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The introduction to Japan of the Titan barnacle, *Megabalanus coccopoma* (Darwin, 1854) (Cirripedia: Balanomorpha) and the role of shipping in its translocation

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The Titan Acorn barnacle, *Megabalanus coccopoma*, a native of the tropical eastern Pacific, has become established in the western Atlantic (Brazil and the northern Gulf of Mexico to the Carolinas), northwestern Europe and the western Indian Ocean (Mauritius), and therefore its dispersal capabilities are well known. This study reports its introduction to Japan and confirms its occurrence in Australia. In an attempt to determine the source of this introduction, phylogeographic techniques, involving cytochrome c oxidase I sequences of various widely separate populations of *M. rosa* and *M. volcano*, were utilized. No significant genetic differentiation or haplotype patterns between widely separated populations of each of the three species were found. Lack of such differentiation indicates recent geographical isolation and thus negates a null hypothesis predicting that the occurrence of one of more of these species in Australia was natural.

Keywords: *Megabalanus coccopoma*; invasive species; alien species; haplotype analysis; phylogeographic distribution; *Megabalanus rosa*; *Megabalanus volcano*

Introduction

Ocean-going vessels can be thought of as 'mobile stepping stones' for fouling species from harbors and estuaries, because they provide a substratum from which attached adults can release larvae in suitable situations (Apte et al. 2000). Fouled ships have plied the oceans since the beginning of oceanic navigation, but changing trading routes and their increasing number, speed and relatively long port residence times have effectively shortened the pathways for biological communities in crossing substantial distances and biogeographical barriers. On modern ships, fouling occurs when the antifouling paint is depleted and is no longer efficient at deterring fouling organisms (Otani et al. 2007; Pettengill et al. 2007). However, even ships that have efficient antifouling paints, may have areas of damage where colonization by fouling organisms can occur, thereby providing the opportunity for the introduction of alien species (Piola and Johnston 2008). It is also suggested that certain antifouling paints may facilitate the recruitment of non-indigenous species, thus providing the potential for further spread of these species (Dafforn et al. 2008).

The dispersal of marine organisms by shipping has long been used in interpreting the biogeography of marine invertebrates, often (in retrospect) long after an alien species is found (Foster and Willan 1979; Carlton and Geller 1993; Zardus and Hadfield 2005). One of the first examples of a well documented introduction of a barnacle by shipping was that of *Austrominius modestus* (= *Elminius modestus*) from New Zealand to England, most likely via convoys during World War II (Southward et al. 1998).

During phylogeographic research on intertidal barnacles in the Indo-West Pacific in 2007, a species of *Megabalanus* distinct from the two northwest Pacific species, *M. rosa* (Pilsbry 1916) and *M. volcano* (Pilsbry 1916), was also found on the southern coast of central Japan, at Shimoda and Senjoujiki on the Izu Peninsula, at Toushima, Tanabe Bay on the Kii Peninsula and from the hulls of two ships dry-docked at Kobe Port (Hyogo) and Mizushima Port (Okayama). This species was identified as *M. coccopoma* (Darwin 1854) and, constitutes the first record of this species in Japan. *M. coccopoma* is native to the tropical eastern Pacific, ranging from Baja California to Guayquil, Ecuador

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Table 1.   List of localities and collection dates for the species of *Megabalanus* examined and sequenced in this study.

Loc. no.	Locality	Date collected	Species (*n*)
1	On buoy: Ogatsu Bay, **Ishinomaki**, Miyagi, Japan, Coll: A Oshino	2007.1	*M. rosa* (20)
2	On buoy: Nabeta Bay, **Shimoda**, Shizuoka, Japan	2007.3.22	*M. coccopoma* (15) *M. rosa* (1)
3	On rock: **Senjoujiki**, Minami-Izu, Shizuoka, Japan	2007.3.19	*M. coccopoma* (8) *M. volcano* (10)
4	On rock: **Toushima**, Shirahama, Tanabe Bay, Wakayama, Japan	2005.3.11	*M. coccopoma* (2) *M. volcano* (9)
5	On hull of 'Ship Y': Kobe Port, Hyogo, Japan (sails between Japan and Australia)	2004.11.5	*M. coccopoma* (10) *M. rosa* (3)
6	On hull of 'Ship Z': Kobe Port, Hyogo, Japan (sails between Japan and Australia)	2004.9.4	*M. coccopoma* (2) *M. rosa* (2)
7	On hull of 'Ship A': Mizushima Port, Okayama, Japan (sails between Japan and Australia)	2007.9.22	*M. coccopoma* (4) *M. rosa* (9) *M. volcano* (4)
8	On rock: **Manly**, Sydney, NSW, Australia	1994.2.6; 2002.5.23; 2007.6.14	*M. coccopoma* (8)
9	**Rio de Janeiro**, Brazil, Coll: PS Young and FB Pitombo	1996.7.14; 2005.3.11	*M. coccopoma* (3)

(Pilsbry 1916; Laguna 1990; Young and Ross 2000). On the eastern Pacific coasts, it spread as far north as San Diego, California during the El Niño of 1982–1983 (Newman and McConnaughey 1988). It is known to have been introduced to Brazil, southeastern USA, northern Europe and the western Indian Ocean. It was recorded as introduced in southern Brazil in the 1970s (Lacombe and Monteiro 1974), but the first record for the north Atlantic was in the western tropical Atlantic (coastal Louisiana, USA) 30 years later (Perreault 2004). Innocenti (2006) reported on specimens collected in 1875 from the Western Indian Ocean (Mauritius), in the collection of the Natural History Museum, University of Florence. The first European record was from a ship's hull in Le Havre, France (Nilsson-Cantell 1932). In the Netherlands, it was found off Terschelling in 1976 and 1977, where it was first reported as *Balanus perforatus* (= *Perforatus perforatus*) (Buizer 1978), and then as *M. tintinnabulum* (Buizer 1980). Wolff (2005) recognized it as *M. coccopoma*. Kerckhof and Cattrijsse (2001) and Kerckhof et al. (2007) have reported the species from the coast of Belgium.

There is no record of *M. coccopoma* from Japan, only *M. rosa* and *M. volcano* had been previously known (Yamaguchi 1973). In addition, re-examination of laboratory collections and new collections confirmed the occurrence of *M. coccopoma*, previously known from a skip at Brisbane, Queensland, Australia (Neil et al. 2005) on natural substrata at Newcastle, Manly, and Sydney on the central coast
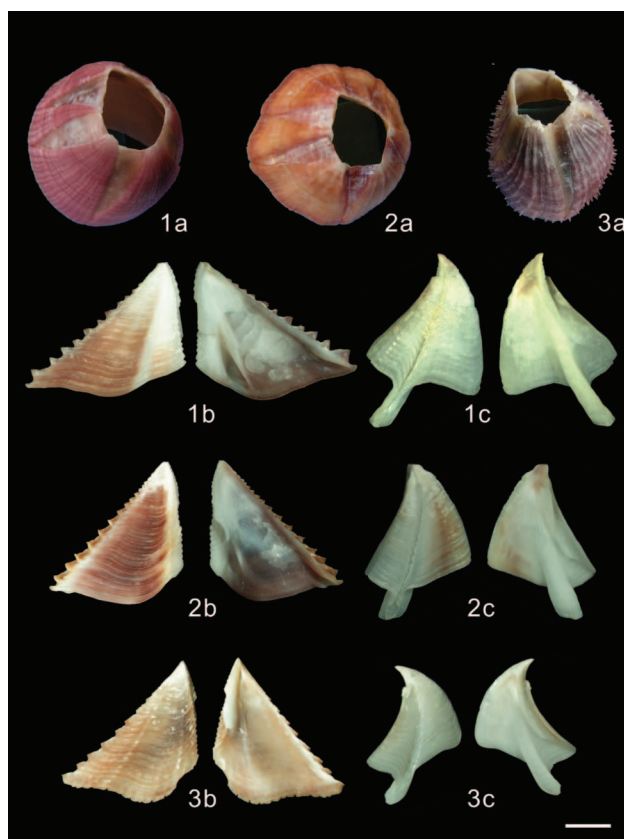

Figure 1.   Morphological appearance of three *Megabalanus* species occuring in Japan. 1 = *M. coccopoma*; 2 = *M. rosa*; 3 = *M. volcano*; a = whole shell, b = scuta (left = external, right = internal),   c = terga   (left = external,   right = internal).

of New South Wales (NSW), following the discovery of *M. coccopoma* on ships in Japan that sailed to and from Newcastle. The present study compares *M. coccopoma* with the two *Megabalanus* species known from Japan using morphological and molecular properties, in an attempt to determine the geographic origin of *M. coccopoma* in Japan.

Materials and methods

Samples examined

Samples used in this study were adult individuals of three species; the alien species *M. coccopoma* and two species native to the northwest Pacific as far as Japan, *M. rosa* and *M. volcano*. Nine groups of samples, designated as populations, were collected from nine localities around Japan (Table 1): Loc. (1) Ogatsu Bay, **Ishinomaki**, Miyagi; (2) Nabeta Bay, **Shimoda**, Shizuoka; (3) **Senjoujiki**, Minami-Izu, Shizuoka; (4) **Toushima**, Shirahama, Tanabe Bay, Wakayama; and from the hulls of three ships: (5) '**Ship Y**' (6) '**Ship Z**' at Kobe Port, Hyogo and (7) '**Ship A**' at Mizushima Port, Okayama. Samples of *M. coccopoma* from (8) **Manly**, Sydney, Australia and (9) **Rio de Janeiro**, Brazil were used to compare species identity and genetic population structure.

Table 2. Morphological comparison between three distinct *Megabalanus* species in Japan.

| Characters	M. coccopoma	M. rosa	M. volcano
Shell			
Parietes	Smooth, rugose or finely ribbed	Smooth	Roughened with ribs, mostly with numerous small spines or spine-like projection
Parietes coloration	Usually deep red, mostly with fine white longitudinal lines or stripes, radii deep purple or reddish purple	Pinkish red to reddish purple, occasionally entirely white, radii darker except those entirely white	Dull reddish or bluish purple or grayish purple; radii similar or darker in color
Radii	Moderately wide	Moderately wide	Moderately wide or rather narrow
Scutum			
Tergal margin	Broad obtusely inflected	Narrow tergal segment acutely inflected	Slightly inflected tergal margin in small individual, broad obtusely inflected in large individual, narrow tergal segment
Occludent margin	Strongly toothed	Toothed	Weakly toothed
Growth ridge	Widely separated strongly developed, longitudinal striae faint or absent	Closely set strongly developed, longitudinal striae usually lacking	Prominent strongly scalloped by well developed longitudinal striae
Scutum coloration	Pinkish gradually white to apex	Usually pinkish on all external surface, white tinged with purple internally on basal margin	Pink or pinkish or bluish purple externally, white tinged with purple internally on basal margin
Adductor ridge	Well developed, prominent extended to basal margin	Blunt confluent with articular ridge often extended to basal margin	Prominent narrow
Pit for lateral depressor muscle	Deep	Deep	Shallow
Tergum			
Shape	Broad, scutal margin relatively straight	Broad, scutal margin relatively straight	Moderately broad, scutal margin inflected with apex rather beaked
Tergum coloration	White	White tinged longitudinaly with pinkish externally	White
Spur	Long narrow, separated by more or les twice own width from basi-scutal angle	Short broad, separated by own width from basi-scutal angle	Moderately long narrow, separated by twice own width from basi-scutal angle
Spur furrow	Closed or nearly so	Closed or nearly so	Closed
Growth ridge	Prominent, widely separated longitudinal striae faint or absent	Prominent, closely set longitudinal striae faint or absent	Prominent on scutal side, longitudinal striae fine
Basal margin of carinal side	Basal margin relatively straight on both side or slightly sloping to spur on both sides	Basal margin slightly sloping on scutal side and sloping on carinal side	Straight on scutal side and strongly sloping on carinal side
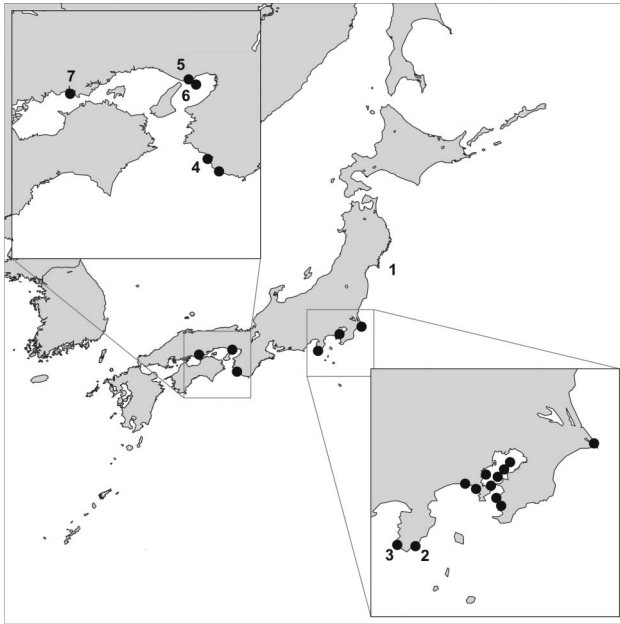
Figure 2. The present geographic distribution of *M. coccopoma* in Japan.

DNA amplification and sequencing

Polymerase chain reaction (PCR) amplifications of mitochondrial cytochrome c oxidase I (COI) were amplified partially (426 bp) using ABI 2720 Thermal cycler. A forward-reverse primer pair and thermocycling protocol, COI-3 5′-GTNTGRGCN CAYCAYATRTTYACNGT-3′ and COI-6 5′-GGR TARTCNSWRTANCGNCGNGGYAT-3′ based on Shimayama et al. (1990), was performed at 94°C for 60 s, 35 cycles at 92°C for 40 s, 50°C for 60 s, 72°C for 90 s and a final extension of 72°C for 7 min.

DNA automated sequencing of PCR products was performed at the Paleobiology Laboratory, Chiba University, Japan, on a ABI PRISM® 310 Genetic Analyzer using BigDye© (Perkin Elmer) terminator chemistry. DNA forward and reverse sequences were then inspected and edited by eye using the BioEdit program (Hall 1999).

Phylogenetic analysis

The COI gene fragments of 426 bp from 110 individuals of three species of *Megabalanus*, viz. *M. coccopoma* ($n = 52$), *M. rosa* ($n = 35$) and *M. volcano* ($n = 23$) were successfully sequenced and aligned using ClustalW (Thompson et al. 1994). Phylogenies were constructed to assess species identity using distance matrix and parsimony approaches with the software package PAUP* 4.0b10 (Swofford 1998), and Bayesian inference (BI) with MrBayes 3.1.2 (Ronquist and

Huelsenbeck 2003), with *Tetraclita japonica* used as the outgroup. For parsimony reconstruction, a maximum parsimony (MP) tree topology was constructed based on heuristic searches. For distance matrix reconstruction, the neighbor-joining (NJ) algorithm (Saitou and Nei 1987) was used to estimate tree topology with the distance set to ML; best-fit models of substitution were selected by Modeltest. For both MP and NJ trees, branches support were evaluated using 50% majority-rule bootstrap from 1000 replicates. For BI, as COI is a protein coding gene, sequences were partitioned based on the codon position, and best-fit models of substitution were selected by Modeltest for each codon position. BI trees were obtained using 1,000,000 generations and a sample frequency of 100. The first 1000 of these were considered as the 'burn in' and discarded. A 50% majority-rule consensus tree of the remaining trees was produced. Best-fit models of nucleotide substitutions used in NJ and BI were selected by performing MODELTEST 3.0 (Posada and Crandall 1998).

Population structure

Haplotypes (h) were determined from mtDNA COI sequence data of *M. coccopoma*. To further demonstrate the relationships among haplotypes, haplotype networks were also constructed using statistical 95% parsimony criterion (Templeton et al. 1992) as implemented in TCS 1.2 (Clement et al. 2000). All alternative connections were examined to determine if they resulted in fundamental changes in the topology. Unique clusters of two or more haplotypes found primarily or exclusively in a single geographical region were color-coded. Haplotype (h) and level of genetic differentiation based on Wright's conventional haplotype frequency (FST) between pairs of populations were calculated using ARLEQUIN 2.0 software (Excoffier et al. 2005). Pairwise genetic distances (FST) were also calculated by considering both haplotype frequency differences and numbers of substitutions between haplotypes. Kimura's two-parameter method was used, which allows for multiple substitutions at a site and for different rates of transitions and transversions. A mantel test was also calculated using Arlequin to find the correlation between the level of genetic differentiation based on Wright's conventional haplotype frequency (FST), and pairwise genetic distances (FST), with pairwise geographic distances (km) among populations. The results suggested whether both (FST) were correlated or inferred by geographic distances. The significance of these statistics was evaluated using 1000 permutations and was considered significant when the corresponding P value was < 0.05.

Results and discussion

A comparison of the morphology of *Megabalanus* species from Japan with previous descriptions (Darwin 1854; Pilsbry 1916; Henry and McLaughlin 1986) indicates the occurrence of three species: *M. coccopoma, M. rosa* and *M. volcano* (Figure 1, Table 2). This is the first report of *M. coccopoma* on the Japanese coast, an introduction most likely associated with ships. It has been found on natural substrata at Senjoujiki (Loc. 3) and Toushima (Loc. 4), and also on two of three ships (Ship 'Y' and 'Z') dry-docked at Kobe Port. The ships fouled by *M. coccopoma* had sailed between Japanese and Australian ports (mainly Newcastle and adjacent area in NSW and Port Hedland and Dampier in Western Australia).

On the basis of a geographic survey of non-indigenous species in Japan, *M. coccopoma* was found in various areas of Tokyo Bay, at Chigasaki in Sagami Bay and Choshi on the northeast of the Boso Peninsula in central Japan (Figure 2, Table 3) in addition to the localities of the samples examined in the molecular study. In Tokyo Bay, *M. coccopoma* was mainly found on light buoys or fishing nets near to the entrance of the bay. In Sagami Bay and at Choshi, it occurred on natural substrata of rocky shores. As far as is known, *M. coccopoma* has to have been established on the Pacific coast of central Japan since 2005, based on the oldest collection from Chigasaki in Sagami Bay. The northern limit of its distribution does not extend to Ishinomaki (Loc. 1) on the Pacific coast of the northern Japan (Figure 2).

Henry and McLaughlin (1986) reported only one *Megabalanus* species, *M. linzei* (Foster 1978), as native to Australian waters, distributed on the central coast of NSW. However, re-examination of laboratory and

Table 3.   List of localities and collection dates of *M. coccopoma* in Japan.

Date	Locality	Ecology	Species	Observer
2005.1.27	Southern Beach Chigasaki, Chigasaki, Kanagawa	Rock (−1 m)	*M. coccopoma*	M. Otani
2005.5.11	Koura Fisheries Port, Minami-Boso, Chiba	Fishing net	*M. coccopoma*	T. Shuto
2007.12.8	South of Cape Myosho-zaki, Kyonanmachi, Awa, Chiba	Fishing net	*M. coccopoma*	M. Kikuchi
2008.4.11	Takasaki Fisheries Port, Iwai, Minami-Boso, Chiba	Fishing net	*M. coccopoma, M. rosa*	T. Yamaguchi, K. Yamada, H. Terasawa, R. Hiramoto, A. Fujimoto
2008.5.7	Togawa Fisheries Port, Choshi, Chiba	Rock	*M. coccopoma*	T. Yamaguchi
2008.6.21	Nagai Fisheries Port, Yokosuka, Kanagawa	Fishing net	*M. coccopoma*	I. Ueda, T. Yamaguchi
2008.7.25	Banzu C Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi, K. Yamada, H. Terasawa, R. Hiramoto, A. Fujimoto
2008.7.25	Kita-Sode 1 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.7.28	Yokosuka Harbor South 1 Light buoy in Tokyo Bay	Buoy	*M. coccopoma, M. rosa*	T. Yamaguchi
2008.8.28	Yokosuka Harbor 3 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.8.28	Yokosuka Harbor 5 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.9.3	Chisaki Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.9.4	Kita-Sode 3 Light buoy in Tokyo Bay	Buoy	*M. coccopoma, M. rosa*	T. Yamaguchi
2008.9.4	Kita-Sode 4 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.9.5	Shonan Harbor, Enoshima Island, Sagami Bay	Buoy	*M. coccopoma*	I. Ueda
2008.9.10	Yokosuka Harbor 1 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T Yamaguchi
2008.9.10	Yokosuka Harbor 6 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.9.17	Funabashi 2 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.9.18	Yokosuka Harbor South 2 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.9.18	Yokosuka Harbor South 3 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.10.3	Kisarazu-Futtsu 3 Light buoy in Tokyo Bay	Buoy	*M. coccopoma*	T. Yamaguchi
2008.10.3	Kisarazu-Futtsu 4 Light buoy in Tokyo Bay	Buoy	*M. coccopoma, M. rosa*	T. Yamaguchi
2008.11.9	Uraga sea route Center 6 Light buoy in Tokyo Bay	Buoy	*M. coccopoma, M. rosa*	T. Yamaguchi

new collections have confirmed the occurrence of *M. coccopoma* on natural substrata at Newcastle, Manly and Sydney, as well as on the central coast of NSW. This occurrence is most likely an introduction. *M. coccopoma* is also known in Australia, from Port Phillip Bay, Victoria (Lewis, personal communication) and Jervis Bay, NSW (mooring at Darling Road Western Australian Muesum Reg. No. 37513 NSW, 35°00′00″S, 15°45′00″E collected at 03/05/2006, by R. Hilliard). The time and location of the first introduction of *M. coccopoma* in Australia is not known.

All collections of *M. coccopoma* from Japan and Australia examined in this study were morphologically identical to those collected from Rio de Janeiro, Brazil. *M. coccopoma* in both Japan and Australia is now rather widespread, suggesting either rapid dispersal since introduction, or the species being present, but not detected. The similarity and wide range of suitable environmental conditions in both hemispheres and the dispersal capabilities of *M. coccopoma* would explain the present wide ranges of this apparently recently introduced species.

International ships (bulk carriers) voyaging to Japan are considered the most likely vector for the translocation of *M. coccopoma*. Shipping is widely considered to be the major vector for the translocation of species between countries, either in ballast water or as hull fouling (Carlton 1985; Cohen and Carlton 1995; Coutts and Taylor 2004; Otani et al. 2007; Pettengill et al. 2007). The source of introduction to Japan could have been on ships directly from within the native range of *M. coccopoma*, or indirectly from other areas of introduction. The occurrence of this species on bulk carriers voyaging between Japan and Australia has linked the populations in these two regions, and is one possible introduction pathway. Otani et al. (2007) previously described the potential risk of introduction of barnacles on ships sailing between these two countries. Molecular properties used in the present study were unable to resolve the source of *M. coccopoma* as no distinct molecular sequences were found in different geographical populations.

For parsimony reconstruction, MP tree topology was constructed based on heuristic searches. The best tree obtained had a consistency index (CI) = 0.7389, homoplasy index (HI) = 0.2611, CI excluding uninformative characters = 0.6974, HI excluding uninformative characters = 0.3026, retention index = 0.9820 and a rescaled consistency index = 0.7257, indicating low level of homoplasy in the COI gene data. For distance matrix reconstruction, the NJ algorithm (Saitou and Nei 1987) was used to estimate the tree topology with the distance set to ML. The evolutionary model selected by Modeltest for all positions of the COI sequence was GTR + I. The model assumes

unequal base frequencies ($A = 0.2919$; $C = 0.2165$; $G = 0.1207$; $T = 0.3709$). The assumed substitution rate differences were found to be $R(a)$ $[A-C] = 0.1161$; $R(b)$ $[A-G] = 10.4196$; $R(c)$ $[A-T] = 0.8891$; $R(d)$ $[C-G] = 0.0000$; $R(e)$ $[C-T] = 3.5084$; and $R(f)$ $[G-T] = 1.0000$. The proportion of invariable sites (I) = 0.6508, and variable sites (G) = equal rates for all sites. Data were bootstrapped for evaluation of the confidence of the results, using the bootstrap option of PAUP, with 1000 replicates for both MP and NJ. For BI, as COI is a protein coding gene, sequences were partitioned based on the codon position, and best-fit models of substitution were selected by Modeltest for each codon position. These are, TrNef + I for the first codon position, F81 for the second codon position, and
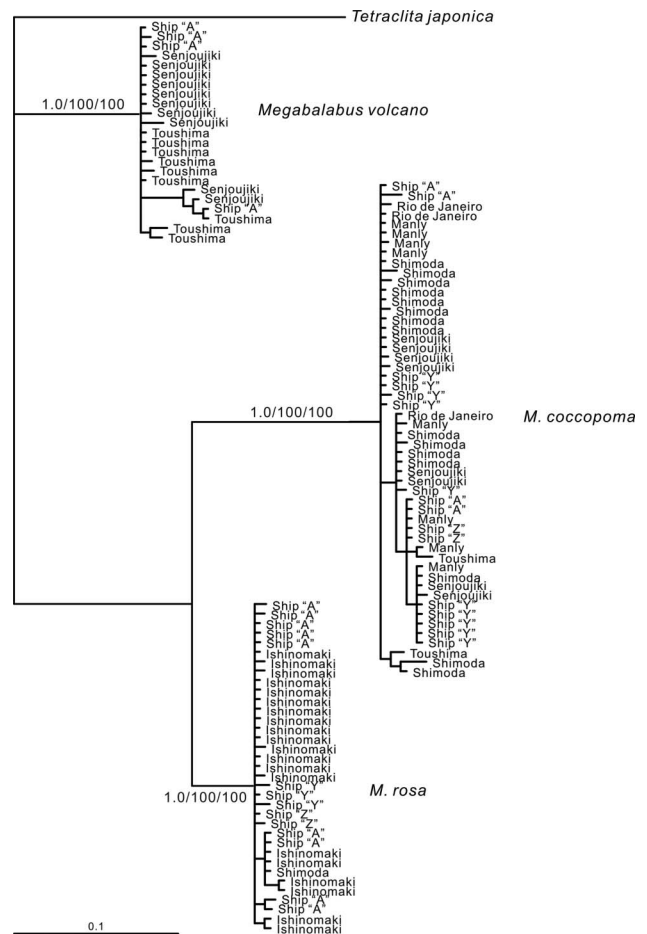

Figure 3. Phylogram derived from Bayesian inference (BI) of three distinct species of *Megabalanus* occuring in Japan using the tetraclitid barnacle *Tetraclita japonica* as an outgroup. Basal branch nodes showing the posterior probability value of BI compared with bootstrap values of the phylogram of neighbor-joining and maximum parsimony, also showing the same topology, are presented in the following order (BI/NJ/MP).

Table 4.   List of haplotypes (*h*) of *M. coccopoma* and number of individuals (*n*) used in this study for each population.

No.	Localities (Populations)							
	2 Shimoda	3 Senjoujiki	4 Toushima	5 Ship Y	6 Ship Z	7 Ship A	8 Manly	9 Rio
1	4	1		3		1	2	1
2					2	2	1	
3						1		
4								1
5	3	2						1
6							1	
7	1	1					1	
8							1	
9							1	
10	1	1		5			1	
11	1							
12	1							
13	1							
14	1							
15	1							
16	1							
17		1						
18		1						
19		1						
20			1					
21			1					
22				1				
23				1				
n	14	9	2	10	2	4	8	3

TrN + G for the third codon position. BI trees were obtained using 1,000,000 generations and a sample frequency of 100. The first 1000 of these were considered as the 'burn in' and discarded. A 50% majority-rule consensus tree of the remaining trees is presented for phylogenetic assessment. The three methods of analysis, MP, NJ and BI, resulted in similar phylogram topology, similar in both major branching and branch length. The difference between trees involved the internal nodes of each *Megabalanus* species.

The phylograms of MP, NJ and BI trees clearly split *M. coccopoma, M. rosa* and *M. volcano* as distinct species, corroborating the opinion of Newman and Ross (1976) that they were not subspecies of *Balanus* (*Megabalanus*) *tintinnabulum tintinnabulum* (Linnaeus 1758), as previously believed. *M. rosa* is in the same lineage as *M. coccopoma*, from which *M. volcano* is divergent. Bootstrap values of consensus trees for MP and NJ and the credibility level for BI are high (Figure 3).

Mitochondrial COI data collected from 52 individuals of *M. coccopoma* from nine populations yielded 23 unique haplotypes of 426 bp. Haplotypic diversity varied within all populations. The majority of haplotypes (78%) were singletons and 50–100% (mean 81%) of the haplotypes in any population was represented by a single individual. The remaining five haplotypes (0.22%) were shared by three or more populations.
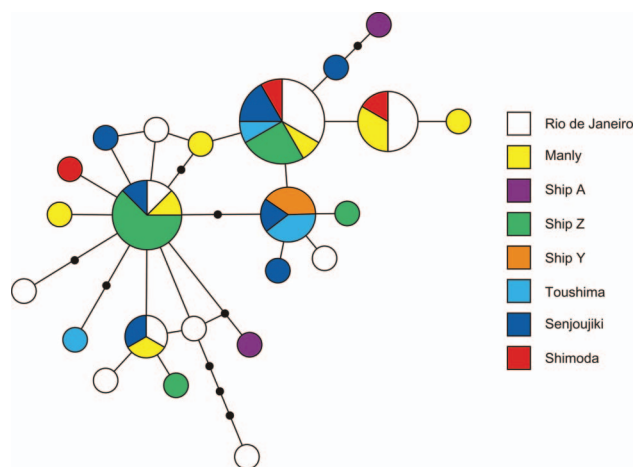

Figure 4.   Statistical parsimony networks of COI sequence data of *M. coccopoma* showing a single network, suggesting no haplotype restriction in any population. The area of a circle is proportional to the number of observed individuals, with each grayscale representing one population as presented in Table 1, and network reticulation showing mutation steps between haplotypes.

Haplotype data for all populations is presented in Table 4. The populations pairwise *FST*, for both levels of genetic differentiation based on Wright's conventional haplotype frequency and genetic distances, show no significance in genetic differentiation or genetic

distance at the 0.05 level. The result suggests that all eight local populations of *M. coccopoma* represent the same, more widespread, population or gene pool.

To demonstrate the relationships among haplotypes, haplotype networks were constructed using statistical parsimony criteria with a 95% cut-off (Templeton et al. 1992) as implemented in TCS 1.2 (Clement et al. 2000). The results suggest that there is only one haplotype network for *M. coccopoma* (Figure 4), indicating close linkage between haplotypes. Despite the vast geographical distances between the Brazilian, Australian and Japanese populations, there is no haplotype restriction in any population, as indicated by the F_{ST} result. However, the molecular analyses in this study could not resolve the source of introduced populations in either Japan or Australia.

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