
南西太平洋ラウ海盆周辺海域の深海熱水生物群集の
起源に関する研究

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(基盤研究(B)) 研究成果報告書

平成18(2006)年3月

研究代表者 山口 寿之
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<はじめに>

この一連の研究は、我々自身が2000年夏にインド洋ロドリゲス島沖の三重合点でインド洋初の深海熱水噴出孔生物群集を発見したことに始まる。インド洋群集が、東・西太平洋、あるいは大西洋に由来する生物群集なのか、あるいは両者の混合なのかという問題は、深海熱水・冷湧水性生物群集の成立過程、分散、種分化、系統進化に関する本質的な重要な課題を含む。

深海熱水・冷湧水性生物群集の主要構成員であるフジツボ類(山口)、軟体動物(長谷川、佐々木、小島)、カニ類(土田)、ハオリムシ類(小島)、シンカイヒバリガイ類(宮崎、橋本、藤倉、藤原)、および寄生性のバクテリアなどを用いて、それら生物集団の起源と進化について研究をし、上記の問題を解明することを試みた。あわせてそれらの生物集団が生息する熱水・冷湧水の化学組成について生息条件の評価として研究をおこなった。

特に、インド洋、東・西太平洋、および大西洋の熱水生物群集のうち、その(フジツボ類)構成要素から南西太平洋・ラウ海盆周辺海域が最も多様性が高く、高次の全4分類群(亜目)がそこに生息し、かつ化石記録をその地域にもつことラウ海盆周辺海域が深海熱水生物の分散や進化の中心と考えた(Yamaguchi & Newman 1995,1997)。

この研究では主として西太平洋からインド洋に至る海域での熱水生物群集の種構成、遺伝子組成などから、ラウ海盆周辺海域の熱水生物群集の成立過程、分散、種分化、進化を明らかにすることを目的とした研究であり、その基礎的なレベルには到達した。

研究組織

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	直接経費	間接経費	合計
平成15年度	7,500,000	0	7,500,000
平成16年度	5,300,000	0	5,300,000
平成17年度	2,600,000	0	2,600,000
総計	15,400,000	0	15,400,000

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[長谷川和範]

なし

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研究成果による工業所有権の出願・取得状況

なし

研究成果

研究の目的

我々は、2000年夏にインド洋ロドリゲス島沖の三重合点でインド洋初の深海熱水噴出孔生物群集を発見した。インド洋群集が、東・西太平洋、あるいは大西洋に由来する生物群集なのか、あるいは両者の混合なのかという問題、それは熱水生物群集の成立過程、分散、種分化、系統進化に関する重要な問題を含む。

深海熱水・冷水生物群集の主要構成員であるフジツボ類(山口)、軟体動物(長谷川、佐々木、小島)、カニ類(土田)、ハオリムシ類(小島)、シンカイヒバリガイ類(宮崎、橋本、藤倉、藤原)、および寄生性のバクテリアなどを用いて、それら生物集団の起源と進化について研究をし、上記の問題を解明することを目的とする。あわせてそれらの生物集団が生息する熱水・冷水の化学組成について生息条件の評価として研究をおこなう。

特に、インド洋、東・西太平洋、および大西洋の熱水生物群集のうち、その(フジツボ類の)構成要素から南西太平洋・ラウ海盆周辺海域が最も多様性が高く、高次の全4分類群(亜目)がそこに生息し、かつ化石記録をその地域にもつことラウ海盆周辺海域が深海熱水生物の分散や進化の中心と考えた(Yamaguchi & Newman 1995, 1997)。

この研究では主として西太平洋からインド洋に至る海域での熱水生物群集の種構成、遺伝子組成などから、ラウ海盆周辺海域の熱水生物群集の成立過程、分散、種分化、進化を明らかにすることを目的とした。

平成15年度報告:

熱水・冷湧水性蔓脚類ミョウガガイ科のミトコンドリア(mt)DNAの16S-12SrRNA遺伝子の塩基配列に基づき系統を解析した。“*Neolepas*”および冷湧水性新属 *Ashinkailepas* は単系統をなした。“*Neolepas*”は、共通の祖先からインド洋集団とその他の集団(エディソン、スندا、マヌス、北伊平屋)とに分岐した。後者の集団は、エディソン(*Lucolepas*)を含み、かつ遺伝的距離が小さいために同属と考えられ、また *Ashinkailepas* の北伊平屋および明神の間で同様に遺伝的に同じであり、遠隔地間でも遺伝子交流の可能性ある。北西太平洋集団はインド洋・南西太平洋集団起源の可能性が高く、ラウ海盆の調査の重要性が増した。

二枚貝類イガイ科シンカイヒバリガイ亜科 *Bathymodiolus* は、世界で16種、日本周辺で6種が熱水噴出孔や冷水湧出帯に知られる。mtDNAのCOIとND4遺伝子の塩基配列を決定し、類縁関係・種分化の過程を考察した。この属は3グループに分かれ、グループ1は日本周辺に生息する4種を含み、グループ2は日本周辺に生息する1種とマヌス海盆産未記載種で形成され、グループ3は日本周辺-インド洋-大西洋-東太平洋に広く分布する7種を含み、西太平洋に生息する種、東太平洋に生息する種、大西洋に生息する種がそれぞれクラスターを形成した。

ラウ海盆および西太平洋から採集された笠貝型腹足類の分類学的検討から、ラウ海盆のそれは、マヌス海盆-マリアナ海溝-海形海山にかけて分布する熱水性生物群集の種と類似性が高いこと、沖縄トラフとマヌス海盆-ラウ海盆に飛び離れて分布する近似種が2種あることが明らかになった。

ハオリムシ類のmtDNAの塩基配列に基づき解析し、ラウ海盆に生息する *Lamellibrachia colomuna* が、南海トラフの同属未記載種と遺伝的に異なるが極めて近縁で同種である可能性が確認された。*Alaysia* と考えられる西太平洋の未記載種個体群は、別種と考えられる4つのグループに分かれ、南から北に向かって分散、分化してきた事が示された。蔓脚類 Neoverrucidae 科でも南から北へ向かって分散、分化した事が示された。

深海熱水生物群集の有機物や微生物などからなる懸濁粒子を摂食する種類について、その栄養源を特定する目的で、海底でそれらを濾過濃縮する装置の開発に成功し、ラウ海盆にて2004年10月に使用する。

平成16年度報告:

2004年9月23日～11月10日南太平洋ラウ海盆およびハブル海盆で「しんかい6500」による潜航調査を行った。

ラウ海盆では、Vai Lili は最高 87.9°C、pH 4.8、H₂S 26.6μM の熱水が認められたが、15年前調査の Desbruyères ら(1994)が記載した活発な活動は無かった。活動の低下に伴い最高温度を記録した噴出孔には、巻貝類、コシオリエビなどが僅か生存し、代わって膨大な量の二枚貝、巻貝、フジツボ類の死殻があった。Hine Hina は最高 2.8°C、pH 7.3、H₂S 18.5μM であったが、エビ、コシオリエビ、イバラガニ、多毛類*、フジツボ類*、二枚貝、笠貝、巻貝などが生息していた(*:ラウ海盆初記録)。他に新しい熱水活動域 Mariner が発見され、最高 360°C、pH 2.39、H₂S 14100μM で、噴出孔に群がったエビ類、多毛類が多く、コシオリエビ、小型巻貝、笠貝、二枚貝シンカイヒバリガイは少なかった。三地点の種類数は、Mariner および Hine Hina は 15 種、Vai Lili は 9 種であった。全生物は 5 (6) 門、7 (12) 綱、23 (29) 科、27 (13) 属、29 (44) 種だった(括弧内は Desbruyères らが報告した数)。

ハブル海盆は有人潜水船として初潜航調査で、Brothers 火山カルデラ北西壁および中央火口丘へ潜航した。前者は、最高 302°C、pH 2.9 で活発な熱水活動が見られ、後者は 69°C、pH 5 の浸みだしだった。生物はハオリムシ(前者のみ)、ウロコムシ、多毛類、シンカイヒバリガイ、笠貝類、巻貝類、コシオリエビ、エビ類、カニ類、フジツボ類、魚ゲンゲなどが採集された。エビ類 2 種、フジツボ類 1 種は Brothers カルデラの全域に非常に多くいた。

以上の分類学的な検討の上、リストを作成し、DNA による遺伝的・系統的解析を試みる。特定の分類群は東太平洋、北西太平洋および南西太平洋、インド洋集団間で比較し、系統や分散、生物地理学的な検討を行う。

平成17年度報告:

2004年9月23日～11月10日南太平洋ラウ海盆およびハブル海盆で「しんかい6500」による潜航調査で得られた生物標本について主に形態・生態・生物地理・分子生物学的研究を行った。本研究ではラウ海盆およびハブル海盆だけでなく、歴史的に我々自身が調査してきた太平洋・インド洋・大西洋の深海熱水・冷湧水噴出孔周辺に生息する生物群集を用いて、それら群集の系統的・地理的分散の起源を明らかにする目的で研究を行った。

その結果次のようなことが明らかになった。

各生物分類グループの科以上の分類群の多様性を見た時、研究対象とした軟体動物二枚貝類、巻貝類、節足動物甲殻類、環形動物多毛類、ハオリムシ類などで、南西太平洋は他の海域の深海熱水・冷湧水噴出孔生物群集と比べて多様性が高い。特に甲殻類の蔓脚類は世界で唯一、全4亜目の分類群がラウ海盆に知られ、次いで西太平洋に3亜目が知られた。

形態的解析から最も原始的な形態を持つと評価された分類群がラウ海盆、ハブル海盆に見出された。特に蔓脚類のミョウガガイ類4属のうち、ハブル海盆周辺で採集された *Ashinkailepas* 属が知られる限り最も原始的な形態を保持すると共に最も広い分布域(南西太平洋から北西太平洋)をもつ。

分子系統学的な解析から、シンカイヒバリガイ、巻貝類、蔓脚類、ハオリムシ類は、知られる限り南半球に生息する分類群が系統的に原始的な位置に来ることが明らかになった。特に蔓脚類とシンカイヒバリガイ類は深海熱水・冷湧水噴出孔生物群集の系統的小および地理的分散の起源が南西太平洋となることが明かになった。

この結論は、形態や生物多様性から見た系統や分散の起源として推定される南西または南太平洋起源と良い一致を示した。

またこれらの成果の一部は、2006年2月23-24日に開催された「しんかいシンポジウム」(横浜国際会議場)にて、口頭およびポスターにて発表した(次ページの研究発表参照)。

また最終年度の報告に当たり、本助成に深く感謝の意を表します。

キーワード: 深海熱水噴出孔、固有種、分類、系統進化、分散、起源、原始性、南西太平洋。

代表者および分担者の個別研究の成果

代表者: 山口寿之 (千葉大学海洋バイオシステム研究センター)

深海熱水噴出孔に棲む原始的な蔓脚類の進化、生物地理、および分散

深海熱水・冷湧水性フジツボ類の発見

深海熱水・冷湧水噴出孔に生息するフジツボ類は、東太平洋海嶺(北緯 20°50′、西経 109°、水深 2600m)ではじめて発見・記載(Newman 1979)されて以来、インド洋-太平洋各地から知られるようになり、蔓脚類の完胸超目の全四亜目に属する熱水域固有の原始的な分類群(いずれも新属新種)が発見された。これらの原始的な熱水・冷湧水性フジツボ類とその進化学的意義および生物地理の特徴についてまとめる。

有柄目ミョウガガイ亜目

カリフォルニア半島の南の海洋底には、東太平洋海嶺と呼ばれる海底火山列がある。そこに潜航した潜水調査船「アルビン号」のダイブ 915 によって採集されたフジツボは筋肉でできた柄部を持つ有柄目ミョウガガイ亜目ミョウガガイ科に属し、*Neolepas zeviniae* Newman (図 1 の①)と命名された(Newman 1979)。ミョウガガイ科は三畳紀初期(2.4 億年前)に繁栄していた Eoscalpellidae 科の唯一の生き残りと考えられ、頭状部の石灰質の殻板数およびそれらの配列によって現存するミョウガガイ亜目の中で、最も原始的な形態を持つ。また、浅海に生息する全てのミョウガガイ科から異なり、深海底のかすかな流れによって運ばれる微細な浮遊物(バクテリアのコロニーなど)を餌として濾しとるのに適した著しく繊細な細く長い付属肢(蔓脚)と餌を咀嚼するための口器を持つ(Newman 1979)。この *Neolepas* の発見後、ニューカレドニア(ジュラ紀初期、2 億年前)から同属の化石(*Neolepas augurata* Buckeridge and Grant-Mackie 1985)が発見され、共産した二枚貝や腕足類などから、ジュラ紀当時の生息環境は浅海性の陸棚域を示すと解釈された。従って、ジュラ紀以後に生息域を深海底の特殊な環境に移動したと推定される。

熱水・冷湧水に固有のミョウガガイ亜目の知られる全分類群のうち東太平洋海嶺(EPR) 19°-21°N の *Neolepas zeviniae* Newman 1979 の他、インド洋ロドリゲス三重合点の *Neolepas* sp.、初島沖(冷湧水)、明神海丘、沖縄トラフの *Ashinkailepas seepiophilia* Yamaguchi, Newman & Hashimoto 2004 および 2005 年 6 月に実施された「アルビン」の調査でニュージーランド沖ハブル海盆の冷湧水噴出孔からこの属の新種が発見された(図 2)。ハブル海盆ブラザーカルデラの熱水噴出孔からは *Vulcanolepas osheai* Buckeridge 2000、ラウ海盆のかつて熱水活動のあったヒネヒネサイト(現在では低温)から *Vulcanolepas* sp.、およびパプアニューギニア・リーヒー島沖(冷湧水) *Leucolepas longa* Southward & Jones 2003 とパプアニューギニア・マヌス海盆(熱水)、インドネシア・スダグ海溝(冷湧水)、沖縄トラフの *Leucolepas* spp.、EPR37°S や PAR38°S 等の資料を入手し、それらを用いたネオレパス科の系統関係を分子生物学的手法を用いて、それらの分子系統を解析した。

4つの属間で現在までに得られている結果は、*Ashinkailepas* が最も原始的で、次に *Vulcanolepas* が派生し、それから *Neolepas* と *Leucolepas* とが分化したという結果が得られている(図 3)。



图 1

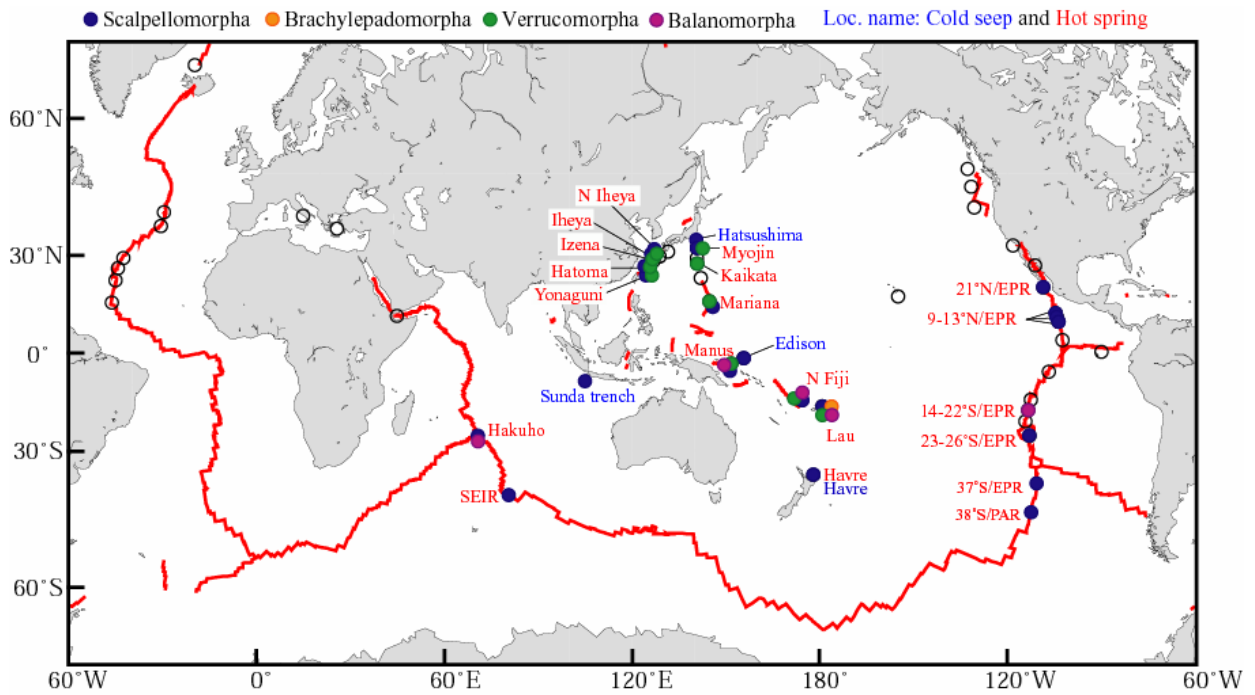


图 2

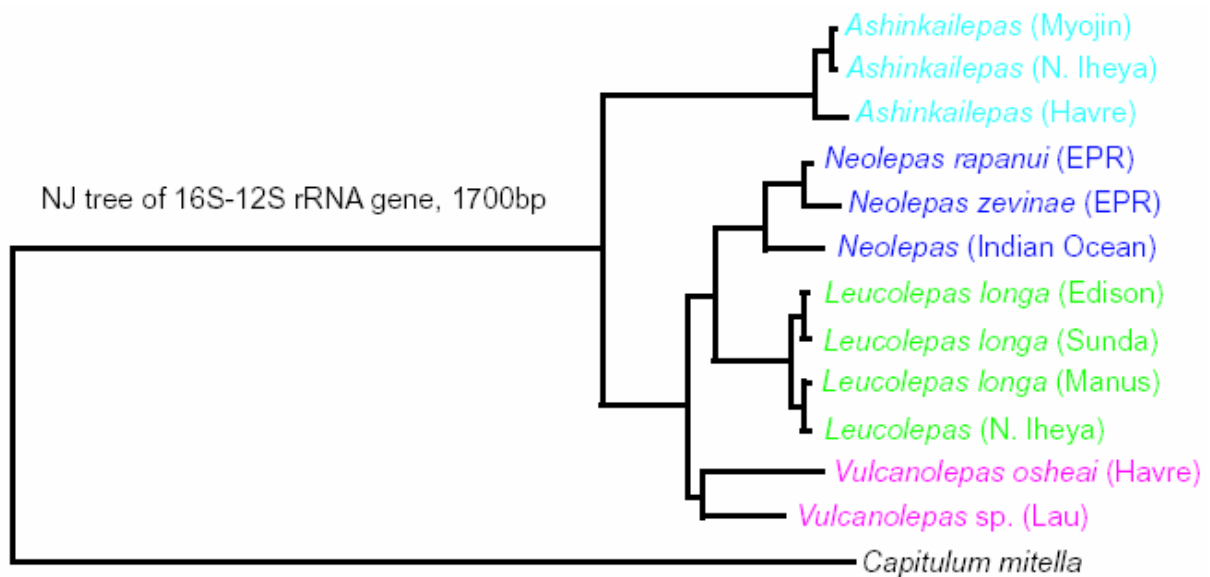


図 3

無柄目ハナカゴ亜目

西太平洋のマリアナ背弧海盆(北緯18°12′、東経144°42′、水深3600m)より、1987年「アルビン号」のダイブ1822、1840、1844によって第2の熱水フジツボが採集された。それは筋肉の柄部を失い、左右非対称の無柄目ハナカゴ亜目の *Neoverruca brachylepadiformis* Newman であった(Newman & Hessler 1989)。その個体発生は筋肉の柄部を持つエボシガイ亜目の段階を経て、最終的に柄部を失うハナカゴ亜目との間を埋めるミッシングリンクとして注目される。この属は左右非対称であることを除けば、種名の *brachylepadiformis* が示すようにジュラ紀後期(1.5億年前)に出現し中新世(1500万年前)に“絶滅した”ブラキレパドモルファ亜目(Brachylepadomorpha)に類似の形態を持っていた。また、殻周辺に多層の付随小殻板を持つことが原始的特徴の一つで、現存するハナカゴ亜目の中で最も原始的な分類群といえる。この属は形態的にも類似の極めてデリケートな付属肢・口器を持つことで *Neolepas* と生態的に類似している。

無柄目フジツボ亜目

北フィジー海盆(南緯16°59′、東経173°55′、水深1990m)から、1987年海洋科学技術センターの調査船「かいよう」、1989年フランスの潜水船「ノーチル号」、そして1991年海洋科学技術センターの潜水船「しんかい6500」が第3番目の熱水フジツボを採集した。それらはいずれも筋肉の柄部を持たない無柄目フジツボ亜目(左右対称)の *Eochionelasmus ohtai* Yamaguchi であった。本属はハワイ沖の水深約400mの熱水噴出域ではない浅海に生存する *Chionelasmus darwini*(Pilsbry)に近縁であるが、多層の付随小殻板や蓋板の筋肉の付着様式から、さらに原始的であると評価される。近縁な化石 *Chionelasmus* がニュージーランドの始新世後期(3800万年前)から知られている(Yamaguchi & Newman 1990)。

無柄目ブラキレパドモルファ亜目

4番目の亜目はブラキレパドモルファ亜目と呼び、第三紀中新世中期に“絶滅した”と考えられた。「ノーチル号」によって1990年西太平洋トンガ諸島沖ラウ海盆から直径約7mmの大変小さな個体ではあるが数個体採集された。それは同亜目を特徴づける左右対称で、かつ一対の median latus と呼ばれる殻板と、多層の付随小殻板を持っていた。当然唯一の現存する分類群であり、最も原始的な現存種となる(Newman & Yamaguchi 1995)。

以上のようにフジツボ類(蔓脚類・完胸超目)のうちの全4亜目(エボシガイ亜目、ハナカゴ亜目、フ

ジツボ亜目、ブラキレパドモルファ亜目)が、深海熱水・冷湧水噴出孔に生息していた。しかもそれらは各亜目の中で最も原始的な形態を持っていた。

フジツボ類の系統進化やこのような特殊な環境に原始的な分類群が生存していることに対して、次のような推論がなされた(Newman 1979, Newman & Hessler 1989, Yamaguchi & Newman 1990, Newman & Yamaguchi 1995)。完胸超目には中生代後期(およそ1億年前)から第三紀初期(5000万年前)にかけて、大規模な適応放散が知られ、筋肉の柄部を持った原始的なエボシガイ亜目から筋肉の柄部を失った新しい分類群のブラキレパドモルファ亜目、ハナカゴ亜目、フジツボ亜目が出現し、さまざまな環境へ適応して行くことになる(図4)。多様性の高い浅海域では多くの種がヒビタットをめぐる競争の結果、フジツボ群集はより進化した分類群へ置き換えられることになる。より進化した分類群によって浅海域から排除されたより原始的なフジツボ類は、深海の熱水・冷湧水噴出孔周辺に用意された避難場所で生き残った。それが熱水・冷湧水フジツボの出現の経緯として考えている。フジツボ類には潜在的に水圧、温度、乾燥、塩分濃度などの環境要因に対する広い耐性が、熱水域でない深海底、潮間帯、河川の下流域、原子力・火力発電所などのプラント設備内への進出を可能にしている。この潜在的な能力および深海熱水・冷湧水域の微小な餌を取るデリケートな蔓脚と口器への修正が、深海の熱水・冷湧水噴出孔で適応につながったと考えられる(Newman 1979, Newman & Hessler 1989, Yamaguchi & Newman 1990, Newman & Yamaguchi 1995)。

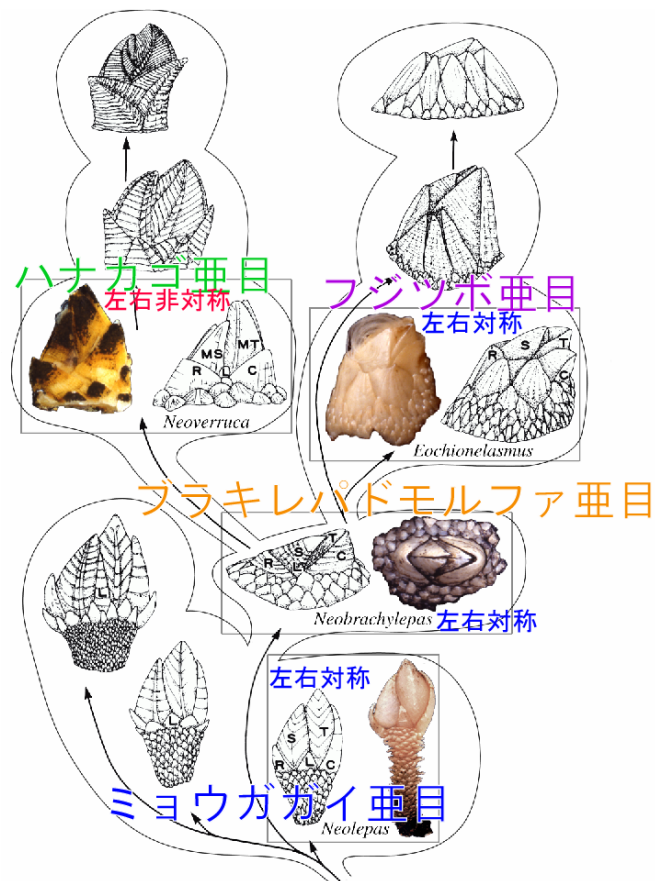


図4

多様性から見た起源

分子系統学的にみたフジツボ類4亜目の系統は単系統を指示する。フジツボ類の最も古い分類群はカンブリア紀から知られるが、熱水・冷湧水固有のフジツボ類の系統の最も古い分類群は現時点でジュラ紀初期(約2億年前)の地層から発見される分類群に起源を持つ。しかしそれらの分類群(*Neolepas augurata* Buckridge and Grant-Mackie 1985)が現在と同様に深海熱水(または冷湧水)

噴出孔という特殊な生態系に生息していたものではないことは、共産する化石の種構成から明かである。従ってジュラ紀以降、深海の熱水・冷湧水噴出孔という特殊な生態系に新たな生息場を求めて移住したことになる。亜目毎に地理的分布を見た時、図2に示されるように、全4亜目がラウ海盆に、ブラキレパドモルファ亜目を除く3亜目が北フィジー海盆、マヌス海盆周辺海域に分布する。すなわち南西から西太平洋にそれらの分布の中心が有ることになる。すなわちその地域が深海熱水・冷湧水噴出孔固有のフジツボ類の起源と考える。

分類群の特徴とその分布から見た起源

唯一のブラキレパドモルファ亜目の現存種は南西太平洋ラウ海盆に知られた (Newman & Yamaguchi 1995)。フジツボ亜目はインド洋、南西・西太平洋およびEPRのイースター島沖にしられ、それらは南半球に限定される。ハナカゴ亜目は北半球にも広く分布するが、南半球にも知られる。ミョウガガイ亜目は最も広い分布域を持ち、最も多様性に富み4つの属からなる。その内形態的に最も原始的と評価した *Ashinkailepas* は南西太平洋から西太平洋、北西太平洋に広く分布する。

以上の多様性や形態・分布の特徴を考慮すると、これらの分類群の起源として南西太平洋を考えることが出来る。

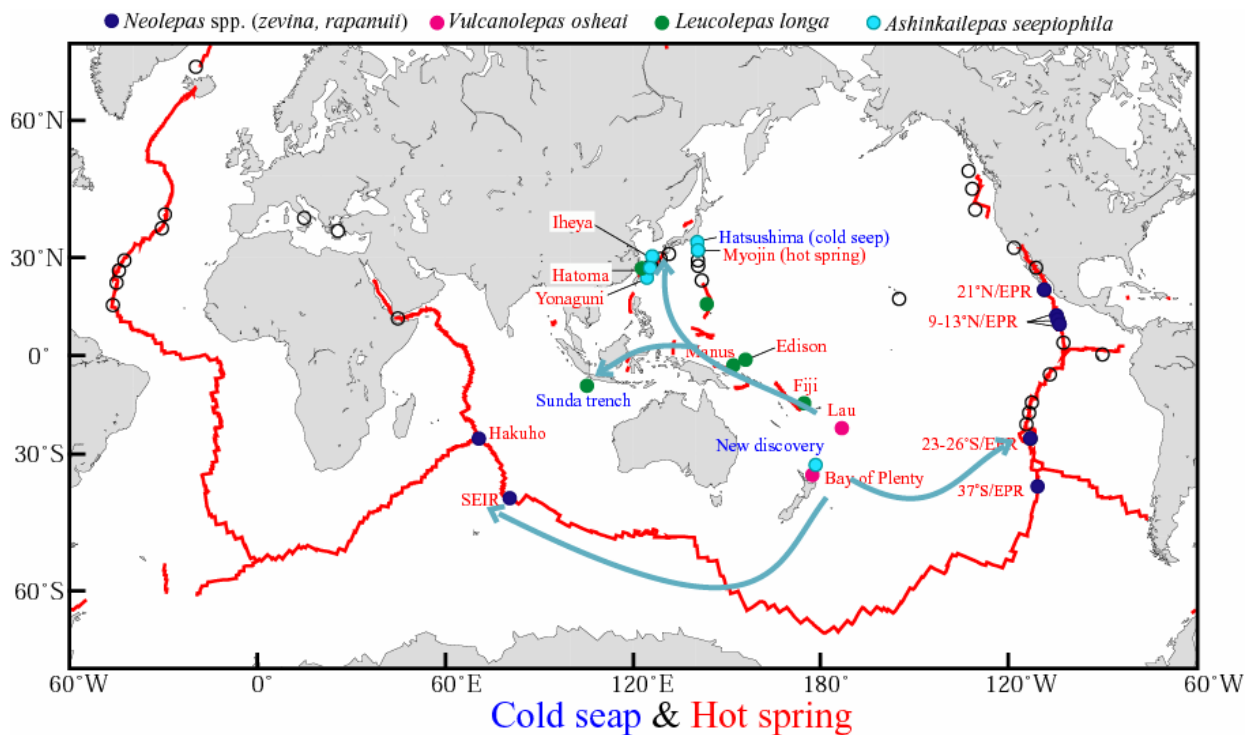


図5

まとめ

深海フジツボ類の多様性、分類群の特徴とその分布、ミョウガガイ科の4属の分子系統から考察した起源と分散過程は次のように整理される。

南西太平洋が起源で、北西太平洋や西太平洋(一部スダ海峡を経てスダ海溝へ)へ分散した。南西太平洋からインド洋中央や東太平洋海嶺へ分散した。

分子系統の研究は、浅海のように卓越する海流が無い深海のような場所での生物の分散過程を調べる上で重要である。既に深海熱水・冷湧水噴出孔からの生物群集が既知の分類群の中で最も原始的で、かつそれらの分類群の研究が既知の分類群の系統関係を考察する上で重要な分類群であることも明らかになっている段階では、次の興味の対象は分散過程の研究である。

謝辞

この一連の研究は、多くの学生の研究や援助によるところが大きい。深海熱水性ミョウガイ類の分子系統学的研究については、元千葉大学大学院生・寒河江美里、上岡雅史、郷戸祐子、林 碧虹、千田愛美による研究が大いにそれらの分類群の理解を深めることとなった。

また深海熱水・冷湧水噴出孔のフジツボ類については、この研究を進めるきっかけを与えてくれた東京大学海洋研究所・太田 秀教授、米国・スクリップス海洋研究所・ビル・ニューマン教授、英国・プリマス海洋研究所・アラン・サウスワード教授、フランス・IFREMER・ミッシェル・セコンザック、ロシア・S・ガクキン博士からはサンプルの提供や意見交換を行った。

また深海の研究は深海研究開発機構(旧海洋科学技術センター)の各種深海探査機を利用させて頂いたまた調査船航海に参加し、フジツボ類採集に際しては多くの援助を頂いた。

以上の学生および研究者の方々にごここで改めて御礼申し上げます。

またこの研究は、明らかに科学研究費補助金の支出がなくては成り立たなかった、ここに記して深く感謝する。

研究分担者:橋本 惇(長崎大学)

南西太平洋ラウ海盆周辺海域の深海熱水生物群集の起源に関する研究

1. 平成 16 年度末に投稿した西太平洋小笠原海域の海形海山 KC 峰中央火口丘で採集された埋没型イガイ科二枚貝の新種記載論文が日本貝類学会誌(*Venus*)で公表した(資料1)。
2. 西太平洋パプアニューギニアのマヌス海盆の熱水活動域で採集されたシンカイヒバリガイ属二枚貝の形態分類学的検討結果を取りまとめ、海洋研究開発機構が主催する第 22 回しんかいシンポジウムで発表した(要旨:資料2)。
発表者:橋本 惇・古田真紀子
タイトル:マヌス海盆熱水活動域に生息するシンカイヒバリガイ類の分類学的検討
発表学会名(場所):第 22 回しんかいシンポジウム(パシフィコ横浜会議センター)
年月日:平成 18 年 2 月 23 日
3. 平成 16 年度に実施された南西太平洋ラウ海盆で潜水調査船「しんかい6500」潜航により採集された 2 個体のシンカイヒバリガイ類の軟体部の詳細観察を行い、北フィジー海盆で採集された同種と形態学的比較を行ったが、形態的差異は認められなかった。上記結果は、南西太平洋ラウ海盆周辺海域に生息する深海熱水生物群集の一グループであるイガイ科二枚貝類の起源について考察するためのデータとして重要ある。
4. 成果公表用消耗品およびシンカイヒバリガイ類のサンプル処理・整理に必要なサンプルビン等を購入した。

研究分担者:小島茂明(東京大学海洋研究所)

ラウ・ケルマディック海域の深海熱水噴出域で採集されたハオリムシ類および Provannidae 科巻貝類の系統分類・系統地理学的研究

1. ハオリムシ類の系統分類学的研究

2004年秋に実施された「よこすか」/「しんかい6500」SWEEPVENTS航海において、ニュージーランド北方に位置するケルマディック島弧の Brothers 海山北西部カルデラ壁で発見された熱水噴出域(水深1600m)で採集された *Lamellibrachia* 属および *Oasisia* 属のハオリムシ類について、形態および分子に基づく系統解析をおこなった。

形態観察は、採集後10%ホルマリンで固定し、70%エチルアルコール中で保存されていた標本(国立科学博物館に収納済み)を用いた。*Lamellibrachia* 属のハオリムシは、本属で唯一薄い棲管を作る点、lamellar sheaths が3対以下である点、vestmentum と trunk に同じ大きさの cuticular plaque を持つ点など、これまでに記載された全ての種と明らかに異なる特徴を有していた。また *Oasisia* 属のハオリムシも唯一の記載種である *O. alvinae* が20対持つ branchial lamellae が8対のみである点で明確に識別された。以上の事から、Brothers 海山で採集された2種のハオリムシは、いずれも未記載種であると判断した。

これら2種のハオリムシ類各3個体について、ミトコンドリアDNA・チトクロームcオキシダーゼI(COI)領域の塩基配列(630塩基対)を決定し、既知のハオリムシ類との系統関係を解析した。Brothers 海山の *Lamellibrachia* は、マヌス海盆・デスモスサイトで採集された未記載種(Kojima et al. (2003) の *Lamellibrachia* sp. L7)と単系統群を形成した。Brothers 海山の *Oasisia* は、東太平洋海膨に生息する *O. alvinae* と単系統群を形成した。いずれについても同属の記載種との間に別種と考えるに足る遺伝的差異のある事が示された。今回の *Oasisia* 属未記載種の発見により、*Oasisia* 属と最も近縁である事が分子系統解析により示されている *Ridgeia* 属と本属を分ける形態形質とされてきた obturacular appendage と chaetal denticles の列数が、これら2属を分ける形質として不十分である事が判明し、今後の分類体系の見直しが必要となった。

以上の結果をまとめた原著論文を Species Diversity 誌に投稿した。

2. *Lamellibrachia* n. sp.の進化と集団構造

Brothers 海山20個体、マヌス海盆・デスモスサイト(「しんかい2000」第621回潜航)4個体および南マリアナ海嶺TOTOサイトで採集された(「しんかい6500」第773回潜航)10個体の *Lamellibrachia* についてミトコンドリアDNA・COI領域の塩基配列(1017塩基対)を決定した。3ヶ所の個体間に共通する配列はなく、それぞれから3、2、1種類の配列が得られた。これら6種類の配列の間の違いは種内変異の範囲内(木村の遺伝的距離で0.002-0.014)であった。TOTOサイトの10個体が全て同じ配列を持ち、デスモスサイトの個体から得られた2種類の配列が互いに最も近縁であるのに対して、Brothers 海山の個体はTOTOサイトの個体に近縁な5個体とデスモスサイトの個体に近縁な15個体から構成されていた。デスモスサイトとTOTOサイトのサンプルがいずれも冷凍されており、状態のいい固定標本が存在しないため、3集団の個体間で形態を詳細に比較する事ができなかったが、現在までのところ大きな形態上の差異は見出されていない(宮崎大学三浦知之教授 私信)。これらの結果から、3ヶ所の熱水域から得られた *Lamellibrachia* を全体として、本研究により現在記載中の種とするのが妥当であると考えられる。

3ヶ所の熱水域の個体と他の全ての *Lamellibrachia* 属ハオリムシ類は、それぞれ単系統群を形成し、本種が *Lamellibrachia* 属ハオリムシ類の進化過程で最初に分岐したグループである事が示された。Brothers 海山、デスモスサイトおよびTOTOサイトの熱水域では、これまで世界で知られている中で最も強酸性(pH2以下)の熱水が噴出しており、他の *Lamellibrachia* 属ハオリムシ類は生息していない。一方、近隣するラウ海盆やマヌス海盆・バックマヌスサイトには、別種の *Lamellibrachia* 属ハオリムシ類が分布している(Southward 1991; Kojima et al. 2003)。本種は薄い棲管を作るなど、強酸性環境に適応した種であると考えられるが、一方でそれほど強酸性でない熱水域では他種との競争により排除されるのかもしれない。Brothers 海山には他に、今回発見された *Oasisia* 属未記載種、デ

スモサイトには *Arcovestia ivanovi* と *Alaysia* 属未記載種が共存しているが、これらはいずれも最近まで熱水域固有とされ、hydrothermal vent-endemic vestimentiferans と呼ばれていたグループ(ハオリムシ類全体から Lamellibrachiidea 科と Escarpiidae 科を除いたものに当たる)に属する。ハオリムシ類が本来持っていた強酸性耐性という形質が、ほとんどの *Lamellibrachia* 属ハオリムシでは、本種の分岐後失われたものと考えられる。

本種の3集団の遺伝的多様性を比較すると Brothers 海山集団が最も高く、他の2つの集団に対して多系統となるので、デスモサイトと TOTO サイトの集団は Brothers 海山の集団から独立に派生した可能性が高いと考えられる。Kojima et al. (2003) は、東太平洋の中央海嶺に固有のハオリムシ類が南太平洋、東太平洋の順に分散、分化した事を示唆している。今回解析した種が上記のような歴史的分散過程を経たとすると、この仮説と整合的である。Hurtado et al. (2002) は、東太平洋海膨南部に分布する *Oasisia* が *O. alvinae* とは種レベルで分化している事を報告しており、これが Brothers 海山の *Oasisia* と近縁あるいは同種である可能性がある。今回 Brothers 海山で発見された2種のハオリムシは、太平洋を東から西へ熱水域固有種を分散させた歴史的要因の存在を示すものなのかもしれない。

以上の結果をまとめた原著論文を前述の記載論文が受理され次第、Journal of marine Biology Association of United Kingdom 誌に投稿する予定で、既に準備が完了している。

3. ラウ海盆で採集された Provannidae 科巻貝類2種の分子系統学的研究

ラウ海盆の Vai Lili サイト(水深 1700m)で採集された Provannidae 科巻貝類 *Alviniconcha* sp. および *Ifremeria nautilei* 各1個体について、ミトコンドリア DNA・COI 領域の塩基配列(696、792 塩基対)を決定した。既知の Provannidae 科巻貝類の塩基配列(Kojima et al., 2000, 2001, 2004)と比較したところ、*Alviniconcha* sp. は他の *Alviniconcha* 属巻貝類のいずれとも大きく異なる配列を示した。一方、*Ifremeria nautilei* の配列は、北フィジー海盆の集団に比較的高頻度に出現するものと一致した。

Alviniconcha 属の巻貝類についてはこれまでに、模式種であるアルビンガイ *Alviniconcha hessleri* がマリアナトラフに分布するのに加え、南太平洋のマヌス海盆および北フィジー海盆に共産する2系統、インド洋中央海嶺の1系統の遺伝的に異なる4つの系統群(Kojima et al., 2001, 2004)の存在が報告されている。今回の研究により、第5の系統の存在が明らかになった。Denis et al. (1993) は、アロザイム分析に基づき北フィジー海盆とマヌス海盆の *Alviniconcha* 属巻貝類の集団が種レベルで、遺伝的に異なっている事を報告しており、今回の結果と一致する。

Provannidae 科巻貝類はシロウリガイ類などと同様に、鰓の細胞中で硫黄酸化細菌と細胞内共生している事が知られている。Urakawa et al. (2005) と Suzuki et al. (2005) は独立に、*Alviniconcha* 属の巻貝類に ϵ -プロテオバクテリアと共生するものがある事を明らかにした。これは深海化学合成生物群集の動物が γ -プロテオバクテリア以外の細菌と細胞内共生している事を報告した初めての事例である。さらに Suzuki et al. (2006a, 2006b) が Provannidae 科巻貝類の共生細菌を網羅的に系統解析した結果、*Ifremeria nautilei* の共生細菌が全て γ -プロテオバクテリアである事、*Alviniconcha* 属巻貝類では、南太平洋の2つの系統群の中のひとつとインド洋の系統群が ϵ -プロテオバクテリアと共生し、他の3つの系統群は γ -プロテオバクテリアと共生している事が明らかになった。同じ Provannidae 科に属する *Provanna* 属では、細菌との細胞内共生は見つかっていない。

Provannidae 科巻貝類における共生細菌の置換が本科の進化過程のどの段階で起きたかを明らかにするため、*Alviniconcha* 属巻貝類各系統1個体およびマヌス海盆と北フィジー海盆の *Ifremeria nautilei* 各1個体について、COI 領域のさらに上流側の塩基配列を決定し、1213 塩基対の配列に基づく系統解析をおこなった。その結果、*Alviniconcha* 属は γ -プロテオバクテリアと共生する3系統と ϵ -プロテオバクテリアと共生する2系統の2つの単系統群から成る事が示された。ただし、 ϵ -プロテオバクテリアと共生する系統群の単系統性を支持するブートストラップ確率はそれほど高くなく(近隣結合法 88%、最大節約法 60%)、核遺伝子の塩基配列等により更に検証する必要がある。

一方、*Ifremeria nautilei* ではこれまでに、ミトコンドリア DNA・COI 領域の塩基配列に基づく解析からマヌス海盆と北フィジー海盆の集団は、完全に遺伝的に分化しているが、その違いはまだ種内レベルに留まっている事が報告されている(Kojima et al., 2000)。*Alviniconcha* 属巻貝類では有意な

集団分化が見られないこれら2つの海盆の間に、*Ifremeria nautilei*では有意な分化が見られる事について、Kojima et al. (2000) は、*Ifremeria nautilei* の幼生分散能力が *Alviniconcha* 属巻貝類に比べて劣る結果である可能性を示唆している。しかし今回の研究結果は北フィジー海盆とラウ海盆の間では逆に *Alviniconcha* 属巻貝類が分化しているのに対し、*Ifremeria nautilei* に遺伝的分化が生じていない事を示している。いずれの属についても幼生型や分散能力に関する直接的な報告はない。Provannidae 科巻貝類の集団間の隔離と分化を支配する他の要因がある可能性が考えられる。

以上の内容を含む原著論文が Marine Ecology Progress Series 誌 (*Ifremeria nautilei*) および Applied and Environmental Microbiology 誌 (*Alviniconcha* sp.) に受理されている。

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熱水の地球化学および有機地球化学的研究

熱水無機化学成分の特徴

2004年9月22日～11月10日に行われたラウ海盆およびケルマデック島弧を調査対象としたYK04-09航海に参加し、「しんかい6500」の潜航調査によって熱水試料を採取して、その化学地球化学的研究を行った。

ラウ海盆ではCVFR(中央ファルファ海嶺セグメント)において、Vai LiliサイトおよびMarinerサイトに熱水試料の採取を行った。その結果であるMgダイアグラムの一部を図1に示す。Vai Liliサイトでは、1989年のフランスによる潜航調査の際と比べて、熱水活動が著しく衰退していることが確認された。熱水の噴出温度は1989年には340°Cと報告されているが、現在では最高でも88°Cの熱水噴出が確認されるにとどまっている。この熱水の化学組成(図中□印)には、硬石膏の沈殿生成を意味するCa, Sr, SO₄の低下が見られ、熱水と浸入してきた海水の混合が海底下で起こっていることも明らかになった。これに対して、最高360°Cの高温熱水噴出が見られたMarinerサイトで採取された熱水試料では、Cl濃度が高い熱水(図中▲印)とCl濃度が低く溶存気体成分に富む熱水(▼)の両者が見出され、海底下での気液二相分離が起こっていることが確認された。また両者ともpH<2.5という強い酸性を示しており、300°C以上の高温熱水でこれだけ強酸性を示す例はこれまでに知られていない。

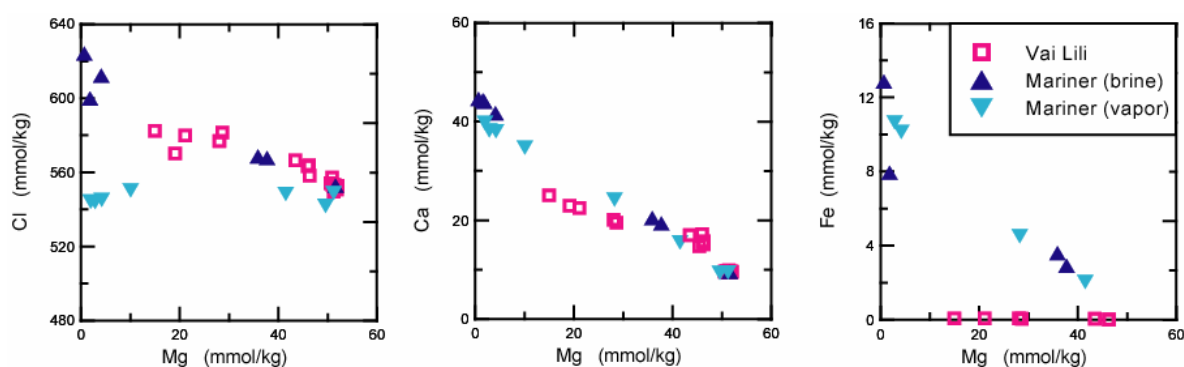


図1 ラウ海盆の熱水活動地帯で採取された試料の化学組成

ケルマデック島弧のBrothers海山においては、北西カルデラ斜面の熱水サイトと中央火口丘の熱水サイトで、熱水試料の採取を行った。北西斜面サイトでは(図中◆印)、最高温度が302°Cに達しCl濃度が高くpH=3.0の熱水が採取され、島弧型の高温熱水活動に共通した特徴を示した。これに対して中央火口丘における熱水は、温度が60°C程度と低く主成分組成も海水とあまり変わらないにもかかわらず、硫黄成分に非常に富みpHが著しく低いという性質を示した(図中●印)。この特異的な特徴は、マグマから脱ガスした揮発性成分を取り込んで変質した海水が、高温熱水岩石反応を経ずに噴出しているとして説明できる。

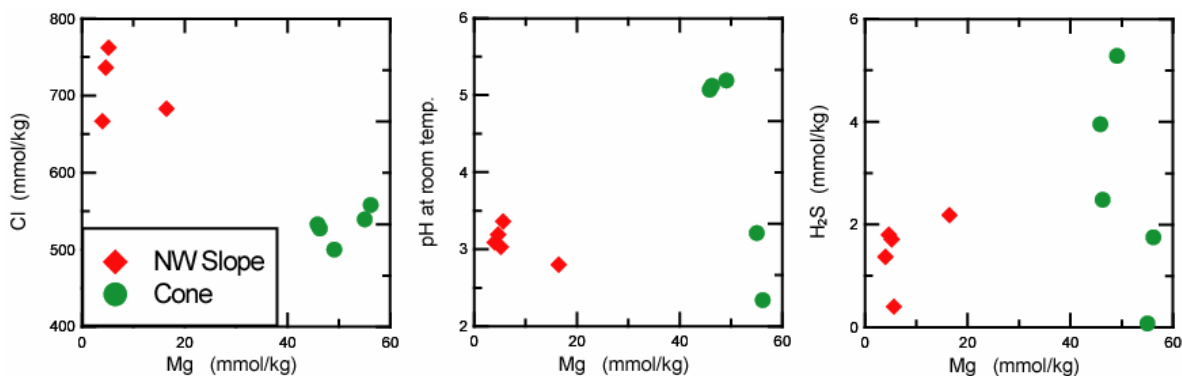


図2 ケルマデック島弧ブラザーズ海山の熱水活動地帯で採取された試料の化学組成

熱水噴出孔周辺堆積物に含まれる有機物の有機地球化学的研究

熱水噴出孔周囲には、生物が皆無な熱水噴出孔のない深海底と対照的に、そこに固有の生物が高密度で生息し、膨大なバイオマスが存在する。それは、熱水とともに硫化水素やメタンといった還元化学種が湧出し、その酸化エネルギーを生育に利用する化学合成微生物が多量の有機物を生産し、その一次生産物によって、巨大なバイオマスが維持されているためと考えられている。実際、様々な微生物が熱水噴出孔周辺に生息していることが微生物学的に示されているが、どういった微生物がどういった割合でどの程度のバイオマスを熱水噴出孔周辺に成しているか、従来の微生物学的手法では必ずしも定量的な評価には至っていない。そこで、熱水噴出孔周辺の堆積物中のバイオマーカー、すなわち有機化合物のうち起源生物の推定に有効な特定の成分を用いて、熱水噴出孔周辺における微生物生態系の解明を試みた。

研究に用いた試料は西太平洋に分布する、南半球のパプアニューギニアマヌス海盆、沖繩トラフ、伊豆小笠原弧の3つの熱水系から潜航調査の際に得られた堆積物を用いた。ラウ海盆は残念ながら堆積物に欠き、試料採取に至っていない。分析したバイオマーカーは主に生物の膜組織の主成分である脂肪酸を対象とした。

分析の結果、陸上から多くの堆積物が供給され、熱水活動域がそういった堆積物で厚く覆われている海域(沖繩トラフ)では、堆積物中の微生物起源のバイオマーカーがあまり顕著に認められないものの、その他の海域では微生物起源バイオマーカーに富み、確かに熱水噴出孔周辺は微生物による一次生産が活発であることが裏付けられた。しかも、微生物起源バイオマーカーの多い海域では堆積物中の全有機炭素量に占める脂肪酸濃度が極めて高く、分解が進行し脂質成分に欠く有機物が大半を占める熱水噴出孔のない海域と明瞭な差が確認された。今後は、検出されたバイオマーカーが示す起源生物であるバクテリアにそのバイオマーカーがどの程度の濃度で含まれるかを培養実験などで検証することで、堆積物から検出されたバイオマーカー濃度を元にバイオマスの見積もられると期待される。

ケルマデック島弧北部に位置する Brothers 海底火山熱水系に生息するハオリムシとミョウガガイのエネルギー源に関する有機地球化学的研究

本研究では、ケルマデック島弧北部に位置する Brothers 海底火山の熱水活動域から採取された熱水依存生物(主に macro benthos)の地球化学分析を通じて、生物の成育を支えている化学エネルギーや生態系に関する情報を得ることを主な目的とした。

分析は、生物軟組織の炭素および窒素安定同位体組成について元素分析計を前処理装置とした連続フロー型安定同位体質量分析計を用いて行った。

分析に用いた生物試料は Brothers 海底火山でしんかい 6500 の潜航調査 (Dive #851, #852, #854) の際採取されたフジツボの中間であるミョウガガイ (*Vulcanolepas osheai*) と2種のハオリムシ (*Lamellibrachia* spp.) について行った。

分析の結果を以下の表に示す。比較のため、沖繩トラフおよび南部マリアナ海嶺の熱水系で採取されたハオリムシとフジツボの分析結果を示す。

ハオリムシは、口も排泄器官も消化器官さえも持たない動物で、その成育のための栄養を体内に多量に共生させた細菌から得ている。この共生細菌は硫化水素を酸化する際に生じる化学エネルギーを利用して一次生産を行う硫黄酸化細菌の一種である。この細菌の炭素固定経路には2種類のもものが知られており、カルビンベンソン回路を用いるものと還元的TCA回路を用いるものがあると考えられている。両者は遺伝子を用いるなど通常の生物学的手法で容易に知ることが出来るが、地球化学的にも炭素同位体比を測定することで区別できる。すなわち、カルビンベンソン回路を用いる細菌は大きな同位体効果を示し、生体の炭素同位対比が用いる炭酸の炭素同位対比より30‰以上軽くなる。一方、還元的TCA回路を用いた場合は、同位体分別は小さく、たかだか15‰以下である。

一方、ミウガガイなどのフジツボは主に懸濁粒子や体に付着した細菌などを蔓脚で集めて摂食していると考えられているが、実際に何を食べているか、具体的な研究は少ない。また、同位体などを用いた地球化学的研究も皆無である。

Brothers海底火山で採取されたハオリムシは、 $\delta^{13}\text{C} = -7.5$ と -12.9 ‰と重く、これらが還元的TCA回路を利用する細菌を共生細菌として持つことが確認された。一方、ミウガガイもその炭素同位対比は非常に重く、 -13 ‰程度である。これらハオリムシとミウガガイは分布が異なり、両者とも十分の距離を置いていることから、ハオリムシの代謝物などをこのミウガガイが濾しとって摂食しているとは考えにくいことから、恐らくミウガガイの周囲にハオリムシの共生細菌同様、還元的TCA回路を利用して一次生産を行う微生物が繁茂し、それを摂餌していると考えられる。

ハオリムシの同位体組成の報告を各海域で見ると、 $\delta^{13}\text{C} = -26 \sim -16$ ‰で、別属と思われるが、アリューシャン列島から報告されているもので $\delta^{13}\text{C} = -47 \sim -29$ ‰、大西洋でも -27 ‰より低い。Brothers火山で発見されたハオリムシ同様に高い炭素同位対比を持つのは、属が異なるが東太平洋から発見された*Riftia pachyptila*である。共生細菌の系統関係と併せてこの同位体組成を考えることで、ハオリムシと共生細菌の関係とその共生関係を支える環境的要因を解明する糸口をつかめるものと期待される。

フジツボに関しては同位体データが少ないが、他の海域で得られたフジツボ類の同位体比と比較すると、沖縄トラフの熱水系で見いだされるミウガガイ(*Ashinkailepas seepiophilia*)では、 $\delta^{13}\text{C} = -22.8$ ‰、南部マリアナ海嶺のハナカゴ類(*Neoverruca brachylepadiformis*)でも、同様に $\delta^{13}\text{C} = -21.9$ ‰である。沖縄トラフでは、ハオリムシとミウガガイが隣接して生息しており、このハオリムシがやはり $\delta^{13}\text{C} = -22.3$ ‰といった値を有する。 $\delta^{13}\text{C} = -22$ ‰という値は、海洋表層における植物プランクトンが一次生産を行った有機物の炭素同位体比($\delta^{13}\text{C} = -25 \sim -18$ ‰)と一致するため、沖縄トラフのミウガガイがハオリムシの代謝物だけに依存しているか断定は出来ない。しかし、窒素同位対比がハオリムシで $\delta^{15}\text{N} = +3$ ‰でミウガガイが $+7$ ‰程度であることから、餌-捕食者間で期待される窒素同位対比の差($+3 \sim +5$ ‰)の範囲内に入る。なお、測定された軟組織の硫黄同位対比は $\delta^{34}\text{S} = -11$ ‰と、通常の海洋生物($\delta^{34}\text{S} = +15 \sim +20$ ‰)に比べ非常に低く、このような値は硫黄酸化細菌にのみ期待される値であることから、沖縄トラフのミウガガイの主たる栄養源がハオリムシの代謝産物である可能性は高い。一方、南部マリアナ海嶺のハナカゴ類では、周囲にはイソギンチャクの群集があるのみで、その炭素・窒素同位対比($\delta^{13}\text{C} = -17.7$ ‰, $\delta^{15}\text{N} = +9.7$ ‰)とも明瞭に異なり、植物プランクトンに類似した炭素同位体比と海洋表層から沈降してくる有機物粒子に匹敵する高い窒素同位対比($\delta^{13}\text{C} = -22$ ‰, $\delta^{15}\text{N} = +10.5$ ‰)から、わずかながら海洋表層から沈降してくる有機物粒子に依存して生育している可能性が高いといえる。

以上の結果より、ハオリムシ、ミウガガイともに同位体比といった地球化学データから見た場合、それぞれ両種は他海域のものと共生細菌の代謝系や摂食する餌の起源が明瞭に異なり、熱水系生物群集の世界的な分布・拡散やその進化を考える上で本海域が重要な位置にあることが示唆された。

表 ケルマデック島弧 Brothers 火山および沖縄トラフ、南部マリアナ海嶺で採取されたフジツボ類、ハオリムシ類、イソギンチャクの同位体組成

Species	Group	Area	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
<i>Vulcanolepas osheai</i>	Barnacle	Brothers volcano	-13.0	7.8	n.d.
<i>Lamellibrachia</i> sp.1	Pogonophora		-7.5	1.9	n.d.

<i>Lamellibrachia</i> sp.2	Pogonophora		-12.9	3.7	n.d.
<i>Ashinkailepas seepiophilia</i>	Barnacle	Okinawa Trough	-22.8	7.2	-11.0
<i>Vestimentiferan</i> tube-worm	Pogonophora		-22.3	2.9	n.d.
<i>Neoverruca brachylepadoformis</i>	Barnacle	South Mariana Ridge	-21.9	10.8	n.d.
<i>Marianactis</i> sp.	Anemone		-17.7	9.7	n.d.

n.d.: not determined.

研究分担者:宮崎淳一(山梨大学)
軟体動物の分子系統

シンカイヒバリガイ類はイガイ科シンカイヒバリガイ亜科 *Bathymodiolus* 属に分類される二枚貝類で、世界で 18 種、日本周辺海域では 6 種が記載され、水深 500~4,000m の熱水噴出孔や冷水湧出帯に生息している。このような地域には硫化水素やメタンが豊富であり、これを利用して一次生産を行う化学合成細菌に依存する化学合成生物群集が形成されている。シンカイヒバリガイ類は化学合成生物群集の優占種のひとつであり、鰓の上皮細胞内に共生するメタン酸化細菌や硫黄酸化細菌からエネルギーを得ることで特殊な環境下において生命を維持している。

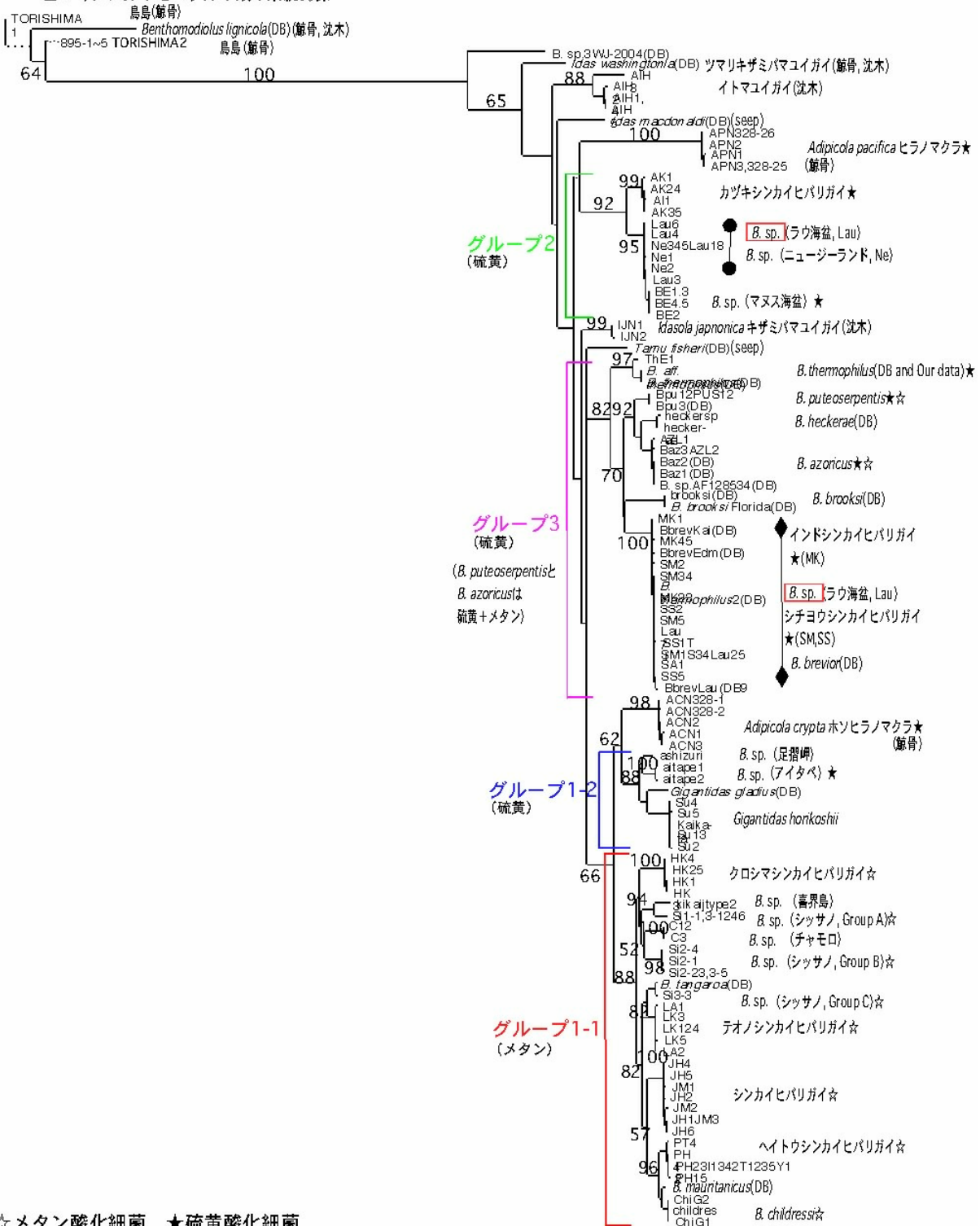
シンカイヒバリガイ類の進化と起源を明らかにするため、ミトコンドリア遺伝子の塩基配列を比較することによって、種間および種内集団間の系統解析を行ってきた。その結果、日本周辺海域に生息するシチヨウシンカイヒバリガイ *B. septendierum*、南太平洋の *B. brevior*、インド洋のインドシンカイヒバリガイ *B. marisindicus* は形態学的研究によって異なる種として記載されたが、遺伝的には非常に近縁であり、現在でも遺伝的交流がある可能性がでてきた。これらの生息地は、直線距離でおおよそ 10,000km も離れており、特に日本周辺海域とインド洋の生息地の間には、大陸や島嶼などが存在し、これらが障壁となって直接的な遺伝的交流は不可能にみえる。したがって、シンカイヒバリガイ類の遺伝的交流は、南太平洋の生息地を介して間接的に行われていると思われる。このことは、シンカイヒバリガイ類の進化過程においても、南太平洋を起源として、インド洋あるいは西太平洋にシンカイヒバリガイ類が分散した可能性を示唆している。

シンカイヒバリガイ類の起源が南太平洋にあり、現在でも南太平洋を介して日本周辺海域からインド洋にわたる遺伝的な交流があることを明らかにするために、シチヨウシンカイヒバリガイおよびインドシンカイヒバリガイの遺伝的な解析を行ってきた。しかし、南太平洋のサンプルが入手できなかったため、それ以上の検討を行うことができなかった。本研究の南太平洋ラウ海盆の調査によって、シンカイヒバリガイ類が 10 個体採集され、解析を行うことが可能となった。

内容:

本研究では、ミトコンドリア NADH デヒドロゲナーゼサブユニット 4 (ND4) の塩基配列を決定し、系統解析および集団遺伝学的解析を行った。その結果、シンカイヒバリガイ類は 3 つのグループに分かれることが明らかとなった (図 1)。グループ 1 は日本周辺海域に生息するシンカイヒバリガイ、ヘイトウシンカイヒバリガイ、クロシマシンカイヒバリガイ、およびテオノシンカイヒバリガイと南太平洋の *B. tangaroa* と大西洋の *B. childressi* および *B. mauritanicus* を含む。これらの種は共生細菌として、メタン酸化細菌をもつ。グループ 2 は日本周辺海域に生息するカヅキシシンカイヒバリガイと南太平洋マヌス海盆産未記載種を含み、これらの種はイオウ酸化細菌をもつ。グループ 3 は日本周辺海域に生息するシチヨウシンカイヒバリガイ、南太平洋の *B. brevior*、インド洋のインドシンカイヒバリガイ、東太平洋の *B. thermophilus* および未記載種、大西洋の *B. brooksi*、*B. heckerae*、*B. puteoserpentis* および *B. azoricus* を含む。これらの種は主にイオウ酸化細菌をもつが、イオウ酸化細菌とメタン酸化細菌の両方をもつものもある。グループ 3 のシチヨウシンカイヒバリガイ、*B. brevior*、インドシンカイヒバリガイは、遺伝的には非常に近縁であり、この 3 種はそれぞれが排他的なクラスターを形成することなく、3 種全体で 1 つのクラスターを形成することが明らかとなった。この結果はミトコンドリアシトクローム *c* オキシダーゼサブユニット I (COI) 遺伝子を用いた系統解析の結果からも支持された。

図1 ミトコンドリアND4遺伝子の塩基配列に基づくシンカイヒバリガイ類の系統関係



ラウ海盆で採集されたシンカイヒバリガイ類 10 個体のうち 7 個体はグループ 2 に含まれる南太平洋マヌス海盆産未記載種に近縁であり、3 個体は予想したとおりグループ 3 のシチヨウシンカイヒバリガイ、*B. brevior*、およびインドシンカイヒバリガイから成るクラスターに含まれた。これらの種の形態学的検討は現在なされているところであるが、グループ 3 に属する未定種の 3 個体を用いて集団遺伝学解析を行った(表1)。シチヨウシンカイヒバリガイは伊豆・小笠原島弧海域の 2 集団(明神海丘と水

曜海山)を用いたが、これらの間の遺伝的な相違(F_{ST})は0.009と非常に小さく、1世代あたり約53個体(Nm)が両地域の間で移動する、すなわち大きな遺伝的交流があったことがわかった。これに比べシチヨウシンカイヒバリガイとインドシンカイシバリガイ、シチヨウシンカイヒバリガイとラウ海盆産シンカイヒバリガイ類、インドシンカイシバリガイとラウ海盆産シンカイヒバリガイ類の間では、遺伝的な相違が大きく、1世代あたりに移動する個体も少なかった。しかし、ラウ海盆産シンカイヒバリガイ類とシチヨウシンカイヒバリガイまたはインドシンカイシバリガイの間では、1世代あたり移動する個体が1以上であり、遺伝的交流があることを示している。シチヨウシンカイヒバリガイとインドシンカイシバリガイの間では、1を若干越えるか1未満であり、遺伝的交流がもしあったとしてもわずかなものであることを示している。この結果はプレリナリーなものであるとはいえ、南太平洋を介して日本周辺海域とインド洋の間で遺伝的交流があること、また深海生物であるにもかかわらず直線距離で約10,000kmもの分散を行うことを示唆しており、非常に興味深い。

表1シンカイヒバリガイ類の遺伝的交流

Nm		F_{ST}		シチヨウ	インド	ラウ
		明神	水曜			
シチヨウ	明神海丘(N=10)		0.009	0.385*	0.050	
	水曜海山(N=11)	53.065		0.327*	-0.146	
インドシンカイヒバリガイ(N=10)		0.798	1.029		0.275*	
ラウ海盆未記載種(N=3)		9.559	inf	1.317		

* $P < 0.05$

1.ラウ海盆およびケルマディック海域熱水噴出孔生物群集の分布と生物相の解析

ニュージーランド北沖のケルマディック海域から、フィジー沖のラウ海盆にある深海熱水噴出孔生物群集を構成するメガベントスの分布と生物相を明らかにするために、「しんかい6500」による潜航調査航海に参加した。ケルマディック海域では Brothers Seamount において熱水噴出孔生物群集を見つけたことができた。しかしながら、参加期間中は、悪天候のためラウ海盆では潜航調査を行うことができなかった。したがって本報告では、ケルマディック海域の熱水噴出孔生物群集におけるマクロ・メガベントスの分布と生物相について述べる。

ケルマディック海域の潜航調査はNorth-West Caldera Wall site (34-51.7'S, 179-03.5'E)とCone site (34-52.7'S, 179-04.3'E)の2地点で行われた。North-West Caldera Wall site (水深1470-1851m)では、第851(2004年10月26日)および852(2004年10月27日)回の計2回の潜航を実施した。Cone site (水深1224-1434m)では、第853(2004年10月28日)および854(2004年11月1日)回の計2回の潜航を実施した。ベントスのサンプリングは、「しんかい6500」に搭載されたスラープガンやマンピュレータで行い、観察はTVカメラ、スティルカメラおよび潜航研究者の肉眼で行われた。本報告で用いる各種の分類群名は、調査船上で便宜的に与えたものも多数ある。

(1)ブラザースカルデラ North-West Caldera Wall site

この地点からは20種のマクロ・メガベントスが採集・同定された。なかでも、2種?の多毛綱ウロコムシ類、小型笠型腹足類、ユノハナガニ類、数種のコシオリエビ類、ハオリムシ類が熱水噴出孔から数m以内に生息していた。2種のエビ類(*Alvinocaris niwa* と *A. longirostris*)も分布しており、一見した分布密度は *A. niwa* のほうが *A. longirostris* より高かった。これら、2種のエビ類は噴出孔のみならず離れた地点の岩石、堆積物上にも認められた。このサイトにおいて優占的に生息していたのが、柄の長いタイプの蔓脚類 *Vulcanolepas osheai* であった。柄の長さは40cmにも達した。

(2)ブラザースカルデラ Cone site

この地点は、North-West Caldera Wall site のように激しい熱水噴出活動は見られず、温水がゆっくり湧出するような環境であった。そして、17種のマクロ・メガベントスが採集・同定された。湧出域の岩石は変色しており、そこには赤黒いウロコムシ類が密集していた。North-West Caldera Wall site と同様、*A. niwa* と *A. longirostris* が分布しており、一見した分布密度は *A. longirostris* のほうが *A. niwa* より高かった。さらには Hippolytidae 科の *Lebbeus* sp.も認められた。他にも2種の笠型腹足類、イバラガニ類 *Paralomis* sp.、ゲンゲ科魚類、そして柄の長いタイプの蔓脚類 *V. osheai* が高頻度に出現した。特に *V. osheai* は、高密度の集団を形成していた (Fig. 1)。



Fig. 1. Close up of a cluster of stalks barnacle *Vulcanolepas osheai*. Shinkai 6500 #854.

ケルマディック海域の North-West Caldera Wall site と Cone site のマクロ・メガベントス生物相は、概して類似していた。しかしながら、分布生態では異なる点も認められ、例えば、2種のエビ類

(*Alvinocaris niwa* と *A. longirostris*) は、両地点に分布しているのも係わらず、意見した密度は、NW Caldera Wall site では *A. niwa* が、Cone site では *A. longirostris* のほうが高くなっていた。また、ゲンゲ科魚類についても、Cone site では高頻度に観察されたが、NW Caldera Wall site では稀であった。これらを含め、生物種の分布パターンの違いが何に起因するのか、今後ビデオや環境因子データを基に解析されるであろう。また、ケルマディック海域のマクロ・メガベントスの分子系統解析も進行し、西太平洋や東太平洋の化学合成生物群集の分散・進化に関する有益なデータが出てくるであろう。

2. 深海化学合成生物群集に関する総合的研究

日本周辺の深海にある化学合成生物群集(熱水噴出孔生物群集、冷水湧出生物群集、鯨骨生物群集)を対象に、群集組成、生態的特性、進化過程を明らかにするための研究を実施した。

(1) 群集組成

軟体動物を中心に、多数の新種記載を行った。また、鹿児島県野間岬沖に沈められたマッコウクジラの死骸からは、植物の根のような構造をもち、それを鯨骨に埋没させて生息する新奇な環形動物 (*Osedax* 属と思われる) が見つかри、今後多方面にわたる研究に着手した。

(2) 生態的特性

ベントスの食性や分布について解析した。あわせてベントスの成長を高精度に測定する手法の開発も行った。

(3) 進化過程

主に軟体動物・環形動物・節足動物を材料に、形態的分類と分子生物学的手法をリンクさせながら系統・生物地理に関する研究を行った。

これらの成果は、各専門分野の科学雑誌等で公表した。また日本ベントス学会誌に特集号が生まれ、これまでの研究のレビューなどを公表した。

研究分担者: 土田真二

西太平洋における熱水性甲殻類の多様性と生物地理学的分布

熱水性短尾類

ユノハナガニ科短尾類は、これまで東太平洋から3属8種、西太平洋～インド洋から1属4種、大西洋から1属1種の計13種が報告されている。いずれも深海の熱水域にのみ生息し、体は白色、甲は丸みを帯び、眼柄は退化して眼窩と癒合するなど生態学的分類学的にも特異な短尾類である。これまで、ユノハナガニの遺伝的変異に関する情報はほとんどなく、その種分化や系統関係は不明である。本年は、2004年11月に行ったケルマディック島弧熱水域における調査により新たに、新種の *Austinograea* 属ユノハナガニが採集された。本研究では、この種類も含め、東～西太平洋～インド洋～大西洋に分布する3属7種のユノハナガニ科短尾類の遺伝的変異明らかにするとともに、他科の短尾類とも比較検討した。

用いた試料は、*Austinograea yunohana* (伊豆・小笠原諸島海域) 5地点、*A. williamsi* (マリアナ舟状海盆)、*A. alayseae* (ラウ海盆、マヌス海盆)、*Austinograea* sp. (ケルマディック島弧)、*A. rodriguezensis* (インド洋中央海嶺) 各1地点、*Segonzacia mesatlantica* (大西洋中央海嶺) 4地点、*Bythograea therymydon* (東太平洋中央海嶺) から得た個体の COI 遺伝子約 660bp および 16SrRNA 遺伝子約 450bp を分析した。

その結果、ユノハナガニ類は単系統群を形成し、今回比較した他科のカニ類とは遺伝的に大きく異なっていた。また、*Austinograea* 属、*Segonzacia* 属、*Bythograea* 属とも単系統群を形成し、それぞれの種も同様に単系統であったが、種内での地域変異は明らかではなかった。種間でみると *A. alayseae* と *A. williamsi* は、7種の中ではもつとも近縁であった。次いで、地理的距離が直線7000km 以上も離れている *A. rodriguezensis* が近縁であった。また、地理的に6000km 以上も離れている *A. yunohana* とケルマディック島弧産の *Austinograea* sp. が近縁であった。*A. yunohana* は *A. williamsi* の分布域と400km 程度しか離れていないにも関わらず、*Austinograea* 属の中ではかなり遺伝的に離れていた。当初、西太平洋のユノハナガニは北から南へと種分化、伝播した可能性が示唆されたが、これらの結果から単に直線的でないことが示唆された。

熱水性エビ類

オハラエビ科に属するエビ類は現在7属19種知られており、その全ての種が海底に存在する熱水噴出域や冷湧水域において特異的に生息し、またその生息域は世界の三洋におよんでいる。その中で日本近海からニューージーランドまでの西太平洋域の熱水域には *Alvinocaris* 属、*Chorocaris* 属、*Opaepele* 属、*Nautirocaris* 属、*Shinkaicaris* 属の5属の生息が確認されている。このうち前者3属は東太平洋や西太平洋においても生息が確認されている。2004～2005年のラウ海盆、ケルマディック島弧および北部マリアナ弧における調査航海により、*Alvinocaris* 属と *Opaepele* 属においては同一種がとても広い範囲で分散している事が明らかになった。*Alvinocaris longirostris* では沖縄、マヌス海盆、ケルマディック島弧の熱水域にも分布し、*Opaepele loihi* についてはハワイ、沖縄、北部マリアナ弧の熱水域にも分布していた。各生息域は数千 km 離れており、同一種を維持するためには大きな分散能力を必要とするはずである。本研究では海洋研究開発機構の潜水調査システムにより採集されたサンプルを元に *Alvinocaris longirostris* と *Opaepele loihi* において分子系統解析 (COI 部分配列) を行った。*A. longirostris* をみると、南西諸島海域の鳩間海丘、第四与那国海丘、伊平屋海嶺北部凹地とマヌス海盆のサンプルにおいて遺伝的な差異が見られなかった。しかし、ケルマディック島弧のサンプルは前者のサンプルに対し遺伝的な差異が見られた。つまり、ケルマディック島弧のものは、形態的には *A. longirostris* に分類されるが、遺伝学的には亜種あるいは別種になることが示唆された。*Opaepele loihi* では、北部マリアナ弧とハワイとを比較した結果、両者は単系統になった。このことから、これらは遺伝学的にも同種であり、広い分布域をもつことが明らかになった。今後は、幼生の浮遊期なども明らかにし両種の分散機構や種分化について考察する。

熱水性異尾類

熱水、冷湧水性の甲殻類として、シンカイコシオリエビ類がしばしば出現する。シンカイコシオリエビは、1属で190種以上を含み、世界中の深海に生息する。熱水、冷湧水に固有なものもこれまで9種知られている。2004年のケルマディック島弧の調査では、同じ地点からシンカイコシオリエビが3種採集され、そのうちの一種は新種であった。また、これまで同所的に3種が生息していた例はなく、ケルマディック島弧の熱水域はシンカイコシオリエビ類の多様性が高いと言える。2005年の北部マリアナ弧の調査では、栄福海山より多数のシンカイコシオリエビが採集された。これらは、伊豆・小笠原海域の明神海丘に生息する未記載種と同種であった。今後は、これまでに得られているシンカイコシオリエビ類の遺伝学的な検討を行い、遺伝的多様性や生物地理学的な検討を行う予定である。

研究分担者:佐々木猛智・長谷川和範
西太平洋における笠型貝類の多様性と地理分布

ラウ海盆の笠型貝類の分類

ラウ海盆から採集された笠型貝類の分類学的研究を行った結果、7種に分類され、それらは全て新種である。そのうち4種は、Acmaeidae の *Bathyacmaea* 属の種である。外套腔内には櫛鰓があり、歯舌は歯尖が褐色で、基底板がある点で、Neolepetopsidae の種とは区別される。

4新種のうち、sp. 1 は純白で殻は厚く、表面には絹目状の彫刻がある。ほぼ全ての個体で殻頂部が著しく浸食され強い段差を生じる。殻表は膜状の付着物に覆われている個体が多い。本種は *Bathyacmaea tertia* Sasaki, Okutani & Fujikura, 2003 に最も類似するが、殻の前後の幅がほぼ等しくなる点で異なっている。sp. 2 は殻は薄く、軟体部が透けて見える。殻頂は全ての個体でほとんど浸食を受けておらず、殻表には付着物がない。本種は *Bathyacmaea jonassoni* Beck, 1996 に近似するが、殻が薄く、殻高が低く、殻表に微細な放射状の彫刻がある点で異なる。sp. 3 は顕著な放射細肋が密にあり、肋の表面は顆粒状である。殻は全体的に厚く、殻縁部のみが薄く透き通って見える。sp. 4 は sp. 3 に類似しているが、放射肋が太く、肋上の顆粒が顕著で、殻形は前側が先細りになる点が異なる。表面は鏽状物質によって覆われている。

残りの3種は上足触角を持ち、殻頂は中央よりも後方に位置しており、古腹足類に属する。sp. 1 の殻は前後に長く、灰白色で、殻頂は後方に著しく偏り、殻の左側を向く。左右の殻縁はほぼ平行である。殻表には微細な放射細肋がある。sp. 2 の殻は円形に近く、表面は平滑である。足の側面は多数の上足突起によって全面的に縁取られている。sp. 3 は *Puncturella* に類似した殻形を持つが、殻頂部には孔がない。顆粒を伴う強い放射肋をもち、殻縁に突出する。3種とも新属新種であると考えられ、科レベルの分類上の位置はさらに検討が必要である。

Bathyacmaea 属の分布

Bathyacmaea 属の分布は、北西太平洋および南太平洋の化学合成生物群集に限定されている。最も多様性の高い海域は、日本列島の周辺である。文献上少なくとも6種がに記録されており(Sasaki et al., 2005)、さらに未記載種が複数存在する。分布域も広く、沖縄トラフ、南海トラフ、相模湾、伊豆諸島の化学合成生物群集から知られている。一方、南太平洋からはエディソン海山およびマヌスから *Bathyacmaea jonassoni* Beck, 1996 が知られているのみであった。しかし、本研究により、ラウ海盆には4新種が分布することが明らかになり、北西太平洋と南太平洋の両海域で分化していることが判明した。不思議なことに北西太平洋と南太平洋をつなぐ海域からは、本属の種は知られていない。この地理的分布のパターンは他の貝類には見られないものである。

Bathyacmaea 属の系統上の位置

Bathyacmaea secunda を解剖した結果、腸と心臓の関係、口球軟骨と筋肉の関係、歯舌の形態、平衡胞の位置によって、他のカサガイ類と区別されることが明らかになった(Sasaki et al. 投稿中)。様々な器官の形態を比較した結果、ツタノハガイ上科および Neolepetopsidae とは共有される派生形質がなく、カサガイ類の中では遠い関係にあることが分かった。一方、Acmaeidea のグループとはいくつか特異的な形質を共有しており、より近縁であると考えられる。Acmaeidea の中での関係は、解剖されていない属があり、比較解剖学の観点からは十分に解明することができなかった。貝殻の微細構造の形質では、semi-foliated 構造からなる殻層を持っており、この点からも Acmaeidea の一部との類縁性が支持される(Fuchigami & Sasaki, 2005)。

Shinkailepas 属の系統上の位置と地理分布

Shinkailepas 属の1種、ミョウジンシンカイフネアマガイを解剖した結果、外套器官、消化器官、排出器官、生殖器官、神経系の構造から、アマオブネガイ目の1群であることが裏付けられた(Sasaki et al., in press)。また、生息器官、感覚器官、外套腔内の腺組織には特異的な形質が見つかった。アマオブネガイ目の様々な科と形態形質を比較した結果、本属をアマオブネガイ上科のユキスズメ科

Phenacolepadidae に置く現在の分類が支持された。ユキスズメ科の中では深海に生息する属 (*Shinkailepas* 属と *Olgasolaris* 属)、および浅海に生息する属 (*Phenacolepas* 属と *Cinnalepeta* 属) がそれぞれ単系統であると考えられる。

Shinkailepas 属の種分類は形態形質では、殻形態、眼柄、上足襞、陰茎、歯舌、蓋の形質によって区別されている(Sasaki et al., in press)。本属は、伊平屋北部海丘、海明神海丘、海形海山、マリアナ、フィジー、マヌス、ラウ海盆から記録され、それぞれの地点で別種である可能性が高い。大西洋中央海嶺から *Shinkailepas briandi* Warén & Bouchet, 2001 が記録されているが、太平洋との種の関係は明らかではない。

古腹足類の笠型貝類の地理分布

西太平洋に生息する古腹足類型の笠型貝類では、*Lepetodrilus* 属が比較的多産する。しかし、*Lepetodrilus* 属はラウ海盆からは採集されなかった。本属は、沖縄トラフの熱水噴出域に特に多く、伊豆小笠原海溝およびマリアナ海溝に沿った熱水噴出域からは記録がなく、マヌスから1種知られているのみである。

上記以外の古腹足類型の笠型貝類は、西太平洋では一般的に多様性が低い。*Puncturella*, *Pseudolimula* 等が記録されているが、種数も産地も限られている(Warén & Bouchet, 2001)。しかし、古腹足類の地理的分布については、今後本研究で発見された新属新種と考えられる種の系統分類上の位置を確定した上で議論しなければならない。

研究分担者:藤原義弘(海洋研究開発機構)

要旨:

1. 化学合成系生物の分散過程に鯨骨が関与しているかどうかを明らかにするため鯨骨生物群集調査を実施した.
2. 化学合成共生イガイ類の起源を探るためにイガイ類共生細菌の系統解析ならびに電子顕微鏡観察を実施した.

内容:

1. 鯨骨ステッピング・ストーン仮説の検証

鯨骨が熱水噴出域などに出現する生物の“ステッピング・ストーン”として機能するかどうかを検証するために、2002年1月、鹿児島県野間岬沖に海洋投入されたマッコウクジラ遺骸周辺の潜航調査を実施した(2003~2005年). 鯨遺骸が海洋に投入されてからわずか1年半後には鯨骨特異的な数多くの底生生物が出現した. 優占種はイガイ科二枚貝のヒラノマクラ *Adipicola pacifica*, ホソヒラノマクラ *A. crypta*, キヌタレガイ科二枚貝のアブラキヌタレガイ *Solemya pervernicosa*, *Osedax* 属多毛類の新種(記載中), *Protodrilus* 属多毛類の1種などであった. これらの近縁種はいずれも熱水噴出域, 冷水湧出域より出現しているが同一種ではない. 先行研究でも鯨骨域に優占種として出現するのは鯨骨固有種であり, 熱水噴出域・冷水湧出域に出現する種はわずかである. また野間岬沖鯨骨域からは, 高次分類群レベルでこれまで化学合成生態系からの報告のないグループが多数出現した. 以上の事実を考え併せると鯨骨が熱水/冷水固有種のステッピング・ストーンとして機能しない可能性が高く, これら生物の分散には別の要因を考える必要がある.

2. 化学合成共生イガイ類の起源に関する研究

シンカイヒバリガイ類は深海産の化学合成共生二枚貝であり, 鰓上皮細胞内に硫黄細菌, メタン酸化細菌を宿す. シンカイヒバリガイ類の起源は浅海にあり, 沈木に付着して生活するタイプのイガイ類が沈木と共に深海底に沈み, それがシンカイヒバリガイ類に繋がったとする説明がなされているが詳細は不明である. そこで本研究では沈木付着イガイ類に近縁とされている鯨骨に付着する2種類のイガイ類(ヒラノマクラ, ホソヒラノマクラ)がシンカイヒバリガイ類とどのような関係にあるのかを, 共生様式および共生細菌の観点から検討した. ホソヒラノマクラの共生システムはシンカイヒバリガイ類と非常に類似しており, 鰓上皮細胞内にシンカイヒバリガイ類共生硫黄細菌と近縁な単一系統の硫黄細菌を宿していた. 他方, ヒラノマクラはこれまでの知見とは大きく異なり, 複数種の細菌を鰓上皮細胞の外側に宿していた. またこれらの細菌は鰓組織上で不完全な住み分けを行っていることを蛍光 *in situ* ハイブリダイゼーションにより確認した. 宿主イガイ類の系統解析からもヒラノマクラはシンカイヒバリガイ類の直近の外群に位置することから, ヒラノマクラの示す細胞外共生系はイガイ類の祖先的形質であると推定した.

Study on the Origin of Deep-sea Hydrothermal Vent Communities at the Lau Back-Arc Basin, Southwest Pacific

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Summary of Research Results

This is report on the morphological, ecological, biogeographic and molecular biological studies for vent communities collected at our expedition to the deep-sea hydrothermal vents at the Lau and Havre Back-arc Basins, Southwest Pacific from September 23 to November 10, 2004 using Japanese submersible “*Shinkai 6500*” and her mother ship “*Yokosuka*.” The main target of this research project is to clarify origin and evolution of the deep-sea hydrothermal vent and cold seep communities of the Southwest Pacific.

The Lau and Havre Back-arc Basins, Southwest Pacific hold communities of high biological diversity in comparison with other such vent and seep communities in the world based on our research of principal component in the deep-sea hydrothermal vent and seep communities such as bivalves, gastropods, crustaceans (including crabs, shrimps and barnacles), worms, tube-worms, etc. Particularly, barnacles in crustacean shown the highest diversity in the known deep-sea vent and seep of the world. We could found all four suborders, Scalpellomorpha, Brachylepadorpha, Verrucomorpha, and Balanomorpha from the Lau Basin. And the peripheral areas of the Lau Basin such as North Fiji and Manus Basins show the second high biological diversity hold three suborders except Brachylepadorpha.

The morphological examination of barnacles collected from the Lau and Havre basins shows that scalpellomorphan barnacle *Ashikailepas* has the most primitive morphological characteristics and also the widest distribution at the West Pacific.

The molecular biology of some principal vent animals collected from the Lau and Havre basins shown primitive phylogenetic position in comparison with the group of the East Pacific, West Pacific (Northwest to Southwest) and Indian Oceans. *Ashikailepas* in comparison with two Japanese and one Havre populations population shows the most primitive Scalpellomorph in the molecular phylogeny. Therefore, the origin of the known vent and seep communities seems to be Southwest Pacific.

Keywords: deep-sea hydrothermal vent; endemic species; taxonomy; phylogenetic evolution; dispersal; origin; primitiveness, Southwest Pacific

A cold seep barnacle (Cirripedia: Neolepadinae) from Japan and the age of the vent/seep fauna

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A new pedunculate barnacle, *Ashinkailepas seepiophila* gen. and sp. nov. has been recovered from a hydrothermal cold seep at 1175 metres of depth off Hatsushima Island, Sagami Bay, central Japan. It is the second record of a Neolepadinae, otherwise known from vents, from a seep, but the two are not closely related. Its coarsely ornamented capitular plates, and primary peduncular armament being in whorls of six plates, distinguish it from all previously known neolepadines, not only as a new genus and species, but also as a distinct clade, and therefore a new tribe is proposed to accommodate it. There is a slight bilateral asymmetry in the proportions of the capitular plates as well as a marked bilateral asymmetry in the arrangement of the peduncular scales related to bending over to one side. This apomorphic characteristic distinguishes the new species from all other Scalpelloomorpha. While leaning over to one side is suggestive of verrucosomorphs, it is not judged a sign of affinity. *Ashinkailepas* is apparently the most primitive of the vent/seep barnacles, which are considered relics largely of the Mesozoic age.

INTRODUCTION

The first barnacle, *Neolepas zeviniae* Newman, 1979, was described from hydrothermal vents at a depth of approximately 2100 m on the East Pacific Rise (EPR) (21°N). Since then three other neolepadine species have been described; *Neolepas rapanuii* Jones, 1993 from near Easter Island (23°S) on the EPR, *Vulcanolepas oshaei* (Buckeridge, 2000) from Brothers Caldera on the Kermadec Ridge, north of New Zealand, and *Leucolepas longa* Southward & Jones, 2003 from off Lihir Island, Papua New Guinea. Four other apparently distinct neolepadine species have also been reported, one from the south-east Indian Ridge (Southward et al., 1997), two from the Lau Basin (Southward & Newman, 1998) and one from the Rodriguez Triple Junction, Indian Ridge (Hashimoto et al., 2001). In addition to these, several other likely new neolepadine species have been collected from hydrothermal vents of the North Fiji Basin, Manus, and Okinawa Back-Arc Basin (Fujikura et al., 2001), Myojin Knoll on the Izu-Marina Ridge, and the south-east EPR (37°S) (Stecher et al., 2002; TY., personal communication). These neolepadines are variously allied, all are from vents except *L. longa* from a seep, and they represent a substantial diversity ranging across the entire Pacific and into the Indian Ocean.

The present report describes a new Neolepadinae from a volcanic cold seep. It is represented by three individuals collected at a depth of 1175 m off Japan (Fujikura et al., 2002), and it constitutes the second record of a neolepadine from a cold seep. The new form is distinguished from the previously described neolepadine from a seep, *L. longa*, as well as all other neolepadines, by numerous characters including coarsely sculptured and somewhat differently shaped capitular plates, whorls of peduncular

plates smaller in number, and by its distinct capitulo-peduncular ratio. There is also a bilateral asymmetry to the capitulum and peduncular plates or scales, something seen in no other scalpellomorph. This apomorphy, related to bending over to one side and presumably for defence and/or feeding, is not included in the generic diagnosis because the modifications associated with it are superimposed on the basic plan. It is the basic morphological differences between the new form and all previously reported vent neolepadines that set it aside not only as a distinct new genus and species, but also as representing a distinct clade. It will therefore be proposed that the Neolepadinae be split into two tribes, the Ashinkailepadini to accommodate the new form and the Neolepadini to accommodate the remainder of the Neolepadinae.

SYSTEMATICS

Order PEDUNCULATA Lamarck, 1818
Suborder SCALPELLOMORPHA Newman, 1987
Family EOLEPADIDAE Buckeridge, 1983

The diagnoses, given in the landmark paper of Southward & Jones (2003), for the Eolepadidae and its subfamilies, the extinct Eolepadinae Buckeridge, 1983 and the extant Neolepadinae Newman, 1996, were based on the capitular plates, six for the former and eight for the latter. These are the unpaired rostrum and carina and the paired scuta and terga (R-S-T-C) in the Eolepadinae, with the addition of a pair of latera (R-S-L-T-C) in the Neolepadinae. It should also be noted that where peduncular plates are known, the basic number per whorl is eight for the former and six for the latter, the reverse of that for the capitulum. The plates include the unpaired subrostrum and subcarina and paired rostrolatus, latus

and carinolatus (sr-rl-l-cl-sc) in the former, but with the pair of latera absent (sr-rl-cl-sc) in the latter (Newman, 1997). Thus the formulae for the two subfamilies are R-S-T-C/sr-rl-l-cl-sc and R-S-L-T-C/sr-rl-cl-sc respectively. It is variations of the peduncular plan of *Archaeolepas* as well as capitular plans that led to the neolepadines as well as higher Scalpellomorpha (Newman & Ross, 1998; Young, 2001). Of the two tribes proposed below, the Neolepadini and Ashinkailepadini, the latter typifies the basic Neolepadinae plan.

Neolepadini tribe nov.

Diagnosis

Neolepadinae having relatively smooth, unornamented capitular plates, an adult peduncular to capitular length ratio of 3:1 or greater, and an adult peduncle with whorls of 12 or more scales which are about as wide as high. *Neolepas* Newman, 1979, *Vulcanolepas* (Buckeridge, 2000) and *Leucolepas* Southward & Jones, 2003.

Ashinkailepadini tribe nov.

Diagnosis

Neolepadinae having capitular plates ornamented by longitudinal ribs or ridges, a peduncular to capitular ratio of 1:1 or less, and a peduncle (if unaltered by bending to one side) with whorls of six scales which are considerably wider than high.

Ashinkailepas gen. nov.

Diagnosis

As for the tribe.

The new species is not only distinguished from Neolepadini by the major diagnostic characteristics given for the tribe but also by less important differences noted in the description. These include a slight but discernible bilateral asymmetry of its capitular plates and a marked asymmetry of the peduncular scales in number as well as proportions, related to individuals bending over sideways towards the substratum. However, early in ontogeny the peduncle of *Ashinkailepas seepiophila* undoubtedly consisted of 6-plated whorls (sr-rl-cl-sc), as in the juvenile peduncle

of *Neolepas* (Newman, 1997). Since the asymmetry is an apomorphy related to predation and/or feeding, it has not been included in the generic diagnosis. This allows symmetrical fossil and extant forms to be included in the genus, should they be discovered. Type, *Ashinkailepas seepiophila* sp. nov.

Ashinkailepas seepiophila sp. nov.

Diagnosis

Monotypic and hence as for the tribe.

Materials examined

Three adult specimens from a hydrothermal cold seep on a seamount at a depth of 1159 m off Hatsushima Island (35°00.11'N 139°13.45'E), near Sagami Bay; Dive no. 585, 19 November, 1991, Japan Marine Science and Technology Center (JAMSTEC) submersible 'Shinkai-2000' (J. Hashimoto, observer).

Deposition of types

Holotype, National Science Museum, Tokyo (NSMT) Cr 15638; first paratype, National Museum of Natural History, Washington, DC (USNM) USNM 1018131; second paratype, Scripps Institution of Oceanography, Benthic Invertebrate Collection (SIO/BIC) C-10608.

Etymology

We would like to name the new genus in honour of the Japanese submersible 'Shinkai 2000'. Barnacle names frequently include *lepas*, Greek for shellfish, but the name *Shinkailepas* Okutani et al., 1989, has already been proposed for a gastropod mollusc. Therefore we propose *Ashinkailepas* for the new form. Since it is a seep barnacle, 'seep' is combined with the Greek *philos* meaning loving or liking and thus *Ashinkailepas seepiophila*.

Habitat

There was no evidence of hot hydrothermal activity in the vicinity of the type locality. To the contrary, the temperature at the seep of 2.84°C was essentially ambient (Fujikura et al., 2002). The sediment surface was white from growths of bacteria. The barnacles were collected from a boulder of breccia measuring 1.5 × 2 m

Table 1. Gross measurements of *Ashinkailepas seepiophila* gen. and sp. nov.

Type designation		TL	CL	PL	Wmax	Wbase	No. of whorls in peduncle
Second paratype	Inside	29.1	16.6	12.5	22.4	11.5	6+
	Outside	29.7	17.8	11.9	21.9	11.2	
Holotype	Inside	28.1	17.5	10.6	21.1	12.8	6+
	Outside	28.4	19.2	9.2	21.1	13.1	
First paratype	Inside	34.1	19.7	14.4	24.2	14.7	8+
	Outside	35.8	21.1	14.7	24.4	14.2	
	Mean inside	30.4	17.9	12.5	22.6	13.0	6.7+
	Mean outside	31.3	19.4	11.9	22.5	12.8	
	R inside	29.1–34.1	16.6–19.7	10.6–14.4	21.1–24.2	11.5–14.7	6–8+
	R outside	28.4–35.8	17.8–21.1	9.2–14.7	21.1–24.4	11.2–14.2	

TL, height of capitulum and peduncle; CL, capitular height; PL, peduncular height; Wmax, maximum rostrum–carina capitular width; Wbase, width at capitulo–peduncular junction. inside, left side; outside, right side, in all three specimens; R, range, measurements in mm.

Table 2. Measurements of the capitular plates of *Ashinkailepas seepiophila* gen. and sp. nov.

	R			S			L			T			C		
	W	L	W/L	W	L	W/L	W	L	W/L	W	L	W/L	W	L	W/L
Second paratype				8.2	14.6	0.56	4.7	4.7	1	10.3	18.4	0.56			
Inside	5.2	10.4	0.5										5.6	14.9	0.38
Outside				7.8	16.2	0.48	4.3	6	0.72	10.6	22.4	0.47			
Holotype				8.2	15	0.55	4.7	3.8	1.24	9.7	18.7	0.52			
Inside	5.9	7.7	0.77										5.6	11.3	0.5
Outside				8.5	16.5	0.52	5.2	5.3	0.98	9.9	20.3	0.49			
First paratype				9.6	16.3	0.59	4	4.9	0.82	11.5	20.6	0.56			
Inside	7.9	9.1	0.87										7.1	14	0.51
Outside				9.9	20.3	0.49	4.6	7.1	0.65	11.3	22.7	0.5			
Mean inside				8.7	15.3	0.57	4.5	4.5	1.02	10.5	19.2	0.55			
Mean outside	6.3	9.1	0.71	8.7	17.7	0.49	4.7	6.1	0.78	10.6	21.8	0.49	6.1	13.4	0.46
Range inside	5.2–	7.7–	0.5–	8.2–	14.6–	0.55–	4.0–	3.8–	0.82–	9.7–	18.4–	0.52–	5.6–	11.3–	0.38–
Range outside	7.9	10.4	0.87	9.6	16.3	0.59	4.7	4.9	1.24	10.3	20.6	0.56	7.1	14.9	0.51
				7.8–	16.2–	0.48–	4.3–	5.3–	0.65–	9.9–	20.3–	0.47–			
				9.9	20.3	0.52	5.2	7.1	0.98	11.3	22.7	0.50			

outside, right side; inside, left side; R, rostrum; S, scutum; L, median latus; T, tergum; C, carina; W, width; L, height, measurements in mm.

Table 3. Differences in the capitulum and peduncle of *Ashinkailepas seepiophila* gen. and sp. nov. and *Neolepas* in general. Notations as in Tables 1 and 2.

		<i>Neolepas</i> spp.	<i>Ashinkailepas seepiophila</i>
Capitulum	Wmax / CL Wmax CL/PL	< 1 (approximately 0.9) narrow < 1 (> 1 in juveniles of <i>N. zeviniae</i> , figure 3, Newman, 1979)	> 1 (approximately 1.21) wide > 1.5
Rostrum	lateral view W/L growth lines longitudinal ridges	curved internally relatively small (<0.6) distinct indistinct or none	straight relatively large (0.7) more or less distinct distinct
Carina	lateral view W/L growth lines longitudinal ridges	curved internally relatively small (<0.3) distinct indistinct or none	straight relatively large (=0.5) more or less distinct distinct
Peduncle	Form Scales	straight and long bilaterally symmetrical and number small and in more than three rows	curved and short bilaterally asymmetrical inside, and number laterals small and in more than three rows in inside, but large and two rows outside

and lying on a gentle slope at the base of a steep slope where other similar large boulders were accumulating. A seep clam, *Calyptogena* sp., and the shrimps, *Alvinocaris* sp. and *Lebbeus* sp., were found on the bottom where the barnacles were collected, and specimens of an ordinary deep-sea crab, *Paralomis multispina*, were found in crevices between the boulders.

Comparative description

In general, *Ashinkailepas seepiophila* resembles all previously reported neolepadines in having eight capitular plates (R-S-L-T-C). The new form can readily be distinguished from them by its coarsely sculptured capitular plates and the notable difference between the peduncular scales of each side due to the bending of the animal to one

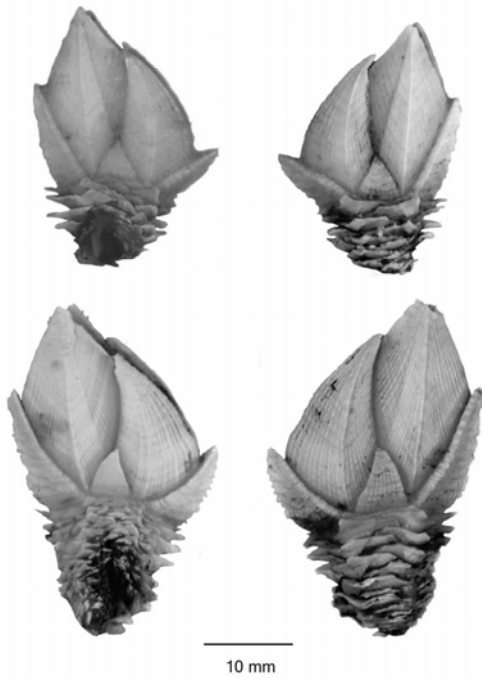


Figure 1. *Ashinkailepas seepiophila* gen. and sp. nov.: upper, holotype; lower, first paratype; left and right sides respectively.

side. On the outside of the bend the peduncular scales are arranged normally; e.g. subrostra, rostrilatera, carinolatera and subcarinal plates (sr-rl-cl-sc), as in juveniles of *Neolepas* (Newman, 1997) and extinct *Archaeolepas* except for the presence of a median lateral (l) tier in the latter. Furthermore, judging from the ontogeny of *Neolepas* as well as of scalpellomorphs in general, it can be inferred that the early juveniles of *Ashinkailepas seepiophila* have a symmetrical peduncle with whorls of six plates or scales.

The three specimens of *A. seepiophila* ranged from 27 to 36 mm in total height (Tables 1 & 2). The height of the capitulum is greater than the length of the peduncle, as it is in juveniles of *N. zeviniae* (Newman, 1979; figure 3), whereas it is less in adult Neolepadini (Table 3). The width of capitulum is approximately 1.5 times wider than the width of peduncle.

All capitular plates are ornamented by fine longitudinal ridges overshadowing the growth lines. The scutum and tergum each have a strong median apico-basal ridge, and the upper margin of the rostrum and carina have a welting at least on the right or outside (Figures 1 & 2). On the other hand, Neolepadini have no ornamentation other than the growth lines, and there is no distinct ridge associated with the change in their direction along the apico-basal axis of the scutum and tergum. The plates of the left or inside of *A. seepiophila* are slightly smaller than

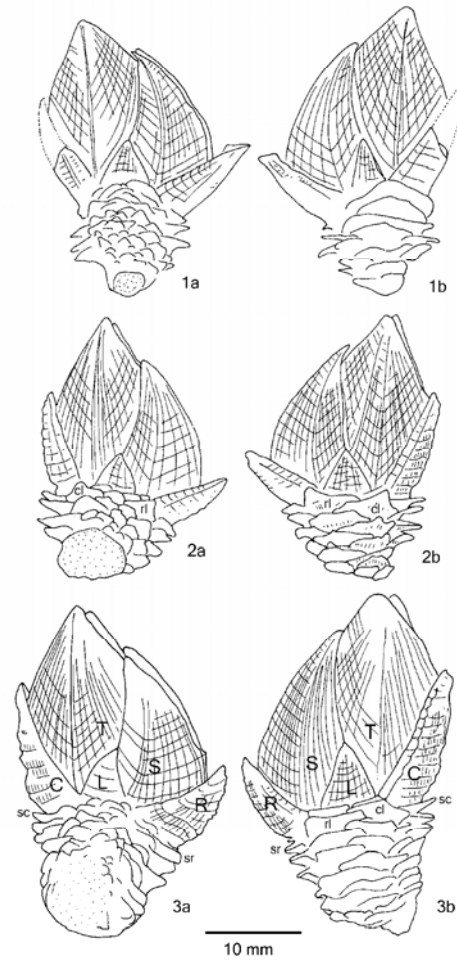
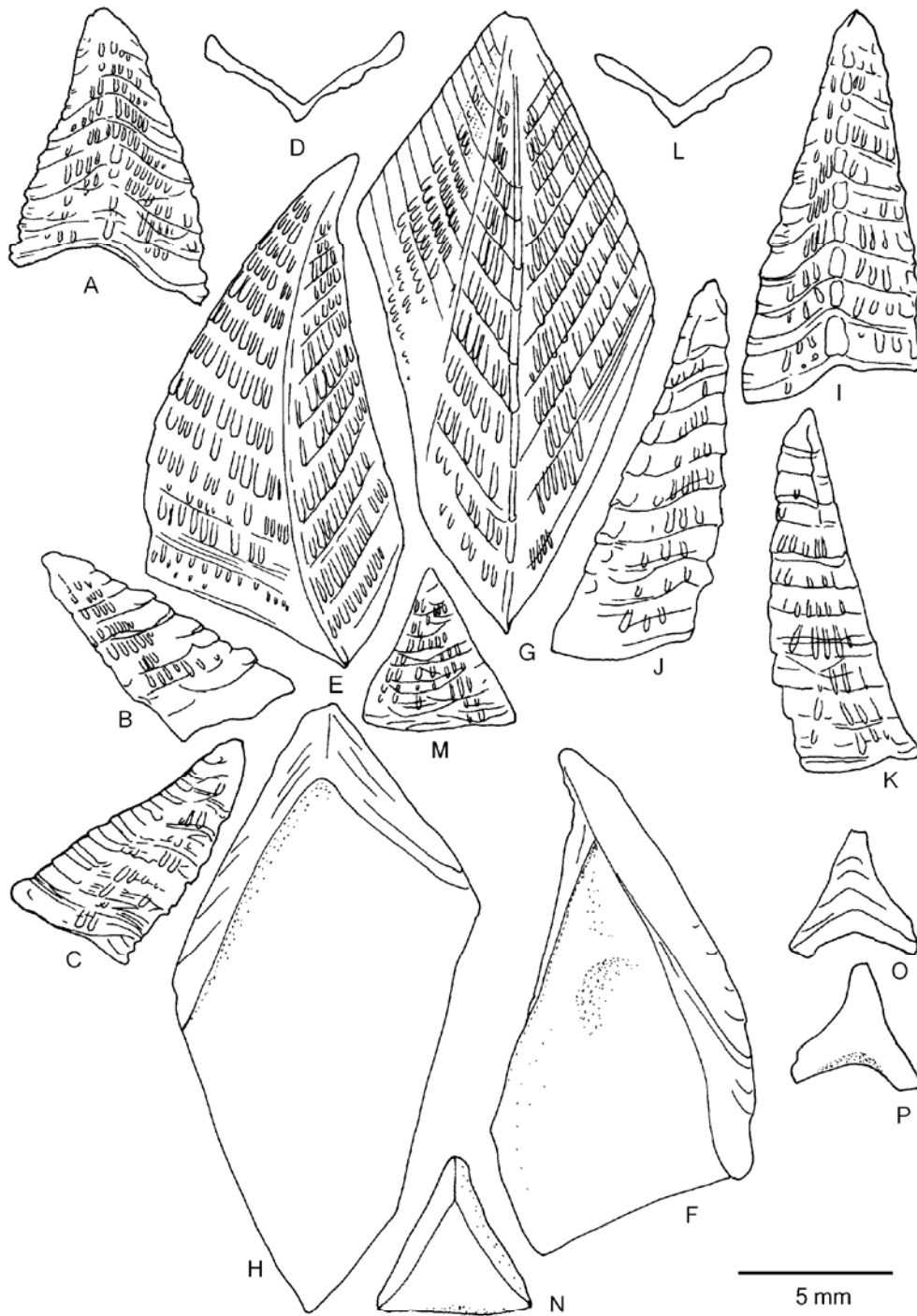


Figure 2. *Ashinkailepas seepiophila* gen. and sp. nov.: 1a–b, second paratype; 2a–b, holotype; 3a–b, first paratype; left (inside) and right (outside) respectively.

those of the outside. However, the difference is by no means as pronounced as that between the plates of the right and left sides of peduncle (Tables 1 & 2).

A comparison of sizes and ratios of heights and widths of some capitular plates of *A. seepiophila* and the Neolepadini

Figure 3. (*Opposite*) *Ashinkailepas seepiophila* gen. and sp. nov.: holotype; disarticulated capitular valves and a peduncular scale of right side. (A–D) Frontal, right lateral, left lateral, and cross-sectional views of rostrum; (E&F) external and internal views of the right scutum; (G&H), external and internal views of the right tergum; (I–L), frontal, left lateral, right lateral, and cross-sectional views of carina; (M&N) external and internal views of right median latus; and (O&P), external and internal views of subcarina.



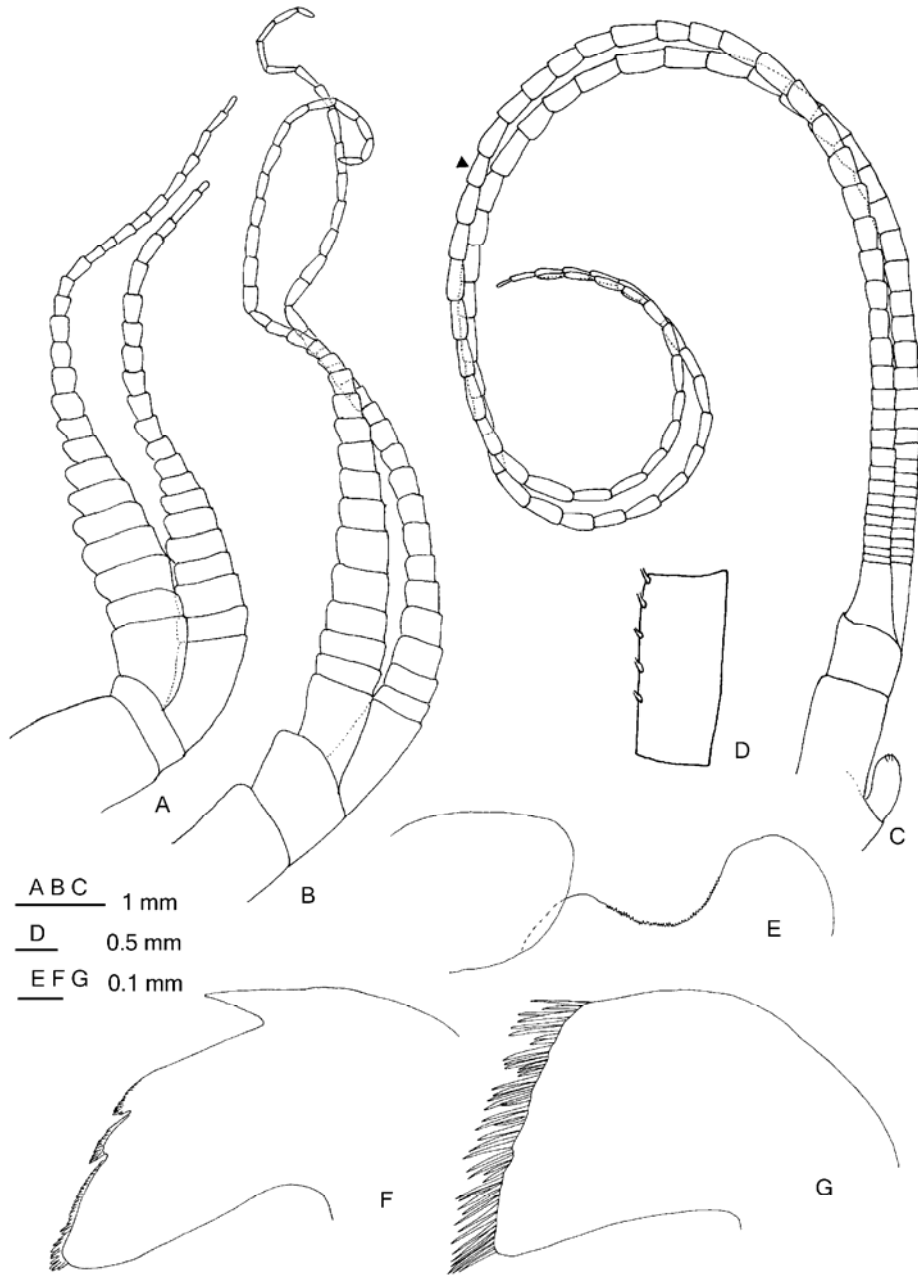


Figure 4. *Ashinkailepas seepiophila* gen. and sp. nov. Holotype: (A) right cirrus I; (B) right cirrus II; (C) right cirrus VI and caudal appendage; (D) 35th article from outer ramus and bases of setae of right cirrus VI; (E) labrum and right palp (setae omitted); (F) right mandible; (G) right second maxilla.

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Table 4. Number of articles of cirri I–VI in *Ashinkailepas seepiophila* gen. and sp. nov.

Specimen		I	II	III	IV	V	VI	c.a.
Second paratype (right)	a	24	26	44	53	55	62	?
	p	24	34	44	54	57	55	
Holotype (right)	a	26	30	48	43+	59	60	1
	p	20	33	45	51	56	56	
	a	29	33+	42	37+	55	60	1
	p	27	29	51	56	60	63	
First paratype (right)	a	31	37	48+	54	54+	55+	1
	p	23+	26+	54	51+	62	55	
	a	31	29	28+	36+	50+	59	1
	p	23+	26	51	58	62	59	

a, anterior ramus, p, posterior ramus, +, articles broken off; c.a., caudal appendage.

are given in Table 3. It will be observed, for example, the R–C width of the capitulum (Wmax), between the rostrum (R) and carina (C) in *A. seepiophila*, is greater than the height between the apex of tergum and the capitulo-peduncular junction (CL) ($W_{max}/CL > 1$). Furthermore, the ratio of the width (W) to the length (L) of the rostrum in *A. seepiophila* is somewhat greater, while that of the carina is considerably greater (Table 3), and these differences further distinguish the new species from the known Neolepadini.

The inner margins of the rostrum and carina of *A. seepiophila* are nearly straight, meet basally with the median ridge of the scutum and tergum respectively (Figures 1 & 2), and their v-shaped cross sections form an angle of approximately 120° (Figure 3D & L). In Neolepadini their inner margins are concave, there is no pronounced median ridge on the scutum or tergum to meet with basally, and at least in *Neolepas zeviniae* the v-shaped cross section of the carina, being approximately 90° (Newman, 1979; figure 4C), is substantially less than in *Ashinkailepas*.

The peduncular scales on the right or outside of *A. seepiophila*, between the subrostral and subcarinal tiers, are large, approximately twice as wide as high, and arranged in two tiers (rl and cl). However, those on the left or inside, between the subrostral and subcarinal tiers, are relatively small, approximately equal in height and width and deployed in more than two tiers; (~three in the holotype and second paratype, and six the first paratype). In mature *N. zeviniae*, *N. rapanuii*, *Vulcanolepas osheai* and *Leucolepas longa*, the number of peduncular scales in each whorl is greater; 12, 30, and 24 respectively (Southward & Jones, 2003). The width of the peduncular scales is approximately the same as their length in *N. zeviniae* and *N. rapanuii*, while it is approximately half their length in *V. osheai*. Scales on the inside of the bend in *A. seepiophila*, between the subrostra and subcarinae, more closely resemble the peduncular scales in *Neolepas zeviniae* and *N. rapanuii* than they do the finer and more numerous scales seen in the apparently more advanced species of *Neolepas* spp., *V. osheai*, *L. longa* and Lau A. While the three specimens of *A. seepiophila* all bend to the left, it is likely that bending to one side or the other may not be fixed, since it is not fixed in the verruciform *Neoverruca* (Newman & Hessler, 1989).

The trophi (labrum, palps, mandibles, and first and second maxillae), cirri and caudal appendages of the new species (Figure 4) are much like those of vent barnacles in general. The mandible has a large superior incisiform tooth followed by two lower teeth with their upper edges supporting a comb of strong short teeth, and an inferior angle supporting a comb of similar short teeth above a shorter comb of somewhat larger terminal teeth. This is relatively generalized compared to the situation in Lau A where the combs have been increased at the expense of the major teeth (Southward & Newman, 1998). The cirri are long, with the distal portions of the rami of first and second pair antenniform while the third to sixth pairs are ctenopod throughout. Cirral counts are given in Table 4, and setal lengths (s) to article widths (a) were 1.35/0.19, 1.44/0.22, and 1.5/0.25, or 7.1, 6.5 and 6.0 (holotype, first and second paratypes, respectively).

Southward & Newman (1998) reported on the extraordinary long setae associated with the farming of bacteria in an undescribed neolepadine, 'Lau sp. A', from the Lau Basin, Tonga. In order to compare different species in this regard, the ratio of the longest seta (s) to the width of article (a) supporting it, from the intermediate region of the sixth cirrus, was chosen as an index. For Lau A, which was s/a 14.1, Southward & Jones (2003, table 9) provided an up-to-date list of ratios for 11 vent taxa and their s/a range is 2.8–14, the mean of which is s/a 6.06. As noted above, *Ashinkailepas seepiophila* has a s/a range of 6.0–7.1 and thus ranks right in among these barnacles, which includes the seep barnacle, *Leucolepas longa* having a mean s/a ratio of 5.2.

Ashinkailepas seepiophila has a well-developed penis and is therefore, like vent barnacles in general, a cross-fertilizing hermaphrodite. The second largest specimen (second paratype) contained approximately 120 ovoid eggs measuring ~300 by 500 µm, the same magnitude in number and size as in *Neolepas zeviniae* (Newman, 1979).

DISCUSSION

Evolutionary significance of the new form

The secondary bilateral asymmetry of *Ashinkailepas seepiophila*, unique among the Scalpellomorpha, is superimposed on the peduncular as well as to some extent the

capitular armament. This apomorphy is the result of individuals bending over to one side, perhaps bringing their cirral nets closer to the substratum to collect food and/or to lower their profile as a defence against predation, as suggested for verrucosomorphs by Anderson (1980) and Newman & Yamaguchi (1995) respectively. Bending over is not limited to scalpellomorphs (cf. *Poecilasma inaequilaterale* Pilsbry, 1907:85 living on the backs of crabs for example). Predatory pressures leading to development of such defences were increasing in the Mesozoic, largely in the form of shell-crushing fish and crabs (Vermeij, 1977) and such pressures may well explain the appearance of the asymmetrical sessile barnacles, the Verrucosomorpha, in the Late Cretaceous (Newman & Yamaguchi, 1995).

It is noteworthy that the new seep form, *Ashinkailepas*, with its relatively formidable armour and sculpturing, is more reminiscent of the heavily armoured scalpellomorphs of the Mesozoic than of the Neolepadini. One might expect a form from seeps, such as *Ashinkailepas*, to more closely resemble the ancestral stock of the Neolepadinae than would members of the Neolepadini, just as *Lamellibrachia* from seeps has proven to be genetically closer to the stem vestimentiferans than say to *Riftia* (Williams et al., 1993; Hurtado, 2002). The arrangement of peduncular scales in the ontogeny of *Neolepas* (and presumably all Neolepadini) is the same as the basic adult ashinkailepadine plan. While it is possible the peduncular armament in *Ashinkailepas* is a reversion to the more primitive type (atavism), an explanation for primitiveness in vent forms offered by Hickman (1983), it is judged unlikely.

It was once hypothesized that bending over in a scalpellomorph gave rise to the verrucosomorphs (Newman et al., 1969, figure 113), but that was before our improved understanding of the brachylepadomorphs and the discovery of *Neoverruca*. It is nonetheless remotely possible a scalpellomorph rather than a brachylepadomorph gave rise to the verrucosomorphs, and if so would the scalpellomorph have been a neolepadine? Not according to the early ontogeny of *Neolepas* (Newman, 1997), and now apparently in *Ashinkailepas*, since the (I) tier of peduncular plates is missing in both. However, the peduncle of the geologically older colepadine, *Archaeolepas* (Upper Jurassic–Lower Cretaceous) includes this tier whereby the whorls consist of eight plates, sr-rl-l-cl-sc (Newman & Ross, 1998; Young, 2001), and all eight are seen in brachylepadomorphs and on the normal (movable) side of neoverrucids (Newman & Yamaguchi, 1995; Newman, 1989, respectively). It is for this reason we now look to some intermediate (R-RL-L-CL-C/sr-rl-l-cl-sc) between *Archaeolepas* (R-RL-CL-C/sr-rl-l-cl-sc) and *Ashinkailepas* (R-RL-L-CL-C/sr-rl-cl-sc) as the ancestor of the verrucosomorphs. Such an ancestral stock would suffice for the verrucosomorphs as well, in the unlikely event it turns out they did not evolve from or share a common ancestor with brachylepadomorphs.

Age of the vent fauna

Hydrothermal activity has apparently existed since the earth cooled sufficiently for the oceans to form, but it has been observed that mid-ocean ridges and basins are ephemeral to varying degrees, and locations shift unpredictably in space and time (Corliss et al., 1979;

Hessler & Lonsdale, 1991; Tamaki & Honza, 1991; Hurtado, 2002). New forms may compensate for extinctions by immigration into hydrothermal habitats, and then most likely from cognate habitats such as seeps (Newman, 1985). Such habitats are generally refugial; e.g. once adapted to them, taxa can persist for extended periods of time and then sometimes long enough for their non-vent/seep ancestors to go extinct.

Faunas of refugial habitats are expected to include taxa of different ages. When it comes to vents and cognate habitats, it was long ago suggested that despite various extinction events some inhabitants may be Mesozoic or even Paleozoic in age (McLean, 1985; Newman, 1985). In addition extinctions due to shifting of vents and/or the Permo-Triassic and Cretaceous–Tertiary calamities, there have been periods of anoxia in the deep sea as recently as 40 million years ago (MYA) (Benson, 1990), or more likely no more recently than 90 MYA (Horne, 1999). Jacobs & Lindberg (1998) suggest '... the possibility that modern deep-sea faunas, both at vents and in the oxygenated realm, did not evolve *in situ* for long periods of time, but colonized the deep-sea habitat after the last major disruptions of these habitats by anoxic events...', the last being ~55 MYA or Paleocene. If so, deep-sea faunas would be no older than Eocene in establishment. In light of all this, what presently can be said regarding the antiquity of the vent/seep fauna?

One might expect the best estimates of age for any group inhabiting vents and seeps would be available for molluscs, since among the higher invertebrates they have one of the best fossil records. But curiously this is not the case for vent gastropods (McLean, 1985; Warén & Bouchet, 2001). McLean (1985) argued that since many of the family-group taxa among the vent gastropods he was dealing with could not be derived from Cenozoic forms, they must be at least Mesozoic or perhaps even Paleozoic in age. On the other hand, Newman's (1985) argument for possible antiquity of the vent fauna, including molluscs, was based on the earliest occurrences of the taxa involved. While Warén & Bouchet (2001) take these authors to task for their suggestions of antiquity, they provide a table of 24 vent gastropods, including two genera, many familial- and a couple of ordinal-group taxa. Age estimates for 22 of the 24 include two possibly Late Paleozoic (Permian) and 11 Mesozoic (several from each of the Triassic, Jurassic and Cretaceous), while six are estimated to be no older than Oligocene and for two no estimate is given. This appears to be largely a Mesozoic-based gastropod fauna.

There is evidence from fossil vent and/or seep deposits of both monoplacophoran molluscs and vestimentiferan worms, ranging in age from the Silurian to the Recent (Little, 2002). The first, the most primitive living molluscs, have no seep representatives, fossil or extant, and they are rarely encountered in the deep sea much less in shallow water. The Paleozoic ones apparently gave way to those of the Mesozoic which then range to the Recent, but that they have continuously inhabited deep-sea vents from the Mesozoic to the Recent is doubted since conceivably they could have reinvaded vents from shallow water. The vestimentiferans are known from seeps as well as vents since the Cretaceous, but it can be argued they too re-entered this deep-sea habitat from shallow water since

then. And so on with the gastropods; trochids have lived at vents since the Jurassic and at seeps since the Tertiary, and cerithioids have occurred at the vents since the Cretaceous. So it is argued that while fossil evidence from vents and seeps favours at least Mesozoic ages for a number of taxa, that they have been there continuously can be doubted (Little, 2002; Little & Vrijenhoek, 2003).

What other lines of evidence are available? While some genetic studies indicate some taxa of vent/seep fauna are young (cf. Shank et al., 1999), other taxa are old. For example, while McArthur & Koop's (1999) study of vent gastropods found that partial 28S rRNA sequences lacked the resolution necessary to resolve Paleozoic radiations, Mesozoic radiations were resolved. Furthermore, the vesicomid clams, previously considered to stem back <50 MYA (Peek et al., 1997), have recently been pushed back to between 100–50 MYA (Goffredi et al., 2003), a period largely falling (70%) within the Cretaceous. Likewise, the vent divergences of the vestimentiferan worms, previously pegged at <100 MYA by Halanych et al. (2001) but recently pushed back to 140–40 MYA (Hurtado, 2002), is again a period largely falling (77%) within the Cretaceous. So unless these revised estimates were somehow biased towards the older end of the spectrum, the odds do not favour an extensive deep-sea anoxia extinction event in the Paleocene. To the contrary, the forgoing molecular phylogenetic as well as some of the fossil evidence supports the notion that some seep/vent forms are at least Mesozoic in age. But what about forms having a reasonable fossil record outside of vent/seep situations and have yet to be looked at genetically?

No fossil evidence for barnacles has been recognized at vents or seeps (Little, 2002), but there are fossil records for closely allied taxa elsewhere (Newman et al., 1969; Buckeridge & Grant-Mackie, 1985; Newman, 1996). Four suborders are represented at vents, and their antiquity in hierarchical order, general habitat preference, next of kin, and relationships to their respective suborders are as follows: (1) the neolepadines, seeps and vents, Indo-Pacific, closest relative is *Archaeolepas* (Upper Jurassic–Upper Cretaceous), most primitive morphology of the living Scalpelloomorpha (Lower Jurassic); (2) a neobrachylepadid, *Neobrachylepas*, vents, Lau Basin, Tonga, only living representative of the Brachylepadomorpha, more similar to *Brachylepas* (Upper Cretaceous) than to more generalized *Pycnolepas* (Upper Jurassic–Miocene); (3) the neoverrucids, vents, West Pacific, most primitive of the living Verrucomorpha, more generalized than eoverrucids (Upper Cretaceous); and (4) *Eochionelasmus*, vents, Indo-West Pacific, most generalized of the living Balanomorph (Eocene).

From the foregoing it is evident that vent/seep barnacles represent either the most primitive or only living members of their respective suborders. All are relics, and all except the eochionelasmatis stem from the Mesozoic. This does not preclude their having entered vents from shallow water habitats where they subsequently became extinct. But there is presently no evidence suggesting such a sequence of events. Even if there were, the fact remains that vents and seeps afford a refuge for a diversity of barnacles having no comparable counterparts in other habitats that we know of. The deep-sea vents and seeps of the south-west Pacific provide a glimpse of barnacle antiquity seen

nowhere else on earth (Newman, 2000), and it would be curious indeed if there were no other such relic taxa found there. Yet, as noted above, some palaeontologists and more recently molecular geneticists have been pushing the notion that, in general, vents and seeps have acquired their faunas relatively recently. This may be, at least in part, but from the barnacles we know it can only hold in large part when the vent field itself is intermittent and/or relatively isolated.

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Phylogenetic relationships of deep-sea mussels of the genus *Bathymodiolus* (Bivalvia: Mytilidae)

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Abstract We examined phylogenetic relationships among three *Bathymodiolus* species in Japanese waters and *Bathymodiolus* spp. from the Manus Basin by two different approaches. Two-dimensional gel electrophoresis allowed us to compare 263 407 (average = 318) proteins, giving comprehensive information on genetic distances among the species. The neighbor-joining tree presented two clusters: (1) *B. japonicus* and *B. platifrons* and (2) *B. septemdirum* and *B. sp.* Members of the first cluster contain methanotrophic endosymbiotic bacteria and members of the second cluster contain thioautotrophic endosymbionts. DNA sequencing of a fragment (415 bp) of mitochondrial cytochrome *c* oxidase subunit I (COI) provided a neighbor-joining tree with the same topology as that derived from protein analysis. Inspection of intraspecific variation in COI in *B. japonicus* and *B. platifrons* revealed no genetic differentiation between mussel populations of either species from cold-water seeps versus hydrothermal vents, suggesting high adaptability of these *Bathymodiolus* species to deep-sea chemosynthetic environments. Our results indicated genetic exchanges between mussels from distant localities, suggesting that a limited dispersal capability of the larvae is not the likely factor leading to speciation events in these *Bathymodiolus* species.

Introduction

Unique biological communities are formed in deep-sea hydrothermal vents and cold-water seeps throughout the world, and mussels of the genus *Bathymodiolus* (Kenk and Wilson 1985) are one of the dominant macroorganisms in these communities. Living in a reductive environment, these mussels depend primarily on energy supplied by the chemosynthesis of bacterial endosymbionts. As other dominant vent and seep macroorganisms such as *Calyptogena* spp. clams and vestimentiferan tubeworms also depend on the energy supplied by symbiotic bacteria, these animal assemblages are called chemosynthesis-based communities. Deep-sea hydrothermal vents on spreading ridges and back-arc basins and cold-water seeps along plate-tectonic subduction zones are separated from one another by various distances. Therefore, it is tempting to study processes like colonization, dispersal, and speciation of these animals. Studies of their adaptation to the reductive environment and establishment of communities are also fascinating. Several hypotheses about the evolution of deep-sea mussels have been proposed. Craddock et al. (1995a) hypothesized, based on multi-locus allozyme data and morphological traits of the Pacific and Atlantic mussels, that the vent mussels arose from seep ancestors and settled by historical progression from shallow-water to deep-water habitats. Distel et al. (2000) suggested, based on phylogenetic analysis of nucleotide sequences of the nuclear 18S rRNA gene, that *Bathymodiolus* spp. mussels made the transition to vent and seep habitats from ancestors that exploited sunken wood and whale bones. Nevertheless, the evolutionary processes of deep-sea animals (their origin, colonization, speciation, and adaptation) are still mysterious and remain to be studied.

Four species of *Bathymodiolus* mussels, *B. japonicus*, *B. platifrons*, *B. septemdirum*, and *B. aduloides* occur in the western Pacific Ocean around Japan (Hashimoto

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and Okutani 1994). The habitats of the *Bathymodiolus* species around Japan are unique (Kojima 2002), because *B. japonicus*, *B. platifrons*, and *B. aduloides* are found in both hydrothermal vents (Okinawa Trough) and cold-water seeps (Sagami Bay), although *B. septemdiarium* is reported only from hydrothermal vents (Izu-Ogasawara Island-arc). In other regions of the world, most chemosynthesis-based species occupy only one type of habitat, either vents or seeps. *B. japonicus* and *B. platifrons* depend on methanotrophic endosymbionts, and *B. aduloides* and *B. septemdiarium*, on thioautotrophic endosymbionts for energy (Fujiwara et al. 2000; and unpublished preliminary data of Fujiwara). Therefore, phylogenetic and ecological studies on deep-sea mussels from Japanese waters could make substantial contributions to our understanding of the deep-sea animals in chemosynthesis-based communities.

Six *Bathymodiolus* species, including the four species mentioned above, from the western Pacific Ocean and six species from the Atlantic Ocean and one species each from the eastern Pacific and Indian Oceans have been described so far (Kenk and Wilson 1985; Cosel et al. 1994, 1999; Cosel and Olu 1998; Gustafson et al. 1998; Hashimoto 2001). Endosymbionts of *Bathymodiolus* mussels have been investigated by 16S rRNA sequencing and electron-microscopy; the *Bathymodiolus* species examined so far contain either methanotrophic or thioautotrophic endosymbiotic bacteria. Only one species from the Atlantic Ocean, *B. puteoserpentis*, has both kinds of bacteria (Distel et al. 1995). Phylogenetic congruence has been shown between host bivalves and endosymbionts (Distel et al. 1994).

Interspecific and intraspecific phylogenetic relationships of deep-sea animals have been investigated in vestimentiferan tubeworms (Black et al. 1997; Kojima et al. 2001a), in *Calyptogena* clams (Kojima et al. 1995; Peek et al. 1997, 2000), in provannid gastropods (Kojima et al. 2000, 2001b), in bresiliid shrimps (Shank et al. 1999), in alvinellid polychaetes (Jollivet et al. 1995), and in amphipods (France and Kocher 1996). However, the phylogeny of *Bathymodiolus* is poorly understood. In the present study, we used two different molecular methods to analyze phylogenetic relationships among *Bathymodiolus* species and populations in the Pacific Ocean around Japan, and we inferred processes that may have affected speciation of these mussels. First, protein constituents of the foot muscle were compared by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). This method is useful for comparing comprehensive genetic differences among organisms for systematic study (Miyazaki et al. 1987, 1988, 1998; Tsubokawa and Miyazaki 1993; Tokita et al. 2002). Second, we determined partial nucleotide sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene to analyze precisely phylogenetic relationships within and among *Bathymodiolus* species.

Materials and methods

Materials

Specimens used in this study are listed in Table 1 and collection sites are mapped in Fig. 1. All the deep-sea mussels of the genus *Bathymodiolus* (Mytilidae: Bathymodiolinae) were collected during the dives of the manned submersible "Shinkai 2000" of the Japan Marine Science and Technology Center (JAMSTEC). The other *Bathymodiolus* species reported from Japanese waters, *B. aduloides*, was not examined in this study because no sample was available for 2D-PAGE and DNA sequencing. *Bathymodiolus* sp. from the PACMANUS site of Manus Basin differs in conchological traits from *B. brevior* and will be described elsewhere. *Septifer virgatus* (Mytilidae: Mytilinae) was collected at the intertidal zone in Nakaminato, Ibaraki, and used as an outgroup for protein analysis. Mussels were frozen at -40°C or -80°C and preserved at -20°C or -80°C .

2D-PAGE of foot muscle proteins

2D-PAGE was carried out according to the procedure of Hirabayashi et al. (1981) and Oh-ishi and Hirabayashi (1988). In brief, the frozen foot muscle from each mussel was homogenized thoroughly in 20 vol of an extraction medium containing 8 M guanidine-HCl. The homogenate was dialyzed against 5 M urea and 1 M thiourea and centrifuged at 60,000 g for 30 min at 4°C . The supernatant (40 or 50 μl) was subjected to first dimension isoelectric focusing with agarose gels for 13,500 V h. Second dimension SDS-PAGE was performed using a running gel of 12% acrylamide and a stacking gel of 3% acrylamide. Proteins were stained with silver and Coomassie brilliant blue R (CBBR).

Comparison of 2D-PAGE patterns was performed by the triplet method, in which three patterns of two different samples (40 μl , each from one individual) and the mixed sample (25 + 25 μl , pooled together from two different samples) were compared by visual inspection (Miyazaki et al. 1987). Pairwise comparisons were made on 15 triplet patterns. Genetic distances were calculated according to the formula of Aquadro and Avise (1981): $D = 1 - 2N_{AB} / (N_A + N_B)$, where D is the genetic distance between specimens A and B, N_{AB} is the number of protein spots shared by A and B, and N_A and N_B are the numbers of protein spots scored for A and B. We used the genetic distances and the program PHYLIP (Felsenstein 1994) to construct dendrograms by the neighbor-joining (NJ) method (Saitou and Nei 1987) and the unweighted pair-group clustering method with arithmetic averages (UPGMA, Sneath and Sokal 1973).

Sequencing of the mitochondrial COI gene

Total DNA was prepared from the frozen foot muscle tissue. A small piece of foot muscle was homogenized in 400 μl of the T10E100 buffer (10 mM Tris-HCl pH 7.2 and 100 mM EDTA). The cellular membrane was dissolved by addition of 20% SDS (20 μl) to the homogenate. DNA was purified by phenol/chloroform and chloroform treatments. The supernatant was mixed with 1/10 vol of 5 M NaCl and 2 vol of 95% ethanol, left on ice for 10 min, and centrifuged at 16,000 g for 15 min at 4°C with a TMA-30 rotor (Tomy Seiko, Tokyo). After washing with 70% ethanol, the pellet was dissolved in 500 μl of the T10E100 buffer and mixed well with 5 μl of RNase A ($10 \mu\text{g} \mu\text{l}^{-1}$), 106 μl of 5 M NaCl, and 151.5 μl of 5% CTAB (cetyltrimethylammonium bromide) in 0.7 M NaCl. After incubation for 30 min at 65°C , DNA was purified by chloroform, phenol/chloroform, and chloroform treatments and precipitated as described above.

To amplify the approximately 710 bp partial fragment of COI, PCR (polymerase chain reaction) was performed in a reaction solution containing template DNA and KOD dash (Toyobo,

Table 1 *Bathymodiolus* spp. Sample list. The samples were used for analysis by two-dimensional gel electrophoresis of foot proteins (P) and DNA analysis by sequencing the mitochondrial cytochrome *c* oxidase subunit I gene (N). Notations for distinguishing individuals used in DNA analysis are presented in parentheses

Species	Sampling site	Depth (m)	Location	Habitat type	Dive no.	Date	Sample
<i>B. japonicus</i>	Off Hatsushima, Sagami Bay	1,170	35 00'00"N; 139 13.50'E	Seep	2K715	16 Nov 1993	P, N (BJH-1-3)
	Minami-ensei Knoll, Mid-Okinawa Trough	718	28 23'50"N; 127 38.50'E	Vent	2K618	6 Jun 1992	N (BJM-1-3)
	Off Hatsushima, Sagami Bay	1,180	35 59'59"N; 139 13.68'E	Seep	2K792	24 May 1995	P, N (BPH-1-w)
<i>B. platifrons</i>	Off Hatsushima, Sagami Bay	1,180	35 00'00"N; 139 13.50'E	Seep	2K715	16 Nov 1993	N (BPH-4)
	North Iheya Ridge, Mid-Okinawa Trough	1,028	27 47'18"N; 126 53.985'E	Vent	2K863	7 May 1996	P, N (BPH-1-2)
<i>B. septemdiarium</i>	Myojin Knoll, Izu-Ogasawara Island-arc	1,288	32 06'27"N; 139 52.18'E	Vent	2K1009	7 May 1998	P, N (BSM-1-1)
	Myojin Knoll, Izu-Ogasawara Island-arc	1,290	32 06'27"N; 139 52.123'E	Vent	2K1115	3 Jul 1999	N (BSM-2)
	Myojin Knoll, Izu-Ogasawara Island-arc	1,346	32 06'30"N; 139 52.043'E	Vent	2K1112	29 Jun 1999	N (BSM-3-4)
	Suiyo Seamount, Izu-Ogasawara Island-arc	1,375	28 34'05"N; 140 38.65'E	Vent	2K627	12 Jul 1992	N (BSS-1)
	Suiyo Seamount, Izu-Ogasawara Island-arc	1,373	28 34'04"N; 140 38.773'E	Vent	2K889	18 Aug 1996	N (BSS-2-3)
	PACMANUS Field E, Manus Basin	1,629	03 43'728"S; 151 40.183'E	Vent	2K1075	22 Nov 1998	P, N (BSP-1)
<i>Bathymodiolus</i> sp.	PACMANUS Field E, Manus Basin	1,627	03 43'731"S; 151 40.188'E	Vent	2K913	3 Nov 1996	N (BSP-2-5)
<i>Septifer virgatus</i>	Nakaminato, Ibaraki			Intertidal zone		Oct 1998	P

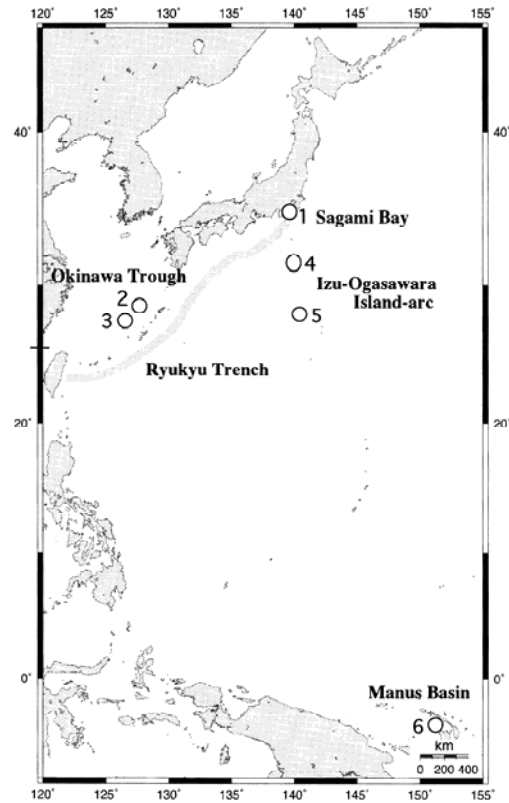


Fig. 1 Collection sites of deep-sea mussels used in this study. Mussels (*Bathymodiolus* spp.) were collected at six localities during the dives of the submersible "Shinkai 2000" [1 off Hatsushima, Sagami Bay (seep, 1,170 and 1,180 m deep); 2 Minami-ensei Knoll, mid-Okinawa Trough (vent, 718 m deep); 3 North Iheya Ridge, mid-Okinawa Trough (vent, 1,028 m deep); 4 Myojin Knoll, Izu-Ogasawara Island-arc (vent, 1,288, 1,290, and 1,346 m deep); 5 Suiyo Seamount, Izu-Ogasawara Island-arc (vent, 1,373 and 1,375 m deep)]; 6 PACMANUS field E, Manus Basin (vent, 1,627 and 1,629 m deep)]. Both *B. japonicus* and *B. platifrons* were obtained from Sagami Bay and the Okinawa Trough. *B. septemdiarium* and *Bathymodiolus* sp. were obtained from the Izu-Ogasawara Island-arc and the Manus Basin, respectively

Osaka), with 30 cycles of 30 s denaturation at 94°C, 5 s annealing at 42°C or 49°C (for *B. septemdiarium* by universal primers) or 55°C (for the others), and 30 s extension at 74°C. Earlier we used universal primers for COI of metazoan invertebrates (Folmer et al. 1994); sense LCO1490 (5'-GGTCAACAATCATAAAGATATTGG-3') and antisense HCO2198 (5'-TAACTTCAGGGTGACCAAAAATCA-3'). However, DNA from *B. septemdiarium* could not be amplified well, and thus we designed internal primers based on the COI sequence of *Bathymodiolus* sp. from the Manus Basin; sense SMANU (5'-GGTTTGTGATCGGGAATAATTGGGAC-3') and antisense ASMANU (5'-CTATTCGCTCCCCTCGCATACTTTC-3'). The amplified fragment was purified by chloroform and diethyl ether treatments and precipitated as described above. Direct sequencing of the purified double-strand PCR product was performed using an ABI PRISM BigDye

terminator cycle sequencing ready reaction kit (Applied Biosystems) and the primers for PCR on a model 377 DNA sequencer (Applied Biosystems) according to the manufacturer's directions.

DNA sequences were edited and aligned with DNASIS (Hitachi Software Engineering) and corrected by visual inspection for phylogenetic analyses. Dendrograms were constructed by the NJ method and the maximum-parsimony (MP) and maximum-likelihood (ML) methods based on genetic distance and character-state matrices, respectively. Genetic distances were computed by Kimura's two-parameter method (Kimura 1980), and the NJ and ML trees were depicted using "Neighbor" in PHYLIP. The MP tree was depicted with PAUP (Swofford 1993), and a majority-rule consensus tree based on 1,000 bootstrap replicates was produced. Sequence data of *Modiolus modiolus* (Mytilidae: Modiolinae) was used for rooting dendrograms (Hoeh et al. 1998, DDBJ accession number U56848).

Results

Phylogenetic relationships among *Bathymodiolus* mussels inferred by 2D-PAGE

Representative 2D-PAGE patterns are shown for comparison of foot muscle protein constituents between *B. japonicus* and *B. platifrons* (Fig. 2). Protein spots (263–407, average = 318) were compared on the basis of 15 sets of triplet patterns (Table 2). Genetic distances among *Bathymodiolus* species (Table 2) ranged from 0.120 to 0.287 (average = 0.206) and were well within the range of genetic distances among congeneric species in various organisms (Miyazaki et al. 1987, 1988, 1998; Tsubokawa and Miyazaki 1993). Genetic distances between *Bathymodiolus* species (Bathymodiolinae) and *Septifer virgatus* (Mytilinae) were 0.239–0.426 (average = 0.356), while that between two specimens of *B. platifrons* from hydrothermal vents of the Iheya Ridge and cold-water seeps off Hatsushima was 0.023.

Based on the genetic distances, two dendrograms were constructed using the outgroup species *S. virgatus*. The NJ tree presented two clusters comprising *B. platifrons* and *B. japonicus* on the one hand and *B. septemdirum* and *Bathymodiolus* sp. on the other (Fig. 3). The UPGMA tree revealed the same topology (data not shown).

Phylogenetic relationships among *Bathymodiolus* mussels inferred by sequencing mtDNA

Partial DNA fragments (415 bp) of mitochondrial COI were sequenced for more than five specimens of each *Bathymodiolus* species. Representative sequences of the four *Bathymodiolus* species and *Modiolus modiolus* were aligned (Fig. 4). Sequence data were deposited in DDBJ, EMBL, and GenBank databases under accession numbers AB101419–101434. No deletions or insertions were found, and 132 out of 415 sites (20 at the first position and 5 at the second position in the codon) were variable. Amino acid substitutions were found at 8 of 138 residues, with 1 fixed replacement in *B. septemdirum* (Ser

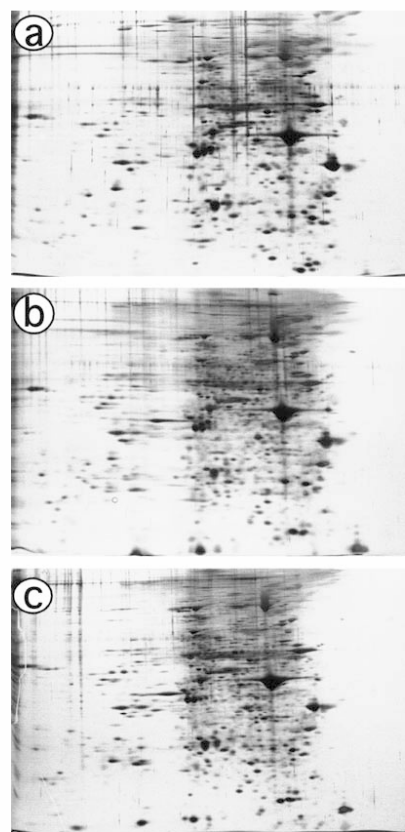


Fig. 2a–c *Bathymodiolus* spp. Representative 2D-PAGE patterns. Mussel foot muscle protein constituents were compared by 2D-PAGE among four *Bathymodiolus* species and *Septifer virgatus* as an outgroup. The 2D-PAGE pattern of *B. japonicus* (a) was compared to that of *B. platifrons* (c), with the mixture pattern from both species (b) by the triplet method

Table 2 *Bathymodiolus* spp. Genetic distances among five mussel species. Genetic distances (above diagonal) calculated according to Aquadro and Avise (1981) and the number of spots (below diagonal) used for comparisons are shown (B.j., *B. japonicus*; B.p., *B. platifrons*; B.s., *B. septemdirum*; B.sp., *Bathymodiolus* sp. from the Manus Basin; S.v., *Septifer virgatus*; h, off Hatsushima, Sagami Bay; I, north Iheya Ridge, mid-Okinawa Trough)

	B.j.	B.p. (h)	B.p. (I)	B.s.	B.sp.	S.v.
B.j.		0.128	0.120	0.265	0.172	0.306
B.p. (h)	334		0.023	0.248	0.287	0.426
B.p. (I)	270	407		0.263	0.222	0.422
B.s.	269	277	317		0.151	0.386
B.sp.	402	403	296	326		0.239
S.v.	263	286	288	298	327	

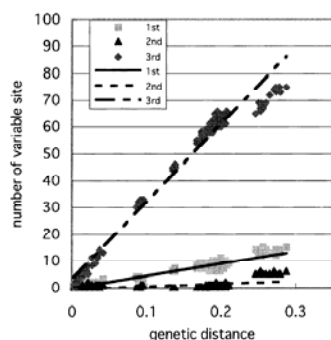


Fig. 5 Correlations of the numbers of variable sites with genetic distances. The numbers of variable sites at the first, second, and third positions in the codon were plotted against genetic distances on the basis of pairwise comparisons to examine whether nucleotide substitutions were saturated or not. Only slight saturation was detected at the third position for intergeneric comparisons

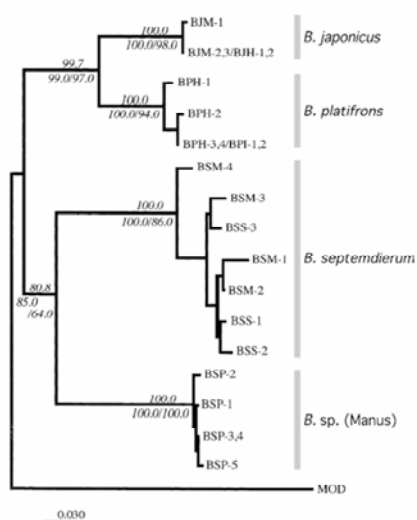


Fig. 6 *Bathymodiolus* spp. Phylogenetic relationships of *Bathymodiolus* species inferred by DNA analysis. The dendrogram was constructed by the neighbor-joining method based on genetic distances calculated by Kimura's two-parameter method. *Modiolus modiolus* was used for rooting. Bootstrap probabilities of 1,000 replicates are presented above branches when they exceed 50%. Bootstrap probabilities of 1,000 replicates for the maximum-parsimony tree (left) and 100 replicates for the maximum-likelihood tree (right) are also presented (below branches). Bar indicates a genetic distance of 0.030

specimens of *B. platifrons* (Fig. 7). Again, haplotype A was shared by two specimens from hydrothermal vents of the Iheya Ridge and two specimens from cold-water seeps off Hatsushima. Two specimens from off

		Site
		11111111222333334444
		11134455599122357994460146790001
Species	Haplotype	1079042602978414982397887691310692
<i>B. japonicus</i>	A	TGTGAGCGTTGATTGTCATTGGCTATTATG
	BA.....
<i>B. platifrons</i>	AA.....C.A.....A.....GA.
	BA.....C.....A.....C.A.....
	CCA.....C.A.....A.....GA.
<i>B. septemdiarium</i>	A	A.CT.ATACC.T...AGT...C...AT.TGA.TCC
	B	A.CT.A.A...T...CAGT...C...T.T.A.TCC
	C	AACA.A.A...T...CAG...C.C...CT.A...C
	D	GA.T...C...T...AG.CCCG...AT.T.G.T.T
	E	A.CT.A.A...T.GCAG.....T.T.A.TCC
	F	G.CT.A.A.....CCG.....T.T.A.TCC
	G	GACC.A.A...TC.CAG...C.C...TCT.A.TCC
<i>B. sp. (Manus)</i>	A	GA.A...C...G...G.CCCG...ATC...G.T.T
	B	GA.A...T...G...G.CCCG...ATC...G.T.T
	C	GA.A...C...G...G.CCCG...ATCT.GCT.T
	D	GA.A...C...AG...G.CCCG...ATC...G.T.T

Fig. 7 *Bathymodiolus* spp. Nucleotides at variable positions in the COI sequence. Nucleotides where intraspecific substitutions were found are shown. Numbers refer to nucleotide positions in the sequences of Fig. 4. *B. japonicus* and *B. platifrons* had two and three haplotypes, respectively. *B. septemdiarium* and *Bathymodiolus* sp. had seven and four haplotypes, respectively

Hatsushima each possessed one of the remaining haplotypes. The substitutions in haplotype C (position 30) led to one amino acid replacement. It seems surprising that the same haplotypes were held in common by specimens obtained from distant localities and highly differentiated environments (hydrothermal vents of the Okinawa Trough and cold-water seeps of Sagami Bay).

On the other hand, all the specimens of *B. septemdiarium* possessed distinct haplotypes (haplotypes A–G in Fig. 7). Seven haplotypes were unique to four specimens from the Myojin Knoll and three specimens from the Suiyo Seamount in *B. septemdiarium*. Substitutions at positions 17, 42, 50, 114, and 124 resulted in five amino acid replacements, and the other substitutions were synonymous. Four haplotypes (haplotypes A–D) were found in five specimens of *Bathymodiolus* sp. (Fig. 7). Haplotype A was shared by two specimens, and three haplotypes (haplotypes B–D) were unique to three specimens. Substitutions at position 59 resulted in one amino acid replacement, and the other substitutions were synonymous.

Discussion

Interspecific phylogenetic relationships of *Bathymodiolus* mussels

The present results showed that three *Bathymodiolus* species from Japanese waters and *Bathymodiolus* sp.

from the Manus Basin were grouped into two lineages, one comprising *B. japonicus* and *B. platifrons* that live in both hydrothermal vents and cold-water seeps and the other comprising *B. septemdiarium* and *Bathymodiolus* sp. that inhabit hydrothermal vents solely. This is the first report on the phylogeny of *Bathymodiolus* mussels from the western Pacific. *B. japonicus* and *B. platifrons* are found in Sagami Bay and the Okinawa Trough, and *B. septemdiarium* is reported from the Izu-Ogasawara Island-arc. *B. septemdiarium* was more closely related to *Bathymodiolus* sp. than to *B. japonicus* and *B. platifrons*, although the straight-line distance from the Myojin Knoll to the Manus Basin is about 12 times longer than that from the Myojin Knoll to Sagami Bay. It is worth mentioning that all the dendrograms obtained by two different approaches, 2D-PAGE (NJ and UPGMA) and DNA sequencing (NJ, MP, and ML), gave the same topology. 2D-PAGE allows comprehensive comparisons of a large number of proteins encoded mainly by nuclear genes and DNA sequencing enables one to compare precisely mitochondrial DNA.

The phylogenetic relationships among *Bathymodiolus* species were supported by the grouping of endosymbiotic bacteria. Fujiwara et al. (2000) showed that endosymbionts of *B. japonicus* and *B. platifrons* from the Iheya Ridge were closely related to methane-oxidizing bacteria (methanotrophs) and those of *B. septemdiarium* from the Myojin Knoll to sulfur-oxidizing bacteria (thioautotrophs). The undescribed *Bathymodiolus* species from the Manus Basin contains thioautotrophic endosymbionts (data will be described elsewhere). The congruency of the phylogeny in *Bathymodiolus* species with that of their endosymbionts is quite reasonable, because the endosymbionts are believed to be transmitted into offsprings through eggs in *Bathymodiolus* mussels (Distel et al. 1994; Cary and Giovannoni 1993). However, evidence of horizontal transmission has been presented by Won et al. (2003b). They suggest that endosymbionts of Atlantic *Bathymodiolus* species are acquired from the environment.

Large differences in depth (approximately 1,000 m) separate two *Bathymodiolus* species living in hydrothermal vents of the Mid-Atlantic Ridge (3,080–3,480 m for *B. puteoserpentis* vs. 869–2,303 m for *Bathymodiolus* sp.), leading Maas et al. (1999) to propose that depth might provide a fundamental barrier to the dispersal of the two Atlantic species. Different vertical stratification is also reported in vesicomid clams (Fujikura et al. 2000; Goffredi et al. 2003). It is suggested that extrinsic restrictive factors for their distributions are related to bathymetrical zonation rather than to environmental types (vents vs. seeps), and the primary intrinsic factor is probably ascribed to the different physiological tolerance of the clams to pressure (Olu et al. 1996; Fujikura et al. 2000). However, such differences in the depth of habitats were not found among the three *Bathymodiolus* from Japanese waters. When the bathymetrical distribution patterns were examined for each species, the vertical distribution ranges overlapped broadly (Fig. 8).

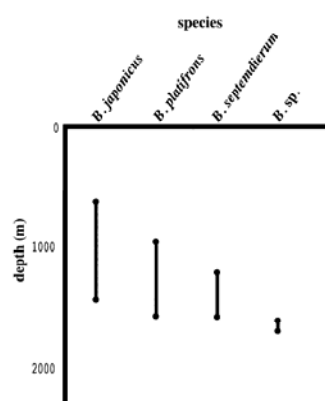


Fig. 8 *Bathymodiolus* spp. Vertical distribution patterns of *Bathymodiolus* species. Bathymetrical distributions of *Bathymodiolus* species were compiled, to the best of our knowledge, and the vertical distribution ranges are presented. The patterns show no vertical habitat segregation.

Therefore, bathymetrical zonation and physiological tolerance to pressure are not likely to be responsible for habitat segregation among them.

Intraspecific relationships in *Bathymodiolus* mussels

The intraspecific genetic distance between two specimens of *B. platifrons* from the Iheya Ridge (Okinawa Trough) and off Hatsushima (Sagami Bay) obtained by 2D-PAGE was only 0.023, nearly one order of magnitude lower than the average of interspecific distances (average=0.206) among *Bathymodiolus* species. Genetic distances among *Bathymodiolus* species obtained by DNA sequencing of the mitochondrial COI gene were 0.087–0.211 (average=0.172). The intraspecific genetic distances were 0.001 and 0.005 for the methanotroph-containing species and 0.004 and 0.025 for thioautotroph-containing species. These results are suggestive of intraspecific genetic exchanges between hydrothermal vents of the Okinawa Trough and cold-water seeps of Sagami Bay (over 1,500 km apart from each other) in *B. japonicus* and *B. platifrons*, but the gene flow between conspecific populations should be evaluated by statistical methods using more specimens and sample locations (e.g. Won et al. 2003a).

As for *B. japonicus* and *B. platifrons*, specimens from the Okinawa Trough and Sagami Bay shared the same haplotypes of COI, while one haplotype was specific to the specimen from the Okinawa Trough in *B. japonicus* and two haplotypes were specific to the specimens from Sagami Bay in *B. platifrons*. Although the vesicomid clam in the chemosynthesis-based communities, *Calyptogena okutanii*, is distributed from the Okinawa Trough to Sagami Bay via the Nankai Trough, one fixed difference (synonymous substitution) in the COI

sequence was detected between specimens from the Okinawa Trough and those from Sagami Bay (Kojima et al. 1995). On the other hand, no fixed difference was found between specimens from the Okinawa Trough and from Sagami Bay in *Bathymodiolus* species. The present results suggest that the capability for larval dispersal is relatively high, and environmental types (vents vs. seeps) are not the primary factor responsible for habitat segregation and speciation in these *Bathymodiolus* species.

Ambient conditions are not, however, entirely identical between hydrothermal vents at the Iheya Ridge and the Minami-ensei Knoll (Okinawa Trough) and those at the Myojin Knoll and the Suiyo Seamount (Izu-Ogasawara Island-arc). It has been shown that the methane concentration was significantly higher in the former than in the latter, and cold-water seeps in Sagami Bay also revealed a significantly higher methane concentration than hydrothermal vents in the Izu-Ogasawara Island-arc (Fujiwara et al. 2000). Fujiwara et al. (2000) suggested that deep-sea mussels harboring methanotrophs were restricted to areas with the high methane concentration. It is likely that the colonization and speciation of deep-sea mussels depend on their preference to one or some specific ambient condition(s). However, this scenario does not explain why *B. septemdiernum* harboring thioautotrophs are localized only in the Izu-Ogasawara Island-arc, although thioautotroph-containing *B. adulooides* can live in both the Okinawa Trough and Sagami Bay, or why *B. adulooides* could not colonize the Izu-Ogasawara Island-arc. Further studies on direction and drift of ocean currents and differences in dispersal abilities among species could provide an answer to the question. It is also conceivable that the hadal zone, e.g. the Ryukyu Trench, may serve as a barrier to dispersal between the Okinawa Trough and the Izu-Ogasawara Island-arc, because *Bathymodiolus* mussels only seem capable of surviving at depths of up to 3,600 m and, thus, may be incapable of settling in transit habitats in the hadal zone during colonization.

In an earlier work (Grassle 1985), substantial genetic differences were revealed between populations of the eastern Pacific mussel, *B. thermophilus*. However, latter studies of allozymes and mitochondrial genes (Craddock et al. 1995b; Won et al. 2003a) identified no comparable geographical subdivision across the region sampled by Grassle (1985), although topographical features such as the Easter Microplate do appear to coincide with dispersal barriers (Won et al. 2003a). The genetic variability of western Pacific *Bathymodiolus* species from the North Fiji and the Manus Basins was seemingly lower than that of their shallow-water counterparts and other deep-sea organisms (Moraga et al. 1994). Lutz et al. (1986) examined larval shell morphologies of mollusks from hydrothermal vents in the eastern Pacific and showed that one mytilid (*B. thermophilus* in Vrijenhoek 1997) had morphology indicative of planktotrophic (actively feeding planktonic larval) development, suggesting that *Bathymodiolus* mussels are highly dispersible. Therefore,

the speciation of *Bathymodiolus* species cannot be ascribed to the poor larval dispersal ability. Nevertheless, examination of more specimens from different localities with faster-evolving genes is needed to assess intraspecific genetic variation.

As pointed out by Vrijenhoek (1997), one has to consider many factors that may contribute to colonization and speciation, such as topography, geological histories, the stability of vents and seeps, oceanic currents at various depths, larval positions in the water column, and settlement cues, as well as the dispersal ability and physiology of mussels, in order to elucidate the evolutionary process for *Bathymodiolus* mussels and other deep-sea organisms.

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太平洋西部・インド洋のシンカイヒバリガイ類の分子系統と分岐年代(予報)
**Phylogenetic Relationships and Divergence Time of Deep-sea
Mussels of the Genus *Bathymodiolus* (Bivalvia, Mytilidae)
in the Western Pacific and Indian Oceans (Preliminary Report)**

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Abstract: There are unique biological communities around deep-sea hydrothermal vents and cold-water seeps throughout the world. Mussels of the genus *Bathymodiolus* (Bivalvia, Mytilidae) are one of the dominant macroorganisms in these communities. *Bathymodiolus* mussels live in a reductive environment, depending on energy supplied by bacterial endosymbionts employing chemosynthesis. Although *Bathymodiolus* mussels are taxonomically classified by conchological traits, their evolution is poorly understood. In this study, phylogenetic relationships of nine *Bathymodiolus* species obtained from the Western Pacific and Indian Oceans were investigated by comparing partial nucleotide sequences of the mitochondrial cytochrome *c* oxidase subunit I gene (COI). These species were divided into two clusters comprising *Bathymodiolus japonicus*, *B. platifrons*, *B. sp.* (short-type, Kuroshima Knoll), and *B. sp.* (long-type, Kuroshima Knoll) on the one hand (group 1) and *B. septemdiarium*, *B. aduloides*, *B. marisindicus*, *B. sp.* (Mariana Back-Arc Basin), and *B. sp.* (Manus Basin) on the other (group 2). The former is a lineage containing methanotrophic endosymbionts and the latter is a lineage containing thioautotrophic endosymbionts (no data for *B. sp.* from the Mariana Back-Arc Basin). *Bathymodiolus septemdiarium*, *B. marisindicus*, and *B. sp.* (Mariana Back-Arc Basin) are closely related with small genetic distances among them (cluster A). Divergence time calculated based on the estimated evolutionary rate of COI (0.014 of sequence divergence/million years) was 12 to 14 million years ago between the two groups and 0.3 to 2.9 million years ago among the three species in cluster A.

Key Words: mtDNA, COI, hydrothermal vent, cold-water seep, bacterial endosymbiont.

はじめに

深海に生息する二枚貝類であるイガイ科シンカイヒバリガイ属 (*Mytilidae*, *Bathymodiolus*) は、シロウリガイ属やハオロムシ類と同様に化学合成生物群集の主要なメンバーの一つであり、これまでに 14 種が記載されている (Kenk & Wilson 1985; Cosel *et al.* 1994; Hashimoto & Okutani 1994; Cosel & Olu 1998; Gustafson *et al.* 1998; Cosel *et al.* 1999;

Hashimoto 2001). シンカイヒバリガイ属は硫酸化細菌やメタン酸化細菌 (あるいはその両方) を細胞内に共生させ、それらが作り出すエネルギーを利用して、光の届かない深海において生命を維持している。化学合成生物群集の生息する熱水域や冷湧水域は海溝や海嶺付近に分散して存在しているため、遺伝的隔離が起こりやすいと考えられるが、シンカイヒバリガイ属では相模湾と沖縄トラフというおよそ 1,600 km も離れた場所に同じ種が生息している (Hashimoto & Okutani 1994)。一方、およそ 500 km 離れた相模湾と伊豆・小笠原島弧ではそれぞれ別種のシンカイヒバリガイ属が生息しており (Hashimoto & Okutani 1994)、このことはシンカイヒバリガイ属の種分化が、単純に地理

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的距離に相関して起こっているのではないことを示している。シンカイヒバリガイ属の幼生期における分散能力は大きいと考えられるが(Lutz et al. 1986), その詳細が分かっていないこともあり、シンカイヒバリガイ属がどのようにして分布を拡げ、種分化をしてきたのか未だに明らかとなっていない。シンカイヒバリガイ属は、殻の形や筋痕・靱帯の位置といった形質を基に分類されているが、その系統関係についての知見は得られていない。

本研究では、シンカイヒバリガイ属の種分化のプロセスを解明するために、太平洋西部・インド洋に生息するシンカイヒバリガイ属の種間の系統関係をミトコンドリアシトクロームcオキシダーゼサブユニットI遺伝子(COI)の塩基配列を比較することによって解析し、分岐年代を推定した。

材料と方法

材料

本研究では、日本周辺に生息する4種、シンカイヒバリガイ(*Bathymodiolus japonicus*)、ヘイトウシンカイヒバリガイ(*B. platifrons*)、シチヨウシンカイヒバリガイ(*B. septemdiarium*)およびカツキンカイヒバリガイ(*B. aduloides*)と未記載種2種、マリアナ背弧海盆の未同定種、マヌス海盆の未記載種、インド洋ロドリゲス三重点のインドシンカイヒバリガイ(*B. marisindicus*)の計9種のシンカイヒバリガイ属を使用した(Table 1, Fig. 1)。解析には各々の種を5個体以上を使用した(雌雄の判別はしなかった)。これらの標本は海洋科学技術センターの深海調査の際に採集された。

分析方法

シンカイヒバリガイ属の足の筋肉から、SDSを用いてタンパク質を、CTABとRNaseAを用いてRNAを取り除き、DNAを精製

した。このDNAをテンプレートとし、COIの断片約710 bpをPCRで増幅した。プライマーは軟体動物のユニバーサルプライマー、センス LCO1490 (5'-ggccaacaatacaagatattgg-3')とアンチセンス HCO2198 (5'-taaactcagggtgacaaaatca-3')を使用した(Folmer et al. 1994)。シチヨウシンカイヒバリガイのCOI断片はユニバーサルプライマーでは増幅されなかったため、マヌス海盆産未記載種のCOI部分塩基配列を基に設計したプライマー、センス SMANU (5'-ggtttgtgacgggaataattggac-3')とアンチセンス ASMANU (5'-ctattcgtccctccatcttcc-3')を用いた(COIの断片

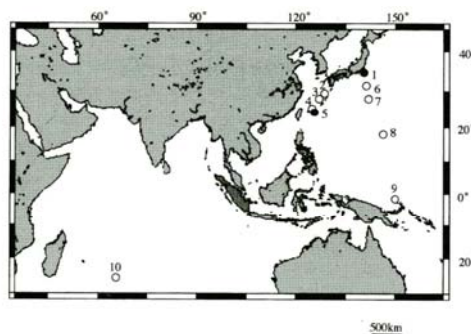


Fig. 1. The ten collection sites of deep-sea *Bathymodiolus* mussels used in this study. 1, Off Hatsushima (seep), Sagami Bay; 2, Minami-Ensei Knoll (vent), Mid-Okinawa Trough; 3, Iheya Ridge (vent), Mid-Okinawa Trough; 4, Hatoma Knoll (vent), Southern Okinawa Trough; 5, Kuroshima Knoll (seep), South of Ishigakijima Island; 6, Myojin Knoll (vent), Izu-Ogasawara Island-arc; 7, Suiyo Seamount (vent), Izu-Ogasawara Island-arc; 8, Mariana Back-Arc Basin (vent); 9, PACMANUS Field E (vent), Manus Basin; 10, Kairei Field (vent), Southern Central Indian Ridge. Open circles denote hydrothermal vent communities. Closed circles denote cold seep communities.

Table 1. Sample list. Each specimen was numbered with a prefix incorporating an abbreviation of its scientific name.

Species	Sampling site (locality number in Fig. 1)	Depth (m)	Habitat type	Dive#	Date	Abbreviation
<i>Bathymodiolus japonicus</i>	Off Hatsushima, Sagami Bay (1)	1,170	seep	2K715	1993.11.16	BJH1, 2
	Minami-Ensei Knoll, Mid-Okinawa Trough (2)	718	vent	2K618	1992. 6. 6	BJM1-3
<i>B. platifrons</i>	Off Hatsushima, Sagami Bay (1)	1,170	seep	2K831	1995.11.25	BPH1-3
	Off Hatsushima, Sagami Bay (1)	1,180	seep	2K715	1993.11.16	BPH4
<i>B. septemdiarium</i>	North Iheya Ridge, Mid-Okinawa Trough (3)	1,028	vent	2K863	1996. 5. 7	BP11, 2
	Myojin Knoll, Izu-Ogasawara Island-arc (6)	1,288	vent	2K1009	1998. 5. 7	BSM1
	Myojin Knoll, Izu-Ogasawara Island-arc (6)	1,290	vent	2K1115	1999. 7. 3	BSM2
	Myojin Knoll, Izu-Ogasawara Island-arc (6)	1,346	vent	2K1112	1999. 6.29	BSM3, 4
	Suiyo Seamount, Izu-Ogasawara Island-arc (7)	1,375	vent	2K627	1992. 7.12	BSS1
<i>B. aduloides</i>	Suiyo Seamount, Izu-Ogasawara Island-arc (7)	1,373	vent	2K889	1996. 8.18	BSS2-4
	Hatoma Knoll, southern Okinawa Trough (4)	1,523	vent	2K1270	2001. 5.20	BST1
	Iheya Ridge, Mid-Okinawa Trough (3)	1,339	vent	3K375	1998. 6.20	BA11
<i>B. marisindicus</i>	Kairei Field, Southern Central Indian Ridge (10)	2,454	vent	6K659	2002. 2.15	BMK1-5
<i>B. sp. (short-type)</i>	Kuroshima Knoll, South of Ishigakijima Island (5)	637	seep	2K1370	2002. 6.30	BSP(A)1-5
<i>B. sp. (long-type)</i>	Kuroshima Knoll, South of Ishigakijima Island (5)	637	seep	2K1370	2002. 6.30	BSP(D)1-5
<i>B. sp.</i>	Mariana Back-Arc Basin (8)	3,600	vent	6K357	1996.12.10	BSP(Mari)1
<i>B. sp.</i>	PACMANUS Field E, Manus Basin (9)	1,629	vent	2K1075	1998.11.22	BSP(Manu)1
	PACMANUS Field E, Manus Basin (9)	1,627	vent	2K913	1996.11. 3	BSP(Manu)2-5

481 bp). PCR は、熱変性 94°C 30 秒、アニーリング 40°C から 55°C 5 秒、伸長 74°C 30 秒という条件で 30 サイクル行った。

精製した PCR 産物をテンプレートとし、ABI PRISM Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) と PCR 用のプライマーを用いてシーケンス反応を行った後、377 DNA Sequencer (Applied Biosystems) を使用して、塩基配列を決定した。

DNASIS (Hitachi Software Engineering) を用いて、得られた塩基配列のアライメントを行った。遺伝的距離は木村の 2 パラメーター法 (Kimura 1980) により算出した。これらの塩基配列データを基に、PHYLIP (Felsenstein 1994) を用いて近隣結合 (NJ) 法と平均距離 (UPGMA) 法および最尤 (ML) 法によって樹形図を構築した。また、PAUP (Swofford 1993) を用いて最大節約 (MP) 法によって樹形図を構築した。樹形の信頼性を調べるために 1,000 回の反復 (最尤樹では 100 回の反復) によるブートストラップ検定を行った。アウトグループとして用いたイガイ科ヒバリガイ属 *Modiolus modiolus* の塩基配列はデータベースより引用した (Hoeh *et al.* 1998; DDBJ accession number U56848)。

結果および考察

系統関係

mtDNA の COI 部分塩基配列 431 bp を基に近隣結合法によって構築した樹形図 (Fig. 2) から、本研究で利用したシンカイヒバリガイ属は 2 つのクラスターに分けられることが明らかとなった。シンカイヒバリガイ *B. japonicus* (BJH1, 2/BJM1-3)、ヘイトウシンカイヒバリガイ *B. plati-frons* (BPH1-4/BPI1, 2)、黒島海丘の未記載種 2 種 (BSP (A)1-5 と BSP (D)1-5) で 1 つのクラスター (グループ 1) を、シチヨウシンカイヒバリガイ *B. septemdiarium* (BSM1-4/BSS1-4/BST1)、カヅキシンカイヒバリガイ *B. aduloides* (BA11)、インドシンカイヒバリガイ *B. marisindicus* (BMK 1-5)、マリアナ背弧海盆の未記載種 (BSP (Mari)1)、マヌス海盆の未記載種 (BSP (Manu)1-5) で別のクラスター (グループ 2) を形成した。最大節約法、UPGMA 法および最尤法を用いても同様の樹形が得られることから、ここで示したシンカイヒバリガイ属の類縁関係は信頼性が高いと考えられる。藤原らの研究から、グループ 1 の 4 種はメタン酸化細菌を、グループ 2 の 4 種は硫酸酸化細菌 (マリアナ背弧海盆の未記載種は不明) を共生細菌として持っており (Fujiwara *et al.* 2000)、シンカイヒバリガイ属と共生細菌のグループ分けが一致した。このことは、シンカイヒバリガイ属の共生細菌は外界から取り込まれるのではなく、親から子へ直接受け継がれることが示されているので (Cary and Giovannoni 1993)、宿主と共生者の共進化を示唆していると思われる。

グループ 1 においては、まず黒島海丘の未記載種 short-type (BSP (A)1-5) が最も根元から分岐し、次にシンカイヒバリガイが分岐した。ヘイトウシンカイヒバリガイと黒島海丘の未記載種 long-type (BSP (D)1-5) が最も最近に分岐

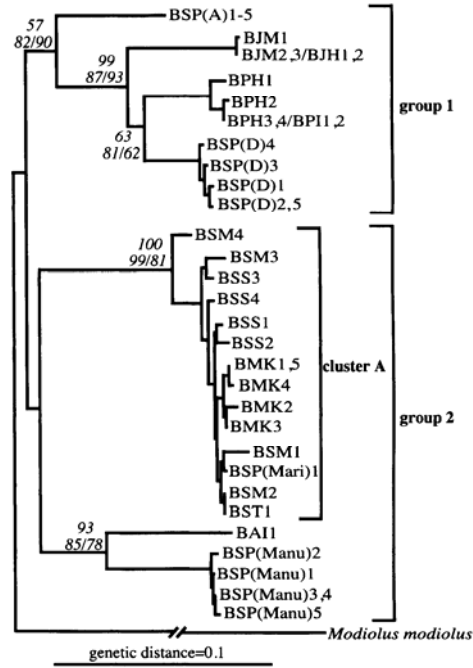


Fig. 2. Phylogenetic relationships of *Bathymodiolus* species inferred by mtDNA analysis. Partial nucleotide sequences (431 bp) of COI were determined for more than five specimens of each *Bathymodiolus* species (for *B. aduloides* and *B. sp.* from the Mariana Back-Arc Basin, only one specimen was available). The sequence of the outgroup *Modiolus modiolus* was obtained from a database. The dendrogram was constructed by the NJ method based on genetic distances calculated by Kimura's two-parameter method. UPGMA, MP, and ML trees presented fundamentally the same topology as the NJ tree. Italic numbers (upper) denote the bootstrap percentages of 1,000 replicates for NJ. Italic numbers (lower) denote those of percentages of 1,000 replicates for MP (left) and 100 replicates for ML (right). Bootstrap probabilities are denoted when they exceed 50%. The bar indicates a genetic distance of 0.1. See Table 1 for abbreviations of *Bathymodiolus* mussels.

した。シンカイヒバリガイとヘイトウシンカイヒバリガイは、冷湧水域の相模湾と熱水域の沖縄トラフに生息するが、冷湧水域と熱水域に生息する個体で生息環境ごとに別々のクラスターを形成しなかった。また、各々の種において両地域に共通するハプロタイプがみられた。このことから、シンカイヒバリガイとヘイトウシンカイヒバリガイでは相模湾と沖縄トラフの間で遺伝的交流があり、生息環境による遺伝的分化は起こっていないと考えられる。

グループ 2 では、カヅキシンカイヒバリガイとマヌス海盆の未記載種が近縁であり、シチヨウシンカイヒバリガイとインドシンカイヒバリガイおよびマリアナ背弧海盆の未

同定種が混在して1つのクラスターを形成した(クラスターA)。クラスターAの種間の遺伝的距離はきわめて小さいので、これらの種は非常に近い過去に種分化したか、現在も日本周辺海域からマリアナ沖を経てインド洋にわたり遺伝的交流を行っているのではないかと考えられる。

このようにシンカイヒバリガイ属は非常に広範囲にわたって遺伝的に交流している可能性がある。特にクラスターAに属する種の間で現在もなおある程度の遺伝的交流があるとすると、シンカイヒバリガイ属の幼生はきわめて大きな分散能力を持つことになる。一方、黒島海丘に生息する未記載種はこれまでに他の地域で確認されておらず、幼生の分散能力は逆に非常に小さいことになる。シンカイヒバリガイ属の幼生の分散についてより深い知見を得るためには、今後生息地域での精査と新しい生息地の探索によって、シンカイヒバリガイ属の分布の状況を正確につかむ必要がある。

シンカイヒバリガイとヘイトウシンカイヒバリガイでは、相模湾と沖縄トラフの間で遺伝的交流が存在すると思われるが、伊豆・小笠原島弧(明神海丘と水曜海山)には

両種は生息していない。シンカイヒバリガイ属はこれまでに3,600 m以深からは報告されておらず、同じく化学合成生物群集に属する二枚貝類であるシロウリガイ属に比べ、深海への適応能力は低いと考えられるので、沖縄トラフと伊豆・小笠原島弧の間に存在する琉球海溝が分布拡大の障壁となっている可能性がある。海溝のような超深海が存在すると中継地を確保できず、シンカイヒバリガイ属の分散が妨げられると思われる。しかし、相模湾と伊豆・小笠原島弧の間には超深海は存在しないので、海流や各々の種の幼生期の分散能力の相違もシンカイヒバリガイ属の分布拡大に関係していると思われる。

分岐年代

COIの進化速度は十脚甲殻類の研究から、0.014 of sequence divergence/million years と見積もられている(Knowlton et al. 1998)。この進化速度を用い、木村の2パラメーター法(Kimura 1980)により算出した遺伝的距離に基づいて、シンカイヒバリガイ属の分岐年代を推定した(Table 2, Fig. 3)。これによると、グループ1とグループ2

Table 2. Genetic distances among deep-sea mussels of the genus *Bathymodiolus*. Genetic distances (%) were calculated by Kimura's two-parameter method. Their averages (above) and ranges (below) are shown. BJ, *Bathymodiolus japonicus*; BP, *B. platifrons*; BS, *B. septemdiernum*; BAI, *B. aduloides*; BSP(A), *B. sp.* (short-type, Kuroshima Knoll); BSP(D), *B. sp.* (long-type, Kuroshima Knoll); BSP(Mari), *B. sp.* (Mariana Back-Arc Basin); BMK, *B. marisindicus*; BSP(Manu), *B. sp.* (Manus Basin).

	BJ	BP	BS	BAI	BSP(A)	BSP(D)	BSP(Mari)	BMK	BSP(Manu)
BJ	0.1 (0~0.23)	8.9 (8.33~ 9.45)	20 (18.43~ 20.85)	17.6 (17.52~ 17.81)	13.1 (12.90~ 13.17)	9.6 (9.38~ 9.91)	20 (19.61~ 19.91)	19 (18.43~ 19.32)	18.6 (18.36~ 19.28)
BP		0.5 (0~1.66)	19 (17.81~ 20.06)	17.4 (16.79~ 17.84)	12.7 (12.38~ 12.73)	6.8 (6.58~ 7.07)	19 (18.40~ 18.92)	19 (18.10~ 19.22)	17.8 (17.46~ 18.56)
BS			2 (0~4.05)	17.5 (15.21~ 18.72)	15.4 (14.80~ 16.51)	18 (16.68~ 19.17)	1.4 (0.47~ 4.04)	2 (0.7~ 4.04)	16.5 (13.00~ 18.17)
BAI				0 0	14.8 (14.78)	16 (16.02~ 16.62)	18 (17.53)	18 (17.53~ 18.11)	11 (10.68~ 11.19)
BSP(A)					0 0	13 (12.94~ 13.51)	15 (15.37)	15 (14.80~ 15.65)	17.8 (17.66~ 17.98)
BSP(D)						0.7 (0~0.93)	18 (17.26~ 17.87)	18 (16.68~ 18.46)	17.5 (16.91~ 18.14)
BSP(Mari)							0 0	1 (0.70~ 1.17)	16.8 (16.68~ 16.99)
BMK								0.7 (0~0.93)	17.3 (16.96~ 17.85)
BSP(Manu)									0.4 (0.23~0.70)

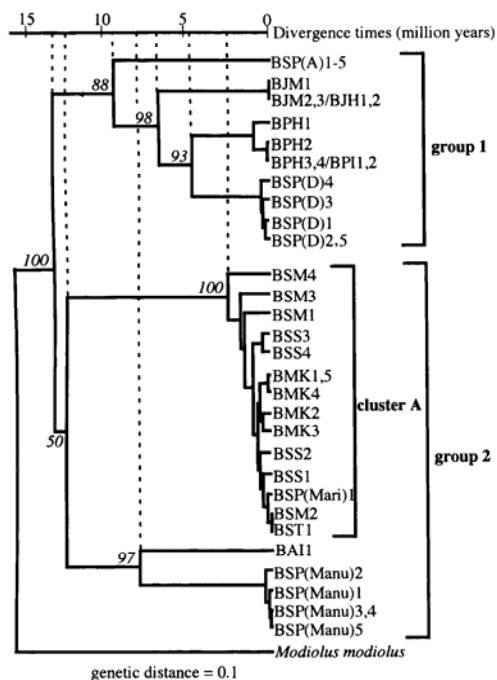


Fig. 3. Divergence time of *Bathymodiolus* species. The UPGMA tree was constructed with *Modiolus modiolus* as an outgroup. Italic numbers denote the bootstrap percentages of 1,000 replicates. Divergence time was calculated based on the estimated evolutionary rate of the COI gene, 0.014 of sequence divergence/million years. The bar (upper) denotes the time scale. The bar (below) indicates the genetic distance of 0.1. See Table 1 for abbreviations of *Bathymodiolus* mussels.

の分岐年代はおおよそ1,400万～1,200万年前と推定され、非常に温暖な時期(1,600万～1,500万年前)の後に両者は分岐したことになる。

グループ1において、黒島海丘の未記載種 short-type とその他の種の共通祖先との分岐年代はおおよそ950万～900万年前、ヘイトウシンカイヒバリガイと黒島海丘の未記載種 long-type の共通祖先とシンカイヒバリガイの分岐年代はおおよそ660万年前、ヘイトウシンカイヒバリガイと黒島海丘の未記載種 long-type の分岐年代はおおよそ490万年前と推定された。グループ2では、クラスターAの共通祖先とカツキシシンカイヒバリガイとマヌス海盆の未記載種の共通祖先の分岐年代はおおよそ1,280万～1,180万年前、カツキシシンカイヒバリガイとマヌス海盆の未記載種の分岐年代はおおよそ790万年前と推定された。この時期は新生代第三紀の中新世(2,400万～500万年前)後半にあたる。中新世の後半は現代型の生物種の発展期で、今日のものに著しく近似したもの、あるいはその直接の祖先などがみられた時代である(八杉ら1996)。

一方、クラスターAに属する種は、290万年前から30万年前に分岐した。この時期は新生代第三紀の鮮新世(約500万～170万年前)後半から第四紀更新世(約170万～1万年前)にあたる。鮮新世は現生種の著しい増加がみられた時代であり、貝化石の総種類に対する現生種の割合は60～70%に達する(八杉ら1996)。また、この時代の後半から気温低下が起こり、第四紀の水河時代を迎える。クラスターAに含まれる種はこのような気候の変動とそれに伴う海面の変化の激しい時期に種分化したと考えられる。

現在、今回得られた種間の系統関係を確かめるため、また、種内集団間の遺伝的交流を綿密に調べるため、mtDNAのNADHデヒドロゲナーゼサブユニット4遺伝子(ND4)の塩基配列の決定を行っている。これまでのところ、今回発表したものとおおむね一致する結果を得ている。

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NOTE

Strontium as a marker for estimation of microscopic growth rates in a bivalveKatsunori Fujikura^{1,*}, Kenji Okoshi², Takeshi Naganuma³¹Marine Ecosystems Research Department, Japan Marine Science and Technology Center (JAMSTEC), 2-15 Natsushima-cho, Yokosuka, Kanagawa 237-0061, Japan²School of Science and Engineering, Ishinomaki Senshu University, 1 Shinmito Minamisakai Ishinomaki, Miyagi 986-8580, Japan³Faculty of Applied Biological Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-hiroshima 739-8528, Japan

ABSTRACT: Microscopic growth increments in bivalve shells were measured by marking the shells of the short-necked clam *Ruditapes philippinarum* with the strontium chloride (strontium marking method, SMM). The results were compared with those achieved by the fluorescent marking method (FMM) using calcein. Because strontium has a higher atomic mass than calcium, strontium-enriched areas in the shells are visible as a bright band in the back-scattered electron image under the scanning electron microscope (SEM). *R. philippinarum* were immersed in strontium chloride solutions of varying concentrations, and the effects of immersion time (17 or 24 h) and concentration (0.72, 1.44 or 2.88 g l⁻¹) were investigated. These shells exhibited clearly visible marks 19 or 20 d post-immersion. Using the SMM, a microscopic (single-digit µm) increment in shell growth could be detected under SEM with a magnification of at least 1200×. Using the FMM, growth increments of only tens of micrometers were detectable under fluorescence optical microscopy up to a magnification of 600×. The SMM is thus superior to previous methods for detecting microscopic increases in shell growth. Marking methods should ideally be based on long-lasting markers that are easily detected and involve simple methodologies. The SMM meets these requirements. It can be used to estimate growth in small bivalve specimens and growth rates in slow-growing species such as deep-sea and polar bivalves.

KEY WORDS: Strontium elemental marking method · Growth rate · Short-necked clam · Microscopic growth

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The growth rate of an organism provides basic ecological data. The growth rates of bivalves have been well studied, since many species are important for the fishing industry (e.g. Shaul & Goodwin 1982, Tanabe 1988, Tian & Shimizu 1997). Many estimation methods have been developed to detect increases in shell growth, including band-analysis (Stevenson & Dickie 1954, Thompson et al. 1980, Sasaki 1981, Richardson

1989), marking methods (mark-release-recapture) using labeling paint, tags, filed notches and fluorescent chemicals (Heald 1978, Jones et al. 1978, Ito 1985, Parsons et al. 1993, Kesler & Downing 1997, Kaehler & McQuaid 1999, McQuaid & Lindsay 2000, Sato-Okoshi & Okoshi 2002), and calculation from age determination using radium-228 chronology (Turekian et al. 1975). Among these, marking methods are efficient in estimating bivalve growth rates because they are inexpensive and relatively easily applied by untrained observers. However, it is difficult to detect microscopic growth increases in shells on a scale of less than tens of micrometers, since such increases must be measured with a sliding caliper or by optical microscopy. One unique marking method uses rust to mark the shell surfaces, making it possible to detect microscopic increases in shell growth of fewer than tens of micrometers with an electron-probe X-ray microanalyzer (Koshikawa et al. 1997). However, at least 14 d of immersion are needed for the rust to be retained by the shell surface.

Recently, artificial marks have been made on otoliths with strontium (Sr) to study the periodicity of otolith growth increments of less than tens of micrometers in fish (gobies) (Iglesias et al. 1997, Hernaman et al. 2000). Using scanning electron microscopy (SEM) Sr-enriched areas in the otolith are detected as bright bands in the back-scattered electron image.

To the best of our knowledge, the use of Sr chloride (SrCl₂) as a growth marker in clam shells has not been previously investigated. In the present study, we assessed the potential of Sr as a marker to estimate microscopic growth rates in bivalve shells. The Sr marking method (SMM) was applied to estimate the growth rate of the short-necked clam *Ruditapes philip-*

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pinarum. This paper describes the practical advantages of the SMM in detecting microscopic growth increments on a single-digit μm scale and compares the results with those of the fluorescent marking method (FMM) using calcein. Collection, immersion, shell preparation, Sr detection and growth rate measurements were as follows.

Materials and methods. Study species: Specimens of the short-necked clam *Ruditapes philippinarum* were collected from the littoral at Nojima-koen, Yokohama, Kanagawa Prefecture, Japan ($35^{\circ}19.4' \text{N}$, $139^{\circ}39.2' \text{E}$) on 7 July 2001, and taken immediately to the Japan Marine Science and Technology Center (JAMSTEC). The shell length of all specimens was measured, and they were then placed in the sea off a pier fronting the JAMSTEC facilities.

Immersion: *Ruditapes philippinarum* shells were marked with SrCl_2 hexahydrate ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, Wako Pure Chemical Industries, Osaka, Japan). For comparison, they were also marked by immersion in the diluted fluorescent chemical calcein ($\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_{13} \cdot \text{HCl}$, Wako Pure Chemical Industries). Clams were placed in static aquaria and submitted to 6 treatments (3 different concentrations for 2 immersion periods of different lengths) with either SrCl_2 or calcein (Table 1). Immersion techniques were standardized among treatments.

SrCl_2 or calcein was dissolved in plastic aquaria containing 2 l of artificial seawater (Rohtemarine, Rei-Sea). Each aquarium was placed in an incubator at 25°C in the dark to prevent light degradation of the

fluorescent chemicals during the immersion period. The chemicals were dissolved at concentrations of 0.72, 1.44 and 2.88 g l^{-1} SrCl_2 and 0.3, 0.4 and 0.7 g l^{-1} calcein. During immersion, small compressors provided air to each aquarium. The clams remained in the aquaria for a treatment period of either 17 or 24 h. Except for the highest concentration, the concentrations and immersion periods for both chemicals were approximately the same as those used in previous studies for marking fish otoliths (Iglesias et al. 1997, Hernaman et al. 2000). After immersion, the clams were reared in ambient seawater (21.5 to 28.0°C) in front of the JAMSTEC pier (water depth approximately 1 to 3 m), and culled 19 to 20 d later. As a control, 10 non-marked specimens were also reared together with the chemically marked specimens. All specimens were retained by nylon nets ($60 \times 30 \times 30 \text{ cm}$).

Shell preparation: After culling, the shells were cleaned of adhering tissue and dried in an oven (60°C) for 24 h. A transverse section was cut across the longest shell axis, and the ventral part was divided into anterior and posterior sections (Fig. 1). Methacrylate-based resin (Technovit 7200 VLC, Heraeus Kulzer) was used as embedding medium. Each shell was first embedded in the resin. For SMM, the cross sections were ground with 600-grit sandpaper and then wet-polished using $9 \mu\text{m}$, $3 \mu\text{m}$ (METADI Diamond Suspension), and finally $0.05 \mu\text{m}$ (Masterprep) polishing suspension. For FMM, embedded shells were sliced into approximately $200 \mu\text{m}$ sections with a lapidary bandsaw (BS-300CP, Exakt Apparatebau). The sections were attached to glass slides and the facings wet-polished with a $9 \mu\text{m}$ polishing suspension (Metadi Diamond Suspension).

Detection of incorporated bands and analysis of Sr concentration in shells:

The embedded shells were carbon-coated using a vacuum-evaporator (JE-400, JEOL). This treatment produced bands of Sr-enriched areas in the shells that were detectable in a back-scattered electron image under SEM (SM-5800L, JEOL). Once the positions of these bands had been determined, the Sr concentrations in the bright band and background (i.e. outside the bright bands) areas were analyzed by spot-analysis using an energy-dispersive X-ray spectrometer (Oxford Lind ISIS 2001, Oxford Instruments) and a detector (Supermini Cup, JEOL). The electron beam was irradiated at an accelerating voltage of 25 kV and a lifetime of 100 s.

Calcein was detected by examining the sectioned shells under fluorescence opti-

Table 1. *Ruditapes philippinarum*. No. of individuals, shell length, chemical concentrations, immersion period, and number of days reared post-treatment for the Sr marking method (SMM) and the fluorescent marking method (FMM)

	No. individuals	Shell length (mm)	Conc. (g l^{-1})	Immersion period (h)	No. days post-treatment
Strontium chloride					
(SMM)	3	26.1–27.5	0.72 ^a	17	19
	3	21.7–28.0	0.72	24	20
	3	22.9–25.4	1.44 ^b	17	19
	3	21.0–22.2	1.44	24	20
	3	23.9–29.5	2.88 ^c	17	19
	3	23.8–25.3	2.88	24	20
Calcein					
(FMM)	3	25.0–26.9	0.3	17	19
	3	21.7–30.4	0.3	24	20
	3	22.3–28.1	0.4	17	19
	3	25.7–25.8	0.4	24	20
	3	22.9–24.6	0.7	17	19
	3	22.7–26.3	0.7	24	20
^a $30 \times$ Sr concentration in natural seawater					
^b $60 \times$ Sr concentration in natural seawater					
^c $120 \times$ Sr concentrations in natural seawater					

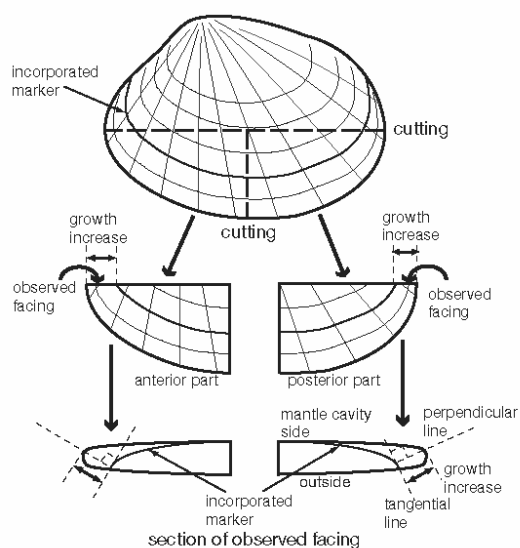


Fig. 1. Shell sectioning for detection of Sr-enriched and fluorescent areas. Shells were prepared to provide transverse section across longest shell axis; ventral portion was divided into anterior and posterior sections. Sr-enriched or fluorescent areas were examined at anterior and posterior margins

cal microscopy (Optiphot, Nikon) fitted with an ultraviolet (UV) light source (HB-10101AF, Nikon) and a fluorescence filter (V-1A, Nikon). In the ultra violet excitation wavelength, calcein appeared as a bright lime-green band in the sectioned shells.

Growth rate: Once the position of a bright band (SrCl_2) or a fluorescent band (calcein) had been determined, the growth increment between the band and the shell margin was measured under SEM for the

SMM or under optical microscopy for the FMM. Growth increments were measured as follows (Fig. 1):

(1) A tangential line was drawn close to an incorporated band on the sectioned shell; (2) a perpendicular line was drawn from a tangential line to the shell margin; (3) the maximum length of the perpendicular line from the tangential line to the shell margin was regarded as the growth increment of the anterior or posterior part of the sectioned shell; (4) the total growth increment equalled the sum of both the anterior and posterior growth increments.

Growth rate was calculated using the formula growth rate ($\mu\text{m d}^{-1}$) = L/D , where L is the total growth increase and D is the number of days post-treatment.

Results. Sr and calcein markings were both successful, but to different degrees.

Sr marking: Clear bright bands were visible on *Ruditapes philippinarum* shells after most immersion treatments, except at a concentration of 1.44 g l^{-1} for 24 h. The Sr-enriched bands tended to become more distinct with longer immersion periods and at higher SrCl_2 concentrations (Fig. 2). The band produced after 17 h immersion in solution with a SrCl_2 concentration of 0.72 g l^{-1} was faint (Fig. 2A). Although no clams died during any 17 h immersion period, 5 of 9 specimens immersed in SrCl_2 solution for 24 h died after being returned to the sea post-immersion. Of 10 control specimens (non-marked) 2 died during rearing in the sea fronting the JAMSTEC pier.

Sr concentration: Energy-dispersive X-ray spectrometer analysis confirmed that the bright bands in shells treated with SrCl_2 were due to greatly enhanced levels of the chemical. The Sr concentration in background areas in parts of the shell not treated with SrCl_2 showed characteristic X-ray counts of approximately 100 counts per 100 s (Fig. 3). However, the Sr concentrations in the bright bands in all treatments were notably higher than those in the background areas, indicating that Sr had accumulated in the bright bands.

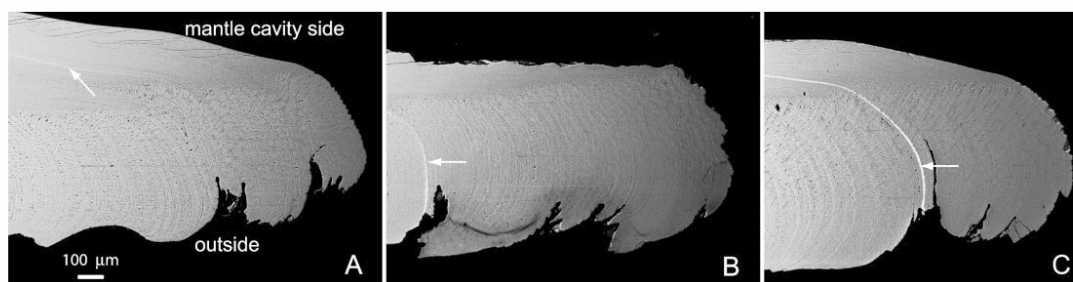


Fig. 2. *Ruditapes philippinarum*. Photomicrographs of back-scattered electron images (SEM) of anterior portions of shell sections after Sr marking. Arrows indicate bright Sr-enriched bands. (A) SrCl_2 conc. = 0.72 g l^{-1} , immersion period = 17 h; (B) SrCl_2 conc. = 2.88 g l^{-1} , immersion period = 17 h; (C) SrCl_2 conc. = 2.88 g l^{-1} , immersion period = 24 h

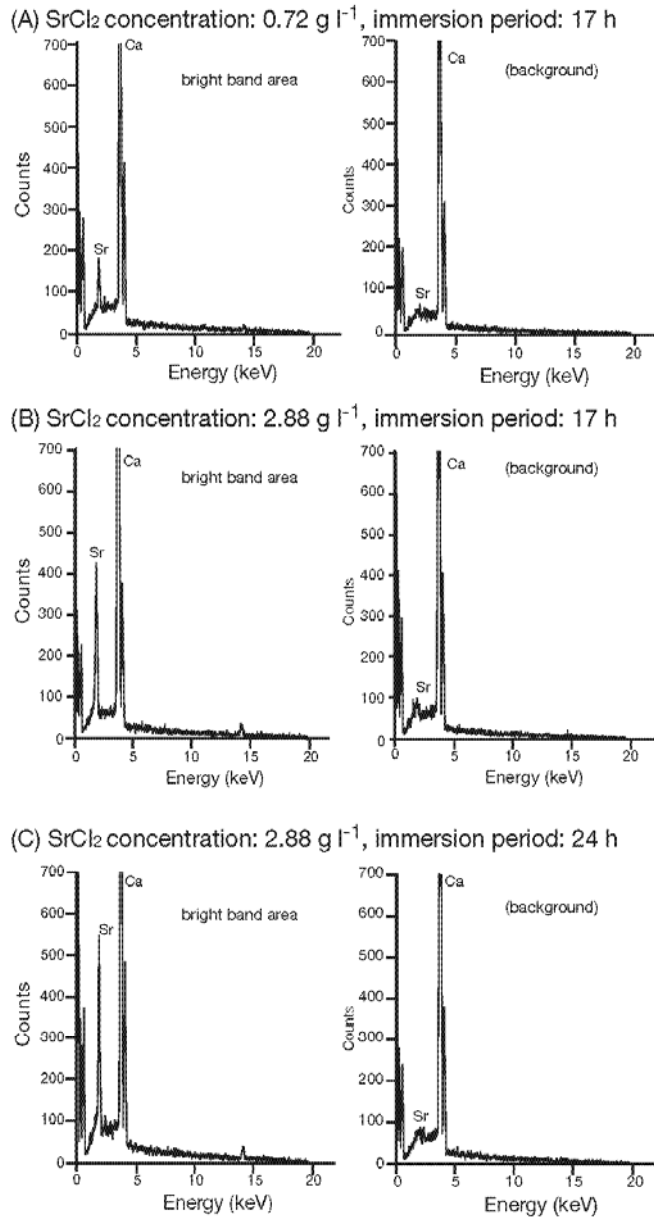


Fig. 3. *Ruditapes philippinarum*. Sr concentration (cps) determined by energy-dispersive X-ray spectrometry of bright band and background band areas in shell sections subjected to 3 different treatments. The Sr concentration in bright-band area is notably higher than that in background area

The Sr concentrations in the bright bands increased with increasing SrCl₂ concentration of the immersion solution (Fig. 3).

Calcein marking: Clams immersed in calcein solution had clearly visible fluorescent growth bands in their shells at all concentrations and exposures (e.g. Fig. 4); 2 specimens died after 24 h immersion in solution at a calcein concentration of 0.3 g l⁻¹. The fluorescent bands appeared more distinct after longer immersion and at higher calcein concentrations.

Higher magnification: The maximum magnification under the fluorescent optical microscope was 600× (eyepiece 15×, objective lens 40×) and the fluorescent bands in the shells could be detected at this magnification (Fig. 5B). In the back-scattered electron image under SEM, however, the Sr-enriched bands were detectable at a magnification of 1200× (Fig. 5A). The boundary between Sr-enriched and non-enriched areas appeared more distinct than that between fluorescent and non-fluorescent areas.

Growth rate measured using SMM: Under SEM, the microscopic growth rate could be estimated in single-digit μm based on the growth increment between an Sr-enriched band and the shell edge. At 19 or 20 d post-immersion, growth rates of *Ruditapes philippinarum* ranged from 13.5 to 116.8 μm d⁻¹; they tended to decrease with increasing shell length (Fig. 6).

Discussion. SrCl₂ was found to be a useful growth marker in the short-necked clam *Ruditapes philippinarum*. After incorporation into the shell, Sr-enriched areas were visible as bright bands in the back-scattered electron image (SEM), and were easily distinguishable from the non-Sr-enriched (background) areas (Fig. 2). Energy-dispersive X-ray spectrometric analysis of the clam shells showed that the bright bands were attributable to high SrCl₂ concentrations. The distinct and narrow bright bands incorporated into the growing margins of the shells at the time of Sr administration were successfully detected.

The SMM (1) allows detection of microscopic growth increases, and (2) requires only shorter post-immersion removal periods. Although the FMM method (Parsons et al. 1993, Kaehler & McQuaid 1999, Sato-Okoshi & Okoshi 2002) has similar advantages, it can generally only measure shell growth increments in tens of micrometers at magnifications between 400 and 600 \times (magnification of 600 \times in the present study [Fig. 5]; magnification of 400 \times for the postlarval giant scallop *Placopecten magellanicus* marked with Alizarin red dye and using compound microscopy in Parsons et al. 1993). Using the SMM, smaller shell growth increments can be detected with the SEM at a magnification of at least 1200 \times (Fig. 5).

Marking methods previously used to estimate bivalves growth rates required post-immersion periods after marker administration ranging from 4 wk to a few years (Shimizu et al. 1988, Parsons et al. 1993, Kesler & Downing 1997, Isono et al. 1998, McQuaid & Lindsay 2000, Sato-Okoshi & Okoshi 2002), whereas the SMM can estimate growth rate at least 19 to 20 d after post-immersion of the Sr marker because it is able to detect smaller microscopic-level shell growth increments.

The growth rates of *Ruditapes philippinarum* estimated with the SMM were similar to those reported in other studies, i.e. estimated growth rates of 13.5 to 116.8 $\mu\text{m d}^{-1}$ in the present study (Fig. 6) compared to approximately 10 to 100 $\mu\text{m d}^{-1}$ from July to August (same season as in the present study) using the FMM (Shimizu et al. 1998). This indicates that immersion in SrCl_2 solution did not impede the

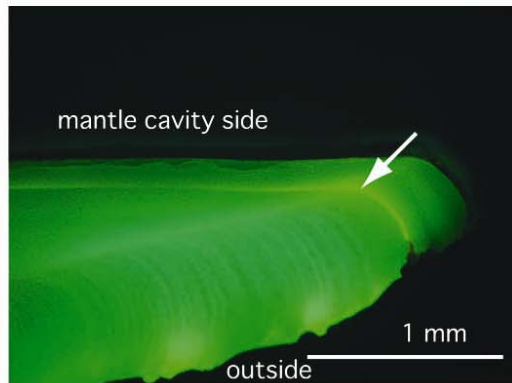


Fig. 4. *Ruditapes philippinarum*. Photomicrograph of shell section after fluorescent marking method (FMM) with calcein. Arrow indicates bright lime-green bands in calcein-enriched area. Photomicrograph was taken under fluorescent optical microscope. Calcein conc. = 0.72 g l $^{-1}$, immersion period = 24 h

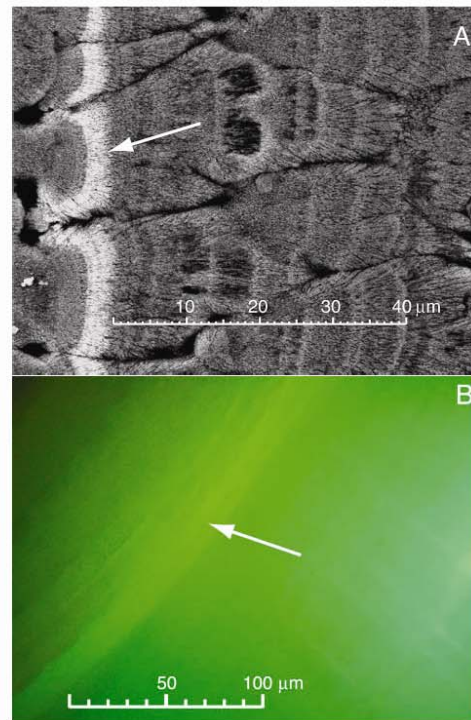


Fig. 5. *Ruditapes philippinarum*. Higher magnification of shell sections. (A) Back-scattered electron image (SEM; original magnification 1200 \times) of shell section marked with Sr; arrow shows Sr-enriched band; this specimen was treated with SrCl_2 concentration of 2.88 g l $^{-1}$ for immersion period of 17 h. (B) Fluorescence optical microscopic image (original magnification 600 \times) of shell section marked with calcein; arrow shows calcein-fluorescent band; this specimen was treated with SrCl_2 concentration of 0.72 g l $^{-1}$ for immersion period of 24 h

growth of *R. philippinarum* even though the experimental clams were immersed in seawater containing high concentrations of dissolved SrCl_2 and accumulated high levels of Sr in their shells. All specimens remained alive during the 17 h immersion period, but 5 of 9 died after 24 h immersion. Of 10 control specimens (non-marked), 2 also died. Whether immersion in SrCl_2 or calcein was fatally toxic to *R. philippinarum* could not be determined because the sample number was too small. The rearing conditions (in the sea in front of the JAMSTEC pier) may have been unsuitable for *R. philippinarum*. To avoid injurious effects, the immersion period should be as short as possible and the SrCl_2 concentration as low as

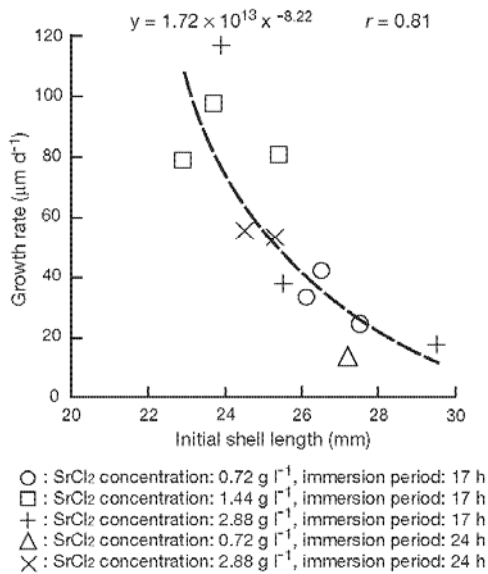


Fig. 6. *Ruditapes philippinarum*. Growth rate estimated by Sr marking method (SMM). After immersion in SrCl₂ in laboratory, the clams were reared in the sea for 19 or 20 d in July 2001

possible. The present experiment indicates that when the SMM is applied to *R. philippinarum* at the same element concentrations and water temperature as in the present study, an immersion period of 17 h (or probably less) is sufficient. Optimal marking conditions, including the minimum chemical concentration and immersion period, will be estimated in a future study.

Marking methods for growth-rate estimations in bivalves should involve long-lasting markers, easy detection, and simple methodologies. Classic marking methods using paint, bonded labels, and tagged labels may damage the marked specimens while exposing them to air, and such markers occasionally peel off the shells. The rust-marking method, while not harmful (Koshikawa et al. 1997), requires at least 14 d to mark shell surfaces. Microscopy employs a variety of fluorescent chemicals that are not easily distinguishable from the very similar natural autofluorescence (Day et al. 1995), and it is also difficult to detect the boundary between fluorescent and non-fluorescent areas, especially at higher magnifications (Fig. 5). The SMM eliminates these problems; however, it involves time-consuming shell preparation, and therefore is not suitable for the analysis of large numbers of samples. Despite this disadvantage, the SMM is simple and can efficiently detect microscopic growth increases (Table 2). Consequently, it can be used to estimate growth rates in small specimens and in slow-growing species such as deep-sea and polar bivalves. In addition, the method is useful for detecting growth rates over short experimental periods: by multiple marking of clam shells using the SMM, it is possible to estimate daily, weekly, monthly, tidal and seasonal growth rate variations in bivalves.

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Table 2. *Ruditapes philippinarum*. Comparison of advantageous characteristics among 4 marking methodologies for growth rate estimation of bivalves. Marker detection: ease of distinguishing between marked and background areas; +++: very good; ++: fairly good; +: poor

Marking	Method Detection	Advantages				
		Detection of microscopic growth increments	Marker longevity	Marking period	Marker detection	Shell preparation
Strontium chloride	Scanning electron microscopy	+++	+++	++	+++	+
Paint labels, tags, notches	Slide caliper	+	+	+++	+++	+++
Fluorescent chemicals	Optical microscopy	++	++	++	++	++
Rust	Electron-probe X-ray microanalyzer	+++	+++	+	+++	+

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「しんかい2000システム」による南西諸島海域鳩間海丘・黒島海丘の 化学合成生態系調査(生物・地球化学統合調査)

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2001年に引き続き、南西諸島海域の化学合成生態系において、生態系を構成する生物群集構造を定量的に把握すること、生物群集をとりまく化学環境を定量的に把握することを目的に、「しんかい2000」システムによる潜航調査を実施した。2002年の調査は、沖縄トラフの鳩間海丘と第四与那国海丘の熱水生態系、八重山諸島沖にある黒島海丘のメタン湧水生態系を対象に実施予定であった。しかしながら、表層の潮流が早く、第四与那国海丘の潜航は実施できなかった。本報告では、2002年に実施した鳩間海丘と黒島海丘における調査航海の概略について述べる。

キーワード: 化学合成生態系, 熱水噴出, メタン湧水, 黒島海丘, 鳩間海丘, 底生動物, 地球化学, バクテリア

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「しんかい2000システム」による南西諸島海域鳩間海丘・黒島海丘の 化学合成生態系調査(生物・地球化学統合調査)

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2001年に引き続き、南西諸島海域の化学合成生態系において、生態系を構成する生物群集構造を定量的に把握すること、生物群集をとりまく化学環境を定量的に把握することを目的に、「しんかい2000」システムによる潜航調査を実施した。2002年の調査は、沖縄トラフの鳩間海丘と第四与那国海丘の熱水生態系、八重山諸島沖にある黒島海丘のメタン湧水生態系を対象に実施予定であった。しかしながら、表層の潮流が早く、第四与那国海丘の潜航は実施できなかった。本報告では、2002年に実施した鳩間海丘と黒島海丘における調査航海の概略について述べる。

キーワード: 化学合成生態系, 熱水噴出, メタン湧水, 黒島海丘, 鳩間海丘, 底生動物, 地球化学, バクテリア

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Report on investigation of vent and methane seep ecosystems by the crewed submersible 'Shinkai 2000' and the ROV 'Dolphin 3K' on the Hatoma and the Kuroshima Knolls, the Nansei-shoto area

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Since 2001, ecological and geochemical investigations were conducted using the submersible 'Shinkai 2000' and the 'Dolphin 3K' at two hydrothermal vent fields (the Hatoma and the Dai-yon Yonaguni Knolls) and a methane seep field (Kuroshima Knoll) in the Nansei-shoto area. The purpose of this investigation was quantitative estimation for deep-sea chemosynthesis-based ecosystems. To accomplish for this purpose, we have some objectives including;

- 1) geochemical analysis of water at vent communities and vent fluids,
- 2) physical environmental factor analysis at vent communities and vent fluids,
- 3) estimation of energy sources for vent ecosystems,
- 4) biodiversity of vent communities,
- 5) estimation of biomass of vent communities,
- 6) estimation of productivity of vent communities,
- 7) relationships of biomass and productivity between microbial communities and benthic communities,
- 8) embryological study of vent benthic species.

Unfortunately, we could not investigate at the Dai-yon Yonaguni Knoll, because water current was too rapid for submersible diving survey. In this paper, we present an outline of the investigation.

Keywords : chemosynthesis-based ecosystem, hydrothermal vent, methane seep, Kuroshima Knoll, Hatoma Knoll, benthic species, geochemical analysis, bacteria

1. はじめに

化学合成生態系は、地球内部から噴き出す硫化物やメタンをエネルギー源として細菌が生産者となりバントスが消費者となった系である。系内におけるエネルギー・物質移動を通じて、とりわけメガバントスの生物量は20-30kg/m²を越す莫大なものになっている。これまで、化学合成生態系を支える熱水や冷湧水の化学物質濃度と細菌現存量・増殖量の関係(例えばKarl et al., 1980; Baross et al., 1984; Naganuma et al., 1989), 化学物質濃度とメガバントスの現存量の関係については研究例がある(例えばHenry et al., 1992; Hashimoto et al., 1995; Ohn et al., 1996; 1997; Sarrazin et al., 1999; Gebruk et al., 2000)。しかしながら、化学合成生態系において一次生産者の生産力と消費者の生産力の相関を統合した研究はない。地球生物圏は光合成と化学合成(地下生物生態系も含む)を基点とした物質循

環系で支えられていることが分かりつつある現状を踏まえ、深海化学合成生態系をモデルとして生態系の物質生産力を評価することは、地球上の生態系の機能を理解する上で有意義である。このような視点から、「化学合成生態系における生物生産力の定量化研究」を提案し、2001, 2002年に「しんかい2000」システムによる調査を実施した。2001年には沖縄トラフの第四与那国海丘および鳩間海丘の潜航調査を実施し、2002年は2001年の成果を踏まえ、得られなかったデータ・サンプルの取得、近傍にある八重山諸島沖の黒島海丘のメタン湧水生態系においても調査研究を実施する予定であったが、第四与那国海丘では表層の潮流が早く潜航できなかった。

本報告では鳩間海丘の熱水生態系、黒島海丘のメタン湧水生態系を対象として、化学物質濃度、生産者の現存量・生産量、消費者の現存量・生産量を定量化し熱水生態系と

冷湧水生態系間で比較することを目的に調査を行った。そして、以下のような課題を主体に調査研究を進めた。

【生態系のエネルギー流に関する研究】

- 化学物質の組成・濃度はどれくらいか
- 生態系のエネルギー源となる主な化学物質は何か(メタン/硫化物)
- メタンおよび硫化物の起源はどこか(地球内部/海水/微生物生産)

【生産者に関する研究】

- 浮遊性細菌の現存量・生産量はどれくらいか
- 共生細菌を保有するホストの共生細菌現存量はどれくらいか
- 附着性細菌の現存量・生産量はどれくらいか

【消費者に関する研究】

- シロカイヒバリガイ類の現存量・生産量はどれくらいか。

調査結果は現在解析中のものがほとんどであるため、本報告では潜航調査結果の概略を報告する。

2. 潜航調査

2002年5月21日と22日の2日間に、黒島海丘において「しんかい2000」の事前調査を目的に「ドルフィン-3K」で5回偵察潜航を行った(Table 1)。偵察潜航の妨げにならない範囲で、測定機器の設置、サンプル採集、観察を行った。2002年5月27日から7月1日にかけては、「しんかい2000」を用いて鳩間海丘で9回、黒島海丘で5回の潜航調査を実施した(Table 2)。うち鳩間海丘の2回(第1353・1361潜航)は、深海版「ミラーの実験」に用いるメンブレンフィルターの現場試験(提案者:藤原義弘)が主目的であった。

3. 研究項目と方法

3.1. 生態系のエネルギー流に関する研究

3.1.1. 地質学的背景・化学物質の組成と濃度

鳩間海丘における熱水活動の特徴を明らかにするため、海底地形、露頭の観察と、岩石および熱水性沈殿物試料の

採取を行った。また、黒島海丘のメタン湧水活動と地質背景を解明するため、海底面直下に生成している炭酸塩クラスト、ガス、湧水、堆積物、岩石のサンプリングを行った。コア試料からは還元層の発達状態や間隙水の化学組成および化学合成生物のエネルギー源となる硫化水素やメタンの起源を解析する。

3.1.1.1. 保圧式採水器による試料の採取

湧水域における溶存ガス濃度は一般に高いが、従来のポンプ式採水器やニスキン採水器を用いた採水器ではガス成分が脱け損失している。従来法の問題点を克服し、より高度な時空間的変動の解析を可能にする保圧式採水器を活用した。この装置は、揮発性成分を多量に含んだ海水試料を採取し、あるいは揮発性成分そのものを損失のない試料保圧容器に保存し、回収することができる。採取された試料は全金属製の保圧容器内に海底における条件のまま保持されるため、船上で回収されるまで揮発成分の散逸は起きないようにしている。保圧式採水器に加えて、ニスキン採水器を用いたその周辺の海水、堆積物中の間隙水の採取も行った。

試料の同位体比は、真空ライン中で開放し脱ガスさせ、その一部を研究室に持ち帰り連続フロー型質量分析法(Tsunogai et al., 1998)により測定する。この方法により低濃度試料や他成分混合試料の同位体定量が可能になる。

3.1.1.2. 熱水試料の化学分析

熱水試料は各種化学分析に供するが、保存がきかない成分(pH, alk., NH₃, H₂S, SiO₂)については、ガラス電極を用いてpH、電位差測定法によってアルカリ度、比色法(蒲生・ギースケス, 1992)によってシリカ、アンモニア濃度を船上で分析した。主成分については、ICP発光分光分析・原子吸光分光分析・イオンクロマトグラフィーなどで分析を行う。

3.1.1.3. 温度計測

保圧式採水器の試料吸入口には白金抵抗測温体のプローブが埋め込まれており、採取する熱水の温度を毎秒測定した。鳩間海丘におけるヘイトウシロカイヒバリガイ成長速

表1 黒島海丘における無人探査機「ドルフィン-3K」の潜航リスト

Table 1 Data relevant to 5 dives by ROV "Dolphin-3K" in the Kuroshima Knoll, the Nansai-shoto area

Dive No.	Date	Start point		End point	
		Lat. Long.	Depth	Lat. Long.	Depth
D3K #554	2002.5.21	24-07.849°N 124-12.068°E	686m	24-07.823°N 124-11.538°E	642m
D3K #555	2002.5.21	24-07.827°N 124-11.584°E	642m	24-07.816°N 124-11.154°E	646m
D3K #556	2002.5.21	24-07.945°N 124-11.564°E	661m	24-07.795°N 124-11.538°E	642m
D3K #557	2002.5.22	24-07.735°N 124-11.802°E	662m	24-07.804°N 124-11.542°E	642m
D3K #558	2002.5.22	24-07.786°N 124-11.600°E	647m	24-07.812°N 124-11.366°E	644m

表2 鳩間海丘および黒島海丘における「しんかい2000」の潜航リスト

Table 2 Data relevant to 12 dives by "Shinkai 2000" in the Hatoma and Kuroshima Knolls, the Nansai-shoto area

Dive No.	Date	Scientist	Pilot Co-pilot	Start point		End point	
				Lat. Long.	Depth	Lat. Long.	Depth
Hatoma Knoll							
2K #1352	2002.5.27	Tsuchida, S.	Chiba, Kazu Mitsufuji, K.	27-47.540'N 126-53.863'E	1520m	27-47.406'N 126-53.958'E	1467m
2K #1353	2002.5.28	Fujiwara, Y.	Ueki, H. Chida, Y.	24-51.470'N 123-50.395'E	1523m	24-51.455'N 123-50.477'E	1488m
2K #1354	2002.5.29	Kato, K.	Mitsufuji, K. Mizui, Y.	24-51.470'N 123-50.395'E	1523m	24-51.452'N 123-50.479'E	1470m
2K #1358	2002.6.5	Fujikura, K.	Ueki, H. Chida, Y.	24-51.495'N 123-50.378'E	1524m	24-51.580'N 123-50.389'E	1492m
2K #1359	2002.6.6	Yamanaka, T.	Chiba, Kazu Mizui, Y.	24-51.461'N 123-50.405'E	1522m	24-51.448'N 123-50.485'E	1480m
2K #1361	2002.6.9	Fujiwara, Y.	Chiba, Kazu Mizui, Y.	24-51.453'N 123-50.472'E	1492m	24-51.591'N 123-50.388'E	1472m
2K #1362	2002.6.10	Toth, L. G.	Ueki, H. Chida, Y.	24-51.547'N 123-50.478'E	1485m	24-51.579'N 123-50.404'E	1512m
2K #1369	2002.6.28	Yamamoto, H.	Chiba, Kazu Chida, Y.	24-51.534'N 123-50.427'E	1509m	24-51.581'N 123-50.427'E	1515m
2K #1371	2002.7.1	Ishibashi, J.	Matsumoto, K. Chida, Y.	24-51.574'N 123-50.432'E	1522m	24-51.612'N 123-50.369'E	1456m
Kuroshima Knoll							
2K #1355	2002.6.1	Fujikura, K.	Chiba, Kazu Mizui, Y.	24-07.728'N 124-11.618'E	652m	24-07.813'N 124-11.755'E	648m
2K #1356	2002.6.2	Mizota, C.	Ueki, H. Chida, Y.	24-07.737'N 124-11.577'E	645m	24-07.826'N 124-11.561'E	636m
2K #1357	2002.6.3	Okoshi, K.	Mitsufuji, K. Chiba, Katsu	24-07.745'N 124-11.508'E	644m	24-07.805'N 124-11.567'E	640m
2K #1360	2002.6.7	Nakayama, N.	Mitsufuji, K. Chiba, Katsu	24-07.763'N 124-11.463'E	642m	24-07.814'N 124-11.150'E	640m
2K #1370	2002.6.30	Miyazaki, J.	Ueki, H. Chiba, Katsu	24-07.813'N 124-11.558'E	641m	24-07.816'N 124-11.375'E	640m

度測定用放流ネットには自己記録式温度計を一緒に設置して係留中の温度データを計測した。この温度計はアレック電子製MDS MARK5である。データ取得は1分ごとに行った。

3.1.2. 生態系のエネルギー源

化学合成生態系の生産者は細菌である。よって、細菌が利用する化学物質が生態系のエネルギー源となる。そこで細菌の分子系統解析から細菌の特性を推定する。具体的には、シンカイヒバリガイ類やハオリムシ類などの共生細菌や、堆積物中の細菌を対象に、16SリボソームRNA遺伝子解析、脂肪酸などバイオマーカー解析や安定同位体組成から、細菌が利用する化学物質を推定する。

3.1.3. 食物連鎖

化学合成生態系の環境水において、メタンおよび硫化水素の取り込み過程の分別効果は無いとされているから、硫黄ないし炭素源となる湧水中のメタンと硫化水素の安定硫黄同位体比はそのまま生物体に持ち込まれる。このことを利用して食物連鎖解析を行う。生物試料は、いくつかの主

要な組織に分け、炭素-窒素同位体組成をDELTA plus質量分析計で測定する。硫黄安定同位体の34S/32S比はSIRA 10質量分析計を用いて行う。

ゴエモンコシオリエビ *Shinkaia crosnieri* の腹側剛毛には、糸状バクテリアマットの付着が認められ、それをゴエモンコシオリエビが摂食している可能性がある。そこで、ゴエモンコシオリエビの胃内容物と腹側剛毛に付着する細菌のDNAを比較し、腹側剛毛に付着する細菌を摂食しているかどうか明らかにする。

3.2. 生産者に関する研究

3.2.1. 浮遊性・付着性細菌の細菌相・現存量・生産量

浮遊性細菌の現存量・細菌相に関しては、ニスキン採水器により熱水域、シンカイヒバリガイ類生息域、コントロール域などから採水した試料を基に、DNA解析、細胞数計数から評価する。生産量は、現場海水をfilter試水とnon-filter試水に分けdiffusion chamberに入れて *in-situ* で培養し両者の差

から推定する(Toth et al., 2000)。付着性細菌の増殖量は、スライドガラスを付着基質にしたトラップを熱水域、シンカイヒバリガイ類生息域に設置し付着する細菌数から評価する。

3.2.2. 共生細菌の細菌相・現存量

シンカイヒバリガイ類やハオリムシ類の共生細菌の組成・現存量を推定するために、脂肪酸やキノンといったバイオマーカー(生物指標性有機化合物)の解析を行う。生物が生産する有機化合物は、その多様性に基いた生理代謝機能の違いを反映し生産する有機化合物も多様である。特に細胞膜などの膜組織の主要構成成分である脂肪酸は微生物種ごとに明確な違いがあり、バイオマーカーとして有効である。ただし、古細菌は脂肪酸を生産しないため、その膜組織に特徴的であるイソプレノイド炭化水素について分析を行う。キノンについては、最近の研究でキノン量から微生物量の評価に有効であることがわかり(Yamamoto et al., 2002)、本研究でも活用する。共生細菌の細菌相については、生態系のエネルギー源を推定する際に行う遺伝子解析から情報が得られる。

3.3. 消費者に関する研究

3.3.1. メガベントスの現存量・増殖量・動物相に関する研究

メガベントスは、マニピュレータにより直接捕獲またはサクショサンプリャーを用いて採集した。また、30×30cmの方形枠もしくは50cmのスケールバーを設置し、その枠内の生物を採集もしくは写真撮影し、定量採集として現存量分析に供した。これらのサンプルは、個体数、湿重量、乾燥重量、炭素量の測定に供せられる。

優占的に生息するベントスについて、成長速度の見積もるために*in-situ*でストロンチウムマーキング法による標識放流実験(mark-release-recapture)を実施した。対象としたベントスは、黒島海丘の*Bathymodiolus* short-typeと*Bathymodiolus long-type*、鳩間海丘のヘイトウシンカイヒバリガイ*Bathymodiolus platifrons*とした。標識放流実験は、現場より採取した実験個体の貝殻にストロンチウムでマーキングし、その個体を現場に再放流、数日から数日後に再捕獲する方法とした。また、シンカイヒバリガイ類の成長過程における貝殻構造や形成特性を把握するために、貝殻各部を走査型電子顕微鏡で観察し貝殻構造を決定する。

3.3.2. その他のメガベントス研究

ハオリムシ*Vestimentifera*の初期生活史を明らかにするために、初期発生の経時的变化および着生、変態過程を追跡することを試みた。採集されたハオリムシの一部を解剖し、卵嚢の中に詰まっている卵を採り、発生段階を観察した。

鳩間海丘に優占的に生息するオハラエビ*Alvinocaris longirostris*、ゴエモンコシオリエビ、シンカイヒバリガイ類などの遺伝子配列を決定する。これまでに得られている試料と比較し、沖縄トラフ内の熱水域に生息する種の遺伝的変異、西太平洋の近縁種とも比較し、種分化や生物地理学的特性について考察する。

4. 結果概要

4.1. 生態系のエネルギー流

4.1.1. 地質学的背景・化学物質の組成と濃度

4.1.1.1. 鳩間海丘

カルデラ状凹地内は安山岩質溶岩によって複雑な地形を呈し、角礫化した溶岩の急崖が随所に認められた。熱水活動は溶岩からなる崖の上方にあることが多く、大きな硫化物マウンドを形成しているように思われる。昨年の航海では硫化物からなるチムニーはほとんど回収されていないが、本航海では黒鉄様硫化物が多く回収された。カルデラ底東側の熱水域は巨大なチムニー群があり、最大比高は約40mであった。ところどころから二酸化炭素と思われる泡が湧出し、この泡はオーバーハングしている岩の下にたまることもあり、



写真1. 岩の隙間に蓄積している二酸化炭素ハイドレート?。
Photo 1. Accumulation of CO₂ hydrate?.

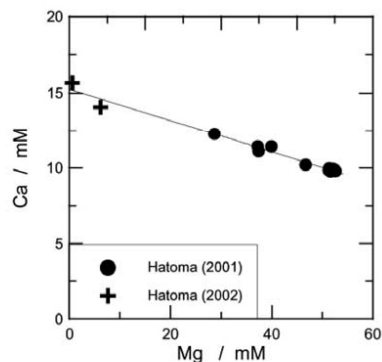


図1. 鳩間海丘の熱水試料のMg濃度とCa濃度の関係。●: 2001年に得られた試料の分析結果、+: 2002年に得られた試料の分析結果。

Fig.1. Relationship between magnesium (Mg) concentration and Calcium (Ca) concentration of vent fluid collected from the Hatoma Knoll. ●: Data in 2001 investigation, +: Data in present investigation.

粘性の高い泡(ハイドレート?)が集まっていた(写真1)。また別の熱水域で比高約20-30mのチムニーがあり、二酸化炭素ハイドレートがストロー状の筒からわき出す様子も観察できた。カルデラ底南側では活発に噴出する熱水を視認し、水温の計測を行ったところ最高で297°Cであった。

熱水試料のマグネシウム濃度とカルシウム濃度の関係をFig.1に示した。マグネシウムは熱水成分の指標物質であり、純粋な高温熱水中の濃度が0であることを利用して試料中の海水混入比を推定するものである。保圧で採取された試料はマグネシウム濃度が0に近いもので純度の高い試料が得られたことがわかる。またすべての試料の分析結果は、図中に示した混合直線のまわりにプロットされていることから、鳩間海丘の高温熱水の化学組成は主成分については一つのエンドメンバーで代表されるものであることが示唆される。また高温熱水の組成は調査が始まった1999年以来ほとんど変動が見られない。またシリカ濃度からは、海底直下

での熱水岩石反応が平衡状態になっていることが示唆されている。熱水噴出孔に採水口を挿入して採水した試料については、塩分が約22前後と低かった(2K #1359 W-3, 2K #1361 W-1,2,3) (Table 3)。また、その時のpHも約5.6と低い値であった。アルカリ度(Alk)は、海水中へ火山性ガスが溶入したことを示すほどには低い値ではなかった。

4.1.1.2. 黒島海丘

海底は一見平坦に見えるが、石灰岩のpavementが海底直下に厚く発達しており、それが複数の亀裂により分割され正断層のようにずれるなどしているためか、非常にでこぼこした海底面をなしていた。エンセイシロウリガイ *Calyptogena solidissima* 死殻分布域およびシンカイヒバリガイ類の分布域にはメタンの発泡現象が見られた。海底表面は僅かに泥質物が堆積しているのみで、その下は貝の死に殻からなる砕屑物が数cm~十数cm被っており柱状採泥器による堆積物の採取は困難であった。

化学環境測定用の海水は、*Bathymodiolus short-type*,

表3 鳩間海丘の熱水域および黒島海丘のメタン湧水域から採水したサンプルの船上分析結果。N: ニスキン採水器, W: 保圧採水器。

Table 3 Results of onboard chemical analysis for water sample from the Hatoma (vent) and the Kuroshima (methane seep) Knolls. N: Niskin water bottle, W: Water and Hydrothermal-fluid Atsuryoku Tight Sampler.

Hatoma Knoll					Kuroshima Knoll				
Dive No.	Sample	Sali.	pH	Alk. (mM)	Dive No.	Sample	Sali.	pH	Alk. (mM)
2K #1352	N-1	35	7.69	2.50	2K #1355	N-1	33	7.38	2.40
	N-2	35	7.67	2.44		N-2	33	7.39	2.35
	N-3	35	7.65	2.45		W-1	32	7.40	2.28
2K #1353	N-1	34	5.75	2.47	2K #1356	W-3	32	7.36	-
	N-2	34	7.51	2.46		N-1	32	7.34	2.35
2K #1354	N-1	32	6.74	2.47	2K #1357	W-1	32	7.34	2.27
	N-2	32	7.29	2.41		W-3	32	7.26	-
2K #1358	N-1	32	6.06	2.36	2K #1360	N-1	33	7.40	2.22
	N-2	34	7.23	2.62		W-2	31	7.37	2.38
2K #1359	N-1	34	7.14	2.53	2K #1362	W-3	32	7.62	-
	N-2	30	5.37	2.15		W-4	33	7.60	-
	W-3	32	6.81	2.51		N-1	32	7.38	2.51
2K #1361	N-1	33	7.06	2.33	2K #1363	N-2	32	7.37	2.39
	N-2	32	6.51	2.37		W-1	31	7.37	2.40
	W-1	22	5.79	-		W-2	31	7.32	2.29
2K #1366	N-1	32	7.12	2.32	2K #1364	W-2	31	7.32	2.29
	N-2	32	6.88	2.48		N-1	32	6.27	2.06
	W-1	21	5.59	2.40		N-2	33	6.23	2.15
2K #1369	W-2	21	5.59	2.44	2K #1367	N-1	35	7.34	2.16
	W-3	22	5.58	2.17		N-2	34	7.37	2.09
	W-1	35	7.37	2.22		W-1	34	7.34	2.16
2K #1369	W-2	35	7.36	-	2K #1367	W-3	35	7.33	2.30
	N-1	35	6.38	2.37		W-1	32	7.322	2.67
	N-2	35	7.29	2.34		W-3	32	7.35	2.36
	W-3	33	5.54	2.67		W-2	35	7.36	2.20

Bathymodiolus long-type, エンセイシロウリガイの各集団上、ガス噴出域など多様な地点で保圧採水器やニスキン採水器により採水した。ガス噴出域ではアルカリ度が高かった (Table 3)。これは、海底または海底下で炭酸カルシウムが溶解したことによるものと推定される。

4.1.2. 食物連鎖解析

生物群集中の環境水からメタンの炭素同位体組成の変動が解析される。これらのデータと、同時に採取したベントス軟組織の炭素・窒素同位体組成との関連を解析することによって、化学合成生態系内のエネルギーの流れが解析できる。また、鳩間および黒島海丘の3種のシンカイヒバリガイ類から成長段階が異なる試料が得られ、成長に伴う栄養摂取様式の変動が解析できる。ゴエモンコシオリエビも採集でき、腹側剛毛の糸状バクテリアマットと胃内容物に含まれる細菌のDNAが比較される。

4.2. 生産者に関する研究

4.2.1. 浮遊性・付着性細菌・共生細菌の細菌相・現存量・増殖量

4.2.1.1. バイオマーカーによる微生物構成とバイオマスの推定

今航海では、昨年は得られなかった黒島海丘の堆積物および岩石と、鳩間海丘の硫化物チムニーをサンプリングすることができたので、これらについて有機成分の抽出を行い、微生物構成およびバイオマスの推定を行う。

Diffusion chamberを用いた浮遊性細菌およびスライドガラスを用いた付着性細菌の増殖量評価のための *in-situ* 実験は、それぞれ回収されたサンプルを解析中である。予察的な観察では、浮遊性細菌の増殖量はとても早いことが示唆された。

本航海において採集した底生生物のうち、先行研究からの類推および船上での解剖の結果、化学合成細菌を共生者する可能性のあるものは以下の通りである。

- ・ヘイトウシンカイヒバリガイ(鳩間海丘)
- ・*Bathymodiolus short-type* (黒島海丘)
- ・*Bathymodiolus long-type* (黒島海丘)
- ・エンセイシロウリガイ(黒島海丘)
- ・オトヒメハマグリ類 *Vesicomysidae* gen. sp. (黒島海丘)
- ・ハオリムシ類(黒島海丘)

このうちシンカイヒバリガイ類3種については、2001年の調査で採集した試料を用いて分子系統解析を実施済みであり、共にメタン酸化細菌を共生者とすることが示唆されている。黒島海丘に生息するエンセイシロウリガイの共生細菌に関する情報は皆無であるが、これまで知られるシロウリガイ類と同様に鰓が乳白色で肥大していること、これまで分子系統の報告されたシロウリガイ類は全て硫黄細菌を共生者とすることから、この海域に生息するシロウリガイ類も同様に硫黄細菌が細胞内共生すると推定する。黒島海丘に生息するハオリムシ類の共生細菌に関する情報も皆無であるが、これまで分子系統解析結果が報告されたハオリムシ類は全て硫黄細菌を共生者とすることから、この海域に生息するハオリムシ類も同様に硫黄細菌が細胞内に共生すると推定する。上記試料については航海終了後、電子顕微鏡観察、分子系統解析および *in-situ* ハイブリダイゼーションを行い、共生細菌

の有無や特徴を明らかにする予定である。また、共生細菌の現存量を推定するために、これらの動物の一部はバイオマーカー(脂肪酸・キノン)分析に供される。

4.3. 消費者に関する研究

4.3.1. メガベントス動物相

4.3.1.1. 黒島海丘

海丘頂部には、ガス噴出がある生物量の非常に多い地域 site A と、炭酸塩チムニーやエンセイシロウリガイの死殻が散乱する生物量の少ない地域 site B (site A の西側約400m) の2タイプの海底環境が確認された。site B には、白色のバクテリアマットらしき変色域が数箇所認められた。site A の豊かな生物群集は、*Bathymodiolus short-type* が優占するものと(写真2)、*Bathymodiolus long-type* が優占するものと(写真3)に分けられた。このシンカイヒバリガイ類の集団は日本周



写真2. 黒島海丘メタン湧水域における *Bathymodiolus short-type* が優占する生物群集。

Photo 2. *Bathymodiolus short-type* dominated community associated with methane seep in the Hatoma Knoll.



写真3. 黒島海丘メタン湧水域における *Bathymodiolus long-type* が優占する生物群集。

Photo 3. *Bathymodiolus long-type* dominated community associated with methane seep in the Hatoma Knoll.

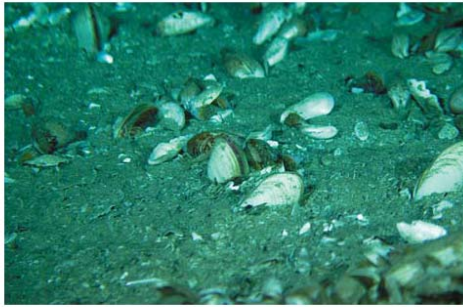


写真4. 黒島海丘メタン湧水域におけるエンセイシロウリガイ。
Photo 4. *Calyptogena solidissima* associated with methane seep in the Hatoma Knoll.

辺では最大規模と思われる。

(1) site Aのうち*Bathymodiolus short-type*が優占する群集

(軟体動物Mollusca) 得られたサンプルには*Bathymodiolus long-type*もわずかに混獲されており、両種は同所的に生息していると思われる。ただし*Bathymodiolus short-type*は、露頭など基質に付着しているが、*Bathymodiolus long-type*は堆積物中に殻の後端部を埋没させることが多い。エンセイシロウリガイの死殻も多く、わずかに生貝が分布する(写真4)。生貝および死殻で形成される複雑な間隙を形成する構築物は、他の生物に棲み場所を提供しており、例えば多毛類のものと思われる白色石灰質? 棲管や小型で白色のヒザラガイの付着も認められた。エンセイシロウリガイは底質に刺さったような状態で生息しており、小型の個体ほど底質に埋まっている部分が多い傾向があった。その他には、ツキガイ類Lucinidae gen. sp.の死殻、オトヒメハマグリ類Vesicomidae gen. sp.、ワタゾコシロアミガサガイモドキ*Bathymacra* sp.、サガミハイカブリナ*Provanna* sp.、エンバイ類Buccinidae gen. sp.、シンカイサンショウガイ*Cantreina jamsteci*、ワタゾコシタダミ類Skeneidae gen. sp.が出現した。エンバイ類は、通常化学合成生物群集にはゲスト種であるが、この群集以外の通常海底からは見つからなかった。

(甲殻類Crustacea) 一見するとユノハナガニ類Bythograeidaeに類似したカニが認められたが、採集された個体はエンコウガニ類Goneplacidaeと思われた。カニを採取するために持ち込んだ餌に、ヨコエビ類Amphipodaが飼集してきた。

(多毛類Polychaeta)*Bathymodiolus short-type*の約半分には外套腔に多毛類が寄生していた。ほかにも複数種出現した。

(有鬚動物Pogonophora) ハオリムシは、活発にガスが噴出しシンカイヒバリガイ類が卓越する地域には生息せず、その周囲の泥質の地域に生息していた。また、確認されたハオリムシは相模湾の*Lamellibrachia* sp.に比べ、いずれも大型個体ばかりで棲管長は1.5m以上になる(写真5)。現在のところ日本最大かもしれない。



写真5. 黒島海丘から得られたハオリムシ。「なつしま」第3ラボラトリーにて撮影。

Photo 5. Vestimentiferan tubeworm from the methane seep area in the Hatoma Knoll.

(棘皮動物Echinodermata) クモヒトア類Ophiuroidea, フクロウニ類Echinothuriidae (オーソンフクロウニ*Araeosoma owstoni*を含む)が出現した。特に、フクロウニ類は個体数も多く、またサイズも15-20cmぐらいと大型であった。

(星口動物Sipuncula) 炭酸塩岩の孔などに生息しており個体数も多かった。

(脊椎動物Vertebrate) メクラウナギ類Myxiniiformes, エイ類Rajiformes, アカグツ類Ogcocephalidaeが散見された。

(2) site Aのうち*Bathymodiolus long-type*が優占する群集

(軟体動物Mollusca) *Bathymodiolus long-type*は、殻の後端部を埋没させ生息している場合と、露頭の基部などに付着しながら生息している場合があった。いずれも、*Bathymodiolus long-type*だけでパッチを形成していた。周辺には*Bathymodiolus short-type*も生息しているが、*Bathymodiolus long-type*のパッチに*Bathymodiolus short-type*が混在することはないように見えた。*Bathymodiolus long-type*は、生殖腺がよく発達していること、鰓が赤いことが特徴的である。エンセイシロウリガイの死殻も多く、ワタゾコシロアミガサガイモドキも散見できた。サンプルには小型の白色のエンバイ類もしくはクダマキガイ類Turridaeも多量に含まれていた。

(多毛類Polychaeta) *Bathymodiolus long-type*の外套腔からは寄生多毛類は認められなかった。

(星口動物Sipuncula) 第1357潜航のサンプルに多量に含まれていた。

4.3.1.2. 鳩間海丘

鳩間海丘の動物相についてはすでに報告されている(土田ほか, 2000; 藤倉ほか, 2001)ので本報告では詳しく述べ

ない。ヘイトウシンカイヒバリガイの成長速度を測定するために昨年設置した3つの放流ネットは、設置点から1~5m離れており、すでにヘイトウシンカイヒバリガイ群集上にはなく砂泥底上に点在していた。この周辺には、大型のゴカクエンゾイバラガニ *Paralomis verrilli* が数個体生息していたことから、これらに放流ネットが運ばれてしまったのかもしれない。熱水域の周辺には、ヘイトウシンカイヒバリガイ、ゴエモンコシオリエビ、オハラエビ、フネカサガイ類 *Lepetodrilus sp.*などを優占種とした生物群集が高密度に分布していた(写真6)。熱水域に接するように分布するヘイトウシンカイヒバリガイ表面には多数のフネカサガイ類が付着していた。ゴエモンコシオリエビの体表にはフネカサガイ類に加え多毛類も付着していた。これまでに、鳩間海丘からは20種以上のメガベントスが出現しており(土田ほか, 2000; 藤倉ほか, 2001)、今回初めて採集した種としては、ウミケムシ類 *Euphrosinidae gen. sp.*、ニシキウズ類 *Trochidae gen. sp.*、星口動物が出現した。

4.3.2. 現存量・生産量

化学合成生物群集域内で30×30cmの定量枠内のステレオカメラ撮影を行い、密度・バイオマスを算出するデータを取得できた。シンカイヒバリガイ類を対象とし、ストロンチウムマーキング法により成長速度の測定実験を試みたが、鳩間海丘より黒島海丘のほうが再捕時の生残率が高かった。実験に供した個体は、走査型電子顕微鏡で観察し放流期間の成長量を見積もる予定である。

シンカイヒバリガイ類の成長過程における貝殻構造に関する研究では、鳩間海丘のヘイトウシンカイヒバリガイ、黒島海丘の *Bathymodiolus short-type* と *Bathymodiolus long-type* では殻皮の剥離状況が異なっていた。ヘイトウシンカイヒバリガイの殻皮剥離(欠損)が殻長50mm前後からはじまるのに対し、*Bathymodiolus short-type* と *Bathymodiolus long-type* では50mm以下でも欠損のある個体が見られ、貝殻の溶解が成長の早い時期からみられることが示唆された。最大個体の殻長は3種とも110-120mm前後で、*Bathymodiolus long-*



写真6. 鳩間海丘の熱水噴出域周辺に分布するヘイトウシンカイヒバリガイとオハラエビの集団。

Photo 6. Dense clusters of the deep-sea mussel, *Bathymodiolus platifrons*, and shrimp, *Alvinocaris sp.*

type は成長途中から腹縁部にくびれが見られるようになっていた。貝殻の厚さはヘイトウシンカイヒバリガイが薄く、他2種は厚い傾向があった。足糸はヘイトウシンカイヒバリガイと *Bathymodiolus short-type* は細く容易に切断されるが、*Bathymodiolus long-type* は太いうえ量も多く付着力も強い傾向にあった。

4.3.3. ハオリムシの初期生活史

黒島海丘においてハオリムシが1個体採集された。解剖により多数の卵が得られたが、未受精卵であったため発生が進まなかった。

5. おわりに

本報告は、2002年度に実施した「しんかい2000」システムによる調査航海クルーズレポートを基に作成した。この航海で得られた成果は、順次専門雑誌や学会に報告されるが、一部については既に学会等で発表されている(小坂ほか, 2002; 溝田ほか, 2002; 大越徳嗣ほか, 2002; 大越和加ほか, 2002; 山中ほか, 2002; Yamanaka et al., 2002など)。最後に、本調査研究は、「しんかい2000」運航チーム並びに「なつしま」乗組員の多大なご協力により成り立った。また、海洋科学技術センター研究業務部の方々、海洋生態・環境研究部の方々、日本海洋事業(株)の方々からもご支援をいただいた。ここに心より深謝する。

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A New Species of *Gigantidas* (Bivalvia: Mytilidae) from a Vent Site on the Kaikata Seamount Southwest of the Ogasawara (Bonin) Islands, Southern Japan

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Abstract: A new species of mytilid mussel, *Gigantidas horikoshii* n. sp., is described. This new species was found burrowing in a sandy bottom from which warm water was seeping out, on the submarine volcano Kaikata Seamount, located southwest of the Ogasawara (Bonin) Islands in Southern Japan. *G. horikoshii* n. sp. is characterized by the absence of a well-developed valvular siphonal membrane, the absence of a continuation of the inner mantle fold across the anterior adductor muscle and by the attachment point of the anterior retractor on the valve being situated out of the umbonal cavity in the same manner as in *G. gladius*, the only other known species of the genus *Gigantidas*. The present species is distinguishable from *G. gladius* by the large swollen umbonal cavity, the markedly concave ventral margin and shell proportions such as shell height and shell width relative to shell length.

Keywords: new species, *Gigantidas*, Kaikata Seamount, hydrothermal vent, burrowing mussels

Introduction

Following the discovery of deep-sea mussels associated with hydrothermalism from the eastern Pacific (Kenk & Wilson, 1985), twenty species of mytilid bivalves belonging to three genera (*Bathymodiolus* Kenk & Wilson, 1985, *Tamu* Gustafson, Turner, Lutz & Vrijenhoek, 1998 and *Gigantidas* Cosel & Marshall, 2003) have been described from the vent and seep sites of the northwestern Pacific, the southwestern Pacific, the southeastern Pacific, and the Atlantic and Indian Oceans (Cosel *et al.*, 1994; Hashimoto & Okutani, 1994; Cosel & Olu, 1998; Cosel *et al.*, 1999; Gustafson *et al.*, 1998; Hashimoto, 2001; Cosel, 2002; Cosel & Marshall, 2003; Okutani *et al.*, 2004).

During a series of biological and geological investigations on the Kaikata Seamount (Fig. 1) with the Japanese submersible *Shinkai 2000*, several large-sized burrowing mussels were found and captured from the sandy bottom where warm water seepage was observed (Hashimoto & Horikoshi, 1989) (Fig. 2). On subsequent expeditions, more mytilid bivalves were collected at the same locality by the *Shinkai 2000*. The specimens were found to be distinguishable from other known large mussels. A description of the new species is given here.

Materials and Methods

All of the specimens were collected during *Shinkai 2000* dives #340, #558, #634 and #691 on the Kaikata Seamount (Fig. 1) using the manipulator of the submersible. Specimens examined were fixed in 10% buffered formalin on board and transferred to 70% ethanol for preservation. Shell length, height and width were measured following the method of Kenk & Wilson (1985). The taxonomic criteria follow Gustafson *et al.* (1998). They include characters of the shell, muscles, gill, mantle margin, foot retractor muscles, byssus retractor muscle and digestive tract. Dissections were performed under a binocular microscope.

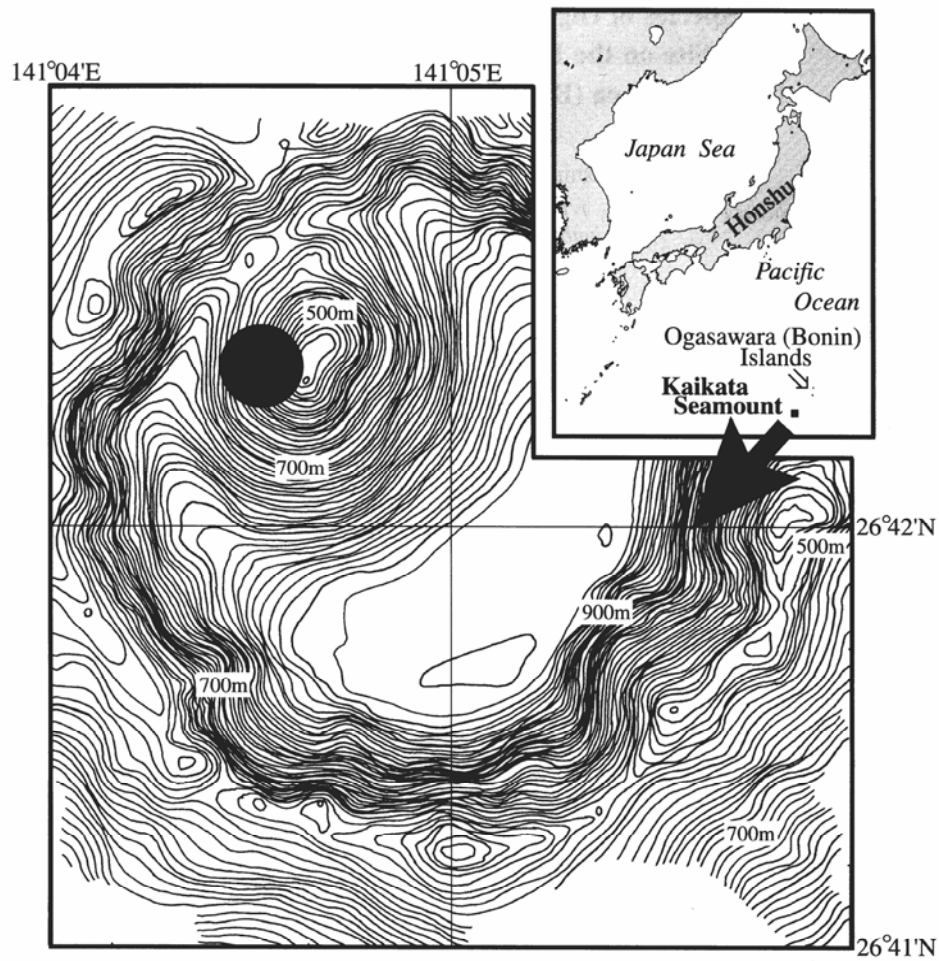


Fig. 1. Sampling site (black circle) of mytilid bivalves in the present study (Kaikata Seamount). (Naka *et al.*, 1989).

Abbreviations used in the text: NSMT – National Science Museum Tokyo; JAMSTEC – Japan Agency for Marine-Earth Science and Technology; UMUT – University Museum, University of Tokyo.

Taxonomy

Family Mytilidae Rafinesque, 1815
 Subfamily Bathymodiolinae Kenk & Wilson, 1985
 Genus *Gigantidas* Cosel & Marshall, 2003



Fig. 2. Habitat of *Gigantidas horikoshii* n. sp. Living mussels are burrowing with about 60-80% of their shells submerged and in a vertical or oblique position. Warm water seepage (approximately 18 °C) was found around their habitat. 26° 42.50' N, 141° 04.50' E, 486 m.

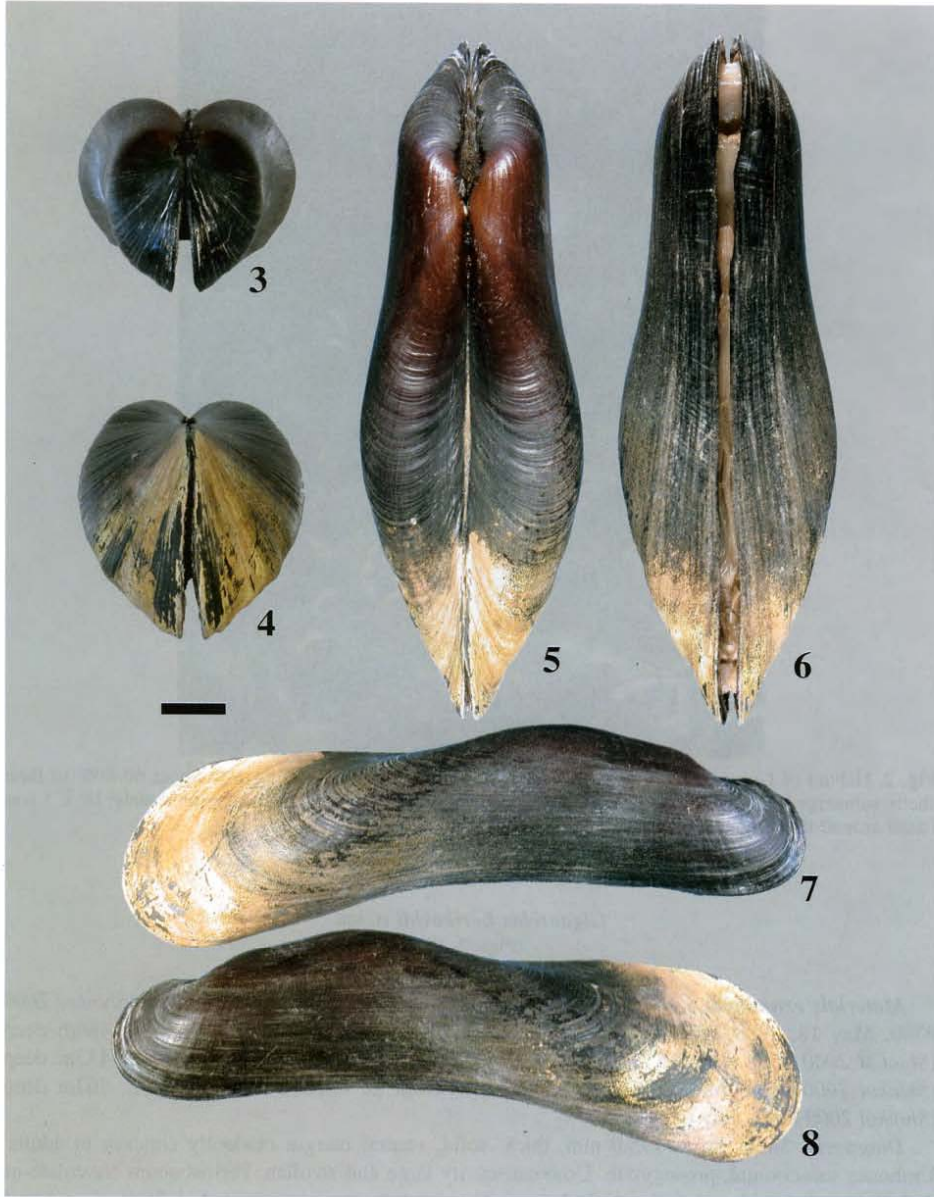
***Gigantidas horikoshii* n. sp.**

(Figs. 2-12)

Materials examined: 8 specimens from 26° 42.30' N, 141° 04.47' E, 480m deep (*Shinkai 2000* #340, May 18, 1988, type locality); 5 specimens from 26° 42.50' N, 141° 04.50' E, 486m deep (*Shinkai 2000* #558, July 15, 1991); 4 specimens from 26° 42.51' N, 141° 04.49' E, 435m deep (*Shinkai 2000* #634, July 23, 1992); 6 specimens from 26° 42.49' N, 141° 04.58' E, 762m deep (*Shinkai 2000* #691, July 04, 1993).

Diagnosis: Shells up to 195.6 mm, thick, solid, ventral margin markedly concave in adults. Umbones subterminal, prosogyrate. Umbonal cavity large and swollen. Periostracum chocolate or dark brown, but umbonal region light brown. Anterior retractor scar situated in front of umbonal cavity. Inner mantle folds entirely separate, terminating anteriorly on anterior adductor. No valvular siphonal membrane.

Description: Shell very large, up to 195.6 mm long, 60 mm high and 57.1 mm broad, thick, solid, aduliform in adults, inflated, equivalve. Outline somewhat variable, curved dorso-ventrally, most inflated at about 60% of shell length from anterior end (Figs. 4-5), height/length ratio 0.28-0.36 in adults, 0.38-0.41 in young specimens (less than 63.7 mm). Umbones subterminal,



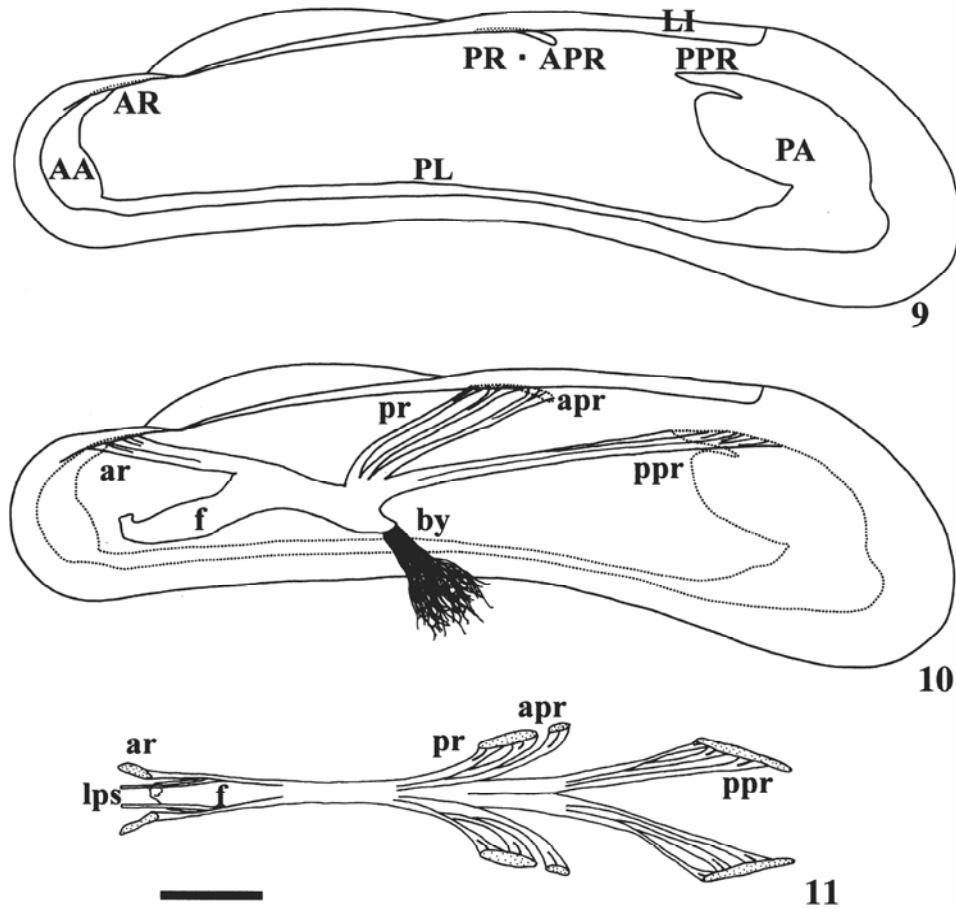
Figs. 3-8. *Gigantidas horikoshii* n. sp. Holotype, NSMT-Mo 73633. **3.** Anterior view. **4.** Posterior view. **5.** Dorsal view. **6.** Ventral view. **7.** Lateral view of right valve. **8.** Lateral view of left valve. Scale bar = 20 mm.

proogyrate, positioned at about 20% of shell length from anterior end in adults, at about 16% of shell length from anterior end in young specimens. Umbonal region extremely elongated and prominent in grown specimens, occupying over 30% of whole dorsal margin, always eroded to ashy whitish pit without periostracum in adult specimens. Blunt rounded ridge running from umbones to postero-ventral corner. Anterior end narrowly rounded, protruding anteriorly, dorsal margin and postero-dorsal margin slightly convex, postero-dorsal corner rather angular, posterior margin broadly rounded, ventral margin nearly straight or slightly concave in young specimens, while markedly concave in subadults and adults. Shell surface rough with well-developed, irregular commarginal growth lines, dull white beneath periostracum. Fine radial striae running from umbones to anterior, ventral and postero-ventral margins, but shell interior smooth. Periostracum strong and smooth, without periostracal hairs, glossy to somewhat dull, yellow-brown in juveniles, chocolate or dark brown in sub-adults and adults; umbonal region light brown in subadults and adults, yellowish to straw-colored in juveniles, mostly covered with yellowish brown layer of ooze-like substance in subadults and adults (Figs. 4–8). Interior nacreous. In some specimens whole shell more or less spirally twisted.

Hinge edentulous, anterior hinge margin protruding ventrally. Ligament opisthodontic, strong, extending about 80–90% of postero-dorsal margin in front of postero-dorsal corner. Ligament plate slightly convex. Posterior end of ligament abruptly terminated in adults (Figs. 9–10), rather tapered in young specimens. Sub-ligamental shell ridge distinct from under umbo to about half of ligament length, then becoming gradually obsolete, visible only from ventral and not from lateral views.

Muscle scars conspicuously distinct. Anterior adductor scar rather large, arched, situated well in front of umbones, near antero-dorsal margin, united with anterior retractor scar in adults, but separated from anterior retractor scar in young specimens (24.8–63.7 mm shell length). Anterior retractor scar situated in front of umbonal cavity in adults, but closer to umbonal cavity in juveniles. Posterior adductor scar large, elliptical, united with scar of posterior bundle of posterior byssus retractor scar. Anterior bundle scar of posterior byssus retractor well separated, situated high in position, at about 65% of ligament length from anterior end of ligament, united with posterior pedal retractor scar. Pallial line somewhat indistinct, almost parallel to ventral margin (Fig. 9).

Ctenidia long and narrow, length more than 75% of shell length, outer demibranch 17 mm broad in the holotype specimen, outer and inner demibranchs nearly equal to each other in length. Ascending lamella of the outer demibranch anteriorly fused to mantle for a very short distance, those of inner demibranch fused to visceral mass. Narrow and somewhat indistinct food groove present on ventral edge of each demibranch, outer surface of ascending lamellae of inner and outer demibranchs with grooves just below free edges and parallel to them. No longitudinal ridge for gill attachment on mantle and visceral mass where the dorsal edges of the ascending lamellae attach. Connection bars between free edges and gill axes absent. Inner mantle folds separate along whole length of ventral margin from anterior adductor to posterior margin, terminating anteriorly over anterior adductor muscle and folding back directly onto adductor muscle but not continuing transversally over it as a rim to meet mantle fold of opposite side. Valvular siphonal membrane absent, but inner mantle fold folded ventrally, becoming very strong and strongly frilled, with deep cleft between right and left inner mantle folds. Foot small, 22 mm (byssus orifice included) in the holotype specimen (Fig. 12). Anterior retractor rather short, inserting just above anterior adductor muscle, near antero-dorsal margin, and well in front of umbonal cavity. Labial palp suspensor slender. Pedal retractor rather slender, arising from anterior side of byssus. Posterior byssus retractor consisting of two diverging bundles with common base at base of byssus. Anterior bundle short and rather slender, arising at about 30 degrees to longitudinal shell axis towards attachment point on shell. Anterior bundle normally forming one scar together with



Figs. 9-11. *Gigantidas horikoshii* n. sp. **9.** Internal view of the right valve illustrating generalized location of the muscle scars, pallial line and ligament. **10.** Lateral view of the musculature system with right valve. **11.** Dorsal view of the musculature system. AA – anterior adductor scar, AR – anterior retractor scar, PR – posterior pedal retractor scar, APR – anterior bundle scar of posterior byssus retractor, PPR – posterior bundle scar of posterior byssal retractor, PA – posterior adductor scar, PL – Pallial line, LI – ligament, ar – anterior retractor, pr – pedal retractor, apr – anterior bundle of posterior byssus retractor, ppr – posterior bundle of posterior byssus retractor, f – foot, by – byssus, lps – labial palp suspensor. Scale bar = 20 mm.

posterior pedal retractor scar, but forming separate scar in some specimens. Posterior bundle very long and rather strong, passing at low angle to longitudinal shell axis towards attachment point above and just in front of posterior adductor. No bundle between anterior and posterior bundles of posterior byssus retractor muscle (Figs. 10-11). Bases of inner and outer labial palp pairs widely separated, inner and outer palp pairs small, narrow-triangular, but outer palp pair smaller than inner one, length of anterior palps *ca.* 4 mm and of posterior palps *ca.* 8 mm in holotype



Fig. 12. *Gigantidas horikoshii* n. sp. Ventral view. Anterior end (left side) showing absence of transverse of inner mantle fold, and ventral, posterior end (right side) showing muscular connection, cleft posteriorly. Scale bar = 20 mm.

specimen. Gut slightly long and nearly straight, somewhat vertically sigmoid-shaped. Stomach indistinct.

Measurements (in mm) of selected type specimens:

Type	Registration number	Shell Length	Shell height	Shell width
Holotype	NSMT-Mo 73633	177.8	49.1	53.6
Paratype #1	NSMT-Mo 73634	159.4	48.6	46.9
Paratype #2	NSMT-Mo 73635	59.9	23.3	17.9
Paratype #3	UMUT RM 28898	173.0	61.6	56.0
Paratype #4	UMUT RM 28899	145.9	48.9	47.9
Paratype #5	UMUT RM 28900	27.3	11.3	8.1
Paratype #6	JAMSTEC 048315	156.9	51.4	47.7
Paratype #7	JAMSTEC 048316	63.7	25.1	19.3
Paratype #8	JAMATEC 048319	171.5	58.7	57.5

Distribution: Hydrothermal vent sites at the Kaikata Seamount, between depths of 435 m and 762 m, located southwest of the Ogasawara (Bonin) Islands, southern Japan.

Ethymology: This new species is dedicated to the late Dr. Masuoki Horikoshi, former Professor of the Ocean Research Institute, University of Tokyo, who contributed greatly to deep-sea biological studies in Japan.

Remarks: *Gigantidas horikoshii* n. sp. looks superficially similar to *Bathymodiolus boomerang* Cosel & Olu, 1999 and *B. tangaroa* Cosel & Marshall, 2003. However, the present new species, is closest to *G. gladius* Cosel & Marshall, 2003, which is the only currently described known species belonging to the genus *Gigantidas*, and is characterized by the absence of a well-developed valvular siphonal membrane at the posterior end, the absence of a continuation of the inner mantle fold across the anterior adductor from one valve to the other, and by the attachment point of the anterior retractor on the valve being situated in front of and not within the umbonal cavity. *G. horikoshii* n. sp. is distinguished from *G. gladius* in having a large swollen umbonal region, markedly concave ventral margin, and different shell proportions such as shell height and width relative to shell length. The mean height/length and width/length ratios of the present new species are 0.28–0.41 and 0.25–0.34, respectively. *G. horikoshii* n. sp. is shorter than *G. gladius* (height/length ratio = 0.16–0.22) and the inflation of the shell in *G. horikoshii* n. sp. is larger than that of *G. gladius* (width/length ratio = 0.15–0.19; Cosel & Marshall, 2003) (Fig.

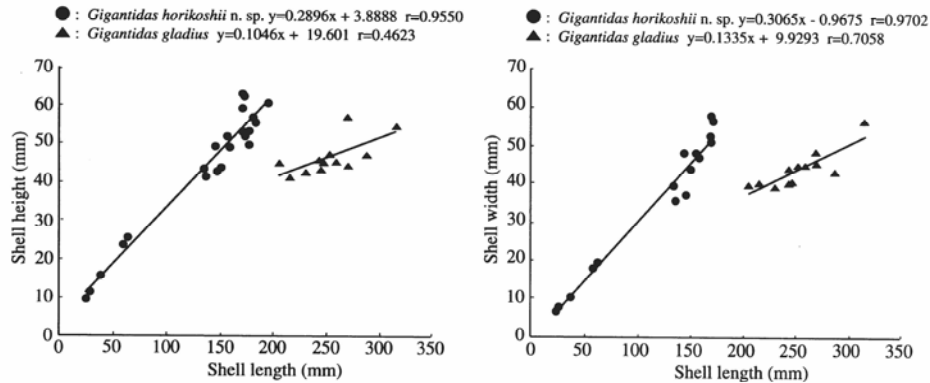


Fig. 13. SL-SH and SL-SW relationship between *Gigantidas horikoshii* n. sp. and *G. gladius* (Cosel & Marshall, 2003).

13). The umbones of *G. horikoshii* n. sp. are situated at about 20% from the anterior end in adult specimens, in *G. gladius* at about 25%. The postero-dorsal corner is rather angular in *G. horikoshii* n. sp., but broadly rounded or not defined in *G. gladius*. The posterior end of the ligament in this new species is abrupt, whereas in *G. gladius*, it is tapering. The interior of the thick-shelled *G. horikoshii* n. sp. is smooth, whereas in the thin-shelled *G. gladius* radial striae that run from the umbones to the anterior, ventral and postero-ventral margins are weakly reflected on the interior, mostly ventrally. The anterior bundle of the posterior byssus retractor in *G. horikoshii* n. sp. is more slender than that in *G. gladius*. In *G. horikoshii* n. sp., there is no additional bundle between anterior and posterior bundle of the posterior byssus retractor, whereas two additional bundles are present in *G. gladius*. The labial palps of this new species are relatively larger (anterior palps about 4 mm long and posterior pair about 8 mm long in the holotype specimen) than those of *G. gladius* (anterior palps 2–5 mm long and posterior pair about 8 mm long in a 270 mm specimen). Symbiotic bacteria were observed also in the new species (Hashimoto & Horikoshi, 1989)

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小笠原海形海山の温水湧出域に生息するオオマユイガイ属（新称）の1新種

橋本 惇・山根崇宏

要 約

Gigantidas horikoshii n. sp. オオマユイガイ（新種）

(Figs. 3-12)

殻は厚く大型で殻長は195.6 mmに達し、厚く硬質。マユイガイ型。両殻の膨らみは中庸で、最大殻幅部は殻の前縁から殻長の約60%後方。殻頂は殻の前縁から殻長の約20%後方、殻頂窩は大きく膨らみ殻長の30%以上を占める。後背隅は丸く、後腹縁は大きく丸い。大型個体の腹縁は大きく凹むが、小型個体は僅かに凹む。靱帯は後位、強靱で殻後背縁の80~90%で後部は截断状。筋痕は明瞭。前閉殻筋痕は大きく丸みを帯びたアーチ状。前牽引筋痕は殻頂窩内になく殻頂の前方で、前閉殻筋痕と繋がり太い逆コンマ形。後閉殻筋痕は大きく楕円形、後足糸牽引筋後束筋痕と繋がり一つの筋痕を形成。套痕はやや不明瞭、ほぼ腹縁に並行。殻長の75%を超す厚く長い鰓を有する。前牽引筋は細長く、収足筋はやや細い。2束の筋肉よりなる後足糸牽引筋前束の前側筋肉は収足筋と繋がる。4束の筋肉よりなる後足糸牽引筋後束は細く、長い。後足糸牽引筋の前束と後束の間に筋束はない。水管弁膜 (valvular siphonal membrane) はなく、外套内襞 (inner mantle fold) は前閉殻筋腹縁で完全に分離。消化管は短く縦方向に弱いS字状を呈する。

分布：小笠原父島小笠原諸島の南西に位置する海形海山の水深435 mから762 mに存在する温水湧出域周辺のみから知られる。

マヌス海盆熱水活動域に生息するシンカイヒバリガイ類の分類学的検討

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ガラパゴス海嶺の化学合成生態系から採集されたイガイ科二枚貝類が、新亜科（Bathymodiolinae）・新属（*Bathymodiolus*）・新種の*Bathymodiolus thermophilus* Kenk and Wilson, 1985（和名：ガラパゴスシンカイヒバリガイ）として記載されて以来、18種のシンカイヒバリガイ（*Bathymodiolus*）属二枚貝類が北西太平洋、南西太平洋、南東太平洋、大西洋、インド洋などから報告されている（Cosel *et al.*, 1994; Hashimoto and Okutani, 1994; Gustafson *et al.*, 1998; Cosel and Olu, 1998; Cosel *et al.*, 1999; Hashimoto, 2001; Cosel, 2002; Cosel and Marshall, 2003; Okutani *et al.*, 2004）。

南西太平洋パプア・ニューギニアのマヌス海盆からは熱水活動が報告されており（Auzende *et al.*, 1996; Gamo *et al.*, 1997）、1996年・1998年・1999年には、BIOACCESS 航海と名付けられ、熱水噴出孔生物群集を主対象とした潜水調査船「しんかい2000」潜水調査が繰り返された（Hashimoto *et al.*, 1999・2000）。潜水調査はPACMANUS及びDESMOSと名付けられた水深1700m～2000mの熱水活動域（図1.）において実施され、計105個体のシンカイヒバリガイ類が採集された。

このシンカイヒバリガイ類の殻は大型でやや厚く硬質であり、膨らみは中庸で、殻頂は殻の前縁から11～18%後方に位置する。殻の前縁はやや狭いが丸く、背縁は僅かに膨らみ、後背隅は幾分角張る。腹縁はやや窪み、後腹縁は丸い。そして、殻高/殻長比は0.40～0.55とやや細長い（図2.）。靱帯は後位、強く、殻頂から後背隅までの約70%で、後部は截断状。筋痕は明瞭。前閉殻筋痕は卵形で殻頂の前に位置する。後閉殻筋痕は丸みを帯びた不等辺四角形、後足糸牽引筋後束筋痕と繋がり一つの筋痕を形成する。前牽引筋痕は殻頂腔の前方、殻頂の前端で側面から観察可能。後足糸牽引筋痕は長円形で高位、靱帯長の前から約65%。套痕は明瞭、中央部が僅かに窪む。外套膜の背側は薄い、腹縁は厚く強い。外套内襞（inner mantle fold）は閉殻筋前縁から後縁までの腹側に沿って分離し、その中央部から後縁が襞状。櫛鰓は厚いが殻長の約70%の長さでやや小さめ。唇弁は非常に小さい。足は小さく平らで、殻長の約20%。前牽引筋は細長く、収足筋はやや太い。後足糸牽引筋前束は太く短い、後束は細く、長い。水管弁膜は短く、狭く、薄く、乳頭状突起はない。消化管は心耳の前方で、背中側から見て反時計回りの小さいループを形成する。

シンカイヒバリガイ属二枚貝類は殻や軟体部の形態的特徴から*B. thermophilus*グループ、*B. breviar*グループ、*B. heckerae*グループおよび*B. childressi*グループの4つのグループに大別されている（Cosel,

Expedition Reveals Changes in Lau Basin Hydrothermal System

PAGES 13, 17

The Valu Fa Ridge (VFR) in the southern Lau Basin—located behind the westward-dipping Tonga-Kermadec subduction zone—was one of the earliest targets to be explored for hydrothermal activity in the southwestern Pacific Ocean (Figure 1). In 1989, the French-German dive program NAUTILAU (*Nautilo-Lau*) discovered active hydrothermal fields venting high-temperature fluids with high acidity accompanied by massive ore deposits in this area [Fouquet *et al.*, 1993].

In September–October 2004, the SWEEP VENTS (SouthWestern Edge of Pacific hydrothermal vents) expedition explored and sampled the hydrothermal systems of the VFR using the deep submergence research vehicle (DSRV) *Shinkai 6500*. The 2004 *Shinkai* dives focused on the geobiological and geochemical character of the deep-sea hydrothermal vent ecosystems of the Valu Fa Ridge and shed new light on these hydrothermal fields 15 years after their discovery. These 2004 dives were the first to revisit this area, and have been followed by further dive programs and continued research.

Fifteen years ago, vigorous hydrothermal activity with fluid temperatures exceeding 340°C was discovered at the Vai Lili field (22°13.0' S, 176°36.5' W, depth = 1720 meters) on the northern section of the central VFR (Figure 1). But the 2004 SWEEP VENTS expedition found only weak shimmering water with a temperature of 88°C discharging from the foot of an oxidized chimney (Figure 2a) as well as diffuse flows associated with yellow patches of iron-oxides (Figure 2b). The lower fluid temperature demonstrates that the activity has declined significantly during the intervening time.

In contrast to the dying Vai Lili field, about four kilometers north SWEEP VENTS located another hydrothermal field, Mariner field (22°10.8' S, 176°35.1' W, depth = 1910 meters), with numerous smokers (Figures 2c and 2d) venting high-temperature fluids ($T = 365^{\circ}\text{C}$) with low pH (2.4–2.8 at room temperature measurement). The contrast between these

two hydrothermal fields may be an indication of the instability of hydrothermal activity in back-arc settings, where magma is located at shallow depths and its significant influence would be expected.

Evolution of Hydrothermal Activity in the Back-Arc Spreading Center

The VFR is the shallowest portion of the Eastern Lau Spreading Center (ELSC) at its

southern end. Petrologic studies have revealed felsic volcanic activity ranging from basalt enriched in lithophile elements to andesite, both of which have abundant vesicles due to the high gas content of the magma. During the 1989 NAUTILAU expedition, three active hydrothermal fields were discovered along the VFR: Hine Hina in the south, Vai Lili in the central VFR, and the White Church fields in the north [Fouquet *et al.*, 1993].

Submarine hydrothermal systems develop where seawater that has penetrated into the crust is heated by magma to ascend along fault lines. The fluid circulation transports to the seafloor not only heat but also substances used by microbes in the hydrothermal environment. As discussed by Kelley *et*

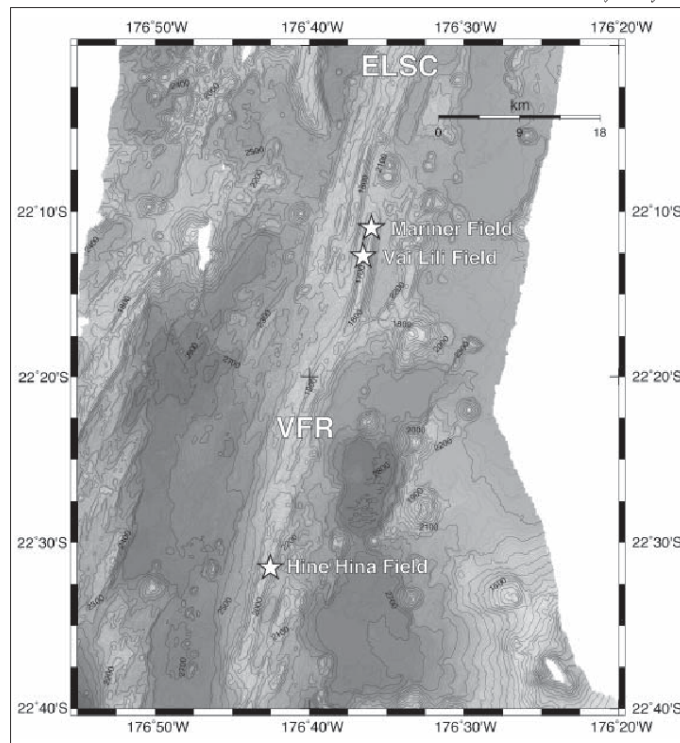


Fig. 1. Bathymetric map of Valu Fa Ridge in the southern Lau Basin (derived from multi-beam swath-mapping survey conducted by the research vessel *Yokosuka*) and locations of active hydrothermal fields.

By J. ISHIBASHI, J. E. LUPTON, T. YAMAGUCHI, J. QUERRELOU, T. NUNOURA, AND K. TAKAI

al. [2002], magmatic events can drastically increase the content of volatile species degassed from magma and incorporated into the hydrothermal fluids, thus forcing a shift in the associated microbial ecosystem. Hydrothermal fields in the central VFR, where the presence of a crustal magma body at shallow depths has been demonstrated by seismic tomographic study [Day *et al.*, 2001], can provide an excellent place to test the influence of magmatic volatiles on the evolution over time of the geochemical and geobiological character of hydrothermal activity. The influence of the significant contribution of magmatic volatiles on the active hydrothermal systems on the VFR has been previously explained in terms of strong acidity and sulfur isotopic composition [Fouquet *et al.*, 1993; Herzig *et al.*, 1998].

During the SWEEP VENTS dive program, which was operated by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) and also designated as the YK04-09 Nirai Kanai cruise of the research vessel *Yokosuka*, 18 dives were conducted on the VFR with the submersible DSRV *Shinkai 6500*. Samples of hydrothermal fluids were collected using the gas-tight WHATS (Water and Hydrothermal-fluid Atsuryoku TIGHT Sampler) fluid sampler and processed on board for gas extraction, as well as for geochemical and microbiological analyses.

Dying Hydrothermal Activity in the Vai Lili Field

The Vai Lili field lies on the upper western flank of the ridge axis on the shallow northern section of the central VFR. When discovered in 1989, this field was reported to have vigorous fluid ventings with abundant black and white suspended materials [Fouquet *et al.*, 1993]. In addition to the numerous smokers with fluid temperatures up to 342°C, extensive diffuse discharge was observed forming a continuous halo of iron and manganese oxides distributed about 400 meters along north-south trending normal faults.

During the 2004 SWEEP VENTS expedition, it was evident that the hydrothermal activity at the Vai Lili field had declined significantly during the intervening 15 years. Diffuse flows ($T = 35\text{--}50^\circ\text{C}$ and $\text{pH} = 6.5$), often accompanied by orange-yellow patches of iron oxides (Figure 2b), were still present throughout the area. The highest fluid temperature was only 88°C, found as a weak shimmering flow issuing from the base of an oxidized chimney in the center of the Vai Lili field (Figure 2a).

In spite of the lower fluid temperature, the hydrothermal reservoir appears to still exist based on fluid chemistry. The collected samples show a linear relationship among the concentrations of chemical species such as chloride, potassium, and total gas versus that of magnesium (Figures 3a–3c), suggesting that both the shimmering fluid and the diffuse flow can be explained adequately as a simple mixture of the hydrothermal fluid end-member and seawater. On the other hand, in the relationship between calcium and mag-

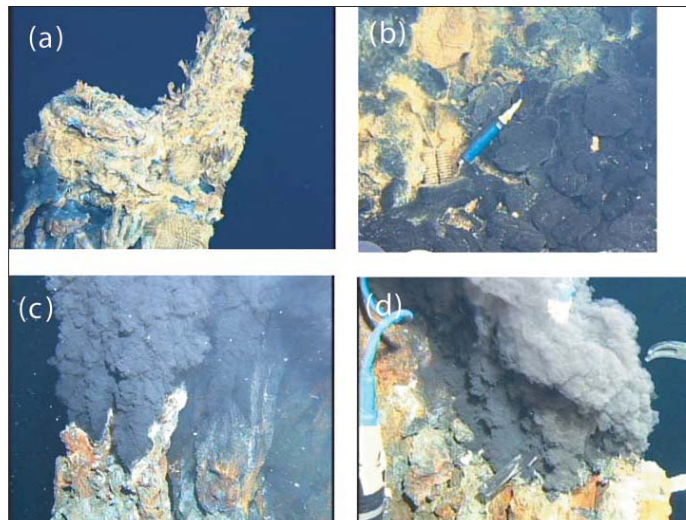


Fig. 2. Photos obtained with Shinkai 6500 showing hydrothermal activity on the Valu Fa Ridge. (a) An oxidized chimney discharging 88°C fluid in the Vai Lili field. (b) Diffuse flow ($T = 40^\circ\text{C}$) in the Vai Lili field. (c) Vigorous venting from a black smoker ($T = 365^\circ\text{C}$) in the Mariner field. (d) Deployment of the ISCS in the vent orifice in the Mariner field.

nesium concentrations (Figure 3d), the weak shimmering fluid ($T = 88^\circ\text{C}$) samples (open squares) and the diffuse flow samples (solid squares) show different trends. Taken together with a similar trend found in strontium and sulfate concentrations, these discrepancies are evidence for subsurface precipitation of anhydrite (CaSO_4) caused by mixing of the hydrothermal fluid with entrained seawater. The low concentrations of iron (70 micromoles per liter ($\mu\text{mol/l}$); Figure 3e) and hydrogen sulfide (30 $\mu\text{mol/l}$; Figure 3f) are consistent with precipitation of sulfide minerals as well as anhydrite.

The depletion of hydrogen sulfide in the Vai Lili hydrothermal fluid should have considerable impact on the microbial and macrofaunal communities. The typical microbial components dominating active hydrothermal fields (such as members of *Thermococcales*, *Methanococcales*, *Aquificales*, and *Proteobacteria*) were not detected by culture-dependent analysis from the samples collected at Vai Lili during the 2004 SWEEP VENTS expedition. However, ribosomal RNA gene sequences of *Thermococcales* (a hyperthermophilic archaea, which grows above 80°C) were recovered by culture-independent molecular analysis from the samples obtained by in situ colonization devices (ISCS) deployed in the diffuse flow. In addition, the archaeal and bacterial ribosomal RNA phylogenies, which had been found specifically in dead chimneys, were also detected in the ISCS samples. Thus, the microbial communities in the diffuse flows at Vai Lili field may contain components representing both active and dead hydrothermal systems.

Newly Discovered Hydrothermal Activity in the Mariner Field

The Mariner field is located about four kilometers north of the Vai Lili field, where the northern end of the central VFR overlaps the northern VFR. During the 2004 *Shinkai* dives, vigorous fluid venting of black and white smokers was found here, together with clear diffuse fluids discharging from the base of chimneys. This activity was clustered within a hydrothermal field less than 100 meters in diameter (Figures 2c and 2d). The highest temperature of 365°C was confirmed at a black smoker in the center of the active field.

As evident in the magnesium diagrams (Figure 3), two distinct hydrothermal end-members are present: a vapor-rich fluid (downward pointing triangle) and a brine-rich fluid (upward pointing triangle). This is clear evidence for phase separation into a vapor and a liquid (brine), possibly reflecting high temperature in the vicinity of the magma. The strong acidity with pH as low as 2.4 (room temperature measurement) together with negative alkalinity (lower than 2 millimoles per liter) are commonly found in brine-rich and vapor-rich fluids, which suggests involvement of a strong acid, probably caused by the injection of magmatic volatiles into the hydrothermal fluid. These geochemical characteristics are similar to those observed at the Vai Lili field 15 years ago [Fouquet *et al.*, 1993], suggesting that both the Vai Lili and Mariner fields have been influenced by significant contributions of magmatic volatiles.

Since the Mariner field is presently active, microbiological analyses of the collected

samples provided a measure of the physiological and phylogenetic diversity of the microbial communities. It was notable that methane-producing archaea were absent and the oxygen-tolerant hyperthermophilic *Aquifex* species were dominant in the chimney structures and even in the samples collected by the ISCS deployed in a 365°C black smoker. These signatures may represent a relatively oxidative and shallow zone of subseafloor biosphere and be in accordance with the idea that the hydrothermal activity is relatively recent.

Other Sites, Water-column Surveys, and Future Work

During the SWEEP VENTS expedition, one dive was devoted to revisiting the Hine Hina field (22°32.3'S, 176°43.1'W, depth = 1850 meters). This dive confirmed that prosperous macrofaunal communities found in 1989 were still present in 2004. Throughout the expedition, the faunal communities associated with hydrothermal activity on the VFR were found to have much lower abundance but similar diversity in species composition (shrimps, crabs, mussels, galatheids, limpets, polychaetes, and gastropods) compared with that reported 15 years ago [Fouquet et al., 1993; Desbruyères et al., 1994].

The ELSC has been selected as one of the Integrated Studies Sites (ISS) of the RIDGE 2000 Program. As part of this Lau Basin ISS work, several systematic water column plume surveys were conducted along the ELSC that either were coincident with the SWEEP VENTS expedition or preceded it. These water column surveys predicted that the activity in the Vai Lili field was dying, and also clearly identified the new robust activity at the Mariner site. Thus, these plume surveys contributed to the success of our *Shinkai* dive program reported here.

In addition to the hydrothermal sites along the Valu Fa Ridge, these plume surveys also identified many additional hydrothermal sources within the Lau Basin. Following the plume surveys, several dive programs have been conducted under the RIDGE 2000 program framework, and research into the findings is still ongoing. Because the Lau Basin spreading center displays large changes in crustal thickness, it can provide an excellent field to test the influence of magmatic volatiles on the evolution of hydrothermal activity. Those ongoing and future investigations using either manned or unmanned submersibles should provide valuable insights into the temporal and spatial variations of hydrothermal systems.

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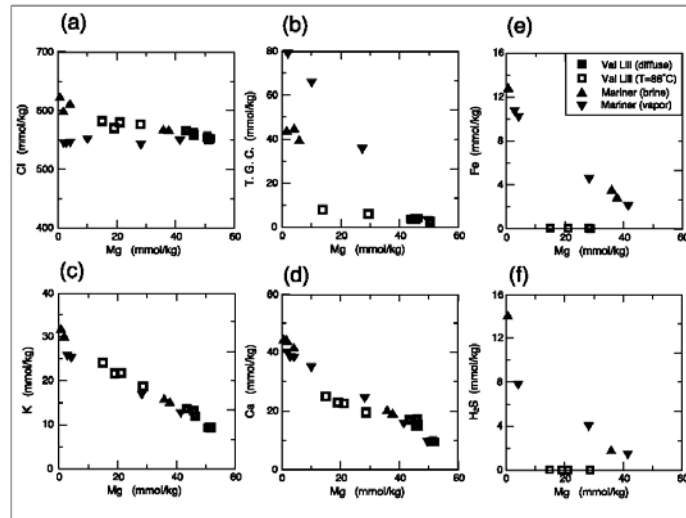


Fig. 3. Chemical composition of the samples from the Vai Lili field (squares) and from the Mariner field (triangles) in the central Valu Fa Ridge. The relationship of (a) chlorine (Cl) versus magnesium (Mg), (b) total gas concentration versus Mg, (c) potassium (K) versus Mg, (d) calcium (Ca) versus Mg, (e) iron (Fe) versus Mg, and (f) hydrogen sulfide (H_2S) versus Mg are plotted. Two distinctive trends among the Mariner fluid suggest phase separation (the vapor-rich fluid shows high Cl, K, Ca, and H_2S concentrations and low total gas concentration compared with the brine-rich fluid). Although the major element composition of the Vai Lili fluid is comparable to that of the Mariner fluid, the Ca concentration of the weak shimmering flow ($T = 88^\circ C$) seems to show a different trend. Substantially low concentrations of Fe and H_2S of the weak shimmering flow are also notable. Those differences are attributed to subseafloor precipitation.

Yokosuka for their skillful operation of the ship and the submersible. We also thank onboard scientists of the YK04-09 cruise for their cooperation. We are grateful to Yves Fouquet of Ifremer, Peter Herzig of the University of Kiel, Ed Baker of the U.S. National Oceanic and Atmospheric Administration (NOAA) Pacific Marine Environmental Laboratory (PMEL), Gary Massoth of the Institute of Geological and Nuclear Sciences, New Zealand, and Fernando Martinez of the School of Ocean and Earth Science and Technology, University of Hawaii at Manoa for providing us with valuable information from their previous cruises. We also thank Charles Langmuir of Harvard University for providing daily updated information of the KM04-17 cruise conducted coincidentally with our expedition, which included results obtained by dives of the autonomous underwater vehicle *ABE* and by water column surveys. We appreciate the efforts of Charles Fisher of the RIDGE 2000 Program in constructing an international cooperative network.

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Abundance and distribution of fatty acids in hydrothermal vent sediments of the western Pacific Ocean

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Abstract

Sediments obtained from three active seafloor hydrothermal areas in the western Pacific Ocean were measured for the abundances and distributions of total fatty acids (TFAs). Approximately 50 fatty acids were identified, with the chain-length ranging from C₉ to C₂₉. The total concentrations of TFAs (Σ TFA) show a wide range from 13.2 to 184 $\mu\text{g/g}$ dry sediment. The values of Σ TFA, over 100 $\mu\text{g/g}$ dry sediment, are significantly higher than those reported for non-hydrothermal seafloor sediments. Furthermore, the observed compositions of TFAs are rich in bacterial biomarkers especially monounsaturated fatty acids, which is common to sediments and symbiotic animal tissues from other sites associated with hydrothermal activities. High ratios of Σ TFA to total organic carbon content (TOC) relative to the non-hydrothermal seafloor sediment may also reflect a large contribution of organic matter produced at the vents. Due to the limited sample availability from these environments, the TFA compositions were not enough to evaluate relatedness between the vent sediment and vent animal community, while the wide diversity of TFAs suggests a large variation in source organisms in these hydrothermal environments. This study confirms the high contribution of bacteria and other vent organisms to organic matter in the sediments.

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1. Introduction

Chemosynthetic-based dense animal communities have been frequently observed around active hydrothermal vents on the deep seafloor. These animals are believed to feed on bacterial primary production. The dense vent animal and bacterial communities can be a dominant source of sedimentary organic matter around the vents. Some of the organic compounds deposited around the vents could be used as biomarkers to understand hydrothermal vent ecology and physiology. Fatty acids are the major constituents of lipids in living

organisms. The myriad structures of fatty acids biosynthesized by organisms, i.e. “biomarkers” (e.g., Bobbie and White, 1980; van Vleet and Quinn, 1979), make them useful tracers of their sources and alteration in particulate matter and sediments (Lee and Wakeham, 1988; Wakeham and Lee, 1993).

Analysis of fatty acid biomarkers in deep-sea hydrothermal environments has been carried out to compare endosymbiotic bacteria and their host species (Conway and Capuzzo, 1991; Ben-Mlih et al., 1992; Fullarton et al., 1995a, b; Pranal et al., 1997). In general, the composition of fatty acids in common marine invertebrates is characterized by the predominance of two polyunsaturated fatty acids: 20:5n-3 and 22:6n-3 (Farrington et al., 1973; Sargent, 1976; Joseph, 1982). This pattern reflects the fact that these organisms utilize organic material produced by phytoplankton, the primary

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producers living in the photic zone (e.g., Sargent, 1976). In contrast, the invertebrates at deep-sea hydrothermal vents contain very small amounts of, or lack, these polyunsaturated fatty acids. They are rich in monounsaturated fatty acids such as 16:1n-7 and 18:1n-7 which are mainly bacterial in origin (Conway and Capuzzo, 1991; Ben-Mlih et al., 1992; Fullarton et al., 1995a, b; Pranal et al., 1997). These monounsaturated fatty acids are considered major components of sulfur-oxidizing and methylotrophic bacteria isolated from marine bivalves (Fullarton et al., 1995a). They have been demonstrated to be the major primary producers doing chemosynthesis in hydrothermal environments (Jannasch, 1983).

Here, we collected deep-sea sediments from three active hydrothermal areas in the western Pacific Ocean, and determined the compositions of total fatty acids (TFAs) in the saponification products of total lipid extracts. The three hydrothermal areas have different tectonic settings (back-arc rift vs. island-arc), sedimentary environments (sediment poor vs. rich), and animal compositions. The results have been used to identify and evaluate the contribution of organic matter of bacterial and other origins to the samples.

2. Materials and methods

Samples of hydrothermal vent sediments were collected from three active hydrothermal areas in the western Pacific, DESMOS caldera of Manus Basin, Myojin knoll caldera of Izu-Ogasawara Island-Arc, and Iheya Ridge of Okinawa Trough, during dive studies of the manned submersible *Shinkai 2000* (Fig. 1). Details of the samples are shown in Table 1. The DESMOS caldera is located close to a back-arc spreading zone in the Manus Basin (Auzende et al., 1996). The samples D916 and D921 were obtained from the 'Onsen' hydrothermal site in the caldera during the BIOACCESS cruise in 1996 (Ohta et al., 1997). At this site, several low-temperature (90–110 °C) white smokers characterized by low-pH and high-sulfide and sulfate fluid venting were observed (Ishibashi et al., 1997). Bottom sediments at the venting site are composed mainly of volcanoclastics with thin muddy sediment. Vestimentiferan tubeworms and giant clams of the genus *Calymptogena* were major invertebrates inhabiting the hydrothermal area (Ohta et al., 1997). The Myojin knoll caldera is one of the active volcanos of the Izu-Ogasawara volcanic front. Samples D1008 and D1011 were obtained from the 'Sunrise' hydrothermal site in 1998. This site was discovered in 1997 and contains a huge sulfide mound (the Sunrise deposit) rich in gold and silver (Iizasa et al., 1998). At this site many high-temperature black-smoker vents (up to 300 °C) have been identified. Bythograeid and galatheid crabs and the deep-sea mussel *Bathymodiulus*

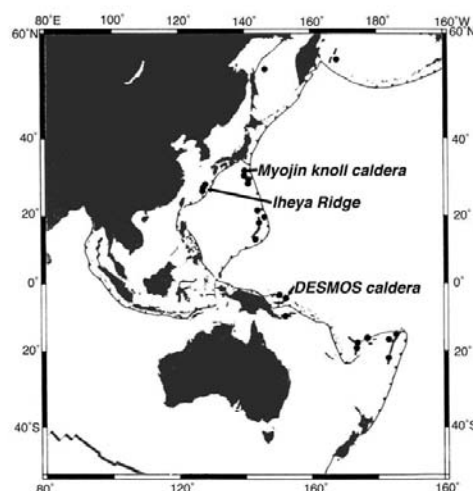


Fig. 1. Map showing sampling sites with plate boundaries and identified active hydrothermal venting (filled circles) in the western Pacific Ocean (Ishibashi and Urabe, 1995).

were major constituents of this community (Iizasa et al., 1998). The Okinawa trough is a back-arc basin and the Iheya Ridge is considered one of the spreading axes in the central Okinawa trough. Samples D857R and D857B were obtained from the hydrothermal site at North Knoll of the Iheya Ridge in 1996, and sample D1030 was obtained from the same hydrothermal site in 1998. This hydrothermal site was discovered in 1995 and described by Chiba et al. (1996). At this site several large-scale chimneys venting high-temperature fluid (up to 300 °C) have been identified. The giant clam *Calymptogena*, the deep-sea mussel *Bathymodiulus*, and vestimentiferan tubeworms were observed as major species of the community (Yamamoto et al., 1999). The Okinawa Trough is covered with thick turbidite derived from the continental shelf of the East China Sea and Yellow Sea (Tsugaru et al., 1991), while the DESMOS caldera and Myojin knoll caldera are not.

Although the sediment sampling was aimed at dense invertebrate colonies and/or thick bacterial mats around the hydrothermal vents, it was difficult to settle *Shinkai 2000* right above the objects. Some samples were collected a few to 20 m from the dense animal colonies. In addition, the sample D857R was collected about 100 m from the hydrothermal area as a reference. The sediment samples were collected with a push core sampler (diameter = 5 cm, length = 30 cm). Samples were frozen immediately in plastic bags, and lyophilized on return to the laboratory. For measurement of total organic carbon contents (TOC), 1N HCl was added to about 0.5 g of the lyophilized samples to remove carbonate and then

Table 1
Sampling sites and other descriptions of the hydrothermal vent sediments

Location	Sample Data	Latitude	Longitude	Depth (m)	Sediment type ^a	Description
DESMOS caldera, Manus Basin, Papua New Guinea	D916 18-Nov-96	3°41.542'S	151°52.064'E	1911	Sandy mud + pumice + sulfur	Interior of a scattered <i>Calyptogenia</i> colony and white bacterial mat.
	D921 24-Nov-96	3°41.582'S	151°51.996'E	1905	Coarse sand + pumice + scoria + volcanic glass fragment + sulfur	Interior of thick white and yellow bacterial mat area and near a tube-worm dense colony
Myojin knoll caldera, Izu-Ogasawara Arc	D1008 6-May-98	32°06.294'N	139°52.083'E	1338	Sandy mud + sulfur	Foot of an active chimney with no significant biological community except a small and thin bacterial mat
	D1011 10-May-98	32°06.238'N	139°52.029'E	1302	Sandy mud + sulfide mineral	No significant biological community except a bacterial mat and several invertebrates
North Knoll of Iheya Ridge, Okinawa Trough	D857R 29-April-96	27°47.178'N	126°54.203'E	1035	Sandy mud	About 100 m apart from an active hydrothermal vent
	D857B 29-April-96	27°47.180'N	126°54.149'E	1049	Sandy mud with white color patch	Interior of a crowded <i>Calyptogenia</i> colony
	D1030 9-Jul-98	27°47.176'N	126°53.823'E	981	Mud	About 20 m apart from a dead <i>Calyptogenia</i> colony

^a Sulfur = small fragments of massive sulfur; sulfide minerals = automorphic sulfide minerals from hydrothermal mineralization.

dried under vacuo. TOC (wt.%) was measured by the dry combustion method using an elemental analyzer (NA2500, CE Instrument, Italy).

Total lipids were extracted from the sediment samples three times with sonification using benzene: methanol (3:1 v/v). The extract was subjected to mild alkaline hydrolysis using 6 ml methanolic 0.2N KOH plus 1 ml distilled water and gentle heating (50 °C, 1 h) using the procedures in Harvey (1994). Samples were cooled, additional water was added, and the neutral fraction containing hydrocarbons, alcohols and sterols was removed by partitioning into hexane: diethyl ether (9:1). The remaining aqueous solution was acidified to pH ≤ 2 , from which all fatty acids were extracted with the same solvent mixture. The entire polar fraction was treated with BF₃-MeOH to form fatty acid methyl esters (FAMES). In order to evaluate the recovery of fatty acids through the whole procedure, the original sediment samples were divided into two portions, and internal standards (10-undecylenic acid and linolenic acid) were added to one of the two prior to sonic extraction with benzene: methanol. Because the amounts of sample D857R and D857B were small, this evaluation was not made for these samples. Elemental sulfur in the extract was removed through an activated copper column using the procedures in Blumer (1975).

FAMES were analyzed by GC/MS (Shimadzu model QP-5000) using a fused silica capillary column of GL Science Inc. Neutrabond-1 (30 m×0.25 mm i.d., film thickness 0.25 μ m). The oven temperature was first held at 60 °C for 5 min., raised to 280 °C at 4 °C/min., and held at that temperature for 80 min. Identification of individual FAMES was based on comparison of their retention times and mass spectra with those of authentic standards.

3. Results and discussion

3.1. Total concentrations of TFAs and organic carbon

Total concentrations of TFAs (Σ TFA) and organic carbon (TOC) contents are shown in Table 2. The values of Σ TFA of samples D857R and D857B were not determined because the internal standards were not added. Those of the other samples range from 13.2 to 184 μ g/g dry sediment (Table 2). High values were observed for the samples from the DESMOS caldera in the equatorial region. Many reports of Σ TFA values in marine sediments have been published and the values often depend on geographic region (e.g. Harvey, 1994; Wakeham et al., 1997). The latitudinal distribution of Σ TFA values of the surface sediments obtained from the Central Pacific along 175°E from 48°N to 15°S ranges from 1.82 to 23.8 μ g/g dry sediment (Ohkouchi, 1995). The highest value was observed at 48°N and the

Table 2
Analytical results of total fatty acids and organic carbon in the hydrothermal vent sediments

	DESMOS Caldera		Myojin Caldera		Iheya Ridge		
	D916	D921	D1008	D1011	D857R	D857B	D1030
<i>Total fatty acids</i>							
Saturated (%)	35.0	49.9	54.1	56.8	77.7	66.8	80.4
Monounsaturated (%)	43.2	34.1	27.1	24.2	13.6	28.7	7.5
Branched (%)	15.4	13.2	13.6	18.3	7.2	3.7	11.4
PUFA (%)	5.3	4.3	1.3	0.8	1.4	0.7	0.4
Total concentration (mg/g dry sediment)	131	184	13.2	42.2			15.7
18:1n-7/ Σ 18:n ^a	0.40	0.22	0.11	0.24	0.06	0.19	0.06
16:1n-7/16:0	1.40	0.50	0.57	0.31	0.13	0.41	0.04
L/H [$\Sigma(<C_{20})/\Sigma(\geq C_{20})$] ^b	3.1	3.7	16.0	3.7	2.5	7.6	1.7
<i>Organic carbon</i>							
Total concentration (10 ⁻² g/g dry sediment)	0.14	0.13	1.30	0.59	–	2.46	1.06
Σ TFA/TOC (mg/g)	94	142	1.0	7.2	–	–	1.5

^a A possible indicator of the contribution of bacterial input (see text).

^b An indicator of the significance of terrigenous input (Fukushima and Ishiwatari, 1984).

average was 7.93 μ g/g dry sediment. The geographical distribution of Σ TFA of those sediments show two maxima, one at high latitudes (>10 μ g/g dry weight: 48°N–35°N) and the other around the equator (ca. 10 μ g/g dry weight: 5°N–12°S), indicating a high accumulation of organic matter in high-latitude and equatorial regions. Although the high values of the DESMOS sediments would reflect this kind of high accumulation, the values of Σ TFA are higher than those reported from the central equatorial regions at 140°W (1.2–32.2 μ g/g dry sediment; Wakeham et al., 1997). From coastal areas of Japan, values of Σ TFA of 28.4 ng/g dry sediment or lower were reported (Naganuma et al., 1996), which were several orders of magnitude lower than those of the vent sediments. The vent sediments consist of hydrothermal precipitation and minerals with pelagic clay, therefore, the production rate of TFA contributing to the vent sediments in this study may be at least several times higher than those of non-vent sediments. It is likely that the vent sediments have organic sources other than those for the non-vent marine sediments. From the hydrothermal vent at Juan de Fuca Ridge distinctly high concentrations of phospholipid fatty acid (PLFA) in muddy sediment of 247 μ g/g dry sediment were reported by Hedrick et al. (1992), while Σ TFA in the vent sediment at 13°N EPR reported by Brault et al. (1984) was only 1.14 μ g/g dry sediment. The wide range of Σ TFA values in the vent sediments may be due to the balance between the rates of production of vent community and of sedimentation of hydrothermal minerals and debris.

Σ TFA is plotted against TOC in Fig. 2. The values of Σ TFA of the vent sediments are inversely related with the values of TOC in the range of 0.13–1.30% TOC (Fig. 2). Usually, TOC and Σ TFA of surface marine

sediments show a weak positive correlation (e.g. Wakeham et al., 1997). The observed range of TOC is comparable with that of non-vent marine sediments in the Pacific ranging from 0.12 to 1.97 μ g/g (Wakeham et al., 1997; Ohkouchi et al., 1997). The present values of Σ TFA are noticeably higher than those in non-vent marine sediments.

The variation between Σ TFA and TOC in Fig. 2 could be given by assuming a relationship between the rates of production at the vents and of sedimentation through the water column. The low value of TOC of the vent sediments could be because a major part of the material consists of volcanoclastic deposits and precipitates lacking in organic matter. It seems more difficult to explain why a wide variety in the ratios of Σ TFA/TOC

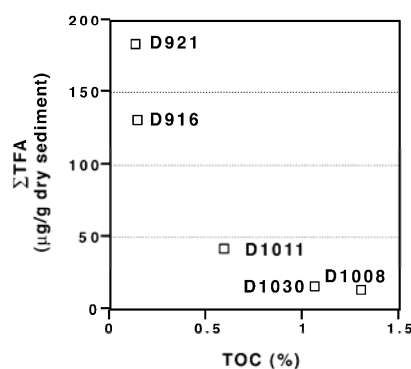


Fig. 2. Relationship between the total concentrations of TFA (Σ TFA) and organic carbon (TOC).

exists between the sediments. In the case of the sample D1030, which was collected from near a dead giant clam colony, Σ TFA of the sample was low. The low Σ TFA may be due to degradation of fatty acids in the sediment after extinction of the clam colony. However, the high TOC with low Σ TFA cannot be interpreted only in terms of degradation of fatty acids because the Okinawa trough is covered by thick sediment containing terrigenous organic matter (Tsugaru et al., 1991). According to the data of Wakeham et al. (1997), the ratios of Σ TFA/TOC of non-vent surface sediments (mean 5.2 mg/g dry sediment) were far lower than those of net plankton in euphotic zone (mean 176 mg/g dry sediment) in the equatorial Pacific Ocean. This difference has been ascribed to selective consumption of fatty acids through biogeochemical degradation in the marine water column. This effect must have lowered significantly the Σ TFA/TOC ratio of the non-vent marine sediment relative to planktonic biomass. In contrast, the effect would be minimal for the vent sediments, because a major fraction of the organic matter was derived from *in situ* chemoautotrophic bacteria and symbiotic invertebrates, both living near seafloor vents. This possibility is supported by the following points: (1) the highest ratio of Σ TFA/TOC of the vent sediments (142 mg/g dry sediment of D921, DESMOS Caldera) falls in the range of those of net plankton (112–232 mg/g dry sediment) calculated from the data of Wakeham et al. (1997), and (2) the concentration of TFAs in the particulate matter from the hydrothermal vent fluid was an order of magnitude higher than that of the non-vent bottom water (Brault et al., 1984). Consequently, it can be predicted that the low TOC values and high Σ TFA/TOC ratios are characterized by a high production in the vent environment and a minor contribution of terrigenous matter and production in the photic zone. The following discussion, based on the compositions of TFAs, supports this view.

3.2. Individual TFA compositions and comparison with previous data from hydrothermal environments

The compositions of TFAs are shown in Table 3. Although approximately 50 FAs were identified at all sites, palmitic acid (16:0) is the most abundant FA in all sediments (10.9–36.4% of TFA), except for sample D916. Other major acids are stearic acid (18:0) and MUFAs with even-carbon numbers (especially, 16:1n-7 and 18:1n-7). PUFAs are very low in concentration and the algal-derived PUFAs (20:5n-3 and 22:6n-3) are absent. Land-plant derived long-chain FAs (≥ 22) were detected in all the samples. The large diversity of FAs except the long-chain FAs in the sediments may reflect high diversity of source organisms or contribution from specific animals in this hydrothermal environment. The following is a review of previous reports of the fatty acid

compositions in sediments, chimney, and animal tissues associated with the deep-sea hydrothermal activities.

Brault et al. (1984) reported the TFA composition of the vent sediment obtained from 13°N EPR, which was abundant in bacterial 18:1 and 16:1 MUFAs (accounting for 20% of TFA) and *iso* and *anteiso* C₁₅ and C₁₇ branched FAs (over 8%). The sediment was collected at the bottom of an inactive chimney; a description of the animal community was not given. The low concentration of Σ TFA in the sample (1.14 μ g/g dry sediment) suggests a small contribution from the vent community. The distribution pattern of TFA, however, was considered to reflect high activity of *in situ* bacterial community because the pattern was significantly different from that of the reference samples obtained far from the vent (40 km from the ridge axis), which had a much lower proportion of the MUFAs and branched FAs. The reference samples had a significant proportion of long-chain FAs, while the vent sediment had a lower proportion of long-chain FAs. A high proportion of short chain FAs (<C₂₂) in the vent sediment indicated high contribution of *in situ* bacterial products at the hydrothermal environment.

Hedrick et al. (1992) reported compositions of PLFAs in a chimney and surrounding sediments obtained from the hydrothermal site at the Juan de Fuca Ridge. The PLFAs in the chimney samples included more than 30 FAs and were rich in MUFAs such as 16:1n-7 and 18:1n-7 (accounting for 26–68% of total PLFA) except one sample which was rich in cyclopropyl 17:0 acids (accounting for 66.4%). The sediment samples, mud and a red-colored bacterial mat, were rich in PUFAs (accounting for 30 and 40%, respectively) with 20:5n-3 predominating, and the MUFAs were also abundant (accounting for 37–54%). The authors concluded that the PUFAs originated in PLFAs were bacterial in origin because some bacteria isolated from the deep seafloor are abundant in PUFAs.

Furthermore, Conway and Capuzzo (1991), Pranal et al. (1997), and Ben-Mlih et al. (1992) reported compositions of PLFAs and/or TFAs in soft tissues of deep-sea mussels and giant clams colonizing hydrothermal environments and bearing symbiotic bacteria. *Bathymodiolus* and *Calyptogena* bivalves obtained from the hydrothermal fields at 13°N EPR and Galápagos Rise contained more than 40 FAs in which MUFAs (16:1n-7, 18:1n-7, 20:1n-7 and 20:1n-9; 22–69% of TFA) and palmitic acid (6–12%) predominate (Ben-Mlih et al., 1992). Since these animals harbor sulfur-oxidizing bacteria in their gill tissues, these MUFAs should be produced by the bacteria. Although several PUFAs, especially 20:2 (2–16%), were detected in these animals, the concentrations of algal-derived 20:5 and 22:6 acids were very low (less than 1% of TFA) except the samples obtained from the hydrothermal field at 13°N EPR (5–9% of TFA). Two *Bathymodiolus* species obtained from

Table 3
Relative abundances of the total fatty acids in the hydrothermal vent sediments

Fatty acid	% of Σ TFA						
	DESMOS caldera		Myojin caldera		Iheya Ridge		
	D916	D921	D1008	D1011	D857R	D857B	D1030
9:0							0.8
10:0	0.1	0.1					0.4
11:0	0.1						0.3
i12:0	0.3						
12:0	1.0	1.7		1.7	0.8	0.5	3.1
i13:0	0.2	0.2	0.2	0.3			0.4
ai13:0	0.1	0.1	0.3	0.1			0.2
13:0	0.1	0.2	0.4	0.2	0.1	0.1	0.9
i14:0	1.6	1.8	2.2	1.6	0.9	0.7	3.5
14:1	0.2	1.2	0.9	0.6			
14:0	4.1	4.4	8.4	5.2	3.2	3.6	6.6
cycl15:0			0.7	1.2			
br15:1	0.6						
i15:0	2.3	1.3	1.7	2.8	0.5	0.3	1.3
ai15:0	3.4	2.4	2.9	3.7	0.8	0.6	2.1
15:1	0.4		0.6	0.1			
15:0	1.3	2.7	4.7	2.5	1.8	1.6	2.6
16:?			1.3	0.4			
i16:1		1.5					
i16:0	2.2	1.1	1.3	1.4			1.6
16:1					0.5	0.4	
16:1n-7	15.2	8.8	11.2	6.7	4.3	14.8	0.8
16:1n-5	4.1	1.9	1.4	1.3		4.7	1.8
16:0	10.9	17.6	19.7	21.8	32.2	36.4	20.4
3-OH 16:0		0.6					
br17:1		2.4					
br17:0	1.9		2.4	4.8	1.5	0.6	0.7
i17:0	0.9	1.1	0.5	0.9	0.3	0.1	0.5
ai17:0	1.7	1.4	1.7	1.6	0.8	0.4	1.1
17:1	1.4	1.0	1.2	1.2		0.2	0.4
17:0	3.0	3.8	2.7	2.0	2.0	1.1	2.2
18:3			0.3	0.4		0.7	0.4
18:2	3.3		0.7	0.4	1.4		
18:1n-9	4.6	9.6	7.8	5.1	7.3	3.9	2.5
18:1n-7	9.7	5.5	2.6	4.5	1.5	4.7	0.9
18:1	1.0						
18:0	5.7	9.9	13.2	8.4	13.7	15.1	11.4
br19:0			0.5	1.1			
19:2	0.7						
19:1	2.1	0.6	1.3	2.1			0.3
19:0	1.3	0.3	1.1	1.4	1.6	0.6	2.1
20:4	0.8	0.9					
20:3	0.8	2.7					
20:2		0.8	0.2				
20:1	3.9	1.6	0.2	2.7			0.7
20:0	1.5	2.1	1.2	2.4	2.1	1.4	4.0
21:0	1.0	1.2	0.3	1.2	2.9	0.4	1.7
br22:0					2.4	1.0	
22:0	1.6	5.1	0.8	2.3	4.5	3.3	5.3
23:0	0.7	0.8	0.3	1.1	3.8	0.5	1.5
24:0	1.8	1.1	0.5	2.6	3.0	1.1	6.4
25:0	0.5	0.3	0.1	0.4	1.5	0.2	1.6
26:0	1.0	0.2		1.3	2.6	0.3	6.5
27:0	0.3	0.1		0.1	0.8	0.2	0.7
28:0	0.5	0.1		0.3	1.2	0.4	2.0
29:0							0.2

i: iso, ai: anteiso, OH: hydroxy, br: methyl branched but the position unidentified.

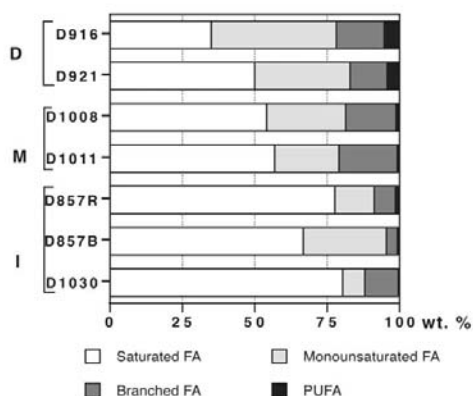


Fig. 3. Distributions of TFAs classified by the structures of alkyl-chains, i.e., straight chain saturated fatty acids (Saturated FAs), straight chain monounsaturated fatty acids (Monounsaturated FA), methyl-branched fatty acids (Branched FAs), and straight-chain polyunsaturated fatty acids (PUFAs). *M*: Myojin caldera, *I*: Iheya Ridge, *D*: DESMOS caldera hydrothermal sites.

the hydrothermal vent areas in Lau and Fiji basins, southwestern Pacific, were also shown to have compositions of PLFAs characterized by higher contents of MUFAs and a lack of algal-derived PUFAs (Pranal et al., 1997). They are also rich in 20:2 acids (11–15% of total PLFA) and 18:3 and 22:2 acids. Pranal et al. (1997) reported the composition of fatty acids in both neutral and polar lipid fractions from the *Bathymodiolus*, finding no significant difference in the distributions of fatty acids between the two lipid fractions. It indicates that the two lipid fractions provide similar information about symbionts and nutritional status.

The characteristics of the TFA composition in the present study, rich in MUFAs and lacking in PUFAs (Fig. 3), is common in the vent sediments and animal samples with the few exceptions mentioned above. We can therefore conclude that the MUFAs such as 16:1n-7 and 18:1n-7 in the samples were derived from bacteria either directly or via symbiotic animals. Although 16:1n-7 acid is also abundant in diatoms and haptophytes (Volkman et al., 1980a; 1989) and those organisms synthesize 18:1n-7 acid via chain-elongation of 16:1n-7, bacterial production of 18:1n-7 acid is noticeably contributed to fatty acid compositions in an intertidal sediment (Volkman et al., 1980b). The dysaerobic surface sediments in the coastal upwelling regimes of Peru and Chile are also rich in 18:1n-7 acid (15% of total fatty acids), where sulfur-oxidizing bacteria, *Thioploca*, constitutes as much as 80% of the biomass (McCaffrey et al., 1989). Therefore, the possible source of 18:1n-7 acid is considered sulfur-oxidizing bacteria, that are a major primary producer in the seafloor hydrothermal environment (Jannasch, 1983).

Table 2 gives the ratios of 18:1n-7 to total C₁₈ fatty acids (18:1n-7/∑18:n), which are expected to indicate the relative contribution of bacterial input. Significantly low ratios (<0.1) are observed for samples D857R and D1030, which is consistent with the lower abundances of MUFAs in the two samples. This result suggests that the contribution of sulfur-oxidizing bacteria to these two sediments is very low. The sample D857R was collected from the non-hydrothermal area as reference (approx. 100 m away from an active vent). The sample D1030 was obtained near a dead *Calymptogena* colony (about 20 m from the colony), where thin sandy mud covered dead shells. The low 18:1n-7/∑18:n ratios of these two samples are in good agreement with these observations suggesting low activity of bacterial production at these sites. The ratios of 16:1n-7 to 16:0 are similar to the 18:1n-7/∑18:n ratio, and the 16:1n-7/16:0 ratios of samples D857R and D1030 can be distinguished from the other samples. This suggests that the 16:1n-7 fatty acid is also bacterial in origin. The ratio of short-chain saturated fatty acids to long-chain saturated acids, i.e., L/H ratio [∑(<C₂₀)/∑(≥C₂₀)] in Table 2, is an indicator of contribution of terrigenous input (Fukushima and Ishiwatari, 1984); the low ratios of these two samples indicate higher contributions of terrigenous organic matter rather than another input. In contrast, higher 18:1n-7/∑18:n ratios of the other samples indicate that these samples are rich in bacterial fatty acids.

Algal-derived PUFAs from the photic zone were absent from all the samples. Some vent animals, shrimps, worms and clams, have been reported to contain the algal-derived PUFAs (Pond et al., 1997, 2002; Allen et al., 2001), these PUFAs rarely reach the sediments due to alteration and/or decomposition during the metabolism in the water column. Although the non-algal PUFAs were also absent from the present samples except those obtained from DESMOS caldera, PUFAs are expected to be preferentially decomposed by heterotrophic bacterial activity (Canuel and Martens, 1993). Methyl branched FAs, such as *iso* and *anteiso* branched, are usually derived from heterotrophic anaerobes (Findlay et al., 1990). Sulfate-reducing bacteria (SRB) are among the plausible source organisms (Taylor and Parkes, 1983; Vainshtein et al., 1992). An abundance of the branched acids suggests that intense decomposition and alteration of organic matter occurred in the sediments. The presence of SRB in the normal to high temperature (>100 °C) sediments around hydrothermal vents has been shown using radiotracer techniques at the hydrothermal site of the Guaymas Basin, Gulf of California (Jørgensen et al., 1992). The samples from DESMOS caldera were collected at the dense *Calymptogena* and tubeworm communities and they include considerable amount of the PUFAs. Therefore, input and/or synthesis of the PUFAs in the DESMOS site was expected to be higher than for the other sites. On the

other hand, at the sampling sites of Myojin caldera the vent animals were sparsely distributed. The PUFAs would be consumed by the heterotrophic bacteria, and as a result the branched FAs, which are synthesized by the heterotrophic bacteria, would be relatively more abundant than for the samples from DESMOS caldera. The sample D857B was collected from a dense *Calyptogena* colony, however, the proportions of PUFAs and branched FAs were much lower than for the same community of the DESMOS site. The sample D1030 collected from a dead *Calyptogena* community included higher amounts of the branched FAs, implying that the dead community may be still providing organic substances for the SRB. Although the source organisms of the PUFAs cannot be identified from the present data alone, preservation of the PUFAs and heterotrophic bacterial activity at the surface sediment may be affected by the high sedimentation rate at the Okinawa Trough.

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