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Journal

Deep Sea Research Part II Topical Studies in Oceanography, 57(24-26)

ISSN

0967-0645

Authors

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Publication Date

2010-12-01

DOI

10.1016/j.dsr2.2010.09.014

Peer reviewed



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Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Integrated molecular and morphological biogeography of the calanoid copepod family Eucalanidae

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ARTICLE INFO

Article history:

Received 18 September 2010

Accepted 18 September 2010

Available online 21 September 2010

Keywords:

Pelagic biogeography

Cryptic species

Mitochondrial 16S rRNA

Nuclear internal transcribed spacer 2

Phylogeography

ABSTRACT

Species range information forms the empirical data of pelagic biogeography. Early descriptions of canonical zooplankton distributions in the Pacific Ocean were based, in part, on distributional data from the planktonic copepod family Eucalanidae. A large-scale molecular survey of this group, covering Atlantic, Pacific, and Indian Oceans (1295 individuals), increased the total diversity from 24 to 39 evolutionarily significant units (ESUs). New biogeographies are presented here for 18 lineages within 10 described species in the genera *Subeucalanus*, *Pareucalanus*, and *Rhincalanus*. Integration of molecular and morphological data on diversity and distribution resulted in three primary outcomes: (1) the morphological species was confirmed to be valid, and the biogeographic distribution remains largely unchanged from prior reports, (2) the species was found to contain multiple ESUs, each of which has a more restricted distribution than the parent taxon, and (3) the species was found to contain multiple ESUs, whose biogeographic distributions remain unclear. *Subeucalanus subtenuis*, *S. mucronatus*, *S. subcrassus*, *Pareucalanus attenuatus*, *P. langae*, and *P. parki* are all valid genetic and morphological species, and prior distribution records from Fleming (1973) and Lang (1965) are confirmed to be accurate. New records in the western Indian Ocean extend the biogeographic range of *S. subtenuis*, *S. mucronatus*, *S. subcrassus*, and *P. langae*. *Subeucalanus pileatus*, *P. sewelli*, and *R. rostrifrons*, all species with Indo-Pacific or circumglobal distributions, consist of genetically divergent, allopatric populations that subdivide the original biogeographic range. *Subeucalanus crassus* and *Rhincalanus nasutus* are species complexes containing 4–8 genetically divergent lineages, whose distributions are inadequately characterized. Although results suggest more restricted pelagic habitats for some eucalanid species, those habitats have been previously described for other zooplanktonic taxa and do not represent new pelagic distributional types.

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1. Introduction

Calanoid copepods of the family Eucalanidae are common members of zooplanktonic assemblages in temperate-boreal, subtropical and tropical waters of the global ocean. Species in this family are primary consumers in epipelagic and upper mesopelagic depths and can occur in high abundance. Due in part to the ecological significance of species in the group, the family has received considerable attention to its systematics and biogeography. Early distributional maps of eucalanid species continue to serve as canonical examples of the basic biogeographic patterns of pelagic organisms in the Pacific Ocean as well as other regions (e.g., McGowan, 1971; Reid et al., 1978; Miller, 2004). Despite substantial prior research, the taxonomy of the

family remains problematic. At present, the group contains 24 nominal species in active use in the literature, within the four genera *Eucalanus*, *Subeucalanus*, *Pareucalanus*, and *Rhincalanus*. However, molecular studies have identified an additional 15 genetic lineages that appear to represent new, undescribed species (Goetze, 2003, 2010). The presence of these undescribed taxa calls into question the biogeographic distributions of described species.

The goal of this work is to provide updated biogeographic distributions for species within the Eucalanidae that integrate prior records from morphological studies with new genetic data. Results on the detection and description of species boundaries in the Eucalanidae, based on mitochondrial and nuclear gene loci, are presented elsewhere (Goetze, 2010). Here we (1) confirm existing records for valid, described species, and provide additional records from throughout the biogeographic range, (2) re-describe the distributions of morphospecies found to contain multiple genetic lineages, identifying areas of the distribution in which taxonomic confusion is likely to occur, and (3) provide

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preliminary biogeographic information for currently undescribed, genetically distinct species. We focus, in particular, on the globally distributed genera *Subeucalanus* and *Pareucalanus*. We also report on the intraspecific phylogeographic structure of *Subeucalanus subtenuis*, *S. mucronatus*, *S. subcrassus*, and *S. pileatus*, all species with widespread distributions in subtropical and tropical waters of the Pacific, Indian and, in some cases, Atlantic Oceans. As will become clear, resolution of the biogeographic range is far from complete for some species. This is particularly true for taxa that are rare or patchy in distribution. Nonetheless, sufficient study has been completed to enable a revised description of the biogeographic range for many species in the Eucalanidae.

1.1. Brief systematic review of the Eucalanidae

The family Eucalanidae contains the four genera *Eucalanus*, *Subeucalanus*, *Pareucalanus*, and *Rhincalanus* (Table 1). There are

currently six species within *Eucalanus*: *E. californicus* Johnson, 1938, *E. bungii* Giesbrecht, 1893, *E. inermis* Giesbrecht, 1893, *E. elongatus* (Dana, 1849), *E. hyalinus* (Claus, 1866), and *E. spinifer* Scott, 1894. With the re-description of *E. spinifer* (Goetze and Bradford-Grieve, 2005), all documented cryptic species in this genus have now been described. The current taxonomy encompasses the genetic and some of the morphological diversity known to be present in the group; some taxa need more complete morphological descriptions (e.g., *E. elongatus*). Although distinct mitochondrial haplotype clades were observed in the North Atlantic and North Pacific populations of *E. hyalinus* s.s., the level of genetic differentiation is not clearly indicative of species-level status (Goetze, 2005). At present there is insufficient information to conclude whether these clades represent genetic diversity at the intraspecific level, or are incipient species undergoing the initial stages of differentiation. Biogeographic distributions of *E. spinifer* and *E. hyalinus* s.s. were reported in Goetze (2005) and Goetze and Bradford-Grieve (2005). Due to broad sympatry of the

Table 1

Authors' provisional list of species and genera comprising the family Eucalanidae, modified from Fleminger (1973, Table 1). Listed are the number of individuals included in the genetic survey (N, 16S rRNA), number of haplotypes observed (H), and revised biogeographic distributions for each species. ** = not reported on in this study (but see Goetze 2010).

Genera and species	N	H	Distribution
Eucalanus			
1 <i>Eucalanus hyalinus</i> (Claus 1866)	**	**	Tropical - subtropical, circumglobal especially in eutrophic waters adjacent to boundary currents
2 <i>Eucalanus spinifer</i> Scott 1894	**	**	Tropical - subtropical, circumglobal especially in oligotrophic central gyre waters
3 <i>Eucalanus elongatus</i> (Dana 1849)	**	**	Tropical, Indian Ocean to western Pacific in equatorial circulation
4 <i>Eucalanus californicus</i> Johnson 1938	**	**	Temperate, North Pacific transition zone
5 <i>Eucalanus bungii</i> Giesbrecht, 1893	**	**	Boreal-subpolar, North Pacific and Bering Sea
6 <i>Eucalanus inermis</i> Giesbrecht, 1893	**	**	Eastern tropical Pacific
Subeucalanus			
subtenuis species group			
7 <i>Subeucalanus monachus</i> (Giesbrecht, 1888)	**	**	Tropical-subtropical, Atlantic Ocean, broadly neritic
8 <i>Subeucalanus longiceps</i> (Matthews 1925)	**	**	Boreal-temperate, circumglobal, Southern Ocean transition zone
9 <i>Subeucalanus subtenuis</i> (Giesbrecht, 1888)	120	106	Tropical - subtropical, circumglobal in eutrophic oceanic waters
10 <i>Subeucalanus mucronatus</i> (Giesbrecht, 1888)	86	23	Tropical, Indian Ocean to western Pacific in equatorial waters
crassus species group			
11 <i>Subeucalanus crassus</i> (Atl) (Giesbrecht, 1888)	9	8	Tropical - subtropical, Atlantic Ocean, in eutrophic broadly neritic waters
12 <i>Subeucalanus crassus</i> (Pac) (Giesbrecht, 1888)	13	8	Tropical - subtropical, Pacific Ocean, in eutrophic broadly neritic waters
13 <i>Subeucalanus</i> sp.	30	16	Tropical - subtropical, Indian Ocean to western Pacific, in eutrophic broadly neritic waters
14 <i>Subeucalanus</i> sp. 2	4	2	Poorly characterized; Agulhas - Benguela region, eutrophic waters
pileatus species group			
15 <i>Subeucalanus pileatus</i> (Atl) (Giesbrecht, 1888)	49	3	Tropical-subtropical, Atlantic Ocean, in coastal - neritic waters
16 <i>Subeucalanus pileatus</i> (Ind-Pac) (Giesbrecht, 1888)	155	24	Tropical-subtropical, Indian and Pacific Oceans, in coastal - neritic waters
17 <i>Subeucalanus</i> sp. 3	12	10	Tropical-subtropical, Indian Ocean, in coastal - neritic waters
18 <i>Subeucalanus subcrassus</i> (Giesbrecht, 1888)	96	24	Tropical, Indo-Pacific, broadly neritic
19 <i>Subeucalanus flemingeri</i> Prusova et al., 2001	**	**	Persian/Arabian Gulf
20 <i>Subeucalanus dentatus</i> (Scott, 1909)	**	**	Tropical, Flores Sea, coastal
Pareucalanus			
21 <i>Pareucalanus attenuatus</i> (Dana 1849)	98	9	Tropical-subtropical, Indo-Pacific, equatorial systems
22 <i>Pareucalanus sewelli</i> (Atl - Ind) (Fleminger, 1973)	25	13	Tropical-subtropical, Atlantic and Indian Oceans
23 <i>Pareucalanus sewelli</i> (Pac) (Fleminger, 1973)	16	1	Tropical-subtropical, Pacific Ocean
24 <i>Pareucalanus</i> sp.	34	18	Tropical-subtropical, circumglobal
25 <i>Pareucalanus langae</i> (Fleminger, 1973)	21	6	Temperate, circumglobal, Southern Ocean transition zone
26 <i>Pareucalanus parki</i> (Fleminger, 1973)	8	4	Temperate, North Pacific transition zone
27 <i>Pareucalanus smithae</i> Prusova, 2007	**	**	Arabian Sea
Rhincalanus			
28 <i>Rhincalanus rostrifrons</i> (EP) Dana, 1852	78	20	Eastern tropical Pacific, extending into central equatorial Pacific
29 <i>Rhincalanus rostrifrons</i> (WP-Ind) Dana, 1852	86	16	Tropical - subtropical in Indian Ocean and western Pacific
30 <i>Rhincalanus cornutus</i> Dana 1849	**	**	Tropical - subtropical in Atlantic Ocean
31 <i>Rhincalanus gigas</i> Brady 1883	**	**	Southern Ocean, circumpolar
32 <i>Rhincalanus nasutus</i> (SU/Ind) Giesbrecht, 1888	**	**	Eutrophic waters of Austral-Asian seas, Indo-west Pacific
33 <i>Rhincalanus nasutus</i> (AQ/Ind) Giesbrecht, 1888	**	**	Red Sea and western Indian Ocean
34 <i>Rhincalanus nasutus</i> (CA) Giesbrecht, 1888	**	**	California Current
35 <i>Rhincalanus nasutus</i> (PE) Giesbrecht, 1888	**	**	Humboldt Current
36 <i>Rhincalanus nasutus</i> (JP) Giesbrecht, 1888	**	**	Kuroshio Current, Philippine Sea
37 <i>Rhincalanus nasutus</i> (SWP/Ind/SA) Giesbrecht, 1888	**	**	Subtropical waters, South Pacific and Indian Ocean
38 <i>Rhincalanus nasutus</i> (NA) Giesbrecht, 1888	**	**	North Atlantic, eutrophic waters
39 <i>Rhincalanus nasutus</i> (SA) Giesbrecht, 1888	**	**	Subtropical waters, Benguela and Agulhas Current region

two species, prior records cannot be confidently ascribed (with the exception of sta. 82, Vervoort, 1963; as noted in Goetze and Bradford-Grieve, 2005). Other described species in the genus appear to be valid genetic species, and previous records are, for the most part, reliable (e.g., Johnson, 1937; Lang, 1965; Fleminger, 1967; Lang, 1967; Fleminger, 1973; De Decker, 1984). New distributional records from this global molecular survey do not substantially alter the described biogeographic distributions of *Eucalanus* species, and new biogeographies are therefore not presented here.

The genus *Subeucalanus* contains three species groups: 'subtenuis', 'pileatus', and 'crassus' (Fleminger, 1973; Goetze, 2010). The subtenuis group includes *S. longiceps* (Matthews, 1925), *S. monachus* (Giesbrecht, 1888), *S. subtenuis* (Giesbrecht, 1888), and *S. mucronatus* (Giesbrecht, 1888), with no additional undescribed species known to be present in the group. The pileatus group includes the smaller-bodied species *S. pileatus* (Giesbrecht, 1888), *S. subcrassus* (Giesbrecht, 1888), *S. flemingeri* (Prusova et al., 2001), *S. dentatus* (Scott, 1909), and an undescribed species, *Subeucalanus* sp. 3 (this paper, Goetze, 2010). The final species group in this genus, 'crassus', was recently designated to include four genetically distinct lineages within the morphospecies *S. crassus* (Goetze, 2003, 2010). These lineages are identified as *S. crassus* (NA), *S. crassus* (PAC), *S. sp.*, and *S. sp. 2*. This paper reports new distributional information for *S. subtenuis*, *S. mucronatus*, *S. pileatus*, *S. subcrassus*, *S. crassus* and all undescribed species in the genus.

Pareucalanus consists of five described species, *P. langae* (Fleminger, 1973), *P. parki* (Fleminger, 1973), *P. attenuatus* (Dana, 1849), *P. sewelli* (Fleminger, 1973) and *P. smithae* Prusova (2007), in addition to one undescribed species, *P. sp.* (Goetze, 2003). *Pareucalanus peruanus* Volkov 1971 has not been reported since its description, and we consider it to be synonymous with *P. langae*. No evidence has been found in this or related works for the presence of an additional South Pacific *Pareucalanus* species with four setae on B2 of the mandibular palp, despite collections from relatively near the type locality of *P. peruanus*. *Pareucalanus sewelli* consists of two distinct genetic lineages, *P. sewelli* (NA/I) and *P. sewelli* (PAC), as does the undescribed species *P. sp.* The species *Pareucalanus smithae* was recently described on the basis

of material from the Arabian Sea (Prusova, 2007), and is confirmed here to be distinct from the undescribed species *P. sp.* New distributional information is reported here for all *Pareucalanus* species, with the exception of *P. smithae*.

The final genus, *Rhincalanus*, contains the described species *R. nasutus* Giesbrecht, 1888, *R. gigas* Brady, 1883, *R. cornutus* Dana, 1849, and *R. rostrifrons* Dana, 1852. A revision of *Rhincalanus* has yet to be undertaken, in contrast to other genera in the family, and the current descriptions are inadequate for identification of the true species in the group. *Rhincalanus nasutus* is a cryptic species complex, containing at least eight distinct genetic lineages, many of which likely merit description as species (Table 1). *Rhincalanus rostrifrons* contains two distinct mitochondrial haplotype groups that appear to have allopatric geographic distributions and may be distinct species (Goetze, 2003, 2010). Geographic distributions of the *R. rostrifrons* genetic clades are described below.

2. Materials and methods

This study was based on a global collection of 70 samples, covering temperate, subtropical and tropical waters of the Atlantic, Pacific, and Indian Ocean basins (Fig. 1, Appendix A). The sample distribution covers much of the global biogeographic distribution of the family, with the notable exception of the South Atlantic. Fig. 1 includes plankton samples used in the present study; additional material was included in related studies on eucalanid species discovery and phylogenetics (Goetze, 2003, 2005, 2010). The plankton samples derive from multiple sources, including six major research cruises and a number of point samples. Samples collected by the first author on four cruises were obtained by oblique tows of either a 0.71-m diameter bongo net or a 1-m ring net (202 - 333 μm mesh) from between 400 -1000 m and the surface (MP3 North Atlantic, VAN10MV Indian Ocean, COOK14MV South Pacific, DRIFT07RR South Pacific). The maximum depth of oblique tows on two additional cruises was 200 m (STAR00 eastern tropical Pacific; Ace-Asia North Pacific). Point samples contributed by other scientists were collected using a variety of sampling gear and methods (see Acknowledgments). A complete

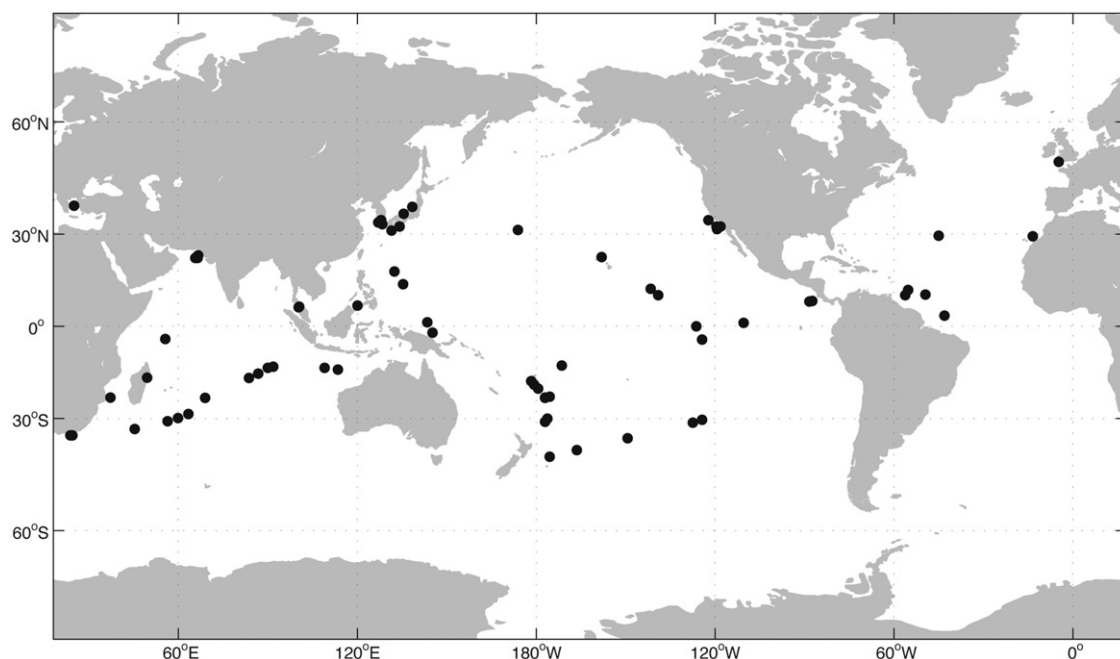


Fig. 1. Sampling locations for specimens included in this study.

listing of collection localities is reported in Appendix A. Bulk plankton samples were preserved and stored in 95% non-denatured ethyl alcohol, changed to new alcohol within 12–24 hours of collection, and maintained at -20°C to prevent DNA degradation. In some cases, collectors provided plankton material preserved in 100% ethyl alcohol or 95% denatured ethyl alcohol, and these preservatives also were successful in many cases.

Eucalanid specimens were removed from bulk plankton samples for identification and DNA extraction. 228 specimens were identified to species following Fleming (1973), Bradford-Grieve (1994), and Lang (1965), including integumental pore analysis of the posterior prosome, as necessary, for accurate species identification (as reported in Goetze 2003). 728 additional specimens were identified as closely as possible to species in the absence of integumental pore information. Species identity was then determined by genetic similarity to previously identified material (details below). This approach was necessary to distinguish *P. attenuatus*, *P. sewelli*, and *P. sp.*, which at the moment are morphologically distinguishable only by integumental pore patterns, as well as *S. pileatus*, *S. subcrassus* and the undescribed *Subeucalanus* and *Rhincalanus* lineages. Species within the *Subeucalanus* 'pileatus' group are particularly difficult to distinguish by morphological traits in the absence of integumental pore information, based on current knowledge. The species overlap broadly in size, and differ only in the shape of the anterior portion of the forehead and the relative width of the genital segment (Lang, 1965). These characters were found to be unreliable for specimens collected across the entire biogeographic range. The undescribed species *S. sp. 3* is apparently morphologically intermediate between *S. pileatus* and *S. subcrassus*, and is broadly sympatric in many areas, making accurate identification challenging.

DNA was extracted from individual copepods using the QIAGEN DNeasy kit or a lysis buffer protocol (Goetze, 2003; Lee and Frost, 2002). The QIAGEN manufacturer's protocol was modified by limiting the 55°C lysis buffer step to 1–1.25 h, reducing the quantity of elution buffer to 100 μl , and increasing the duration of the elution step to 10 min.

A fragment of the mitochondrial gene 16S rRNA was amplified by polymerase chain reaction (PCR). Amplification reactions used the universal primers 16SAR [5' – CGCCTGTTTATCAAAAACAT – 3'] and 16SCB [5' – ATT CAA CAT CGA GGT CAC AA – 3'], as well as the genus-specific internal primers 16S_PAR1 [5' – GCT AAG GTA GCA TAA TAA TTA GTT – 3', for *Pareucalanus*] and 16S_SUB2 [5' – AAG TGC TAA GGT AGC ATA AT – 3', for *Subeucalanus*] (Goetze, 2003; Braga et al., 1999). These primer combinations amplify a ca. 380 bp fragment of the 16S rRNA gene. Use of a short gene fragment has the advantage that it can be consistently amplified from plankton material collected and preserved by diverse scientists using varied methods, enabling comprehensive geographic coverage of the sample distribution. As reported in Goetze (2010), this short gene fragment contains sufficient phylogenetic information to resolve species-level relationships with high confidence. Amplifications were carried out in 25 or 50 μl reactions, with 1–5 μl of genomic DNA as template. PCR conditions began with 30 sec at 95°C for denaturation, followed by 40 cycles of 30 sec denaturing at 95°C , 30 sec annealing at 50°C , and 1 min extension at 72°C , with a final 5 min extension step at 72°C . PCR products were cleaned with the QIAGEN PCR purification kit, and sequenced directly using BigDye Terminator cycle sequencing chemistry. PCR primers were used in sequencing reactions.

DNA sequences were edited using Sequencher (Genecodes), Vector NTI (Invitrogen), or Genelous (Biomatters) software and base calls were confirmed by aligning both strands. Sequences are available under GenBank accession numbers GU259687–GU260651. Multiple sequence alignments for all specimens in each genus were completed in ClustalX (Thompson et al., 1997), followed by minor adjustments by eye in MacClade (Maddison and Maddison,

2000). Alignments for *Rhincalanus* and *Pareucalanus* were unambiguous and no nucleotide sites were removed prior to phylogenetic analysis. Fifty-eight poorly aligned sites in 7-indel regions were identified and removed from the *Subeucalanus* alignment using Gblocks (Castresana, 2000). Final genus-level alignments were 278, 356, and 288 bp in length for *Pareucalanus*, *Rhincalanus* and *Subeucalanus* respectively (further details in Goetze, 2010). Phylogenetic relationships among specimens were inferred using neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods. All phylogenetic analyses were conducted in PAUP 4.0b10 (Swofford, 2002; as described in Goetze, 2010). Upon completion of the phylogenetic analysis, the species identity of all specimens was verified by placement in the 16S rRNA phylogenies. Placement of individuals to mtDNA clades was unambiguous, with high levels of bootstrap support for distinct monophyletic mitochondrial clades in all analyses. These verified species identifications were used in biogeographic mapping.

Intraspecific genetic diversity and population structure were examined for four eucalanid species with widespread or global distributions in subtropical and tropical waters. For each species, nucleotide diversity and sums of squared haplotype frequencies were calculated in Arlequin v2.001 (Schneider et al., 2000). Unbiased estimates of haplotype diversity were calculated according to Nei (1987, eq. 8.4). Genetic differentiation among population samples was tested using a hierarchical analysis of molecular variance (AMOVA), with 1000 permutations of haplotypes among populations, as well as Fisher's exact tests (as implemented in Arlequin v2.001). Statistical parsimony gene networks were estimated using TCS 1.21 (Clement et al., 2000), and genetic distances among sequences were calculated in MEGA v4.0 (Tamura et al., 2007).

Maps of species distributions were generated in Matlab (The Mathworks) using the M_Map package (Pawlowich, 2006). Collecting locations for all specimens of each species were plotted from the molecular study, in addition to as many records as possible from prior morphological studies by Fleming (1973) and Lang (1965). Those two comprehensive revisions of the genus *Eucalanus* or the entire eucalanid family, respectively, include sampling coverage from all ocean basins. Geographic coordinates of 126 collecting locations for specimens of each species were published in Fleming (1973). Unfortunately, comparable data are unavailable in Lang's (1965) dissertation. Lang published a list of all examined samples as well as distributional maps, but did not report the species present in each sample. In attempting to reconstruct Lang's distributional maps, all eucalanid specimens from Lang's dissertation that are currently held in the Scripps Institution of Oceanography Pelagic Invertebrates Collection were plotted. However, this extant collection of identified specimens is clearly a subset of the original material used in producing the distributional maps in her dissertation, even if one considers that a portion of the Lang material was subsequently examined for integumental pores and reported in Fleming (1973). Therefore, it is important for the reader to note that distributional records from Lang (1965) reported here represent only a subset of the original material, and indicate collecting locations that can be confirmed based on existing material. Summary distributional maps from Lang's work are included, where appropriate, to provide additional information regarding the species' biogeographies as delineated by Lang.

3. Results: biogeography and phylogeography

3.1. *Subeucalanus*

This genus is in need of systematic revision. It contains five new ESUs that are cryptic, putative new species that cannot

currently be distinguished by morphological criteria (Goetze, 2010). Discovery of these genetic lineages requires revision of the biogeographic distributions of described *Subeucalanus* species in some, but not all cases. Species in this genus range from being oceanic, with widespread or circumglobal distributions, to being broadly neritic, with localized or patchy distributions.

3.1.1. The 'subtenuis' species group

Subeucalanus subtenuis is a valid morphological and genetic species. Current sampling indicates both an absence of highly divergent intraspecific genetic clades, as well as an absence of strong genetic structure at the population level in the Pacific Ocean. A genetic survey of 120 individuals collected at 17 locations in the Pacific and Indian Oceans identified 106 mt16S

rRNA haplotypes (Fig. 2, Table 2). The survey sampled populations in the eastern tropical Pacific and western Pacific and Indian Oceans, but did not include the North Atlantic population of this species (Fig. 2). The average genetic distance among all *S. subtenuis* DNA sequences was 1.6% (Kimura 2-parameter distance, K2P), with a maximum genetic distance of 4% (K2P) observed between the most divergent haplotypes. Phylogenetic analysis of all *S. subtenuis* sequences did not identify any prominent intraspecific mitochondrial clades with high bootstrap support (maximum bootstrap support 51%, results not shown). In an analysis of five population samples from the eastern and western Pacific (97 individuals, Table 2, Fig. 2), haplotype and nucleotide diversities were high, ranging from 0.96–1.00 and 0.014–0.018, respectively. No strong signal of genetic structure between eastern and western Pacific populations was observed

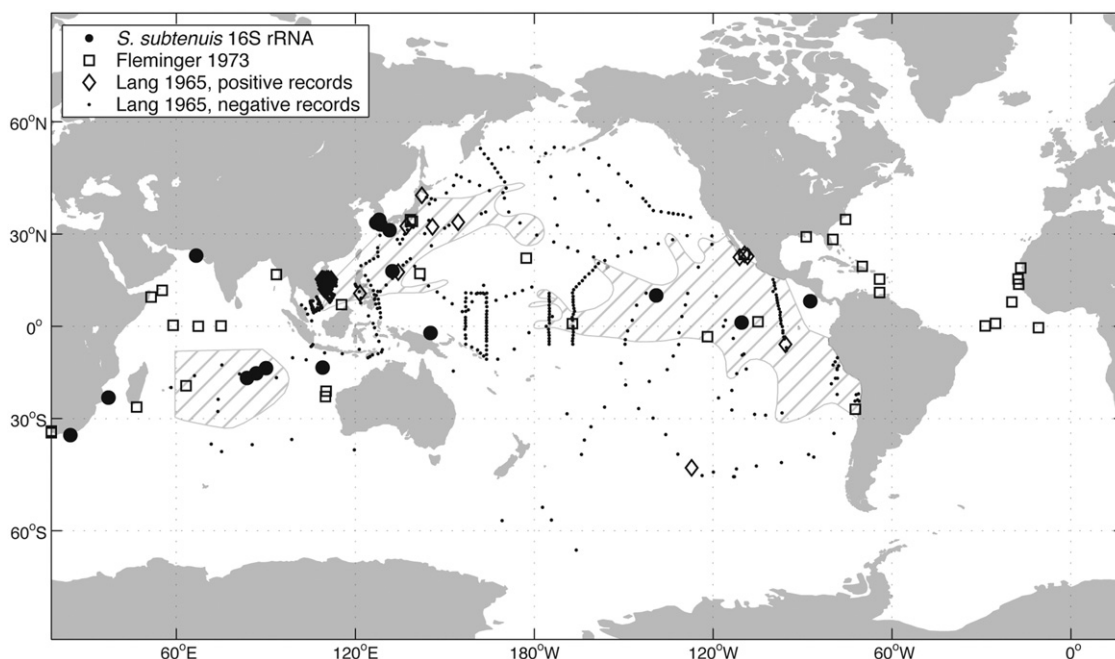


Fig. 2. Biogeographic distribution of *Subeucalanus subtenuis*, a circumglobal species in subtropical and tropical waters. Solid circles mark sampling locations of specimens whose species identity was verified by DNA sequencing of 16S rRNA. The species distribution from Lang (1965) is outlined with hatched lines. Records from morphological studies by Lang (1965) and Fleming (1973) are marked as in the legend. Note: The 'positive' records plotted from Lang (1965) are a subset of her original material (see Materials and Methods for details).

Table 2
Phylogeography of *Subeucalanus subtenuis*. (A) Population samples and summary statistics of mtDNA genetic diversity. (B) Pairwise Φ_{ST} estimates between population samples in A. **Bold** numbers indicate significant values ($\alpha=0.05$). DNA sequences from an additional 23 individuals not included. Column headings for this and all subsequent tables: N=number of individuals included in the genetic survey (16S rRNA), H=number of haplotypes observed.

(A)	Population samples	N	H	H/N	haplotype diversity (<i>h</i>)	nucleotide diversity (π)
a	ETP-M0096	23	22	0.96	0.974	0.014
b	ETP-M0077	20	19	0.95	0.969	0.017
c	ETP-M0051	19	19	1.00	0.973	0.015
d	Korea	21	21	1.00	0.976	0.014
e	WTP-COOK11	14	14	1.00	0.957	0.018
	Total	97				
(B)	Pairwise Φ_{ST} estimates between samples					
	a	b	c	d	e	
a	0.000					
b	-0.009	0.000				
c	0.009	0.004	0.000			
d	0.006	-0.010	0.014	0.000		
e	0.069	0.057	0.089	0.039	0.000	

(Table 2). Although pairwise Φ_{ST} estimates between the western and eastern tropical Pacific samples were statistically significant, they included the sample with the smallest sample size ($N=14$, Table 2A, B). No significant differentiation was observed between samples from Korean waters and other population samples. Exact tests for genetic differentiation were non-significant in all cases. These results confirm Fleming's (1973) report of an absence of significant population structure within ocean basins for this species, based on an analysis of population variability in integumental pores on the exoskeleton. However, Fleming did find significant differentiation between ocean basins in the total number of perforations in the dorsal integument, with the Indian Ocean population intermediate between the Pacific and Atlantic populations. More complete genetic sampling, in the Indian Ocean in particular, would be required to test the hypothesis of genetic structure between ocean basins.

Subeucalanus subtenius is a circumglobal species inhabiting subtropical and tropical waters of the Indian, Pacific, and Atlantic Oceans. Given genetic cohesiveness of the morphological species, prior distribution records by Fleming (1973) and Lang (1965) are confirmed as accurate (Fig. 2). *Subeucalanus subtenius* is abundant in equatorial and tropical waters of the eastern Pacific Ocean (e.g., Lang, 1965; Grice, 1962; Chen, 1986), and Lang reports this eastern tropical Pacific (ETP) population as discontinuous from the population in the western Pacific. New records from this study in the ETP, Kuroshio Current, and East China and Yellow Seas are within the distributional range reported by Lang (Fig. 2). The tropical western Pacific sample (2.24 °S 145.22 °E) with *S. subtenius* is in a region that Lang reports as being dominated by the sister species *S. mucronatus* (Appendix A). New records from the eastern, central and southwestern Indian Ocean complement the sample coverage of Fleming (1973), and in combination, suggest that *S. subtenius* is present throughout the Indian Ocean, occurring over a wide range of oceanographic environments (Fig. 2, Appendix A). The absence of genetic data from the North Atlantic was due to a lack of specimens in our material (June cruise, subtropical North Atlantic, Fig. 1); Lang also notes that this species is rarely recorded in the North Atlantic and

may occur in low abundance there. See Lang (1965) for additional comments on early distribution records of this species in the Indian and Pacific Oceans (Cleve, 1901; Wolfenden, 1905; Carl, 1907; Scott, 1909; Sewell and British Museum (Natural History), 1947; Vervoort, 1949; Wilson, 1950). Early records may be confused with the congeneric species *S. mucronatus*, in part due to Giesbrecht's (1893) original description of *S. subtenius* as having two setae on the second segment of the basipod of the mandible (Md B₂), while three setae is the normal state for it.

Subeucalanus mucronatus is also a valid species, not found to contain distinct genetic lineages that are likely cryptic species. A genetic survey of 86 individuals collected at 12 sites throughout its distributional range identified a total of 23 16S rRNA haplotypes (Figs. 3 and 4). Genetic distance among *S. mucronatus* sequences was 0.8% on average, and ranged up to a maximum of 3% (K2P-corrected distance). Two mitochondrial clades in *S. mucronatus* were supported by bootstrap values of 97 – 99% (NJ/MP, Fig. 4), but were separated by a relatively short branch. Clade A contains haplotypes found throughout the range of the species. Clade B haplotypes were sampled at five sites across the Indian Ocean from the Agulhas region to the Sulu Sea, but were not found in the western Pacific. These two mitochondrial clades were also present in the strict consensus tree of the most parsimonious trees found for these data (8 parsimony-informative sites in the alignment). Given the relatively small genetic distance between clades (3%, K2P), they are interpreted as intraspecific mitochondrial clades rather than likely cryptic species. A comparison of five samples from the western Pacific and Indian Ocean found preliminary evidence for population genetic structure in this species (Table 3). Eight out of 15 pairwise Φ_{ST} comparisons were significant, despite relatively low haplotype and nucleotide diversities (Table 3). Few haplotypes were found in each sample (Table 3), in contrast to *S. subtenius*, and differentiation was largely due to changes in frequency of the most common haplotype, H9, as well as the restricted geographic distribution of clade B. Exact tests of genetic differentiation among samples were highly significant (overall, $P < 0.001$), with 6 of 15 pairwise comparisons also significant.

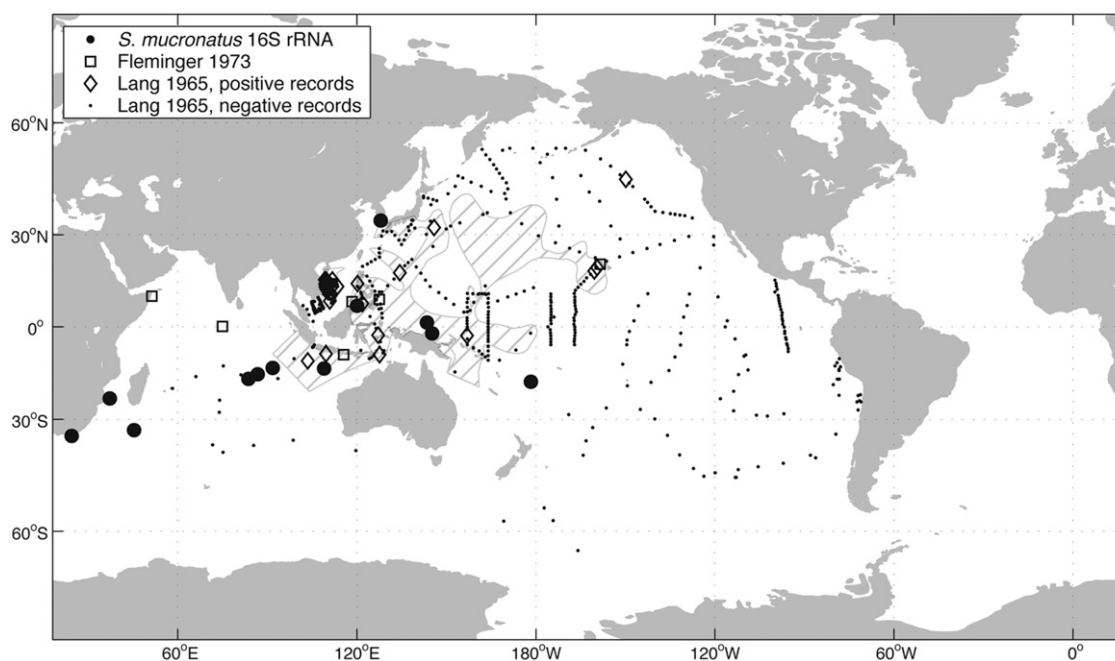


Fig. 3. Biogeographic distribution of *Subeucalanus mucronatus*, an Indo-West Pacific species. Solid circles mark sampling locations of specimens whose species identity was verified by DNA sequencing of 16S rRNA. Symbols and lines as in Fig. 2. Lang's 'positive' records as described in Fig. 2 and Materials and Methods.

Subeucalanus mucronatus is an Indo-West Pacific species, occurring in subtropical and tropical waters of the western Pacific and Indian Oceans (Fig. 3). In this study, the species was not found east of 178.28 °E in the Pacific Ocean (Appendix A), although Lang records it as far east as the Hawaiian Islands. New records in the Pacific Ocean fall largely within the reported range of the species (Fig. 3). In contrast, new records from the central and western Indian Ocean extend the species distribution considerably farther south and west than reported in prior work. *Subeucalanus mucronatus* was sampled in the Agulhas Current region and western Indian Ocean subgyre, in addition to oligotrophic waters in the central Indian Ocean at 83.68 – 91.77 °E (Appendix A). This species may occur throughout much of the Indian Ocean rather than having a center of abundance in the Malay Archipelago with

extensions into the northern Indian Ocean and Kuroshio Current region, as suggested by Lang (1965). Lang (1965) reports the bathymetric distribution of this species as being primarily below 200 m, with congener *S. subtenuis* occurring in the upper 150 m in areas where they co-occur.

3.1.2. The 'crassus' species group

Subeucalanus crassus is a species complex containing four distinct ESUs (Goetze, 2003, 2010). Two lineages are sister clades that likely represent Atlantic and Pacific populations of *S. crassus* s. s. (Fig. 5). The remaining two lineages are highly divergent genetically and are putative new species (Fig. 6, see below; Goetze, 2003, 2010). The morphological and genetic variability contained in *S. crassus* s. l. is inadequately described in this or prior work, as the species is difficult to sample consistently due to its patchy distribution (Lang, 1965). The taxonomic complexity within *S. crassus* s. l. is not entirely unexpected; Lang (1965) reported individual variability in the presence of fine spinules over the dorsal surface of the body ('hairy' vs 'non-hairy'), as well as in body shape. Bradford-Grieve (1994) also noted that her South Pacific specimens were covered in fine spinules, although Farran (1929) described specimens as having a smooth integument. The type locality of *S. crassus* s. l. is ambiguous, as Giesbrecht (1888, 1893) reports this species from both the Pacific and Atlantic Oceans in his original description (west of South America between 14 – 26° S, and further 175° W – 138° E, 19 – 20° N; 38° W 20° S; Rio de Janeiro; Pisco). Subsequent taxonomic revision will require fixing the name-bearing lineage, *S. crassus* s. s., as well as identifying a type locality for this species.

Subeucalanus crassus s. l. was described as a subtropical-tropical circumglobal species in eutrophic neritic waters (Fleminger, 1973). Although current sampling is insufficient to accurately map the distribution of each *S. crassus* ESU, it appears unlikely that *S. crassus* s. s. has such a broad distribution. The Atlantic *S. crassus* lineage was sampled both near Plymouth, U.K. and in the Agulhas region (Fig. 5), suggesting that it may well be present throughout the Atlantic Ocean. The Pacific *S. crassus* lineage was sampled only in the subtropical South Pacific (Fig. 5). Prior distribution records for *S. crassus* from Fleminger and Lang cannot be accurately ascribed to any of the ESUs within *S. crassus* s. l., and it is unknown how eastern and central Pacific populations observed by Lang (1965) relate to the genetic lineages sampled by us.

Subeucalanus sp. and *Subeucalanus* sp. 2 are putative cryptic species within *S. crassus* s. l. (Fig. 6, Goetze, 2010). Fig. 6 illustrates

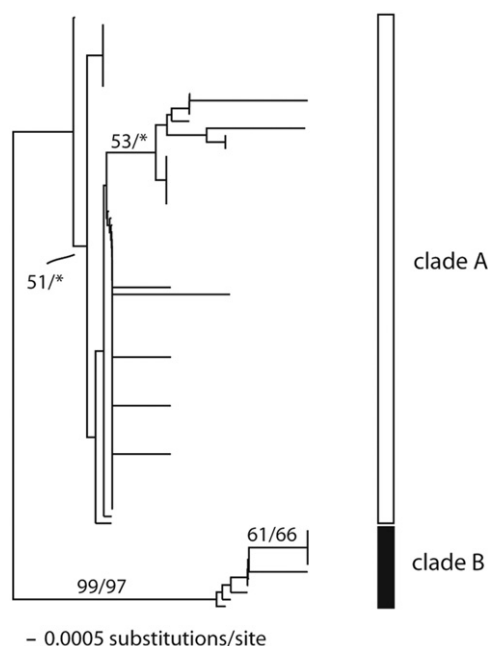


Fig. 4. Unrooted neighbor-joining gene tree of all *S. mucronatus* 16S rRNA sequences, with clades A and B as marked. Bootstrap support values are listed by each node from neighbor-joining (NJ) and parsimony (MP) analyses in the format NJ/MP. Genetic distances used in neighbor-joining analyses were corrected for multiple substitutions with the Kimura-2-parameter model. Clade A haplotypes were sampled throughout the species range; clade B haplotypes occurred in the Indian Ocean between the Agulhas region and the Sulu Sea.

Table 3
Phylogeography of *Subeucalanus mucronatus*. (A) Population samples and summary statistics of mtDNA genetic diversity. (B) Pairwise Φ_{ST} estimates between population samples in A. **Bold** numbers indicate significant values ($\alpha=0.05$). DNA sequences from an additional 18 individuals not included.

(A)	Population samples	N	H	H/N	haplotype diversity (h)	nucleotide diversity (π)
a	S-WTP	13	3	0.23	0.492	0.000
b	N-WTP1	14	3	0.21	0.455	0.002
c	N-WTP2	18	6	0.33	0.610	0.003
d	CW-IND	15	5	0.33	0.728	0.012
e	SW-IND	8	6	0.75	0.833	0.010
	Total	68				
(B)	Pairwise Φ_{ST} estimates between samples					
	a	b	c	d	e	
a	0.000					
b	0.263	0.000				
c	0.107	-0.018	0.000			
d	0.478	0.490	0.497	0.000		
e	0.117	0.062	0.104	0.230	0.000	

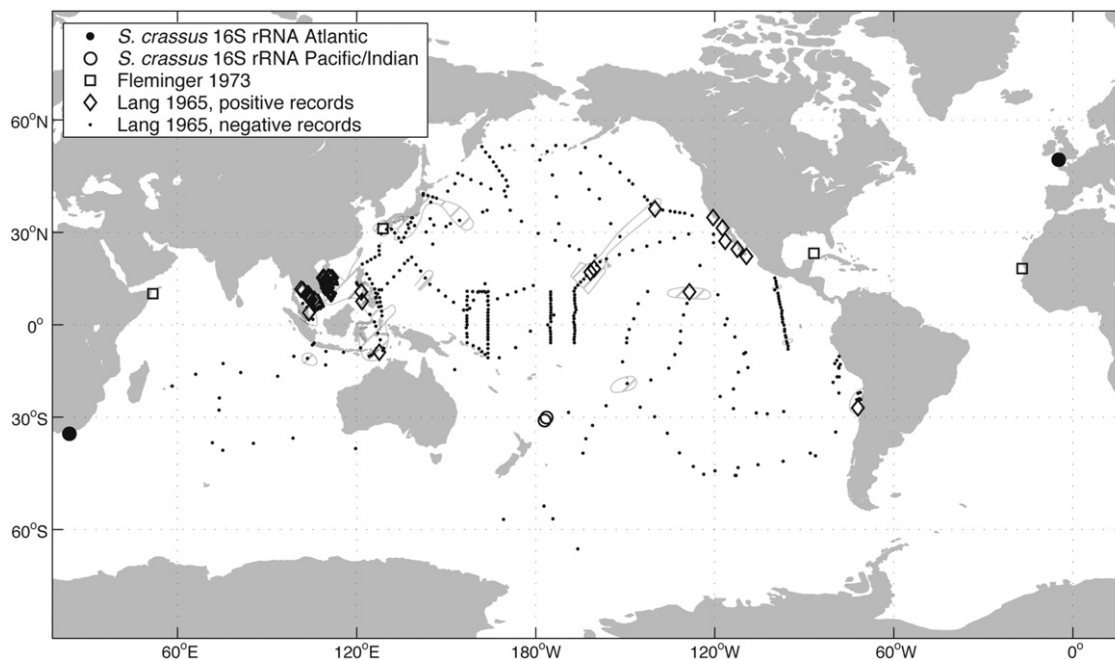


Fig. 5. Biogeographic distribution records for *Subeucalanus crassus* (Atl) and *Subeucalanus crassus* (Pac), two distinct genetic lineages within the nominal species *Subeucalanus crassus* s.l. *Subeucalanus crassus* was described by Fleminger (1973) as a subtropical-tropical, circumglobal species in eutrophic or broadly neritic waters. Current sampling of new genetic lineages is inadequate to describe the geographic distribution of these distinct taxa. Solid and open circles mark sampling locations of specimens whose species identity was verified to be *S. crassus* (Atl) or *S. crassus* (Pac), respectively, by DNA sequencing of 16S rRNA. Other symbols and lines as in Fig. 2. Lang's 'positive' records as described in Fig. 2 and Materials and Methods.

all that is currently known regarding the biogeographic distributions of these distinct species, and includes only distributional records from the genetic survey. A total of 30 individuals of *S. sp* were sampled at five sites throughout the Indian Ocean and western Pacific (Fig. 6A). The species appears to have an Indo-West Pacific distribution. Based in part on this limited biogeographic information, we hypothesize that *S. sp.* may correspond to *Eucalanus oculanus* Marukawa, 1921. This species was described on the basis of material collected at 37° 35' N, 144° 40' E in Japanese waters (Marukawa, 1921), but was subsequently considered synonymous with *S. crassus* and the name is not in active use (Tanaka, 1956; Mori, 1937; Vervoort, 1949). *Subeucalanus sp.* is the only *S. crassus* s.l. genetic lineage sampled in the region near the type locality for *E. oculanus*. Four specimens of *Subeucalanus sp. 2* were sampled from a single location in the Agulhas region. This species may be rare, or it may have a geographically restricted distribution in the Agulhas-Benguela region off southern Africa.

3.1.3. The 'pileatus' species group

Study of this group was focused on the widespread species *S. pileatus* and *S. subcrassus*, with additional genetic results for *S. flemingeri* reported in Goetze (2010). The only remaining described species, *Subeucalanus dentatus*, is restricted to coastal waters of Austral-Asian seas, and no specimens were found in our material.

Accurate morphological discrimination of *S. pileatus* and *S. subcrassus* in the absence of integumental pore information was difficult, if not impossible, in many ocean regions. Lang (1965) described morphological differences between *S. pileatus* and *S. subcrassus*. These differences include the shape of the anterior portion of the forehead, the presence/absence of a marked dorsal constriction between the insertion of the antenna and mandible, and in the extent to which the genital double somite is broad and onion-shaped. However, Lang also noted the presence of two distinct forms within *S. subcrassus*, 'small' and

'large', that broadly intergrade in these traits between *S. pileatus* and *S. subcrassus*. Bradford-Grieve (1994) and Vervoort (1963) also noted variability in head shape among specimens identified as *S. pileatus*. In this study, a genetically distinct cryptic species, *S. sp 3*, was found within the 'pileatus' species group and is thought to be morphologically intermediate between *S. subcrassus* and *S. pileatus* in head and genital segment shape characters (Fig. 6C). This makes accurate identification using these non-integumental pore characters tenuous at best. Species identification by DNA sequence placement in the phylogeny was required for areas where *S. sp 3* occurs in sympatry with *S. subcrassus* and/or *S. pileatus* (Indian Ocean). It is unknown whether the cryptic species *S. sp 3* shares the integumental pore pattern of *S. pileatus* or *S. subcrassus*, as genetic and integumental pore analyses were completed on separate specimens (the entire specimen was required for DNA extraction). Below, we discuss results for the genetic species *S. subcrassus*, *S. pileatus*, and *S. sp 3*.

The molecular survey sampled 96 individuals of *S. subcrassus* from 12 sites throughout the species range in the Indian and Pacific Oceans (Fig. 7). A total of 24 16S rRNA haplotypes were identified. The genetic distance between sequences was 0.6% on average, and ranged up to a maximum of 2.1% between the most divergent haplotypes (K2P-corrected distance). Gene trees of *S. subcrassus* sequences contained a number of clades with low to moderate bootstrap support (8 clades, 50 – 86% NJ bootstrap, results not shown). As in *S. mucronatus* above, given low genetic distances among haplotypes, these clades are not likely to be cryptic taxa, but rather intraspecific mitochondrial clades. Significant genetic differentiation was observed among 7 population samples in the Indian Ocean and western Pacific (Table 4). Nineteen out of 21 pairwise Φ_{ST} estimates were significant, and the overall Fisher's exact test for genetic differentiation was also highly significant ($P < 0.001$). These results are preliminary, given the small sample sizes, but they suggest genetic differentiation among populations in distinct areas of the species distribution. Additional study is warranted.

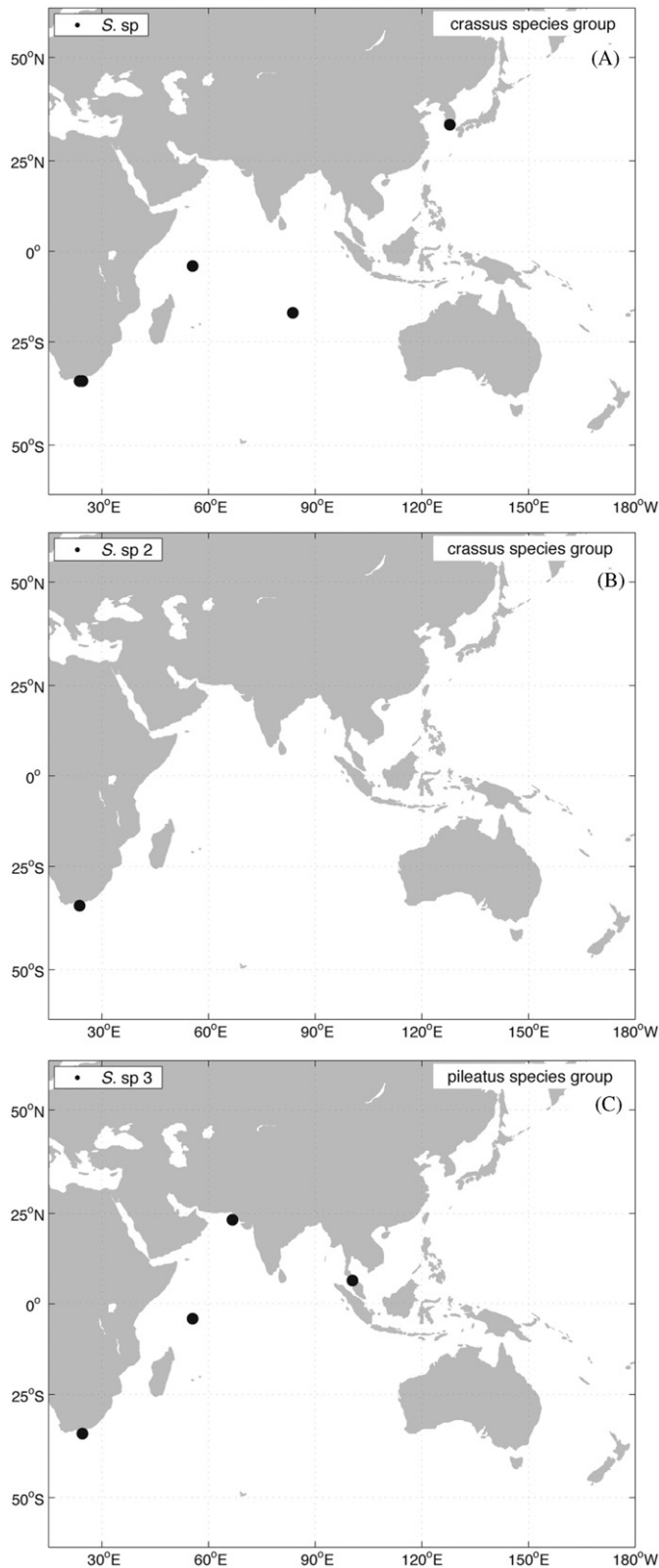


Fig. 6. Biogeographic distribution records for undescribed species in the genus *Subeucalanus*: (A) *S. sp.* in the 'crassus' species group, (B) *S. sp. 2* in the 'crassus' species group, and (C) *S. sp. 3* in the 'pileatus' species group. This figure includes all that is known, or can be inferred from prior records, regarding the distribution of these species.

Subeucalanus subcrassus is an equatorial and tropical species in the Indian and Pacific Oceans. New distribution records from this study fall broadly within the previously described species range in

the eastern tropical Pacific, western tropical Pacific and as far north as the Yellow Sea (Fig. 7; Lang, 1965, Fleminger, 1973). New records in the western and southwestern Indian Ocean extend the species distribution into subtropical waters. These animals may be transported south in the Agulhas Current outside of their normal tropical habitat, as also appears to occur in the Kuroshio region in the North Pacific. Lang (1965) reports *S. subcrassus* as the predominant eucalanid species in shallow waters of the Indo-Pacific region, with the distribution extending into oceanic equatorial waters of the Pacific (Fig. 7). Both the 'small' and 'large' morphological forms were found in coastal waters of the South China Sea, with only the 'large' form occurring in oceanic Pacific waters and in coastal regions of the Americas (Lang, 1965). Given the observed biogeographic distributions of the genetic species *S. subcrassus* and *S. sp. 3* (Figs. 6C and 7), we hypothesize that the 'large' form corresponds to *S. subcrassus* of this study, and the 'small' form likely includes primarily *S. sp. 3* specimens. Following morphological re-description of these distinct species, it may therefore be possible to ascribe some of Lang's records to the correct species based on extant information (e.g., Table 5 Naga cruise S-10 records, Lang, 1965). Distinguishing the correct genetic species for Fleminger's (1973) *S. subcrassus* and *S. pileatus* records will be possible only by re-examination of extant material.

Subeucalanus pileatus consists of two highly divergent ESUs that are identified here as *S. pileatus* (Atlantic) and *S. pileatus* (Pacific/Indian; Figs. 8, 9 and 10, Goetze, 2003, 2010). Giesbrecht (1888, 1893) reports *S. pileatus* s. l. from both the Atlantic and Pacific Oceans (24 – 38 °W, 8 °N – 20 °S, West of South America) in his initial description of this species. Subsequent systematic revision will require fixing a type locality for the name-bearing lineage *S. pileatus* s.s., as the geographic origin of the material illustrated in Giesbrecht (1893) is unclear. The genetic survey sampled 204 individuals from throughout the species circumglobal range (Fig. 8). A total of 27 16S rRNA haplotypes were identified, eight of which were represented by more than one individual (Fig. 9). Haplotypes H20 – H22 were found exclusively in the tropical Atlantic, and haplotypes H15 – H19 were restricted to the eastern tropical Pacific (Figs. 9 and 10). The remaining haplotypes were sampled in the Indian and western Pacific Oceans (Fig. 10). Significant population genetic structure was observed among 12 sampling sites (exact test, $P < 0.001$, Table 5). Pairwise Φ_{ST} estimates were also significant in 40 out of 66 cases (Table 5), and indicated strong genetic differentiation of eastern tropical Pacific (ETP) and North Atlantic populations, in particular. The tropical Atlantic population is characterized by an endemic, highly divergent haplotype group, and does not share haplotypes with any other populations. The ETP population also consists entirely of a distinct haplotype group (H15 – H19) that is not shared with other regions. However, ETP haplotypes are 5 – 8 mutational steps away from western Pacific and Indian Ocean haplotypes, in contrast to the North Atlantic haplotype group, which differs by 25 – 26 mutational steps. Contemporary gene flow is absent among North Atlantic, ETP, and other *S. pileatus* populations. Five pairwise Φ_{ST} estimates also were significant among western Pacific and Indian Ocean samples, suggesting that there may be mild genetic structure among populations in this region. The Seychelles Islands and western Pacific samples, in particular, had a higher fraction of singleton haplotypes relative to other samples in the Indian Ocean (Fig. 10). Indian Ocean and western Pacific populations were dominated by the most common haplotype H1, with H3 occurring in lower frequency, as well as a number of additional singleton haplotypes that were 1–2 mutational steps away from these two dominant haplotypes (Figs. 9 and 10).

Subeucalanus pileatus s. l. was described as a circumglobal coastal-neritic species in tropical and subtropical waters (Fleminger, 1973).

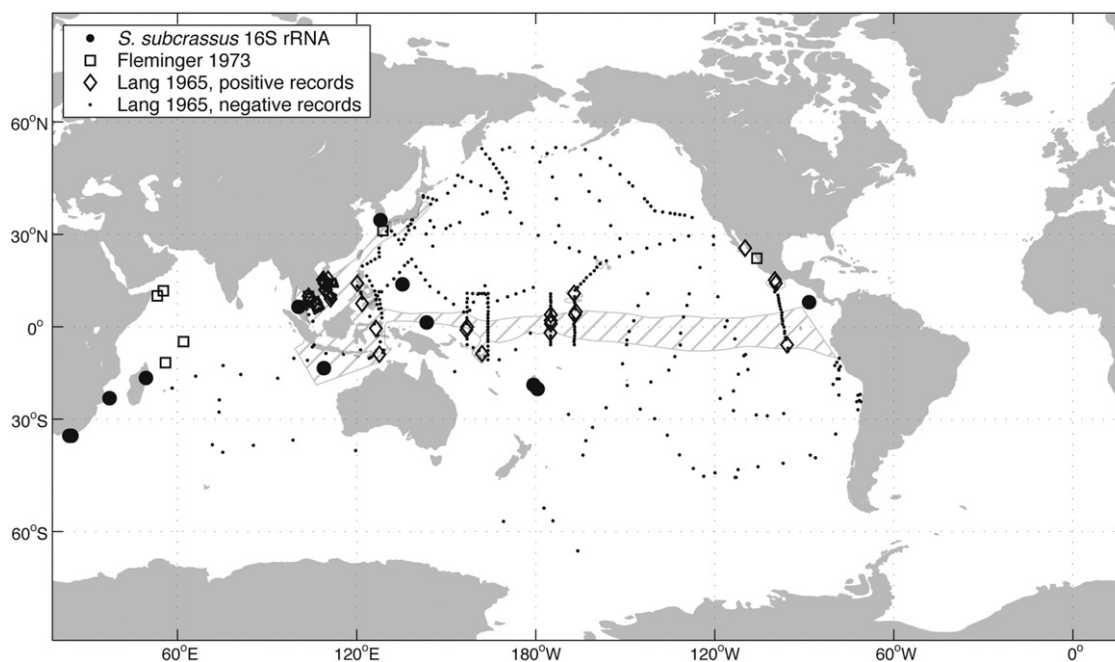


Fig. 7. Biogeographic distribution of *Subeucalanus subcrassus*, an equatorial species in the Pacific and Indian Oceans. Solid circles mark sampling locations of specimens whose species identity was verified by DNA sequencing of 16S rRNA. Symbols and lines as in Fig. 2. Lang's 'positive' records as described in Fig. 2 and Materials and Methods.

Table 4

Phylogeography of *Subeucalanus subcrassus*. (A) Population samples and summary statistics of mtDNA genetic diversity. (B) Pairwise Φ_{ST} estimates between population samples in A. **Bold** numbers indicate significant values ($\alpha=0.05$). DNA sequences from an additional 11 individuals not included.

(A)	Population samples	N	H	H/N	haplotype diversity (<i>h</i>)	nucleotide diversity (π)	
a	AGUL	23	5	0.22	0.383	0.001	
b	W-IND1	9	3	0.33	0.392	0.002	
c	SEYCHEL	14	6	0.43	0.772	0.012	
d	E-IND	15	1	0.07	0.000	0.000	
e	MALAYSIA	8	7	0.88	0.900	0.006	
f	WTP-1	8	3	0.38	0.567	0.004	
g	WTP-2	8	2	0.25	0.233	0.002	
	Total	85					
(B)	Pairwise Φ_{ST} estimates between samples						
	a	b	c	d	e	f	g
a	0.000						
b	0.551	0.000					
c	0.522	0.495	0.000				
d	-0.005	0.729	0.521	0.000			
e	0.791	0.758	0.170	0.840	0.000		
f	0.093	0.439	0.299	0.222	0.583	0.000	
g	0.806	0.823	0.243	0.924	0.465	0.572	0.000

The two *S. pileatus* lineages have distinct biogeographies, with one species endemic to coastal – neritic waters of the Atlantic and a second species in similar habitats of the Pacific and Indian Oceans (Figs. 8 and 10). The Pacific – Indian species also consists of at least two populations, one in the western Pacific and Indian Oceans (classic Indo-West Pacific distribution) and a second in the eastern tropical Pacific (Table 5, Figs. 9 and 10). The absence of gene flow among eastern and western Pacific populations of *S. pileatus* supports the inference of a distribution tightly linked to coastal waters. In contrast, the congener *S. subcrassus* shares haplotypes among eastern and western Pacific populations, and gene flow may occur through oceanic equatorial waters in the Pacific (compare Figs. 7 and 8). The distribution of *S. pileatus* in the North Atlantic has been poorly characterized; new records from

this study extend the distribution into coastal waters of South America (Fig. 8). The ETP, western tropical Pacific, and Strait of Malacca records are within the known distribution of the species. Samples containing *S. pileatus* from the Mozambique Channel and Agulhas region illustrate that the distribution extends south into subtropical waters in the western Indian Ocean, beyond prior records for this species (Fig. 8). Given genetic and biogeographic results, *S. pileatus* populations are expected to be contiguous throughout coastal regions of the Indian Ocean and western Pacific.

Subeucalanus sp. 3 is an Indo-West Pacific coastal – neritic species in subtropical and tropical waters. All that is known regarding the distribution of this undescribed, cryptic species is presented in Fig. 6C. It was sampled at six sites throughout the Indian Ocean, from the Agulhas region to the Malay Archipelago.

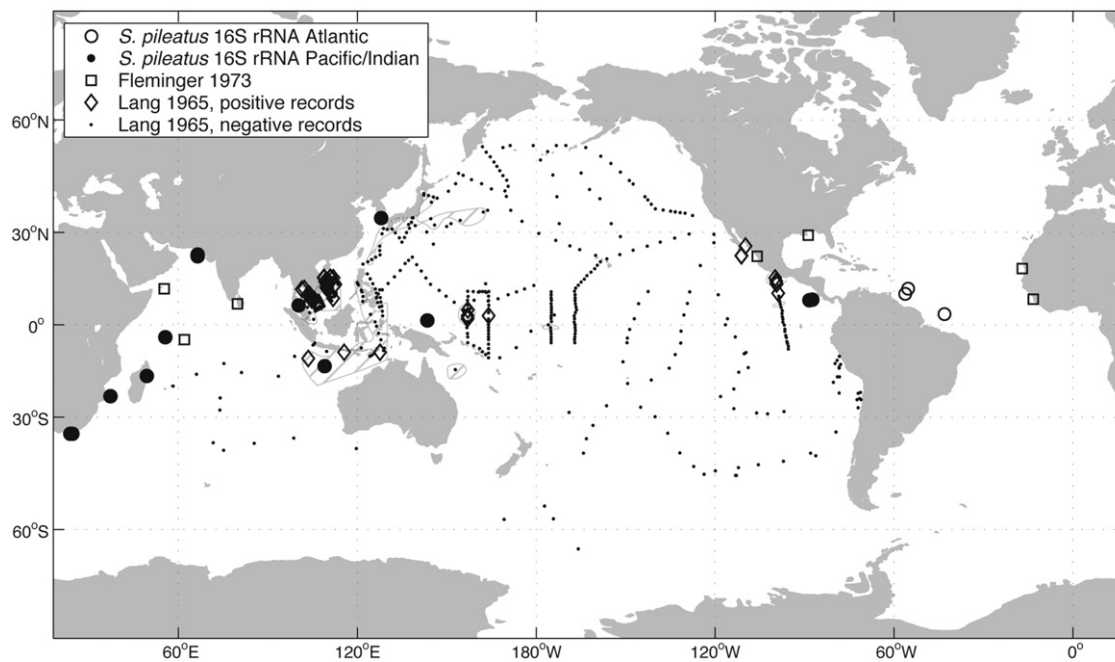


Fig. 8. Biogeographic distribution of *Subeucalanus pileatus*, a circumglobal species in eutrophic or broadly neritic waters. This nominal species contains two distinct genetic lineages. Solid and open circles mark sampling locations of specimens whose species identity was verified to be *S. pileatus* (Atl) or *S. pileatus* (Pac/Ind), respectively, by DNA sequencing of 16S rRNA. Other symbols as in Fig. 2. Lang's 'positive' records as described in Fig. 2 and Materials and Methods. *Subeucalanus pileatus* is under-sampled in the Atlantic Ocean in this and prior studies.

As described above, we hypothesize that this genetic species corresponds to Lang's 'small' form of *S. subcrassus*, and may be present and relatively abundant in coastal waters of Southeast Asia. Lang reports that the small and large forms of *S. subcrassus* occur in approximately equal abundance in the South China Sea, with *S. pileatus* being quite rare. Additional description of this species' distribution awaits morphological characterization of the distinct species.

3.2. *Pareucalanus*

This genus consists of five described species and one additional genetic ESU that is a putative new species (*P. sp.*). We focused on the broadly distributed species *P. attenuatus* and *P. sewelli*, with a small amount of new data reported on *P. langae* and *P. parki*. The genus includes species that are widespread in subtropical and tropical waters, as well as transition zone species in northern and southern hemispheres.

Pareucalanus attenuatus is a valid species, and does not contain multiple genetic lineages. The genetic survey sampled 98 individuals collected at 20 locations throughout the species distribution in Indian and Pacific Oceans (Fig. 11). This species is relatively abundant where it occurs, and can be sampled consistently throughout its range. Genetic diversity in *P. attenuatus* was low, with a total of nine 16S rRNA haplotypes identified. Four haplotypes were observed in more than a single individual, and 82 individuals shared the most common haplotype (results not shown). Maximum sequence divergence among *P. attenuatus* haplotypes was 1.1 % (K2P), with an overall average for all sequences of 0.038 %. The most common haplotype was sampled at all 20 sites. Little intraspecific clade structure was observed in phylogenetic analyses; three haplotype pairs or groups separated by short branches were supported with low to moderate bootstrap support (50–65%, NJ).

Pareucalanus attenuatus is an Indo-Pacific species in tropical and subtropical waters (Fig. 11). New distribution records from this study complement Fleming's (1973) sample coverage and

extend the range into subtropical waters in both the Pacific and Indian Oceans. In the Pacific Ocean, the species was found in equatorial waters of the ETP, the oligotrophic North Pacific central gyre, and tropical waters of the western Pacific, with extensions north into the Kuroshio region (Fig. 11, Appendix A). The species was present throughout diverse oceanographic environments in the Indian Ocean, including the Agulhas current region, Mozambique Channel, Arabian Sea, and oligotrophic central and eastern tropical waters (Fig. 11).

Pareucalanus sewelli s. l. consists of two ESUs (Fig. 12; Goetze, 2003, 2010). The type locality for *P. sewelli* (38° 46' S 83° 20' W) is in the SE Pacific (Fleming, 1973), suggesting that *P. sewelli* (Pacific) should be the name bearing lineage, *P. sewelli* s. s., in the case of systematic revision. The genetic survey sampled 41 individuals of *P. sewelli* s. l. from 15 locations throughout the circumglobal distribution. Twenty-five of these were of the *P. sewelli* (Atlantic-Indian) lineage and 16 were of *P. sewelli* (Pacific). Within the *P. sewelli* (Atlantic-Indian) lineage, genetic distance between DNA sequences averaged 0.3%, up to a maximum of 1.5% (K2P). All *P. sewelli* (Pacific) individuals were of the same haplotype. No intraspecific clades were present within either lineage, and based on current sampling there do not appear to be additional populations that are genetically distinct.

Pareucalanus sewelli s. l. has been previously described as a circumglobal species in subtropical – tropical waters (Fleming, 1973). As in *S. pileatus*, the two *P. sewelli* lineages appear to have distinct biogeographies, with *P. sewelli* (Atlantic-Indian) present in the Atlantic and Indian Oceans and *P. sewelli* (Pacific) restricted to the Pacific Ocean. This Atlantic-Indian distribution is relatively uncommon, but is observed in a few other holoplanktonic species (e.g., *Phronima solitaria*, *Desmopterus papilio*; van der Spoel and Heyman, 1983). *Pareucalanus sewelli* (Atlantic – Indian) was sampled at five sites in the subtropical – tropical North Atlantic and Mediterranean Sea, ranging from the Aegean Sea to coastal waters of South America (Fig. 12, Appendix A). Given the genetic similarity of North Atlantic and Indian Ocean populations, the South Atlantic population is also expected to correspond to this

genetic lineage. *Pareucalanus sewelli* s. l. has been recorded at sites as far south as 29.11 °S 40.60 °W in the South Atlantic (Fig. 12; Fleminger, 1973). In the Indian Ocean, *P. sewelli* (Atlantic-Indian) was present in the Agulhas Current region, the oligotrophic central gyre, and eastern tropical waters (Fig. 12). This genetic lineage was also sampled in the Yellow Sea (34.25 °N 127.90 °E), indicating that the distribution extends into the Indo-West

Pacific. The two *P. sewelli* s. l. lineages may overlap in distribution in Austral-Asian Seas, as *P. sewelli* (Pacific) was sampled as far west as the Sulu Sea. *Pareucalanus sewelli* (Pacific) was also sampled in the equatorial ETP, subtropical waters of the South Pacific and the Gulf of California, and it is likely present in eutrophic waters throughout the Pacific Ocean.

Pareucalanus sp. is an undescribed species with a circumglobal distribution in subtropical – tropical waters of Atlantic, Indian, and Pacific Oceans (Fig. 13). This species is highly divergent genetically from other *Pareucalanus* taxa (Goetze, 2003, 2010). Although we initially hypothesized that *P. sp.* could be synonymous with the recently described *P. smithae*, we confirm here that these are distinct species following examination of integumental pore patterns of genotyped specimens. Thirteen specimens of *P. sp.* from Indian and Pacific Oceans were examined for integumental pore number and distribution on the posterior prosome and urosome, with DNA sequence data obtained from tissue of the anterior prosome. None had 6 pores on the dorsal surface of the anal segment, as described for *P. smithae* by Prusova (2007). The majority had a total of 5 pores, as described for *P. sewelli* (Fleminger, 1973), with 2 on the anterior margin of the segment and 3 distal of this pair, above the caudal furca. A few specimens had 4 or 3 pores, with pores absent in sites noted to be variable by Fleminger (1973). This observation suggests that either *P. sp.* is an additional undescribed species, or the integumental pore characters described to differ between *P. smithae* and *P. sewelli* are not reliably distinct throughout the biogeographic range.

A total of 34 individuals of *P. sp.* were sampled from 13 sites throughout the global distribution (Fig. 13). This species was not particularly abundant in our material, but was consistently present in samples from diverse oceanographic regions. Gene trees of *P. sp.* sequences included two haplotype clades supported by bootstrap values of 86% and 90% in NJ and MP analyses, respectively. These two clades were present in the strict consensus tree of the 10 most parsimonious trees for *P. sp.* sequences. One clade contained haplotypes collected in Indian and western Pacific Ocean samples; a second clade contained

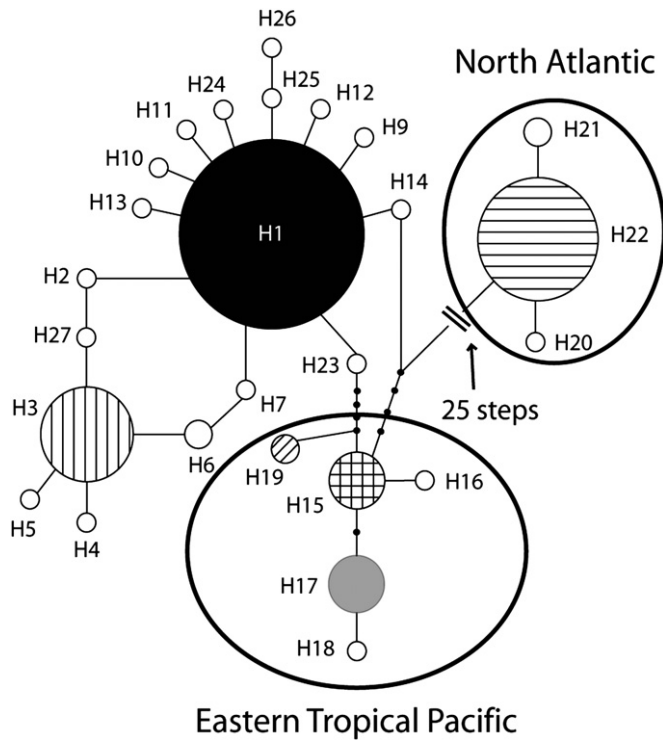


Fig. 9. Statistical parsimony haplotype network for *Subeucalanus pileatus*. Shading and pattern as in Fig. 10; Population samples as listed in Table 5.

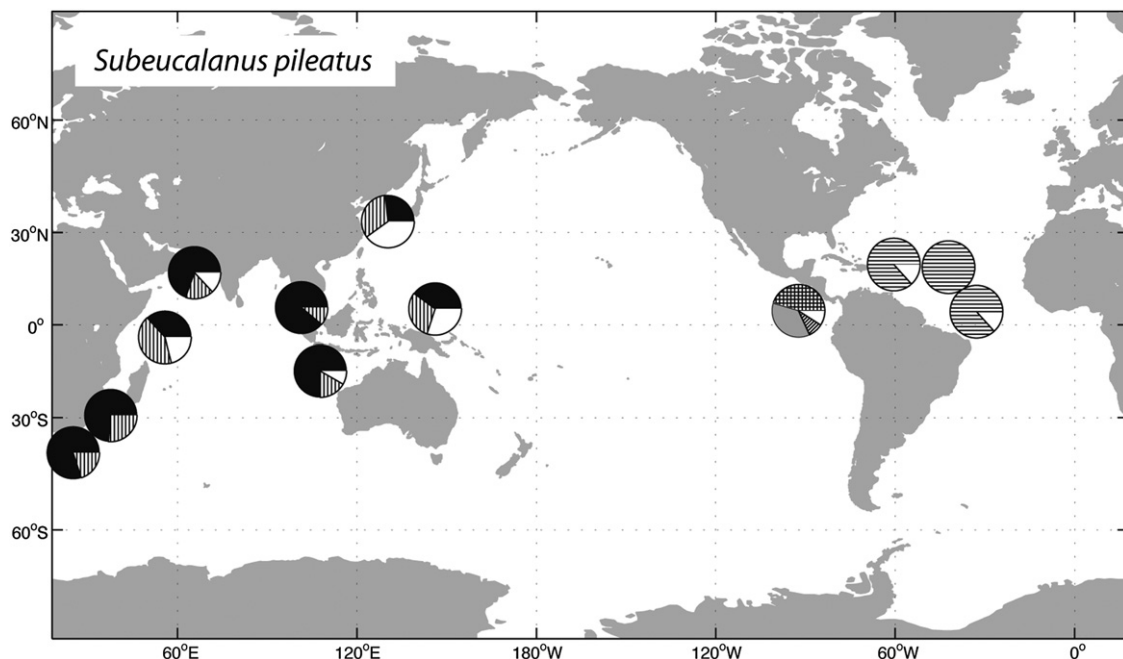


Fig. 10. Frequencies of 16S rRNA haplotypes in *Subeucalanus pileatus* population samples from the Indian, Pacific and Atlantic Oceans, with shading and pattern as in Fig. 9. Pairwise Φ_{ST} estimates and population summary statistics in Table 5.

Table 5
Phylogeography of *Subeocalanus pileatus*. (A) Population samples and summary statistics of mtDNA genetic diversity. (B) Pairwise Φ_{ST} estimates between population samples in A. **Bold** numbers indicate significant values ($\alpha=0.05$).

(A)	Population samples	N	H	H/N	haplotype diversity (h)	nucleotide diversity (π)	Ocean Basin
a	Agulhas region	15	2	0.13	0.331	0.001	Indian
b	Mozambique Channel	19	2	0.11	0.398	0.002	Indian
c	Seychelles Is.	19	6	0.32	0.694	0.004	Indian
d	Arabian Sea	23	5	0.22	0.491	0.002	Indian
e	Malaysia	18	2	0.11	0.203	0.001	Indo-west Pacific
f	Eastern Indian Ocean	14	3	0.21	0.418	0.002	Indian
g	Korea	15	8	0.53	0.818	0.005	Pacific
h	Western tropical Pacific	10	5	0.50	0.758	0.004	Pacific
i	Eastern tropical Pacific	22	5	0.23	0.664	0.006	Pacific
j	North Atlantic 1	15	3	0.20	0.248	0.000	Atlantic
k	North Atlantic 2	19	1	0.05	0.000	0.000	Atlantic
l	North Atlantic 3	15	2	0.13	0.239	0.001	Atlantic
	Total	204					

(B)	Pairwise Φ_{ST} estimates between samples											
	a	b	c	d	e	f	g	h	i	j	k	l
a	0.000											
b	-0.052	0.000										
c	0.101	0.062	0.000									
d	-0.044	-0.021	0.121	0.000								
e	-0.033	0.020	0.205	-0.027	0.000							
f	-0.058	-0.031	0.111	-0.041	-0.039	0.000						
g	0.022	0.008	-0.008	0.043	0.090	0.024	0.000					
h	0.089	0.051	-0.037	0.099	0.206	0.090	-0.023	0.000				
i	0.856	0.861	0.838	0.859	0.869	0.842	0.805	0.821	0.000			
j	0.992	0.991	0.981	0.987	0.994	0.991	0.975	0.983	0.968	0.000		
k	0.995	0.993	0.985	0.990	0.997	0.994	0.980	0.987	0.972	0.016	0.000	
l	0.990	0.989	0.979	0.986	0.993	0.989	0.973	0.981	0.966	-0.045	0.095	0.000

B. (see legend above).

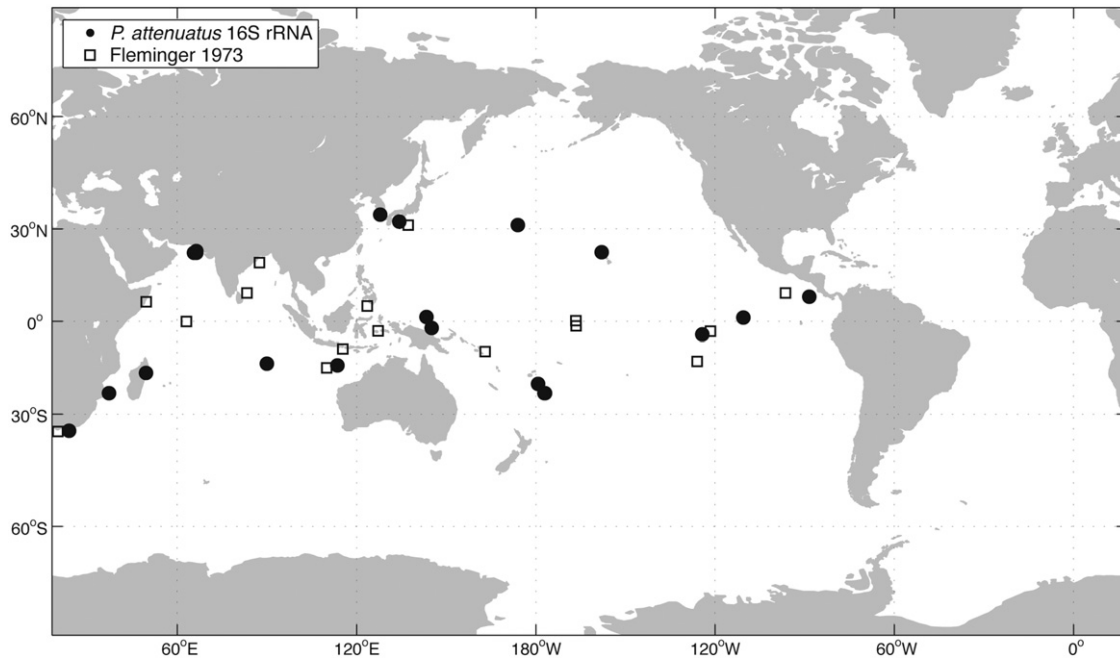


Fig. 11. Biogeographic distribution of *Pareucalanus attenuatus*, a subtropical-tropical species in the Indian and Pacific Oceans. Solid circles mark sampling locations of specimens whose species identity was verified by DNA sequencing of 16S rRNA. Fleming (1973) records of this species are plotted as open squares.

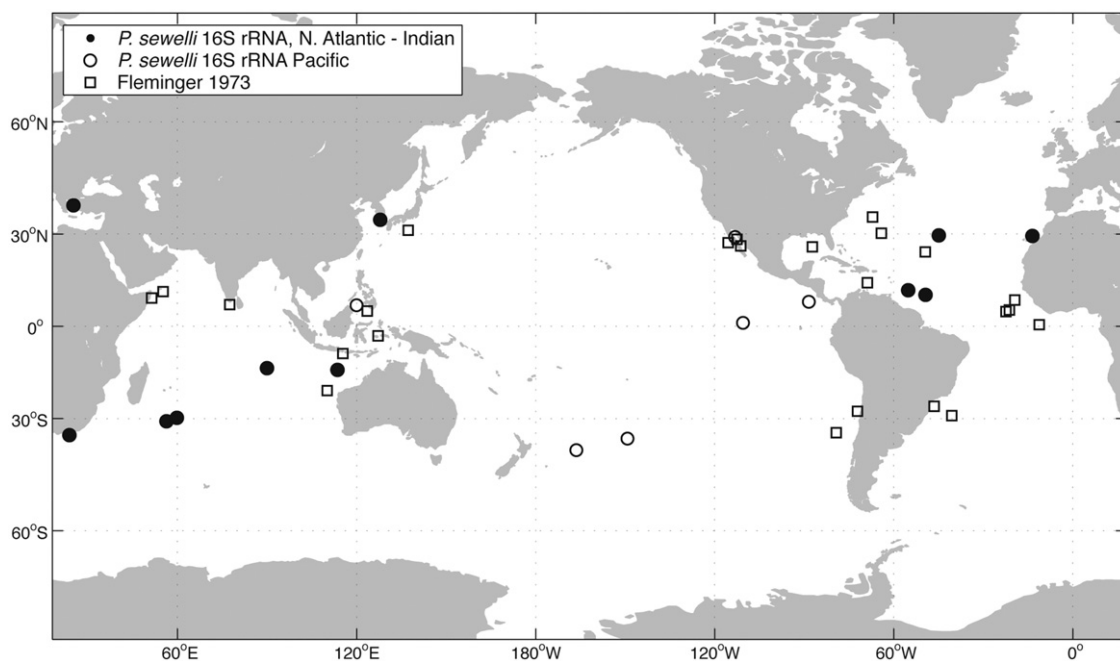


Fig. 12. Biogeographic distribution of *Pareucalanus sewelli*, a circumglobal species in subtropical and tropical waters. This nominal species contains two distinct genetic lineages. Solid and open circles mark sampling locations of specimens whose species identity was verified to be *P. sewelli* (Atl/Ind) or *P. sewelli* (Pac), respectively, by DNA sequencing of 16S rRNA. Fleming (1973) records for *P. sewelli* s. l. are plotted as open squares.

haplotypes from all three ocean basins (Fig. 14). Average genetic distance between all *P. sp.* 16S rRNA sequence pairs was 0.9% (K2P). The maximum observed distance between haplotypes, 3% (K2P), was comparable to that between Atlantic–Indian and Pacific lineages of *P. sewelli*. The taxonomic significance of these clades in *P. sp.* is unclear. *Pareucalanus sp.* was sampled in the tropical-subtropical North Atlantic, throughout the Indian Ocean, and in the western Pacific (Fig. 13). The curious absence of the species in the eastern Pacific may indicate an Atlantic and Indo-West Pacific distribution similar to the Atlantic – Indian lineage of *P. sewelli*. Alternatively, given that the species was relatively

rare in our material, the absence may be due to insufficient sampling.

Pareucalanus langae is a circumglobal transition zone species in the southern hemisphere (Fig. 15). DNA sequence data were obtained from 21 individuals sampled at 9 sites in the Indian and Pacific Oceans. This species is also present in the South Atlantic transition zone, but no suitably-preserved material was available from that region for inclusion in this study. A total of six 16S rRNA haplotypes were observed, with 3 present in more than a single individual. No significant intraspecific clade structure was observed in phylogenetic analyses, and Indian and

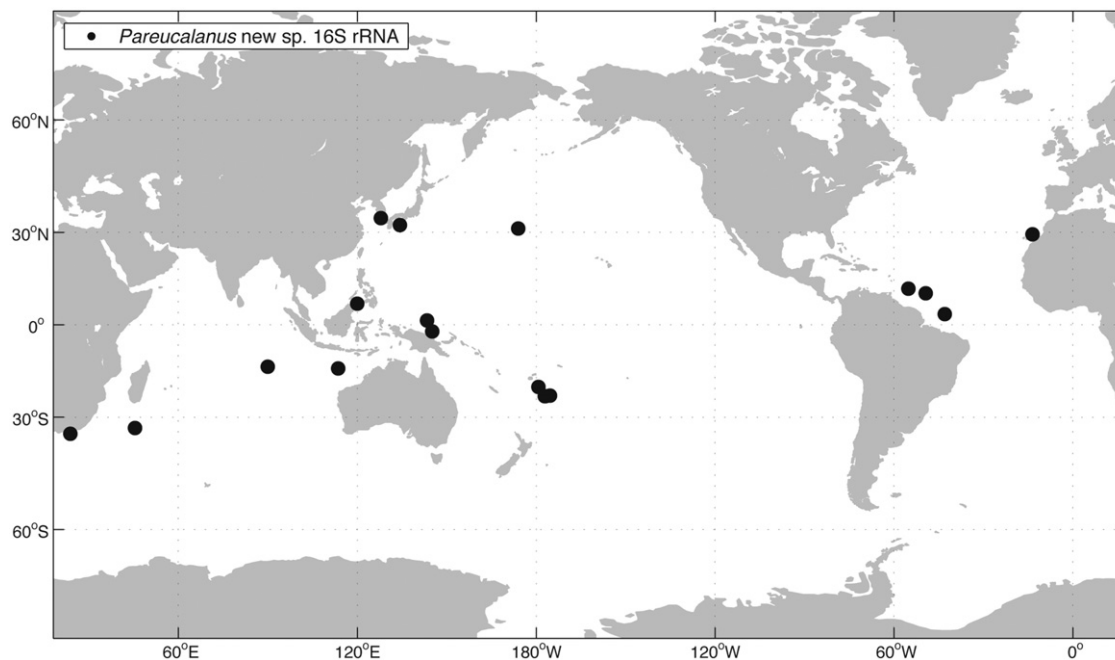


Fig. 13. Biogeographic distribution of the undescribed species *Pareucalanus* sp., a circumglobal species in subtropical and tropical waters. Solid circles mark sampling locations of specimens whose species identity was verified by DNA sequencing of 16S rRNA.

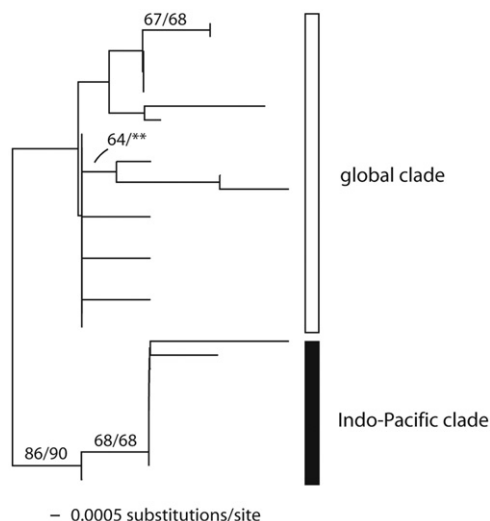


Fig. 14. Neighbor-joining gene tree of all *Pareucalanus* sp. sequences, with bootstrap support for nodes listed as NJ/MP. Global clade haplotypes were sampled in all three Ocean basins; Indo-Pacific clade haplotypes were collected in western Pacific and Indian Oceans. ** indicates this node was not supported in the analysis.

Pacific specimens were not highly divergent genetically. Maximum genetic distance among all haplotypes was 0.7% (K2P, overall average 0.3%). New distribution records augment the sample coverage of [Fleminger \(1973\)](#) and [Lang \(1965\)](#) in temperate latitudes of the western sector of each ocean basin, and confirm the prior inference of a transition zone distribution for this species. A few individuals were sampled as far north as 20.52 °S 179.29 °W in the South Pacific, well within subtropical waters (Appendix A).

Pareucalanus parki is a North Pacific transition zone species ([Fig. 16](#)). DNA sequence data were obtained from eight individuals collected at 6 sites in eastern and western boundary currents of the North Pacific. Four 16S rRNA haplotypes were observed. Specimens collected in eastern and western boundary currents were not highly divergent genetically. New records from this study are from the southern end of the distribution in the

California Current; records from [Fleminger \(1973\)](#) illustrate the oceanic distribution of this species ([Fig. 16](#)).

3.3. *Rhincalanus*

The genus *Rhincalanus* is the only contemporary genus of the Eucalanidae not treated by [Fleminger \(1973\)](#), and it is in need of systematic revision. Of the four described species, *R. nasutus* [Giesbrecht, 1888](#), *R. gigas* [Brady 1883](#), *R. cornutus* [Dana 1849](#), and *R. rostrifrons* [Dana, 1852](#), two, *R. nasutus* and *R. rostrifrons*, have been found to contain multiple ESUs. The genetic survey focused on the widely-distributed subtropical – tropical species *R. nasutus* and *R. rostrifrons*. Taxonomic complexity within *R. nasutus* is not yet fully resolved, and results for this nominal species are not reported here. New biogeographies for Indo-Pacific *R. rostrifrons* s. l. are presented in [Fig. 17](#).

Rhincalanus rostrifrons s. l. contains two reciprocally monophyletic mitochondrial lineages with distinct biogeographic distributions ([Fig. 17](#); [Goetze, 2003, 2010](#)). The genetic survey sampled 164 individuals from 15 sites throughout the Indo-Pacific distribution ([Fig. 17](#)). Of these, 86 specimens were of *R. rostrifrons* (WP-Ind) and 78 were of *R. rostrifrons* (EP). Totals of 16 and 20 haplotypes were observed within *R. rostrifrons* (WP-Ind) and *R. rostrifrons* (EP), respectively. The genetic distance between *R. rostrifrons* (WP-Ind) sequences averaged 0.2% and ranged up to a maximum of 1.1% between the most divergent haplotypes (K2P-corrected distance). Comparable values for *R. rostrifrons* (EP) were 0.3% and 1.7% (K2P-corrected), respectively. Comparison of five population samples of *R. rostrifrons* (WP-Ind) and four population samples of *R. rostrifrons* (EP) found moderate genetic diversity; haplotype diversities ranged from 0.281 to 0.763 and nucleotide diversities from 0.002 to 0.004, with the eastern Pacific lineage more diverse than the western Pacific – Indian lineage ([Table 6](#)). No genetic structure at the population level was observed within either *R. rostrifrons* (WP-Ind) or *R. rostrifrons* (EP; [Table 6](#)). Exact tests for genetic structure and pairwise Φ_{ST} estimates were non-significant in all cases ([Table 6B, D](#)). These results suggest that the lineages are both panmictic within their range.

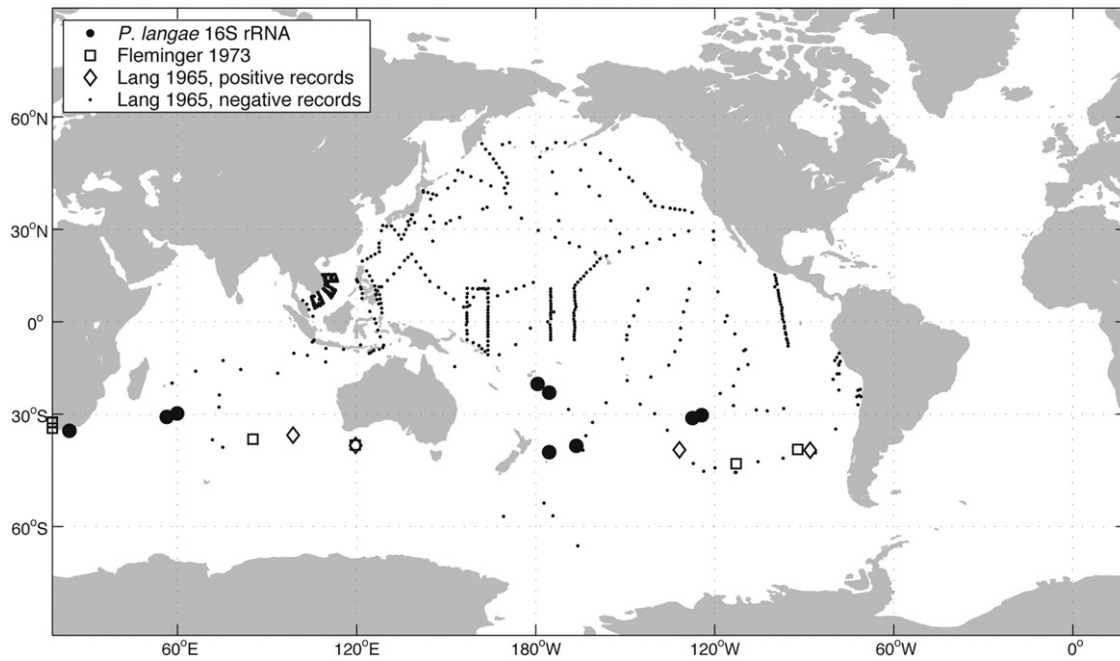


Fig. 15. Biogeographic distribution of *Pareucalanus langae*, a circumglobal transition zone species in the southern hemisphere. Solid circles mark sampling locations of specimens whose species identity was verified by DNA sequencing of 16S rRNA. Fleminger (1973) records of this species are plotted as open squares. Lang records of 'cold-water form' specimens are plotted; data from Fig. 10, Lang (1965, p. 57).

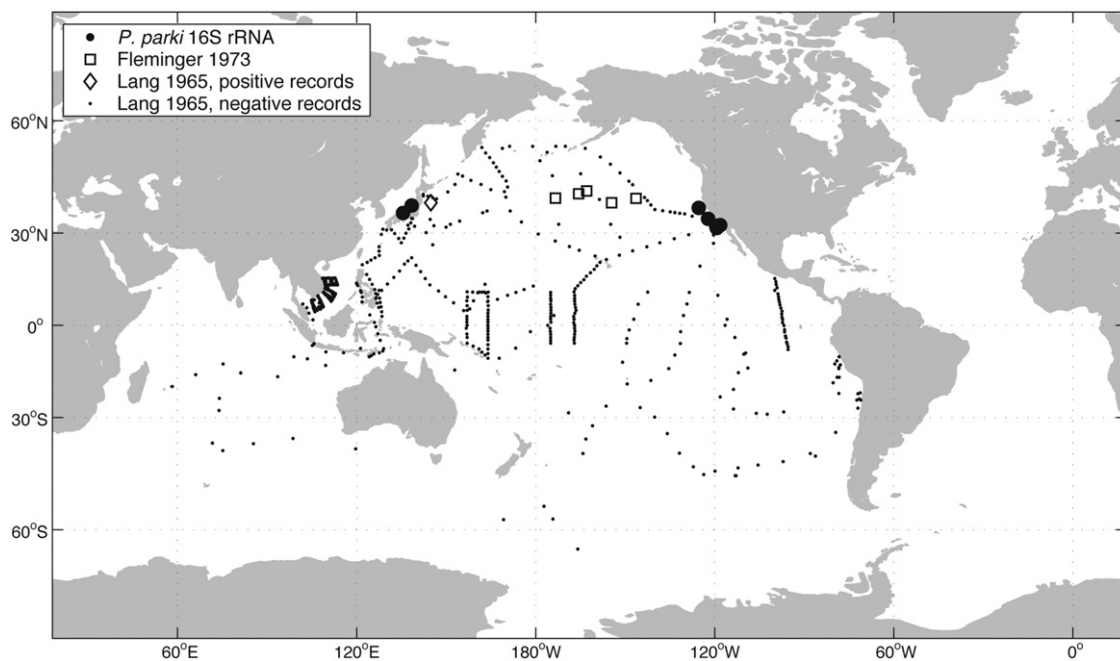


Fig. 16. Biogeographic distribution of *Pareucalanus parki*, a transition zone species in the North Pacific. Fleminger (1973) records of this species are plotted as open squares. Lang records of 'cold-water form' specimens are plotted; data from Fig. 10, Lang (1965).

Rhincalanus rostrifrons s. l. is well known as an Indo-Pacific species in tropical and subtropical waters (Schmaus and Lehnhofer, 1927; Lang, 1965; Bradford-Grieve, 1994). The two mitochondrial lineages within this nominal species have quite distinct biogeographies, with the *R. rostrifrons* (WP-Ind) lineage having an Indo-West Pacific distribution, and *R. rostrifrons* (EP) an eastern tropical Pacific endemic (Fig. 17). No geographic overlap between lineages was observed in our material, and the biogeographic boundary that separates the lineages occurs between 171.43 °W and 145.22 °E in the western tropical Pacific. The distribution of *R. rostrifrons* s. l. is continuous across this region (Lang, 1965), and it

is currently unknown whether the lineages show an abrupt transition or broadly co-occur in this area. Dana (1852) reports the Sulu Sea as the collection locality in the original description of *R. rostrifrons* s. l. Given the distributions reported in Fig. 17, it is likely that Dana examined material of the *R. rostrifrons* (WP-Ind) lineage. New records of *R. rostrifrons* (WP-Ind) in the Agulhas Current region and in the central subtropical Indian Ocean extend the reported distribution of the species farther west and south than reported by Lang (1965). Schmaus and Lehnhofer (1927) also reported *R. rostrifrons* s. l. throughout the northern Indian Ocean and Arabian Sea; these records likely correspond to the

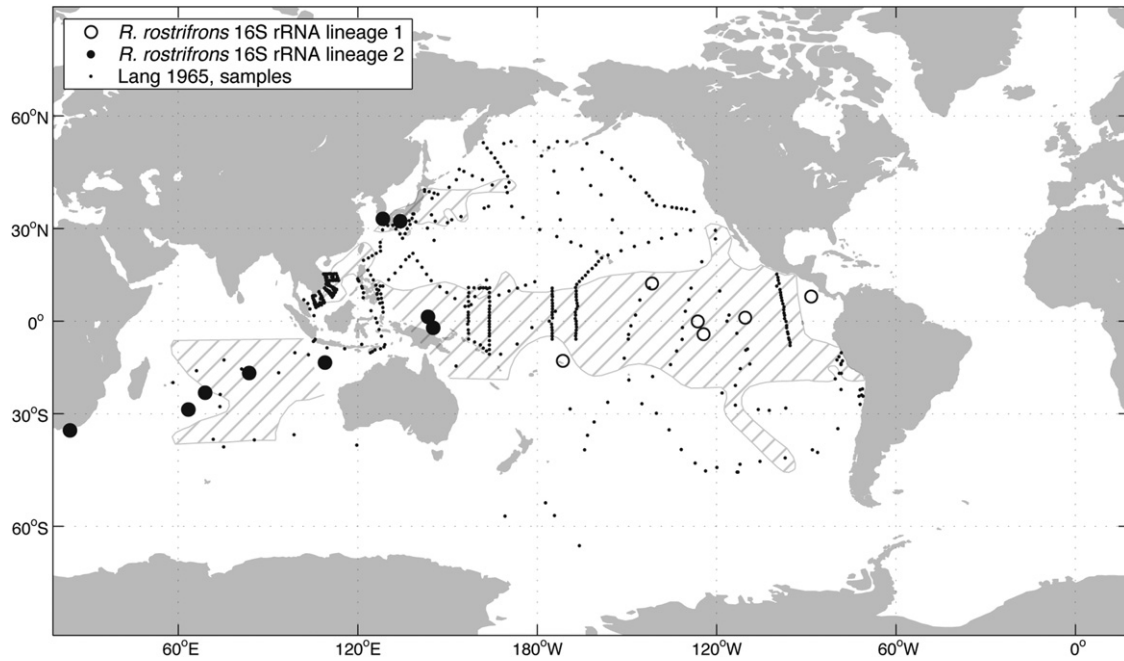


Fig. 17. Biogeographic distributions of two genetic lineages in *Rhincalanus rostrifrons*. Lineage 1 is endemic to the eastern tropical Pacific; Lineage 2 occurs throughout the Indian Ocean and western Pacific. The species distribution from Lang (1965) is outlined with hatched lines, and the sample distribution on which it was based is indicated in the legend. Fleming (1973) did not report on the genus *Rhincalanus*.

Table 6
Phylogeography of *Rhincalanus rostrifrons*. (A) Population samples and summary statistics of *R. rostrifrons* (WP-Ind) mtDNA genetic diversity. (B) Pairwise Φ_{ST} estimates between population samples in A. **Bold** numbers indicate significant values ($\alpha=0.05$). DNA sequences from an additional 6 individuals not included. (C) Population samples and summary statistics of *R. rostrifrons* (EP) mtDNA genetic diversity. (D) Pairwise Φ_{ST} estimates between population samples in C. **Bold** numbers indicate significant values ($\alpha=0.05$). DNA sequences from an additional 5 individuals not included.

(A)	Population samples	N	H	H/N	haplotype diversity (<i>h</i>)	nucleotide diversity (π)
a	W-IND	10	4	0.40	0.420	0.003
b	CW-IND	17	6	0.35	0.391	0.002
c	CE-IND	15	5	0.33	0.333	0.003
d	E-IND	12	5	0.42	0.319	0.003
e	W-PAC	26	8	0.31	0.281	0.002
	Total	80				
(B)	Pairwise Φ_{ST} estimates between samples					
	a	b	c	d	e	
a	0.000					
b	-0.048	0.000				
c	-0.029	0.027	0.000			
d	-0.025	-0.037	0.051	0.000		
e	-0.031	-0.021	-0.023	0.005	0.000	
(C)	Population samples	N	H	H/N	haplotype diversity (<i>h</i>)	nucleotide diversity (π)
a	ETP1	16	7	0.44	0.669	0.004
b	ETP2	19	5	0.26	0.711	0.003
c	ETP3	25	11	0.44	0.754	0.004
d	WTP	13	7	0.54	0.763	0.004
	Total	73				
(D)	Pairwise Φ_{ST} estimates between samples					
	a	b	c	d		
a	0.000					
b	0.000	0.000				
c	-0.010	-0.023	0.000			
d	-0.013	0.004	-0.006	0.000		

R. rostrifrons (WP-Ind) mitochondrial lineage. Other new records from our study fall within the described range of the nominal species *R. rostrifrons* s. l.

4. General discussion

The systematics and biogeography of marine holozooplankton was an area of intense research focus for biological oceanographers in the late 1950s, 1960s and early 1970s. Little research has been conducted on these topics since the advent of molecular ecology in the subsequent decades. This study presents one of the first attempts to re-examine, on a global spatial scale, the diversity and distribution of a holozooplanktonic group using both molecular and morphological approaches. Two primary observations emerge from this work. First, the number of evolutionarily significant units (ESUs) within the Eucalanidae is approximately twice as high as the number of species described based on morphological criteria. Many of these ESUs are likely cryptic species. This observation is significant in light of the fact that this family is one of the calanoid copepod families best characterized in previous systematic and biogeographic works. Second, while molecular data enable description of the biogeographic distributions of these putative new species, the results do not significantly alter our view of the distributional patterns of pelagic animals. The fundamental pelagic biogeographies have been described. This observation is in contrast to results for planktonic protists, in which molecular tools have revealed a new level of ecological specialization and habitat restriction in pelagic environments (de Vargas et al., 1999, 2002, 2004; Darling et al., 2004).

Integration of molecular and morphological data for 24 nominal eucalanid species resulted in three distinct outcomes: (1) the species was confirmed to be a valid genetic species, and the biogeographic distribution remains largely unchanged, (2) the species was found to contain multiple genetic lineages, each of which has a more restricted distribution than the parent taxon, and (3) the species was found to contain multiple genetic lineages, the distributions of which are currently unclear due to insufficient sampling. Examples of the first outcome include *S. subtenuis*, *S. mucronatus*, *S. subcrassus*, *P. attenuatus*, *P. langae*, and *P. parki*. Each of these species is a valid taxon, and prior records from morphological studies by Fleming (1973) and Lang (1965) are confirmed to be accurate. New records in the western Indian Ocean extend the previously described ranges for *S. subtenuis*, *S. mucronatus*, *S. subcrassus*, and *P. langae*, in some cases quite significantly beyond prior biogeographies for these species. Examples of the second outcome include *S. pileatus*, *P. sewelli*, and *R. rostrifrons*. In each case, the described species each consist of multiple ESUs, which appear to unambiguously occupy only a portion of the original, widespread distribution. The third and final outcome, in which multiple genetic lineages are present in the described species, but the biogeography of each lineage is

poorly characterized, is observed in *S. crassus* and *R. nasutus*. Both are species complexes containing 4–8 highly divergent lineages, and both are insufficiently resolved in present and earlier works on the systematics and biogeography of the group.

A number of biogeographic ambiguities remain for the species included in this work. First, the genetic relationship among Atlantic and Indian-Pacific populations of *S. subtenuis* remains to be characterized. Second, the undescribed species *S. sp. 3* appears to be widespread throughout the Indian Ocean, occurring in sympatry with both *S. subcrassus* and *S. pileatus* over much of its range. Higher resolution sampling would identify regions in which it occurs in higher abundance and confirm whether the distribution is continuous throughout coastal waters of the Indian Ocean. Third, resolution of the taxonomic status of the mitochondrial lineages in *R. rostrifrons* is needed to confirm whether these distinct biogeographies correspond to reproductively isolated populations. The genetic identity of *R. rostrifrons* specimens in the central-western Pacific also remains to be determined. And finally, a more extensive global collection of material is required to understand the diversity and distribution of the *S. crassus* species complex. These species can be very abundant in eutrophic and coastal waters (e.g., Smith, 1995; Smith et al., 1998), and resolving the taxonomy of this morphospecies should be a high priority in subsequent work.

Acknowledgments

A number of people generously contributed ethanol-preserved samples from around the globe for inclusion in this study. We gratefully acknowledge the sampling assistance of: I. Prusova and F. Al-Yamani, F. Ferrari, X. Irigoien, R. Harris and T. Smith, D. Conway, E. Carpenter and A. Subramaniam, G. Mitchell and S. Storms, M. Landry and C. Allen, C. Whitcraft, Y.-S. Kang, S. Ohtsuka, V. Loeb, P. Fiedler and V. Philbrick, A. Hays, V. Farstey, P. Hidalgo Diaz and R. Escibano, B. Frost (and through Frost: P. Ward, N. Iguchi, J. Bower), V. Andersen, D. Field, B. Lavaniegas, F. Yusoff and P. Kuppan, R. Machida and S. Nishida, R. Shreeve, S. Gasparini and L. Mousoult, M. Galbraith, S. Hernandez Trujillo, J. Grieve, G. Harkins, M. Gibbons, and C. van der Lingen, P. Ayon, R. Wilson and T. Baiz, and R. Barthélémy. C. Grover provided laboratory assistance. We thank A. Townsend for assistance with material in the Pelagic Invertebrates Collection, SIO – UCSD, and J. Bradford-Grieve and C. Miller for their careful reviews of the manuscript. This research was funded by NSF OCE02-21063 and OCE04-17616 (California Current Ecosystem LTER); the Natural History Museum, London provided access to additional molecular facilities.

Appendix A

See Table A1.

Table A1

List of plankton samples used in molecular studies, with the cruise or region, station number, latitude, longitude, and species present in the sample. Species identifications verified by placement of mt16S rRNA sequences in the molecular phylogeny. Species names as in Table 1.

Sample	Cruise or Region	Station Number	Lat (°)	Long (°)	Species present
1	COOK11MV	02	18.19	132.44	subtenuis
2	COOK11MV	03	14.03	135.28	subcrassus
3	COOK11MV	04	1.43	143.43	mucronatus, pileatus, subcrassus, rostrifrons (WP/IND), attenuatus, P. sp.
4	COOK11MV	05	−2.24	145.22	subtenuis, mucronatus, rostrifrons (WP/IND), attenuatus, P. sp.
5	HOT	ALOHA	22.75	−158.00	attenuatus
6	Korea	205/03	34.25	127.90	subtenuis, mucronatus, S. sp., pileatus, subcrassus, attenuatus, P. sp., sewelli (NA/IND)

Table A1 (continued)

Sample	Cruise or Region	Station Number	Lat (°)	Long (°)	Species present
7	Korea	314/01	33.00	128.40	subtenuis, rostrifrons (WP/IND)
8	Korea	314/04	33.50	127.00	subtenuis
9	Arabian Sea	56101#12A	23.29	66.70	subtenuis, S. sp 3
10	Arabian Sea	56101#12B	23.17	66.42	pileatus, attenuatus
11	Arabian Sea	56116#12	22.54	66.37	pileatus, attenuatus
12	Arabian Sea	56136#9	22.52	65.60	attenuatus
13	California Current	77.70	34.23	–122.15	parki
14	CalCOFI	90.0 60.0	32.25	–119.58	parki
15	CalCOFI	93.3 40.0	32.31	–118.13	parki
16	CalCOFI	93.3 60.0	31.51	–119.34	parki
17	Ace-Asia	AA-19	32.26	134.23	rostrifrons (WP/IND), attenuatus, P. sp
18	Ace-Asia	AA-8	31.24	173.92	attenuatus, P. sp
19	Aegean Sea	AS1	38.47	25.05	sewelli (NA/IND)
20	Canary Islands	CAN1	29.37	–13.48	P. sp, sewelli (NA/IND)
21	COOK14MV	COOK14MV-01	–18.16	178.28	mucronatus
22	COOK14MV	COOK14MV-02	–19.32	179.27	subcrassus
23	COOK14MV	COOK14MV-03	–20.52	–179.29	subcrassus, attenuatus, P. sp, langae
24	COOK14MV	COOK14MV-05-01	–23.53	–177.06	attenuatus, P. sp
25	COOK14MV	COOK14MV-05-02	–23.52	–177.05	attenuatus, P. sp
26	COOK14MV	COOK14MV-06	–23.31	–175.42	P. sp, langae
27	COOK14MV	COOK14MV-20	–30.13	–176.35	crassus (Pac)
28	COOK14MV	COOK14MV-21	–31.05	–177.05	crassus (Pac)
29	COOK14MV	COOK14MV-41	–13.17	–171.43	rostrifrons (EP)
30	DRFT07RR	DRFT07RR-04	–30.40	–124.46	langae
31	DRFT07RR	DRFT07RR-05	–31.21	–127.50	langae
32	DRFT07RR	DRFT07RR-11	–36.05	–149.29	sewelli (Pacific)
33	DRFT07RR	DRFT07RR-15	–39.37	–166.42	langae, sewelli (Pacific)
34	DRFT07RR	DRFT07RR-19	–41.24	–175.40	langae
35	Gulf of California	Est:14 Sta:335			sewelli (Pacific)
36	Central California Coast	Fm9			parki
37	North Pacific, transition zone	FROST-2	38.10	138.41	parki
38	North Pacific, transition zone	FROST-4	36.10	135.51	parki
39	Western Indian Ocean	Isle St Marie 6	–17.06	49.49	pileatus, subcrassus, attenuatus
40	Western Indian Ocean	KN162-10	–23.48	37.16	subtenuis, mucronatus, pileatus, subcrassus, attenuatus
41	STAR00	M00-49	12.49	–141.59	rostrifrons (EP)
42	STAR00	M00-51	10.30	–139.11	subtenuis
43	STAR00	M00-61	–0.10	–126.32	rostrifrons (EP)
44	STAR00	M00-65	–4.44	–124.33	rostrifrons (EP), attenuatus
45	STAR00	M00-77	1.12	–110.49	subtenuis, rostrifrons (EP), attenuatus, sewelli (Pacific)
46	STAR00	M00-94	8.22	–88.47	pileatus, subcrassus, rostrifrons (EP), attenuatus, sewelli (Pacific)
47	STAR00	M00-96	8.45	–87.38	subtenuis, pileatus
48	MP3	MP3-12-06-00	29.57	–45.03	sewelli (NA/IND)
49	MP3	MP3-14-01-00	12.04	–55.26	pileatus (NA), P. sp, sewelli (NA/IND)
50	MP3	MP3-18-01-00	10.57	–49.39	P. sp, sewelli (NA/IND)
51	MP3	MP3-23-02-00	10.32	–56.32	pileatus (NA)
52	MP3	MP3-34-03-00	3.59	–43.07	pileatus (NA), P. sp
53	Plymouth, UK	PLYMOUTH-1	50.42	–4.83	crassus (Atl)
54	Malaysia	Pulau Payar	6.58	100.46	S. sp 3, pileatus, subcrassus
55	Western Indian Ocean	Seychelles Is	–4.20	55.51	S. sp, S. sp3, pileatus
56	Sulu Sea	ST. 26	7.00	120.00	mucronatus, P. sp, sewelli (Pacific)
57	North Pacific	ST-3	31.16	131.52	subtenuis
58	VANC10MV	VANC10MV-01	–35.05	23.73	S. sp, crassus (Atl), S. sp 2, pileatus, subcrassus, rostrifrons(WP/IND), attenuatus, P. sp, langae, sewelli (NA/IND)
59	VANC10MV	VANC10MV-02	–35.07	24.50	subtenuis, mucronatus, S. sp, S. sp 3, pileatus, subcrassus
60	VANC10MV	VANC10MV-07	–33.30	45.36	mucronatus, P. sp
61	VANC10MV	VANC10MV-10	–30.85	56.30	langae, sewelli (NA/IND)
62	VANC10MV	VANC10MV-11	–29.85	59.84	langae, sewelli (NA/IND)
63	VANC10MV	VANC10MV-12	–28.74	63.39	rostrifrons (WP/IND)
64	VANC10MV	VANC10MV-13	–23.59	69.01	rostrifrons (WP/IND)
65	VANC10MV	VANC10MV-18	–17.18	83.68	subtenuis, mucronatus, S. sp, rostrifrons (WP/IND)
66	VANC10MV	VANC10MV-19	–15.76	86.78	subtenuis, mucronatus
67	VANC10MV	VANC10MV-20	–13.96	89.94	subtenuis, attenuatus, P. sp, sewelli (NA/IND)
68	VANC10MV	VANC10MV-21	–13.49	91.77	mucronatus
69	VANC10MV	VANC10MV-25	–13.85	109.04	subtenuis, mucronatus, pileatus, subcrassus, rostrifrons (WP/IND)
70	VANC10MV	VANC10MV-26	–14.49	113.46	attenuatus, P. sp, sewelli (NA/IND)

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