200	27-Jan-2008	53°8.19'S	130°8.19'E	eastern Antarctica
L. multicristata	KC782538	300-600	24-Apr-2009	35°0.25'N	139°20'E	Sagami Bay (Japan)
L. multicristata	KC782539	300-600	24-Apr-2009	35°0.25'N	139°20'E	Sagami Bay (Japan)



Figs 1–6. 1–4. *Eudoxia macra*: **1**, **2**. Lower view of bract (gastrozooid broken off in Fig. 1). **3**. Right lateral view of gonophore. **4**. Upper view of gonophore. **5**, **6**. *Lensia cossack* (right lateral view). Collected in Japanese waters (south of Kamogawa (1), east of Oshima Island (2), off north-eastern Japan (3, 4), and north of Okinawa (5, 6). Scale bar = 1 mm.

Bayesian analysis was performed on MrBayes version 3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) under a General Time Reversible (GTR) model, with 1 million generations, a sampling every 1000 generations, and a burn-in period of 5000 generations. The convergence of the data was verified using Tracer version 1.5 (Rambaut & Drummond, 2003). The consensus tree was analysed in FigTree version 1.4.0 (Rambaut, 2007). A neighbour-joining analysis was performed using MEGA version 5.05 (Tamura *et al*., 2011), with complete gap deletion. Bootstrap support was estimated over 1000 replicates.

Artwork was created from photographs of preserved specimens using Adobe Illustrator CS5.1.

Results

The *Eudoxia macra* material conformed to that described and illustrated by Totton (1954), being elongate, without any marked teeth or ridges. The bracts (Figs 1, 2) were conical, with a smooth transition between headpiece and neck shield, about 2 mm tall. The distal margin showed a small, shallow central notch in some specimens. The hydroecium, reaching to 80% of the bract height (from distal edge of the neck shield), was enclosed by large lateral flaps of the neck shield. The phyllocyst, of variable size, was ovoid, extending from the roof of the hydroecium nearly to the apex of the bract. The gonophores (Figs 3, 4) were elongate, 5 mm

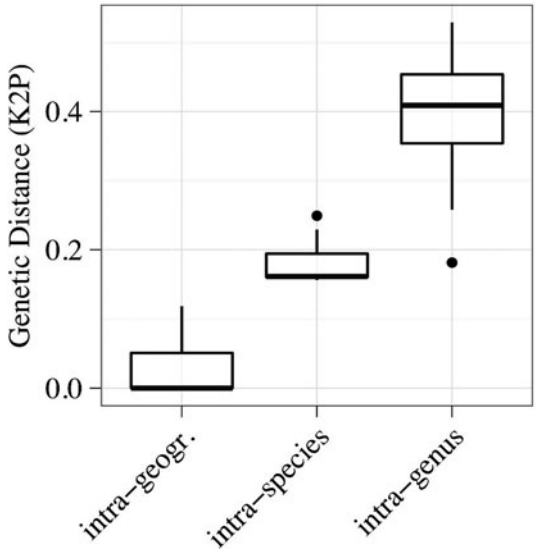


Fig. 7. Quantile boxplot of the genetic distance (Kimura-2 parameter) between species ('intra-genus'); between individuals of the same species from different geographic locations (intra-specific, inter-geographic: 'intra-species'); and between individuals of the same species from the same geographic location (intra-specific, intra-geographic: 'intra-geogr.').

tall on average, without ridges or mouthplate but with large longitudinal furrows, and a large bluntly pointed proximal projection directed towards the lower side. Small hydroecial flaps bordered the flat hydroecial surface of the gonophore, at the proximal end, but were undeveloped at the distal (ostial) end. The pedicular canal extended from the gonophore surface to one side of the apex of the nectosac, as shown in Fig. 4); it gave rise to four radial canals which each passed distally down the nectosac to the ostial region of the gonophore. The nectosac was 40% thinner and 15% shorter than the nectophore.

Due to the morphology of *Eudoxia macra*, we considered *Lensia* species without marked ridges to be a possible polygastric stage of it. However, because the eudoxids of multistriate *Lensia* species have yet to be described, a couple of representatives of this group were also included in the study. Following the results of a study using the mitochondrial COI gene (Ortman *et al.*, 2010), seven-ridged *Lensia* species were expected to be closely related, and *L. achilles* Totton, 1941 was chosen to represent the five-ridged *Lensia*, and placed as the outgroup.

A total of 26 mitochondrial 16S sequences were obtained, of which four belonged to *E. macra*. All sequence data have been deposited on GenBank (Table 1). The presence of a barcoding gap between all but the multistriate *Lensia* species was verified (Fig. 7). The phylogenetic tree (Fig. 8) showed a perfect discrimination between the seven considered species (Bayesian posterior probabilities = 100%, neighbour-joining bootstrap $\geq$ 99%). Three main morphologically dissimilar *Lensia* subgroups could be discerned, with the five-ridged *L. achilles* placed as the outgroup: the seven-ridged group containing *L. havock* Totton, 1941 and *L. multicristata* (Moser, 1925); the multistriate group with *L. exeter* Totton, 1941 and *L. grimaldi* Leloup, 1933, and, finally, *Lensia* without marked ridges: *L. campanella* (Moser, 1925) and *L. cossack* Totton, 1941. Additionally, for the three species for which specimens from the Southern Ocean were obtained, the sequences formed distinct clades according to their geographic origin, and the intra-specific variation between samples from different geographic areas (intra-specific, inter-geographic) was on average six times higher than that between specimens from the same geographic area (intra-specific, intra-geographic) (Fig. 7). Nucleotide differences between sequences varied from 9.5% to 21% for inter-species comparisons, from 8% to 12.2% for inter-geographic intra-species comparisons, and from 0% to 6.6% for intra-geographic intra-species comparisons.

All *Eudoxia macra* sequences formed a statistically supported clade containing all four *Lensia cossack* polygastric stage specimens (Fig. 8). This clade was subdivided into two sub-groups, with lower posterior probability values, only one of which contained the *L. cossack* samples. These two groups corresponded to small-scale geographic variations, the group containing the *L. cossack* samples being from waters south of mainland Japan, while the two *E. macra* samples forming the second group were from off the north-eastern shores of the main island of Japan. The genetic distances between sequences from these two groups were of 0.061 at their highest, well below the average inter-species distances observed for this group (Fig. 7). Microscope observations of the sequenced *E. macra* samples did not uncover sufficient morphological differences to discriminate two distinct species.

Discussion

First described from the Indian and Atlantic Oceans (Totton, 1954), *Eudoxia macra* has since been found in all tropical and temperate waters (Totton, 1965; Patriti, 1970; Alvariño, 1980, 1981; Zhang, 1984; Daniel, 1985; Alvariño *et al*., 1990; Zhang & Lin, 1997; Kitamura, 2000; Gao *et al*., 2002; Zhang, 2005; Xu *et al*., 2008; Kitamura, 2009). In the present material from along the north-eastern and south-eastern shores of Japan, the bracts and gonophores, of similar size to those reported from the western Pacific (Zhang, 1984), were up to twice as long as those described by Totton (1954) from the Indian ocean, or the ones collected in the Sulu and Celebes Seas during the present study. The samples collected south of Japan in March had undeveloped gonads, while samples collected in May and June from similar locations possessed mature or spent gonads. No pigmentation was observed in any of the live material from Japanese waters. This could possibly represent a geographic variation from the specimens from the Atlantic and eastern coast of Africa described by Totton

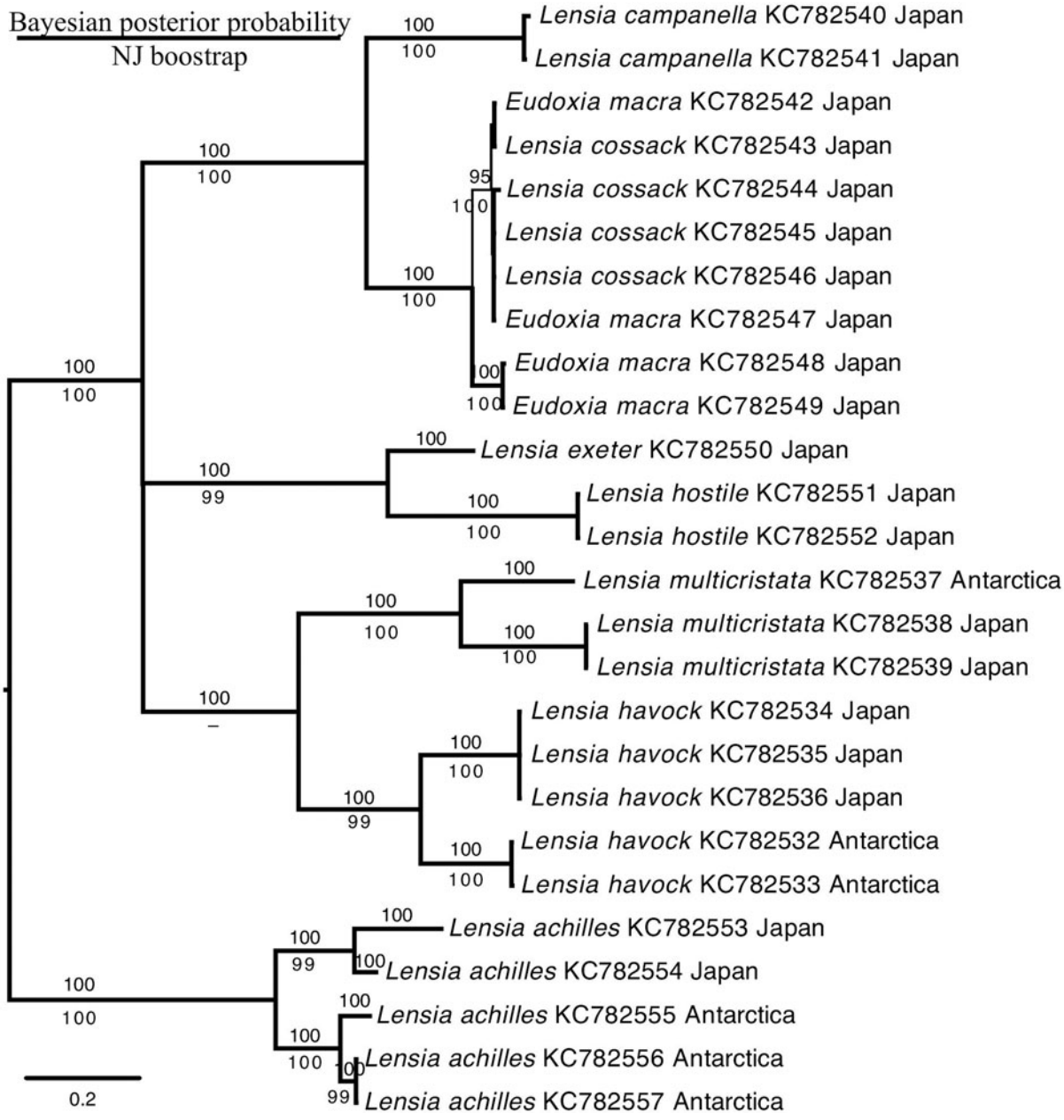


Fig. 8. Bayesian consensus tree based on the mitochondrial 16S gene. Bayesian posterior probabilities (top) and neighbour-joining bootstrap values (bottom) in%. Terminal posterior values, and those inferior to 95% not shown. Scale represents 0.2 substitutions per site.

(1954: 118), which were described to have a manubrium with a "deeply pigmented tip".

Based on the sequences of a fragment of the 16S ribosomal RNA gene, *Eudoxia macra* formed a well-supported clade with the non-ridged *Lensia cossack* (Fig. 8). The latter were about 4.5 mm tall, without marked ridges (Figs 5, 6), and without the characteristic apical twist observed in preserved *L. campanella*. The somatocysts were of the same general shape as the phyllocysts of *E. macra* bracts. Both *E. macra* and *L. cossack* are present in all oceans, if not very common in the Pacific, mostly in the tropical to subtropical zone, and primarily in the upper 500 m of the water column (Margulis, 1971; Zhang, 2005).

Although only two non-ridged *Lensia* were successfully sequenced, *Lensia campanella* and the remaining three valid species, *L. asymmetrica* Stepanjants, 1970, *L. meteori* (Leloup, 1934) and *L. subtilis* (Chun, 1886) all have known eudoxid stages, described by C. Carré (1968), Pugh & Pagès (1997), Gamulin & Kršinić (2000), and Totton (1932), respectively. A eudoxid stage associated with *Lensia cossack* was briefly described and illustrated by Daniel (1985), but the illustration does not resemble *Eudoxia macra*, and, as

the listed characters are insufficient to assign it to another species, this record is considered a *nomen nudum*.

Daniel (1985) tentatively assigned *Eudoxia macra* to *Sulculeolaria chuni* (Lens & van Riemsdijk, 1908). However, rearing experiments of the latter by Claude Carré (1979) showed this species released only the gonophores of the cormidia, the bracts and gastrozooids remaining attached to the stem. Additionally, *Sulculeolaria* cormidia differ morphologically from *Eudoxia macra*, the bracts being flattened as in *Chuniphyes*, curled around the stem so as to form a cone, with marked basal teeth. No phyllocyst has so far been described in *Sulculeolaria* bracts, while figure 4 in C. Carré (1979) shows two lateral bracteal canals. The gonophores display clear ostial teeth (Vogt, 1854; C. Carré, 1979).

The three genetically and morphologically dissimilar groups found within the genus *Lensia* corresponded to those previously observed using the mitochondrial COI gene (Ortman *et al.*, 2010), and may, once a sufficiently representative genetic database is established, serve as a solid base for a splitting of the genus *Lensia* in order to obtain new, monophyletic genera. Another important result from the present study was the quantification of intra-specific distances observed amongst taxa from different geographic regions, and the separation of the sequences of a given species into distinct clades according to their geographic origin, and this even at a regional scale. Small-scale geographic differences may also explain the higher genetic distance (0.12) found between the two Japanese *L. achilles* samples. Indeed, although both were sampled in Sagami Bay, KC782554 was collected between 400 and 600 m, a depth at which waters of northern origin such as the Oyashio Intermediate Water (Senjyu *et al.*, 1998) periodically intrude into the Bay, while KC782553 was collected between 750 and 800 m, a more hydrographically stable layer. The depth of the inter-geographic divergences observed in *L. achilles*, *L. havock* and *L. multicristata*, with K2P genetic distances between 0.16 and 0.25, and between 8.0% and 12.2% nucleotide differences, were similar to the inter-specific variations found between the multistriate *Lensia*, and those between *L. campanella* and *L. cossack*, and may reflect the existence of cryptic species rather than intra-specific variations. More diverse and detailed sampling would be needed to study the extent of regional, water-mass specific and large-scale inter-geographic genetic variation, and the existence of cryptic species complexes within the genus *Lensia*. For DNA barcoding to be a useful tool for the reliable identification of Siphonophora by non-experts, not only do a representative number of species need to be present in the databases, these should also cover the widest geographic ranges possible, in order to allow the highest matching probabilities.

Within medusozoan Cnidaria, the mitochondrial 16S gene shows promise in linking different life stages via genetic barcoding. For instance, this approach has been used to show that the minute, putative hydrozoan, *Microhydrula limopsicola* Jarms & Tiemann, 1996, is really a distinct life stage of the stauromedusa *Haliclystus antarcticus* Pfeffer, 1889 (Miranda *et al.* 2010), and an intensive study of the Leptothecata family Sertulariidae using this gene allowed both cryptic species complexes and potential synonymies to be identified (Moura *et al*., 2011). For the first time, DNA barcoding using the mitochondrial 16S gene was successfully applied to the taxonomic identification of cnidarians of the order Siphonophora. This technique allowed the eudoxid stage known as *Eudoxia macra* to finally be linked with its polygastric stage, *Lensia cossack*. As the database of well-characterized medusozoan samples associated with the mitochondrial 16S gene grows, we expect further improvement in this group's systematics.

Acknowledgements

We are grateful to the reviewers for critical and constructive comments on the manuscript and nomenclature employed. We also thank Drs Katsunori Fujikura and Hiroyuki Yamamoto of the BioGeos Research team, JAMSTEC, for their support, and Dr Jun Nishikawa of the Atmosphere and Ocean Research Institute of Tokyo University for his help in collecting many of the samples. This study is a contribution to the Census of Marine Zooplankton (CMarZ), an ocean realm field project of the Census of Marine Life, and the International Network for Scientific Investigations of Deep-Sea Ecosystems (INDEEP). This work was partially funded by Japan Society for the Promotion of Science (JSPS) KAKENHI, grant number 24248032, and MEXT Grant-in-Aid for Scientific Research on Innovative Areas TAIGA (20109003). AGC acknowledges the Smithsonian Laboratories of Analytical Biology, where some of the sequences were generated.

References

Alvariño, A. 1980. The relation between the distribution of zooplankton predators and anchovy larvae. *CalCOFI Reports* **21**, 150–160.

Alvariño, A. 1981. Siphonophorae. In: Boltovsky, D., Ed., *Atlas del zooplankton del Atlántico Suddocidental y métodos de trabajo con zooplankton marino*. Publicatión especial del Instituto Nacional de Investigación y Desarollo Pesquero (INIDEP), Mar del Plata, Argentina, pp. 383–441.

Alvariño, A., Wojtan, J.M. & Martinez, M.R. 1990. Antarctic siphonophores from plankton samples of the United States Antarctic Research Program. In: Kornicker, L.S., Ed., *Biology of the Antarctic Seas XX*. 49, pp. 1–436.

Carré, C. 1968. L'eudoxie de *Lensia campanella* Moser, 1925, avec des precisions sur le stade polygastrique (siphonophore calycophore Diphyidae). *Bulletin du Museum d'Histoire Naturelle* **50**, 438–445 (in French).

Carré, C. 1979. Sur le genre *Sulculeolaria* Blainville, 1834 (Siphonophora, Calycophorae, Diphyidae). *Annales de l'Institut Océanographique de Paris* **55**, 27–48 (in French).

Carré, D. 1972. Etude du développement des cnidocytes dans le gastrozoïde de *Muggiaea kochi* (Will, 1844) (siphonophore calycophore). *Comptes Rendus de l'Académie des Sciences de Paris* **275**, 1263–1266 (in French).

Collins, A.G., Bentlage, B., Lindner, A., Lindsay, D.J., Haddock, S.H.D., Jarms, G., Norenburg, J.L., Jankowski, T. & Cartwright, P. 2008. Phylogenetics of Trachylina (Cnidaria: Hydrozoa) with new insights on the evolution of some problematic taxa. *Journal of the Marine Biological Association of the U.K.* **88**, 1673–1685.

Cunningham, C. & Buss, L. 1993. Molecular evidence for multiple episodes of Paedomorphosis in the family Hydractiniidae. *Biochemical Systematics and Ecology* **21**, 57–69.

Daniel, R. 1985. *The fauna of India and the adjacent countries. Coelenterata: Hydrozoa, siphonophora*. Zoological Survey of India, Calcutta, India.

Fujikura, K., Lindsay, D.J., Kitazato, H., Nishida, S. & Shirayama, Y. 2012. Marine biodiversity in Japanese waters. *Public Library of Science One* **5**, e11836.

Gamulin, T. 1966. Contribution to the knowledge of *Lensia fowleri* (Bigelow) (Siphonophora, Calycophorae). *Pubblicazioni della Stazione Zoologica di Napoli* **35**, 1–6.

Gamulin, T. & Kršinić, F. 2000. Calycophores (Siphonophora, Calycophorae) of the Adriatic and Mediterranean Seas. *Fauna Natura Croatica* **9**, 1–198.

Gao, S., Hong, H. & Zhang, S. 2002. Phylum Cnidaria, class Hydrozoa subclass Siphonophora, and Class Scyphomedusae. In: Gao, S., Hong, H. & Zhang, S., Eds., *Fauna sinica (Invertebrata)* 27, Science Press, Beijing, China, pp. 1–177 (in Chinese).

Grossmann, M.M., Lindsay, D.J. & Fuentes, V. 2013. A redescription of the post-larval physonect siphonophore stage known as *Mica micula* Margulis 1982, from Antarctica, with notes on its distribution and identity. *Marine Ecology* **34**, 63–70.

Huelsenbeck, J.P. & Ronquist, F. 2001. MrBayes, Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755.

Kitamura, M. 2000. Taxonomy and distribution of planktonic Cnidaria around Japan. Ph.D. thesis, Tokyo University of Fisheries, Tokyo, Japan.

Kitamura, M. 2009. Vertical distribution of planktonic Cnidaria in three sites of northwestern Pacific. *Kaiyo Monthly* **41**, 382–392 (in Japanese).

Kitamura, M., Tanaka, Y., Ishimaru, T., Mine, Y., Noda, A. & Hamada, H. 2001. Sagami Bay research report, Improvement of multiple opening/closing net, IONESS (Intelligent Operative Net Sampling System). *Journal of the Tokyo University of Fisheries* **10**, 149–158 (in Japanese).

Mapstone, G.M. 2009. *Siphonophora (Cnidaria: Hydrozoa) of Canadian Pacific waters*. NRC Research Press, Ottawa, Canada.

Margulis, R.Ya. 1971. Distribution of siphonophores of the genus *Lensia* (suborder Calycophorae) in the Atlantic. *Okeanologiya* **11**, 80–84.

Miranda, L.S., Collins, A.G. & Marques, A.C. 2010. Molecules clarify a cnidarian life cycle – the "hydrozoan" *Microhydrula limopsicola* is an early life stage of the staurozoan *Haliclystus antarcticus*. *Public Library of Science One* **5**, e10182.

Moura, C.J., Cunha, M.R., Porteiro, F.M. & Rogers, A.D. 2011. The use of the DNA barcode gene 16S mRNA for the clarification of taxonomic problems withing the family Sertulariidae (Cnidaria, Hydrozoa). *Zoologica Scripta* **40**, 520–537.

Nishikawa, J., Matsuura, H., Castillo, L.V., Campos, W.L. & Nishida, S. 2007. Biomass, vertical distribution and community structure of mesozooplankton in the Sulu Sea and its adjacent waters. *Deep Sea Research II* **54**, 114–130.

Ortman, B.D., Bucklin, A., Pagès, F. & Youngbluth, M. 2010. DNA barcoding the Medusozoa using mtCOI. *Deep-Sea Research II* **59**, 2148–2156.

Patriti, G. 1970. Aperçu systématique de la faune de siphonophores des zones supperficielles des eaux du large de Tulear (S.W. de l'Océean Indien, Madagascar). *Recueil de Travaux de la Station Marine d'Endoume*. Suppl. **10**, 285–303. (in French)

Pugh, P.R. & Pagès, F. 1997. A redescription of *Lensia asymmetrica* Stepanjants, 1970 (Siphonophorae, Diphyidae). *Scientia Marina* **61**, 153–161.

Rambaut, A. 2002. *Se-Al, sequence alignment editor* [updated at http://tree.bio.ed.ac.uk/software/seal/, accessed 12 July 2013].

Rambaut, A. 2007. *FigTree, a graphical viewer of phylogenetic trees* [updated at http://tree.bio.ed.ac.uk/software/figtree/, accessed 12 July 2013].

Rambaut, A. & Drummond, A. 2003. *Tracer 1.5* [updated at http://tree.bio.ed.ac.uk/software/tracer/, accessed 12 July 2013].

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes, Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Russell, F.S. 1938. On the development of *Muggiaea atlantica* Cunningham. *Journal of the Marine Biological Association of the U.K.* **19**, 73–81.

Senjyu, T., Asano, N., Matsuyama, M. & Ishimaru, T. 1998. Intrusion events of the Intermediate Oyashio Water into Sagami Bay, Japan. *Journal of Oceanography* **54**, 29–44.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5, Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Totton, A.K. 1932. Siphonophora. *Scientific Reports of the Great Barrier Reef Expedition* **4**, 317–374.

Totton, A.K. 1954. Siphonophora of the Indian Ocean together with systematic and biological notes on related specimens from other oceans. *Discovery Reports* **27**, 145–168.

Totton, A.K. 1965. *A synopsis of the Siphonophora*. British Museum (Natural History), London, UK.

Vogt, C. 1854. Recherches sur les animaux inférieurs de la Méditerranée. 1. Sur les Siphonophores de la mer de Nice. *Mémoires de l'Institut National Genèvois* **1**, 1–164 (in French).

Wiebe, P.H., Morton, A.W., Bradley, A.M., Backus, R.H., Craddock, J.E., Barber, V., Cowles, T.J. & Flierl, G.R. 1985. New development in the MOCNESS, an apparatus for sampling zooplankton and micronekton. *Marine Biology* **87**, 313–323.

Xu, Z., Lin, M. & Gao, Q. 2008. Causal analysis of the diversity of medusae in the East China Sea. *Frontiers of Biology in China* **3**, 300–307.

Zhang, J.B. 1984. The Calycophorae (Siphonophora) from tropical waters of the western Pacific Ocean. In: *Proceedings on Plankton from the Tropical Region of the Western Pacific Ocean*. China Ocean Press, Beijing, China, pp. 52–85 (in Chinese with English abstract).

Zhang, J.B. 2005. *Pelagic Siphonophora in China Seas*. China Ocean Press, Beijing, China (in Chinese).

Zhang, J.B. & Lin, M. 1997. Study on the ecogeography of Siphonophora in the South China Sea. *Acta Oceanologica Sinica* **19**, 121–131 (in Chinese).

Associate Editor: Joanne Porter