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**Molecular phylogenetics and population structure derived from
mitochondrial DNA sequence variation in the edible goose
barnacle genus *Pollicipes* (Cirripedia, Crustacea)**

Van Syoc, Robert Jay, Ph.D.

University of California, San Diego, 1994

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

UNIVERSITY OF CALIFORNIA, SAN DIEGO

Molecular Phylogenetics and Population Structure Derived from
Mitochondrial DNA Sequence Variation in the Edible Goose Barnacle
Genus Pollicipes (Cirripedia, Crustacea)

A dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy in Marine Biology

by

Robert J. Van Syoc

Committee in Charge:

Professor William A. Newman, Co-chair
Professor Margo G. Haygood, Co-chair
Professor Ronald S. Burton
Professor Nicholas Holland
Professor Richard H. Rosenblatt
Professor Christopher Wills

1994

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The dissertation of Robert Jay Van Syoc is approved, and it
is acceptable in quality and form for publication on microfilm:

Richard H. Rosenblatt

Ronald S. Burton

Nicholas D. Holland

Christy Wills

Margo G. Haygood

Co-Chair

William D. Murawski

Co-Chair

University of California, San Diego

1994

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VITA

Robert Jay Van Syoc

Department of Invertebrate Zoology & Geology
California Academy of Sciences
Golden Gate Park
San Francisco, CA 94118-4599

Phone: 415-750-7082 FAX: 415-750-7090
email: bvansyoc@calacademy.org

EDUCATION AND DEGREES:

University of California, San Diego, Scripps Institution of
Oceanography Sept. 1988-March 1994
Ph. D. in Marine Biology March 1994

San Francisco State University Jan. 1977-May 1983
B.A. in Marine Biology May 1978
M.A. in Marine Biology May 1983

University of Michigan Sept. 1969-May 1973
B.A. in Journalism May 1973

GRANTS AND FELLOWSHIPS:

University of California: Regents Fellow, 1988-89.

Pauley Foundation: Crustacean DNA research at Univ. of
Hawaii, 1989.

National Science Foundation: CAS collection/facilities grants,
Co-principal investigator, 1985-
present.

PROFESSIONAL APPOINTMENTS:

California Academy of Sciences: Collection Manager, 1983-
present
Invertebrate Zoology and Geology Curatorial Asst., 1979-1983

University of California, San Diego:	Teaching Asst., (Genetics, Evolution, General Biology) 1990-91
San Francisco State University:	Lecturer in Biology, 1978-79
Association of Systematics Collections:	Staff Asst. and Report Editor, Council on Curatorial Methods, voucher specimen conference, 1981

PUBLICATIONS:

- 1981. A conference on voucher specimen management. ASC Newsletter 9(2): 26-27.
- 1981. Voucher specimen conference progress report. ASC Newsletter 9(3):46.
- 1986. The occurrence of *Lepas anatifera* on *Mirounga angustris* and *Zalophus californica*. California Department of Fish & Game Bulletin 72(2):124-126. (with Jan Roletto.)
- 1988. Description of *Membranobalanus robinae*, a new species of sponge barnacle from Baja California, with a key to the genus. Proceedings of the Biological Society of Washington. 101(4): 832-837.
- 1992. Living and fossil populations of a western Atlantic barnacle, *Balanus subalbidus* Henry, 1974, in the Gulf of California region. Proc. San Diego Soc. Nat. Hist. 12:1-7.
- 1992. Genetic divergence between reproductive types in northern and southern populations of the edible goose barnacle, *Pollicipes*. p.210-211. In: California Sea Grant Biennial Report of Completed Projects, 1988-1990. (with W. A. Newman)
- 1993. PCR primers for the CO1 gene in barnacle mtDNA. Newsl. Crust. Mol. Tech. 4:1-2.
- 1994. The barnacles of Rocas Alijos. In: R. Schneider (ed.) The Rocas Alijos. in press.
- In prep. A molecular phylogeny of the goose barnacle genus *Pollicipes*: zoological remnants of the Tethys Sea. In: F. Schram

and J. Hoeg (eds.), *New Frontiers in Barnacle Biology*. A. A. Balkema, Rotterdam, Netherlands.

WORKSHOPS AND MEETINGS ATTENDED:

- 1981. Association of Systematics Collections and National Science Foundation sponsored Conference on Voucher Specimen Management. University of Maryland Conference Center.
- 1984. Smithsonian Institution sponsored workshop on Developing, Managing and Maintaining Collections. Washington, D.C.
- 1986. Western Society of Naturalists. Annual Meeting, Hilo, HI.
- 1988. Association of Systematic Zoologist, Crustacean Society, Western Society of Naturalists, Joint Meeting, San Francisco, CA.
- 1990. Colloquium on benthic macro-crustaceans of the Eastern Tropical Pacific. Universidad Nacional Autonoma de Mexico, Mazatlan, Mexico.

FIELD TRIPS AND EXPEDITIONS:

- 1981-present: California coast including several SCUBA surveys of the Channel Islands sponsored by the Tatman Foundation
- 1984 and 1985: Baja California, joint CAS/CISESE Expeditions
- 1985: Kingdom of Tonga, CAS Expedition
- 1985: Canada, Vancouver Island, CAS Expedition
- 1985: California, Gulf of the Farallones, U.S.E.P.A. dump site survey
- 1986: Solomon Islands, CAS Expedition
- 1987: Baja California, Islas San Benito, San Martin and Cedros, CAS/SIO Expedition
- 1987: Republic of Palau (Belau), CAS Expedition
- 1988: Intertidal biological survey of San Clemente Island, sponsored by Los Angeles County
- 1988: Philippines and East Malaysia, CAS Expedition
- 1989: California Borderland Basin studies, SIO Expedition
- 1989: Hawaii
- 1990: Baja California, Rocas Alijos, Sponsored by Cordell Expeditions

ABSTRACT OF THE DISSERTATION

Molecular Phylogenetics and Population Structure Derived from
Mitochondrial DNA Sequence Variation in the Edible Goose Barnacle
Genus Pollicipes (Cirripedia, Crustacea)

by

Robert Jay Van Syoc

Doctor of Philosophy in Marine Biology
University of California, San Diego, 1994

Professor William A. Newman, Co-chair
Professor Margo G. Haygood, Co-chair

Individuals within a geographically widespread species may face considerably different environmental and ecological conditions depending on which part of the range they inhabit. This is especially true for species with long latitudinal distributions. Natural selection pressure may lead to genetic divergence and eventual speciation if the homogenizing effects of broad based gene flow are insufficient to prevent it. Therefore, clinal variation may be due either to local environmental influence on gene expression or to natural selection and reduced gene flow at the edges of the distribution.

Mitochondrial DNA sequence data and morphological characters for three species of edible goose barnacles are compared in the examination of three situations which exhibit varying

degrees of potential gene flow. The first of these is possible genetic clinal variation within an apparently continuous population of Pollicipes polymerus, having a 3,300 km latitudinal distribution. The second is genetic divergence between seemingly geographically separate sub-populations of a congener, Pollicipes elegans, with a 4,400 km latitudinal distribution. The last question concerns the genetic relationships between these two eastern Pacific Ocean species, and a third, geographically isolated congener from the eastern Atlantic Ocean, Pollicipes pollicipes.

Surprisingly, the present data do not reveal a genetic discontinuity or latitudinal gradient in Pollicipes polymerus, despite the fact that the distribution of this species crosses a major marine biogeographic boundary between the Oregonian and Californian faunal provinces. This finding contradicts the hypothesis that differing reproductive types north and south of Pt. Conception are due to reduced gene flow and genetic divergence.

The disjunct (paramphitropical) sub-populations of Pollicipes elegans have a net nucleotide sequence divergence of about 1.2%. Calculated estimates for the timing of this genetic divergence range from 292,500 to 1,260,000 years before present. A divergence of this magnitude coincides with Pleistocene Epoch cooling periods and the narrowing or disappearance of the warmer sea surface temperatures forming the north equatorial barrier between present day sub-populations. This "ice age" timing for genetic interchange is not unexpected. However, the present data offer evidence for multiple transgressions of the equatorial barrier in both directions,

rather than a single limited exchange.

Pollicipes, represented by three extant species, has a Tethyan distribution. Curiously, Pollicipes elegans, from the eastern Pacific, is more similar to Pollicipes pollicipes, the eastern Atlantic species, than it is to P. polymerus from the northeastern Pacific. Estimated time of genetic divergence for these two species is about 37 million years before present, near the Eocene/Oligocene boundary, when the Tethys Sea was uninterrupted and the Atlantic was significantly narrower than it is today.

CHAPTER 1

General Introduction

The modern synthetic theory of evolution was born in the 1930's and 1940's when the "naturalist" theory of evolution via small, gradual changes in species, was effectively combined with experimental genetics and Gregor Mendel's rediscovered genetic observations (Huxley 1942, see Mayr 1982 for a recent historical review). Until this time, the naturalists, enlisting only parts of Charles Darwin's (1859) theory of natural selection, had considered that speciation could only occur gradually, as natural selection acted upon, or even caused, small morphological variations among geographically isolated individuals of a species. In the early days of experimental genetics, de Vries and Morgan had argued that saltational genetic mutation alone, resulting in discontinuous and sudden changes in phenotype, could cause speciation. Only when the geneticists came to appreciate the importance of genetic diversity within and among populations and the potential effect of differential selection in local environments on this diversity did the naturalists accept the fact that genetic mutation and subsequent inheritance play an important role in speciation and evolution.

The synthesis led to consideration of the effect of gene flow within and among populations on the speciation process. Subsequently, Sewell Wright (1931, 1932), Dobzhansky (1937) and Simpson (1944) noted the importance of limited gene flow or genetic isolation and genetic drift as well as natural selection in

producing genetic divergence and speciation events.

Today, phylogenies are built and population structures examined through studies of genetic variability by analyses of variation in morphological, physiological and molecular level attributes of organisms. These attributes, along with biogeographic patterns, are often used to determine distinct populations or groups (races, varieties or subspecies) within species, as well as evolutionary relationships among species. Environmental influences on gene expression can result in broad variation in organismal attributes which may be used to identify and classify species (e.g. barnacle morphology, Van Syoc 1992). The study of within-species variation in relation to common ancestry has been termed population systematics.

However, attributes used to classify and categorize organisms may or may not be directly related to lines of descent. For example, convergent or parallel evolution can produce similarities in physical characters for unrelated taxa (e.g. Gosliner and Ghiselin 1984, parallelism in the mollusca). Recognizing the potential difficulties in resolving questions of genetic relationships and descent solely by analysis of phenotypic characters, the present work uses molecular data to augment morphological and physiological evidence. Three different situations are examined, each with a different degree of gene flow, with the potential for genetic divergence due to natural selection and genetic drift. They are as follows:

- 1) Possible latitudinal clinal variation within an apparently

continuous population having a long latitudinal distribution (Pollicipes polymerus, Chapter 3).

2) Genetic divergence between seemingly geographically separate subpopulations of a congener with a long latitudinal distribution (Pollicipes elegans, Chapter 4).

3) Genetic divergence between these two species and a third geographically distant congener (Pollicipes pollicipes, Chapter 5). The three species are obviously different but exhibit a potential conflict between evolutionary relationships as judged by existing morphological similarities and those based upon geographic proximity.

To accomplish these analyses on barnacles, I developed molecular tools which could be used effectively to examine all three levels of genetic variation. Chapter Two reviews general molecular techniques commonly used for the study of genetic divergence in barnacles as well as other taxa. It also summarizes my search for appropriate methods and materials, and development of new molecular tools specifically designed for working with crustacean DNA. The remaining chapters deal with various applications of the newly developed materials and methods.

Specifically, Chapter Three makes molecular genetic comparisons within one barnacle species (Pollicipes polymerus) to determine the genetic distance between potential clinal genetic variants of a continuous population having different reproductive cycles (Cimberg 1981) on either side of a major latitudinal transition zone between faunal provinces of the northeastern

Pacific. Cimberg (1981) hypothesized that these differences in reproductive physiology are due to natural selection, promoting or at least contributing to genetic divergence between populations at the extremes of the latitudinal range. In other words, gene flow within the unbroken range of P. polymerus may be influenced by selection for and against particular genotypes related to brooding.

Chapter Four compares sub-populations of a related but distinct amphitropical species, Pollicipes elegans, which are more clearly geographically isolated from each other on opposite sides of the equator in the tropical eastern Pacific. Here gene flow may be disrupted by environmental impediments to dispersal and colonization, with genetic drift and possible founder effects causing measurable divergence between the sub-populations.

In Chapter Five the analysis is extended to include a species which is morphologically very similar to the latter, Pollicipes pollicipes, isolated from the previous two species by the width of the Atlantic Ocean as well as the land barrier formed by the Isthmus of Panama. Despite the morphological similarities among the three species of Pollicipes, the separation of the eastern Pacific and eastern Atlantic species has likely existed for many millions of years, since Pollicipes is not known to have been part of the tropical marine fauna surrounding Panama and the Caribbean (Newman, et al. 1969, Southward and Newman 1977, Donovan and Davis-Strickland 1993, Quintas pers. comm.). Therefore, the present distributions of the barnacles are in conflict with morphological evidence and further explanation for them is sought through

historical biogeography and genetic distance data.

Chapter Six summarizes the findings presented in the previous chapters and concludes with a brief discussion of the study of oceanic biodiversity.

CHAPTER 2

Molecular techniques and methodology for determining genetic variation and building gene phylogenies

2.1 Molecular techniques

2.1.1 Overview

Today's scientist can study variation with a wide array of molecular tools. Protein analyses employ immunological tests and protein electrophoresis to examine variation in proteins. DNA analyses utilize DNA-DNA hybridization, restriction fragment polymorphisms (RFLPs), random amplified polymorphic DNA analyses (RAPDs) and DNA or RNA sequencing to study variation. Each of these methods has earned a place in the molecular tool kit of modern evolutionary biologists. Choice of a particular technique generally depends on the question posed and the potential level of genetic divergence within and between the subject groups (Hillis and Moritz 1990).

Protein analyses examine the expressed products of genes coded for by nucleotide sequences. Such products are subject to the pressures of natural selection on the individuals bearing them. Theoretically, this would potentially eliminate the proteins from use as neutral markers of gene flow and phylogeny. In contrast, DNA analyses offer the promise of direct examination of the genome for both "neutral" changes and those "constrained" by natural selection. In practice, however, enzyme variability has often been an effective tool in the study gene flow within and among populations of barnacles and the study of local or regional

genetic variation (Hedgecock 1979, Flowerdew 1983, Laguna 1988).

Electrophoresis of proteins offered one of the first biochemical techniques for determining the genetic distance between populations or species (Lewontin and Hubby 1966). This method can help delimit population genetic structure and biogeographic patterns (Southward 1983, Piper 1984, Huber 1985, Burton 1986, Laguna 1988, Rosenblatt and Waples 1986, for example).

Protein electrophoresis has several advantages for population studies. Foremost among these are speed of sample processing and small expense when compared to other methods. One can run a large number of individual samples and examine a large number of gene loci relatively cheaply. In addition to being less expensive than DNA work, the ability to look at a large number of samples quickly enables an analysis of various gene frequencies both within and between population segments.

Protein electrophoresis results are visualized as a pattern of bands from fast and slow running alleles. These bands represent changes in amino acid sequence that change either the size or the net electrical charge of the molecule. Therefore, protein electrophoresis can detect size variations resulting from amino acid substitutions and changes in the net polarity as produced by the additive charges of any of the five charged amino acids. Because only nucleotide substitutions which result in amino acid changes (non-synonymous substitutions) can be detected, synonymous base changes can not be detected by protein electrophoresis. Therefore,

some levels of genetic change can not be studied with protein electrophoresis.

In some circumstances, sequencing of homologous nucleic acid fragments can be a more appropriate method of obtaining information on the genetic variation within and between species. This was the method of choice in the present study, which concerns the phylogenetic relationships of species with a potentially high degree of genetic divergence and requires the study of preserved material. As with enzyme electrophoresis, the results can be quantified and used to calculate gene flow and amount of divergence or "evolutionary distance" (Nei 1987, Hudson, et al. 1992).

2.1.2 Methods of obtaining data for DNA sequence comparisons

Individual nucleotide sequencing is not the only way to collect comparative sequence data for analysis of genetic variation, molecular evolution, statistical manipulation and phylogenetic tree building. DNA-DNA hybridization or restriction endonuclease fragment patterns and site maps are other popular techniques for DNA sequence comparison.

Various vertebrate populations have been examined and phylogenies have been constructed with data from DNA-DNA hybridization melting points (Benveniste and Todaro 1976, humans; Hoyer, et al. 1972, hominids; Bonner, et al. 1980, primates; Sibley and Alquist 1984, birds). The great advantage of this technique is in employing the entire genome, or a very large part of it, in the

analysis. Although hybridization has provided relatively good data for estimation of genetic distance, it gives only a single datum point (the DNA hybridization melting temperature) per species pair. A few researchers have applied this method to invertebrates (Hall, et al. 1980, sea urchins; Hunt, et al. 1981, fruit flies; Palumbi and Metz 1991, sea urchins).

Much of what we know about mitochondrial DNA variation in natural populations comes from comparisons of restriction site maps or restriction fragment patterns on electrophoretic gels. This information allows for phylogenetic analysis and study of population dynamics (Brown and Simpson 1981, Ferris, et al. 1981, Templeton 1983, Wilson, et al. 1985, Wu and Li 1985, DeSalle, et al. 1986, Cann, et al. 1987, Hale and Singh 1987, Shields and Wilson 1987, Bowen, et al. 1989). Although algorithms have been developed which allow analysis of RFLP patterns (Nei 1987), restriction length polymorphisms can't demonstrate the details of nucleotide substitutions. These details, transitions vs. transversions and synonymous vs. nonsynonymous changes, are often of interest.

In addition, restriction endonucleases recognize only specific four or six base sequence sites and therefore they only test for the presence or absence of those sites. So, the actual number of nucleotides sampled may be small (four or six multiplied by the number of fragments per genome), and of ambiguous location.

As noted above, sequencing homologous DNA fragments offers larger data sets for analysis. It is also less susceptible than DNA hybridization or RFLPs to problems stemming from repetitive

sequences (usually, but not always, in non-coding regions).

Repetitive sequences should be much less of a problem when using the mitochondrial genome rather than the nuclear genome.

A disadvantage in the application of nucleotide sequencing data for population studies is that it can be much more expensive and time consuming than RFLP or protein electrophoresis studies. This factor tends to limit the number of individuals sampled within and among populations. Protein electrophoresis studies routinely sample dozens or hundreds of individuals from each sub-population. It would be extremely costly and labor intensive to acquire a similar amount of nucleotide sequence data.

2.1.3 Mitochondrial DNA

It is generally recognized that the mitochondrial DNA molecule offers a relatively simple genetic marker for studies of evolution and biogeography (Wilson et al. 1985). The animal mitochondrial genome is small (often about 16,000 nucleotides), almost always circular and often can be quickly and easily extracted from a few micrograms of tissue. Although the arrangement of genes varies between major taxa, the molecule always contains genes for two rRNAs, 22 tRNAs, and thirteen proteins (Brown 1983, Wilson et al. 1985). Nuclear chromosomal DNA can also be extracted quite easily, but the size and number of the molecules involved may present researchers with a more complex task when attempting to isolate homologous sequences for comparison.

Although the polymerase chain reaction (PCR, see following

section) allows homologous nuclear DNA sequences to be targeted with specific oligonucleotide primers and amplified to swamp out any other fragments, the mitochondrial genome copy number is much higher than most nuclear genes. This high copy number simplifies the process of successfully amplifying homologous fragments to generate nearly pure samples of template DNA for nucleotide sequencing or RFLPs. Furthermore, whereas nuclear DNA is represented in the diploid condition (usually) and is subjected to recombination during sexual reproduction or by crossing-over in meiosis, the haploid mtDNA molecule is almost always acquired solely via egg cytoplasm (Birky, et al. 1983, Wilson, et al. 1985).

In addition, there is evidence that mtDNA is more sensitive than nuclear DNA for tests of recent genetic divergence (Avise, et al. 1979). The mitochondrial genes of most vertebrates evolve about 10 times faster than the nuclear genes (Brown, et al. 1979; however, Martin et al. 1992 found that this rate was lower for sharks than for mammals). Despite the fact that the rate differential is only about two times faster in Drosophila (Powell, et al. 1986, Satta, et al. 1987), the generally faster rate of sequence divergence in mtDNA makes it especially appropriate for studies of population divergence or recent speciation events where relatively little time has elapsed to allow for nucleotide substitution events. For these reasons, mtDNA became popular for genetic studies particularly for those on endothermic vertebrates (e.g. Avise, et al. 1983, Southern, et al. 1988, Avise, et al. 1989).

Much work has been done using mtDNA comparisons to examine the genetic variation and distribution of terrestrial species (e.g. Avise, et al. 1979, gophers; Brown and Simpson 1981, rats; Hale and Singh 1987, fruit flies), and marine vertebrates (e.g. Southern, et al. 1988, dolphins; Bowen, et al. 1989, sea turtles; Rosel 1992, porpoises and dolphins). Relatively few groups of marine invertebrates have been studied in this manner (e.g. Saunders, et al. 1986, horseshoe crabs; Palumbi and Wilson 1990, sea urchins; Reeb and Avise 1990, oysters). Recently, however, marine crustacean taxa have been studied using mtDNA (e.g. Brasher, et al. 1992a, lobster; Knowlton, et al. 1993, shrimp; Burton and Lee ms, copepods).

Mitochondrial DNA also offers a variety of regions with relatively conserved nucleotide sequences. These regions, tRNAs and some sections of rRNAs and protein coding genes, can serve as sites for designing complementary oligonucleotides for PCR and nucleotide sequencing reaction primers. The tRNAs are especially attractive for this purpose because they can act as sites for primers on either side of a more variable region such as a protein coding gene or the control region. Unfortunately, if the gene arrangement for a particular taxon is not well known, tRNAs become less attractive primer sites.

2.1.4 The Polymerase Chain Reaction

The preparation of pure samples of specific DNA fragments has become commonplace in many laboratories. Clones can be made by ligating target DNA fragments into vector genomes which are

then replicated by growing them out in bacterial colonies (see Sambrook, et al. 1989). However, an increasingly popular technique is the more direct, labor- and time-saving method of the polymerase chain reaction (PCR) (Mullis and Faloona 1987). These techniques even allow the researcher to use partially degraded, dried or formalin/alcohol preserved material, something that is not possible with protein or RNA analyses. PCR is especially useful once specific portions of the target DNA are sequenced. These oligonucleotide sequences can then be used to design primers for PCR. PCR requires much less pure DNA than cloning, moreover, dried, ethanol or formalin preserved material is acceptable (Pååbo 1985, Mullis and Faloona 1987).

2.1.5 Cytochrome c oxidase subunit I gene

For practical reasons, the entire mitochondrial genome cannot be sequenced for each individual. Some portion of the molecule must be chosen. The gene for cytochrome c oxidase subunit 1 (CO1) has several properties which make it attractive for this study. First, it has large regions of highly conserved amino acid sequence. These are necessary for PCR primer design and function. Second, there are also some regions of variable amino acid sequence, which may permit comparisons of non-synonymous base differences. This is most important for comparisons between highly divergent groups. Third, with several stretches of about 300 and 500 bases between highly conserved regions (used as primer sites), its size is appropriate for PCR, sequencing and subsequent data analysis.

The CO1 gene has the additional advantage, shared by many protein coding genes with regions of conserved amino acid sequence, of relatively easy sequence alignment and analysis. More variable sequences such as the control region (D - loop) can be difficult to align and subsequent analysis may be compromised by misalignment or undiscovered gaps in sequence data. Although ribosomal DNA may be more highly conserved, its loops may present problematic aligning of homologous sequences. The well known functional constraints of nucleotide placement within codon triplets in the protein coding regions result in conserved bases at the first and second positions that aid in aligning homologous sequences. Translating nucleotide sequence data to amino acid sequence can also reveal gaps and typographical or technical errors in the data set. The resulting amino acid sequences may also be useful in determining the phylogenetics of highly divergent taxa.

Two other mtDNA protein coding genes which are commonly used for phylogenetic studies are cytochrome b and NADH dehydrogenase (e.g. De Salle, et al. 1987, Meyer and Wilson 1990). In vertebrates, cytochrome b is slightly more variable than CO1 in amino acid sequence, while the NADH dehydrogenase subunits are extremely variable and thus lack many conserved regions of amino acid sequence for PCR primer sites. The CO1 gene has sites (synonymous nucleotide positions) that evolve nearly as quickly as pseudogenes, which are the fastest changing sequences known. These positions can therefore be effective for determining population structure and building phylogenies for closely related

species. The other nucleotide sites (non-synonymous nucleotide positions) that change very slowly can be used for PCR and DNA sequencing primer location, as noted above.

2.2 Methods for working with barnacle DNA

2.2.1 Extracting DNA

Total genomic DNA, both nuclear and mitochondrial, was easily extracted from whole organisms or small tissue samples. Whole specimens were either frozen or preserved in ethanol or isopropanol prior to subsequent DNA extraction (see Appendix 1 for original preservatives and time between preservation and DNA extraction). Tissues preserved by any of these methods or in saturated NaCl, 20% DMSO solution (see Rosel 1992) yielded DNA which satisfactory for PCR. Small pieces (50-100 µg) of muscle tissue were dissected from specimen peduncles with a sterile razor blade or jeweler's forceps, macerated briefly in a 1.5 ml Eppendorf tube with 400 µl homogenization buffer. Although sodium dilaurel sulfate (SDS) was customarily added (to a final concentration of 1%) to the homogenate, I discovered that SDS is not necessary for effective digestion of small amounts of tissue. Residual SDS in genomic DNA preps can interfere with PCR, so avoiding use of SDS in tissue digestions may be preferable. Finally, 4 µg of protinase K and 4 µg RNase were added and the digestions were incubated at 55-65° C for 2-12 hours. Shorter digestion times produced DNA of higher molecular weight.

Equilibrated buffered phenol (Sambrook et al. 1989) was added in an equal volume to the digested homogenate. The

Eppendorf tube was gently tilted from end to end for a few seconds to form an emulsion and spun in a table top microfuge for a few minutes. The supernatant was removed to another 1.5 ml Eppendorf tube and subjected to a second phenol extraction, as above. The resulting supernatant was removed and an equal volume of chloroform added, mixed to form an emulsion and microfuged. (A mixture of phenol:chloroform:isoamylalcohol was occasionally used in place of individual phenol and chloroform emulsions.) The remaining supernatant was removed and added to three times its volume of absolute ethanol, gently mixed, allowed to stand for a few minutes and microfuged for 5-15 minutes or until a DNA pellet or precipitate was visible in the base of the tube. The supernatant was pipetted from the tube and discarded. The pellet was washed with 400-500 μ l of 70% ethanol. The tube was spun briefly in a microfuge to bring the pellet down and the alcohol pipetted off again. The pellet was then dried in a Speedvac vacuum dryer centrifuge. The dried DNA was resuspended in 50-100 μ l of Tris EDTA (TE).

Alternatively, specimens were extracted in hexadecyltrimethylammonium bromide (CTAB). In comparison to proteinase K extraction, this had the advantage of having only one emulsion step with chloroform:isoamylalcohol rather than three with phenol (twice) and chloroform. CTAB is commonly helpful when working with tissues from mollusks or coelenterates which exude copious amounts of mucous or when polysaccharides co-precipitate with DNA.

The quality of the template DNA is an important factor for successful PCR. Clean, intact, high molecular weight DNA template performs substantially better than "dirty", fragmented, low molecular weight DNA in PCR. Also, purified clones of DNA fragments containing PCR template genes can yield PCR products with poorly matched primers, whereas genomic DNA preparations contaminated with proteins or polysaccharides may not give good products even with perfectly matched primer pairs.

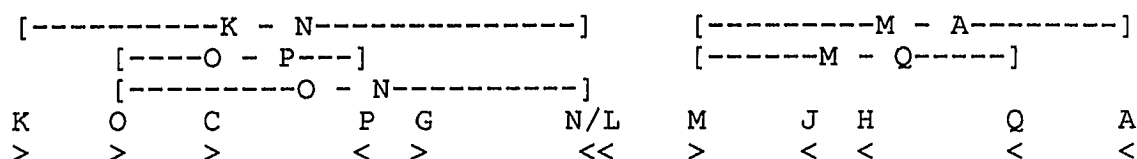
2.2.2 PCR and sequencing primers

I started experimenting with various "Universal" primers (e.g. Kocher et al. 1989, Palumbi et al. 1991) for mitochondrial ribosomal DNA during a two week visit to Stephen R. Palumbi's lab at the University of Hawaii in the summer of 1989. While these primers worked well with "sheep crab" (Loxorhynchus grandis) DNA, they didn't consistently result in PCR products from my barnacle genomic DNA extractions. It therefore became necessary to design primers that were more specific to barnacle DNA.

When I began to work on primers for barnacle mtDNA, very little crustacean DNA sequence had been published or submitted to GenBank. Most available sequences were for familiar lab animals (e.g. sea urchins and fruit flies) or mammals. However, two clones of Artemia salina (brine shrimp) mtDNA were obtained from a group working in Madrid, Spain (see Batuecas, et al. 1988) via Dr. Miles Brennen (then at Scripps Clinic in La Jolla, CA). Using published sequence data for Artemia (Batuecas, et al. 1988), Drosophila yakuba (Clary and Wolstenholme 1985) and

Strongylocentrotus (Jacobs, et al. 1988), conserved regions were located in the CO1 gene for potential primer sites. This search led to the design of two primers (these were designated CO1G and CO1H, Fig. 1). These primers worked to amplify and sequence a part of one of the Artemia clones obtained from Batuecas, et al. (1988). In addition, they primed very well for PCR on my barnacle genomic DNA, yielding bright, clear bands of the correct size. Unfortunately, subsequent sequencing of the barnacle DNA PCR products and attempted alignment with known sequence from Drosophila and the newly derived Artemia sequence revealed that the DNA amplified from the barnacle genomic DNA was not from the CO1 gene. In fact, the sequence obtained could not be aligned with any known sequence in GenBank! This suggests to me that researchers using PCR products for non-sequencing projects (RFLPs, gene specific probes, etc.) might want to try and sequence a small portion of their product to make a positive identification of the DNA fragment they have amplified. I should note that the sequence in question was extremely clear and very readable. Brasher and Ovenden (1992) had a similar experience in their attempt to sequence PCR products from krill DNA.

Despite the lack of sequence from barnacle DNA, the additional sequence from Artemia gave enough data to allow for the location of other conserved regions and the design of another PCR primer set. This primer set (CO1K and CO1J, Fig. 1) gave PCR products from barnacle DNA. Unfortunately, one of the primers appeared to anneal effectively at two sites. This mispriming



SENSE STRAND PRIMERS (> DIRECTION)

	5'	3'
K	(1729)	GAGCTCCAGATATAGCATTC
O	(1834)	GCACTGGATGAACTGTATACCC
C	(2018)	TCGTCTGATCCGCTTTGTCAC
G	(2146)	GATCCCATCCTTTATCAACAT
M	(2272)	GGAACATTAGGAATAATTTATGC

ANTI-SENSE STRAND PRIMERS (< DIRECTION)

	5'	3'
P	(2087)	GTAATAGCTCCTGCTAGAACTGG
N	(2234)	TGAGAAATTATTCCGAAGGCTGG
L	(2234)	TGAGAGATTATTCCAAATCC
J	(2536)	CAATACCTGTGAGTCCTCCTA
H	(2672)	CCAGTAAATAATGGGTATCAGTG
Q	(2746)	GCTAATCCTAAGAAGTGTTGGGG
A	(2791)	AGTATAAGCGTCTGGGTAGTC

Fig. 1: PCR and sequencing primers designed for crustacean cytochrome c oxidase subunit 1, mtDNA. Numbers for 5' end position are in reference to Drosophila yakuba sequence (Clary and Wolstenholme 1985). See text for details on relative effectiveness of the various primers. Primers A and C from The Simple Fool's Guide to PCR (Palumbi et al. 1991). All other primers designed by the author. PCR products for nucleotide sequencing reactions are shown above primer positions.

produced two products, one having the expected length of about 800 bases, the other shorter.

The 800 bp product was isolated by running PCR products on a 1.2% low melting temperature agarose TAE gel and removing the larger product with a sterile blade. The gel slices were then melted in about 200 µl of sterile water. The products were re-amplified by using 1 µl of the melted gel slice as template in a new reaction with primers CO1K and CO1J.

The K-J products were then sequenced for Pollicipes polymerus, Pollicipes elegans and a clone of balanomorph barnacle (Balanus glandula) mtDNA obtained from Dr. Lani West (Hopkins Marine Station, Pacific Grove, CA). This sequence helped to locate new potential primer sites that led to design and publication of primers CO1N and CO1M (Fig. 1 herein, Van Syoc 1993).

Much of the sequence data presented here was obtained using the two primer sets of K-N and M-A. Note that primer CO1 A was published in the Simple Fool's Guide (Palumbi et al. 1991) and was not designed by myself for barnacle PCR.

A few species resisted PCR with either the K-N or M-A primer set. Therefore, I designed primers CO1 O, CO1 P and CO1 Q (Fig. 1). The O-P or O-N sets and M-Q set gave PCR products for the remaining species in the analysis.

Although I haven't tried these primers with crustaceans other than Loxorhynchus and cloned mtDNA from Artemia, the K-J set has worked with harpacticoid copepods (Tigriopus californica, Burton and Lee, ms).

In general, primer design involved the following steps:

- 1) Locate regions of conserved amino acid sequence. It is best to consider several points when selecting primer sites. The sites should be far enough apart to yield a sufficient amount of data (300-400+ bases), yet close enough together to allow for successful amplification from partially degraded DNA samples. That is, primers sited 400-500 bases apart may successfully amplify fragmented template DNA, when those 1000-1200 bases apart cannot.
- 2) Examine the codon specificity of amino acids at the sites. Some amino acids offer very little flexibility in codon usage. This means that a more conserved nucleotide sequence will be required for the sequence of amino acids. Amino acids with high codon specificity are trp (W), met (M), phe (F), asn (N), lys (K), and cys (C). Those with very low codon specificity are leucine (L), arginine (R) and ser (S). Sequences with many high codon specific amino acids are more likely to possess more highly conserved nucleotide sequences and so make better sites for PCR primers.

A corollary of this point is that the codons of some amino acids have a higher guanosine or cytosine content than others. Guanosine and cytosine pair with three hydrogen bonds rather than the two hydrogen bonds which pair adenosine and thymine. Therefore, G-C pairs anneal at a higher temperature and melt at a higher temperature than A-T pairs. For this reason, it is advisable to look for sites with proline (P), glycine (G) or alanine (A) on the 3' end (see number 5 below).

- 3) Primer sequences of about equal A + T and G + C content are desirable.
- 4) Avoid sequences with many nucleotide repeats or palindromes.
- 5) It's best for the 3' end of the primer to have three or four guanosines or cytosines. This offers a better chance of tight annealing at the end of the primer where the DNA polymerase begins to add dNTPs to extend the complementary sequence of the template DNA (see Kwok et al. 1990).
- 6) Primers should be about 20-23 bases in length. Shorter primers have a greater chance of mispriming and longer primers are generally not required and are more expensive to synthesize.
- 7) Primers sited on protein coding regions should end on the second base of a codon, this is the most conserved position of any codon. If this is not possible, end the primer on the first base of the codon. Never end the primer sequence on the third base of the codon. This position is usually highly variable and will very likely result in a mismatch at the 3' end of the primer (also see number 5 above).

2.2.4 PCR Buffers, cycle temperatures and other conditions

It seems that there are nearly as many recipes for PCR buffers as there are labs using PCR and manufacturers of TAQ DNA polymerase. Some recommend adding non-ionic detergents (TritonX-100 or NP-40), gelatin and organic solvents (PEG or DMSO). Although, commercially available DNA polymerases may work better with buffers supplied by the manufacturer, I've found that a simple PCR buffer recommended by Kary Mullis (see Palumbi et al.

1991) works well with Perkin Elmer-Cetus TAQ DNA polymerase. Despite the variety of PCR buffers, all must contain MgCl_2 . The recommended concentration of MgCl_2 in 10x buffer ranges from 1.5 mM to 10 mM. For nearly all of my reactions, 4 mM concentrations of MgCl_2 PCR buffer consistently produced good PCR products without multiple size bands.

PCR was run at the standard 94°C melting and 72°C extension temperatures on both the Perkin-Elmer Cetus and the Coy thermal cyclers. Annealing temperatures were varied from 42°C to 50°C depending on condition of template and primer combination. If an accurate template/primer bond was assured, higher annealing temperatures were found to work better. If the template/primer match was uncertain or poor, lower annealing temperatures were most productive. Times at each temperature were 1.5 minutes at melting temperature, 2 minutes at annealing temperature and 2 minutes at extension temperature. Typically, PCRs were run for 35-40 cycles and ended with a 7 minute final extension step at 72°C before a 4°C hold.

2.2.5 DNA sequencing

Double stranded PCR products were made into single stranded DNA (ssDNA) in preparation for solid-phase sequencing reactions by using Biotin labeled primers in PCR (Hultman, et al. 1989). Biotin binds with high affinity to Avidin, a basic glycoprotein, and Streptavidin, a similar molecule with no carbohydrate. This property enables the separation of the DNA strands of PCR products prior to running sequencing reactions.

Briefly, the PCR primers are tagged on the 5' end with amino-linker during synthesis. Half of the newly synthesized primer is then labeled with Biotin. Only one of the two primers for each PCR is Biotin labeled. This is the strand which is bound to the Streptavidin coated magnetic beads for solid phase sequencing.

The PCR products are mixed in a buffer solution with Streptavidin coated iron beads. The Biotin labeled primer strand adheres to the beads. The covalent Biotin-Streptavidin bond is much stronger than the hydrogen bonds between the DNA strands. This allows the stripping of the other strand by adding NaOH to break the hydrogen bonds. The Eppendorf tubes containing the samples on the beads are placed into a specially designed holder with a magnet on one side. The beads are pulled to one side of the Eppendorf tube and the strand which is not to be sequenced is washed away from the beads and the tightly bound ssDNA to be sequenced. The resulting ssDNA can be sequenced without removal from the Streptavidin coated iron beads. Complementary strands were retained and prepared for sequencing reactions by washing with TE and double distilled water and filtering out primers and dNTPs with Centricon or Microcon tubes (available from Amicon, Inc. see Lee and Vacquier 1993). Although Lee and Vacquier (1993) suggested Centricon tubes for this technique, Microcon tubes are preferable as they are cheaper and retain about 5 μ l of retentate which is very close to the 6 μ l required for sequencing reactions. This eliminates the need for the lyophilizing of 100 μ l retentates from Centricon tubes down to 3-5 μ l.

Dideoxy sequencing techniques (Sanger, et al. 1977) with internal labeling were followed. U.S. Biochemical's Sequenase version 2.0 enzyme kit and suggested protocols were employed with S³⁵ labeled dATP. Samples were heat denatured in heat blocks at 75-90° C and 2.5 µl loaded onto 8% polyacrylamide wedge sequencing gels, run at power settings from 60-80 watts for 1 1/2 to 4 hours depending on length and location of sequence desired. Gels were fixed in 10% methanol, 10% glacial acetic acid for 30 to 60 minutes and dried in a vacuum dryer for about 60 to 90 minutes, until dry. Dried gels were exposed on x-ray film for 12 hours to several days.

2.2.6 Protein electrophoresis

In addition to DNA sequencing sixteen specimens of P. polymerus for the study of potential latitudinal population structure, 117 specimens were examined for enzyme polymorphisms by starch gel electrophoresis. This technique enables one to process a large number of specimens in a very short time and was used here as a screening procedure to check results from the smaller sample of individuals for which DNA sequence data was obtained. Details on methods used are given in Chapter 3.

2.2.7 Specimen acquisition and disposition

Appendix 2 lists specimen collection localities and catalog numbers at the California Academy of Sciences where voucher specimens were deposited.

P. polymerus specimens were collected by the author from Salt Point, Sonoma County and the pier at Scripps Institution of

Oceanography, UCSD, La Jolla. P. polymerus from Vancouver Island, British Columbia were collected and sent to the author by Dr. William Austin. Prof. William A. Newman obtained forty specimens of P. elegans via Albertina Kameya Kameya, Instituto del Mar del Peru. Additional specimens of P. elegans were obtained from the Pacific Ocean side of Cabo San Lucas, Baja California, Mexico.

Specimens of P. pollicipes were donated to the author by Teresa Cruz of the Universidade de Evora, and Professor Luiz Saldanha at the Universidade de Lisboa, Portugal. Calantica villosa specimens from New Zealand were collected and forwarded by Dr. Keith Probert. Capitulum mitella specimens were donated by Prof. Toshiyuki Yamaguchi of Chiba University, Japan.

All specimens are now in the collections of the Invertebrate Zoology and Geology Department at the California Academy of Sciences (CAS), preserved in 75% EtOH or frozen at -70° C. Catalog numbers for specific specimen lots are noted in the text where appropriate. Each specimen analyzed is additionally labeled with an "RVS" number which corresponds to the author's field numbers. A photocopy of notes corresponding to these numbers is on file at CAS.

2.3 Data analysis

2.3.1 Overview

Although the basic logic of DNA sequence comparison is simple (align the sequences, compare variable positions and calculate extent of diversity within populations and divergence between populations or construct phylogenies), statistical analysis

of DNA sequence data can be complicated by multiple substitutions (Lewontin 1989) and genetic polymorphism in the ancestral species (Pamilo and Nei 1988) as well as different rates of substitution at different nucleotide positions for a variety of reasons (e.g. Gillespie 1984, Ayala 1986, Martin and Palumbi 1993)

2.3.2 Calculation of genetic variation within and between populations

Formulae for the calculation of genetic variation within and between populations of a species are generally based on averages of differences observed among the expressed alleles of individuals or the actual DNA sequence differences among individuals.

Genetic variation, or polymorphisms, among individuals within populations must be quantified before meaningful comparisons between populations can be made. Nei (1987) suggests that, for DNA sequence data, nucleotide polymorphism (d_x) between haplotypes within a population may be expressed by the following formula:

$$d_x = n_x / (n_x - 1) \sum_{ij} x_i x_j d_{ij}$$

"where n_x is the number of sequences samples and d_{ij} is the number of nucleotide substitutions per site between the i th and j th haplotypes", with x_i and x_j representing the sample frequencies of the i th and j th haplotype in population x (Nei 1987).

Nei (1987) further explains that d_{ij} may be calculated using the formula,

$$d_{ij} = - \frac{3}{4} \log_e (1 - \frac{4}{3} p),$$

where p = the number of variable nucleotides between two

haplotypes (designated i and j in this case).

2.3.3 Building phylogenetic trees from DNA sequence data

The three major types of phylogenetic tree building methods are distance matrix methods, maximum likelihood and maximum parsimony methods (see Nei 1987 for review). Distance methods calculate genetic distance between each species pair and uses these values to build the tree. The two remaining methods use discrete character data, that is, the original sequence data. Maximum likelihood computes a likelihood score for each tree. Parsimony minimizes the number of nucleotide changes as an optimality criterion.

Parsimony methods for tree-building are probably the most popular. Parsimony models are generally considered to be the most robust of the currently available methods for sequence comparison of closely related groups with most nucleotide variation coming from transitions in synonymous positions (Hillis et al. 1992, Nei 1991). However, some computer simulations (Nei 1991) with representative data from known trees have shown that comparisons of more divergent sequences might benefit from an analysis by distance methods (such as Neighbor Joining) which can take into account variation in the probabilities of nucleotide replacement at different positions. Despite this, maximum parsimony allows evaluation of near-optimal trees and generally performs better than the other methods if one increases the number of taxa compared (only six were used in Nei's 1991

simulation) or the number of bases in the sequences compared. Nei (1991) found that, when six taxa with sequences greater than 1,200 bases long are compared, maximum parsimony is better than other methods in selecting the correct tree. This was true until the number of substitutions per site (U) reached 0.5 (50% sequence divergence). Even when only 600 bases of sequence data from six taxa were compared Nei, (1991) found that maximum parsimony was about as good as distance methods for obtaining the correct tree (79% compared to 82%, when $U = 0.5$).

Given that the U values for substitutions per site for the barnacle species compared in the present paper are much lower than 0.5, maximum parsimony was considered to be the best method for phylogenetic tree construction using DNA sequence data. However, trees were also generated by neighbor-joining methods using distance matrix data calculated with the Jukes-Cantor (1969) and the Tamura-Nei (1993) corrections for multiple substitutions.

2.3.4 DNA sequence alignment and phylogenetic tree-building

All sequences were aligned by eye. Trees were constructed from all available comparable sequence data. Parsimony phylogenies were built using the Phylogenetic Analysis Using Parsimony (PAUP 3.1, Swofford 1993). Parsimony phylogeny robustness was examined with both the bootstrap resampling procedure in PAUP 3.1 and skewness of total tree-length distribution (Hillis 1991). Distance data trees were constructed

with the Molecular Evolutionary Genetics Analysis ver. 1.0 package distributed by The Pennsylvania State University (Kumar et al. 1993). Genetic diversity values within populations and genetic divergence values between populations are estimated using the Program for Computing the Standard Errors of Nucleotide Diversity (c) and Nucleotide Divergence (d) written by Li Jin, 1988 (see Nei and Jin 1989).

CHAPTER 3

A test for a possible latitudinal cline or a dichotomy in genetic population structure in the Northeastern Pacific edible goose barnacle, Pollicipes polymerus

3.1 Introduction

The pedunculate barnacle Pollicipes polymerus ranges middle to high intertidal zones of exposed rocky shores from British Columbia to Baja California del Sur, across the Californian Transition Zone (Pilsbry 1907, Newman 1979, Newman and Abbott 1980, Cimberg 1981, Newman and Killingley 1985). Pollicipes polymerus has evidently adjusted its breeding cycle on either side of the Californian Transition Zone, to the conditions of the Oregonian and Californian Faunal Provinces (Cimberg 1981). Cimberg (1981) has, therefore, suggested that northern and southern elements of the Pollicipes polymerus population might exhibit genetic divergence despite apparent opportunities for significant gene flow across the transition zone via planktonic larvae.

There is some evidence from other marine species for latitudinal genetic variation and divergence. Regional genetic variation has been described in coastal populations of a marine arthropod with epibenthic larvae, Limulus polyphemus, the horseshoe crab, in the western Atlantic (Saunders, et al. 1986, mtDNA restriction fragment length polymorphisms). Tigriopus californicus, an intertidal harpacticoid copepod with planktonic larvae, has been shown to exhibit high levels of genetic structure among populations along the California coast (Burton and Feldman

1981, protein electrophoresis; Burton and Lee ms; mtDNA, nDNA and allozymes).

An examination of population structure in Pollicipes polymerus would show what genetic variation might be expected in a seemingly uninterrupted population of a wide ranging sessile marine species with planktonic larvae. Such information could be of great practical value for the development of management strategies for a potential P. polymerus fishery. The congeneric goose barnacle species of Peru (P. elegans) and the Iberian peninsula (P. pollicipes) support highly valued commercial fisheries (Casas 1990). Although a similar fishery has not been developed in North America, small scale experimental harvests have been exported to Europe from British Columbia (W. Austin pers. comm.) and populations in Washington state have been harvested for local sale and consumption (K. McDonnell pers. comm., Anon. 1987).

3.2 Materials and Methods

Protein electrophoresis and DNA sequencing are employed to study genetic variation and latitudinal population structure in Pollicipes polymerus. The two molecular methods were used for different reasons. As pointed out in Chapter Two, protein electrophoresis can be more cost effective for examining large numbers of individuals in a population. This is especially true with species which are easily obtained fresh. On the other hand, DNA sequencing has the advantage of enabling the researcher to work with material which may be difficult to collect or poorly preserved.

DNA sequence variation data can also be recorded within

the populations and species for later comparison with other population samples, congeners, and related genera. It also allows some measurement of within species variation which can then be considered when making interspecies sequence comparisons (Nei 1987). Additionally, results from protein electrophoresis and DNA sequencing techniques can be compared with each other in an effort to determine relative sensitivity of the methods for population level questions. However, it is generally accepted that DNA sequences have the theoretical potential to give greater resolution than protein electrophoresis because they record synonymous nucleotide changes (Hillis and Moritz 1990, also see Chapter 2 herein).

3.2.1 Starch gel protein electrophoresis

Tissues from 112 specimens of P. polymerus from north and south of the California Transition Zone (Newman 1979) were prepared for and subjected to enzyme starch gel horizontal electrophoresis. Barnacles used in the study were collected from the pier at Scripps Institution of Oceanography, La Jolla, San Diego County, California (62 specimens) and from Salt Point, Sonoma County, California (56 specimens). Remaining portions of the specimens used are housed in the collections of the Department of Invertebrate Zoology and Geology at the California Academy of Sciences (see Appendix 2 for collection details and CAS catalog numbers).

Cross-section slices of barnacle peduncles were taken with a sterile razor blade, the ovary and muscle tissues (about 500 mg)

were stripped of the outer skin and plates of the peduncle and homogenized with a clean phillips head screwdriver in 1.5 ml Eppendorf tubes in enzyme electrophoresis homogenization buffer.

The homogenate was then drawn into hematocrit capillary tubes which were plugged with clay. Samples were then frozen at -70° C overnight and run on starch gels the next day. Gel slices were stained for aspartate aminotransferase (AAT-1), malate dehydrogenase (MDH-1 and MDH-2), isocitrate dehydrogenase (IDH-1), gly-leu peptidase, leu-gly-gly peptidase-1, phosphoglucomutase (PGM), glucose-6-phosphate isomerase (GPI), mannose-6-phosphate isomerase (MPI), phosphogluconate dehydrogenase (PGDH), and superoxide dismutase (SOD-1). Many of these enzymes were either represented as monomorphic alleles or activity levels were too low to produce readable stains in gel slices. The allozymes for the readable polymorphic enzymes were scored for data analysis. Protein allozyme data were analyzed for genetic population divergence using Wright's (1951) F-statistics.

3.2.2 DNA sequencing

PCR and DNA sequencing methods are those previously discussed in Chapter 2, herein. Sequences were obtained from eight individuals collected from the Scripps Institution of Oceanography Pier in La Jolla, California and eight taken from the outer coast of Vancouver Island, British Columbia (see Appendix 2 for a comprehensive list of specimen collection data). PCR and sequencing primers designated CO1K and CO1N for the cytochrome C oxidase subunit 1 gene of mtDNA produced at least 403 bases of

comparable sequence data from each individual for analysis (see Fig. 1 in Chapter 2 for positions and sequences of primers K and N).

Sequences were aligned by eye and translated to amino acid sequence to check for errors in alignment, mistakes in the reading of autoradiographs, typographical errors when transcribing data from data sheets to word processing files used in sequence alignment, and gaps in the readable sequences.

Genetic diversity within populations and divergence between populations was calculated with the Standard Errors of Nucleotide Diversity and Nucleotide Divergence (SEND) computer program written by Li Jin in 1988 (see Nei and Jin 1989 for algorithms used, free copies of the program are available from M. Nei, Pennsylvania State University). This IBM-PC compatible software uses either p-distance values or Jukes-Cantor (1969) corrected genetic distances for distances both within and between populations. Genetic distances were derived using the Molecular Evolutionary Genetics Analysis program (MEGA ver. 1.0, Kumar et al. 1993).

Summarizing Nei's (1987) explanation, the Jukes and Cantor (1969) correction method estimates this number (d_{ij}) as

$$d_{ij} = -3/4 \log_e (1 - 4/3 p_{ij}),$$

where p_{ij} is the proportion of nucleotides which differ between the i th and j th haplotypes. The values of d_{ij} are then multiplied by the sample frequencies of the i th and j th haplotype (x_i and x_j) for each pair of haplotypes in the sub-population, summed and multiplied by the ratio of the number of sequences sampled to one less than

the number of sequences. The formula can be written as

$$d_x = n_x / (n_x - 1) \sum x_i x_j d_{ij}.$$

These calculations are made for each sub-population (d_x and d_y) and can then be used to derive an average number of nucleotide substitutions between DNA haplotypes (d_{xy}) from the two sub-populations.

$$d_{xy} = \sum_{ij} x_i y_j d_{ij}$$

From these three values (d_x , d_y and d_{xy}), the number of net substitution differences (d_A) between the two populations can be derived, as follows.

$$d_A = d_{xy} - (d_x + d_y)/2$$

3.3 Results

3.3.1 Protein electrophoresis

Only the gels stained for GPI, PGDH and MPI showed readable, polymorphic alleles. However, PGDH was polymorphic for only one individual (La Jolla population). The allele frequencies for all three polymorphic loci are summarized in Table 1.

The F_{ST} for the MPI alleles is 0.0229 and for GPI it's 0.00102 (Table 2). Because of the extremely low level of polymorphism for PGDH, F_{ST} was not calculated for that locus. Only the chi-square value for the MPI locus F_{ST} is significant (Table 2, $p < 0.05$).

3.3.2 DNA sequencing

DNA sequences of at least 393 comparable nucleotides from the Cytochrome C oxidase subunit 1 mitochondrial gene were obtained from each of 16 individuals. Only 5 variable positions were found among the six haplotypes (Table 3) and only 3 of these

MPI

Site	N	Genotype frequency						Allele frequency		
		AB	BB	BC	AA	AC	CC	A	B	C
La Jolla (SIO Pier)	47	.53	.32	.06	.09	0	0	.35	.62	.03
SaltPoint (Sonoma Co.)	43	.19	.60	.12	.05	.02	.02	.15	.76	.09

GPI

Site	N	Genotype frequency					Allele frequency			
		BB	BC	AC	CC	CD	A	B	C	D
La Jolla (SIO Pier)	62	.00	.02	.02	.80	.16	.01	.01	.90	.08
SaltPoint (Sonoma Co.)	50	.02	.12	.00	.78	.08	.00	.08	.88	.04

PGDH

Site	N	Genotype frequency		Allele frequency	
		BB	AB	A	B
La Jolla (SIO Pier)	48	.98	.02	.01	.99
SaltPoint (Sonoma Co.)	43	1.00	.00	.00	1.00

Table 1: Genotype and allelic frequencies for Pollicipes polymerus samples from Scripps Pier, La Jolla, San Diego County and Salt Point, Sonoma County.

MPI

$$\begin{array}{ll} F_{IS} = -0.1019 & X^2 = 0.934 \text{ n.s.} \\ F_{ST} = 0.0229 & X^2 = 4.12 (p < .05) \end{array}$$

GPI

$$\begin{array}{ll} F_{IS} = -0.0567 & X^2 = 0.36 \text{ n.s.} \\ F_{ST} = 0.0010 & X^2 = 0.228 \text{ n.s.} \end{array}$$

Table 2: Wright's F - statistic values for MPI and GPI loci in Pollicipes polymerus, with X^2 and levels of significance. Alleles were scored B/not B for MPI and C/not C for GPI.

	Relative nucleotide number				
	18	66	261	270	390
La Jolla					
Haplotype A (186-1, 202-3, 202-8)	G	G	G	T	A
Haplotype B (186-2)	G	G	G	T	G
Haplotype C (202-1)	G	A	A	A	A
Haplotype D (202-7)	G	A	G	A	A
Haplotype E (202-9)	A	G	G	T	A
Vancouver Island					
Haplotype A (203-4, 203-5, 203-12)	G	G	G	T	A
Haplotype C (203-1, 203-15)	G	A	A	A	A
Haplotype D (203-11)	G	A	G	A	A
Haplotype F (203-2, 203-7)	G	G	A	T	A

Table 3: Variable nucleotide positions among the haplotypes in sampled Pollicipes polymerus, mtDNA CO1 gene sequences.

variable positions were phylogenetically informative within the species.

Four of the five nucleotide differences are transitional (all guanine - adenine changes), only one (Position 278) is transversional (thymine - adenine changes). All of the substitutions represented in the data set are synonymous changes at the third base position in codons; they do not result in amino acid replacements.

No fixed nucleotide position differences between northern and southern sub-populations were found. The nucleotide change at position 269 represents the only frequency difference between sub-populations. All other variable positions have either a single individual with a different nucleotide (positions 25 and 399) or show considerable variation within both sub-populations (positions 73 and 278).

Nucleotide polymorphism (p) was about the same within and between populations (Table 3). The value for the La Jolla, SIO pier sample was 0.546% (SE 0.2475%), for Vancouver Island 0.3876% (SE 0.2153%) and the pooled sample polymorphism was 0.4643% (SE 0.2098%). The net genetic divergence (d_A) between the two samples was negative.

Genetic divergences calculated with the Jukes-Cantor correction, were nearly the same as the p -distance values (Tables 4 and 5). The average number of nucleotide substitutions for the La Jolla, SIO pier sample was 0.5464% (SE 0.2493%), for Vancouver Island 0.3857% (SE 0.2149%) and the pooled sample polymorphism

Sample	Polymorphism (p)
La Jolla, SIO Pier	0.546% (SE 0.247%)
Vancouver Island	0.3876% (SE 0.215%)
Combined	0.4643% (SE .210%)

Table 4: Nucleotide polymorphism (p) values for sampled sub-populations of Pollicipes polymerus from the Scripps Institution of Oceanography pier, La Jolla, CA and the outer coast of Vancouver Island, British Columbia and the pooled sampled sub-populations (Combined). Standard errors (SE) are enclosed by parentheses following the polymorphism values.

Sample	Genetic distance (d)
La Jolla, SIO Pier	0.546% (SE 0.249%)
Vancouver Island	0.386% (SE 0.215%)
Combined	0.459% (SE 0.204%)
Between	0.462% (SE 0.211%)

Table 5: Genetic diversity (d) values for sampled sub-populations of Pollicipes polymerus from the Scripps Institution of Oceanography pier, La Jolla, CA and the outer coast of Vancouver Island, British Columbia, the total diversity for both of the sampled sub-populations (Combined), and between the sub-populations. Values were calculated with the Jukes-Cantor correction (1969). Standard errors (SE) are enclosed by parentheses following the distance values.

was 0.4594% (SE 0.2044%). Again, d_A was a negative value.

A consensus DNA sequence for P. polymerus has been submitted to GenBank.

3.4 Discussion

3.4.1 Levels of genetic diversity

The genetic diversity levels exhibited in the sampled populations of P. polymerus (0.3857%, Vancouver Island and 0.5464%, La Jolla) fall within the range of mtDNA sequence variation found among the extremely limited number of marine invertebrate species recorded to date. Ovenden (1990) surveys intraspecific genetic diversity and divergence in marine organisms using data from studies based on mitochondrial DNA comparisons.

His lists include only three species of marine invertebrates, two of these are sea urchins in the genus Strongylocentrotus, the third was an oyster, Crassostrea virginica. The sea urchins vary widely in genetic diversity, from a low of 0.11% in S. droebachensis to measures in S. purpuratus of 0.48% and 0.99%. The oyster work (Reeb and Avise (1990) reports a genetic discontinuity and gave a level of genetic divergence of 2.6%. Brasher et al. (1992b) record within species diversity of mtDNA sequences (RLFPs) in the rock lobster genus Jasus. The genetic diversity within the five species examined range from 0.33% to 0.99%.

Intraspecific genetic variation in marine vertebrates also encompasses the values measured here for P. polymerus

populations (Ovenden 1990, from 0.004% to 2.4% in various fish species; Rosel 1992, 0.96% in common dolphins and 1.6% among harbor porpoise populations).

3.4.2 Lack of genetic discontinuity

Although two of the mtDNA haplotypes south and one north of Pt. Conception and the Californian Transition Zone were unique, the mtDNA sequence data set gives no discernable indication that net genetic divergence exists between the sampled northern and southern subsets of Pollicipes polymerus. Similarly, although the allozyme data show some allelic frequency differences, no fixed differences exist and therefore no firm evidence can be currently presented for a latitudinal genetic cline or dichotomy for the population around or through the Californian Transition Zone.

This has also been found for another intertidal barnacle, Tetraclita rubescens (Ford and Mitten 1993, protein electrophoresis). Tetraclita rubescens, often found along side P. polymerus in the upper intertidal zone, has a range from Baja California to near San Francisco, clearly traversing the California Transition Zone centered around Pt. Conception. Another species with a long latitudinal range which shares the intertidal habitat with Pollicipes and Tetraclita is Mytilus californianus, the California mussel (Newman and Abbott 1980, Haderlie and Abbott 1980). Like its two barnacle neighbors in the intertidal zone, M. californianus shows no north/south genetic divergence at Pt. Conception (Levinton and Suchanek 1978, protein electrophoresis). In contrast to this developing pattern of genetic homogeneity

among individuals within three species of intertidal invertebrates, genetic or morphological divergence is found between populations on either side of Pt. Conception in two species of shallow subtidal fishes (Davis et al. 1981, painted greenling Oxylebius pictus, protein electrophoresis; Love and Larson 1978, kelp rockfish Sebastes atrovirens, protein electrophoresis).

3.4.3 Environmental factors: sea surface temperatures and currents

The present data suggest that Cimberg's (1981) results are not due to restricted gene flow and genetic divergence. In fact, the mtDNA sequence data suggest that the species is panmictic throughout its range or has been in the recent past. If this is the case, the brooding temperature differences recorded by Cimberg may be ecotypic, that is, due to variable ecological or environmental parameters influencing the similar genotypes in different ways.

The environmental differences between the northern sample areas (the west coast of Vancouver Island and the exposed coast of northern Sonoma County) and the southern sample site (Scripps Institution of Oceanography pier at La Jolla) are numerous, with perhaps the most obvious being the seasonal sea surface water temperatures. Differences in summer ocean temperatures could lead to regional changes in brooding physiology and behavior.

These temperature changes could affect enzyme allele patterns if one allele is more efficient than others in a particular temperature regime (Coppes and Somero 1990). DiMichele and Powers (1982) found that allelic variants of lactate dehydrogenase-

B in the killifish, Fundulus could profoundly affect swimming performance and development in different temperature regimes. The alleles were also linked to environmental temperatures in Fundulus populations in the southeastern United States (DiMichele and Powers 1991).

If purifying selection is at work here, preventing the survival and reproduction of immigrant genotypes, it appears that it is not strong enough to produce a disruption in gene flow or diversification of the gene pool to favor distinctly different genotypes for the change of conditions through the transition zone. However, the present set of enzyme allele data combined with the mtDNA sequence data do allow for the possibility of P. polymerus acclimatizing its brooding behavior to local environmental conditions.

The broad latitudinal range of Pollicipes polymerus, through a major biogeographical boundary without a major disturbance in apparent gene flow, is unusual. Many marine invertebrates ranging along the length of the eastern coast of North America exhibit population genetic subdivision from one biotic province to the next along the Atlantic coast of Florida (e.g. Saunders, et al. 1986, Limulus polyphemus mtDNA; Reeb and Avise 1990, Crassostrea virginica mtDNA).

Although marine organisms on the eastern seaboard of the United States may experience a much steeper latitudinal thermal gradient (about 15° C) than those on the western coast (about 10° C) with larger seasonal changes, the oceanographic conditions at Pt.

Conception are broadly analogous to those present along the coast of northern Florida where the warm waters of the Gulf stream shear away from the shore. The California current flows south from the northeastern Pacific down along the northern California coast. As it passes Pt. Conception, the current maintains its southerly direction, rather than going southeast to conform to the coastline. Instead it flows past the Channel Islands, generally bypassing the southern California Bight. This has two immediate consequences. First, the northernmost Channel Islands, Santa Rosa and San Miguel, have a cool temperate fauna similar to that north of Pt. Conception. Second, the southern California Bight, from San Diego north to Santa Barbara, has warmer surface water temperatures due in part to a gyre of recirculating coastal water. This gyre may act to entrain larvae within its boundaries and help to sustain the warm water biota. All of this would suggest that larval transport may not commonly occur directly across the temperature and current gradient at Pt. Conception. It was this scenario that Cimberg (1981) invoked to explain differences brooding temperatures, which suggested the possibility of two distinct genetic races of P. polymerus on opposite sides of Pt. Conception.

Despite this circumstantial evidence for a discontinuity in gene flow, the present mtDNA sequence data indicates that P. polymerus does not exhibit genetic divergence between populations north and south of the Pt. Conception boundary. It is noteworthy that two other intertidal invertebrate species, mentioned above, which occupy nearly identical habitat and niche space, show a

similar lack of latitudinal genetic subdivision.

Perhaps the explanation for this paradox lies not in an analysis of "normal" current patterns and surface temperatures, but in those occasional occurrences of "unusual" currents and water parcel movement such as those generated by El Niño/Southern Oscillation (ENSO) events.

Although the immediate cause of ENSO events is not known, the biotic results of these physical changes in both atmospheric and oceanic current patterns have been well documented, especially in the last decade. For the eastern Pacific Ocean, the biotic results include the northerly movement of southern forms along with warm water parcels from Baja California to the Canadian border (see Wooster and Fluharty 1985 for various aspects of the 1982-1983 El Niño, see Norton, et al. 1985 for specifics on current changes).

The 1982-83 ENSO event has been positively correlated with the movement of pelagic red crabs (Pleuroncodes planipes), euphausiids (Nyctiphanes simplex), and other marine species from their "normal" range off the western coast of Baja California northward to northern California, Oregon, and Washington (Smith 1985, Van Syoc pers. obs., red crabs; Schoener and Fluharty 1985, many species; Brodeur 1986, euphausiids). This northward movement of planktonic organisms offers evidence for a potential northward gene flow via planktonic larvae. This gene flow may retard genetic divergence in marine organisms which, like P. polymerus, exhibit broad latitudinal ranges that cross major

biogeographic boundaries.

Perhaps this warrants returning to the analogy, made earlier in this section, of the California Current in the eastern Pacific with the Gulf Stream in the western Atlantic along the northern coast of Florida. The genetic sub-divisions of species noted by Saunders (1986, horseshoe crabs) and Reeb and Avise (1990) coincide with general water parcel movement with the Gulf Stream offshore in northern Florida. No major shifts in current patterns on the scale of ENSO events in the eastern Pacific are known to occur in the western Atlantic. While "warm rings" do occasionally spin off from the Gulf Stream, carrying tropical pelagic organisms far to the north of their usual range, it may be that these sporadic water parcel movements cannot match the larval delivery rate or volume of the ENSO altered currents in the eastern Pacific. Also, current direction is always south to north in the northwestern Atlantic, contrasting sharply with the opposing currents or reversal of currents and perhaps gene flow that occur in the eastern Pacific.

3.5 Conclusions

Although Pollicipes polymerus exhibits a very long latitudinal range which spans a major biogeographic boundary, the present data offer no evidence to suggest a genetic divergence or latitudinal gradient. This finding coincides with those for two other intertidal invertebrates with similar ranges, Tetraclita rubescens (a barnacle, Ford and Mitten 1993) and Mytilus californianus (a mussel, Levinton and Suchanek 1978). Changes in coastal current patterns due to ENSO events and a concomittant wide-spread

north and south dispersal of planktonic larvae may play a role in high gene flow throughout the range of Pollicipes polymerus, resulting in a reduced chance of genetic divergence between north and south sub-populations through either natural selection or genetic drift.

CHAPTER 4

Genetic population structure within and between paramphitropical sub-populations of the edible, eastern Pacific goose barnacle Pollicipes elegans

4.1 Introduction

The long latitudinal range (about 3,300 km) of the North American goose barnacle, Pollicipes polymerus, has previously led to an inference that some underlying genetic population structure might be the cause of regional variations in seasonal brooding (Cimberg 1981). Although this has not been proven to be the case (Ch. 3, herein), another eastern Pacific goose barnacle with a long latitudinal range exhibits a disjunct distribution of sub-populations (Newman 1992) this may be indicative of genetic subdivision. This species, Pollicipes elegans, inhabits the Panamic Faunal Province; that is, it ranges from northwestern Mexico (where it is sympatric with P. polymerus) south across the equator to southern Peru, a distance of about 4,400 km (Newman and Killingley 1985, Kameya and Zeballos 1988, Laguna 1990). However, the current distribution of P. elegans is apparently paramphitropical; e. g., while it is a locally abundant intertidal form at the northern and southern ends of its range, it is infrequently encountered in the intervening tropical areas of the eastern Pacific north of the equator (Newman and Foster 1987, Laguna 1990, Newman 1992, Fig. 2 herein).

This observed disjunction between the north and south populations of P. elegans allows two hypotheses: 1) the two



Fig. 2: Distribution of *Pollicipes elegans*. Sample sites are marked with asterisks (*).

populations have been genetically separated for some time and thus should be genetically divergent, or 2) they are in continuous genetic contact via planktonic larval drift or adult rafting across the uninhabited equatorial region between the two sub-populations and thus should share genetic diversity equally between the two groups.

If the first hypothesis is correct, the genetic split should be reflected in genetic diversity and divergence and in phylogenetic trees constructed with DNA sequence data from individual barnacles from the two sub-populations. To test this hypothesis, the levels of genetic diversity within and net genetic divergence between the two sub-populations are estimated from comparisons of mitochondrial DNA sequences from fourteen specimens of P. elegans from Peru and Baja California. In addition, phylogenetic trees are constructed both from these distance data and a parsimony analysis of compared individual DNA sequences.

4.2 Materials and methods

PCR and DNA sequencing methods are those previously discussed in Chapter 2, herein. Sequences were obtained from seven individuals from Cabo San Lucas, Mexico and seven of the Peruvian specimens received from Albertina Kameya Kameya (see Appendix 2 for a complete list of specimen collection data). PCR and sequencing primers designated CO1K and CO1N (see Chapter 2, Fig. 1, for primer sequences and their relative positions) for the cytochrome c oxidase subunit 1 gene of mtDNA produced 312 bases of comparable sequence data from each individual for analysis.

DNA data analysis was performed using the computer programs contained in Molecular Evolutionary Genetics Analysis (MEGA ver. 1.0, Kumar et al. 1993) and the Program for Computing the Standard Errors of Nucleotide Diversity and Nucleotide Divergence (SEND, written by Li Jin 1988, see Nei and Jin 1989 for explanation of the algorithm used). The SEND program calculates genetic distance between individuals within and between populations. These data were then used to calculate net genetic divergence between populations. SEND and MEGA also execute a neighbor-joining tree building algorithm using the individual genetic distance data.

Sequences were initially aligned by eye. The sequence data were then entered into MEGA format and the program was used to translate them into amino acid sequence to check for errors in alignment, mistakes in the reading of autoradiographs, typographical errors when transcribing data from data sheets to word processing files used in sequence alignment, and gaps in the readable sequences.

The Jukes-Cantor method was used to calculate the number of nucleotide substitutions per site between DNA haplotypes in the two sub-populations. Confidence limits can be placed on genetic diversity and divergence by an analysis of co-variance (Nei 1987, Nei and Jin 1989). These variances are given as standard errors (SE) for all distance calculations.

The phylogenetic tree based on the distance matrix data set was constructed from 500 bootstrap resamplings. Gaps in sequence

data were ignored (using a pairwise deletion process) rather than treated as a fifth character state in the analyses. Phylogenetic trees were also constructed by parsimony analysis (PAUP ver. 3.1.1, Swofford 1993).

4.3 Results

4.3.1 Sequence variation

Comparable DNA sequence data from the cytochrome c oxidase subunit 1 (CO1) mitochondrial gene were obtained from each of 14 individuals. Among the 312 nucleotide positions compared, 19 variable positions were found (Table 6), 14 of these are phylogenetically informative within the species sample. Seven of the variable positions show frequency differences between the two sub-population samples. Five of the 19 nucleotide differences represent changes in only one individual each. Of these, three are found in the individual identified as 177-6 (Peru), one in 177-7 (Peru) and one in 208-3 (Mexico).

Fifteen of the 19 nucleotide differences are transitional (9 cytosine - thymine and 6 guanine - adenine changes), only 4 of the differences are transversional (3 guanosine - cytosine and 1 cytosine - adenine changes). All of the substitutions represented in the data set are synonymous changes; they do not result in amino acid replacements. One of the differences appears at the first base position, the remaining 18 are at the third base position.

Nucleotide polymorphism (p, Table 7) for the Cabo San Lucas, Mexico sample was 1.43% (SE 0.406%), for Peru 1.92% (SE 0.509%), and the total genetic polymorphism for both samples (the

Specimen	Relative nucleotide position in compared sequences																		
	2	3	5	7	7	1	1	1	1	1	2	2	2	2	2	2	3	3	
(RVS no.)	6	7	9	4	2	8	1	5	0	2	9	1	6	1	0	2	8	0	1
					*	*				*				*	*	*		*	*
177-5	C	T	A	A	T	G	C	C	A	C	T	A	G	A	A	T	C	C	C
177-6	.	.	.	.	C	C	.	.	.	T	C	G	.	.	.	C	A	T	T
177-7	.	.	.	.	C	C	.	G	.	.	.	.	C	.	G	.	.	.	.
177-8	T	C	.	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.
177-9	.	.	G	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.
177-10	.	.	G	.	.	.	.	.	.	.	.	.	.	.	G	.	.	-	.
177-12	T	C	.	.	C	.	.	.	.	.	.	.	C	.	G	.	.	.	.
208-1	.	.	.	.	C	C	.	.	G	T	.	.	.	G	.	C	.	T	T
208-2	.	.	.	.	C	C	.	.	.	T	.	.	-	G	.	C	.	T	T
208-3	.	.	.	G	C	C	T	.	G	T	.	.	.	G	.	C	.	T	T
208-4	.	.	.	.	C	C	T	.	G	T	.	.	.	G	.	C	.	.	T
208-5	.	.	.	.	C	C	.	.	.	T	.	.	A	G	.	C	.	T	T
208-6	.	.	.	.	C	C	T	.	.	T	.	.	.	G	.	C	.	T	T
208-7	T	C	.	.	C	C	.	.	.	.	.	.	C	.	G	.	.	.	.

Table 6: Variable nucleotide positions in Pollicipes elegans haplotypes. Frequency differences are marked (*). RVS numbers 177-x refer to individuals from Peru, 208-x refer to individuals from Cabo San Lucas, Mexico.

Sample locality	Polymorphism (p)
Mexico	1.43% (SE 0.406%)
Peru	1.92% (SE 0.509%)
Combined	2.29% (SE 0.539%)

Table 7: Nucleotide polymorphism (p) values for sampled sub-populations of Pollicipes elegans from Peru and the outer coast of Cabo San Lucas, Baja California, Mexico and the pooled sampled sub-populations (Combined). Standard errors (SE) are enclosed by parentheses following the polymorphism values.

entire population) was 2.29% (SE 0.539%).

A consensus DNA sequence for P. elegans has been submitted to GenBank.

4.3.2 Phylogenetic trees

The mtDNA sequences from the fourteen individuals sampled show 19 variable nucleotide positions resulting in 14 different haplotypes (Table 6). The haplotype phylogeny represented by the parsimony tree generated by PAUP (Fig. 3, branch-and-bound bootstrap) reveals two main evolutionary clusters. The two clusters correlate strongly with the geographic origin of the specimens. That is, nearly all of the Peru specimens (RVS numbers beginning with 177) cluster together and most of the Mexican specimens (RVS numbers beginning with 208) cluster together. However, one individual from each locality exhibits a haplotype which clusters within the opposite group. Individual 208-7 (Cabo San Lucas) has a haplotype which clusters closely with two of the Peruvian individuals, 177-8 and 177-12. The Peru specimen (177-6) that groups with the Mexican specimens does not exhibit clustering affinity to any subset of those haplotypes. A similar phylogenetic tree was generated from the Jukes-Cantor derived genetic distances (Fig. 4). Again, two strongly defined main clusters are apparent, with individual branch placement corresponding to that noted above for the parsimony tree. The bootstrap confidence level for the branch node of the two main clusters is 99%. Other bootstrap confidence levels are printed at the branch nodes (Fig. 4). All of these values for the Mexican clade

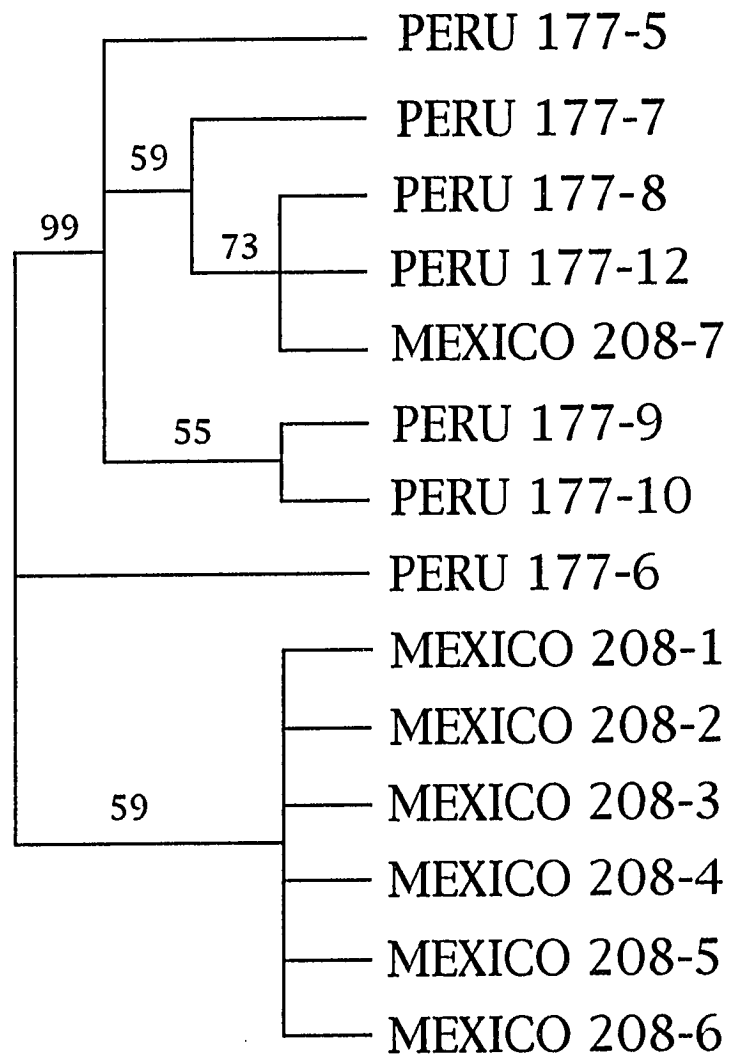


FIG. 3: Branch-and-bound bootstrapped parsimony tree of *Pollicipes elegans* haplotypes. Bootstrap confidence levels over 50% are noted on branches.

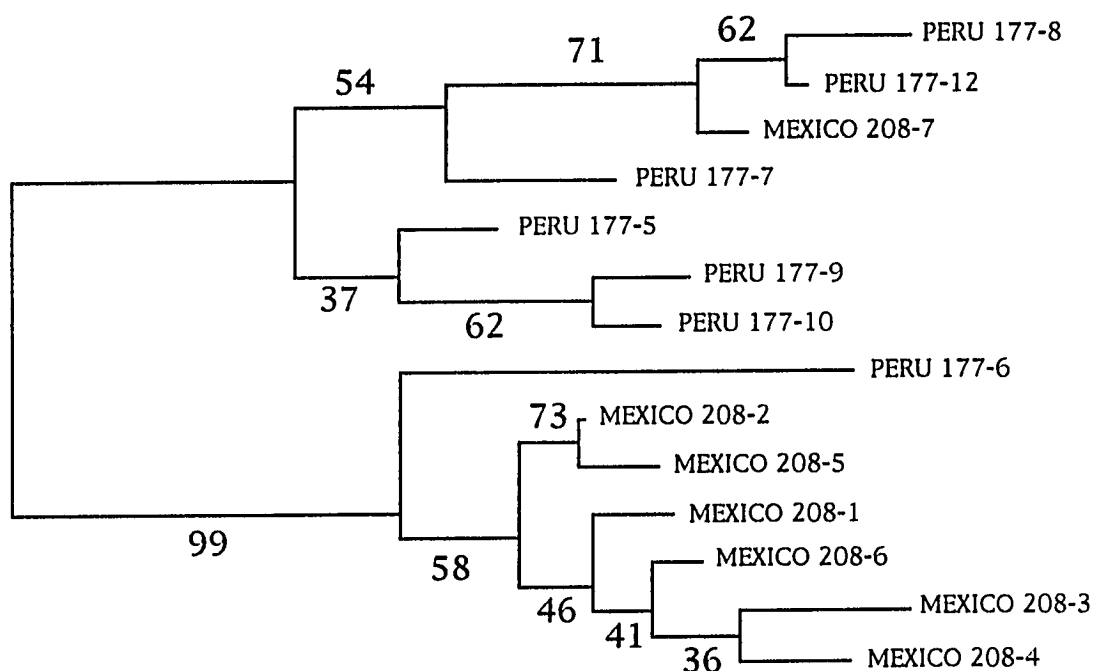


Fig. 4: Bootstrapped tree from genetic distance data (Jukes-Cantor) among *Pollicipes elegans* haplotypes. Neighbor-joining tree building method. Bootstrap confidence levels over 50% are noted on branches. 500 bootstrap replications. Scale: each cm equal to genetic distance of 0.00176.

(including Peru 177-6) are below 60% and, therefore, indicate ambiguous branching patterns and little confidence in internal relationships among the members of the clade. However, the Peruvian clade (including Mexico 208-7) does show somewhat higher BCLs for nodes within the clade, reflecting the higher level of genetic diversity within the Peru population sample.

4.3.3 Sequence diversity and divergence

Genetic diversity values, are near those of other marine organisms (see following Discussion section herein, and Ovenden 1990) within sub-populations but show a distinct north/south divergence in the pooled sub-population data samples (Table 8). Genetic diversity within the Peru sub-population (Table 8) is 1.95% (SE 0.524%) and 1.45% (SE 0.415%) within the Mexican sub-population sample (Table 8). The between population diversity (Table 8) is 2.89 (SE 0.709%). The total population nucleotide diversity is 2.34% (SE 0.547%). The net nucleotide divergence between the two sub-populations (d_A) is estimated to be 1.18% (SE 0.412%).

4.4 Discussion

4.4.1 Overview

The distinct genetic divergence of the two clusters of *P. elegans* haplotypes and the net genetic divergence between them strongly suggests that the sampled sub-populations of *P. elegans* are genetically separated. Therefore, the hypothesis of a high level of continuous, uninterrupted gene flow between the two sub-populations via dispersal across the uninhabited equatorial region

Sample	Genetic distance (d)
Mexico	1.45% (SE 0.415%)
Peru	1.95% (SE 0.514%)
Combined	2.34% (SE 0.547%)
Between	2.89% (SE 0.709)
Net Divergence (dA)	1.18% (SE 0.412)

Table 8: Genetic diversity (d) values for sampled sub-populations of Pollicipes elegans from Peru and the outer coast of Cabo San Lucas, Baja California, Mexico, the pooled sampled sub-populations (Combined). Values were calculated by the Jukes-Cantor method (1969). Standard errors (SE) are enclosed by parentheses following the distance values.

can be rejected. However, the presence of one haplotype from each sub-population within the cluster containing all of the individual haplotypes from the other locality (Figs. 3 and 4) indicates that there is some low level of genetic exchange between the Peru and Mexico sub-populations.

4.4.2 Present barriers to gene flow

The most obvious meso-scale physical environmental factors which might inhibit dispersal of genetic material across the uninhabited area just to the north of the equator in the Eastern Pacific are sea surface temperatures and generally prevailing offshore currents (Fig. 5). It may be that the shallow water temperatures are too warm for P. elegans to colonize this fully tropical equatorial region. Additionally, the offshore currents could carry larvae and rafting adults out to sea where they could not find favorable conditions for survival and genetic dispersal. However, nearshore eddies and countercurrents may help to prevent offshore entrainment of all larvae.

The sea surface temperatures in the uninhabited north equatorial region average between 26-28° C (Halpert and Ropelewski 1989). The specimen database records of the Benthic Invertebrate Collection at Scripps Institution of Oceanography (SIO) and the California Academy of Sciences, Department of Invertebrate Zoology and Geology (CASIZG) list specimens of P. elegans collected from the Mexican States of Baja California (Punta Santa Domingo, Migrino and Cabo San Lucas), Sinaloa (Mazatlan), Jalisco (Puerto Vallarta) and Colima (Manzanillo), La Libertad in El Salvador, and

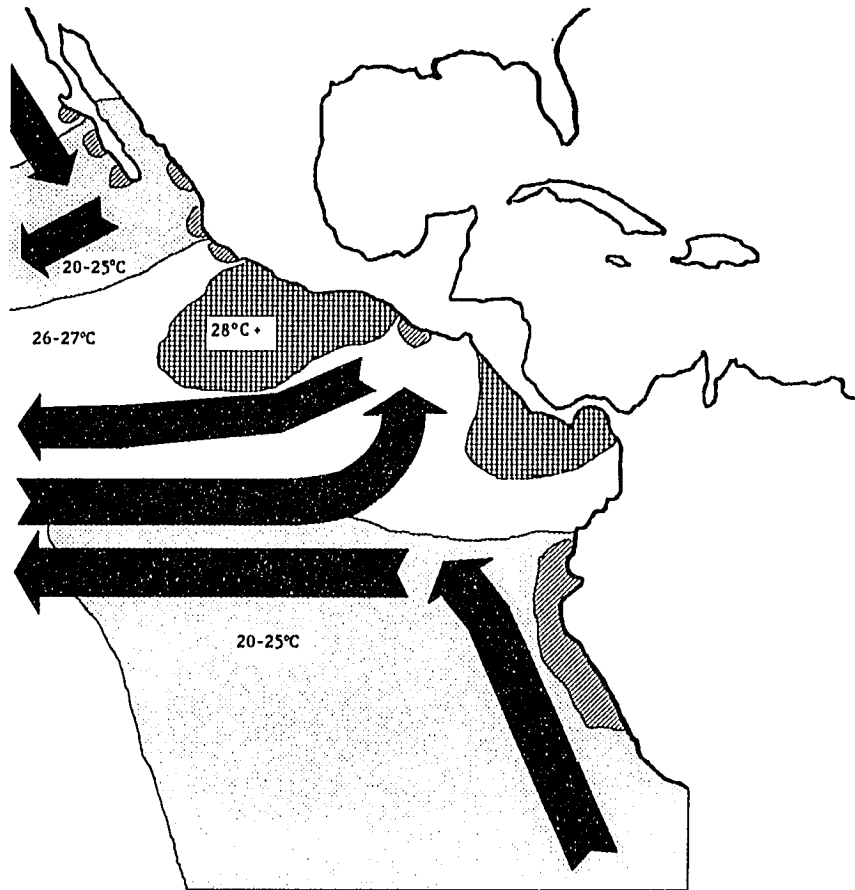


Fig. 5: Distribution records of *Pollicipes elegans* (lined coastal regions). Records taken from specimen catalogs of the Benthic Invertebrate Collection at Scripps Institution of Oceanography and the Department of Invertebrate Zoology, California Academy of Sciences, see text for place names of collection localities. Isotherms (multi-year averages during Austral summer months, after Halpert and Ropelewski 1989) and major currents in the Eastern Tropical Pacific also shown.

various localities in Peru. The localities of these records coincide with regions of sea surface temperatures between 20° C and 25° C (Fig. 5). These regions exclude two major coastal areas of Meso-America. One of these extends from the area just south of the Mexican state of Colima to Guatemala, the second encompasses the Pacific coast of Nicaragua, Costa Rica, Panama and northern Colombia.

The El Niño/Southern Oscillation (ENSO) phenomenon may act to spread the sub-populations further away from each other and the northern equatorial "barrier" by increasing sea surface temperatures north and south from the equator in the eastern Pacific (Newman and Foster 1987). ENSO events create a lessening of the equatorial current and a concomitant increase in flow from the western Pacific, warming coastal waters in the eastern Pacific. During ENSO phenomena the average sea surface temperatures between the Mexican state of Jalisco and northern Peru rise from 26 to 27° C with the coastal "hot spots" in southern Mexico and Costa Rica/Panama rising above 28° C (Halpert and Ropelewski 1989).

Evidence of the effect of these elevated sea surface temperatures is given by Kameya and Zeballos (1988) with their record of a significant reduction in local Peruvian populations of P. elegans during the 1982-83 El Niño. An increase in sea surface temperatures can, therefore, be correlated with the effective exclusion of Pollicipes elegans from the tropical coastal areas immediately north of the equator in the eastern Pacific.

4.4.3 Dispersal, vicariance, or both?

The general pattern of genetic divergence just observed may be due to, 1) a cessation of gene flow among geographically isolated individuals from a once continuous population ranging along the rocky intertidal areas of the tropical eastern Pacific from Peru to Mexico, 2) a colonization event, via either larval drift or rafting adults, from one hemisphere to the other during a relatively short period of favorable temperature and current patterns, or 3) a more complex series of events involving an initial vicariant or dispersal event with subsequent periods of genetic exchange across the uninhabited region north of the equator.

To elaborate further on this last point, the sub-populations may be in tenuous genetic contact now, after a lengthy separation in the past. Alternatively, the populations could have been split, connected briefly in the recent past via dispersal across a narrowed, more easily crossed, tropical region and split more effectively again when the equatorial barrier to gene flow widened. If this last hypothesis is correct, this sequence of events (splitting, reuniting, and splitting again) may have been replayed several times during the history of the disjunction between the sub-populations.

A single dispersal event leading to the colonization of either the southern or northern population of P. elegans could produce a genetic bottleneck. This founder effect would reduce the effective genetic population size of the newly established colony to a very small number due to the low probability of larvae or rafting adults

successfully crossing the wide zone of uninhabitable area north of the equator and colonizing the new distant habitat. The result of this bottlenecking of the gene pool would be a pattern of low genetic diversity within one of the two sub-populations, the newly established colony.

Because clones of parental mitochondria are almost exclusively inherited through the egg cytoplasm, mitochondrial DNA data is especially susceptible to bottleneck effects (Wilson et al. 1985). This can severely affect genetic distance calculations as the number of mtDNA haplotypes in the newly colonized population would be very small. For example, if colonization recently took place via larval transport from the south to the north, the Mexican sample would have a very low diversity index. The haplotypes hatching from a single egg mass have an extremely high probability of being identical. The low genetic diversity of a recently founded population ($d_x = 0$ in the extreme case of a single haplotype) artificially inflates the genetic divergence between it and the ancestral population. This effect is eventually blurred with the passing of time as point mutations in the newly established population occur and a number of new haplotypes evolve. However, if rates of nucleotide substitution based on other organisms can be used here, a considerable amount of time must pass before enough mutations accumulate in the population to develop a diversity level approaching that of either the Peru or Mexican sub-populations of P. elegans.

In contrast to this hypothesized homogeneity or lowering of

genetic diversity, the present mtDNA sequence data show unique haplotypes for every individual from both sub-populations of P. elegans. Also, the genetic diversity within each of the two populations (Peru = 1.61%, Mexico = 0.915%) is near that of the marine species for which mtDNA sequence data are available (Ovenden 1990, Brasher et al. 1992). Indeed, the genetic diversity for P. polymerus, the northeastern Pacific congener of P. elegans, is much lower (0.459%, Table 5, Chapter 3, herein) than those within either population of P. elegans. Therefore, little evidence can be found to support a hypothesis of a recent population bottleneck due to a single founder event. In spite of this, the diversity values do provide some data in support of a larger effective population size in Peru than in Mexico.

4.4.4 Time of genetic divergence

From the calculated d_A (1.17%, Table 8) we can derive a time of effective genetic divergence of the two sub-populations. Using the formula presented by Nei (1987),

$$d_A = 2 \lambda T$$

where T is time and λ is the rate of nucleotide change in each sequence. Nei (1987) lists the average rate of nucleotide change for synonymous positions in various protein coding nuclear DNA genes as 4.65×10^{-9} . A generally used average rate of nucleotide change at all positions in mtDNA is 2% per million years or 20×10^{-9} (Wilson et al. 1985; based on mammalian mtDNA restriction maps). However, there is mounting evidence that, in poikilotherm mtDNA, nucleotide sequences diverge somewhat more slowly (see Martin

and Palumbi 1993 for review): rates are generally less than 1% per million years (10×10^{-9}), many being less than 0.5% per million years (5×10^{-9}). For these rates for nucleotide substitution, times of genetic divergence range from 292,500 YBP to 1.26 MYBP.

Admittedly, the estimates given above are subject to a high degree of error since the very broadly derived rates of change are being applied to a new set of data with no calibration for any rates of DNA sequence change within the Cirripedia in general or the synonymous base positions in the goose barnacle mtDNA CO1 gene in particular. Indeed, the possible reasons for the broad range of variation in rates in different taxa and different genes at different times have been widely discussed (e.g. Gillespie 1984, episodic rate changes; Ayala 1986, different rates in different genes; Martin and Palumbi 1993, rate differences based on metabolic rates and body size). In spite of this caveat, the above calculations may be considered to be relatively conservative as an estimate of a rather broad range of time since genetic divergence. Therefore, let us briefly consider some potential causes of a past disruption in gene flow and conversely, an intermittent genetic connection across the tropics.

4.4.5 Genetic divergence and reconnection

The presence of "foreign" haplotypes in each sub-population of P. elegans suggests that there has been some relatively recent gene transport across the "barrier" of the equatorial region to mix gene pools on either side of the equator. The time scale of this crossing (or crossings) is not known. However, Newman and Foster

(1987) note that Darwin (1859) speculated that cooling of tropical sea surface temperatures during the ice ages of the Pleistocene could allow for a trans-tropics faunal exchange.

The earliest time of divergence for the two sub-populations of P. elegans is about 1.26 MYBP, the lower Pleistocene. The lower end of the range of time of divergence, 292,500 YBP, correlates with the upper Pleistocene. The Pleistocene epoch is marked by a series of continental glaciation periods, the "ice ages", times of cooling at high latitudes and compression of the tropics, especially in the northern hemisphere. These ice ages were interspersed by interglacial periods of warming, during which tropical and sub-tropical organisms could extend their ranges poleward.

The calculated time since genetic divergence would allow for a major genetic interchange between the two sub-populations of P. elegans sometime during the Pleistocene. Such an interchange may have taken place several times during the epoch. The fact that the haplotype of one individual (208-7, Fig. 3 and 4) from the Mexican sub-population cannot be statistically separated from several of the Peruvian haplotypes suggests that its ancestor(s) arrived from Peru relatively recently. This lends further credence to the multiple genetic exchange hypothesis. The two main clusters would both show closer affinities within themselves if a major vicariant event had stopped gene flow or if a single dispersal had taken place. The "interlopers" in each cluster, Peru 177-6 and Mexico 208-7 indicate recent dispersal across the tropics.

4.4.6 Other disjunct distributions in the eastern Pacific

The disjunct distribution of P. elegans is not unique among the marine crustacean fauna of the eastern Pacific. The sand crab, Emerita analoga, ranges from Kodiak Island, Alaska to Bahia Magdalena, Baja California, and it also occurs in Peru and Chile. Other geographically isolated populations have been described from the head of the Gulf of California and Caleta Falso, Argentina (Haig and Abbott 1980). Emerita's usual distribution roughly corresponds to the two eastern Pacific species of Pollicipes. It ranges into latitudes north of Oregon only during El Niño periods (Hart 1982). Despite some differences in habitat requirements (sand crabs are limited to sandy beach habitats rather than the rocky substrata necessary for settlement of Pollicipes larvae), both species are presently excluded from the tropical eastern Pacific. Initial rough estimates of genetic divergence between amphitropical sub-populations of Emerita (about 2%, Kit and Kornfield, ms) are relatively close to the value of divergence between the two sub-populations of P. elegans (about 1.2%).

In addition, Cancer porteri, a species of Cancroid crab with a similarly disjunct range, was only recently split in two taxonomically with the re-evaluation of the northern population as a distinct species (Cancer johngarthi, Carvacho 1989). The two species of crabs exhibit low-latitude submergence, inhabiting deeper water (along a broadly defined latitudinal isotherm) as they near the equatorial region (Garth 1960). A study of genetic

divergence between these closely related species could be compared with the divergence data for sub-populations of both sand crabs and goose barnacles.

Hubbs (1952) noted that many species of fish, sea lions, right whales and giant kelp Macrocystis are amphitropical, present in higher latitudes in both northern and southern hemispheres, but absent in warmer, tropical regions. Randall (1981) later added many other species of fishes to this list.

4.4.7 Southern hemisphere endemism

The paramphitropical distribution exhibited by P. elegans has been discussed by Newman and Foster (1987) in their analysis of amphitropicality as a potential pre-condition for present day patterns of southern hemisphere endemism. They suggested that shallow water marine organisms living in eastern boundary areas of the Atlantic and the Pacific Oceans would be susceptible to population division by changes in current patterns and sea surface warming in the tropics. These divided populations would then be subject to extinction in the northern hemisphere.

The data presented here generally support a hypothesis of genetic division of the paramphitropical disjunct sub-populations of P. elegans. These data are also consistent with Newman and Foster's hypothesis in the sense that the northern hemisphere sub-population has a lower genetic diversity than the southern hemisphere. As mentioned above, this lower diversity suggests that it has a smaller effective population size than its southern hemisphere counterpart. If this is the case, the small northern

populations would be much more sensitive to local habitat disruptions or environmental changes which might effect a local extinction, leading to southern hemisphere endemism.

4.5 Conclusions

The paramphitropical sub-populations of Pollicipes elegans have a net nucleotide sequence divergence of about 1.2%. Initial calculated estimates for the timing of this divergence range from 292,500 YBP to 1.26 MYBP. This range of time coincides with the Pleistocene epoch and periods of cooling and warming which may have allowed for a series of expansions and contractions of P. elegans populations in the eastern tropical Pacific. It is further hypothesized that these expansions and contractions represent alternating periods of genetic exchange and isolation of the two populations. This may be tested by genetic analysis of some of the many other organisms with similar disjunct distributions.

CHAPTER 5

A molecular phylogeny of the edible goose barnacle genus Pollicipes, a Tethyan relict

5.1 Introduction

Despite a rich fossil record in Europe (Darwin 1851a, Withers 1953) and western Australia (Buckeridge 1983) dating from the Upper Cretaceous and perhaps as early as the Jurassic, only three species remain extant in the edible goose barnacle genus, Pollicipes (Foster 1978, Newman and Killingley 1985, see Fig. 6 herein). Two of these species inhabit wave swept rocky intertidal zones along the open coast of the eastern Pacific margin. Specifically, P. polymerus ranges from Punta Abreojos, Baja California to British Columbia (Newman and Abbott 1980); P. elegans, the other eastern Pacific species, ranges from central Peru to Punta Abreojos, Baja California, with a distinct range gap along the Pacific coast of Central America, just north of the equator (Newman 1992, Kameya and Zeballos 1988); and the third species, P. pollicipes, is found in similar habitats in the eastern Atlantic from the Cape Verde Islands (northern Africa) to the Iberian peninsula and, occasionally, southern England (Newman and Killingley 1985).

Darwin (1851b) was the first to note that P. elegans of the eastern Pacific and P. pollicipes of the eastern Atlantic are very closely related. It has been hypothesized that P. pollicipes and P. elegans represent relic elements of the Tethys Sea fauna which have become restricted to the eastern boundary conditions of the

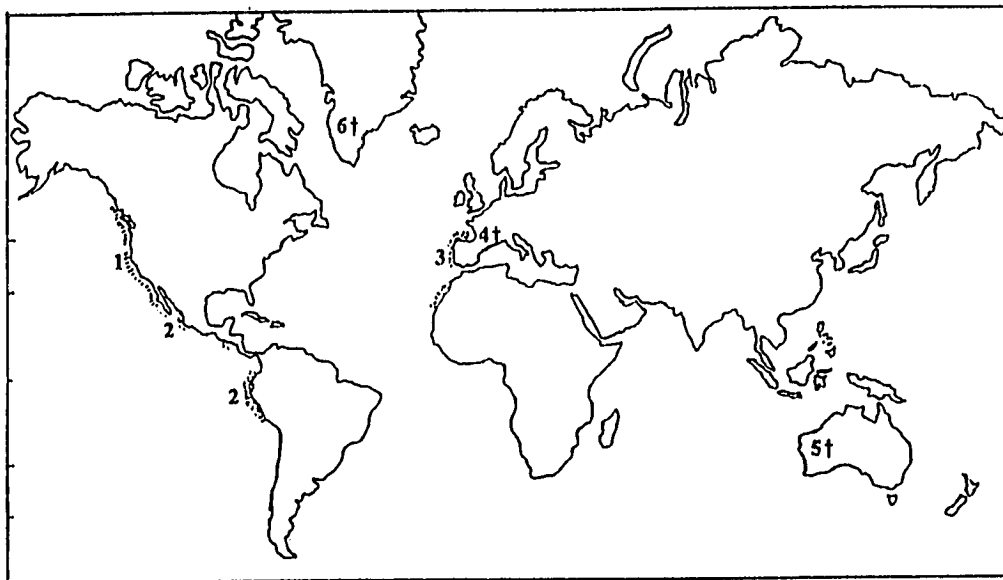


Fig. 6: Distribution of extant and fossil species in the barnacle genus Pollicipes.

1 = P. polymerus, extant, northeastern Pacific

2 = P. elegans, extant, paramphitropical eastern Pacific

3 = P. pollicipes, extant, northeastern Atlantic

4† = several fossil species, southern Europe

5† = fossil species, western Australia

6† = fossil species, Greenland

Atlantic and Pacific Oceans (Newman and Killingley 1985, Newman and Foster 1987, Newman 1992). The present distributions of the three species therefore suggest that P. polymerus diverged from an ancestral pollicipedine prior to the divergence of P. pollicipes and P. elegans. In order to test this hypothesis, molecular phylogenies of 1) the three species of Pollicipes and two ecologically similar but distantly related species, Capitulum mitella, Calantica villosa, from within the Scalpellomorphs and 2) the five species from the first analysis, and an additional barnacle species, Lepas anatifera a lepadomorph, are presented. These molecular analyses are also compared with a newly constructed phylogeny of the 5 scalpellomorph species based on a suite of morphological characters.

5.2 Materials and methods

5.2.1 General methods and specimen acquisition and deposition

Barnacle specimens were obtained from a variety of sources (see Chapter 2 and Appendix 2). Most specimens were received preserved in ethyl or isopropyl alcohol, but some tissues were kept frozen until after DNA was extracted (see Chapter 2 and Appendix 1). All specimens from which tissues were extracted for analysis are housed in the collections of the California Academy of Sciences, Department of Invertebrate Zoology and Geology.

5.2.2 Morphological characters and analysis

Many of the characters chosen for the morphological study were based on various shell and appendage characters which have

been previously used by others to describe or diagnose the species in the genus Pollicipes, the subfamily Pollicipedinae, and the other two scalpellomorphs used in the analysis (Darwin 1851b, Withers 1928, Foster 1978, Zevina 1981). These characters are listed below as numbers 1-7, 9, 11, 14, and 16-18. The remaining characters (numbers 8, 10, 12, 13, 15, and 19-25) were defined by myself from personal examination of several specimens from each species. Characters are scored 1 if they are present in a species and 0 if they are lacking. Characters used were defined as follows:

- 1) Less than 19 capitulum plates in hermaphrodite.
- 2) Imbricating capitular plates condensed into a single whorl.
- 3) 2 or more distinct whorls of capitular imbricating plates.
- 4) Incremental growth lines sculpturing major plates of the capitulum.
- 5) Thick "hirsute" cuticular integument on the peduncle and capitulum.
- 6) Absence of filamentary appendages.
- 7) Multi-articulate caudal appendages.
- 8) Caudal appendages not setose on upper margins.
- 9) Absence of complemental males.
- 10) Peduncular scales smaller than 1 mm in width.
- 11) Anterior rami of the sixth cirri with no more than 1.5 times the number of segments present on the anterior rami of the first cirri.
- 12) Upper latus nearly same length as carina.
- 13) Excluding the upper latus, all latera of about equal size.
- 14) Peduncle and/or opercular plates reddish.

- 15) Peduncle scales point outward rather than up toward capitulum.
- 16) Height of capitulum greater than rostrum-carina (R-C) diameter.
- 17) Peduncle scales spine-like or spindle shaped.
- 18) Tuft of spines at end of caudal appendages.
- 19) Scutum triangular.
- 20) Carina triangular.
- 21) Carina with sub-equal diamond shape.
- 22) Peduncular scales flexed, sub-equal oval shape.
- 23) Peduncular scales overlapping by about 1/2 scale length.
- 24) Peduncular scales flattened, blunt-tipped and triangular in shape.
- 25) Rows of peduncular scales overlapping by over 1/2 scale length.

The most parsimonious tree was constructed from these characters using maximum parsimony analysis (Swofford 1993, PAUP v. 3.1.1). A branch-and-bound bootstrapping routine was repeated 200 times to determine the stability of internal branch nodes.

5.2.3 DNA sequence data acquisition and analyses

DNA extraction, PCR and sequencing procedures are detailed in Chapter 2. Homologous portions of the cytochrome c oxidase subunit 1 (CO1) gene of mtDNA genome were sequenced and compared. DNA data analysis was performed using the computer programs contained in Molecular Evolutionary Genetics Analysis (MEGA ver. 1.0, Kumar et al. 1993). These programs execute a neighbor-joining tree building algorithm from genetic distances

between taxonomic units. Sequences were initially aligned by eye. The sequence data were then entered into MEGA format and the program was used to translate them into amino acid sequence to check for errors in alignment, mistakes in the reading of autoradiographs, typographical errors when transcribing data from data sheets to word processing files used in sequence alignment, and gaps in the readable sequences.

Phylogenetic trees were constructed from sequence data from all 6 barnacle species and, because of the small data set for Lepas anatifera, only the 5 scalpellomorph barnacle species. Data for the construction of trees was derived from maximum parsimony methods (Swofford 1993, PAUP v. 3.1.1), the neighbor-joining method, using distance data calculated from either the Juke-Cantor or the Tamura-Nei method (Kumar et al. 1993, MEGA v. 1.0). The Juke-Cantor method was chosen as the most generally appropriate method for calculating genetic distances for the present data. This decision was based upon recommendations made by Nei (1991) in his analysis of the accuracy of several distance estimating and tree-building methods under various circumstances. The Tamura-Nei method was used to select and analyze a reduced data set containing only transversional differences among the sequences compared. Transversions were weighted twice transitions in the parsimony (PAUP) analyses. Reasons for favoring transversion data in the analyses are explained further in the results section. Assumptions, logic and formulae for the Juke-Cantor method are presented in a brief review of the various distance estimating

methods contained in Chapter 2.

For each of the data sets analyzed with distance calculating methods, 500 bootstrapping replicates were performed for the calculation of each distance matrix and the construction of the accompanying tree. Gaps in sequence data were ignored in pair-wise comparisons rather than treated as a fifth character state in the analyses.

5.3 Results

5.3.1 Morphological comparisons

The morphological character data set gave trees that clearly link P. elegans and P. pollicipes together to the exclusion of the other three species (Fig. 7). The most parsimonious tree places P. polymerus in the branch below the P. elegans/P. pollicipes node (Fig. 7). The bootstrap analysis of the morphological data gives very strong support to the node of P. elegans and P. pollicipes (Fig. 8, 98% bootstrap confidence level, BCL) but only moderate support for the node linking all three species of Pollicipes (Fig. 8, 83% BCL).

A list of the morphological characters and their states for each species is given in Table 9.

5.3.2 Molecular comparisons

5.3.2.1 Sequence variation and statistics

Comparable DNA sequence data from the CO1 gene were obtained from at least two individuals for each species (see Appendices 1 and 2 additional information). Homologous sequences of 540 nucleotides were obtained from every species except Lepas anatifera. Only 279 nucleotides of confirmed sequence data are

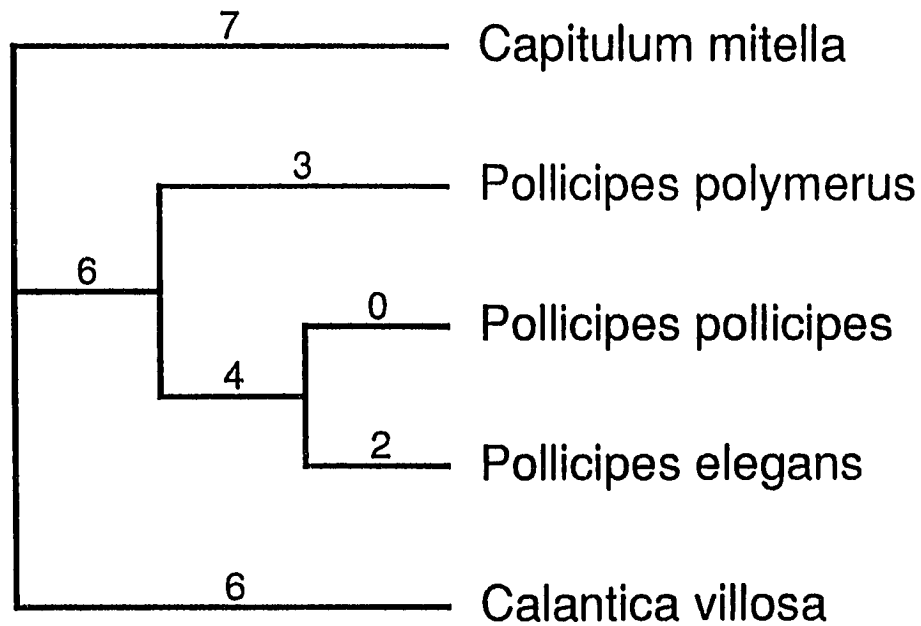


Fig. 7: Most parsimonious tree (28 steps) for morphological character data set of the three species of Pollicipes, Capitulum mitella and Calantica villosa. See Table 9 for character data matrix.

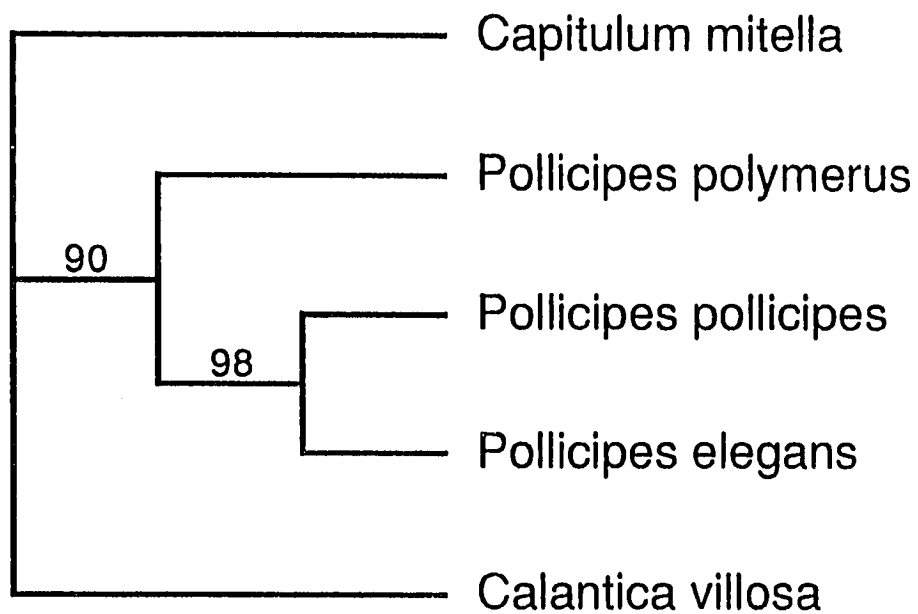


Fig. 8: Branch-and-bound bootstrap tree of 3 species of Pollicipes, Capitulum mitella and Calantica villosa, morphological data set (see text for character description, Table 9 for matrix and states).

Species	Character number																								
	1111111111										222222														
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5
<u>Pollicipes polymerus</u>	0	0	1	0	0	0	0	1	1	1	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0
<u>Pollicipes pollicipes</u>	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0
<u>Pollicipes elegans</u>	0	1	0	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0
<u>Capitulum mitella</u>	0	0	0	1	0	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1
<u>Calantica villosa</u>	1	0	0	0	1	1	0	1	0	1	1	0	1	0	1	0	1	0	1	0	1	1	0	0	0

Table 9: Morphological character data matrix for three species of Pollicipes, Capitulum mitella and Calantica villosa. See text for description of characters and character state definitions.

available for Lepas (Appendix 3).

The 5 taxon scalpellomorph sequence comparison shows 200 variable positions (Appendix 3), 75 of which are phylogenetically informative, 70 positions are two-fold degenerate positions, and 80 are four-fold degenerate positions. Transition to transversion ratios are no more than 1.811 for each species pair (Table 10).

The addition of the lepadomorph barnacle (Lepas anatifera) sequence data resulted in 208 variable positions (Appendix 3). Sixty-five of the variable positions are phylogenetically informative, 33 positions are two-fold degenerate and 42 are four-fold degenerate positions (Appendix 3). The species pair comparisons gave transition/transversion ratios from over 1.8 to less than 0.8 (Table 11).

Consensus DNA sequences have been submitted to GenBank.

5.3.2.2 Phylogenetic trees

5.3.2.2.1 Parsimony trees

The scalpellomorph barnacle haplotype phylogeny represented by a 433 step most parsimonious tree generated by an exhaustive search on PAUP (Swofford 1993) groups the three species of Pollicipes, with P. elegans and P. pollicipes branching off from P. polymerus together (Fig. 9). The next shortest tree (435 steps) swaps the branches of P. polymerus and Capitulum mitella. Calantica villosa is an outlier in both of these trees. A bootstrap analysis shows bootstrap confidence level (BCL) support for the P. elegans/P. pollicipes branch at 76% and all three species of

OTU	2	3	4	5
1	1.390	1.838	1.585	0.885
2		1.545	1.349	1.016
3			1.439	0.857
4				1.053

Table 10: Transition/transversion ratios for CO1 sequence data pair-wise comparisons among 5 scalpellomorph barnacle species.

OTU Labels:

- 1.. Pollicipes polymerus
- 2.. Pollicipes elegans
- 3.. Pollicipes pollicipes
- 4.. Capitulum mitella
- 5.. Calantica villosa

OTU	2	3	4	5	6
1	1.450	1.811	1.585	0.869	1.031
2		1.688	1.341	1.016	0.811
3			1.439	0.857	0.938
4				1.053	1.059
5					0.903

Table 11: Transition/transversion ratios for CO1 sequence data pair-wise comparisons among 6 barnacle species.

OTU Labels:

- 1.. Pollicipes polymerus
- 2.. Pollicipes elegans
- 3.. Pollicipes pollicipes
- 4.. Capitulum mitella
- 5.. Calantica villosa
- 6.. Lepas anatifera

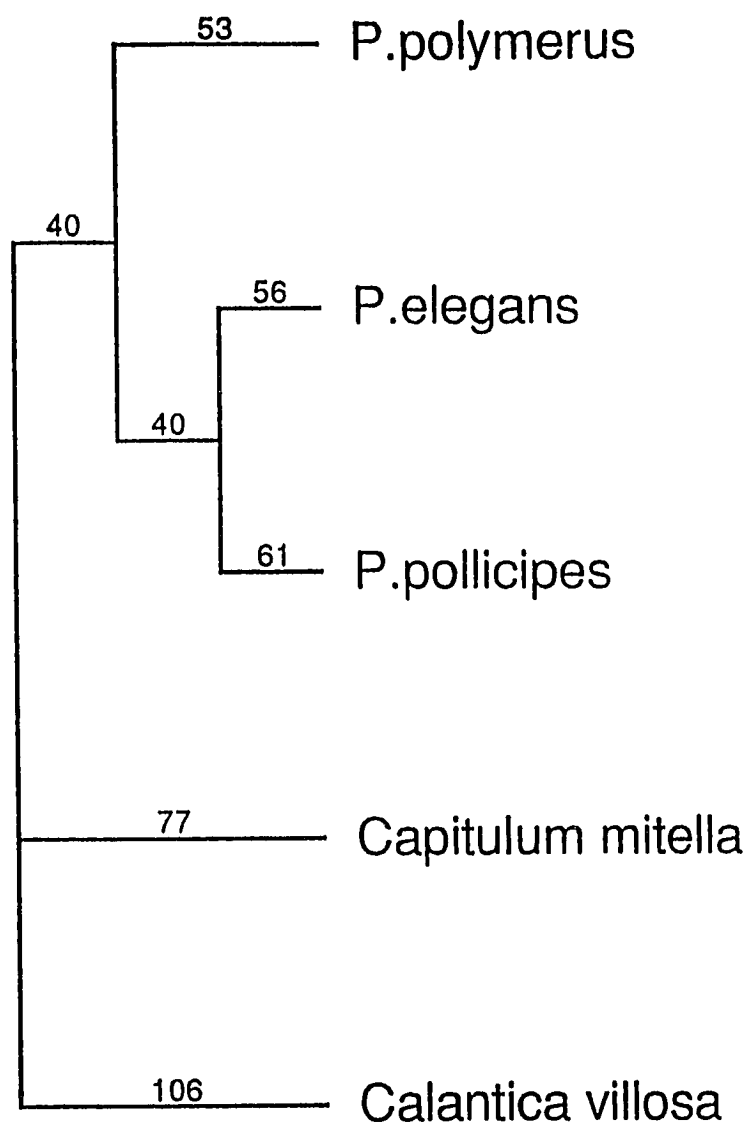


Fig. 9: 433 step most parsimonious tree constructed from an exhaustive search of sequence data from CO1 gene of 3 species of Pollicipes, Capitulum mitella and Calantica villosa. Transversion weighted twice transitions, $g1 = 0.033519$, next shortest tree 435 steps.

Pollicipes at 67% (Fig. 10). No other groups could be supported above the 50% BCL by the data.

The most parsimonious tree representing the phylogeny of the scalpellomorphs and Lepas (the additional outgroup species) also groups P. elegans and P. pollicipes (Fig. 11, 652 steps). In fact, the tree is identical in form to the five species tree with Lepas anatifera at the base. The g_i for the shortest tree, -0.780828, is well below the critical value for 6 taxa with 100-250 four-state characters (Hillis and Huelsenbeck 1992). A branch-and-bound bootstrap test shows support for the P. elegans/P. pollicipes grouping at the 79% BCL (Fig. 12). The grouping of the P. elegans/P. pollicipes node with P. polymerus is supported at the 56% BCL (Fig. 12).

5.3.2.2.2 Genetic distances and distance trees

Distance values among the 5 scalpellomorph taxa are given in Table 12 (p-distance values) and Table 13 (Jukes-Cantor method). The Jukes-Cantor distance values between species pairs range from 0.1803 (P. elegans and P. pollicipes) to 0.2824 (P. elegans and Calantica villosa). When Lepas anatifera is added to the pair-wise comparison calculations, the p-distance and Jukes-Cantor distance values are very similar, but slightly higher (Tables 14 and 15). The Jukes-Cantor corrected genetic distances for the 6 species data set range from a low of 0.1828 (once again P. elegans and P. pollicipes) to the high of 0.3084 (P. elegans and C. villosa).

Pollicipes elegans and P. pollicipes are paired together in all of the trees generated by both data sets (Figs. 9, 10, 11, 12). The

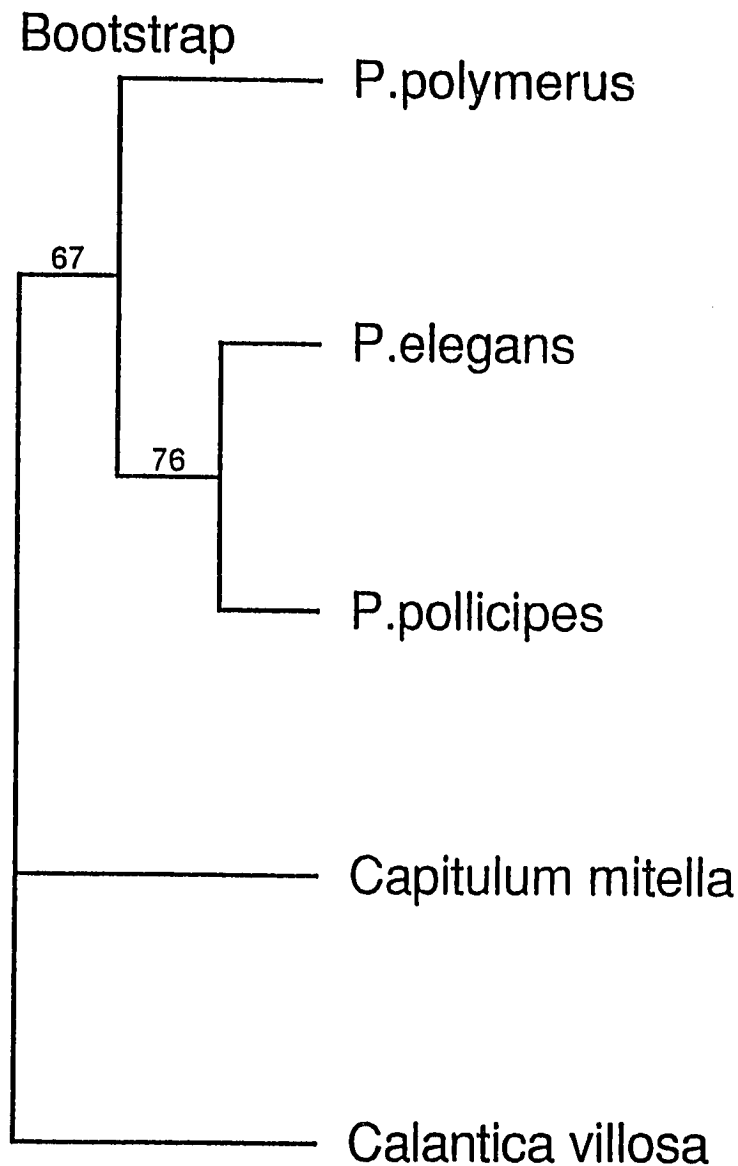


Fig. 10: Branch-and-bound bootstrap parsimony tree constructed from sequence data from CO1 gene of 3 species of Pollicipes, Capitulum mitella and Calantica villosa. Transversion weighted twice transitions, 100 replicates.

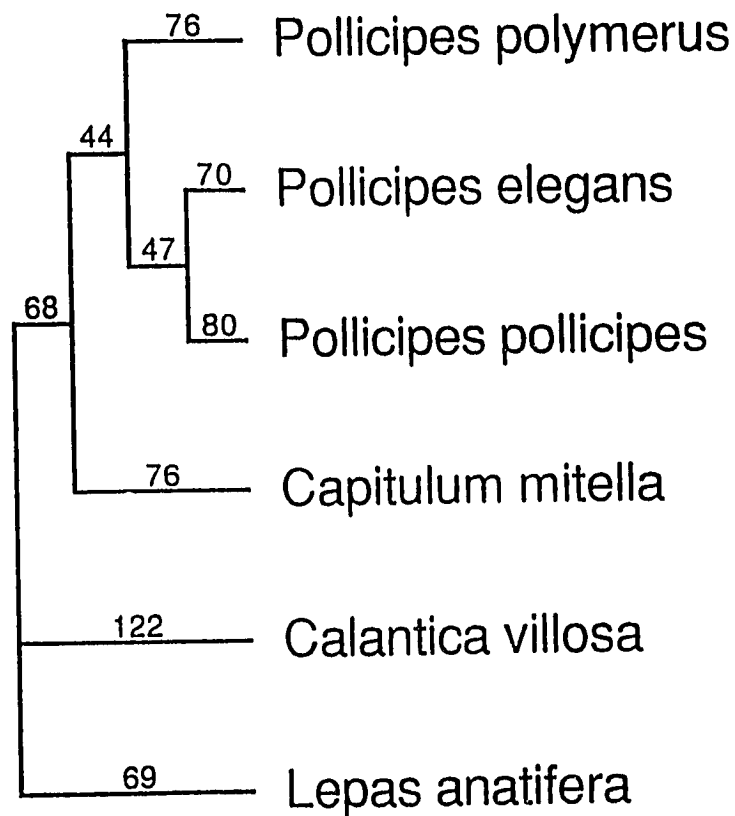


Fig. 11: Most parsimonious tree of 3 species of Pollicipes, Capitulum mitella, Calantica villosa, and Lepas anatifera for CO1 sequence data set. 540 positions compared. 652 steps, next shortest 655 steps. Transversions weighted twice transitions, $g1 = -0.7808$

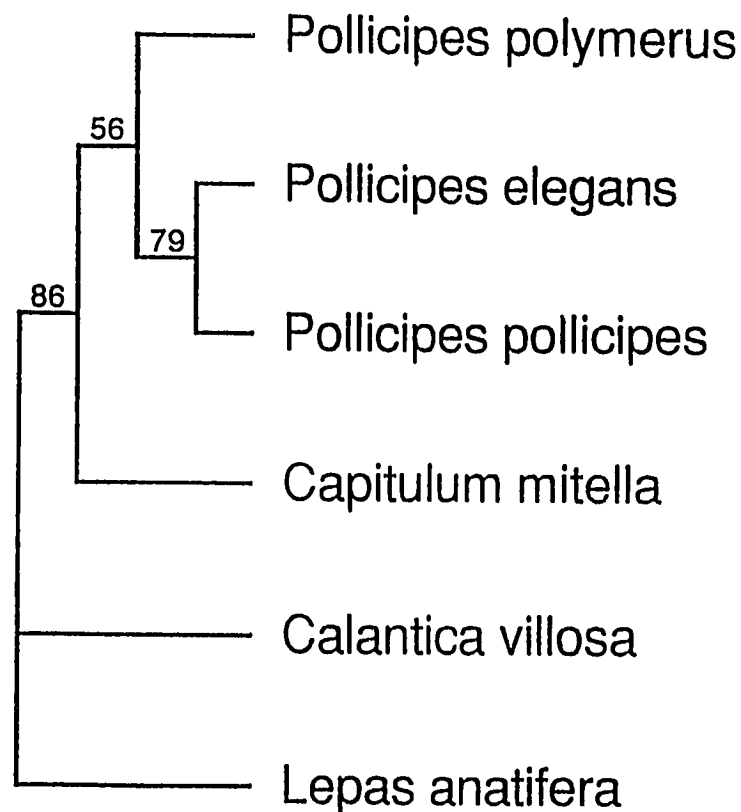


Fig. 12: Branch-and-bound bootstrap tree of 3 species of Pollicipes, Capitulum mitella, Calantica villosa, and Lepas anatifera for CO1 sequence data set. 540 positions compared. Transversions weighted twice transitions. 100 replications

OTUs	1	2	3	4	5
1		0.1835	0.1996	0.2103	0.2154
2	0.0168		0.1588	0.2000	0.2332
3	0.0174	0.0159		0.2000	0.2216
4	0.0182	0.0178	0.0179		0.2317
5	0.0178	0.0183	0.0181	0.0188	

Table 12: Genetic p-distance values for 5 scalpellomorph barnacle species analysis. Distances in the upper-right matrix, standard errors in lower-left matrix.

OTU Labels:

- 1.. Pollicipes polymerus
- 2.. Pollicipes elegans
- 3.. Pollicipes pollicipes
- 4.. Capitulum mitella
- 5.. Calantica villosa

OTUs	1	2	3	4	5
1		0.2105	0.2321	0.2468	0.2539
2	0.0222		0.1784	0.2326	0.2793
3	0.0238	0.0202		0.2326	0.2627
4	0.0252	0.0243	0.0244		0.2771
5	0.0250	0.0265	0.0257	0.0272	

Table 13: Genetic distance values for 5 scalpellomorph barnacle species analysis, Jukes-Cantor method. Distances in the upper-right matrix, standard errors in lower-left matrix.

OTU Labels:

- 1.. Pollicipes polymerus
- 2.. Pollicipes elegans
- 3.. Pollicipes pollicipes
- 4.. Capitulum mitella
- 5.. Calantica villosa

OTUs	1	2	3	4	5	6
1		0.1839	0.1981	0.2107	0.2139	0.2355
2	0.0168		0.1626	0.2040	0.2369	0.2410
3	0.0174	0.0160		0.2000	0.2216	0.2279
4	0.0182	0.0179	0.0179		0.2317	0.2574
5	0.0178	0.0184	0.0181	0.0188		0.2122
6	0.0255	0.0257	0.0254	0.0265	0.0245	

Table 14: Genetic p-distance values for 6 barnacle species analysis. Distances in the upper-right matrix, standard errors in lower-left matrix

OTU Labels:

- 1.. Pollicipes polymerus
- 2.. Pollicipes elegans
- 3.. Pollicipes pollicipes
- 4.. Capitulum mitella
- 5.. Calantica villosa
- 6.. Lepas anatifera

OTUs	1	2	3	4	5	6
1		0.2109	0.2300	0.2474	0.2518	0.2827
2	0.0222		0.1832	0.2380	0.2848	0.2907
3	0.0236	0.0205		0.2326	0.2627	0.2717
4	0.0253	0.0246	0.0244		0.2771	0.3152
5	0.0248	0.0268	0.0257	0.0272		0.2495
6	0.0372	0.0378	0.0365	0.0404	0.0342	

Table 15: Genetic distance values for 6 barnacle species, Jukes-Cantor method. Distances in the upper-right matrix, standard errors in lower-left matrix

OTU Labels:

- 1.. Pollicipes polymerus
- 2.. Pollicipes elegans
- 3.. Pollicipes pollicipes
- 4.. Capitulum mitella
- 5.. Calantica villosa
- 6.. Lepas anatifera

bootstrap of the distance data calculated by the Jukes-Cantor method supports the branch shared by these two species at the 76% BCL for the 5 scalpellomorph species tree. The C. mitella linking node is supported at 57% BCL. The trees constructed by employing standard error calculations give higher levels of confidence, with 88% confidence probability (CP) for the P. elegans and P. pollicipes node and 61% CP for the C. mitella node joining with the former two species. When Lepas is added to the bootstrapped comparisons, confidence level support for the P. elegans and P. pollicipes node is 68% and for C. mitella is only 44%. Standard error trees give confidence probability levels of 88% and 61%, respectively. Note that the 44% BCL for the C. mitella and P. pollicipes/P. elegans node is so low that it is essentially meaningless.

All of the trees resulting from distance data comparisons group P. polymerus and Capitulum mitella with P. pollicipes and P. elegans to the exclusion of Calantica villosa and Lepas (Figs. 13 and 14, 94% BCL for Fig. 14). However, the scalpellomorph only tree offers a relatively weak link between Capitulum and the two most closely related species of Pollicipes with a (non-significant) BCL value of 54%. The 6 species tree also groups Capitulum here. However the BCL is only 56% which, as mentioned above, is not a significant value. Therefore, the branching pattern between the P. elegans/P. pollicipes node, P. polymerus and Capitulum cannot be resolved with the present distance "unfiltered" data set. For this reason, an attempt was made to filter some of the "noise" from data set.

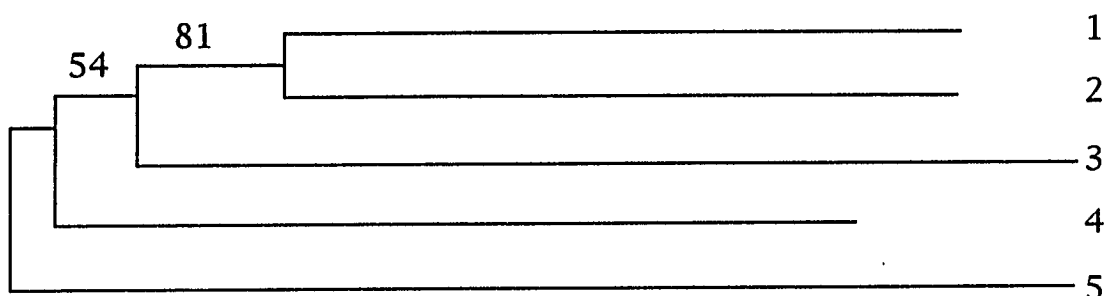


Fig. 13: Bootstrapped tree for CO1 mtDNA sequence data from 5 scalpellomorph barnacle species, Pollicipes elegans (1), P. pollicipes (2), Capitulum mitella (3), P. polymerus (4), and Calantica villosa (5). Tree constructed from genetic distance data estimates (Jukes-Cantor) using the neighbor-joining method. 500 bootstrap replications. 540 nucleotide positions compared. Bootstrap confidence levels over 50% noted on branches. Branch lengths reflect genetic distances. Scale: each cm equal to genetic distance of 0.013605.

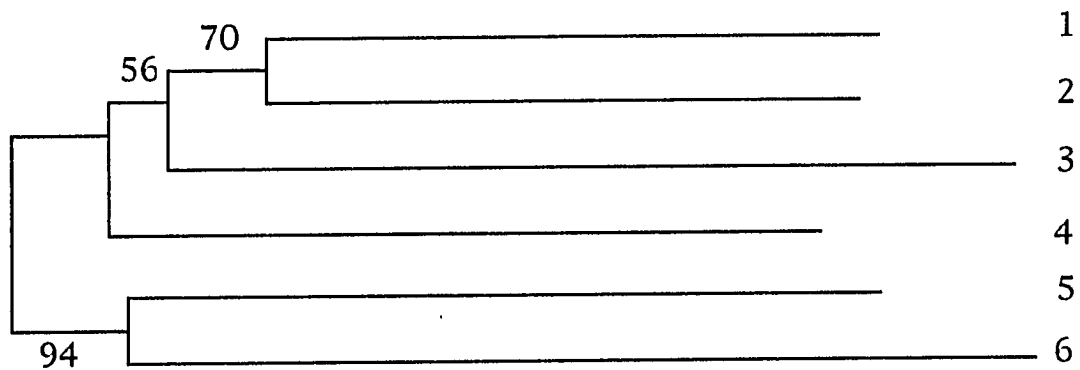


Fig. 14: Bootstrapped tree for CO1 mtDNA sequence data from 6 barnacle species, Pollicipes elegans (1), P. pollicipes (2), Capitulum mitella (3), P. polymerus (4), Calantica villosa (5), and Lepas anatifera (6). Tree constructed from genetic distance data estimates (Jukes-Cantor) using the neighbor-joining method. 500 bootstrap replications. 540 nucleotide positions compared. Bootstrap confidence levels over 50% noted on branches. Branch lengths reflect genetic distances. Scale: each cm equal to genetic distance of 0.013765.

5.3.2.2.3 Trees resulting from transversion data set analyses

It is now generally accepted that, when genetic isolation and divergence between species or populations first begins, the so-called transitional point mutations accumulate faster than transversional changes (DeSalle, et al. 1987, mtDNA NADH gene nucleotide sequences in Drosophila; Irwin et al. 1991, mtDNA cytochrome b gene nucleotide sequences in mammals). Transitions, the changes from purine to purine (adenine and guanine) or pyrimidine to pyrimidine (thymine and cytosine) are favored over transversions (any other change) (Topal and Fresco 1976). This is because transitional changes allow for better base pairing with the bases in the complementary DNA strand. Although most base pairing follows the Watson and Crick adenine-thymine and guanine-cytosine pairing, "mispairs" between a purine and a pyrimidine (A-C and G-T) are possible. If one of the paired bases is in a minor tautomeric form, the "mispair" will conform precisely to the Watson and Crick model of DNA geometry. However, substitutions that form pyrimidine-pyrimidine pairs are not allowed (by model building) and purine-purine pairs require a 9 degree distortion of the glycosyl bond angle (Topal and Fresco 1976).

Because early transitional changes tend to "saturate" the positional differences, analysis of highly divergent sequence data is less biased if this difference in substitution rates of transitions and transversions is considered. The transition/transversion ratios in

the present data set (Tables 10 and 11) are relatively low, none higher than about 1.8 with many near or below 1.0. Therefore, an analysis of genetic distance values was made using only transversional sequence data. This comparison used the Tamura-Nei method for calculating sequence divergence (Tamura and Nei 1993 as presented in Kumar et al. 1993, MEGA v. 1.0).

When only transversions are considered, P. polymerus groups with the other two species of Pollicipes (Figs. 15 and 16). C. mitella branches off below the Pollicipes node. The BCL values for P. elegans and P. pollicipes are still relatively high (Figs 15 and 16, 85% and 81% respectively). However, the P. polymerus connection is not convincing with BCLs of only 71% (Fig. 15) and 62% (Fig. 16). Despite this low level of confidence, limiting the data analysis to transversional differences only does yield a shift in the branching pattern between P. polymerus, C. mitella and the P. elegans/P. pollicipes node.

5.3.2.3 Genetic distances and sequence divergence

The number of net nucleotide substitutions calculated with the Jukes-Cantor method between P. elegans and P. pollicipes is calculated as 18.03% with a standard error (SE) of 2.1% when the 5 scalpellomorph species are compared (Table 13). The divergence value increases slightly to 18.28% with a much higher SE of 2.89% when sequence data from Lepas anatifera is included in the analysis (Table 15).

Levels of genetic distance between all other pairs of scalpellomorph species are all over 20% in both data sets (Tables 13

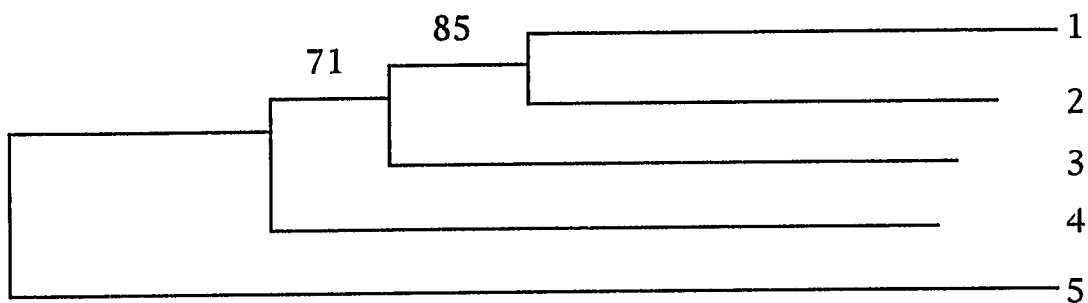


Fig. 15: Bootstrapped tree for CO1 mtDNA sequence data from 5 scalpellomorph barnacle species, Pollicipes elegans (1), P. pollicipes (2), P. polymerus (3), Capitulum mitella (4) and Calantica villosa (5). Tree constructed from genetic distance data estimates (Jukes-Cantor) using the neighbor-joining method. 500 bootstrap replicates. 540 nucleotide positions compared. Bootstrap confidence levels over 50% noted on branches. Branch lengths reflect relative genetic distances. Transitional changes not included in analysis. Scale: each cm equal to genetic distance of 0.00657.

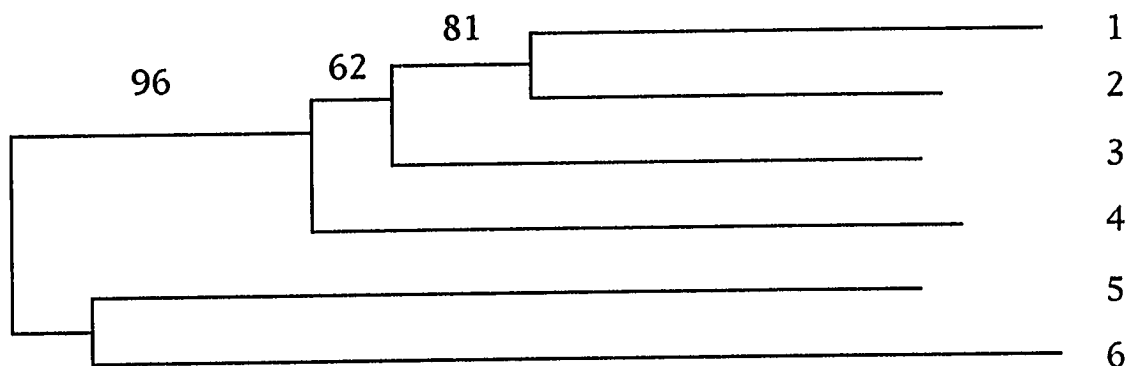


Fig. 16: Bootstrapped tree for CO1 mtDNA sequence data from 6 barnacle species, Pollicipes elegans (1), P. pollicipes (2), P. polymerus (3), Capitulum mitella (4), Calantica villosa (5), and Lepas anatifera (6). Tree constructed from genetic distance data estimates (Jukes-Cantor) using the neighbor-joining method. 500 bootstrap replications. 540 nucleotide positions compared. Bootstrap confidence levels over 50% noted on branches. Branch lengths reflect relative genetic distances. Transitional changes not included in analysis. Scale: each cm equal to genetic distance of 0.007075.

and 15). Nearly all of the distances are lower in the 5 taxon, scalpellomorph only, data set. The only exception to this is the distance values between Calantica villosa and the other four scalpellomorphs. These values are lower in the 6 taxon distance matrix.

Examining the remaining distance values species by species, for both data sets, P. polymerus is closer to P. elegans than any other species (0.2065 and 0.2275, Tables 13 and 15). Distances between P. polymerus and the other species continue in ascending order from P. pollicipes (0.2310 and 0.2378), C. mitella (0.2477 and 0.2641), C. villosa (0.2534 and 0.2430) and, lastly, L. anatifera (0.2915).

C. mitella is very slightly closer to P. pollicipes than the other two species in the genus with distance values of 0.2337 (Table 13, 5 taxa) and 0.2378 (Table 15, 6 taxa) compared with 0.2365 (Table 13) and 0.2535 (Table 15) to P. elegans and 0.2477 (Table 13) and 0.2641 (Table 15) to P. polymerus. The distance values between C. mitella and the other two taxa higher in both data sets, with Lepas furthest away (0.3084, Table 15).

C. villosa is clearly an outlier in the analysis of the 5 scalpellomorph taxa only. However, in the 6 taxon data set an anomolous value appears. The distance between C. villosa and P. polymerus in the 6 taxon distance matrix (0.2430, Table 15) is lower or only slightly higher than the distance between C. mitella and the three species of Pollicipes (Table 15).

5.4 Discussion

5.4.1 Phylogenetic relationships within the *Pollicipedinae*

Clearly, the paramphitropical eastern sub-tropical Pacific species, *P. elegans*, and the eastern sub-tropical Atlantic species, *P. pollicipes*, are more closely related to each other than either is to their congener in the eastern warm-temperate Pacific, *P. polymerus*. All of the phylogenetic trees, those based on molecular data as well as the tree derived from morphological data and the calculated genetic distances, support this relationship among the three species in the genus. *Pollicipes elegans* and *P. pollicipes* sequence comparisons also show a higher ratio of transitions to transversions than those between either of these species and *P. polymerus*. This indicates a shorter time of genetic divergence for the *P. elegans* and *P. pollicipes* species pair as transitional differences are known to be present in higher proportions to transversions during the initial stages of genetic divergence (DeSalle et al. 1987).

Based upon the present molecular data sets and analyses, the closest living relative of the three species of the genus *Pollicipes*, the western Pacific goose barnacle *Capitulum mitella*, cannot be confidently removed from the branch containing the genus *Pollicipes*. It clusters either with or within the three species of *Pollicipes* in all trees. Despite this close relationship to *Pollicipes*, the shift in the branches of *P. polymerus* and *C. mitella* which resulted from analysis of the "filtered" data set, containing only

transversions, does suggest that Capitulum is an outgroup to Pollicipes, as do the morphological data. The exact relationship of C. mitella to Pollicipes might be resolved by analyzing additional molecular data sets. This might be accomplished by either the addition of more sequence data from transversional changes within the CO1 mtDNA gene and other protein coding mtDNA genes, or by analysis of sequence data from a more slowly evolving gene, such as one of the ribosomal RNA genes.

5.4.2 Levels of genetic divergence

Despite the relative similarity of the DNA sequences of the two sub-tropical species of Pollicipes, they exhibit a very large percentage of divergent nucleotide sequence for congeners, about 18%. Although no mtDNA sequences have been published for barnacles, values for variation within genera are available for a variety of other taxa, including some crustaceans. Intra-generic differences in CO1 mtDNA gene sequences among some species within the snapping shrimp genus Alpheus from Panama are as high as those found among the barnacles in the present study (Knowlton et al. 1993). Trans-isthmusian differences within and between species of these shrimp range from a low of 6.6% between A. paracrinitus in the Caribbean and A. rostratus in the eastern Pacific, to a high of 19.7% between Caribbean and Pacific populations of A. cristulifrons. The high degree of divergence found between populations of the same species on either side of the isthmus is quite remarkable and unique. It is quite possible that these populations represent sibling species.

Cunningham et al. (1992) used the more slowly evolving 16s rRNA gene to study the relationship of the Alaskan king crab to hermit crabs. They found genetic divergence levels from 0.5 to 11% between species within the hermit crab genus Pagurus. The great disparity among these values may result more from paraphyly than genetic variation within the genus Pagurus (A. Harvey pers. comm.).

However, levels of divergence above 10% are present within other crustacean genera. Palumbi and Benzie (1991) found that two congeneric species of penaeid shrimp (Penaeus stylirostris and P. vannamei) differed at 11% of the compared nucleotides in the CO1 mtDNA gene. The divergence between these species in 12s rRNA sequences was 9.6%. They noted that this level of divergence within a genus is very high and can be compared with levels of divergence among orders of mammals.

Indeed, most other available data comparisons come from within the Mammalia. The high level of divergence within crustacean genera in general and barnacle genera in particular may be due to greater time since evolution of genus level morphological characters. For example, the barnacle genus Pollicipes is at least 55 million years old (Newman et al. 1969). The shrimp genus Penaeus dates to about 70 MYBP (Glaessner 1969). These dates coincide with the late Cretaceous/early Tertiary radiation of the placental mammalian orders (Carroll 1988).

5.4.3 Estimated time and potential causes of the genetic divergence of the Tethyan relict species of Pollicipes

The final closure of the isthmus of Panama, which occurred about 3 to 3.5 MYBP (Saito 1976, Duque-Caro 1990), is the most obvious physical barrier between the Pacific and Atlantic species of Pollicipes. However, the physical separation of the eastern Pacific and eastern Atlantic species has likely existed for substantially more than 3 million years, since the genus apparently has been excluded from the deep tropical areas surrounding Panama (Laguna 1985, Newman and Killingley 1987) and no fossil record of the genus in the tropical America or the Caribbean exists (Donovan and Davis-Strickland 1993).

The geographically widespread and speciose fossil record of species reputed to be Pollicipes in Europe, Greenland and western Australia indicates a broad radiation during the Lower Eocene, about 55 MYBP. During this epoch, the warm waters of the Tethys Sea covered much of what is now southern Europe and Asia, stretching from a broad opening into the western margins of the tropical Pacific to a narrowing shallows in central Europe and what is now the eastern Atlantic Ocean (Tozer 1989, Fig. 17 herein). The opening of the southern Atlantic Ocean basin between South America and Africa began during the Cretaceous Period, about 125 million years ago (van Andel 1979). However, the northern margins of those continents remained relatively close to each other until the early Oligocene or late Eocene, about 30-40 MYBP. By that

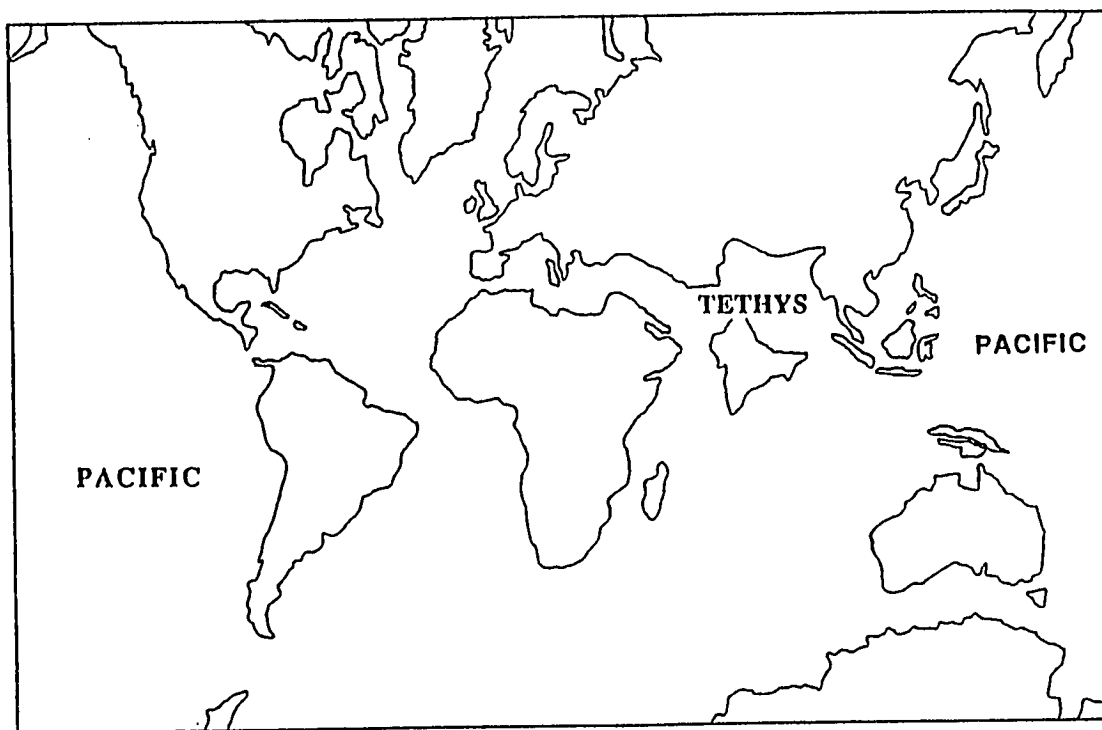


Fig. 17: Palaeogeographic reconstruction of the continents and oceans during early Tertiary time, about 50 MYBP (after Open University 1988). Note that continental margins are approximate present-day outlines, not meant to coincide precisely with ancient coastlines.

time the Atlantic may have become an effective barrier to gene flow via the planktonic larvae of shallow water coastal barnacle species. Van Andel (1979) suggests that a slowing of equatorial currents at the boundary of the Eocene/Oligocene epochs could have resulted in a drop in productivity along the equatorial tropical convergence zones. This slowing of the equatorial current across the widening Atlantic Ocean could correspond to a break in gene flow between populations of the ancestral stock of P. pollicipes and P. elegans. This stoppage of gene flow across the Atlantic correlated with the slowing of equatorial currents could be accompanied by extinction of Pollicipes populations in the western Atlantic or Caribbean coast of South America, from a drop in regional productivity at that time (Vermeij 1989).

The calculated genetic distance between P. pollicipes and P. elegans is about 18%. Converting this value into a date for genetic divergence requires an estimation of rate of nucleotide substitution over a relatively long period of time. Molecular clock variations have been well documented in several taxa; however, and general smoothing of curves over long time scales may allow for an estimation of a general range for dates of genetic divergence. Alternatively, we might consider a possible Tethyan origin of the genus Pollicipes and genetic divergence between P. pollicipes and P. elegans at, or near, the time of final closure of the Panamic seaway (mid to late Pliocene). This scenario implies a presence of Pollicipes in the western Atlantic just prior to the closure of the Panama seaway. Unfortunately for this hypothesis, no fossil evidence of

this presence exists, and the western Caribbean has very little suitable settlement substrata (Southward and Newman 1977). It is also possible that sea surface temperatures were too high for Pollicipes by that time. Therefore, despite the aberrantly high levels of divergence recorded for Alpheus cristulifrons on either side of the isthmus (19.7%, Knowlton et al. 1993), the sequences of P. pollicipes and P. elegans are too different for such a recent time of genetic divergence.

5.5 Conclusions

The eastern boundary distribution exhibited by the members of the genus Pollicipes, in both the Pacific and Atlantic Oceans, is relatively rare. This subtle pattern has been noted in but a few other organisms (Garth 1968, van den Hoek 1984, Vermeij 1986, crabs, red algae, and some mollusks, respectively). Newman (1992) has suggested that these are relicts of a once broader, Tethyan distribution. The present data support this hypothesis.

Specifically, the ranges of the extant species Pollicipes and their closest living relative, when combined with the fossil records for the genus, show a Tethyan distribution. Pollicipes pollicipes, the eastern Atlantic species, and Pollicipes elegans, the paramphitropical eastern Pacific species, are the most recently diverged of the three species in the genus. From the current distribution of the two species and the amount of calculated genetic distance between them, it is estimated that they diverged from each other near the Eocene/Oligocene boundary.

CHAPTER 6

Discussion and conclusions

6.1 Measuring biodiversity: variation within and among species

Current discussion regarding attempts to catalog the biological diversity present in the world can be considered a scaled-up continuation of the efforts of people through time to name and classify living organisms. Taxonomic classifications invariably stress the definition of certain groups of organisms (usually species) as morphological entities. Morphological variants within species were once described typologically as "forms" or "varieties". Modern taxonomists usually include the range of morphological variation among individuals for each taxon they describe as well as a suite of differences between the new taxon and similar taxa.

The mutability of species and the potential role of within species morphological variation in the process of natural selection are essential components of Darwin's The Origin of Species (1859). The merging of evolutionary theory with taxonomic study allowed for the development of classification schemes based on common descent rather than simply by grouping morphologically similar organisms.

These systematic classifications, or phylogenies, constructed from the distribution of similarities and differences in the morphological, physiological and molecular level attributes among the organisms under study, connote lines of descent. However, the attributes by which we classify and organize living things

taxonomically may or may not reflect a common ancestry. In fact, the most obvious attributes may be more directly associated with similar niche requirements or with local environmental conditions than with a shared lineage. For example, certain aspects of shell morphology in some species of barnacles, characters which taxonomists and systematists often employ to identify and classify species, can be affected by local environmental conditions (Bullock 1964, Van Syoc 1992). This plasticity of morphological and physiological characters under varying local environmental conditions could lead to erroneous conclusions when using exclusively phenotypic characters for phylogenetic analyses.

Despite the potential influence of environment, the phenotypic attributes of organisms are expressions of their genetic make-up. Newly available techniques can produce evidence for the inference of common descent by studying organisms at the molecular level. These data may confirm or refute older concepts based on studies of morphological characters alone.

The data from which molecular phylogenies are constructed, along with biogeographic patterns and the trees themselves, can also be effectively used to determine distinct sub-populations or groups (races, varieties or subspecies) within species (e.g. Brasher et al. 1992, Baker et al. 1993). Molecular data are also useful in the study of the major components of modern evolutionary theory: gene flow (Avice et al. 1988, Palumbi and Wilson 1990), natural selection (e.g. Lee and Vacquier 1992, Karl and Avice 1992,), population divergence (e.g. Rosel 1992, Brasher et

al. 1992), and speciation (Harrison 1991).

The range of differences among groups of organisms, usually at either the species level or above, is now called biodiversity. However, a study of global biodiversity could properly include the examination of the potential range of phenotypic and genetic characters within each species or population of organisms. Measuring variation of these characters within as well as among species can help us define not only the relative positions of groups of organisms within a family tree, but whether or not the tips of the tree branches have recently sprouted new growth.

6.2 Genetic diversity within the genus Pollicipes

There is considerable difference in the levels of genetic polymorphism within species in the genus Pollicipes. Pollicipes polymerus, the northeastern Pacific Ocean species, ranges along most of the western coast of North America, a latitudinal distance of over 3,300 km. However, mtDNA CO1 gene sequence data from P. polymerus exhibits no discernible latitudinal dichotomy or cline in genetic population structure and total genetic diversity levels were moderately low (0.5%).

Pollicipes elegans is a paramphitropical species with disjunct populations north and south of the north equatorial tropics region of the eastern Pacific. Despite the hypothesized genetic contact between the sub-populations within the last several thousand years (Van Syoc herein), they have a net genetic distance of 1.2% between sub-populations. The sampled populations of P.

elegans have a combined nucleotide polymorphism of 2.34%. In addition, each sub-population has higher genetic diversity levels (1.61% for Peru, 0.915% for Mexico) than P. polymerus.

Genetic distances between pairs of the three extant species of the genus Pollicipes range from about 18% (P. elegans and P. pollicipes) to 24% (P. polymerus and P. pollicipes). Although these distances are relatively high for within genus genetic divergence, they are also rather close to each other. This suggests a series of isolating events over a relatively short time followed by a long period of genetic divergence.

6.3 Biodiversity in the oceans

The data presented above are not unlike previous findings on genetic diversity within and among populations, and among closely related species. The levels of population- and species-level mtDNA diversity recorded here for goose barnacles are within the range of variation for the relatively few marine species which have been studied to date. Ovenden (1990) lists intraspecific divergence values for 13 marine species with a mean divergence of 1.2% and a range of 0.19% to 2.6%. Billington and Hebert (1993) compiled intraspecific sequence divergence values for 10 species of marine fishes which had a mean divergence of 1.01% and a range from 0.2% to 4.4%. However, a few marine species possess very great intraspecific genetic variation (e.g. shrimp, Alpheus cristulifrons, 19.7%, Knowlton et al. 1993). In addition, some catadromous and anadromous fishes have extremely little diversity (e.g. catadromous Atlantic eel, Anguilla rostrata, 0.1%, Avise et al. 1986; anadromous

striped bass, Morone saxatilis, 0.004%, Wirgin et al. 1989). The data tabulated by Billington and Hebert (1993) have a mean intraspecific divergence value of 0.54% for anadromous fishes. About half of the mean (1.01%) of the data they presented from marine species.

Low levels of intraspecific mtDNA diversity may be attributed to population bottlenecks and a reduction in genetic polymorphism (Wilson et al. 1985). The low genetic diversity values noted above for striped bass and Atlantic eel may reflect genetic bottlenecking effects associated with anadromy and catadromy. Extremely high levels of mtDNA sequence difference usually reflect genetic divergence between or among populations that have been isolated from each other for a very long time. In some cases these divergent populations may be morphologically conservative (and therefore undescribed) sibling species. In fact, Knowlton et al. (1993) used a combination of mating experiments and molecular comparisons to reveal that mating behavior and reproductive success was negatively correlated with genetic distance measures for populations of shrimp. The populations with the highest levels of genetic distance exhibit complete reproductive isolation (Knowlton et al. 1993). Therefore, if these two populations are subjected to the criteria of Mayr's biological species concept, they can be considered cryptic sibling species. Given the experience of past workers (e.g. Dando and Southward 1980, Laguna 1988), a detailed examination of these two shrimp populations may yield morphological characters which could

distinguish them from each other and thus help establish them as separate species.

Levels of genetic divergence among the species within a genus also vary greatly. The genetic distances within crustacean genera range from about 18 - 24% between species in the barnacle genus Pollicipes and 11% in the shrimp genus Penaeus (mtDNA CO1 sequences, Palumbi and Benzie 1991), to 2.6 - 8% in the lobster genus Jasus (mtDNA RFLPs, Brasher et al. 1992). Amazingly, Burton and Lee (ms) have found intraspecific divergence levels in CO1 nucleotide sequences of over 25% between closely approximated populations of the Tigriopus californicus, an intertidal copepod in the eastern Pacific Ocean. However, there is also some evidence of hybrid breakdown in the descendents of crosses between these divergent populations (Burton 1990).

Palumbi and Benzie (1991) have pointed out that these greatly differing amounts of genetic divergence among populations of a species, or species of a genus, may reflect differing rates of change in morphological characters used to define these taxon levels. The tremendous range of values for measured genetic variation within species and genera point to the danger of attempting to equate a particular taxonomic level with some arbitrary amount of evolutionary distance or time of existence. That is, populations within a species, or species within a genus, may exhibit varying degrees of genetic and morphological difference.

There is mounting evidence, however, that accumulating sequence data for divergence rates of different taxa and varying

taxonomic levels will allow for the construction of many different molecular clocks that may function very well within certain constraints. The data contained herein is, therefore, a beginning toward the collection of data that could well eventually lead to the calibration of a mtDNA sequence rate clock for barnacles.

APPENDIX 1

Original preservation of specimens and time until DNA extraction

R. Van Syoc numbers (RVS XXX-X) refer to field notes, copies of which are archived at the California Academy of Sciences, San Francisco, CA. This table contains information only for sequenced specimens. See Appendix 1 for catalog numbers, collection localities and collection dates of voucher specimens lots. IsOH = isopropanol
EtOH = ethanol

Species and specimen number	Original Preservative	Time until DNA extraction
<u>Pollicipes polymerus</u>		
RVS 186-1	frozen (-20°C)	3 months
RVS 186-2	frozen (-20°C)	3 months
RVS 202-1	frozen (-20°C)	4 days
RVS 202-3	frozen (-20°C)	4 days
RVS 202-4	75% EtOH	4 days
RVS 202-7	75% EtOH	2 months
RVS 202-8	75% EtOH	2 months
RVS 202-9	75% EtOH	2 months
RVS 203-1	95% IsOH	3 weeks
RVS 203-2	95% IsOH	3 weeks
RVS 203-4	95% IsOH	3 weeks
RVS 203-5	95% IsOH	3 weeks
RVS 203-7	95% IsOH	13 weeks
RVS 203-11	95% IsOH	13 weeks
RVS 203-12	95% IsOH	13 weeks
RVS 203-15	95% IsOH	13 weeks

Pollicipes elegans

RVS 177-5	frozen (-70°C)*	3 yrs. 8 months
RVS 177-6	frozen (-70°C)	1 yrs. 10 months
RVS 177-7	frozen (-70°C)*	3 yrs. 8 months
RVS 177-8	frozen (-70°C)	1 yrs. 10 months
RVS 177-9	frozen (-70°C)	1 yrs. 11 months
RVS 177-10	frozen (-70°C)	1 yrs. 11 months
RVS 177-12	frozen (-70°C)	1 yrs. 11 months
RVS 208-1	95% EtOH	4 days
RVS 208-2	95% EtOH	4 days
RVS 208-3	95% EtOH	4 days
RVS 208-4	95% EtOH	4 days
RVS 208-5	95% EtOH	4 days
RVS 208-6	95% EtOH	4 days
RVS 208-7	95% EtOH	10 months

*All frozen RVS 177-x specimens were preserved in 75% ethanol (EtOH) 2 years after collection. Therefore, subsequent DNA extractions were made from ethanol preserved tissues.

Pollicipes pollicipes

RVS 205-4	EtOH (?)	7 months
RVS 205-5	EtOH (?)	7 months
RVS 205-6	EtOH (?)	7 months
RVS 205-15	EtOH (?)	1 yr. 7 months
RVS 205-20	EtOH (?)	1 yr. 7 months

Capitulum mitella

RVS 204-3	EtOH (?)	2 weeks
RVS 204-5	EtOH (?)	2 weeks
RVS 204-7	EtOH (?)	2 weeks
RVS 204-A	EtOH (?)	1 yr. 6 months

Calantica villosa

RVS 209-1	EtOH (?)	4 months
RVS 209-2	EtOH (?)	4 months

Lepas anatifera

LAA-1	EtOH (?)	9 months
LAA-2	EtOH (?)	9 months

APPENDIX 2

Specimen collection localities and archival deposition

All specimens have been deposited at the California Academy of Sciences, Golden Gate Park, San Francisco, California. CAS catalog number followed by R. Van Syoc number (if any) and collection locality.

Pollicipes polymerus

CAS 091955, RVS-202, pier at Scripps Institution of Oceanography, La Jolla, California, 29 March 1991, R. Van Syoc. (DNA sequencing)

CAS 081295, RVS-203, Seppings Island, Barkeley Sound, Vancouver Island, British Columbia, Canada, 13 June 1991, W. Austin. (DNA sequencing)

CAS 091956, Salt Point, Sonoma County, California, 3 November 1992, R. Van Syoc. (Protein electrophoresis)

CAS 091957, pier at Scripps Institution of Oceanography, La Jolla, California, October 1992, E. Vetter. (Protein electrophoresis)

Pollicipes elegans

CAS 081299, RVS-177, Callao, Peru, June 1989, A. Kameya via W. A. Newman. (DNA sequencing)

CAS 091958, RVS-208, north end of beach on west side of Hotel Finistierra, Cabo San Lucas, Baja California del Sur, Mexico, 16 May 1992, R. Van Syoc. (DNA sequencing)

Pollicipes pollicipes

CAS 091959, RVS-205, mid-tidal zone, Cabo de Sines, southwest coast of Portugal, 24 Oct 1991, T. Cruz. (DNA sequencing)

Calantica villosa

CAS 091960, RVS-209, Warrington, near Dunedin, New Zealand, 3 May 1992, K. Probert. (DNA sequencing)

Capitulum mitella

CAS 081300, RVS-204, Jogashima, Misaki, Kanagawa, Japan, 8 October 1991, T. Yamaguchi. (DNA sequencing)

Lepas anatifera

CAS 086774, attached to board washed up on beach, Rhosilli Bay at Hillend, Gower Peninsula, Glamorgan, Wales, 12 September 1992, R. Lawson. (DNA sequencing)

APPENDIX 3

DNA sequence data used for all analyses contained in the text.

Pollicipes polymerus population data, Chapter 3

				111	111	111	122	222	222	222	333	333	333	444	444	444	455	555
123	456	789	012	345	678	901	234	567	890	123	456	789	012	345	678	901	234	
186-1	TTA	CCC	CCT	GCC	TTA	ATG	TTA	TTA	ATT	AGA	GGA	TCA	CTA	GTA	GAA	GCT	GGG	GGC
186-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-
202-3	...	...	...	...	...	...	...	...	...	...	...	...	...	?	...	...	...	...
202-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-
202-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-	...	...	...
202-8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-	...	...	-
202-9	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	-	...	...	-
203-1	...	...	...	...	...	...	...	...	???	...	...	...	...	...	...	...	...	-
203-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-
203-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-
203-5	...	...	...	...	...	...	...	?	???	...	...	...	...	...	...	...	...	-
203-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-
203-11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-
203-12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-
203-15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-

186-1	TTA	GGA	GCT	ATC	AAC	TTC	ATA	TCC	ACA	GTA	ATT	AAT	ATA	CGA	GCT	GAA	ACT	TTA
186-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-8	...	...	...	...	...	...	...	...	...	...	...	...	...	-	...	...	...	...
202-9	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-2	-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-4	-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-5	-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-7	-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-11	...	...	...	...	...	-	...	...	...	...	...	...	...	...	...	...	...	...
203-12	-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

186-1	CTA	TTA	TTA	TCT	TTA	CCT	GIG	TTA	GCC	GGA	GCC	ATT	ACT	ATG	CTT	TTA	ACT	GAC
186-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
202-9	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
203-15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222
777 777 777 777 777 777 777 777 777 777 777 777 777 777 777 777 777 777 777
123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234

186-1
186-2
202-1
202-3
202-4
202-7
202-8
202-9
203-1
203-2
203-4
203-5
203-7
203-11
203-12
203-15

186-1
186-2
202-1
202-3
202-4
202-7
202-8
202-9
203-1
203-2
203-4
203-5
203-7
203-11
203-12
203-15

186-1	TAC	CAA	CAC	TTA	TTC	333	333	333	333	333
186-2	...	...	...	..G	...	788	888	888	889	999
202-1	...	...	...	...	...	901	234	567	890	123
202-3	...	...	...	...	...					
202-4	...	...	...	...	...					
202-7	...	...	...	...	...					
202-8	...	...	...	...	...					
202-9	...	...	...	...	...					
203-1	...	...	...	...	...					
203-2	...	...	...	...	...					
203-4	...	...	...	...	...					
203-5	...	...	...	...	...					
203-7	?-.	...	...	...	...					
203-11	...	...	...	...	...					
203-12	...	...	...	...	?					
203-15	...	...	...	...	...					

Pollicipes elegans population data, Chapter 4

				111	111	111	111	122	222	222	222	333	333	333	444	444	444
	123	456	789	012	345	678	901	234	567	890	123	456	789	012	345	678	
Peru 177-5	GTT	TAC	CCG	CCG	TTA	GCC	AGA	AAC	ATT	GCC	CAT	TCA	GGA	GCC	TCT	GTT	
Peru 177-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Peru 177-7	...	?C	-	...	...	...	...	...	...	...	...	...	...	...	...	...	
Peru 177-8	...	..T	...	...	...	...	...	...	.C	...	...	...	...	...	...	...	
Peru 177-9	...	...	...	...	...	...	...	...	...	...	...	...	.G	...	...	...	
Peru 177-10	...	...	...	...	...	...	...	...	...	...	...	...	.G	...	...	...	
Peru 177-12	...	..T	...	...	...	...	...	...	.C	...	...	...	...	...	...	...	
Mexico 208-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Mexico 208-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Mexico 208-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Mexico 208-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Mexico 208-5	...	...	...	...	...	...	...	...	...	...	...	...	...	..	-	...	
Mexico 208-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Mexico 208-7	...	..T	...	...	...	...	...	...	.C	...	...	...	...	...	...	...	

Peru 177-5	455	555	555	556	666	666	666	666	777	777	777	777	788	888	888	889	999	999
Peru 177-6	GAT	TTA	TCA	ATT	TTT	TCC	TTA	CAT	TTA	GCG	GGA	GCA	TCT	TCT	ATT	CTA		
Peru 177-7	??.	...	-...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-8	?	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-9	...	...	-...	...	...	-	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-1	...	...	-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-3	...	...	.G	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-5	?	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-7	...	...	-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Peru 177-5	GGA	GCT	ATT	AAC	TTC	ATA	TCA	ACG	GTT	ATT	AAT	ATA	CGA	GCC	GAA	ACT
Peru 177-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-9	-.	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...
Peru 177-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Peru 177-12	---	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-5	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...
Mexico 208-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mexico 208-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

[illegible]

[illegible]

Peru 177-5	222	222	222	222	223	333	333	333	333
Peru 177-6	899	999	999	999	990	000	000	000	111
Peru 177-7	901	234	567	890	123	456	789	012	
Peru 177-8	GGA	GGA	GGG	GAC	CCA	ATT	TTA	TAC	
Peru 177-9	...	...	...	...	...	...	...	...	...
Peru 177-10	...	...	...	...	...	...	...	...	...
Peru 177-11	...	...	...	...	...	...	...	...	...
Peru 177-12	...	...	...	...	...	...	...	...	...
Mexico 208-1	...	...	...	...	...	...	...	...	...
Mexico 208-2	...	...	...	...	...	...	...	...	...
Mexico 208-3	...	...	...	...	...	...	...	...	...
Mexico 208-4	...	...	...	...	...	...	...	...	...
Mexico 208-5	...	...	...	...	...	...	...	...	...
Mexico 208-6	...	...	...	...	...	...	...	...	...
Mexico 208-7	...	...	...	...	...	...	...	...	...

Pollicipes species with Capitulum and Calantica, Chapter 5

	111	111	111	122	222	222	222	333	333	333	444
123	456	789	012	345	678	901	234	567	890	123	456
Pollicipes polymerus	TAC	CCA	CCT	CTA	GCC	AGC	AAT	ATC	GCA	CAC	TCA
Pollicipes elegans	..T	..G	..G	T..	A	..C	..T	..C	..T	...	...
Pollicipes pollicipes	..T	A	--.	T	..C	..T	..T	..T	T	..G	..A
Capitulum mitella	---	---	---	---	---	---	---	---	---	..G	..AT
Calantica villosa	..T	..T	T	..A	..A	...	..T	..T	..T	T	...

444	444	455	555	555	666	666	666	777	777	777	788
345	678	901	234	567	890	123	456	789	012	345	678
Pollicipes polymerus	GTA	GAC	CTC	TCT	ATT	TTT	TCA	TTA	CAC	TTA	GCG
Pollicipes elegans	..T	..T	T.A	..A	C	C	C	C	C	A	..T
Pollicipes pollicipes	T	T.A	..A	..C	C	C	..T	A	A	A	..A
Capitulum mitella	T	T.A	A.C	..C	..C	..C	C.T	G	..T	..G	...
Calantica villosa	..T	..T	T.A	A..	C	C	C.T	..T	T	G	...

111 111 111 111 111 111 111 111 111 111
 888 889 999 999 999 000 000 000 011 111 111 112 222 222
 567 890 123 456 789 012 345 678 901 234 567 890 123 456
 TCT ATT TTA GGA GCT ATC AAC TTC ATA TCC ACA GTA ATT AAT
 C..TA ..G ..T
 C.. ..- ..T-
 C.T ..GT ..T ..GC
 ..AT ..TA
 Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa

111 111 111 111 111 111 111 111 111 111 111 111 111
 222 333 333 344 444 444 445 555 555 555 666 666 666
 789 012 345 678 901 234 567 890 123 456 789 012 345 678
 ATA CGA GCT GAA ACT TTA ACA TTC GAC CGT TTA CCT TTA TTT
CC.GTA C.C ... C...
CC.TTA ..A ...
ACTA ..C- ... -..
G T.GATA A.T ..A ...C

Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa

111 111 111 111 111 111 111 111 111 111 122 222 222 222
 677 777 777 778 888 888 888 999 999 999 900 000 000 001
 901 234 567 890 123 456 789 012 345 678 901 234 567 890
 GTA TGA AG- GTA TTT GTG ACG GTG ATT CTT CTA TTA TTA TCT
A ..TC ..C ..A ... T.A T.. C.G ..G ...
 ..T ..G ..A ..C ..CC ..A ... T.A T.. C.T ...C
A ..T ..- ..A ..C ..AC ..T C.T ..C
 ..AAC ..C ..AG ..C
 Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa

222 222 222 222 222 222 222 222 222 222 222 222 222 222 222
 111 111 111 111 222 222 222 222 222 222 222 222 222 222 222
 123 456 789 012 345 678 901 234 567 890 123 456 789 012
 TTA CCT GTG TTA GCC GGA GCC ATT ACT ATG CTT TTA ACT GAC
 C.C ..G ..T C.. ..AA ..C ..CC.T
 C.CTG ..GC ..C ..AC.T
 C.TT C.C ..ACA ..AC.T ..C ..T
 C.. ..A ..TA ..T ..TA T.A C.. ..A ...

Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa

222 222 222 222 222 222 222 222 222 222 222 222 222 222
 555 555 566 666 666 667 777 777 777 888 888 888 899 999
 345 678 901 234 567 890 123 456 789 012 345 678 901 234
 CGG AAT CTT AAT ACA TCA TTT TTC GAC CCT ACA GGG GGA GGA
 ..A ..CCT ..C ..T ..T ..A ..C ..AG
 ..TAC ..CTT T..G
- ..ACTC ..A
 ..A ... T.ACT ..T ..CT ..T ...

Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa

222 223 333 333 333 333 333 333 333 333 333 333 333 333
 999 990 000 000 000 111 111 111 122 222 222 223 333 333
 567 890 123 456 789 012 345 678 901 234 567 890 123 456
 GAC CCT ATT TTA TAC CTA GGA TTT T-A TTT TTA TTT ACA ATT
ACTTTG.G
 A.. ..C ..CTC CT. ... C..G.A
C.T ..TTC.TG.G
 A.. ..C ..C ..G ... T.. ... CTT ... C.T ..- ..T G.A

Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa

Pollicipes species with Capitulum, Calantica and Lepas, Chapter 5

	123	456	789	012	345	678	901	234	567	890	123	456	789	012
Pollicipes polymerus	TAC	CCA	CCT	CTA	GCC	AGC	AAT	ATC	GCA	CAC	TCA	GGA	GCC	TCT
Pollicipes elegans	.T	.G	.TG	T..	...	.A	.C	...	.C	.T	...	...	...	...
Pollicipes pollicipes	.T	...	.A	--	...	.T	.C	.T	.T	...	.T	.G	.A	.C
Capitulum mitella	---	---	---	---	---	---	---	---	---	---	.G	...	.AT	.A
Calantica villosa	.T	.T	...	.T	.A	.A	...	.T	.T	.T	.T	...	.T	...
Lepas anatifera	---	---	---	---	---	---	---	---	---	---	.C	.G	.A	...

	444	444	455	555	555	556	666	666	666	666	777	777	777	788	888
Pollicipes polymerus	GTA	GAC	CTC	TCT	ATT	TTT	TCA	TTA	CAC	TTA	GCG	GGA	GCT	TCC	
Pollicipes elegans	.T	.T	T.A	.A	...	...	.C	...	.T	...	...	...	.A	.T	
Pollicipes pollicipes	...	.T	T.A	.A	.C	...	.C	C..	.T	...	.A	...	.A	.A	
Capitulum mitella	...	.T	T.A	A.C	.C	.C	.C	C.T	...	.G	.T	.G	...	...	
Calantica villosa	.T	.T	T.A	A..	...	...	.C	C.T	.T	...	.T	...	.G	...	
Lepas anatifera	...	.T	T.G	...	.C	.C	.C	C.T	.T	...	.T	.T	.C	.A	

333 333 333 333 333 333 333 333 333 333 333 333 333 333 333
 333 444 444 444 444 455 555 555 556 666 666 666 777 777 777
 789 012 345 678 901 234 567 890 123 456 789 012 345 678
 GGA GGA TTA -CA G-A GFA GTC TTA GGC AAT TCG TCC CTA GAT
 G.G A.. .G. .T. .T.C .T. -.T.C
 ..T ... G.T A.. .G. .T. .T.C ..A .T. .T. ..C
 ..G ... G.. A.. .G.T C.CC .T. .T. ..C ..C
 ..T ..T A.T A.. -G.TT ..T T... ..C
 --- --- --- .- -.- --- --- --- --- --- --- --- ---

Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa
 Lepas anatifera

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 901 234 567 890 123 456 789 012 345 678 901 234 567 890
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TC ..CTCT ...
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Pollicipes polymerus
 Pollicipes elegans
 Pollicipes pollicipes
 Capitulum mitella
 Calantica villosa
 Lepas anatifera

REFERENCES

- Anonymous, 1987. Barnacles. *Seafood Leader* 7(1):222-226.
- Avise, J. C., G. S. Helfman, N. C. Saunders and S. L. Hales. 1986. Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern.
- Avise, J. C., R. M. Ball and J. Arnold. 1988. Current versus historical population sizes in vertebrate species with high gene flow: A comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. *Mol. Biol. Evol.* 5:331-334.
- Avise, J. C., B. W. Bowen, T. Lamb, A. B. Meylan and E. Bermingham. 1991. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the testudines. *Mol. Biol. Evol.* 9:457-473.
- Avise, J.C., C. Giblin-Davidson, J. Laerm, J.C. Patton and R. A. Lansman. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, Geomys pinetis. *Proc. Natl. Acad. Sci. USA* 76:6694-6698.
- Avise, J.C., J.R. Shapira, S.W. Daniel, C.F. Aquadro and R.A. Lansman. 1983. Mitochondrial DNA differentiation during the speciation process in Peromyscus. *Mol. Biol. Evol.* 1:38-56.
- Ayala, F. J. 1986. On the virtues and pitfalls of the molecular evolutionary clock. *J. Hered.* 77:226-235.
- Baker, C. S., A. Perry, J. L. Bannister, M. T. Weinrich, R. B. Abernethy, J. Calanbokidis, J. Lien, R. H. Lambertsen, J. Urban Ramirez, O. Vasquez, P. J. Clapham, A. Alling, S. J. O'Brien and S. R. Palumbi. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc. Natl. Acad. Sci. U. S. A.* 90:8239-8243.

- Batuecas, B., R. Garesse, M. Calleja, J.R. Valverde and R. Marco. 1988. Genome organization of Artemia mitochondrial DNA. *Nucleic Acids Res.* 16(14):6515-6529.
- Benveniste, R.E. and G.J. Todaro. 1976. Evolution of type C viral genes: evidence for an Asian origin of man. *Nature* 261:101-108.
- Billington, N. and P. D. N. Hebert. 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. *Can. J. Fish. Aq. Sci.* 48:80-94.
- Birky, C.W., Jr., T. Maruyama, and P. Fuerst. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Genetics* 103:513-527.
- Bonner, T.I., R. Heinemann and G.J. Todaro. 1980. Evolution of DNA sequences has been retarded in Malagasy primates. *Nature* 286:420-423.
- Bowen, B.W., A. B. Meylan and J. C. Avise. 1989. An odyssey of the green sea turtle: Ascension Island revisited. *Proc. Natl. Acad. Sci. USA* 86:573-576.
- Brasher, D. J. and J. R. Ovenden. 1992. PCR amplification of krill mtDNA. *Newsletter Crust. Mol. Tech.* 3:3-4.
- Brasher, D. J., J. R. Ovenden, J. D. Booth and R. W. G. White. 1992a. Genetic subdivision of Australian and New Zealand populations of Jasus verreauxi (Decapoda: Palinuridae) - preliminary evidence from the mitochondrial genome. *N.Z. J. Mar. Freshw. Res.* 26:53-58.
- Brasher, D. J., J. R. Ovenden and R. W. G. White. 1992b. Mitochondrial DNA variation and phylogenetic relationships of Jasus spp. (Decapoda: Palinuridae). *J. Zool., Lond.* 227:1-16.
- Brodeur, R.D. 1986. Northward displacement of the euphausiid Nyctiphanes simplex Hansen to Oregon and Washington following the El Niño event of 1982-83. *Journal of Crustacean*

Biology 6(4):686-692.

- Brown, W. M. 1983. Evolution of animal mitochondrial DNA. p. 62-88 In: M. Nei and R. K. Koehn (eds.), Evolution of genes and proteins. Sinauer Assoc. Inc., Sunderland, Massachusetts.
- Brown, W.M., M. George and A.C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967-1971.
- Brown, G.G. and M.V. Simpson. 1981. Intra- and interspecific variation of the mitochondrial genome in Rattus norvegicus and Rattus rattus: restriction enzyme analysis of variant mitochondrial DNA molecules and their evolutionary relationships. Genetics 97:125-143.
- Buckeridge, J. S. 1983. Fossil barnacles (Cirripedia: Thoracica) of New Zealand and Australia. New Zealand Geological Survey Paleontological Bulletin 50, Wellington, 151 p., 13 pl.
- Bullock, J.A. 1964. Variation in the commensal cirriped, Octolasmis cor (Aur.), in relation to its position in the branchial chamber of Scylla serrata Forskal. Federation Museums Journal N.S. 9:84-94.
- Burton, R. S. 1983. Protein polymorphisms and genetic differentiation of marine invertebrate populations. Mar. Biol. Lett. 4:193-206.
- Burton, R. S. 1986. Evolutionary consequences of restricted gene flow among natural populations of the copepod, Tigriopus californicus. Bull. Mar. Sci. 39(2):526-535.
- Burton, R. S. 1990. Hybrid breakdown in physiological response: a mechanistic approach. Evolution 44(7):1806-1813.
- Burton, R. S. and M. W. Feldman. 1981. Population genetics of Tigriopus californicus. II. Differentiation among neighboring populations. Evolution 35(6): 1192-1205.
- Burton, R. S. and M. W. Feldman. 1982. Population genetics of

- coastal and estuarine invertebrates: does larval behavior influence population structure? p. 537-551 In: V. S. Kennedy (ed.) Estuarine comparisons. Academic Press, New York.
- Burton, R. S. and B. -G. Lee. submitted ms. Discordance between allozyme and DNA sequence data across a major phylogeographic break in the copepod Tigriopus californicus.
- Carroll, R. L. 1988. Vertebrate paleontology and evolution. W. H. Freeman and Co., New York, pp. 698.
- Carvacho, A. 1989. Cancer johngarthi, n. sp. and Cancer porteri (Bell) (Crustacea, Decapoda)- comparisons and hypothesis. Proc. Biol. Soc. Wash. 102(3):613-619.
- Casas, P. 1990. Galicia's hard-to-come-by barnacles. New York Times. July 29:6 and 25.
- Cimberg, R. L. 1981. Variability in brooding activity in the stalked barnacle Pollicipes polymerus. Biol. Bull. 160:31-42.
- Clary, D. O. and D. R. Wolstenholme. 1985. The mitochondrial DNA molecule of Drosophila yakuba: nucleotide sequence, gene organization, and genetic code. J. Mol. Evol. 22:252-271.
- Coppes, Z. L. and G. N. Somero. 1990. Temperature-adaptive differences between the M4 lactate dehydrogenases of stenothermal and eurythermal sciaenid fishes. J. Exp. Zool. 254(2):127-131.
- Cunningham, C. W., N. W. Blackstone and L. W. Buss. 1992. Evolution of king crabs from hermit crab ancestors. Nature 355(6360):539-542.
- Dando, P.R. and A.J. Southward. 1980. A new species of Chthamalus (Crustacea: Cirripedia) characterized by enzyme electrophoresis and shell morphology; with a revision of other species of Chthamalus from the western shores of the Atlantic Ocean. J. Mar. Biol. Ass. U.K. 60:787-831.
- Darwin, C.R. 1851a. A monograph of the fossil Lepadidae, or,

- pedunculated cirripedes of Great Britain. Palaeontogr. Soc. Monograph. London, pp. 1-88 + pls. 1-5.
- Darwin, C. R. 1851b. A monograph on the sub-class Cirripedia with figures of all the species. The Lepadidae; or, pedunculated cirripedes. The Ray Society, London, pp. 400, pl. X.
- Darwin, C.R. 1859 (1872). The Origin of Species by Means of Natural Selection (Reprint of 6th edition). Modern Library, New York, pp. 386.
- Davis, B. J., E. E. DeMartini and K. McGee. 1981. Gene flow among populations of a teleost (Painted Greenling, Oxylebius pictus) from Puget Sound to Southern California. Marine Biol. 65:17-23.
- DeSalle, R., T. Freedman, E. M. Prager and A. C. Wilson. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian Drosophila. J. Mol. Evol. 26:157-164.
- DeSalle, R., L. Val Giddings and K.Y. Kaneshiro. 1986. Mitochondrial DNA variability in natural populations of Hawaiian Drosophila. II. Genetic and phylogenetic relationships of natural populations of D. silvestris and D. heteroneura. Heredity 56:87-96.
- DiMichele, L. and D. A. Powers. 1982. The physiological basis for swimming endurance differences between LDH-B genotypes of Fundulus heteroclitus. Science 216:1014-1016.
- DiMichele, L. and D. A. Powers. 1991. Allozyme variation, developmental rate, and differential mortality in the teleost Fundulus heteroclitus. Physiol. Zool. 64(6):1426-1443.
- Dobzhansky, T. 1937. Genetics and the origin of species. Columbia University Press, New York.
- Donovan, S. K. and E. R. Davis-Strickland. 1993. A possible lepadomorph barnacle from the Maastrichtian (Upper Cretaceous) of Jamaica, West Indies. J. Paleont. 67(1):158-159.

- Duque-Caro, H. 1990. Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South America and the evolution of the Panama Seaway. *Palaeog. Palaeocl. Palaeoecol.* 77(3-4):203-234.
- Ferris, S.D., A.C. Wilson and W.M. Brown. 1981. Evolutionary tree for apes and humans based on cleavage maps of mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* 78:2432-2436.
- Flowerdew, M. W. 1983. The circumboreal barnacle Balanus balanoides (L.) and its subpopulations. p. 75-84 In: G. S. Oxford and D. Rollinson (eds.), Systematics Association Special Volume No. 24, Protein polymorphism: adaptive and taxonomic significance. Academic Press, London and New York.
- Ford, M. J. and J. B. Mitten. 1993. Population structure of the pink barnacle, Tetraclita squamosa rubescens, along the California coast. *Mol. Mar. Biol. Biotech.* 2(3):147-153.
- Foster, B. A. 1978. The marine fauna of New Zealand: barnacles (Cirripedia: Thoracica). New Zealand Oceanographic Institute Memoir 69:1-160.
- Garth, J. S. 1960. Distribution and affinities of the brachyuran Crustacea. In: Symposium: The biogeography of Baja California and adjacent seas, Part II. Marine biotas. *Syst. Zool.* 9:105-123.
- Garth, J. S. 1968. Globopilumnus xanthusii (Stimpson), n. comb., a stridulating crab from the west coast of tropical America, with remarks on discontinuous genera of brachyrhynchous crabs. *Crustaceana* 15(3):312-318.
- Gillespie, J. H. 1984. The molecular clock may be an episodic clock. *Proc. Natl. Acad. Sci. USA* 81:8009-8013.
- Glaessner, M. F. 1969. Decapoda. p. R399-R533. In: R. C. Moore (ed.), Treatise on invertebrate paleontology, part R, Arthropoda 4. Geological Society of America, Inc. and

University of Kansas, Lawrence.

- Gosliner, T.M. and M.T. Ghiselin. 1984. Parallel evolution in opisthobranch gastropods and its implications for phylogenetic methodology. *Syst. Zool.* 33:255-274.
- Haderlie, E. C. and D. P. Abbott. 1980. Bivalvia: the clams and allies. p. 355-411. *In:* R. H. Morris, D. P. Abbott and E. C. Haderlie (eds.), *Intertidal invertebrates of California*, Stanford University Press, Stanford, California.
- Haig, J. and D. P. Abbott. 1980. Macrura and Anomura: The ghost shrimps, hermit crabs, and allies. p. 578-593. *In:* R. H. Morris, D. P. Abbott and E. C. Haderlie (eds.), *Intertidal invertebrates of California*, Stanford University Press, Stanford, California.
- Hale, L.R. and R.S. Singh. 1987. Mitochondrial DNA variation and genetic structure in populations of Drosophila melanogaster. *Mol. Biol. Evol.* 4(6):622-637.
- Hall, T.J., J.W. Grula, E.H. Davidson and R.J. Britten. 1980. Evolution of sea urchin non-repetitive DNA. *J. Mol. Evol.* 16:95-110.
- Halpert, M. S. and C. F. Ropelewski. 1989. Atlas of tropical sea surface temperature and surface winds. NOAA Atlas No. 8. Silver Spring, Maryland, National Oceanic and Atmospheric Administration, U.S. Dept. of Commerce.
- Harrison, R. G. 1991. Molecular changes at speciation. *Ann. Rev. Ecol. Syst.* 22:281-308.
- Hart, J. L. 1982. Crabs and their relatives of British Columbia. *Brit. Colum. Prov. Mus. Handbook No.* 40.
- Hedgecock, D. 1979. Biochemical genetic variation and evidence of speciation in Chthamalus barnacles of the tropical eastern Pacific Ocean. *Mar. Biol.* 54:207-214.
- Hillis, D. M. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. p. 278-294 *In:* M. M. Miyamoto and J. Craycraft (eds.), *Phylogenetic analysis of*

- DNA sequences. Oxford University Press, New York and Oxford.
- Hillis, D. M. and J. P. Huelsenbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *J. Heredity* 83:189-195.
- Hillis, D. M., J. J. Bull, M. E. White, M. R. Badgett and I. J. Molineux. 1992. Experimental phylogenetics: generation of a known phylogeny. *Science* 255:589-592.
- Hillis, D. M. and C. Moritz. 1990. Molecular systematics. Sinauer Assoc. Sunderland, Mass. 588 pp.
- Hoyer, B.H., N.W. Van de Velde, M. Goodman and R.B. Roberts. 1972. Examination of hominid evolution by DNA sequence homology. *J. Hum. Evol.* 1:645-649.
- Hubbs, C.L. 1952. Antitropical distribution of fishes and other organisms. Symposium on problems of bipolarity and of pantemperate faunas. Proceedings of the Seventh Pacific Science Congress 3:324-329.
- Huber, M.E. 1985. Population genetics of eight species of Trapezia (Brachyura: Xanthidae). *Mar. Biol.* 85:23-36.
- Hudson, R. R., M. Slatkin and W. P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. *Genetics* 132(2):583-589.
- Hultman, T., S. Stahl, E. Hornes and M. Uhlen. 1989. Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *NAR* 17:4937-4945.
- Hunt, J.A., T.J. Hall and R.J. Britten. 1981. Evolutionary distances in Hawaiian Drosophila measured by DNA reassociation. *J. Mol. Evol.* 17:361-367.
- Huxley, J. S. 1942. Evolution, the modern synthesis. Allen and Unwin, London.

- Irwin, D. M., T. D. Kocher and A. C. Wilson. 1991. Evolution of the cytochrome b gene of mammals. *J. Mol. Evol.* 32:128-144.
- Jacobs, H. T., D. J. Elliott, V. B. Math, A. Farquharson. 1988. Nucleotide sequence and gene organization of sea urchin mitochondrial DNA. *J. Mol. Biol.* 202:185-217.
- Jukes, T. H. and C. R. Cantor. 1969. Evolution of protein molecules. p. 21-132 In: H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, New York.
- Kameya, A. and J. Zeballos. 1988. Distribucion y densidad de percebes Pollicipes elegans (Crustacea: Cirripedia) en el mediolitoral Peruano (Yasila, Paíta, Chilca, Lima). *Bol. Inst. Mar Peru-Callao* 12(1):6-22.
- Karl, S. A. and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science* 256:100-102.
- Kocher, T.D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci.* 86:6196-6200.
- Knowlton, N., L. A. Weigt, L. A. Solórzano, D. K. Mills, and E. Bermingham. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* 260:1629-1632.
- Kumar, S., K. Tamura and M. Nei. 1993. MEGA: Molecular evolutionary genetics analysis, version 1.0. The Pennsylvania State University, University Park, PA.
- Kwok, S., D. E. Kellogg, N. McKinney, D. Spasic, L. Goda, C. Levenson and J. J. Sninsky. 1990. Effects of primer - template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. *NAR* 18(4):999-1005.

- Laguna G., J. 1985. Systematics, Ecology and Distribution of Barnacles (Cirripedia; Thoracica) of Panama. Master's thesis, University of California, San Diego. 234 pp.
- Laguna, J. E. 1988. Euraphia eastropacensis (Cirripedia, Chthamaloidea), a new species of barnacle from the Tropical Eastern Pacific: Morphological and electrophoretic comparisons with Euraphia rhizophorae (deOliveira) from the Tropical Western Atlantic and molecular evolutionary implications. *Pacific Science* (1987) 41(1-4):132-140.
- Laguna, J. E. 1990. Shore barnacles (Cirripedia, Thoracica) and a revision of their provincialism and transition zones in the tropical eastern Pacific. *Bull. Mar. Sci.* 46(2):406-424.
- Lee, Y. H. and V. D. Vacquier. 1992. The divergence of species-specific abalone sperm lysins is promoted by positive Darwinian selection. *Biol. Bull.* 182:97-104.
- Lee, Y. H. and V. D. Vacquier. 1993. A method for obtaining high-quality sequences from the non-biotinylated, free ssDNA remaining after solid-phase sequencing. *Biotechniques* 14(2):191-192.
- Levinton, J. S. and T. H. Suchanek. 1978. Geographic variation, niche breadth and genetic differentiation at different geographic scales in the mussels Mytilus californianus and M. edulis. *Marine Biol.* 49:363-375.
- Lewontin, R. C. 1989. Inferring the number of evolutionary events from DNA coding sequence differences. *Mol. Biol. Evol.* 6:15-32.
- Lewontin, R. C. and J. L. Hubby. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation in natural populations of Drosophila pseudoobscura. *Genetics* 54:595-609.
- Love, M. S. and R. J. Larson. 1978. Geographic variation in the occurrence of tympanic spines and possible genetic differentiation in the kelp rockfish. *Copeia* 1978:53-59.

- Martin, A. P., G. J. P. Naylor and S. R. Palumbi. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature* 357:153-155.
- Martin, A. P. and S. R. Palumbi. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl. Acad. Sci. USA* 90:4087-4091.
- Mayr, E. 1942. *Systematics and the Origin of Species*. New York: Columbia University Press.
- Mayr, E. 1982. *The growth of biological thought. Diversity, evolution, and inheritance*. The Belknap Press of Harvard University Press. Cambridge, Mass. 974 pp.
- Meyer, A. and A. C. Wilson. 1990. Origin of tetrapods inferred from their mitochondrial DNA affiliation to lungfish. *J. Mol. Evol.* 31:359-364.
- Mullis, K. and F.A. Faloona. 1987. Specific synthesis of DNA in Vitro via a polymerase-catalyzed chain reaction. *Meth. Enzymol.* 155:335-350.
- Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York, 512 pp.
- Nei, M. 1991. Relative efficiencies of different tree-making methods for molecular data. p. 90-128. *In*: M. M. Miyamoto and J. Cracraft (eds.), *Phylogenetic analysis of DNA sequences*. Oxford University Press, New York.
- Nei, M. and L. Jin. 1989. Variances of the average numbers of nucleotide substitutions within and between populations. *Mol. Biol. and Evol.* 6:290-300.
- Newman, W. A. 1979. Californian transition zone: significance of short-range endemics. p. 399-416 *In*: J. Gray and A. Boucot (eds.), *Historical biogeography, plate tectonics and the changing environment. The thirty-seventh annual biology colloquium, (Apr. 23-24, 1976)*. Oregon State Univ., Corvallis.

- Newman, W. A. 1992. Biotic cognate of eastern boundary conditions in the Pacific and Atlantic: relicts of Tethys and climatic change. *Proc. San Diego Soc. Nat. Hist.* 16(1):1-7.
- Newman, W. A. and D. P. Abbott. 1980. Cirripedia: the barnacles. p. 504-535 *In*: R. H. Morris, D. P. Abbott, and E. C. Haderlie (eds.), *Intertidal invertebrates of California*. Stanford University Press, Palo Alto.
- Newman, W. A. and B. A. Foster. 1987. Southern hemisphere endemism among the barnacles: explained in part by extinction of northern members of amphitropical taxa? *Bull. Mar. Sci.* 41(2):361-377.
- Newman, W. A. and J. S. Killingley. 1985. The north-east Pacific intertidal barnacle Pollicipes polymerus in India? A biogeographical enigma elucidated by ^{18}O fractionation in barnacle calcite. *J. Nat. Hist.* 19(6):1191-1196.
- Newman, W.A., V.A. Zullo and T.H. Withers. 1969. Cirripedia. p.R206-R295 *In*: R. C. Moore (ed.), *Treatise on invertebrate paleontology, part R, Arthropoda 4*. Geological Society of America, Inc. and University of Kansas, Lawrence.
- Norton, J., D. McLain, R. Brainard, and D. Husby 1985. The 1982-83 El Niño event off Baja and Alta California, and its ocean climate context. *In*: W.S. Wooster & D.L. Fluharty (eds.). *El Niño North*. pp. 44-72. Seattle; University of Washington.
- Open University Course Team. 1988. *Ocean basins: Their structure and evolution*. Open University, Milton Keynes, UK. Pergamon: New York.
- Ovenden, J. R. 1990. Mitochondrial DNA and marine stock assessment: a review. *Aust. J. Mar. Freshwater Res.* 41:835-853.
- Pääbo, S. 1985. Molecular cloning of ancient Egyptian mummy DNA. *Nature* 314:644-645.

- Palumbi, S. R. and J. Benzie. 1991. Large mitochondrial DNA differences between morphologically similar Penaeid shrimp. *Mol. Mar. Biol. Biotech.* 1(1):27-34.
- Palumbi, S. R., A. Martin, S. Romano, W. O. McMillan, L. Stice and G. Grabowski. 1991. The simple fool's guide to PCR. University of Hawaii, Honolulu.
- Palumbi, S. R. and E. C. Metz. 1991. Strong reproductive isolation between closely related tropical sea urchins (genus Echinometra). *Mol. Biol. Evol.* 8(2):227-239.
- Palumbi, S. R. and A. C. Wilson. 1990. Mitochondrial DNA diversity in the sea urchins Strongylocentrotus purpuratus and S. droebachiensis. *Evolution* 44:403-415.
- Pamilo, P. and M. Nei. 1988. Relationships between gene trees and species trees. *Mol. Biol. Evol.* 5:568-583.
- Pearse, J. S., V. B. Pearse and K. K. Davis. 1986. Photoperiodic regulation of gametogenesis and growth in the sea urchin Strongylocentrotus purpuratus. *J. Exp. Zool.* 237:107-118.
- Pilsbry, H. A. 1907. The barnacles (Cirripedia) contained in the collections of the U. S. National Museum. Smithsonian Institution United States National Museum Bulletin 60, Washington, D. C., Government Printing Office.
- Piper, S.C. 1984. Biology of the intertidal mollusc Nuttalina, with special reference to intertidal zonation, taxonomy, and biogeography. Ph.D. dissertation, Univ. Calif. San Diego, 672 p.
- Powell, J. R., A. Caccione, G. D. Amato and C. Yoon. 1986. Rates of nucleotide substitution in Drosophila mitochondrial DNA and nuclear DNA are similar. *Proc. Natl. Acad. Sci. USA* 83: 9090-9093.
- Randall, J.E. 1981. Examples of antitropical and antiequatorial distribution of Indo-West Pacific fishes. *Pacific Science* 35:197-209.

- Reeb, C. and J. C. Avise. 1990. A genetic discontinuity in a continuously distributed species - mitochondrial DNA in the American oyster, Crassostrea virginica. *Genetics* 124(2):397-406.
- Rosel, P. E. 1992. Genetic population structure and systematic relationships of some small cetaceans inferred from mitochondrial DNA sequence variation. Ph.D. dissertation, Univ. Calif. San Diego, 191 p.
- Rosenblatt, R.H. and R.S. Waples. 1986. A genetic comparison of allopatric populations of shore fishes from the eastern and central Pacific Ocean: dispersal or vicariance. *Copeia* 1986(2):275-284.
- Saito, T. 1976. Geologic significance of coiling direction in the planktonic foraminifera Pulleniatina. *Geology* 4(5):305-309.
- Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning. A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sanger, F., S. Nicklen and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* 74:5463-5467.
- Satta, Y., H. Ishiwa and S. I. Chigusa. 1987. Analysis of nucleotide substitutions of mitochondrial DNAs in Drosophila melanogaster and its sibling species. *Mol. Biol. Evol.* 4(6): 638-650.
- Saunders, N. C., L. G. Kessler, and J. C. Avise. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, Limulus polyphemus. *Genetics* 112:613-627.
- Schoener, A. and D. L. Fluharty. 1985. Biological anomalies off Washington in 1982-83 and other major Niño periods. p. 211-225. In: Wooster, W. S. and D. L. Fluharty (eds.) *El Niño north: Niño effects in the eastern subarctic Pacific Ocean*. 312 p. Washington Sea Grant program, University of Washington,

Seattle.

- Shields, G. F. and A. C. Wilson. 1987. Calibration of mitochondrial DNA evolutions in geese. *J. Mol. Evol.* 24:212-217.
- Sibley, C.G. and J.E. Alquist. 1984. The phylogeny of the hominoid primates, as indicated by DNA-DNA hybridization. *J. Mol. Ecol.* 18:387-404.
- Simpson, G. G. 1944. *Tempo and mode in evolution.* Columbia University Press, New York.
- Smith, P.E. 1985. A case history of an Anti-El Niño to El Niño transition on plankton and nekton distribution and abundances. p. 121-142. In: W.S. Wooster & D.L. Fluharty (eds.), *El Niño North.* Seattle, University of Washington.
- Southern, S.O., P.J. Southern and A.E. Dizon. 1988. Molecular characterization of a cloned dolphin mitochondrial genome. *J. Mol. Evol.* 28:32-42.
- Southward, A. J. 1983. A new look at variation in Darwin's species of acorn barnacles. *Biol. J. Linn. Soc. London* 20:59-72.
- Southward, A.J. and W.A. Newman 1977. Aspects of the ecology and biogeography of the intertidal and shallow-water balanomorph Cirripedia of the Caribbean and adjacent sea-areas. *FAO Fisheries Report* 200:407-425.
- Swofford, D. L. 1993. *PAUP: Phylogenetic Analysis Using Parsimony, Ver. 3.1.1.* Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.
- Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10:512-526.
- Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37(2):221-244.

- Topal, M. D. and J. R. Fresco. 1976. Complementary base pairing and the origin of substitution mutations. *Nature* 263:283-289.
- Tozer, E. T. 1989. Tethys, Thetis, Thethys, or Thetys - What, where, and when was it. *Geology* 17(10):882-884.
- van Andel, T. H. 1979. An eclectic overview of plate tectonics, paleogeography, and paleoceanography. p. 9-25 In: J. Gray and A. J. Boucot (eds.), *Historical biogeography, plate tectonics, and the changing environment. Proc. of the Annual Biology Colloquium and selected papers.* Oregon State University, Corvallis.
- van den Hoek, C. . 1984. World-wide latitudinal and longitudinal seaweed distribution patterns and their possible causes, as illustrated by the distribution of rhodophytan genera. *Helgolander Meeresuntersuchungen* 38:227-257.
- Van Syoc, R. J. 1992. Living and fossil populations of a western Atlantic barnacle, Balanus subalbidus Henry, 1974, in the Gulf of California region. *Proc. San Diego Soc. Nat. Hist.* 12:1-7.
- Van Syoc, R. J. 1993. PCR primers for the CO1 gene in barnacle mtDNA. *News. Crust. Mol. Tech.* 4:1-2.
- Vermeij, G. J. 1986. Survival during biotic crises: The properties and evolutionary significance of refuges. p. 231-246 In: D. K. Elliot (ed.), *Dynamics of extinction.* John Wiley & Sons, New York.
- Vermeij, G. J. 1989. Interoceanic differences in adaptation: effects of history and productivity. *Mar. Ecol. Prog. Ser.* 57:293-305.
- Wilson, A.C., R.L. Cann, S.M. Carr, M. George, U.B. Gyllenstein, K.M. Helmbjuchowski, R.G. Higuchi, S.R. Palumbi, E.M. Prager, R.D. Sage and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26:375-400.

- Wirgin, I. I., R. Proenca and J. Grossfield. 1989. Mitochondrial DNA diversity among populations of striped bass in the southeastern United States. *Can. J. Zool.* 67:891-907.
- Withers, T.H. 1953. Catalogue of Fossil Cirripedia in the Department of Geology. 3 (Tertiary):1-396 + pls. 1-64. *Brit. Mus. (Nat. Hist.)*, London.
- Wooster, W. S. and D. L. Fluharty (eds.) 1985. *El Niño North*. Seattle, University of Washington.
- Wright, S. 1931. Evolution in Mendelian populations. *Genetics* 16:97-159.
- Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. *Proc. 6th Intl. Cong. Genetics, Ithaca* 1:356-366.
- Wright, S. 1951. The genetical structure of populations. *Ann. Eugen.* 15:323-354.
- Wu, C.-I. and Li, W.-H. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natl. Acad. Sci. USA* 82:1741-1745.
- Zevina, G.B. 1981. Barnacles of the suborder Lepadomorpha (Cirripedia, Thoracica) of the world ocean. Part 1: Family Scalpellidae. *Guides to the Fauna of the USSR: Zoological Institute of the Academy of Sciences of the USSR* 127:406 pp. Leningrad.