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Research Article

Molecular taxonomy and naming of five cryptic species of *Alviniconcha* snails (Gastropoda: Abyssochrysoidea) from hydrothermal vents

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Large symbiont-hosting snails of the genus *Alviniconcha* (Gastropoda: Abyssochrysidae) are among the dominant inhabitants of hydrothermal vents in the Western Pacific and Indian oceans. The genus was originally described as monotypic, but unique DNA sequences for mitochondrial genes revealed six distinct evolutionary lineages that we could not distinguish based on external morphology. Subsumed under the name *Alviniconcha hessleri* Okutani & Ohta, the distinct allopatric and sympatric lineages have been assigned placeholder epithets that complicate scientific communications. Based on the present multi-gene sequence data, we hereby describe five *Alviniconcha* species (in the order of their discovery) – *A. kojimai* sp. nov., *A. boucheti* sp. nov., *A. marisindica* sp. nov., *A. strummeri* sp. nov. and *A. adamantis* sp. nov. Thus, we restrict application of the name *A. hessleri* to specimens that are genetically similar (≥95% for *COI*) to those found at localities in the Mariana Trough. Single distinct *Alviniconcha* species inhabit vent fields along the Central Indian Ridge, the Mariana volcanic arc, and the Mariana back-arc basin, whereas vents in the Manus, Fiji and Lau back-arc basins may host two or three additional species. Formal recognition of these species facilitates future attempts to assess their physiological differences and symbiont associations. Furthermore, their reported distributions have significant biogeographic implications, affecting estimates of the diversity within and overlap among Indo-Pacific vent localities.

http://zoobank.org/urn:lsid:zoobank.org:pub:1E4B2E71-9F1D-479E-9A9A-22A9E303AAE5

Key words: Abyssochrysoidea, cryptic species, DNA-barcode, hydrothermal vent, deep-sea

Introduction

Deep-sea organisms are woefully undersampled because of the great depths, unpredictable oceanographic conditions and great expense associated with exploring the vast abyssal areas that cover nearly 70% of Earth's surface (McClain & Hardy, 2010). Consequently, our understandings of deep-sea biodiversity and biogeography are relatively poor, even for faunas that have received considerable attention, such as those inhabiting hydrothermal vents and other chemosynthesis-based environments (e.g. Bachraty, Legendre, & Desbruyères, 2009; Rogers et al., 2012; Sibuet & Olu, 1998; Van Dover, 2002). Cryptic species (distinct evolutionary lineages with a common morphological phenotype) frequently occur among the invertebrate taxa inhabiting these habitats (Vrijenhoek, 2009). Failure to recognize evolutionarily distinct lineages results in underestimates of species diversity at local scales and overestimates of species overlap at greater geographic scales (Matabos et al., 2011). Conversely, incomplete sampling of size series and discrete life-history stages (e.g. larvae, juveniles and adults) for many deep-sea taxa can result in failures to link discrete developmental stages or to recognize phenotypically plastic morphotypes within single species. Consequently, species descriptions based on traits other than genetic differences can lead to overestimates of local species diversity and underestimates of geographic overlap. Reliable assessments of diversity and reconstructions of historical biogeography require solid foundations in taxonomy and phylogenetics that often are lacking for undersampled faunas. Molecular systematic methods have helped to remedy

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Table 1. *Alviniconcha*: prior epithets, localities, new species names, *COI* reference sequences and origin.

Prior epithet	Ref.	Locality	Species name	GB #	Authority	Distr.[1]
A. hessleri	(Okutani et al., 1988)	Mariana Back-Arc Basin	*A. hessleri sensu stricto*	AB235216& AB051765-790	Okutani	MT, PV, FV
A. sp. "type I"	(Kojima et al., 2001; Kojima et al., 1998)	Lau Basin	*A. kojimai* sp. nov.	AB235211& AB051792-803	Johnson et al.	MB, WR, WL, TC, TM
A. sp. 'type II'	(Kojima et al., 2001; Kojima et al., 1998)	Lau Basin	*A. boucheti* sp. nov.	AB235212 & AB051804-806	Johnson et al.	MB, WR, WL, MH, KM
A. aff. *hessleri*	(Okutani et al., 2004; Van Dover et al., 2001)	Central Indian Ridge	*A. marisindica* sp. nov.	AB162121-123 & AB235213	Okutani	ED, KA
A. sp. 'Lau' (= type III)	(Beinart et al., 2012; Suzuki et al., 2006a)	Lau Basin	*A. strummeri* sp. nov.	AB235215	Johnson et al.	TM
A. sp. 'type IV'	This study, (Fujiwara et al., 2013)	Izu-Bonin- Mariana Arc	*A. adamantis* sp. nov.	AB856040	Johnson et al.	DS, SS

[1] Known distributions according to the numbered localities in Figs. 1, 5.2 and Table 2.

many of these problems for deep-sea faunas by exposing cryptic species complexes and identifying phenotypic plasticity in a broad range of vent molluscs and annelids (reviewed in Vrijenhoek, 2009). Analysis of DNA sequences provides an objective basis for assessing degrees of divergence among geographic populations of nominal species and examining the coupling (or lack thereof) between distinct evolutionary lineages and morphologically defined species.

The *Alviniconcha hessleri* species complex (Gastropoda: Abyssochrysoidea, previously Provannidae) provides a noteworthy example of cryptic species. *Alviniconcha hessleri* Okutani & Ohta, 1988, was initially described from the Mariana Back-arc Basin. Subsequent collections from other Indo-Pacific locations, however, revealed five additional evolutionary lineages (Table 1, Fig. 1) (Kojima et al., 2001; Kojima, Suguru, Fujiwara, Fujikura, & Hashimoto, 1998; Okutani, Hashimoto, & Sasaki, 2004; Okutani & Ohta, 1988; Suzuki et al., 2006a; Van Dover et al., 2001). Sequencing of mitochondrial cytochrome-*c*-oxidase subunit I (*COI*) identified a highly divergent lineage from the Central Indian Ridge, *Alviniconcha* aff. *hessleri* (Hashimoto et al., 2001; Van Dover et al., 2001). Okutani and coworkers (2004) indicated that the lineage warrants recognition as a distinct species, but morphological differences were not identified. Genetically distinct lineages are also found at western Pacific locations in the Manus, North Fiji and Lau basins (Kojima et al., 2001; Suzuki et al., 2006b). We identified a sixth western Pacific lineage from the East Diamante Seamount in the Mariana volcanic arc with sequence data, from the shallowest vents known to be inhabited by *Alviniconcha*, at a depth of 357 m. To date, these unnamed lineages have proved to be indistinguishable, based on external morphology, from *A. hessleri sensu lato* (Desbruyères, Segonzac, & Bright, 2006; Hasegawa, Fujikura, & Okutani, 1997; Kojima et al., 2001; Warén & Bouchet, 1993), and thus remain unnamed. Efforts to identify diagnostic morphological traits are impeded in many cases by limited samples, ontogenetic changes in conchology and apparent phenotypic variability. These large snails produce relatively thin shells, typically covered with a hairy periostracum that is completely abraded in some cases (Fig. 2). Because these snails often inhabit high temperature vents with low pH, the calcareous component of the shell can be severely degraded or entirely absent (Fig. 2), leaving just a silvery organic matrix. Presently, DNA sequences provide the most reliable diagnostic method for discriminating these lineages.

Informal epithets for the unnamed *Alviniconcha* lineages continue to proliferate through the literature, creating instability of formal taxonomy and impediments to communication about the unique geographic distributions, ecological and physiological differences and symbiont associations of the species. To remedy this 'nomenclatural housekeeping problem' (Brower, 2010, p. 488) we used three mitochondrial (mt) markers, Cytochrome-c-oxidase subunit one (*COI*), 12S ribosomal RNA gene (*12S mt rRNA*), 16S ribosomal RNA gene (*16S mt rRNA*) and three nuclear markers, Histone-3 (*H3*), 18S ribosomal RNA gene (*18S rRNA*), and domains 1 and 6 of 28S ribosomal RNA gene (*28S-D1 rRNA* and *28S-D6 rRNA*) for species diagnoses. Genetic differentiation and phylogenetic relationships allowed us to recognize six *Alviniconcha* species. We recommend that the name *A. hessleri sensu stricto* is applied to snails that are genetically

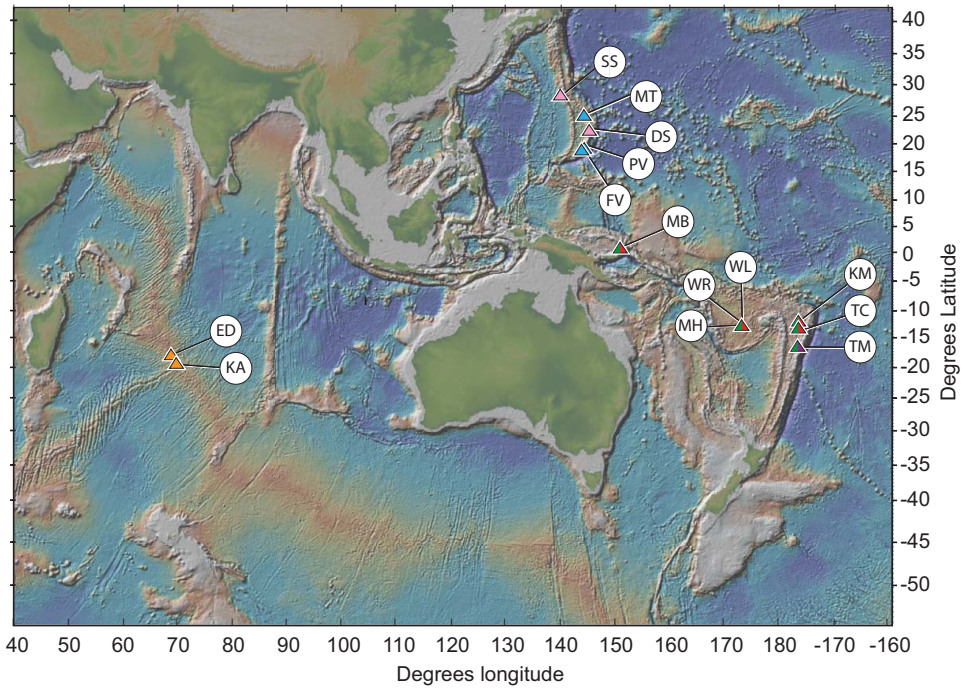

Fig. 1. Sample localities of Indo-Pacific *Alviniconcha* species. Central Indian Ridge: Edmunds vent field (ED); Kairei vent field (KA). Mariana Back-arc Basic: Mariana Trough (MT); Piracy vents (PV); Forecast vent field (FV). Izu-Bonin-Mariana Volcanic Arc: East Diamante Seamount (DS); Suiyo Seamount (SS). Manus Basin: Solwara 1, 8 and South Su (MB). North Fiji Basin: White Rhino (WR); White Lady (WL); Mussel Hill (MH). Lau Basin: Kilo Moana (KM); Tow Cam (TC); Tui Malila (TM). Colours indicate species presence, blue: *A. hessleri s.s.*; green: *A. kojimai* sp. nov.; red: *A. boucheti* sp. nov.; orange: *A. marisindica* sp. nov.; purple: *A. strummeri* sp. nov.; and pink: *A. adamantis* sp. nov.

similar (as subsequently defined) to *A. hessleri* from the 'Alice Springs' and 'Forecast' vent fields in the Mariana Trough (Kojima et al., 2001). The five additional species were from Western Pacific and Indian oceans.

The field of taxonomy is rapidly evolving to accept 'molecular' species descriptions in the absence of other reliable characters (Cook, Edwards, Crisp, & Hardy, 2010). Many arguments have been levied against the practice of molecular species descriptions – e.g. DNA sequencing is expensive, requires great expertise and is less accessible for developing nations. Yet, the costs have fallen recently and accessibility is now widespread. Acquiring and analysing sequence data also encounters perils and pitfalls, but when properly applied, molecular species delineation can provide reliable, replicable characters for cryptic species complexes (Vogler & Monaghan, 2007; Vrijenhoek, 2009). Ideally, DNA taxonomists should follow a uniform, character-based description method. For consistency, we followed the methods of Jörger and Schrödl (2013) who noted three points: (1) The same rules of traditional taxonomy should be applied with respect to the deposition of museum specimens, whether it is DNA or tissue, and accessibility of data; (2) the quality of alignments is critical to determine and extract diagnostic characters and should be carefully tested; and (3) as data are added, alignments can change. For better traceability, the position should be noted either on a reference genome or in a deposited reference sequence ideally generated from type material.

Materials and methods

Samples

Expeditions conducted between 1993 and 2008 explored various segments of the North Fiji, Lau, Manus and Mariana back-arc basins, the Mariana Volcanic Arc and the Central Indian Ridge (Fig. 1, Table 2). Collections were conducted with the remotely operated vehicles (ROVs) *Jason II* and *Ropos*, with the ST212 trenching ROV, and with the human-occupied vehicle (HOV) *Shinkai 6500*. Snails were sampled with scoops, nets or directly with robotic manipulators. Samples were placed in insulated 'bioboxes' containing ambient seawater at 2–4 °C. Upon recovery of the vehicles, most samples were stored temporarily in refrigerated seawater (4 °C) prior to dissection or preservation. A common problem during prolonged oceanographic expeditions is determining what to save, especially for large species like *Alviniconcha* that occupy much of the highly limited and

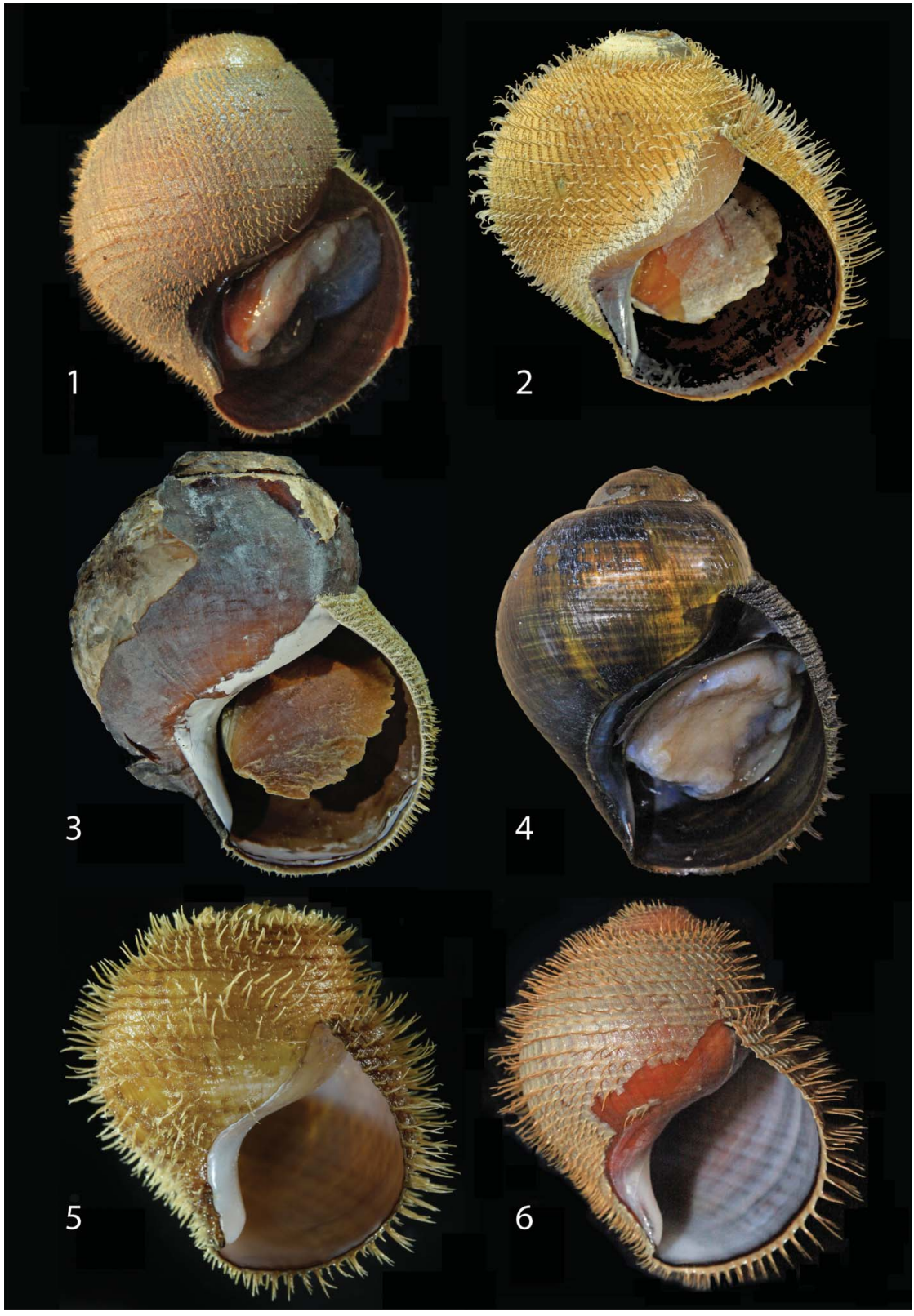

Fig. 2. Images of *Alviniconcha* species holotypes: (2.1) *A. hessleri s.s.*, Mariana Back-Arc Basin, Piracy Vents; (2.2) *A. kojimai* sp. nov. Holotype SMNH type coll. 8577, 54 mm shell height, Lau Basin, Tow Cam; (2.3) *A. boucheti* sp. nov. Holotype SMNH type coll. 8575, 98 mm shell height. Fiji Back Arc Basin, White Lady. Very large specimen from a precipice washed by hydrothermal effluents. Shell almost completely decalcified; (2.4) *A. marisindica* sp. nov. Holotype SMNH type coll. 8572, 61 mm shell height. Central Indian Ridge, Kairei vent field; (2.5) *A. strummeri* sp. nov. Holotype SMNH type coll. 8573, 30.5 mm shell height. Lau Basin, Tui Malila; (2.6) *A. adamantis* sp. nov. Holotype SMNH type coll. 8574, 55.5 mm shell height. Mariana Volcanic Arc, E. Diamante Seamount. Images document morphological variability but are not meant to differentiate among species, since interspecific variation in shape and periostracum structure was not correlated with genetic differentiation amongst species.

Table 2. *Alviniconcha* and *Ifremeria nautilei* (out-group) sample localities.

Locality	Dive No.[1]	Latitude	Longitude	Depth (m)	Date
1. Central Indian Ridge					
Edmunds vent field (ED)	J2-301	23°31.80'S	69°21.6'E	3289	23.4.2001
Kairei vent field (KA)	J2-297	25°52.98'S	70°35.82'E	2432	9.4.2001
2. Mariana Back-Arc Basin					
Mariana Trough (MT)	S185	18°13.00'N	144°42'E	3589	21.10.1993
Piracy Vents (PV)	J2-42	12°57.25'N	143°37.20'E	2863	29.3.2003
Forecast vent field (FV)	J2-185	13°23.68'N	143°55.21'E	1447	21.4.2006
3. Mariana Volcanic Arc					
E. Diamante Seamount	R787	15°56.57'N	145°40.88'E	351-357	4.2.2004
Five Towers (DS)	J2-193				30.4.2006
4. Manus Basin (MB)					
Solwara 8-2	ST 28/30	3°43.82'S	151°40.46'E	1710	28−29.7.2008
Solwara 1-4 †	ST 9	3°47.44'S	152°5.47'E	1530	18.7.2008
Solwara 1-5 †	ST 11	3°47.37'S	152°5.78'E	1490	19.7.2008
Solwara 1-6 †	ST 17	3°47.37'S	152°5.62'E	1480	23.7.2008
South Su-7	ST 38	3°48.56'S	152°6.14'E	1300	4.8.2008
South Su-8	ST 40	3°48.49'S	152°6.19'E	1350	6.8.2008
5. North Fiji Basin					
White Rhino † (WR)	J2-153	16°59.44'S	173°54.86'E	1978	31.5.2005
White Lady † (WL)	J2-150	16°59.39'S	173°54.95'E	1970	29.5.2005
Mussel Hill † (MH)	J2-151/152	16°59.41'S	173°54.97'E	1973	30−31.5.2005
6. Lau Basin					
Kilo Moana (KM)	J2-140	20°3.22'S	176°8.01'W	2620	15.5.2005
Tow Cam † (TC)	J2-142	20°19.08'S	176°8.26'W	2714	19.5.2005
Tui Malila † (TM)	J2-143	21°59.43'S	176°34.15'W	1845	20.5.2005
Tui Malila † (TM)	J2-144	21°59.26'S	176°34.07'W	1900	21.5.2005

[1] J2, ROV *Jason II*; R, ROV *Ropos*; S, HOV *Shinkai 6500*; ST, ROV ST212.
† Sources also include *Ifremeria nautilei* used as outgroup for phylogenetic analyses.

fought-over space in freezers or require large quantities of chemical preservatives. Therefore, most of the present specimens were represented only as samples of gill and foot tissues that were frozen at −80 °C or preserved in 95% ethanol. Nonetheless, for morphological comparisons we had access to approximately 200 complete specimens representing the full range of sample localities, although usually fixed in formalin. Voucher specimens were preserved in 4% seawater-buffered formaldehyde solution. *Ifremeria nautilei*, the abyssochrysoid species most closely related to the genus *Alviniconcha* (Johnson et al., 2010) was used as outgroup for the phylogenetic analyses of all the loci examined in this study.

DNA methods

DNA extraction and purification, PCR conditions, amplicon purification and DNA sequencing used methods that were previously reported for abyssochrysoid snails (Table 3) (Johnson et al., 2010). The *COI* locus was sequenced for all available species (sample sizes for each locality in Fig. 5.2). For nuclear and mt ribosomal markers that can be less variable than *COI*, six randomly chosen individuals that encompassed the species ranges were sequenced. DNA templates for PCR were diluted in sterile water to 1:10 and 1:100 concentrations. PCR primers and protocols are listed in Table 3. Amplicons were diluted in 40−50 μl of sterile water and purified with a Multiscreen HTS PCR 96 vacuum manifold system (Millipore Corp. Billerica, MA). Amplicons were sequenced bi-directionally on an ABI3130XL or an ABI3500XL sequencer with BigDye Terminator v3.1 chemistry (Life Technologies Corp., Carlsbad, CA) and primers (Table 3) used in PCR.

Species identification of formalin-preserved type specimens of *A. boucheti* sp. nov. and *A. strummeri* sp. nov. required special DNA extraction procedures. Tissues were rinsed three times in phosphate-buffered saline (1xPBS), then heated to 150 °C for 10 minutes in the liquid cycle of an autoclave. Genomic DNA was extracted with a modified protocol that used the Qiagen DNeasy™ DNA extraction kit. Initially, 95 μl ATL buffer (DNeasy™)

Table 3. PCR primers and amplification methods.

Locus	Primers	Methods	Length (bp)	References
Cytochrome-c-oxidase subunit-I	HCO/LCO, COIF/R	[1]	~650 ~1200	(Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994; Nelson & Fisher, 2000)
Cytochrome-c-oxidase subunit-I (for formalin fixed specimens)	*Alvini*-HCO, HCO1708[2]	[1]	~150	Current manuscript[2]
16S mitochondrial RNA	16SAR/BR	Fast PCR[3]	~500	(Palumbi, 1996)
12S mitochondrial RNA	12SF/R	Fast PCR[3]	~440	(Kocher et al., 1989)
28S ribosomal RNA subunit-D1	28SD1F/R,	Fast PCR[3]	~350	(Colgan, Ponder, & Eggler, 2000)
28S ribosomal RNA subunit-D6	28SD6F/R	Fast PCR[3]	~450	(McArthur & Koop, 1999)
18S ribosomal RNA	18S1F/4R	Fast PCR[3]	~550	(Giribet, Carranza, Baguna, Riutort, & Ribera, 1996)
Histone-3	H3F/R	Fast PCR[3]	~330	(Colgan et al., 2000)

[1] PCR program: 95 °C/10 min; 35 × [94 °C/1 min, 55 °C/1 min; 72 °C/1 min], extension at 72 °C/7 min.
[2] *Alvini*-HCO: 5′-GATCTGGNTTAGTYGGTACM -3′, HCO1708: 5′-GGGAASGCTATATCTGGRGC-3′.
[3] Touchdown and Fast PCR: Amplitaq Gold Fast PCR Master Mix, UP (Life Technologies Corp., Carlsbad, CA) and the protocol for the *Taq* supplied by manufacturer of Veriti thermal cycler with an annealing temperature at 50 °C (Life Technologies Corp., Carlsbad, CA).

was added to autoclaved tissues then put in a water-bath at 55 °C for 24 hours. Then, each day for 7 days, 5 μl of Proteinase-K was added to the sample and mixed with a vortex mixer. Fifty additional microlitres of ATL buffer were added on days 3 and 5. We then added 300 μl of AL buffer (DNeasy^{TM}) and 300 μl 100% ethanol to the digestion mixture. The extraction was stored at −20 °C overnight. Except for the volume of final elution step (reduced to 100 μl), the remainder of the extraction procedure followed the manufacturer's protocol. PCR and sequencing protocols followed the procedures outlined in Table 3.

Molecular statistics

Bidirectional sequence traces were edited, assembled and aligned using Geneious software (v.6.1.6, Biomatters: Available from http://www.geneious.com/). New sequences were added to existing alignments (from Johnson et al., 2010) using the Muscle (Edgar, 2004) software and default settings. Model selection was estimated with jModelTest with the BIC selection criterion (Darriba, Taboada, Doallo, & Posada, 2012; Guindon & Gascuel, 2003; Posada, 2008). Bayesian phylogenetic analyses were conducted with the MrBayes program v.3.1 (Altekar, Dwarkadas, Huelsenbeck, & Ronquist, 2004; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) for each locus independently, and in a final concatenated analysis using the program parameters such as run-time, burn-in/convergence and sampling frequencies previously defined by Johnson et al. (2010). Resulting trees were visualized with FigTree software v.1.4 (Rambaut, 2010). Network Publisher v.2.0.0.1 (©Fluxus Technology Ltd) was used to construct median joining networks for each locus. Indices of diversity and Kimura-2-parameter (K2P) distance matrices were calculated with the Arlequin program v.3.5.1.3 (Excoffier & Lisher, 2010) and Mega v.5.10 (Tamura et al., 2011). Sequences (Table 1) of *COI* were compared with published *Alviniconcha* sequences for reference species identifications. However, only a subset of the published sequences was used in statistical analyses because of length discrepancies and unverifiable polymorphisms.

Following the methods of Jörger and Schrödl (2013) we used the Characteristic Attribute Organization System (CAOS) (Bergmann, Hadrys, Breves, & Schierwater, 2009; Sarkar, Planet, & Desalle, 2008; Sarkar et al., 2002) to provide diagnostics for each evolutionary lineage. The diagnostic characters were unique nucleotides that are fixed in a particular species. Positions of the diagnostic nucleotides, based on our alignments, included reference sequences for each OTU from GenBank for *COI* (Table 4). For the *28SD6* fragment, base pair positions 57−85 and 315−351 were excluded from CAOS analyses due to ambiguities in the alignment.

Results

COI haplotype networks and diversity

Haplotype networks for mitochondrial *COI* sequences clearly identified six evolutionary lineages of *Alviniconcha* (Fig. 3). The number of mutational steps between the species ranged from 17 to 47. Genetic distances among the species reflect the same scale of divergence (Table 5). The lowest degree of *COI* divergence (4.68%) occurred between *A. hessleri s.s.* and *A. kojimai* sp. nov.

Within-species divergence was substantially lower than the between-species divergence. Genetic distances for *COI* ranged from a low of 0.14% to a high of 0.76% (Table 5). The haplotype networks (Fig. 3) also reflect differences in *COI* diversity among the six species (Table 6). Haplotype diversity (h) ranged from 0.51 to 0.96 and

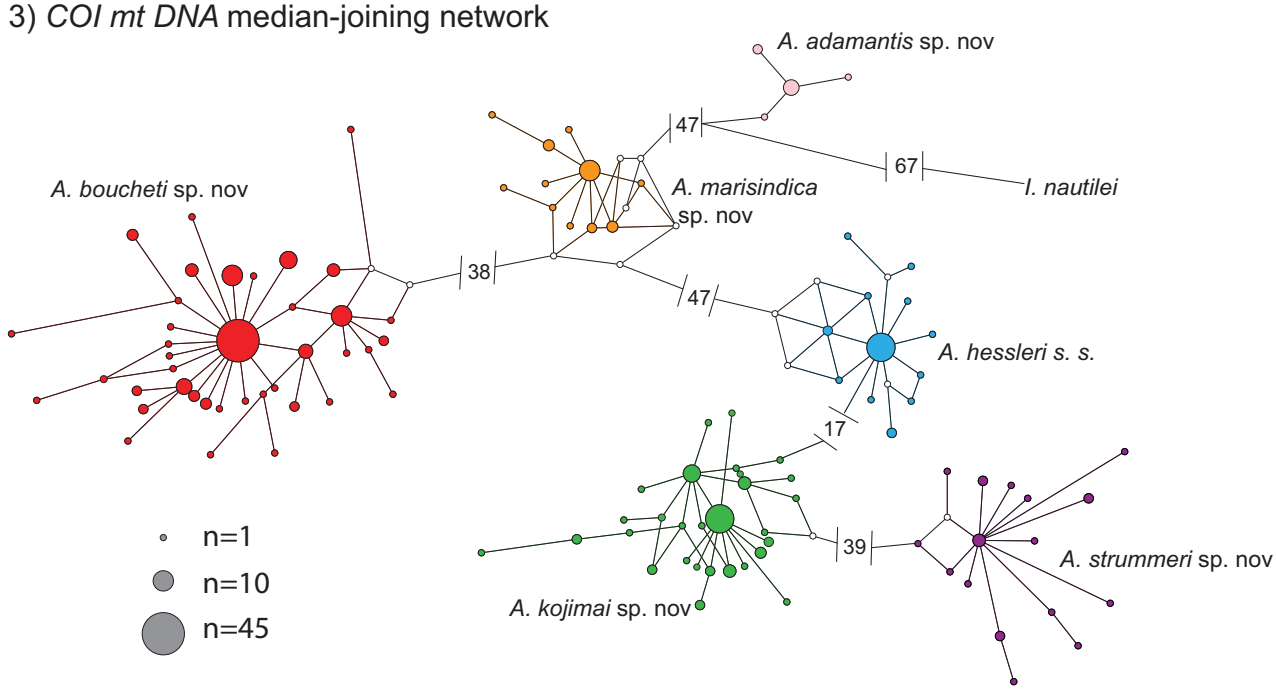

Fig. 3. *Alviniconcha COI* network rooted with *Ifremeria nautilei*. Sizes of coloured circles indicate relative allelic frequencies. White circles indicate missing alleles. Numbers in gaps indicate the mutational steps between species and length of branches indicates mutational steps within a species.

nucleotide diversity (π) ranged from 0.02 to 0.05. Although *A. adamantis* sp. nov. had the smallest sample size, and lowest diversity measures, h and π were uncorrelated with sample size (N) overall ($P = 0.31$ and 0.34 respectively); however, the number of polymorphic sites (k) and absolute number of haplotypes (H) were correlated with N ($P = 0.03$ and 0.009 respectively). Each species exhibited a single 'leading' (most frequent) *COI* haplotype (Fig. 3) surrounded by star-like distributions of low frequency and singleton haplotypes.

Multi-locus concordance

With few exceptions, the multi-locus data corroborate the species diagnostics based on mitochondrial *COI* (Fig. 4). Not surprisingly, mitochondrial *12S* and *16S mt RNA* exhibited the same pattern as *COI*. *Alviniconcha strummeri* sp. nov. did not amplify for *16S*, but the other five species were reciprocally monophyletic for both loci. Additionally, *A. hessleri s.s.* and *A. kojimai* sp. nov. were the most closely related species, and *A. boucheti* sp. nov. had the greatest within-species diversity.

Table 4. GenBank Accession numbers of newly generated sequences. ND designates missing data due to failed amplification.

	COI	*16S*	*12S*	*28SD1*	*28SD6*	*18S*	*H3*
A. hessleri s.s.	KF467922-955	KF467613	KF467628	KF467658	DF467642	KF467655	KF467639
A. kojimai sp. nov.	KF467676-741	KF467618-619	KF467621, KF467625	DF467661-663	ND	KF467648-650	KF467633-635
A. boucheti sp. nov.	KF467742-874	KF467616-617	KF467623, KF467626-627	KF467664	KF467645	KF467653	KF467636-637
A. marisindica sp. nov.	KF467897-921	KF467615	KF467629	KF467660	KF467644	KF467651	KF467640
A. strummeri sp. nov.	KF467875-896 & KJ027398	ND	KF467624	KF467659	ND	KF467652	KF467638
A. adamantis sp. nov.	KF467666-675	KF467614	KF467622	KF467657	KF467643	KF467654	KF467632

Table 5. Genetic distances (K2P%) for the mitochondrial *COI* gene fragment within (diagonal) and among (lower matrix) *Alviniconcha* species.

	hessleri	*kojimai*	*boucheti*	*marisindica*	*strummeri*	*adamantis*
A. hessleri s.s.	0.14					
A. kojimai sp. nov.	4.68	0.41				
A. boucheti sp. nov.	15.67	14.98	0.34			
A. marisindica sp. nov.	12.68	14.41	9.82	0.50		
A. strummeri sp. nov.	11.90	11.42	15.92	16.34	0.76	
A. adamantis sp. nov.	16.66	16.56	13.08	13.97	14.66	0.40

The four nuclear gene segments exhibited varying degrees of incomplete lineage sorting among the species. Neither *A. strummeri* sp. nov. or *A. kojimai* sp. nov. amplified for *28SD6 rRNA*. Nonetheless, the nuclear segments were broadly concordant with the mitochondrial loci. Varying patterns of lineage sorting occurred among the species for different loci. For *H3*, the species-pairs *A. boucheti* sp. nov./*A. marisindica* sp. nov. and *A. hessleri* s.s./*A. kojimai* sp. nov. shared common alleles. For *18S*, *A. boucheti* sp. nov. and *A. adamantis* sp. nov. shared an allele. For *28S D1*, *A. hessleri* s.s. and *A. kojimai* sp. nov. shared an allele. Incomplete lineage sorting is not unexpected for slowly evolving nuclear gene segments. Although the *28S-D6 rRNA* segment was not amplified for all six species, it exhibited relatively high levels of divergence among four species compared with the *28S-D1* fragment.

To assess rates of interpopulational gene flow within several broadly distributed *Alviniconcha* species, we have also examined DNA sequences from four additional nuclear genes *H3*, *ATPSα*, *ATPSβ*, and *EF1α* (Johnson & Vrijenhoek, pers. comm.). Reporting the details is beyond the scope of the present study but, to date, the Hardy−Weinberg analyses have provided no indication of deviations from random mating within species, or evidence for hybridization between species in cases of sympatry. Furthermore, we found no genetic evidence for parthenogenesis, selfing, or other systematic deviations from random mating within *Alviniconcha* species (SBJ, unpublished data). An earlier allozyme study of *A. boucheti* sp. nov. and *A. kojimai* sp. nov. samples from the Lau and N. Fiji basins also indicated that the populations were in Hardy−Weinberg equilibrium (Denis, Jollivet, & Moraga, 1993).

Table 6. Measures of within-species diversity for *COI*.

	N	*k*	*H*	*h*	SD	π	SDx100
A. hessleri s.s.	34	17	10	0.51	0.11	0.02	0.16
A. kojimai sp. nov.	66	28	25	0.81	0.05	0.05	0.30
A. boucheti sp. nov.	132	34	48	0.95	0.01	0.04	0.28
A. marisindica sp. nov.	25	12	11	0.83	0.07	0.03	0.20
A. strummeri sp. nov.	22	23	16	0.96	0.03	0.05	0.30
A. adamantis sp. nov.	10	4	3	0.51	0.16	0.03	0.14

Abbreviations: N = sample size per locus; H = number of haplotypes; k = number of polymorphic sites; h = haplotype diversity; π = nucleotide diversity; SD = one standard deviation.

Species tree

Despite incomplete lineage sorting for three of the nuclear gene segments, Bayesian phylogenetic analyses of the concatenated loci clearly revealed six evolutionary lineages of *Alviniconcha* snails (Fig. 5.1). Each species received strong support (*=1.00 posterior probability). Several higher-level groupings also received strong support. One group consisted of *A. kojimai* sp. nov. and *A. hessleri s.s.*, the most closely related species. A second group consisting of *A. boucheti* sp. nov. and *A. marisindica* sp. nov. nested within a larger grouping that included *A. adamantis* sp. nov. Relationships with *A. strummeri* sp. nov. were unresolved. Further research with additional genes may provide better resolution of basal relationships among these species.

Systematics

Abyssochrysoidea

Remarks. Classification of the Abyssochrysoidea was discussed by Johnson et al. (2010) wherein the family Provannidae that includes the genus *Alviniconcha* is paraphyletic including the family Abyssochrysidae. It is beyond the scope of the present study to verify this assignment; thus, we leave *Alviniconcha* in the Abyssochrysoidea with no reference to family epithets.

Alviniconcha Okutani & Ohta, 1988

Type species. *Alviniconcha hessleri* Okutani and Ohta, 1988, by original designation, from hydrothermal deep-sea vents in the Mariana Trough.

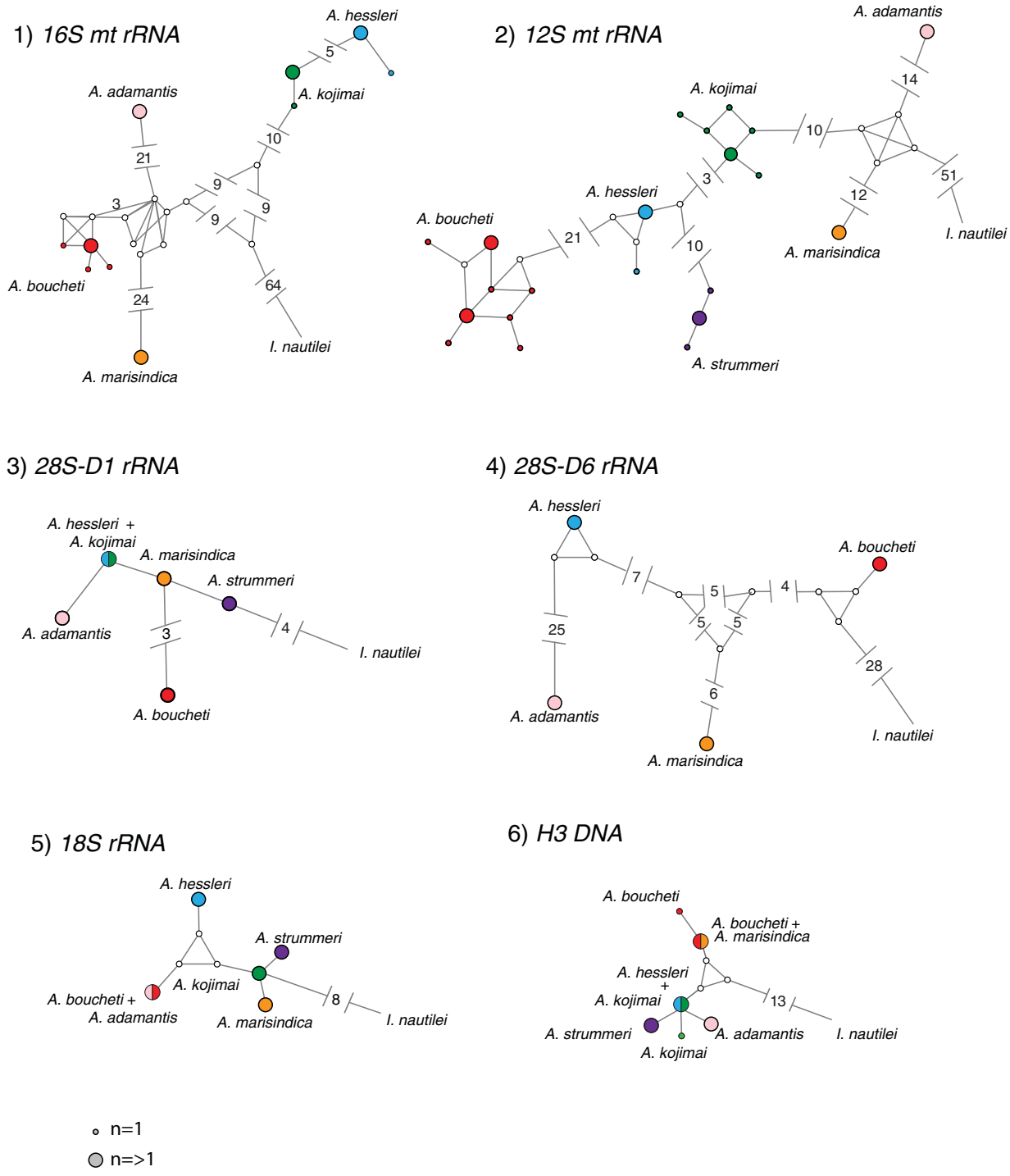

Fig. 4. Rooted *Alviniconcha* networks: (4.1) *16S mt* rRNA; (4.2) *12S mt* rRNA; (4.3) *28S-D1* rRNA; (4.4) *28S-D6* rRNA; (4.5) *18S* rRNA; and (4.6) *H3* DNA. Small circles indicate singleton alleles, larger circles indicate alleles that occurred more than once, and white circles indicate missing alleles. Numbers in gaps indicate the mutational steps between species.

Remarks. Genetic differences between seemingly identical specimens of *Alviniconcha* from the Fiji and Lau Basins were previously recognized by Denis et al. (1993) but did not result in any conclusions on nomenclature. A number of previous studies noted that *Alviniconcha* populations from the Indian Ocean and the Mariana Trough differed in the manner in which they house symbiotic bacteria (Sasaki, Warén, Kano, Okutani, & Fujikura, 2010;

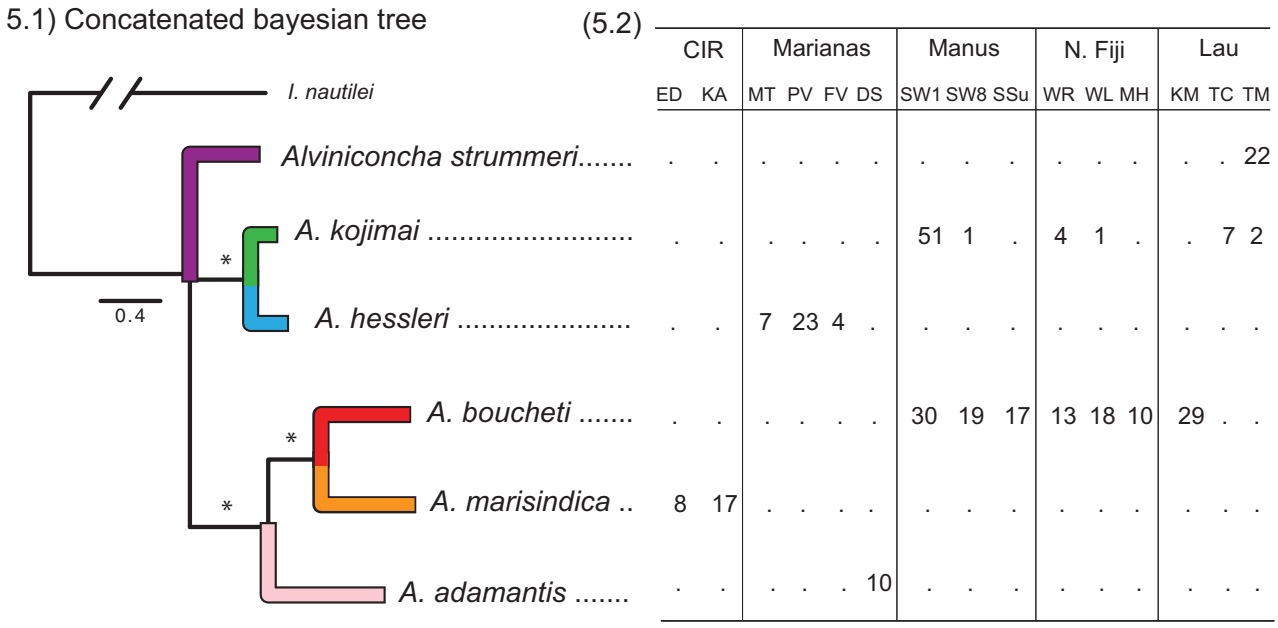

Fig. 5. (5.1) Concatenated Bayesian phylogeny based on the following evolutionary models for each gene segment (in parentheses): *COI* (GTR+SS), *16S* (HKY+G), *12S* (GTR+G), *28S-D1* (GTR+I+G), *28S-D6* (GTR+I+G), *18S* (JC+I) and *H3* (GTR+I+G). *Indicates a posterior probability value of 1.0. (5.2) Regional distribution of *Alviniconcha* species as identified by *COI*-barcodes; numbers indicate sample size.

Suzuki et al., 2005a; Suzuki et al., 2005b). The differences might result from associations with different endosymbiont types (ε-versus γ-proteobacteria) (e.g., Beinart et al., 2012), but resolution of this problem is significantly beyond the scope of the present study.

We observed considerable variation in several shell characters, especially the height of the spire (Fig. 2) and the density and arrangement of the periostracal hairs. However, these characters did not correspond with sequence data for the present species, further illuminating the broad phenotypic variability displayed by members of this genus. Due to a lack of suitably preserved specimens, we were unable to genotype a number of snails that exhibited extreme conditions for these characters. We previously noted a striking variation as shell shape of very young specimens (cf. Warén & Bouchet, 1993, fig 44A, B), but such specimens were rare and only available in the formalin-fixed samples. Because we were unable to sequence these museum specimens, we cannot exclude the possibility that diagnostic differences in shell morphology or soft anatomy might exist.

Alviniconcha hessleri sensu stricto Okutani & Ohta, 1988
Fig. 2.1

Alviniconcha hessleri Okutani & Ohta, 1988
Mariana snail (Stein et al., 1988)
Hairy snail (Hessler, Lonsdale, & Hawkins, 1988)
HOLOTYPE: NSMT MO 64489.
TYPE LOCALITY: 'Snail Pits' site (18°10.95′N, 144°43.20′W, 3670−3680 m depth) on dive A1835, 26 April 1987; and 'Alice Springs' site (18°12.59′N, 144°42.43′E, 3630−3655 m depth) on dive A1845, 6 May 1987.
PARATYPES: NSMT MO 64490−91, USNM 859328−29.
NEW MATERIAL EXAMINED: Molecular analyses included samples from three localities in the Mariana Back-Arc basin including the Mariana Trough, Piracy and Forecast vent fields (Tables 2, 4 & Fig. 5.2).
REVISED DIAGNOSIS: *Alviniconcha hessleri s.s.* Differs from each of the newly described *Alviniconcha* species at multiple sites for mitochondrial *COI*. Moreover, it differs from all the new species at a single *COI* barcode site: 66C (Table 7). It also differs from all other *Alviniconcha* species for *18S rRNA*: 145T and 154T (Table 8). *Alviniconcha hessleri s.s.* is very closely related to *A. kojimai* sp. nov., but differs diagnostically for the following loci: *COI*, *12S mt rRNA* and *18S rRNA*.
COMMENT: *Alviniconcha hessleri s.s.* was originally described in the family Trichotropoidae but soon after Warén and Bouchet (1993) described *Alviniconcha* in more detail and transferred the genus to the family Provannidae. *Alviniconcha hessleri* has been found to harbour gamma-proteobacteria endosymbionts within vacuoles in the ctenidia (Ohta, Endow, & Hessler, 1988; Suzuki et al., 2005b).

Table 7. Fixed differences (highlighted in grey) for each *Alviniconcha* species for COI barcode sequence. Position refers to base pair position in alignment. There were no amino acid changes isolated to a single species, however, two amino acid changes occurred, one due to a third-positional change at position 152 *A. hessleri* s.s. + *A. kojimai* sp. nov. = M (Methionine) and *A. boucheti* sp. nov. + *A. adamantis* sp. nov. + *A. marisindica* sp. nov. + *A. strummeri* sp. nov. = I (Isoleucine). A first-positional change occurred at bp 429: *A. hessleri* s.s. + *A. kojimai* sp. nov. = V (Valine) and *A. boucheti* sp. nov. + *A. adamantis* sp. nov. + *A. marisindica* sp. nov. + *A. strummeri* sp. nov. = I (Isoleucine).

	Position																					
TAXA	8	14	41	50	66	68	74	80	83	95	116	134	149	155	158	171	209	212	218	230	245	255
A. hessleri s.s.	G	T	A	A	C	A	T	T	A	T	T	A	G	G	G	T	T	T	T	T	T	T
A. kojimai sp. nov.	S	T	A	A	T	A	C	T	A	T	T	A	G	G	G	T	T	T	T	T	T	C
A. boucheti sp. nov.	T	C	A	A	T	A	T	T	G	C	C	G	G	G	A	T	T	T	T	T	T	T
A. marisindica sp. nov.	G	T	A	A	T	A	T	T	A	T	T	A	G	A	A	T	T	T	T	T	A	T
A. strummeri sp. nov.	G	T	A	A	T	G	A	C	A	T	T	A	G	G	A	T	T	T	A	T	T	T
A. adamantis sp. nov.	A	T	G	G	T	A	T	T	A	T	T	A	A	G	C	C	C	C	T	C	T	T

COI cont.	272	293	296	299	302	314	317	321	323	326	350	419	452	455	473	531	560	587	614	615	626	632
A. hessleri s.s.	T	T	T	A	A	T	T	T	A	T	A	T	A	G	T	T	A	T	T	T	T	T
A. kojimai sp. nov.	T	T	T	A	A	C	T	T	A	T	A	C	A	G	T	T	A	T	T	T	T	T
A. boucheti sp. nov.	C	C	A	A	A	T	C	T	A	A	A	T	A	A	T	C	A	T	T	T	T	T
A. marisindica sp. nov.	A	A	G	A	A	T	T	T	A	A	A	T	A	G	T	T	A	T	T	T	C	C
A. strummeri sp. nov.	T	T	T	A	G	T	T	C	A	T	S	T	A	G	A	T	A	T	T	T	T	T
A. adamantis sp. nov.	T	T	T	G	A	T	T	T	G	G	T	T	G	G	T	T	T	C	C	C	T	T

DISTRIBUTION: Only known from hydrothermal vents at depths greater than 1400 m in the Mariana Back-arc Basin, where it is the only species of the genus.

Alviniconcha kojimai sp. nov.
Fig. 2.2

Alviniconcha sp. type I. (Beinart et al., 2012; Kojima, Fujikura, Okutani, & Hashimoto, 2004; Kojima et al., 2001; Kojima et al., 1998; Sievert, Hügler, Taylor, & Wirsen, 2008; Suzuki et al., 2006a; Urakawa et al., 2005).
Alviniconcha cf. *hessleri* (Tufar, 1990).
Alviniconcha sp. 1 (Erickson, Macko, & Van Dover, 2009; Hidaka, Watanabe, Kano, & Kojima, 2012; Waite et al., 2008).
Alviniconcha spp. (Collins, Kennedy, & Van Dover, 2012; Denis et al., 1993; Hashimoto & co-authors, 1999; Henry, Childress, & Figueroa, 2008; Kim & Hammerstrom, 2012; Kyuno et al., 2009; Podowski, Ma, Luther, & Wardrop, 2010; Sen et al., 2013; Van Dover et al., 2001).
Alviniconcha aff. *hessleri* (Podowski, Moore, Zelnio, Luther, & Fisher, 2009; Van Dover et al., 2007).

Table 8. Fixed differences (highlighted in grey) for each *Alviniconcha* species for nuclear (*H3*) and three *rRNA* sequence segments. Position refers to base pair position in current alignment.

	Position														
	H3		*28S-D1*						*28S-D6*		*18S*				
TAXA	83	174	52	53	77	192	195	335	418	426	145	146	154	202	204
A. hessleri s.s.	C	C	T	A	A	G	C	A	T	T	T	T	T	G	C
A. kojimai sp. nov.	C	C	T	A	A	G	C	A			A	A	C	G	C
A. boucheti sp. nov.	C	C	T	A	C	T	G	T	T	T	G	T	C	G	C
A. marisindica sp. nov.	C	C	T	A	A	G	C	A	T	T	G	T	C	G	T
A. strummeri sp. nov.	T	C	T	G	A	G	C	A			G	T	C	T	C
A. adamantis sp. nov.	C	T	C	A	A	G	C	A	C	C	G	T	C	G	C

* *Alviniconcha kojimai* sp. nov. and *A. strummeri* sp. nov. did not amplify for the *28S-D6* locus.

Table 9. Fixed differences (highlighted in grey) for each *Alviniconcha* species for *mt RNA* sequence data. Position refers to base pair position in current alignment.

	16S mt rRNA																												
TAXA	13	16	24	48	153	161	234	237	240	243	246	253	254	257	263	267	279	306	316	328	337	358	360	399	416	430	459		
A. hessleri s.s.	-	A	A	A	A	T	A	T	A	G	C	T	T	G	-	T	C	A	T	A	A	T	A	A	C	A	A		
A. kojimai sp. nov.	-	A	A	A	A	T	A	T	A	G	C	T	T	G	-	T	C	A	T	A	A	T	A	A	C	R	A		
A. boucheti sp. nov.	-	T	G	A	A	T	A	C	A	G	A	A	G	A	-	A	C	A	T	A	A	T	G	A	C	A	A		
A. marisindica sp. nov.	-	A	A	A	A	T	G	T	G	A	T	-	A	A	-	-	T	A	C	A	A	T	A	A	C	T	T		
A. adamantis sp. nov.	T	A	A	G	G	A	C	T	A	G	T	T	G	-	T	A	C	G	T	G	T	C	A	G	T	A	A		
	12S mt rRNA																												
TAXA		27	34	126	128	151	156	219	221	259	283	286	303	310	315	317	318	351	362										
A. hessleri s.s.		T	A	T	G	T	T	T	T	A	A	T	A	A	S	T	T	A	T										
A. kojimai sp. nov.		C	A	T	G	T	T	T	T	A	A	T	A	A	G	T	T	A	T										
A. boucheti sp. nov.		T	A	T	G	T	C	T	G	A	A	R	A	G	A	C	T	A	T										
A. marisindica sp. nov.		T	T	T	G	T	T	T	T	A	T	G	A	A	G	T	T	A	T										
A. strummeri sp. nov.		T	A	T	G	T	T	T	T	A	A	C	A	A	G	T	T	A	T										
A. adamantis sp. nov.		T	C	C	A	C	T	C	T	G	A	T	G	A	G	T	C	G	C										

**Alviniconcha strummeri* sp. nov. did not amplify for the *16S* locus.

Alviniconcha hessleri (Corbera & Segonzac, 2010; Desbruyères, Alayse-Danet, Ohta, & the Scientific Parties of the BIO-LAU and STARMER Cruises, 1994; Galkin, 1997; Gollner, Fontaneto, & Martínez Arbizu, 2010; Warén et al., 1993; Windoffer & Giere, 1997).

HOLOTYPE: SMNH type coll. 8577, preserved in 95% ethanol. GenBank # KF467685.

TYPE LOCALITY: Tow Cam vent site, 20°19.076′S, 176° 8.258′W, 2714 metres depth in the Lau basin. ROV *Jason II* dive 142 (Table 2).

PARATYPES: Four SMNH type coll. 8578−8581, same preservation and location. GenBank # KF46781−84.

ZOOBANK ID: 689FFDC7-D852−4ADA-A23E-D24D154DC8F9.

ETYMOLOGY: Named in honour of University of Tokyo researcher Shigeaki Kojima, who first recognized this species from its unique *COI* sequences.

DIAGNOSIS: The species differs from all other *Alviniconcha* species by the following combination of character states for its mitochondrial *COI* barcode: 74C, 255C, 314C and 419C (Table 7). It is also distinct from all other *Alviniconcha* species for *18S rRNA*: 145A and 146A (Table 8); and *12S mt RNA*: 27C (Table 9). No reliable conclusions about morphological differences could be drawn from our material because most of our specimens were dissected for gill and foot with the remainder of the tissue discarded.

COMMENT: *Alviniconcha kojimai* harbours both epsilon- and gamma-proteobacterial endosymbionts within vacuoles in the ctenidia (Beinart et al., 2012; Suzuki et al., 2006a; Urakawa et al., 2005).

DISTRIBUTION: Known from hydrothermal vent localities at 1480- to 2700 m depths in the Manus, Fiji and Lau basins, in the Western Pacific.

Alviniconcha boucheti sp. nov.

Fig. 2.3

Alviniconcha sp. type II. (Kojima et al., 2004; Kojima et al., 2001; Kojima et al., 1998; Konishi et al., 2013; Sievert et al., 2008; Suzuki et al., 2006a).

Alviniconcha sp. 2 (Hidaka et al., 2012; Thaler et al., 2011).

Alviniconcha cf. *hessleri* (Tufar, 1990).

Alviniconcha spp. (Collins et al., 2012; Denis et al., 1993; Hashimoto et al., 1999; Henry et al., 2008; Kim & Hammerstrom, 2012; Kyuno et al., 2009; Podowski et al., 2010; Podowski et al., 2009; Sen et al., 2013).

Alviniconcha aff. *hessleri* (Johnson et al., 2010; Van Dover et al., 2007).

Alviniconcha hessleri (Corbera & Segonzac, 2010; Desbruyères et al., 1994; Galkin, 1997; Gollner et al., 2010; Mullaugh, Luther, Ma, Moore, & Yücel, 2008; Becker et al., 2008; Warén & Bouchet, 1993; Windoffer et al., 1997)

HOLOTYPE: SMNH type coll. 8575, Fixed in 4% seawater-buffered formaldehyde, stored in 80% ethanol. GenBank # KF467804.
TYPE LOCALITY: Mussel Hill vent site, 16° 59.41′S, 173° 54.97′E, 1973 metres depth in the North Fiji Basin. ROV *Jason II* dive J2152 (Table 2).
PARATYPE: SMNH type coll. 8576, same fixation and location.
ZOOBANK ID: 0451EF7F-BA74-4493-A654-FCD6ADE79890
ETYMOLOGY: Named in honour of Philippe Bouchet, who is a major contributor to deep-sea gastropod systematics.
DIAGNOSIS: The species differs from all other *Alviniconcha* species by the following combination of character states of its mitochondrial *COI* barcode: 14C, 83G, 95C, 116C, 134G, 272C, 293C, 296A, 317C, 455A, 531C (Table 7). It also is distinct from all other *Alviniconcha* species in the respective alignments for *12S mt RNA*: 156C, 221G, 310G, 315A, 317C (Table 9); *28SD1 rRNA*: 77C, 192T, 195G, 335T (Table 8); and *16S mt RNA*: 16T, 24G, 237C, 246A, 253A, 360G (Table 9). No reliable conclusions about morphological differences could be drawn from our material because only limited tissue remained for most of our specimens.
COMMENT: *Alviniconcha boucheti* harbours epsilonproteobacterial endosymbionts within vacuoles in the ctenidia (Beinart et al., 2012; Suzuki et al., 2006a).
DISTRIBUTION: Known from hydrothermal vent localities at 1300 to 2700 m depths in the Manus, Fiji and Lau basins, in the Western Pacific.

Remarks. Both the paratype and the holotype were fixed in formalin but yielded enough genetic information to ascertain their identity based on comparisons with the other 135 ethanol-preserved tissue specimens. Only the gill and foot tissues of other specimens were preserved in ethanol.

Alviniconcha marisindica sp. nov. Okutani
Fig. 2.4

Alviniconcha aff. *hessleri* (Goffredi, Jones, Ehrlich, Springer, & Vrijenhoek, 2008; Okutani et al., 2004; Sievert et al., 2008; Suzuki et al., 2005a; Suzuki et al., 2005b; Suzuki et al., 2009)
Alviniconcha sp. Indian Ocean. (Beedessee et al., 2013; Cubelio, Tsuchida, & Watanabe, 2008; Kojima et al., 2004)
Alviniconcha sp. nov. (Van Dover, German, Speer, Parson, & Vrijenhoek, 2002; Van Dover et al., 2001)
Alviniconcha sp. 3 (Hidaka et al., 2012)
HOLOTYPE: SMNH type coll. 8572, preserved in 95% ethanol. GenBank # KF467897.
TYPE LOCALITY: Kairei vent field, 23°52.98′S, 69°35.82′W, 2432 metres depth on the Central Indian Ridge. ROV *Jason II* dive 297 (Table 2).
PARATYPES: Ten specimens, SMNH type coll. 8571, same fixation and location. Genbank # KF467898−907.
ZOOBANK ID: 2911ACD7-D70F-4F40−8F8D-3234808A4B5A
ETYMOLOGY: Latin adjective (*maris* = sea; *indica* = Indian) for the geographical type locality, as originally suggested by T. Okutani.
DIAGNOSIS: The species differs from all other *Alviniconcha* species by the following combination of character states of its mitochondrial *COI* barcode: 155A, 245A, 272A, 293A, 296G, 626C, 632C (Table 7). It also is distinct from all other *Alviniconcha* species for *18S rRNA*: 204T (Table 8); *12S mt RNA*: 34T and 283T (Table 9); and *16S mt RNA*: 234G, 240G, 243A, 253gap, 254A, 267gap, 279T, 316C, 430T, 459T (Table 9). No reliable conclusions about morphological differences could be drawn from our material.
COMMENT: Based primarily on the unique *COI* sequences first identified in Van Dover et al. (2001), Okutani et al. (2004) recognized the CIR specimens from the Kairei vent field as a distinct species; however, the species was not formally described in either publication. We confirm that Professor Takashi Okutani suggested the name *A. marisindica* in a letter to A. Warén in 2001 (Okutani et al., 2004). *Alviniconcha marisindica* harbours epsilonproteobacterial endosymbionts within vacuoles in the ctenidia (Suzuki et al., 2005a).
DISTRIBUTION: Only known from hydrothermal vent localities at 2400 to 3300 m depths on Central Indian Ridge, in the Indian Ocean: Kairei and Edmunds vent fields (Table 2), where it seems to be the only member of the genus.

Alviniconcha strummeri sp. nov.
Fig. 2.5

Alviniconcha type III (Beinart et al., 2012)
Alviniconcha spp. (Henry et al., 2008; Podowski et al., 2009; Sen et al., 2013)
Alviniconcha Lau Basin (Suzuki et al., 2006a)
Alviniconcha sp. 4 (Beedessee et al., 2013; Hidaka et al., 2012)
Alviniconcha hessleri (Gollner et al., 2010)
HOLOTYPE: SMNH type coll. 8573. Fixed in 4% seawater-buffered formaldehyde, stored in 80% ethanol. GenBank # KJ027398.
TYPE LOCALITY: Tui Malila vent site, 21°59.431′S, 176°34.146′W, 1845 metres depth in the Lau Basin. ROV *Jason II* dive J2144 (Table 2).
ZOOBANK ID: 6B72E9C2-CB4E-481E-B87B-A389D8721381.

ETYMOLOGY: Named in honour of Joe Strummer, the lead vocalist and guitarist from *The Clash*, a British punk band. The name highlights the 'hardcore' nature of *Alviniconcha* snails, that inhabit the hottest, most acidic and most sulphidic microhabitats at Indo-Pacific hydrothermal vents. The name also recognizes the surface of *Alviniconcha* shells: the spiky periostracum resembles the fashion of punk rock bands.
DIAGNOSIS: The species differs from all other *Alviniconcha* species by the following combination of character states of its mitochondrial *COI* barcode: 68G, 74A, 80C, 218A, 302G, 321C, 350G/C, 473A (Table 7). It also is distinct from all other *Alviniconcha* species for *18S rRNA*: 202T (Table 8); *28SD1 rRNA*: 53G (Table 8); *H3*: 83T (Table 8); and *12S mt RNA*: 286C (Table 9). No reliable conclusions about morphological differences could be drawn from our material because only limited tissue remained for most of our specimens.
COMMENT: *Alviniconcha strummeri* harbours gamma- and epsilon-proteobacterial endosymbionts within vacuoles in the ctenidia (Beinart, Nyholm, Dubilier, & Girguis, 2014; Beinart et al., 2012; Suzuki et al., 2006a).
DISTRIBUTION: Only known from hydrothermal vent localities at ~1850 m depth in the southern Lau basin, especially at the Tui Malila vent site.

Alviniconcha adamantis sp. nov.
Fig. 2.6

Alviniconcha sp. (Fujiwara, Okutani, & Kimura, 2013)
HOLOTYPE: SMNH type coll. 8574, Frozen and stored in 95% ethanol. GenBank # KF467672.
TYPE LOCALITY: East Diamante Seamount, 15°56.57′N, 145°40.88′E, ~350 metres depth in the Mariana volcanic basin. ROV *Jason II* dive 193 (Table 2).
PARATYPES: Five, SMNH type coll. 8570, ROV *Jason II* dive 193, same preservation and location. GenBank # KF467670−71, KF467673−75.
ZOOBANK ID: 910D807C-1791-4A3D-9164-68EB9E447AD7.
ETYMOLOGY: Named after the seamount locality at which it was initially discovered. *adamas*, Latin for diamond; *adamantis* is the genitive form.
DIAGNOSIS: The species differs from all other known *Alviniconcha* species by the following combination of character states of its mitochondrial *COI* barcode: 8A, 41G, 50G, 149A, 158C, 171C, 209C, 212C, 230C, 299G, 323G, 326G, 350T, 452G, 560T, 587C, 614C, 615C (Table 7). This species is also distinct from other *Alviniconcha* species for, *28SD1 rRNA*: 52C (Table 8); *H3 nDNA*: 174T (Table 8); *28SD6 rRNA*: 418C, 426C (Table 8); *12S mt RNA*: 34C, 126C, 128A, 151C, 219C, 259G, 303G, 318C, 351G, 362C (Table 9); and *16S mt RNA*: 13T, 48G, 153G, 161A, 234C, 257gap, 263T, 306G, 328G, 337T, 358C, 399G, 416T (Table 9). No reliable conclusions about morphological differences could be drawn from the material in our possession.
COMMENT: This species occupies the shallowest known vent locality for an *Alviniconcha* species. *Alviniconcha adamantis* harbours gamma-proteobacterial endosymbionts within vacuoles in the ctenidia (SBJ, unpublished data).
DISTRIBUTION: Known from hydrothermal vents at ~350 m depth on East Diamante Seamount, 15°56.57′N, 145°40.88′E. In addition, a single specimen of *A. adamantis* sp. nov. was sampled from the Suiyo Seamount (JAMSTEC Hyper-Dolphin dive #675: 28°34.29′N, 140°38.30′E, 1380 m depth, on the Izu-Bonin arc) (Fujiwara et al., 2013).

Discussion

Concordance among the various gene trees provided a robust means for assessing the evolutionary independence (Avise & Ball, 1990) of *Alviniconcha* lineages, justifying their status as distinct species. Nonetheless, the *COI* sequences alone are diagnostic, providing convenient DNA-barcodes for distinguishing among the six externally cryptic species. It is true that in most cases the application of these species names will require sequencing, but the names will facilitate discussion and draw attention to their evolutionary independence. This attention, we hope, may ultimately result in the discovery of morphologic characters suitable for species demarcation. The known geographic distributions of each species are listed in Fig. 5.2. Four species appear to be restricted to single non-overlapping regions: *A. marisindica* on the Central Indian Ridge; *A. strummeri* in the southern Lau Basin; *A. hessleri* at deep sites in the Mariana back-arc basin; and *A. adamantis* on shallow seamounts in the Mariana Volcanic Arc. In contrast, *A. boucheti* and *A. kojimai* occur sympatrically at sites in the Manus, North Fiji and Lau basins where *Ifremeria nautilei* is also abundant. There have also been a few individuals of *A. kojimai* collected at the Tui Malila site in the southern Lau basin, although this site is dominated by *A. strummeri*.

Cryptic species

As molecular techniques become more economical and accessible, additional discoveries of cryptic species will contribute markedly to our overall assessment of biodiversity (Bickford et al., 2007). Both alpha- and beta-level diversity may be significantly underestimated at hydrothermal vents, where cryptic species are common (Matabos et al., 2011; Tunnicliffe, Koop, Tyler, & So, 2010; Vrijenhoek, 2009). Yet, it seems unlikely that divergence for so many genetic markers accumulated randomly while morphological phenotypes of these *Alviniconcha* species

would remain cryptic. Indeed, the present lineages displayed considerable morphological variability in size, the height of spires, presence or absence of a shell, coloration, and the length of periostracal 'hairs'. Yet none of these characters allowed us to reliably differentiate amongst the species. In contrast, *Alviniconcha*'s closest relative, *Ifremeria nautilei*, also occurs in the Manus, N. Fiji and Lau basins but has not undergone the same genetic diversification (Thaler et al., 2011).

Denis et al. (1993) first suggested that the sympatric species of *Alviniconcha* appear to segregate according local-scale geochemical conditions. We also found that *A. boucheti* was more frequent in the N. Fiji basin and at the Kilo Moana site in Lau basin, whereas *A. kojimai* was more frequent at southern Lau basin sites (Fig. 5). The N. Fiji and Kilo Moana sites are hotter with higher sulphide levels than southern Lau sites (Beinart et al., 2012; Denis et al., 1993). Beinart et al. (2012) hypothesized that host-symbiont interactions affected by local-scale geochemistry drive habitat partitioning among the co-occurring species. They reported that *A. boucheti* (their type-I), *A. kojimai* (type-II), *A. strummeri* (type-III) and corresponding endosymbiont strains, segregate in the Lau Basin according to local and regional scale differences in vent geochemistry. The host species and symbionts also segregate geographically, with *A. boucheti* hosting epsilon-proteobacteria in the northern Lau Basin and *A. strummeri* hosting gamma-proteobacteria dominating the southern Lau Basin. *Alviniconcha kojimai*, which hosts epsilon- and gamma-proteobacteria, is found throughout the Lau basin. The mechanisms generating these apparent host-symbiont associations remain unknown.

Conclusions

The common occurrence of morphologically cryptic species among deep-sea chemosynthetic faunas creates vexing problems for taxonomists, ecologists and biogeographers. The formal naming of species provides a means for communicating the unique properties of distinct evolutionary lineages, even if they are morphologically indistinguishable. The absence of morphological data or traits should not preclude publication of a species description with only DNA-sequence data as a diagnosis (Cook et al., 2010). The formal names introduced here link distinct *COI*-barcodes to type specimens, known geographic distributions and symbiont associations. Molecular identifications must be employed at localities where the cryptic species co-occur, because distinct lineages might occupy different microhabitats (Beinart et al., 2012). The species typically host endosymbiont assemblages capable of exploiting spatially variable geochemical environments. Future investigations of these snails should include geochemical measurements in concert with endosymbiont characterizations and host identifications. In addition, more comprehensive sampling of ontogenetic stages and proper preservation for morphological and molecular analyses should be a goal of future deep-sea expeditions in the Indo-Pacific region. We hope that with greater awareness, morphological traits will emerge so that *Alviniconcha* snails can be easily identified in the field or from video recordings; however, until then, DNA sequences provide reliable, repeatable traits to differentiate these cryptic species.

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Availability of supporting data

All sequence data including alignments are published on GenBank Accession #'s KF467613-KF468008 and KJ027398 (Table 4). The underlying research materials for this article can be accessed at http://www.ncbi.nlm.nih.gov/popset/633283479, http://www.ncbi.nlm.nih.gov/popset/633283470, http://www.ncbi.nlm.nih.gov/popset/633283460, http://www.ncbi.nlm.nih.gov/popset/633283455, http://www.ncbi.nlm.nih.gov/popset/633283435, http://www.ncbi.nlm.nih.gov/popset/633283425, and http://www.ncbi.nlm.nih.gov/popset/633283417

Author contributions

RCV, CGW, CLVD, VT, AW and SBJ led or participated in oceanographic expeditions that collected the snail specimens. AW examined the snails morphologically. SBJ conducted the DNA sequencing (along with TS), performed all the statistical analyses, and composed the first drafts of the manuscript. RCV, AW, and VT contributed to later drafts of the manuscript. All authors read and approved the final manuscript.

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