The introduction to Japan of the Titan barnacle, *Megabalanus coccopoma* (Darwin, 1854) (Cirripedia: Balanomorpha)

Toshi Yamaguchi*, Y. Ohshiro, A. Fujimoto, M. Kiuchi, M. Otani, I. Ueda, and H. Kawai

*present address: Kanagawa University

Biofouling, Vol. 25, No. 4, May 2009, 325–333.

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

Taylor & Francis
Taylor & Francis Group

T Yamaguchi[1], Prabowo RE[1,2], Ohshiro Y[3], Shimono T[4], Jones D[5], Kawai H[4], Otani M[6], Oshino A[7], Inagawa S[1], Akaya T[3], and Tamura I[3]

Biofouling The Journal of Bioadhesion and Biofilm Research

The type materials of *Megabalanus coccopoma* from Panama wasn't used in this paper.

Biofouling
Vol. 25, No. 4, May 2009, 325–333

Taylor & Francis
Taylor & Francis Group

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

Toshiyuki Yamaguchi[a]*, Romanus Edy Prabowo[a,b], Yuu Ohshiro[c], Takaki Shimono[d], Diana Jones[e], Hiroshi Kawai[d], Michio Otani[f], Akio Oshino[g], Shou Inagawa[a], Tomoyoshi Akaya[c] and Itsuro Tamura[c]

[a]*Department of Earth Sciences, Graduate School of Science, Chiba University, Chiba, Japan;* [b]*Universitas Jenderal Soedirman, Purwokerto, Indonesia;* [c]*Department of Earth Sciences, Faculty of Science, Chiba University, Chiba, Japan;* [d]*Kobe University Research Center for Inland Seas, Kobe, Japan;* [e]*Western Australian Museum, Perth, Western Australia;* [f]*Marine Ecological Institute Inc, Toyonaka, Japan;* [g]*Miyagi Prefecture Fisheries Research and Development Center, Ishinomaki, Japan*

(*Received 22 September 2008; final version received 9 January 2009*)

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

*Corresponding author. Email: tyamaguc@earth.s.chiba-u.ac.jp

ISSN 0892-7014 print/ISSN 1029-2454 online

DOI: 10.1080/08927010902738048
http://www.informaworld.com

Megabalanus coccopoma

Buoys for seaweed culture were established at entrance of Tokyo Bay for 6 months from Sep 2008 to Feb 2009.

Megabalanus coccopoma **reaches to maturity within 6 months.**

On *Megabalanus coccopoma* (Darwin 1854)

Taxonomy:

Darwin (1854): Described **new variety** *Balanus tintinnabulum* var. (7) *coccopoma* Darwin in 11 varieties of *B. tintinnabulum*.

Pilsbry (1917): Described **new subgenus and subspecies** *Balanus* (*Megabalanus*) *tintinnabulum coccopoma* Darwin in 7 subspecies of subgenus *Megabalanus*.

Yamaguchi (1973): Described **species level taxonomy** 0n Japanese megabalanids *B. (M.) rosa* and *B. (M.) volcano* **based on their reproductive isolation**.

Newman (1979): Described **new genus *Megabalanus*** separated from genus *Balanus*.

Geographic Distribution:

Historically known at the East Pacific from Baja California to Ecuador

Known **History of Invasion**:

1980s: **South Brazil**

1987: San Diego, California, USA (Newman & McConnaughey 1987), **Belgium** (Kerckhof & Cattrijsse 2001)

2002～2006s: Louisiana, N. Florida, Georgia, N. Carolina, **East USA**

Iron Carrier *Ship A*

MIZUSHIMA Port, OKAYAMA, JAPAN, Sep. 21, 2007

Sep. 21, 2007 at Mizushima Port

Amphibalanus amphitrite
Amphibalanus eburneus
Amphibalanus improvisus
Amphibalanus reticulatus **(Japan+)**
Austrobalanus imperator **(Australian)**
Balanus trigonus
Chirona amaryllis
Chthamalus challengeri **(Japan+)**
Fistulobalanus albicostatus **(Japan+)**
Lepas anserifera
Megabalanus coccopoma
Megabalanus rosa **(Japan+)**
Megabalanus volcano **(Japan+)**
Tesseropora sp.
Tetraclita japonica **(Japan+)**
Yamaguchiella coerulescens

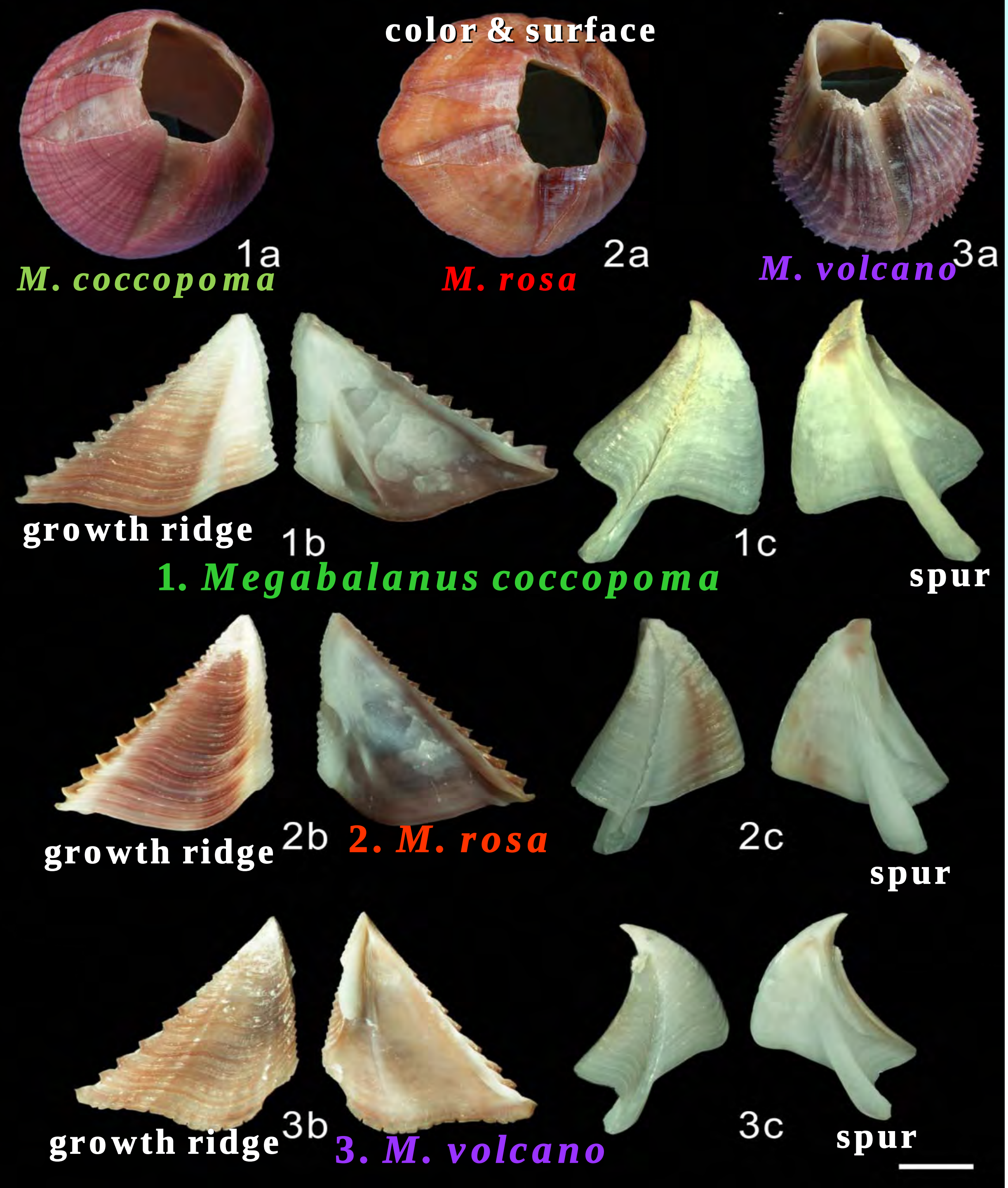
underline: world wide

Jan. 5, 2009 at Chiba Port

Megabalanus coccopoma
Megabalanus rosa **(Japan+)**
Megabalanus volcano **(Japan+)**
Megabalanus occator **(Japan+)**

Cruise data of *Ship A*

Japan				
Dock at Tsu, Mie	4/25/2005	5/4/2005	Port Hedland	W Australia
Mizushima	5/17/2005	6/5/2005	Port Walcott	W Australia
Mizushima	7/1/2005	8/11/2005	Guaiba	Brazil
Mizushima	9/26/2005	10/24/2005	Port Walcott	W Australia
Fukuyama	11/7/2005	11/23/2005	Port Walcott	W Australia
Fukuyama	12/17/2005	1/6/2006	Port Walcott	W Australia
Osaka & Mizushima	2/2/2006	3/5/2006	Gladstone	E Australia
Mizushima	3/24/2006	4/17/2006	Abbot Point	E Australia
Mizushima	5/5/2006	6/2/2006	Gladstone	E Australia
Mizushima	6/15/2006	7/8/2006	Newcastle	E Australia
Kawasaki & Mizushima	7/25/2006	8/13/2006	Robert Bank	Canada
Kawasaki & Fukuyama	9/7/2006	9/28/2006	Gladstone	E Australia
Mizushima & Kawasaki	10/23/2006	11/17/2006	Gladstone	E Australia
Fukuyama	12/9/2006	12/23/2006	Dampier	W Australia
Fukuyama	1/22/2007	2/3/2007	Port Walcott	W Australia
Mizushima	3/10/2007	3/26/2007	Port Hedland	W Australia
Mizushima	4/23/2007	5/6/2007	Port Hedland	W Australia
Mizushima	5/10/2007	6/11/2007	Port Hedland	W Australia
Fukuyama	7/5/2007	7/17/2007	Port Hedland	W Australia
Mizushima	8/20/2007	8/31/2007	Dampier	W Australia



Megabalanus coccopoma was described from the Pacific coast of Panama and is well known as the allied species in the Atlantic Ocean. Yamaguchi *et al.* (2009) is the first report from the western Pacific.

Megabalanus rosa is the endemic species known from Japan since the Miocene.

Megabalanus volcano is also the endemic species known from Japan, Taiwan, Hong Kong. The Holocene fossil was found in Japan.

Shell walls: color and nature of surface are different.
Growth ridges of scutum and spur of tergum are different.

After Yamaguchi et al. (2009)

Information of DNA amplification and sequencing

Polymerase chain reaction (PCR) amplifications of mitochondrial cytochrome c oxidize

COI were amplified partially (383 bp) using ABI 2720 Thermal cycler. A

forward-reverse primer pair and thermocycling protocol,

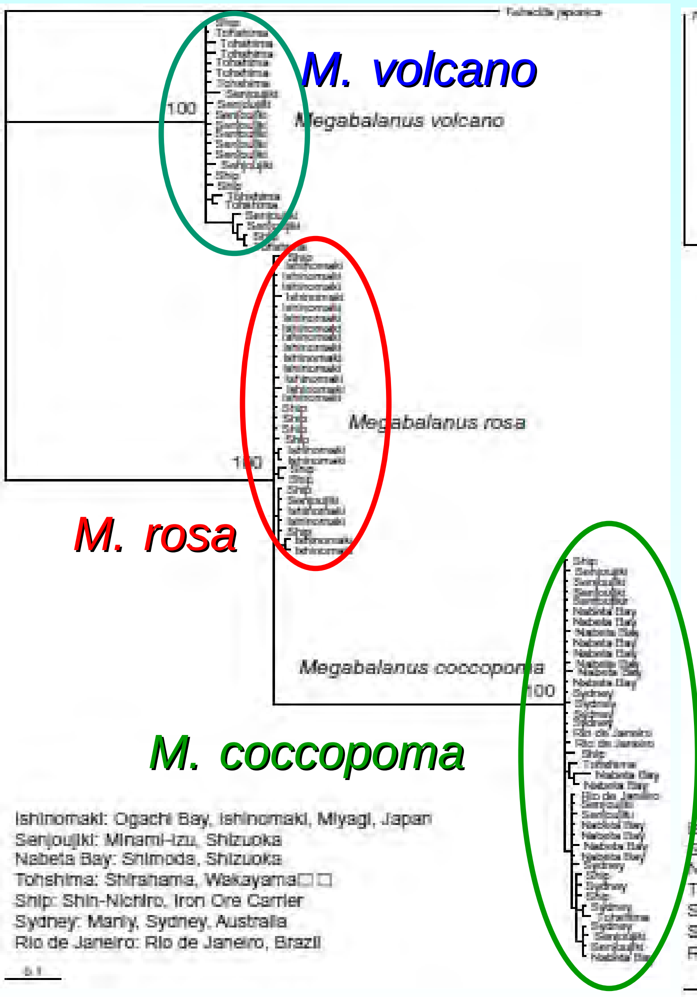
BF2 5'-TGTAATTGTTACTGCTCATGC-3' and

BR2 5'-ACCAAARAAYCAGAATAAGTGTTG-3'

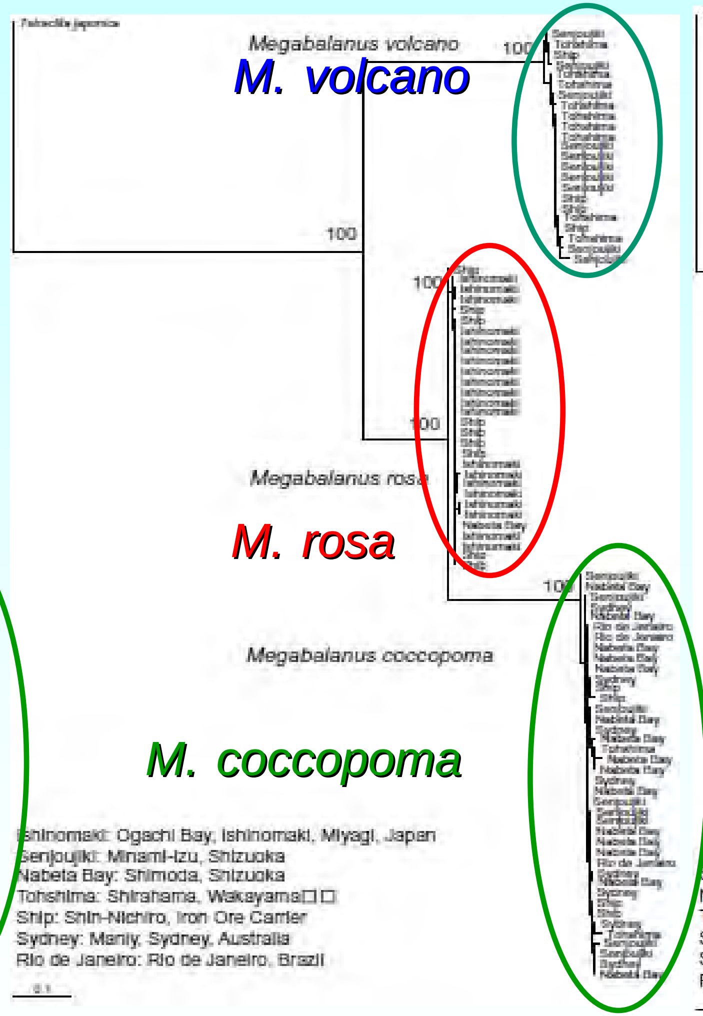
based on **Sotka et al. (2004), was performed at 94°C for 60 s, 40 cycles at 92°C for**

40 s, 52°C for 60 s, 72°C for 90 s and a final extension of 72°C for 7 min.

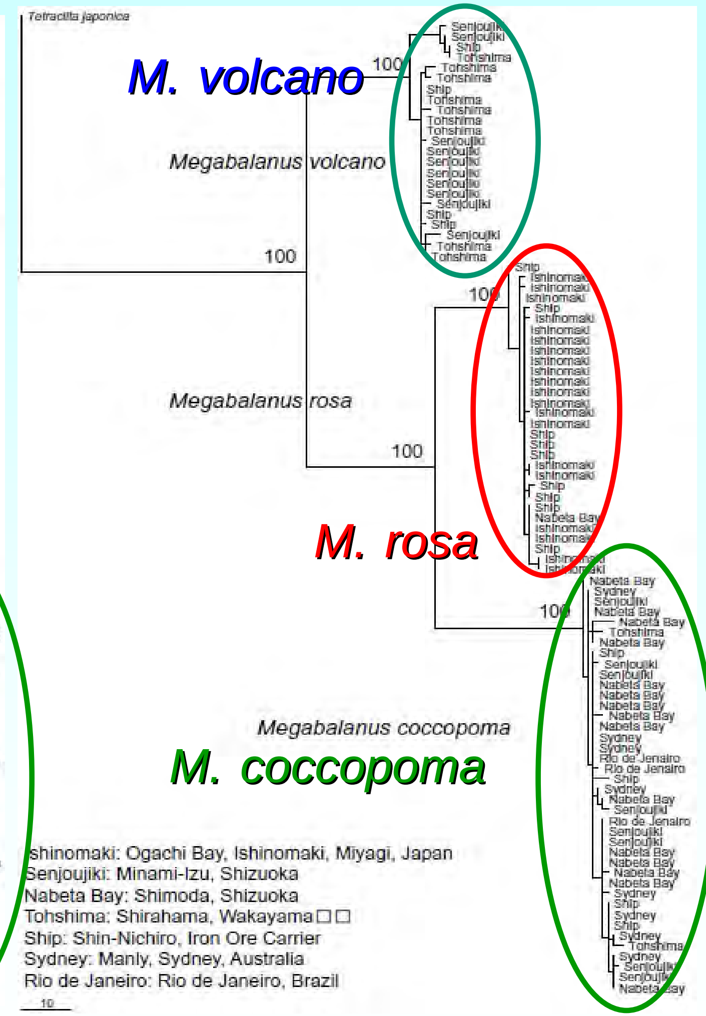
Three phylogenetic trees



Bayesian Inference (BI)



Neighbor Joining (NJ)



Maximum Parsimony (MP)

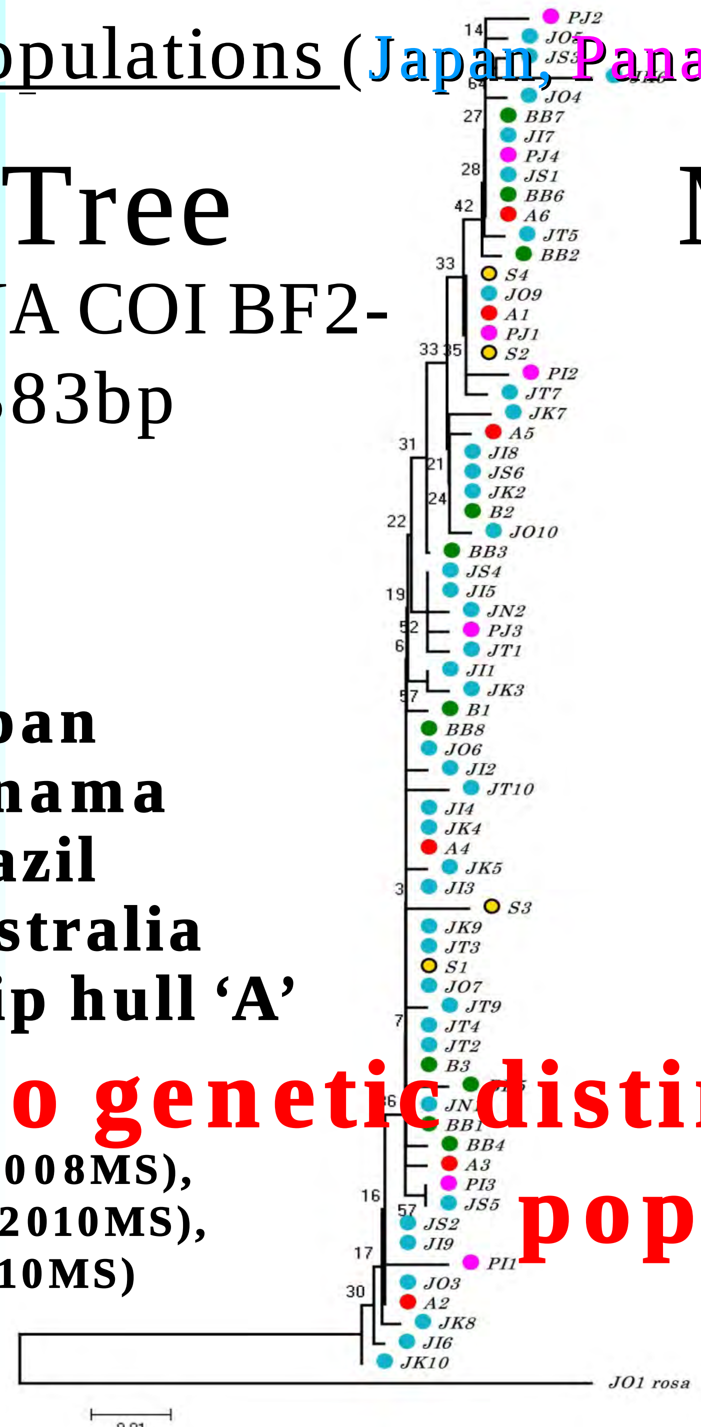
mtDNA COI gene 426 bp

Yamaguchi et al. 2009 Outgroup: *Tetraclita japonica* Pilsbry

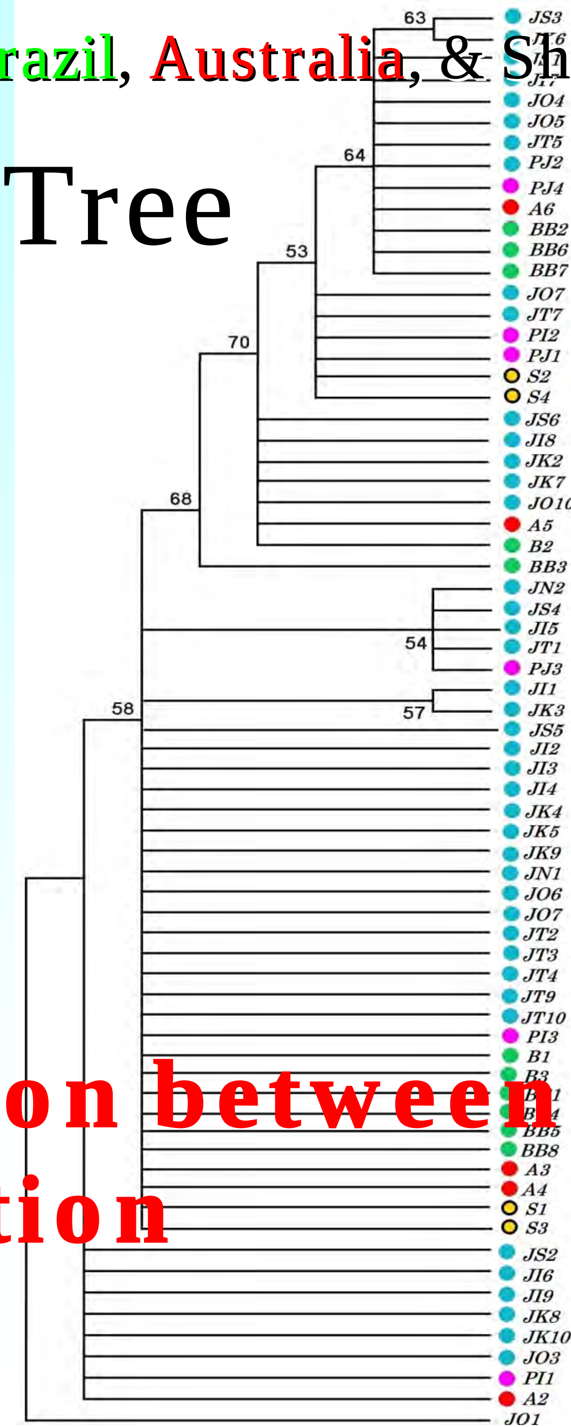
Five Populations (Japan, Panama, Brazil, Australia, & Ship hull 'A')

NJ Tree

mtDNA COI BF2-BR2 383bp



ML Tree



Japan

Panama

Brazil

Australia

Ship hull 'A'

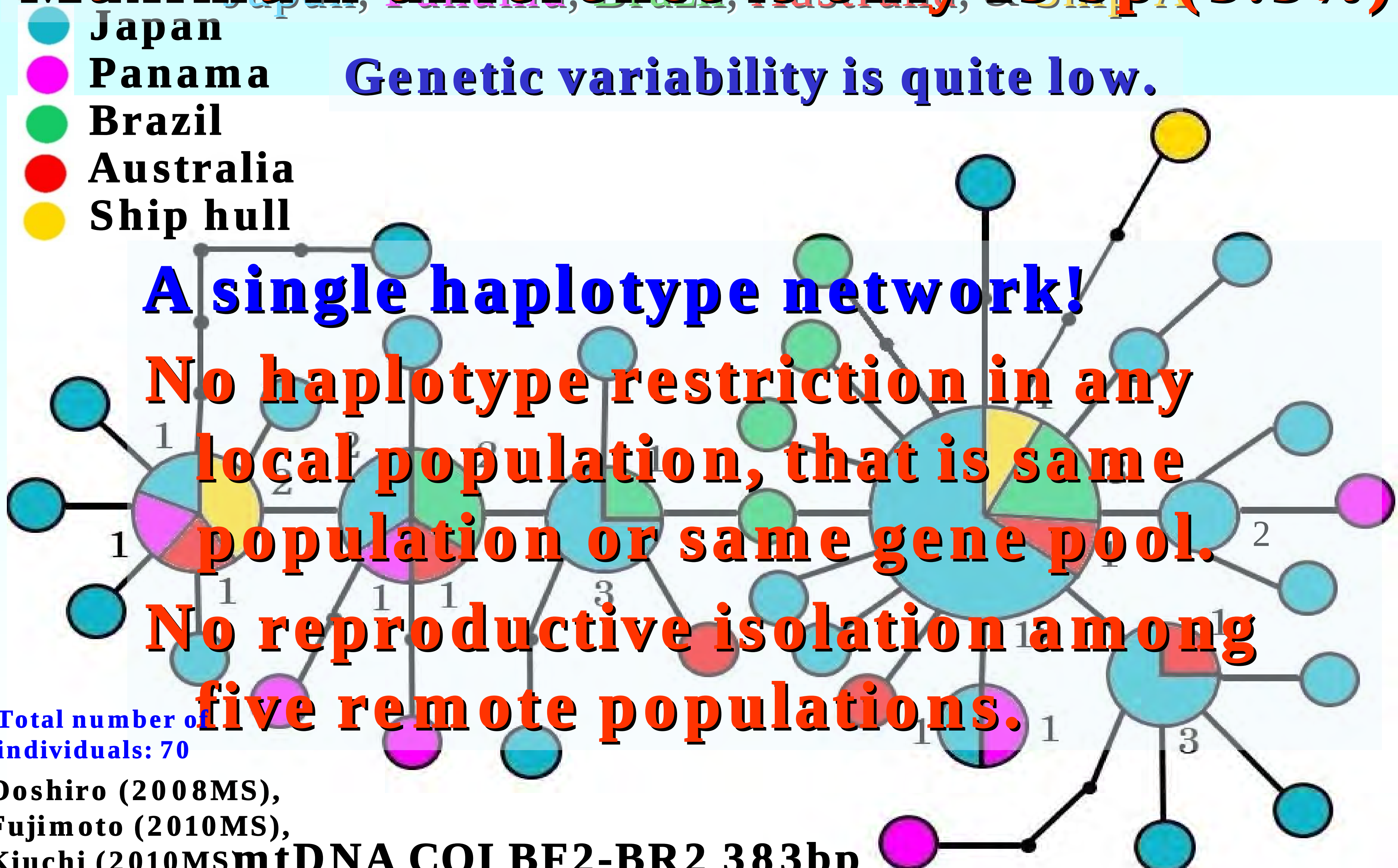
No genetic distinction between any population

Ooshiro (2008MS),
Fujimoto (2010MS),
Kiuchi (2010MS)

Haplotype networks among five populations of
Japan, Panama, Brazil, Australia, & Ship A

Maximum difference is only 13 bp (3.3%).

Genetic variability is quite low.



A single haplotype network!

No haplotype restriction in any local population, that is same population or same gene pool.

No reproductive isolation among five remote populations.

Total number of individuals: 70

Ooshiro (2008MS), Fujimoto (2010MS), Kiuchi (2010MS),

mtDNA COI BF2-BR2 383bp

Life history: planktic and sessile stages

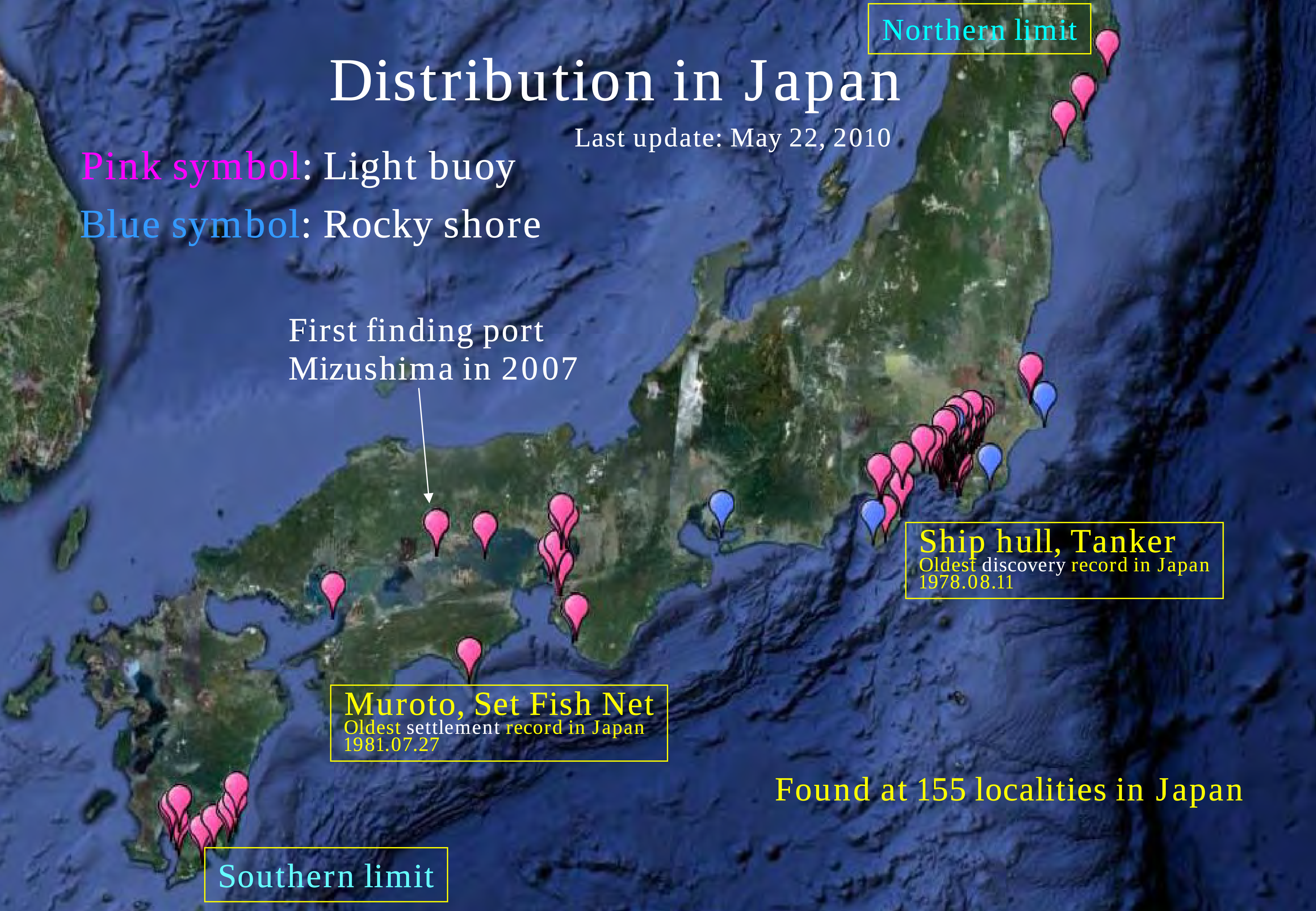
Migration in sessile stage is depend on moving of ships or floating materials as substrata of barnacles.

Larvae: Planktic
Natural dispersal

Juveniles to Adults: Sessile
No migration by themselves, but transfer with movement of substratum, such as ship hull

Image of life history is from HP of Professor K. Okano, Akita University.

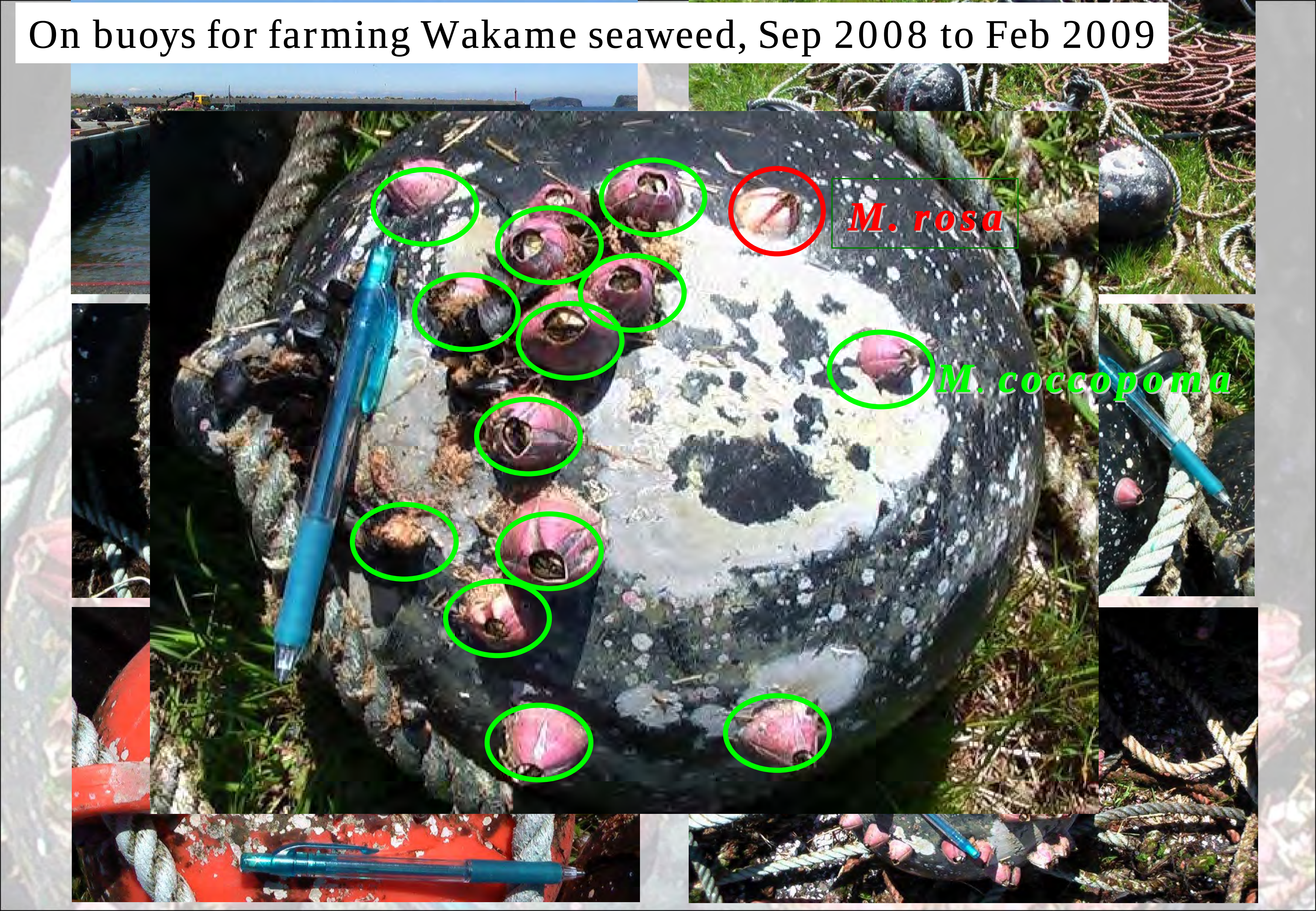
Panama Canal
Panamá
20080917-01
Isla Taboga, Panama
20080918-01
Taboga
Isla Taboga, Panama
Isla Taboga, Panama



Distribution in Japan
Last update: May 22, 2010
Pink symbol: Light buoy
Blue symbol: Rocky shore
Northern limit
First finding port
Mizushima in 2007
Ship hull, Tanker
Oldest discovery record in Japan
1978.08.11
Muroto, Set Fish Net
Oldest settlement record in Japan
1981.07.27
Found at 155 localities in Japan
Southern limit


Pink symbol: Light buoy
Blue symbol: Rocky shore
Tokyo Bay
Sagami Bay
Pacific Ocean
Image © 2010 Digital Earth Technology

On buoys for farming Wakame seaweed, Sep 2008 to Feb 2009



Life history and Ecology

Reported by: J. Masterson, Smithsonian Marine Station, Page last updated: December 1, 2007

Temperature: Cold temperate to tropical

Salinity: High saline to brackish (Kerckhof 2002)

Growth: Rapid growth (Tibbetts 2007)

Our understanding:

General characteristics above menti0ned are identical with those of other invasion species.

No report on Reproduction and larvae development. However, we can see juveniles on light buoys of Tokyo Bay in a whole year, unlike *M. rosa* in winter and *M. volcano* in summer in Japan.

Megabalanus coccopoma is naturalized in Japan.

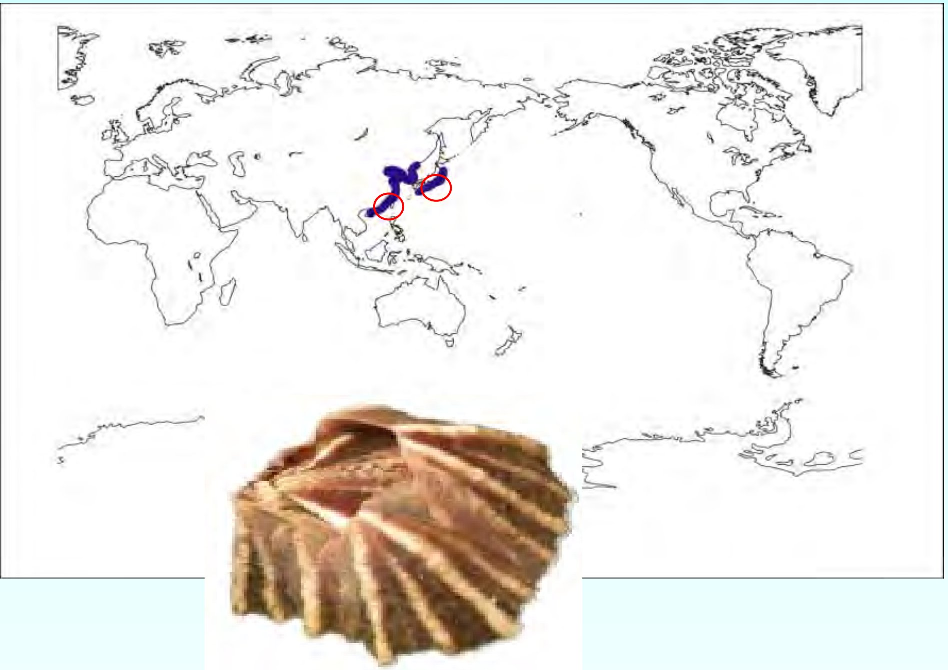
Balanus amphitrite complex
including genera *Amphibalanus* & *Fistulobalanus*

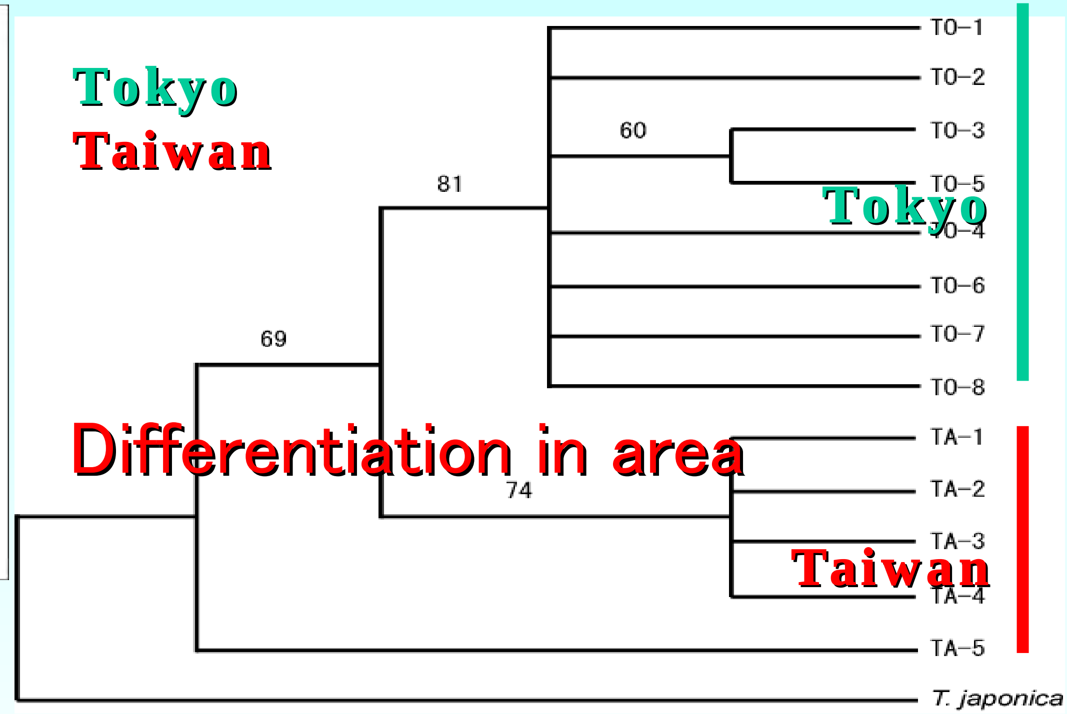
Amphibalanus amphitrite is an introduced species well known in whole world.

The other are the endemic at SE Asia of Indo-West Pacific.

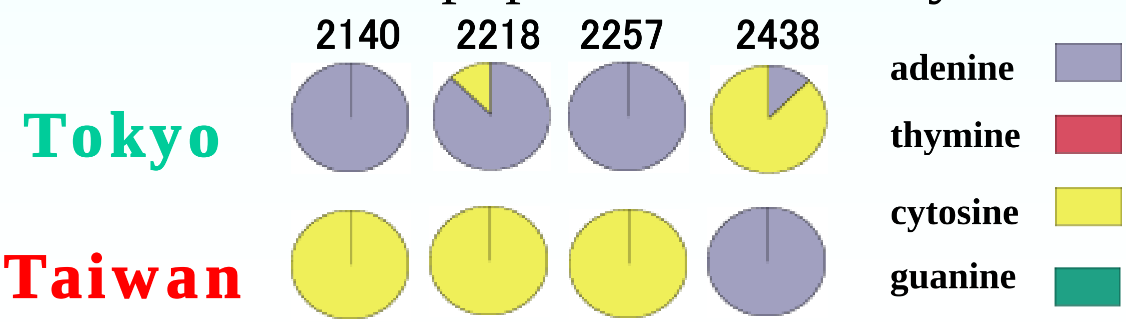
Native *Fistulobalanus albicostatus*

Genetic comparison among 2 populations using COI gene 405 bp





There are four differences (sites) of substitution between two local populations of Tokyo and Taiwan.

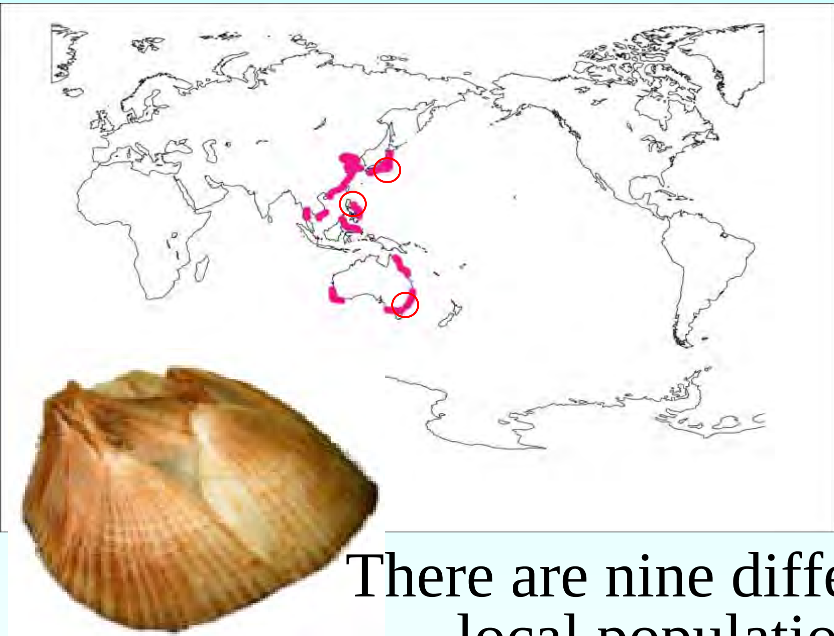


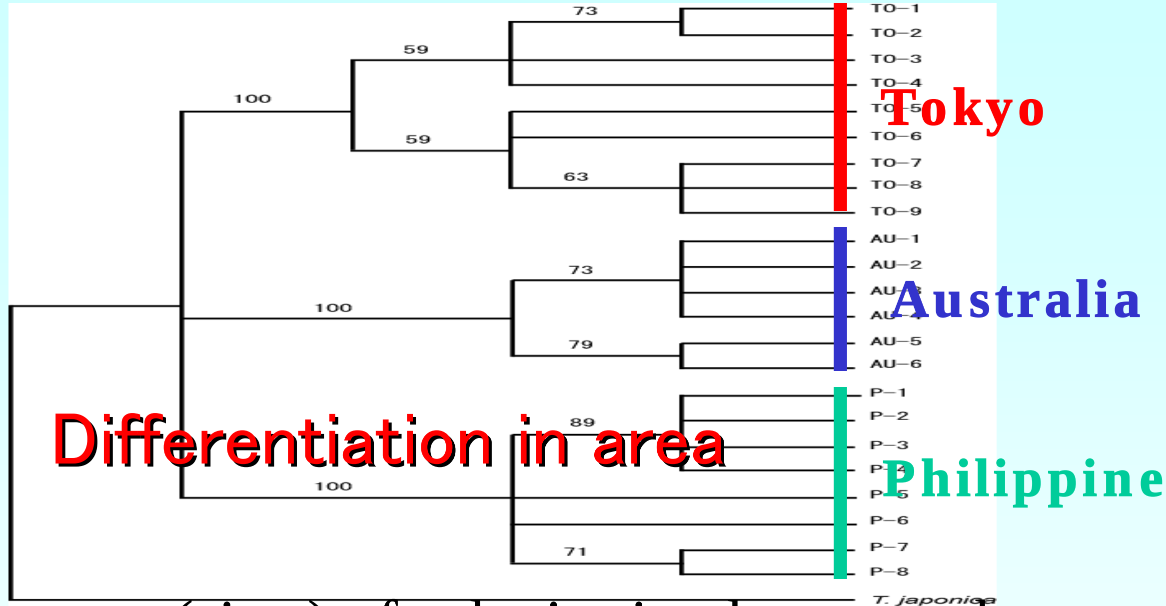
The four substitutions separated two local groups.

Unpublished data by my student S. Inagawa

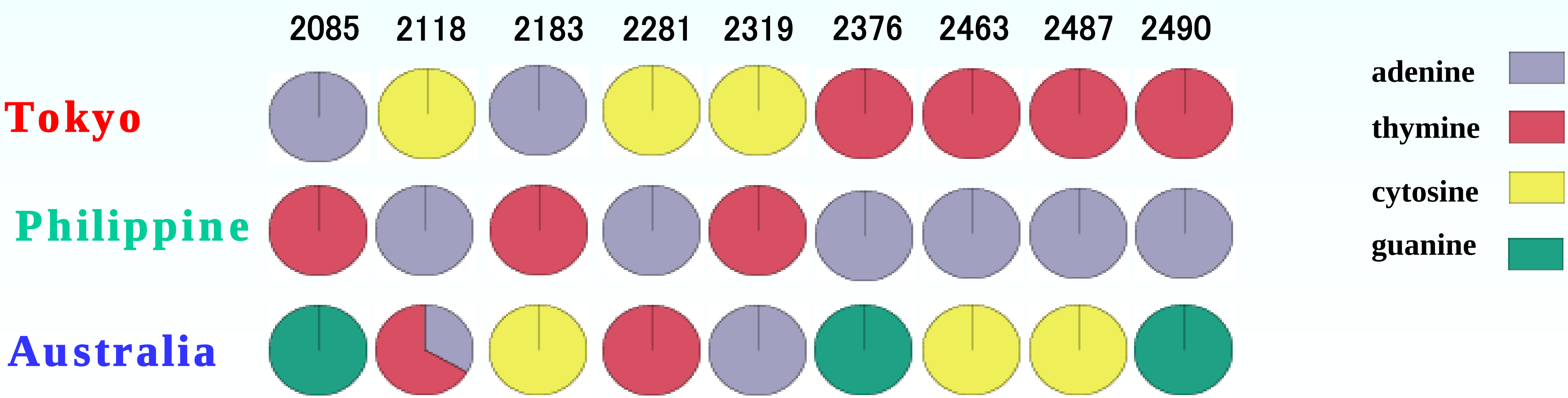
Native *Fistulobalanus kondakovi*

Genetic comparison among 3 populations using COI gene 433 bp





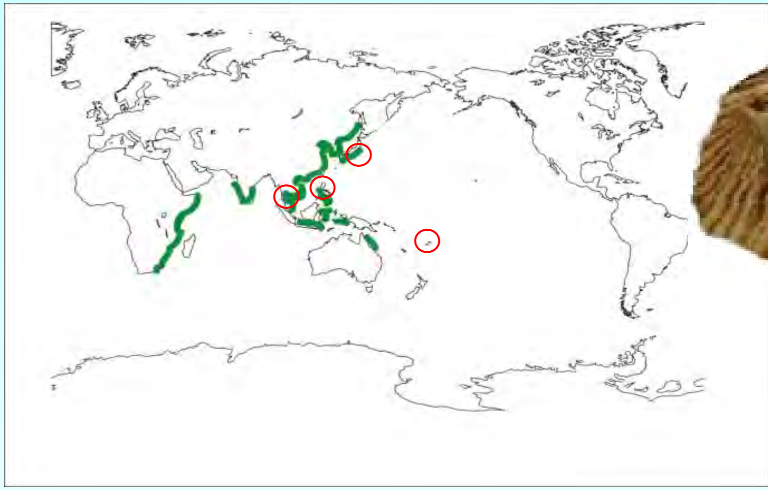
There are nine differences (sites) of substitution between three local populations of Tokyo, Philippine, and Australia.

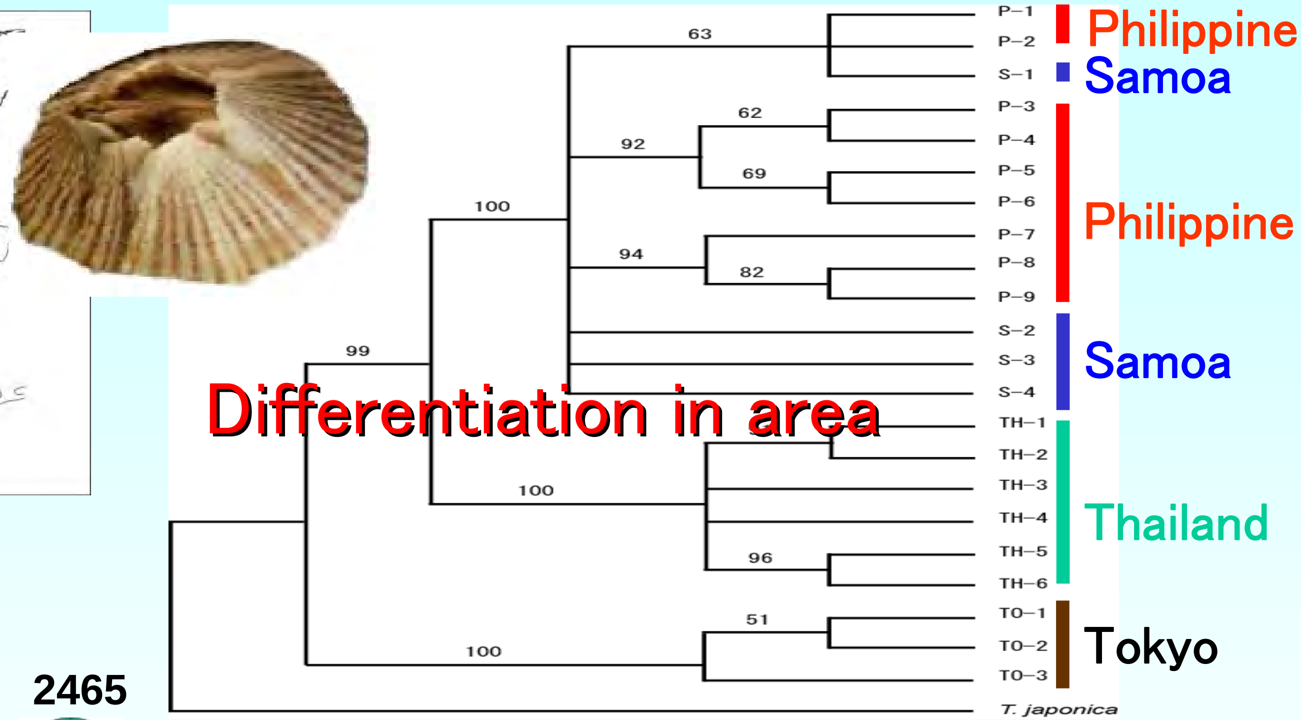


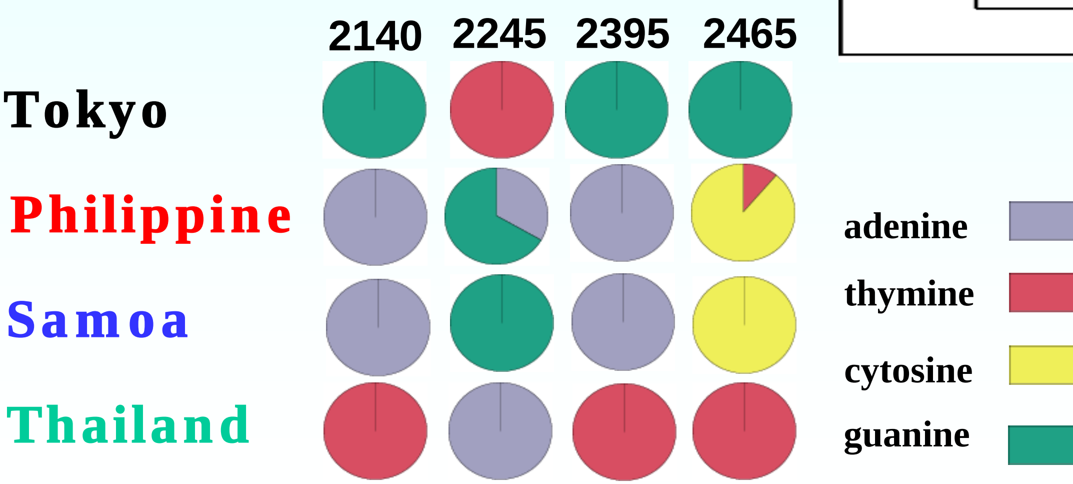
Unpublished data by my student S. Inagawa

Native *Amphibalanus reticulatus*

Genetic comparison among 4 populations using COI gene 407 bp





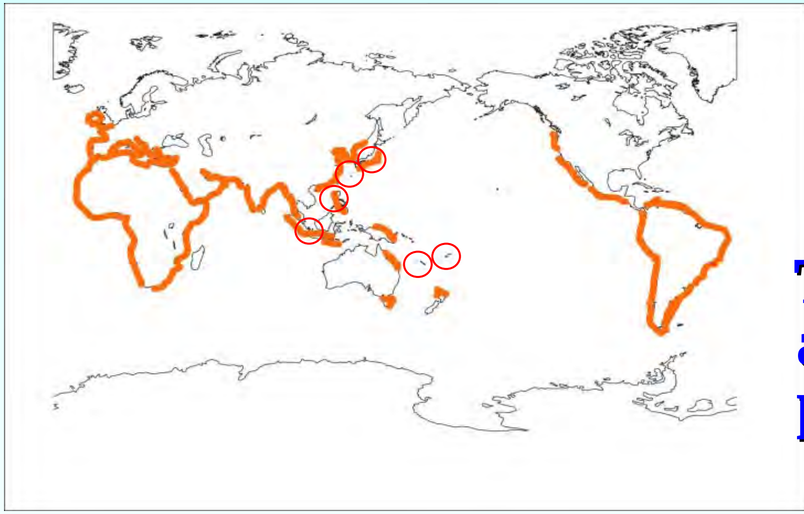


Four local populations are separated to three groups of Tokyo, Thailand, and group of Philippine and Samoa by four substitution.

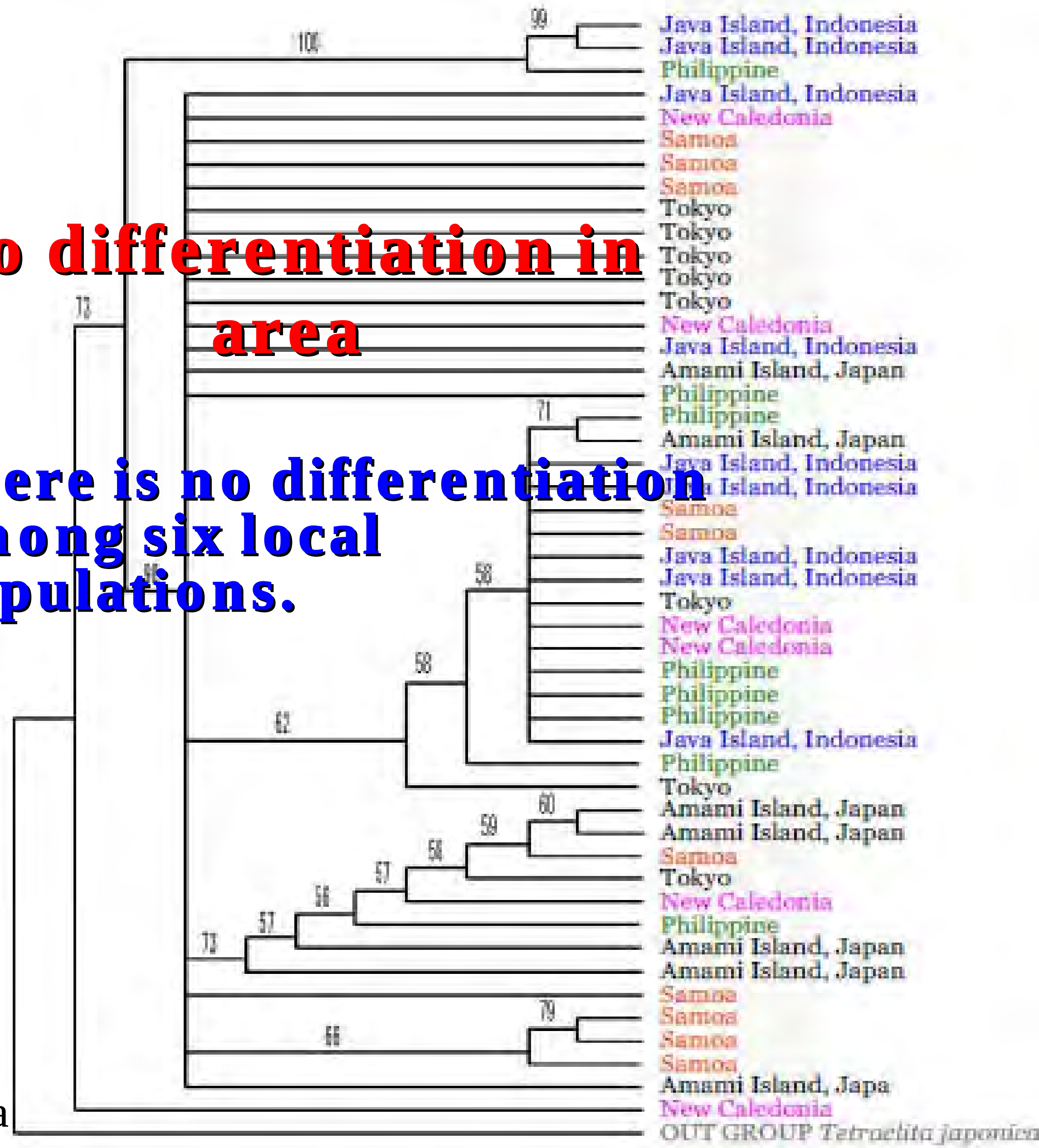
Unpublished data by my student S. Inagawa

Introduced species *Amphibalanus amphitrite*

Genetic comparison among <u>6 populations</u> using COI gene 384 bp



Amami Island, Japan
Tokyo, Japan
Java Island, Indonesia
New Caledonia
Philippine
Samoa



No differentiation in area

There is no differentiation among six local populations.

Unpublished data by my student S. Inagawa

Conclusion

In spite of the vast geographical distances between five populations of Panama, Brazil, Australia, Japan and Ship hull, the haplotype analysis of *M. coccopoma* suggests that there is single haplotype network. There is no haplotype restriction in any local population.

All populations of *M. coccopoma* examined seem to be same population or same gene pool.

The genetic characteristics of *M. coccopoma* seems to be keeping by the multiple-introduction and transportation of a large number of barnacles by ships.

M. coccopoma has bee naturalized in Japan now.