Journal of
Molluscan Studies

The Malacological Society of London

Journal of Molluscan Studies (2014) **80**: 35–42. doi:10.1093/mollus/eyt038
Advance Access publication date: 4 December 2013

TAXONOMIC REVIEW, MOLECULAR DATA AND KEY TO THE SPECIES OF CRESEIDAE FROM THE ATLANTIC OCEAN

REBECA GASCA[1] AND ARIE W. JANSSEN[2]

[1]*El Colegio de la Frontera Sur (ECOSUR), Unidad Chetumal, Av. Centenario km 5.5, Chetumal, Quintana Roo 77014, Mexico; and*
[2]*Naturalis Biodiversity Center, PO Box 9517, NL-2300 RA Leiden, The Netherlands*

Correspondence: R. Gasca; e-mail: rgasca@ecosur.mx

(Received 20 February 2013; accepted 5 September 2013)

ABSTRACT

Distinguishing the various Recent taxa of the pteropod genus *Creseis*, especially the nominal species *Creseis acicula* (Rang, 1828), *C. clava* (Rang, 1828), *C. virgula* (Rang, 1828) and *C. conica* Eschscholtz, 1829, has long been problematic. Based on misinterpretations of the shell morphology, some of these were deemed to represent subspecies or forms, or were synonymized with other species in this group. Shell-morphological and nomenclatural evidence has recently been provided demonstrating that *C. clava* and *C. acicula* in fact represent a single species, for which the name *C. clava* is valid. Both *C. conica* and *C. virgula* represent independent species, and the same is true for *C. chierchiae* (Boas, 1886). This study recapitulates the background to the taxonomic problem, presents a summary of previously published morphological characters to identify species, provides diagnoses of these species and analyses new and previously published sequences from the cytochrome oxidase I barcoding gene, thus showing the utility of this gene to identify species. The data demonstrate that four Recent species should be recognized: *C. clava*, *C. conica*, *C. virgula* and *C. chierchiae*, but the last of these and its f. *constricta*, although clearly differing by shell-morphological characteristics, should be studied further by molecular techniques. *Hyalocylis striata* (Rang, 1828) and *Styliola subula* (Quoy & Gaimard, 1827), traditionally included in the Creseidae, are easily recognized on shell characteristics. A key to the identification of members of this family is provided.

INTRODUCTION

Shelled pteropods are a group of holoplanktonic gastropod molluscs belonging to the order Thecosomata. They are adapted to life in the plankton by having their foot modified into a pair of wing-like structures (parapodia) that function for swimming (Lalli & Gilmer, 1989). Their current taxonomy relies largely on the form of their aragonitic shell. Much information about this group has been recovered from the fossil record (Janssen, 2007a, b, 2012 and references therein) and fossil pteropods in Quaternary sequences are sometimes used for palaeoclimatic reconstruction. Living assemblages are covered by a wide range of literature based on plankton surveys (van der Spoel, 1967; Bé & Gilmer, 1977; van der Spoel & Dadón, 1999 and references therein; Panchang *et al.*, 2007). The group is widely distributed and has distinct distributional patterns depending on temperature, salinity, ocean currents and diel cycles. Because of their sensitivity to environmental changes, they have been proposed as indicators of oceanographic conditions, including ocean acidification (Blank, 2007; Comeau *et al.*, 2009; Roger *et al.*, 2011).

The family Creseidae is a group that has recently been revised with genetic tools and the results of Klussmann-Kolb & Dinapoli (2006) and Corse *et al.* (2013) suggest that this taxon is polyphyletic. In this study, we focus on the genus *Creseis* Rang, 1828. On the basis of molecular data presented here, we recognize three valid nominal taxa: *C. clava* (Rang, 1828), *C. virgula* (Rang, 1828) and *C. conica* Eschscholtz, 1829. In addition, and based on shell-morphological characters, we discuss *C. chierchiae* (Boas, 1886), *C. virgula constricta* Chen & Bé, 1964, *Hyalocylis striata* (Rang, 1828) and *Styliola subula* (Quoy & Gaimard, 1827), all traditionally included in the Creseidae.

This genus has a particularly complex taxonomic history (Janssen, 2007b). Since their original descriptions, species of *Creseis* have been successively synonymized or separated into specific, subspecific or infrasubspecific forms by different authors (see Janssen, 2006, 2007b, 2012). They have been reported in the literature as, for example, *C. acicula*, *C. acicula* (f.) *acicula*, *C. clava*, *C. acicula* (f.) *clava*, *C. conica*, *C. virgula*, *C. virgula* (f.) *clava*, *C. virgula* (f.) *conica*, *C. virgula* (f.) *constricta*, *C. virgula* (f.) *virgula*, *C. chierchiae* and/or *C. chierchiae* (f.) *constricta*. In the classic literature these species and (infra) subspecific forms were distinguished mainly by patterns of morphologic and morphometric characters, including the shape and general proportions of the shell and protoconch, its curvature, apical angle and surface ornamentation (Tesch, 1913; van der Spoel, 1967; van der Spoel & Dadón, 1999 and references therein). Recent shell-morphological studies (Janssen, 2007a, b, 2012) have solved a number of questions concerning systematics and nomenclature and led to the conclusion that four Recent species should be recognized within the genus *Creseis*: *C. chierchiae*, *C. clava*, *C. conica* and *C. virgula*. Apart from the first of these, all are common and distributed in tropical and subtropical water masses worldwide. It is, however, a group with a very complicated systematic history and in need of molecular evidence to test current systematics. In the present paper, we show that the cytochrome oxidase I (COI) barcoding gene effectively delimits these species and can be used to identify damaged specimens.

DNA barcoding based on the analysis of the COI mitochondrial gene has been a useful, reliable tool for separating closely related or morphologically similar species in different taxa of marine plankton including molluscs (e.g. Jennings *et al*., 2010; Bucklin, Steinke & Blanco-Bercial, 2011; Maas, Blanco-Bercial & Lawson, 2013). In these three works and in a recent study by Corse *et al*. (2013), genetic data are provided for some *Creseis* species, but the validity of names used for *Creseis* species was not evaluated. Based on the COI analysis of specimens collected from the western Caribbean Sea and on a comparative analysis of sequences from GenBank (Jennings *et al*., 2010), we provide molecular evidence to evaluate recent statements (Janssen, 2012) that the taxa *C. acicula* and *C. clava* in fact represent the same species and that *C. conica* and *C. virgula* are distinct. We had no fresh material available for *C. chierchiae*, but this taxon is easily distinguished on shell-morphological evidence.

Taxonomic history of Creseis *species*

The genus *Creseis* was introduced by Rang (1828) who considered it a subgenus of *Cleodora* and included nine nominal species, five of which were new. Several of the species included in *Creseis* by Rang have since been referred to other genera. *Creseis gadus* (Montagu, 1803), for instance, is now recognized as a scaphopod (genus *Cadulus*), while others are included in the genera *Vaginella*, *Styliola*, *Cuvierina* and *Hyalocylis*. Only Rang's (1828) *C. acicula*, *C. clava* and *C. virgula* have remained in the genus *Creseis*. A fourth species was introduced shortly afterwards, as *C. conica* Eschscholtz, 1829. There has been some uncertainty about the type species of *Creseis* (see Janssen, 2012), but recently it was found that Pelseneer (1888: 45) designated *C. acicula* as the type species.

Discriminating between *Creseis* species has been hampered by uncertainties and incorrect interpretations, partly the result of vague original descriptions. For example, Rang (1828: 318) described his *C. acicula* as "plus grêle" than the foregoing species (*C. clava*) and reaching only half the size. Both these characters were erroneously interpreted in later papers, where *C. acicula* was generally considered to be larger and more slender than *C. clava*. The word 'grêle' does not mean 'slender', but 'irregular' or 'pock-marked', which agrees with the illustrations given by Rang, which show no difference in shell width between the two, but just a slight irregularity in the shell of *C. acicula*.

The general confusion in the literature led Boas (1886) to conclude that *C. acicula* and *C. virgula* were "most probably" one and the same species. He found them reasonably easy to distinguish in the Atlantic, but found intermediate forms in the Indian and Pacific Oceans. He ignored the taxon *C. conica*, while *C. clava* was interpreted as a synonym of *C. acicula* (Boas, 1886: 59), although Rang (1828) had clearly stated that he considered *C. acicula* "plutôt une variété" ("rather a variety") of *C. clava*. Additionally, Boas (1886: 62) introduced another species, *C. chierchiae*, differing in having clear annulations, absent in the other species.

Tesch (1904) recognized three species as valid, *C. acicula*, *C. conica* and *C. virgula*, but stressed the occurrence of intermediate forms among all three. Contrary to Boas (1886), he considered *C. clava* to be a synonym of *C. virgula*. The species *C. chierchiae* apparently was not present in his material. Later on, the same author (Tesch, 1913) advocated a different taxonomy, distinguishing three species: *C. virgula*, *C. acicula* and *C. chierchiae*. Contrary to his earlier opinion he no longer treated *C. clava* as a synonym of *C. virgula*, but believed it to be a subspecies of *C. acicula*, while stating differences in size and shape between these two that contradicted Rang's original descriptions. *Creseis conica* was treated there as a subspecies of *C. virgula*, whereas *C. chierchiae* was accepted as a separate species. A further species, mentioned by Tesch (1913) as *C. caliciformis* Meisenheimer, 1905, was much later recognized as a juvenile shell of *Cuvierina*.

Curiously, Tesch (1946) distinguished *C. virgula* and *C. acicula*, but treated *C. clava*, again contrary to his earlier opinion, as a variety of *C. virgula*, while ignoring *C. conica*. Only 2 years later the same author agreed "with all authors" that *virgula*, *conica* and *clava* all belong to one and the same species, for which the name *C. virgula* was considered valid (Tesch, 1848). The accompanying illustrations of *C. clava* (Tesch, 1948: fig. 5L, M), however, are very different from Rang's original illustrations of that species and those labelled *C. conica* (Tesch, 1948: fig. 5H–K) represent typical *C. virgula*.

Subsequently, Frontier (1965) succeeded in demonstrating a distinct biometrical difference between *C. conica* and *C. virgula*, but still considered them to be different only at subspecific level. Apparently basing his opinion on the existing literature, he supposed that *C. acicula* and *C. clava* "ne semblent pas être réunies par une série d'intermédiaires" ("do not seem to be connected by a series of intermediate forms") (Frontier, 1965: 14) and therefore considered both to be valid species.

These various misunderstandings and erroneous interpretations were reflected in the monograph of the pteropods by van der Spoel (1967), as he explained in his introduction to the creseids (van der Spoel, 1967: 57). He followed the concepts of Tesch (1913) and therefore distinguished: *C. acicula* with forms *acicula* and *clava*, and *C. virgula* with forms *virgula* and *conica*. *Creseis chierchiae* was interpreted as the larval shell of *H. striata* (Rang, 1828), an opinion later demonstrated to be erroneous by Richter (1976), but still retained by van der Spoel & Dadón (1999). van der Spoel (1976: 189) also designated lectotypes for *C. acicula* and *C. clava*. The forms distinguished by van der Spoel (1967) were widely accepted by later authors, although sometimes interpreted as subspecies (e.g. Pastouret, 1970; Almogi-Labin & Reiss, 1977; Bé & Gilmer, 1977; van der Spoel & Dadón, 1999).

That the taxonomy of *Creseis* was still unsatisfactory is clear from the more recent paper by Rampal (1985), who again discussed the genus. Although she still interpreted *C. clava*, against Rang's original description, to be smaller and less slender than *C. acicula*, she was the first to doubt a specific distinction between these two taxa. She not only pointed to differences in adult shell shapes for the other species, but also in the protoconchs, concluding that there were four valid species: *C. acicula*, *C. virgula*, *C. conica* and *C. chierchiae*, without accepting subspecies or forms. Her conclusion, however, was not generally accepted, as is clear from the list of recognized species given by Lalli & Gilmer (1989: 151) in which *C. conica* is not mentioned. Later on, Rampal (2002) maintained the four taxa she had recognized in her 1985 paper, continuing to suggest that *C. clava* was not a valid species. In a discussion on the *acicula*/*clava* problem her erroneous interpretation of the original statements of Rang (1828) was repeated (Janssen, 2007b: 71) and the validity of the taxon *C. clava* remained unresolved. She introduced two new subspecies, *C. conica falciformis* Rampal, 2002 and *C. virgula frontieri* Rampal, 2002. The former represents the slightly curved morph of *C. conica* and was rejected by Janssen (2006). The latter subspecies was based on full-grown specimens in which the shell shows a slight dorsal curvature. Both occur sympatrically with the typical form of the species.

Finally, the question of whether *C. acicula* and *C. clava* can indeed be distinguished or not was answered by Janssen (2007b: 69, fig. 7) who illustrated the lectotypes of these taxa, concluded that they were synonyms and adopted *C. clava* as the valid name.

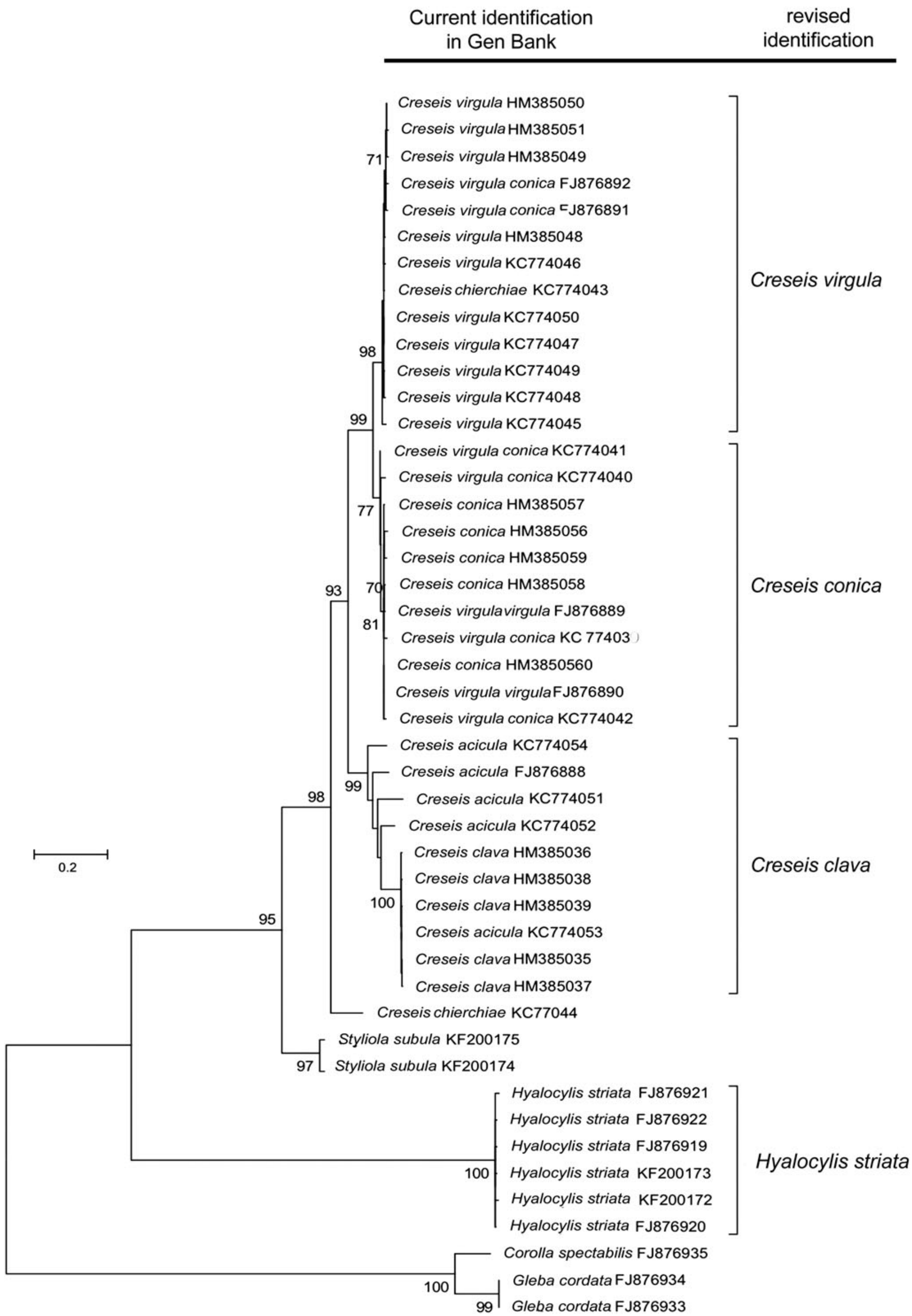

Figure 1. Maximum-likelihood tree based on K2P distance, including the species names as given in GenBank and (for new samples beginning HM and KF) as identified by us. Corrected species names are given to the right. BS support, if above 70%, is shown on branches.

So, based on morphological evidence we conclude that there are four valid Recent species in the genus *Creseis*, each with well-defined shell characters, as follows:

Creseis clava (Fig. 2C): shell straight or slightly irregular, not curved; apical angle *c.* 3°; protoconch hardly or not separated; shell surface with growth lines only

Table 1. Specimens used in the molecular analysis. Specimens with codes beginning HM and KF were newly sequenced and identified according to the criteria in Appendix 1; those with codes beginning FJ were published by Jennings *et al*. (2010) and those with codes beginning KC by Corse *et al*. (2013).

	Collection date	Original identification	Corrected name	Lat	Long	Ocean region	Cruise
HM385035	21-Jan-2007	*Creseis acicula* f. *acicula*	*Creseis clava*	18.79 N	87.14 W	Caribbean	GU0701
HM385036	23-Jan-2007	*Creseis acicula* f. *acicula*	*Creseis clava*	18.76 N	87.76 W	Caribbean	GU0701
HM385037	19-Jan-2007	*Creseis acicula* f. *acicula*	*Creseis clava*	20.08 N	87.25 W	Caribbean	GU0701
HM385038	18-Jan-2007	*Creseis acicula* f. *acicula*	*Creseis clava*	20.00 N	87.23 W	Caribbean	GU0701
HM385039	26-Jan-2007	*Creseis acicula* f. *acicula*	*Creseis clava*	19.12 N	87.06 W	Caribbean	GU0701
HM385048	24-Jan-2007	*Creseis virgula* f. *conica*	*Creseis virgula*	18.71 N	87.66 W	Caribbean	GU0701
HM385049	25-Jan-2007	*Creseis virgula* f. *conica*	*Creseis virgula*	17.90 N	87.47 W	Caribbean	GU0701
HM385050	25-Jan-2007	*Creseis virgula* f. *conica*	*Creseis virgula*	16.91 N	87.66 W	Caribbean	GU0701
HM385051	29-Jan-2007	*Creseis virgula* f. *conica*	*Creseis virgula*	18.12 N	87.81 W	Caribbean	GU0701
HM385057	15-Jan-2007	*Creseis acicula* f. *clava*	*Creseis conica*	20.98 N	86.77 W	Caribbean	GU0701
HM385058	18-Jan-2007	*Creseis acicula* f. *clava*	*Creseis conica*	19.60 N	87.37 W	Caribbean	GU0701
HM385060	27-Jan-2007	*Creseis acicula* f. *clava*	*Creseis conica*	16.57 N	87.98 W	Caribbean	GU0701
HM385059	27-Jan-2007	*Creseis acicula* f. *clava*	*Creseis conica*	16.49 N	87.95 W	Caribbean	GU0701
HM385056	14-Jan-2007	*Creseis acicula* f. *clava*	*Creseis conica*	21.51 N	86.27 W	Caribbean	GU0701
KF200174	29-Jan-2007	*Styliola subula*	*Styliola subula*	18.18 N	87.75 W	Caribbean	GU0701
KF200175	24-Jan-2007	*Styliola subula*	*Styliola subula*	17.89 N	87.88 W	Caribbean	GU0701
KF200172	16-Jan-2007	*Hyalocylis striata*	*Hyalocylis striata*	20.81 N	86.18 W	Caribbean	GU0701
KF200173	23-Jan-2007	*Hyalocylis striata*	*Hyalocylis striata*	18.25 N	87.00 W	Caribbean	GU0701
FJ876919	19-Apr-2006	*Hyalocylis striata*	*Hyalocylis striata*	24.99N	59.99 W	NW Atlantic	RHB0603
FJ876920	11-Nov-2007	*Hyalocylis striata*	*Hyalocylis striata*	3.21 N	14.6 W	NE Atlantic	PS-ANT-24-1
FJ876921	11-Nov-2007	*Hyalocylis striata*	*Hyalocylis striata*	3.21 N	14.6 W	NE Atlantic	PS-ANT-24-1
FJ876922	17-Nov-2007	*Hyalocylis striata*	*Hyalocylis striata*	13.42 S	0.65 W	NE Atlantic	PS-ANT-24-1
FJ876888	17-Nov-2006	*Creseis acicula*	*Creseis clava*	16.02 S	119.32 E	SE Indian	Galathea_2006
FJ876889	23-Apr-2006	*Creseis virgula virgula*	*Creseis conica*	19.76 N	54.61 W	NW Atlantic	RHB0603
FJ876890	16-Apr-2006	*Creseis virgula virgula*	*Creseis conica*	29.88 N	70.07 W	NW Atlantic	RHB0603
FJ876891	17-Nov-2007	*Creseis virgula conica*	*Creseis virgula*	1 S	9.01 W	SE Atlantic	PS-ANT-24-1
FJ876892	17-Nov-2007	*Creseis virgula conica*	*Creseis virgula*	3.21 N	14.06 W	NE Atlantic	PS-ANT-24-1
KC774039	*	*Creseis virgula conica*	*Creseis conica*	*	*	Mediterranean	ANTEDON
KC774040	*	*Creseis virgula conica*	*Creseis conica*	*	*	Mediterranean	ANTEDON
KC774041	*	*Creseis virgula conica*	*Creseis conica*	*	*	Caribbean	ECOSUR
KC774042	*	*Creseis virgula conica*	*Creseis conica*	*	*	Mediterranean	TARA St 18
KC774043	*	*Creseis chierchiae*	*Creseis virgula*	*	*	Indian Ocean	TARA Stn 42
KC774044	Jan 2007	*Creseis chierchiae*		*	*	Caribbean	GU0701
KC774045	*	*Creseis virgula*	*Creseis virgula*	*	*	Caribbean	ECOSUR
KC774046	*	*Creseis virgula*	*Creseis virgula*	*	*	Red Sea	TARA St. 34
KC774047	*	*Creseis virgula*	*Creseis virgula*	*	*	N Indian Ocean	TARA St 42
KC774048	*	*Creseis virgula*	*Creseis virgula*	*	*	Red Sea	TARA St. 34
KC774049	*	*Creseis virgula*	*Creseis virgula*	*	*	Red Sea	TARA St 34
KC774050	*	*Creseis virgula*	*Creseis virgula*	*	*	Red Sea	TARA St. 32
KC774051	*	*Creseis acicula*	*Creseis clava*	*	*	Indian Ocean	TARA St. 52
KC774052	*	*Creseis acicula*	*Creseis clava*	*	*	Gulf of Aden	TARA St 41
KC774053	*	*Creseis acicula*	*Creseis clava*	*	*	Caribbean	CER 2
KC774054	*	*Creseis acicula*	*Creseis clava*	*	*	Mediterranean	ANTEDON
FJ876935	14-Nov-2006	*Corolla spectabilis*		16.44 S	119.92 E	Indian Ocean	Galathea 2006
FJ876934	19-Apr-2006	*Gleba cordata*		24.99 N	59.99 W	NW Atlantic	RHB0603
FJ876933	19-Apr-2006	*Gleba cordata*		25 N	59.95 W	NW Atlantic	RHB0603

*Not available.

Creseis conica (Fig. 2A): shell straight or gently curved throughout its length; apical angle somewhat variable, up to 11°; protoconch very slightly swollen; shell surface with growth lines only

Creseis virgula (Fig. 2B): shell strongly curved for its posterior one third; apical angle initially more than 10° to *c*. 15° but decreasing to almost 0° (i.e. cylindrical) towards aperture; protoconch slightly swollen with 2 weak constrictions; shell surface with growth lines only

Creseis chierchiae (Fig. 3C, D): shell straight or (rarely) very slightly curved; apical angle of teleoconch *c*. 15°; protoconch swollen, clearly separated from teleoconch; teleoconch surface completely or partly covered with annulations [if annulations are totally missing (f. *constricta* Chen & Bé, 1964; Fig. 3D) the species is easily recognized by the shape of the protoconch]

A key to the species of Creseidae is presented in Appendix 1.

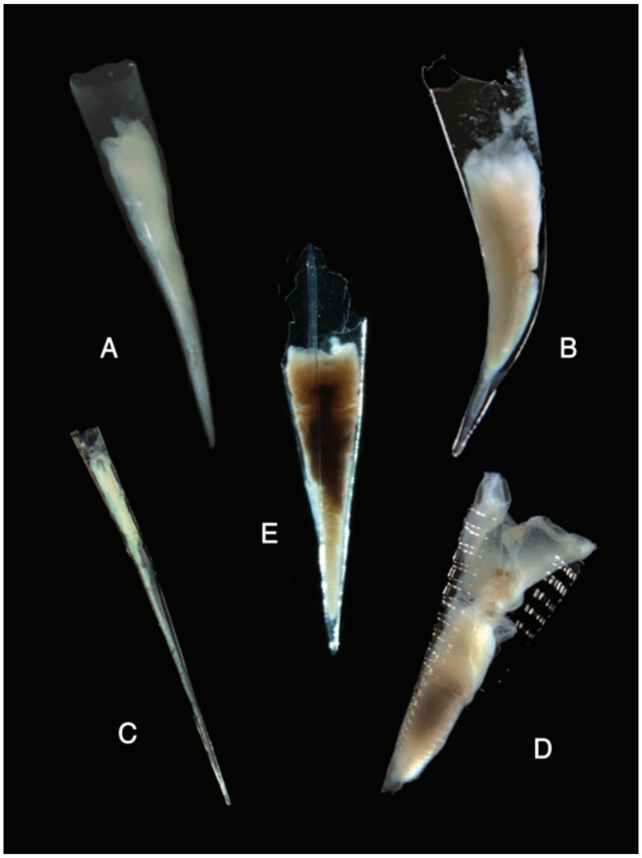

Figure 2. Photographs of the specimens analysed. **A**. *Creseis conica* (height (*H*) = 4.5 mm). **B**. *C. virgula* (*H* = 3.2 mm. **C**. *C. clava* (*H* = 9.3 mm). **D**. *Hyalocylis striata* (*H* = 5 mm). **E**. *Styliola subula* (*H* = 5.7 mm). These are all subadult specimens (Photographs by Ana Parra-Flores, ECOSUR).

MATERIAL AND METHODS

We examined zooplankton samples collected on January 14–30, 2007 during an oceanographic survey by R/V *Gordon Gunter* (GU0701 MASTER Cruise, NOAA) from the Caribbean coast of Mexico. A total of 18 complete, undamaged specimens of pteropods of the species *Creseis clava*, *C. virgula*, *C. conica*, *Styliola subula* and *Hyalocylis striata* (See Appendix 1 for characters used for identification) were selected and processed for genetic analysis. An ecological analysis of material from this same cruise was previously published by Parra-Flores & Gasca (2009), using the names *C. virgula* f. *conica*, *C. acicula* f. *clava* and *C. acicula* f. *acicula* for the species here named *C. virgula*, *C. conica* and *C. clava*.

A small piece (about 1 mm^3) of tissue was removed from each specimen and placed in 100% ethanol. The remainder of each organism was kept as a reference voucher in the Zooplankton Collection of El Colegio de la Frontera Sur, Chetumal Unit (ECO-CH-Z). DNA barcoding was carried out in the Biodiversity Institute of Ontario using standard protocols (Hajibabaei *et al.*, 2005). Tissue samples were digested overnight at 56°C in invertebrate lysis buffer with proteinase K. Genomic DNA was subsequently extracted using a membrane-based approach (Ivanova, de Waard, & Hebert, 2006) and an AcroPrep 96 1 ml filter plate with 1.0 μm PALL glass fibre media (Ivanova *et al.*, 2006). The primer pairs dgLCO-1490_t1 and dgHCO-2198_t1 were used to amplify a DNA fragment of the mitochondrial COI gene. The 12.5 μl PCR reaction mixture included 6.25 μl of 10% trehalose stabilizer, 2 μl of ultrapure water, 1.25 μl of 10× PCR buffer, 0.625 of $MgCl_2$ (50 mM), 0.125 μl of each primer (0.01 mM), 0.0625 μl of each dNTP (0.05 mM), 0.625 μl of Taq polymerase (New England Biolabs or Invitrogen) and 2.0 μl of DNA template. Amplification protocols followed those described in earlier publications (Ward *et al.*, 2005). PCR amplicons were sequenced bidirectionally using the BigDye© Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems), as described by Hajibabaei *et al.* (2005), on an ABI 3730 capillary sequencer. Sequence data, electropherograms, primer details, photographs and collection localities for specimens are available within the project Pteropods from Caribbean Sea [THEC] on the Barcode of Life Data System (see http://www.barcodinglife.org).

COI sequences were edited using the SeqApp v. 1.9 sequence editor and aligned with CLUSTAL W (using default parameters). Sequence data are available from the BOLD project and GenBank; accession numbers for GenBank specimens are provided in Table 1. Additional GenBank sequences identified as *H. striata*, *C. acicula*, *C. virgula* f. *virgula* and *C. virgula conica* (from Jennings *et al.*, 2010) and *C. virgula conica*, *C. chierchiae*, *C. acicula* and *C. virgula* (from Corse *et al.*, 2013) were used in the analysis. Sequences of two specimens of *Gleba cordata* Forsskål in Niebuhr, 1776 and one of *Corolla spectabilis* Dall, 1871 (from Jennings *et al.*, 2010), both belonging to the family Cymbuliidae, were included as the outgroup in the analysis (Table 1). Based on these data, a maximum-likelihood tree was constructed using the Kimura two-parameter (K2P; Kimura, 1980) model (Saitou & Nei, 1987) and MEGA5 software (Kumar, Tamura & Masatoshi, 2004). Selected specimens were photographed and deposited in the collection of Zooplankton at El Colegio de la Frontera Sur, Chetumal, Mexico (ECO-CH-Z).

RESULTS

The 18 newly sequenced specimens analysed yielded five clades, corresponding to the species *Hyalocylis striata*, *Styliola subula* and three species of *Creseis*: *C. conica*, *C. virgula* and *C. clava* (these are the specimens distinguished by the codes HM and KF in Fig. 1 and Table 1). The cluster here identified as *C. virgula* (Fig. 2B) also contains specimens previously published as *C. virgula* f. *conica* (Jennings *et al.*, 2010), plus a specimen possibly misidentified as *C. chierchiae* from the Indian Ocean (Corse *et al.*, 2013) and has a bootstrap (BS) value of 98%. The second cluster, identified as *C. conica* (Fig. 2A; BS = 77%), also includes specimens originally published as *C. virgula* f. *virgula* (Jennings *et al.*, 2010) and *C. virgula conica* (Corse *et al.*, 2013) (Table 1). Specimens grouped in the first cluster are separated from the second cluster by an average K2P genetic distance of 0.062 (Table 2). The third group of *Creseis* in the tree is *C. clava* (Fig. 2C; BS = 99%) and includes specimens originally published as *C. acicula* (Jennings *et al.*, 2010; Corse *et al.*, 2013). This third group includes four singletons from the Mediterranean and Indian Ocean, each of them showing K2P genetic distances above 0.09 with respect to each other and *C. clava*. A fourth lineage includes the only other specimen available in GenBank identified as *C. chierchiae* and was originally published by Corse *et al.* (2013). Two more clusters were obtained, one including two sequences identified as *S. subula* (Fig. 2E; BS = 97%) and the other six sequences of *H. striata* (Figs 2D, 3A, B; BS = 100%). In the outgroup, *Gleba cordata* and *Corolla spectabilis* were separated, as published originally by Jennings *et al.* (2010).

DISCUSSION

Recent surveys of the pteropod fauna of the Caribbean Sea and adjacent areas of the northwestern Atlantic (Suárez-Morales, 1994; Suárez-Morales & Gasca, 1998; Parra-Flores & Gasca, 2009), as well as from other geographical regions (Oliveira &

Table 2. Average K2P genetic distances using COI sequence data. Pairwise comparisons between groups of specimens of Creseidae.

	C. cl	C. co	C. vi	C. ch	C. 54	C. 52	C. 51	C. 88	S. s	H. s	G. c
Creseis clava											
Creseis conica	0.196										
Creseis virgula	0.195	0.062									
Creseis chierchiae	0.204	0.2	0.217								
Creseis acicula KC774054	0.12	0.169	0.172	0.208							
C. acicula KC774052	0.093	0.159	0.157	0.21	0.119						
C. acicula KC774051	0.14	0.215	0.206	0.248	0.122	0.112					
C. acicula FJ876888	0.117	0.17	0.181	0.207	0.107	0.083	0.122				
Styliola subula	0.309	0.346	0.343	0.314	0.353	0.286	0.363	0.326			
Hylaocylis striata	1.286	1.391	1.401	1.288	1.379	1.268	1.425	1.282	1.214		
Gleba cordata	1.794	1.813	1.784	1.792	1.843	1.672	1.805	1.866	1.736	1.821	
Corolla spectabilis	1.6	1.743	1.77	1.661	1.658	1.542	1.821	1.666	1.551	1.799	0.213

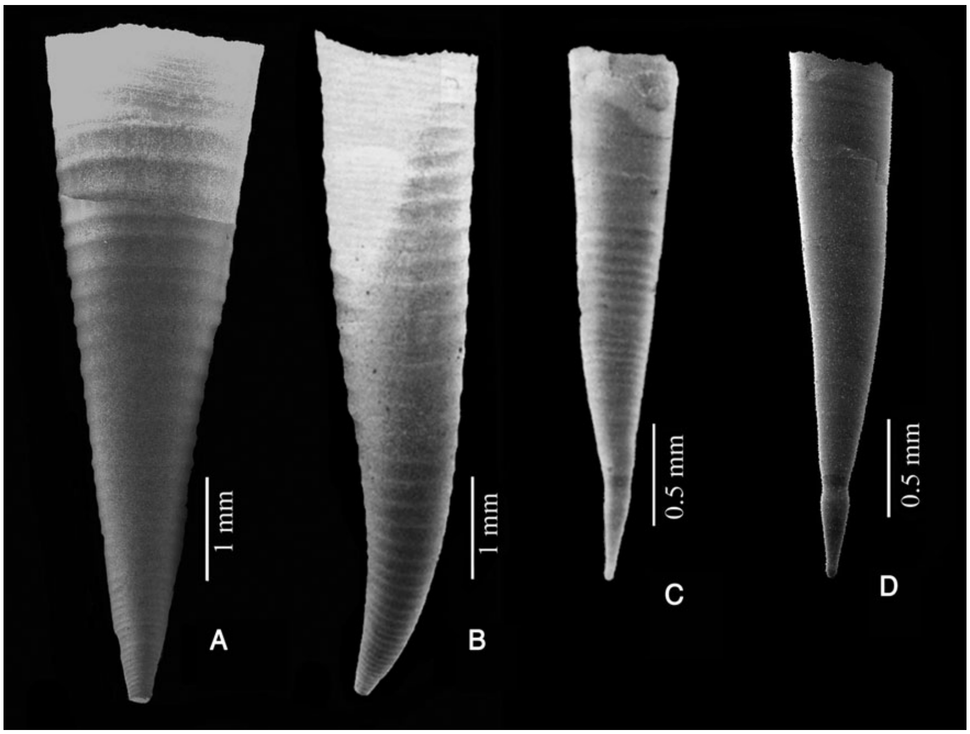

Figure 3. A, **B**. *Hyalocylis striata* from the Red Sea. **C**. *Creseis chierchiae* from the Red Sea. **D**. *C. chierchiae* f. *constricta* from the Red Sea. (SEM images by A.W. Janssen from material in Naturalis Biodiversity Center, Leiden, The Netherlands, registration numbers RGM 540429, 540430, 540419, 540421, respectively).

Larrazábal, 2002; Larrazábal & Oliveira, 2003; Nigro & Seapy, 2008; Bucklin *et al*., 2010; Angulo-Campillo, Aceves-Medina & Avedaño-Ibarra, 2011; Corse *et al*., 2013) have often used outdated, invalid or misspelled names. This is also true of the widely used reference site/database WoRMS (accessed March 2013). Hopefully, the evidence reviewed and presented here should clarify some of the confusion surrounding the genus *Creseis*. Correction of taxonomy also implies that ecological and biogeographic interpretations (i.e. patterns of distribution, abundance and vertical migration) of these species may need to be reevaluated. In pteropods, among other zooplankters, allopatric speciation on a geographical scale has been proposed within some apparently widely distributed morphospecies (e.g. Hunt *et al*., 2010; Jennings *et al*. 2010). However, in the tree resulting from our analysis (Fig. 1; Table 1) *Creseis* specimens from different geographical areas including the Atlantic Ocean, Mediterranean Sea, Indian Ocean, Red Sea and Gulf of Aden grouped closely together in the species-level clusters. An exception to this pattern was the singletons within the third group, identified as *C. acicula*. It is considered unlikely that each of these divergent samples represents a different species. This situation could be the result of sequencing errors, but further analyses will be required to investigate these divergences. Since we detected no interoceanic differences among the available specimens, differentiation of these widespread species among ocean basins is not supported by the molecular data so far available, although this is a topic that deserves further study.

Combining the morphological data provided by Janssen (2007a, b, 2012) and the present results from the barcode analysis allows us to state that the correct name for specimens usually identified as *C. acicula* f. *acicula* (following van der Spoel, 1967 and van der Spoel & Dadón, 1999: fig. 6.44) is *C. clava*

(Rang, 1828). *Creseis acicula* f. *clava* (as depicted by van der Spoel & Dadón, 1999: figs 6.45, 6.49) and *C. virgula* f. *virgula* (van der Spoel, 1967: fig. 32) should be known as *C. conica*. Specimens illustrated as *C. virgula* f. *conica* by van der Spoel (1967: fig. 31) and by van der Spoel & Dadón (1999: fig. 6.47) are *C. virgula*.

The DNA sequences obtained were useful for the classification of these species and confirm the results of shell-morphological studies. These sequences can now be compared with those of additional museum specimens, thus allowing further advancement in the taxonomic and ecological study of this widespread and ecologically important group of zooplankters.

ACKNOWLEDGEMENTS

We thank the crew of the R/V *Gordon Gunter* for obtaining the plankton samples from the western Caribbean. Lourdes Vásquez-Yeomans and John Lamkin kindly allowed us to examine these plankton samples through the project 'Larval fish and physical oceanography survey of the Mesoamerican reef system', supported by the NOAA-SEFSC. We thank Ana Parra for sorting, identifying and processing the pteropods for DNA analysis. We also appreciate help from Leocadio Blanco-Bercial (University of Connecticut), who sent us photographs of GenBank specimens for comparison. Arely Martínez Arce and Manuel Elías Gutiérrez from the ECOSUR node of the National Laboratory for Barcodes, supported by the Mexican Barcode of Life (MEXBOL-CONACYT) network, provided help and advice in processing the COI sequences. The National Oceanic and Atmospheric—Southeast Fisheries Science Centre provided funds for obtaining the samples. The Comisión Nacional para el Conocimiento de la Biodiversidad (CONABIO), Mexico, provided additional support for processing of samples through project HC007. Useful comments from two anonymous reviewers and from Suzanne Williams (Natural History Museum, London) are deeply appreciated.

REFERENCES

ALMOGI-LABIN, A. & REISS, Z. 1977. Quaternary pteropods from Israel, 1. Holocene and Pleistocene pteropods from the Mediterranean continental shelf and slope of Israel; 2. Pteropods from recent sediments in the Gulf of Elat. *Revista Española de Micropaleontologia*, **9**: 5–48.

ANGULO-CAMPILLO, O., ACEVES-MEDINA, G. & AVEDAÑO-IBARRA, R. 2011. Holoplanktonic mollusks (Mollusca: Gastropoda) from the Gulf of California, Mexico. *Check List*, **7**: 337–342.

BÉ, A.W.H. & GILMER, R.W. 1977. A zoogeographic and taxonomic review of euthecosomatous Pteropoda. In: *Oceanic Micropaleontology*, Vol. 1. (A.T.S. Ramsay, ed.), pp. 733–807. Academic Press, London.

BLANK, N. 2007. *Impacts of ocean acidification on shelled pteropods in the Southern Ocean. Biogeochemistry and pollutant dynamics*. Institute of Biogeochemistry and Pollutant Dynamics, Zurich.

BOAS, J.E.V. 1886. Spolia atlantica. Bidrag til pteropodernes. Morfologi og Systematik samt til Kundskaben om deres geografiske Udbredelse. *Videnskabernes Selskabs Skrifter, 6 (naturvidenskabelig og mathematisk Afdeling)*, **4**: 1–231.

BOUCHET, P. 2013. Limacinoidea. In: *World register of marine species*. Available at http://www.marinespecies.org/ accessed 23 February 2013.

BUCKLIN, A., ORTMAN, B.D., JENNINGS, R.M., NIGRO, L.M., SWEETMAN, C.J., COPLEY, N.J.C., SUTTON, T. & WIEBE, P.H. 2010. A "Rosetta Stone" for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean). *Deep-Sea Research II*, **57**: 2234–2247.

BUCKLIN, A., STEINKE, D. & BLANCO-BERCIAL, L. 2011. DNA barcoding of marine Metazoa. *Annual Review of Marine Science*, **3**: 47–508.

COMEAU, S., GORSKY, G., JEFFREE, R., TEYSSIÉ, J.L. & GATTUSO, J.P. 2009. Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*). *Biogeosciences*, **6**: 1877.

CORSE, E., RAMPAL, J., CUOC, C., PECH, N., PEREZ, Y. & GILLES, A. 2013. Phylogenetic analysis of Thecosomata Blainville, 1824 (holoplanktonic Opisthobranchia) using morphological and molecular data. *PLoS ONE*, **8**: e59439.

CURRY, D. 1965. The English Palaeogene pteropods. *Proceedings of the Malacological Society of London*, **36**: 357–371.

EBERL, R., COHEN, S., CIPRIANO, F. & CARPENTER, E.J. 2007. Genetic diversity of the pelagic harpacticoid copepod *Macrosetella gracilis* on colonies of the cyanobacterium *Trichodesmium* spp. *Aquatic Biology*, **1**: 33–43.

ELÍAS-GUTIÉRREZ, M., MARTÍNEZ JERÓNIMO, F., IVANOVA, N.V., VALDEZ-MORENO, M. & HEBERT, P.D.N. 2008. DNA barcodes for Cladocera and Copepoda from Mexico and Guatemala, highlights and new discoveries. *Zootaxa*, **1839**: 1–42.

FRONTIER, S. 1965. Le problème des *Creseis*. *Cahiers ORSTOM-océanographie*, **3**: 11–17.

HAJIBABAEI, M., DEWAARD, J.R., IVANOVA, N.V., RATNASINGHAM, S., DOOH, R.T., KIRK, S.L., MACKIE, P.M. & HEBERT, P.D.N. 2005. Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences*, **360**: 1959–1967.

HUNT, B., STRUGNELL, J., BEDNARSEK, N., LINSE, K., NELSON, R.J., PAKHOMOV, E., SEIBEL, B., STEINKE, D. & WÜRZBERG, L. 2010. Poles Apart: The "bipolar" pteropod species *Limacina helicina* is genetically distinct between the Arctic and Antarctic Oceans. *PLoS ONE*, **5**: e9835.

IVANOVA, N.V., DE WAARD, J.R. & HEBERT, P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, **6**: 998–1002.

JANSSEN, A.W. 2006. Notes on the systematics, morphology and biostratigraphy of fossil holoplanktonic Mollusca, 16. Some additional notes and amendments on Cuvierinidae and on classification of Thecosomata (Mollusca, Euthecosomata). *Basteria*, **70**: 67–70.

JANSSEN, A.W. 2007a. Holoplanktonic Mollusca (Gastropoda) from the Gulf of Aqaba, Red Sea and Gulf of Aden (late Holocene-Recent). *Veliger*, **49**: 149–195.

JANSSEN, A.W. 2007b. Holoplanktonic Mollusca (Gastropoda: Pterotracheoidea, Janthinoidea, Thecosomata and Gymnosomata) from the Pliocene of Pangasinan (Luzon, Philippines). *Scripta Geologica*, **135**: 29–178.

JANSSEN, A.W. 2012. Late Quaternary to recent holoplanktonic Mollusca (Gastropoda) from bottom samples of the eastern Mediterranean Sea: systematics, morphology. *Bollettino Malacologico*, **48**(suppl.): 1–105.

JANSSEN, A.W. & PEIJNENBURG, K.T.C.A. 2013. Holoplanktonic Mollusca: development in the Mediterranean Basin during the last 30 Ma and their future. In: *The Mediterranean Sea. Its history and present challenges* (S. Goffredo & Z. Dubinsky, eds), pp. 341–362, figs 19-1/15. Springer, Dordrecht.

JENNINGS, R.M., BUCKLIN, A., OSSENBRUGGER, H. & HOPCROFT, R.R. 2010. Analysis of genetic diversity of planktonic gastropods from several ocean regions using DNA barcodes. *Deep-Sea Research, II*, **57**: 2199–2210.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**: 111–120.

KLUSSMANN-KOLB, A. & DINAPOLI, A. 2006. Systematic position of the pelagic Thecosomata and Gymnosomata within Opisthobranchia (Mollusca, Gastropoda)—revival of the Pteropoda. *Journal of Zoological Systematics and Evoltionary Research*, **44**: 118–129.

KUMAR, S., TAMURA, K. & MASATOSHI, N. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**: 150–163.

LALLI, C.M. & GILMER, R.W. 1989. *Pelagic snails: the biology of holoplanktonic gastropod mollusks*. Stanford University Press, Palo Alto.

LARRAZÁBAL, M.E. & OLIVEIRA, V. 2003. Thecosomata e Gymnosomata (Mollusca, Gastropoda) da cadeia Fernando de Noronha, Brasil. *Revista Brasileira de Zoologia*, **20**: 51–360.

MAAS, A.E., BLANCO-BERCIAL, L. & LAWSON, G.L. 2013. Reexamination of the species assignment of *Diacavolinia* pteropods using DNA barcoding. *PLoS ONE*, **8**: e53889.

MEYER, C. 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*, **79**: 401–459.

MIYAMOTO, H., MACHIDA, R.J. & NISHIDA, S. 2010. Genetic diversity and cryptic speciation of the deep sea chaetognath *Caecosagitta macrocephala* (Fowler, 1904). *Deep-Sea Research II*, **57**: 2211–2219.

NIGRO, D.T. & SEAPY, R.R. 2008. Diel patterns of vertical distribution in euthecosomatous pteropods of Hawaiian waters. *Veliger*, **53**: 19–209.

OLIVEIRA, V.S. & LARRAZÁBAL, M.E.L. 2002. Pteropoda (Gastropoda, Thecosomata e Gymnosomata) coligidos ao largo dos arquipélagos de Sao Paulo, costa nordeste, Brasil. *Revista Brasileira de Zoologia*, **19**: 21–227.

PANCHANG, R., NIGAM, R., RIEDEL, F., JANSSEN, A.W. & HLA, U.K.Y. 2007. A review of the studies on pteropods from the northern Indian Ocean region with a report on the pteropods of Irrawaddy continental shelf off Myanmar (Burma). *Indian Journal of Marine Sciences*, **36**: 384–398.

PARRA-FLORES, A. & GASCA, R. 2009. Distribution of pteropods (Mollusca: Gastropoda: Thecosomata) in surface waters (0–100 m) of the western Caribbean Sea (winter, 2007). *Revista de Biología Marina y Oceanografía*, **44**: 647–662.

PASTOURET, L. 1970. étude sédimentologique et paléoclimatique de carottes prélevées en Méditerranée orientale. *Téthys*, **2**: 227–266.

PELSENEER, P. 1888. Report on the Pteropoda collected by H.M.S. Challenger during the years 1873–1876, 2. The Thecosomata. *Reports on the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873–1876*, **23**: 1–132.

RAMPAL, J. 1985. Systématique du genre *Creseis* (Mollusques, Thécosomes). *Rapport de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée*, **29**: 259–263.

RAMPAL, J. 2002. Biodiversité et biogéographie chez les Cavoliniidae (Mollusca, Gastropoda, Opisthobranchia, Euthecosomata). Régions faunistiques marines. *Zoosystema*, **24**: 209–258.

RANG, P.C.A.L. 1828. Notice sur quelques mollusques nouveaux appartenant au genre cléodore, et établissement et monographie du sous-genre créseis. *Annales de la Société des Sciences Naturelles*, **13**: 302–319.

RATNASINGHAM, S. & HEBERT, P.D.N. 2007. BOLD: the barcode of life data system. *Molecular Ecology Notes*, **7**: 355–364.

RICHTER, G. 1976. *Creseis chierchiae* Boas, eigene Art oder Jugendstadium von *Hyalocylis striata* (Rang)? *Archiv für Molluskenkunde*, 107: 145–148.

ROGER, L.M., RICHARDSON, A.J., McKINNON, A.D., BRENTON, K., MATEAR, R. & SCADDING, C. 2011. Comparison of the shell structure of two tropical Thecosomata (*Creseis acicula* and *Diacavolinia longirostris*) from 1963 to 2009: potential implications of declining aragonite saturation. *ICES Journal of Marine Sciences*, **69**: 465–474.

SAITOU, N. & NEI, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**: 406–425.

SPOEL, S. VAN DER. 1967. *Euthecosomata: a group with remarkable developmental stages (Gastropoda, Pteropoda)*. J. Noorduijn en Zoon N.V., Gorinchem.

SPOEL, S. VAN DER. 1976. *Pseudothecosomata, Gymnosomata and Heteropoda (Gastropoda)*. Bohn, Scheltema & Holkema, Utrecht.

SPOEL, S. VAN DER, BLEEKER, J. & KOBAYASI, H. 1993. From *Cavolinia longirostris* to twenty-four *Diacavolinia* taxa, with a phylogenetic discussion (Mollusca, Gastropoda). *Bijdragen tot de Dierkunde*, **62**: 127–166.

SPOEL, S. VAN DER & DADÓN, J.R. 1999. Pteropoda. In: *South Atlantic zooplankton* (D. Boltovskoy, ed.), pp. 649–706. Backhuys Publishers, Leiden.

SUÁREZ-MORALES, E. 1994. Distribución de los pterópodos (Gastropoda: Thecosomata y Pseudothecosomata) en el Golfo de México y zonas adyacentes. *Revista de Biología Tropical*, **42**: 522–530.

SUÁREZ-MORALES, E. & GASCA, R. 1998. Thecosome pteropod (Gastropoda) assemblages of the Mexican Caribbean Sea (1991). *Nautilus*, **112**: 43–51.

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.

TESCH, J.J. 1904. The Thecosomata and Gymnosomata of the Siboga Expedition. *Uitkomsten op Zoologisch, Botanisch, Oceanographisch en Geologisch Gebied, verzameld in Nederlandsch OostIndië 1899–1900 aan boord H.M. Siboga*, **52**: 1–92.

TESCH, J.J. 1913. Mollusca, Pteropoda. In: *Das Tierreich. Eine Zusammenstellung und Kennzeichnung der rezenten Tierformen* (F.E. Schulze, ed.), pp. i-xvi, 1–154.36. Friedberger & Sohn, Berlin.

TESCH, J.J. 1946. The thecosomatous pteropods. I. The Atlantic. *Dana Reports*, **28**: 1–82.

TESCH, J.J. 1948. The thecosomatous pteropods. II. The Indo-Pacific. *Dana Reports*, **30**: 1–45.

WARD, R.D., ZEMLAK, T.S., INNES, B.H., LAST, P.R. & HEBERT, P.D.N. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society Series B*, **360**: 1847–1857.

APPENDIX

Key to the Recent species of Creseidae

1A	Shell straight, apical angle *c.* 12–15°, with obliquely situated dorsal furrow (sulcus) forming a rostrum at apertural margin; protoconch well separated, sharply pointed	*Styliola subula* (Quoy & Gaimard, 1827) (Fig. 2E)
1B	Shell straight or curved, no dorsal furrow; protoconch with rounded tip	2
2A	Shell slightly curved in posterior third, elliptical in transverse section and aperture, with annulated ornament on teleoconch surface; protoconch usually absent; height to 8 mm	*Hyalocylis striata* (Figs 2D, 3A, B)
2B	Shell straight or curved, circular in transverse section, surface unornamented or with transverse annulations (in latter case height < 3 mm); height to 35 mm	3
3A	Shell small, height <3 mm; apical angle of teleoconch *c.* 15°; protoconch clearly separated and swollen	4
3B	Shell much larger; unornamented (apart from growth lines); protoconch not clearly separated or swollen	5
4A	Shell partly or completely ornamented with annulations	*Creseis chierchiae* (Boas, 1886) (Fig. 3C)
4B	Shell without annulations	*C. chierchiae* f. *constricta* Chen & Bé, 1964 (Fig. 3D)
5A	Shell needle-shaped, apical angle *c.* 3°; height to > 30 mm	*Creseis clava* (Rang, 1828) (Fig. 2C)
5B	Shell apical angle > 3°	6
6A	Shell straight or gently curved throughout its height, apical angle somewhat variable, to *c.* 11°	*Creseis conica* Eschscholtz, 1829 (Fig. 2A)
6B	Shell curved in posterior third only, apical angle > 10° to almost 15°, shell becoming almost cylindrical towards aperture in adults	*Creseis virgula* (Rang, 1828) (Fig. 2B)