Journal of the Marine Biological Association of the United Kingdom, 2013, 93(4), 1017–1024. © Marine Biological Association of the United Kingdom, 2012 doi:10.1017/S0025315412000823

# *Iheyaspira bathycodon* new species (Vetigastropoda: Trochoidea: Turbinidae: Skeneinae) from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean

VERITY NYE<sup>1</sup>, JON COPLEY<sup>1</sup>, KATRIN LINSE<sup>2</sup> AND SOPHIE PLOUVIEZ<sup>3</sup>

<sup>1</sup>Ocean & Earth Science, National Oceanography Centre Southampton, University of Southampton Waterfront Campus, European Way, Southampton, SO14 3ZH, UK, <sup>2</sup>British Antarctic Survey, High Cross, Madingley Road, Cambridge, CB<sub>3</sub> oET, UK, <sup>3</sup>Nicholas School of the Environment, Duke University Marine Laboratory, 135, Duke Marine Lab Road, Beaufort, NC 28516, USA

Iheyaspira bathycodon sp. nov. is described from the Von Damm Vent Field on the world's deepest spreading centre, the Mid-Cayman Spreading Centre (MCSC), Caribbean, at 2300 m depth. The new species is defined and illustrated from 11 specimens, with brief notes on habitat and known distribution. Molecular phylogenetic data from partial COI mDNA, 16S rDNA and nuclear 18S rDNA regions are used to analyse the species' phylogenetic position and its morphology is compared with previously described skeneid and vent taxa. The new species is distinguished from the most closely allied vent species, Iheyaspira lequios Okutani, Sasaki & Tsuchida, 2000 by morphological differences in radula diagnosis and appendage structure of the head-foot. Iheyaspira bathycodon sp. nov. is the tenth turbinid to be described from a hydrothermal-vent environment and the second species to be named from recently discovered hydrothermal vents on the MCSC. Determining the faunal composition of assemblages at the vent fields of the MCSC will help to elucidate the vent biogeography of the region.

Keywords: Gastropoda, Vetigastropoda, Trochoidea, Turbinidae, Skeneinae, *Iheyaspira bathycodon*, new species, Cayman, hydrothermal vents

Submitted 6 May 2011; accepted 11 May 2012; first published online 13 August 2012

## INTRODUCTION

Gastropoda are one of the most species-rich macrofaunal taxa from hydrothermal vents (Warén *et al.*, 2006), and form a dominant component of assemblages at vent fields in western Pacific back-arc basins and on the Central and South-west Indian Ridges (e.g. Desbruyères *et al.*, 1994; Galkin, 1997; Kojima *et al.*, 2001; Van Dover *et al.*, 2001; Tao *et al.*, 2012). A substantial research effort has sought to elucidate the systematics and higher phylogeny of vent/seep gastropods and to characterize their diversity, biogeography and life-history biology (see Sasaki *et al.*, 2010 for recent review).

More than 200 species of gastropods from at least 100 genera and 35 families have been recorded from deep-sea chemosynthetic environments of the world's oceans (Sasaki *et al.*, 2010). Some of these species also inhabit non-chemosynthetic environments but have been recorded in much greater densities from chemosynthetic assemblages (see Sasaki *et al.*, 2010), but others are endemic to vents and seeps at the species and higher taxonomic levels (e.g. Warén & Bouchet, 2001).

Skeneinae Clark, 1851 was originally treated as a separate family ('Skeneidae') in Trochoidea Rafinesque, 1815 (e.g. Hickman & McLean, 1990). More recent anatomical

**Corresponding author:** V. Nye Email: vn205@noc.soton.ac.uk and molecular studies showed, however, that 'Skeneidae' was polyphyletic and that many genera should be reassigned (e.g. Bouchet *et al.*, 2005; Kano, 2008). Bouchet *et al.* (2005) ranked Skeneinae as a subfamily of the Turbinidae Rafinesque, 1815, an arrangement maintained by Williams *et al.* (2008) in the newly defined Turbinidae. The rank of the Skeneinae remains uncertain and is under discussion (Williams, in press).

To date, all turbinids endemic to deep-sea chemosynthetic environments belong to either the Skeneinae or the Margaritinae Thiele, 1924 (Sasaki *et al.*, 2010; see Table 1). Some colloniids, such as *Cantrainea* Jeffreys, 1883, also live in seeps and vents (e.g. Warén & Bouchet, 1993, 2001; Sasaki *et al.*, 2010). The family Colloniidae Cossmann, 1917 was classified as a subfamily within the Turbinidae (e.g. Hickman & McLean, 1990; Bouchet *et al.*, 2005), but was moved recently to familial rank in the superfamily Phasianelloidea Swainson, 1840 (Williams *et al.*, 2008).

The Von Damm Vent Field is an active, high-temperature hydrothermal system, situated in a unique off-axis setting on the upper slopes of an oceanic core complex at 2300 m depth (Connelly *et al.*, 2012). The Von Damm Vent Field supports an abundant faunal assemblage that is dominated by dense aggregations of the shrimp *Rimicaris hybisae* Nye, Copley & Plouviez, 2012 and includes small skeneimorph gastropods. During a recent research cruise to the Mid-Cayman Spreading Centre a piece of sulphide chimney was sampled from the Von Damm Vent Field. On the surface of the sampled sulphide

Subfamily	Species	Site(s)	Depth (m)	Habitat	Primary references
Margaritinae	Gaza fisheri	GoM: Louisiana Slope; Caribbean Sea: off St Lucia	600-1061	Seep	Dall, 1889; Warén & Bouchet, 1993, 2001
	Margarites huloti	Off Central Chile (36°S)	843-728	Seep	Vilvens & Sellanes, 2006
	Margarites ryukyensis	OT: North knoll of Iheya Ridge	968–1053	Vent	Okutani, Sasaki & Tsuchida, 2000; Sasaki <i>et al.</i> , 2005
	Margarites shinkai	OT; SB	1110-1340	Vent/seep	Okutani, Tsuchida & Fujikura, 1992; Okutani <i>et al.</i> , 1992, 1993; Sasaki <i>et al.</i> , 2005
Skeneinae	Bruceiella athlia	Aleutian Trench	$\sim_{4800}$	Seep	Warén & Bouchet, 2001; Kiel, 2004
	Bruceiella globulus	LB; NFB	1750-2443	Vent	Warén & Bouchet, 1993, 2001; Warén <i>et al.</i> , 2006
	Bruceiella wareni	CIR: Kairei	2422-2443	Vent	Okutani, Hashimoto & Sasaki, 2004
	Fucaria mystax	Edison Seamount	1483	Vent	Warén & Bouchet, 2001; Warén <i>et al.</i> , 2006
	Fucaria striata	JdFR: Middle Valley	2425	Vent	Warén & Bouchet, 1993; Warén & Bouchet, 2001; Warén <i>et al.</i> , 2006
	Iheyaspira bathycodon sp.	MCSC: Von Damm	2300	Vent	This paper
	nov.	OT: North Ireall of Ihave Didge		Vont	Okutani Casaki & Taushida agaa
	Ineyaspira iequios	O1: North knoll of theya kidge	968-1053	Vent	Okutani, Sasaki & Tsuchida, 2000
	Protolira thorvaldssoni	MAR: Menez Gwen to Snake Pit & Ashadze; off south-western Iceland	850-4080	vents and whale bone	Waren, 1996; Waren & Bouchet, 2001; Warén <i>et al.</i> , 2006; Fabri <i>et al.</i> , 2011
	Protolira valvatoides	MAR: Menez Gwen to Lucky Strike, Snake Pit	850-3478	Vent	Warén & Bouchet, 1993, 2001; Warén <i>et al.</i> , 2006

 Table 1. Turbinid gastropods described from extant hydrothermal vents/cold seeps up to the end of 2011 (confirmed locations and fully described species only).

chimney were several small gastropods of one species, *Iheyaspira bathycodon* sp. nov., which is described herein. In addition to enhancing existing knowledge about biodiversity, characterizing the composition of faunal assemblage at Mid-Cayman Spreading Centre vents has the potential to elucidate the factors determining vent biogeography of this region.

#### MATERIALS AND METHODS

Specimens were collected from the Von Damm Vent Field (2300 m) at the Mid-Cayman Spreading Centre, Caribbean, during the 44th voyage of RRS 'James Cook' (April 2010). All specimens were picked from the surface of a sample of sulphide chimney, collected by the hydraulic grab of HyBIS (Hydraulic Benthic Interactive Sampler), a manoeuvrable TV grab sampler. Specimens for molecular analysis were immediately placed in 100% ethanol and the shell and operculum were subsequently removed. Specimens for morphological study were fixed in 10% neutralized formalin, subsequently transferred to 90% Industrial Methylated Spirits and measured to the nearest 0.1 mm using Vernier callipers (see Table 2).

Specimen [NHMUK 20120076] was dissected for scanning electron microscopy (SEM) of shell, operculum and radula. The shell and operculum were placed in an ultrasonic cleaning bath for three minutes. The mantle tissue was dissolved in potassium hydroxide diluted in water to expose the radula. The shell, operculum, radula and ctenidium were mounted uncoated onto an aluminium stub and micrographs were taken with a Hitachi TM3000 tabletop microscope. For SEM of soft parts, specimen [NHMUK 20120070] was dehydrated through a graded ethanol series, critical point dried and sputter coated with gold palladium prior to examination with a FEI Quanta 200 scanning electron microscope at accelerating voltage of 10 kV.

Genomic DNA was extracted from eight specimens using the cetyltrimethyl ammonium bromide (CTAB) extraction procedure (Doyle & Dickson, 1987). A region of mitochondrial cytochrome oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR) performed in 20  $\mu$ l final volume using universal primers (Folmer *et al.*, 1994) and the following conditions: 1X buffer reagent (200 mM Tris pH 8.8, 500 mM KCl, 0.1% Trixton X-100, 2 mg/ml bovine serum albumen), 2 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.5 mM of each primer, 1 U Taq DNA polymerase (Bioline), 5  $\mu$ l of template DNA and sterile H<sub>2</sub>O to final volume. Thermal cycling conditions were: 94°C/2 minutes; followed by 5 cycles at (94°C/35 seconds; 45°C/35 seconds; 72°C/1:20 minutes) and 35 cycles at (94°C/35 seconds; 50°C/35 seconds; 72°C/1:20 minutes) with a final extension of 72°C/10 minutes.

For the 16S ribosomal DNA gene (16S), PCR amplifications were performed in 20  $\mu$ l final volume using 16Sar and 16Sbr primers (Palumbi, 1996) and the following conditions: 1X buffer reagent (same as for COI), 2.5 mM MgCl<sub>2</sub>, 0.13 mM of each dNTP, 0.38 mM of each primer, 1 U Taq DNA polymerase (Bioline), 2.5  $\mu$ l of template DNA and sterile H<sub>2</sub>O to final volume. Thermal cycling conditions were: 94°C/4 minutes; 30 cycles at (94°C/30 seconds; 52°C/1 minute; 72°C/2 minutes) and 72°C/5 minutes.

Polymerase chain reaction amplifications of the 18S ribosomal DNA gene (18S) were performed using the primer pair 5'-CACAGTGAAACTGCGAATGG-3' and 5'-CAAATGCTT TCGCTGTAGGG3' (this study) in a 20 µl final volume amplification mixture as described for COI. Thermal cycling

CIR, Central-Indian Ridge; GoM, Gulf of Mexico; JdFR, Juan de Fuca Ridge; LB, Lau Basin; MAR, Mid-Atlantic Ridge; MCSC, Mid-Cayman Spreading Centre; NFB, North Fiji Basin; OT, Okinawa Trough; SB, Sagami Bay.

Table 2.	Morphological	variation	in Ihe	eyaspira	bathyco	odon sp.	nov.
----------	---------------	-----------	--------	----------	---------	----------	------

Catalogue no.	Type status	Shell height (mm)	Shell diameter (mm)	No. whorls	Operculum diameter (mm)	Operculum no. rings
NHMUK 20120068	Holotype	4.3	3.6	4.3	1.8	11
NHMUK 20120069	Paratype	6.7	5.9	4.5	1.8	11
NHMUK 20120070**	Paratype	5.2	4.3	4.5	2.1	13
NHMUK 20120071	Paratype	5.4	5.1	4.2	1.8	14
NHMUK 20120072	Paratype	5.9	5.5	4.3	2.2	17
NHMUK 20120073	Paratype	4.8	4.2	4.3	2.3	12
NHMUK 20120074	Paratype	3.7	3.6	3.6	1.4	9
NHMUK 20120075*	Paratype	6.9	5.6	4.6	3.3	16
NHMUK 20120076**	Paratype	6.7	6.5	4.6	2.6	13
NHMUK 20120077	Paratype	6.4	5.0	4.6	2.5	14
NHMUK 20120078	Paratype	7.2	6.1.	4.4	2.8	14

\*, soft parts dissected out of shell; \*\*, soft parts dissected out of shell and used for scanning electron microscopy.

conditions were:  $95^{\circ}C/5$  minutes followed by 30 cycles at  $(94^{\circ}C/1)$  minute;  $60^{\circ}C/1$  minute;  $72^{\circ}C/2$  minutes;  $72^{\circ}C/2$  minutes).

Purifications and sequencing were performed as described by Nye et al. (2012). Sequence strands were proofread and assembled with CodonCode Aligner, version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), to produce a continuous fragment. The 16S and 18S partial rDNA sequences were compared with those of other gastropods available in GenBank using the BLAST program (NCBI Basic Alignment Search Tool). The COI partial sequence of the new species was also compared with those of other trochoids of Suzanne William's published and unpublished dataset of deep-sea gastropods (Williams et al., 2008; S. T. Williams, personal communication). Phylogenetic trees were constructed with MEGA5 (Tamura et al., 2011) using both maximumlikelihood (ML) (Kimura, 1980) and neighbour-joining (NJ) (Saitou & Nei, 1987) methods on 425- and 803-base pair (bp) alignments for 16S and 18S respectively. Bootstrap values were calculated on 1000 re-sampling replicates.

The GenBank accession numbers for the partial sequences of COI, 16S and 18S regions from the new species are JQ306326, JQ306327 and JQ306328 respectively.

## SYSTEMATICS

Order VETIGASTROPODA Salvini-Plawen, 1980 Superfamily TROCHOIDEA Rafinesque, 1815 Family TURBINIDAE Rafinesque, 1815 Subfamily SKENEINAE Clark, 1851 Genus *Iheyaspira* Okutani, Sasaki & Tsuchida, 2000 *Iheyaspira bathycodon* sp. nov. Nye, 2012 (Figures 1-5)

#### TYPE MATERIAL

Holotype and paratypes deposited in the Natural History Museum, UK (NHMUK) [NHMUK 20120068–20120078]. All type material collected from the surface of a piece of sulphide chimney sampled from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean ( $18^{\circ}$  22.605'N  $81^{\circ}$  47.875'W), water depth 2300 m.

#### DESCRIPTION

Shell (Figure 1). Rounded, skeneiform, sturdy, height greater than width (see Table 2); maximum dimensions 7.2 mm

height, 6.1 mm width [NHMUK 20120078]. Surface smooth, lacking pigmentation, with thin, beige-white periostracum. Surface and apical region, including the protoconch and early teleoconch, are corroded in most specimens. Protoconch too corroded for any details to be seen (Figure 1 E, F). Teleoconch whorls more than 2.5 in number, body whorl large. No nacre or lustre visible on exterior or interior of the shell. Umbilicus open and deep, clearly visible in basal view. Peristome smooth. Aperture large and circular with smooth outer lip.



Fig. 1. *Iheyaspira bathycodon* sp. nov., shell, from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. (A) Holotype [NHMUK 20120068]; (B) holotype, lateral view [NHMUK 20120068]; (C) holotype, basal view [NHMUK 20120068]; (D) paratype [NHMUK 20120072]; (E) holotype, apical view [NHMUK 20120068]; (F) holotype, sub-apical view [NHMUK 20120068]. Scale bars: A - F = 1 mm.



**Fig. 2.** *Iheyaspira bathycodon* sp. nov., paratype o8 [NHMUK 20120076] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. (A) Operculum; (B) radula; (C) radula: lateral teeth; (D) radula: marginal teeth. Scale bars: A = 1 mm;  $B = 100 \text{ }\mu\text{m}$ ; C,  $D = 50 \text{ }\mu\text{m}$ .

Operculum (Figure 2A) moderately thin, corneous, and yellowish-brown; multispiral with a central nucleus and short growing edge, with a good fit to the aperture. Opercula retraction is deep.

Soft parts (Figures 3–5). Animal pale white in colour. Head quite large, snout cylindrical, terminating in a broad tip with mouth positioned at the midline. One pair of cephalic tentacles of similar size to each other and equal in length to the snout; cephalic tentacles densely papillated with what appear to be sensory papillae (Figure 5D). Eyestalks subequal in length and width to cephalic tentacles, without visible papillae and eyes. Right eyestalk approximately one-half length of right cephalic tentacle (Figure 4A, B, D), left eyestalk approximately one-third length of left cephalic tentacle (Figure 5A, B). Cephalic lappets absent. Neck lobes arise from both basal sides of the head. Right neck lobe (Figure 4D) divided into



**Fig. 3.** *Iheyaspira bathycodon* sp. nov., paratype 02 [NHMUK 20120070] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. Scanning electron microscopy micrographs of head-foot. (A) Dorsal view; (B) anterior view; (C) right side, lateral view; (D) left side, anterolateral view. Abbreviations used: ft, foot; me, mantle edge; op, operculum; pp, parapodium. Scale bars: A = 1 mm; B-D = 500  $\mu$ m.



**Fig. 4.** *Iheyaspira bathycodon* sp. nov., paratype 02 [NHMUK 20120070] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. Scanning electron microscopy micrographs of head-foot, right side. (A) Head, dorsolateral view; (B) head appendages, anterolateral view; (C) close-up of cephalic tentacle, lateral view; (D) head appendages and neck lobes, anterolateral view; (E) epipodial tentacles 1–3, dorsolateral view; (F) epipodial tentacles 4–5, lateral view; (G) epipodial tentacle 3, anterolateral view; (H) papillae on epipodial tentacle 3. Abbreviations used: ct, cephalic tentacle; es, eyestalk; et, epipodial tentacle; ft, foot; me, mantle edge; nl, neck lobe; op, operculum; pp, parapodium. Scale bars: A, D, F = 200  $\mu$ m; B, E = 100  $\mu$ m; C, G = 50  $\mu$ m; H = 20  $\mu$ m.

elongate anterior (nl1) and posterior (nl2, nl3) tentacles. Left neck lobe composed of at least one undivided tentacle beneath the left eyestalk (Figure 5A, B). Foot equipped with five epipodial tentacles on both sides. Right side: first, second and third epipodial tentacles clustered together, similar in size, with dense papillae except on second (Figure 4E); fourth and fifth isolated from first three in middle part of epipodium between lobes of epipodial skirt, densely papillate (Figure 4F). Left side: first, second and third epipodial tentacles clustered together; first and third epipodial tentacles of similar size with dense papillae; second slightly smaller, without visible papillae (Figure 5E); fourth and fifth isolated from first three in middle part of epipodium, densely papillate (Figure 5F). Ctenidium monopectinate, attached along its whole length, with bursicles.



**Fig. 5.** *Iheyaspira bathycodon* sp. nov., paratype 02 [NHMUK 20120070] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. Scanning electron microscopy micrographs of head-foot, left side. (A) Head, dorsolateral view; (B) head appendages, lateral view; (C) tip of cephalic tentacle, lateral view; (D) papillae on cephalic tentacle; (E) epipodial tentacles 1–3, dorsolateral view; (F) epipodial tentacles 4–5, dorsolateral view. Abbreviations used: ct, cephalic tentacle; es, eyestalk; et, epipodial tentacle; ft, foot; me, mantle edge; nl, neck lobe; op, operculum. Scale bars: A = 200  $\mu$ m; B, E, F = 100  $\mu$ m; C = 50  $\mu$ m; D = 20  $\mu$ m.

Radula (Figure 2B, D). Rhipidoglossate, bilaterally symmetrical, with the formula  $\infty$  -9 -1 -9 - $\infty$  (>20). Length  $\sim$  3.1 mm, width  $\sim$  384  $\mu$ m, with at least 60 transverse rows along total length in paratype 08 [NHMUK 20120076]. Central tooth differentiated in form from lateral teeth; smooth-sided, bell-shaped, wider proximally than distally, with a single incurved central cusp (Figure 2B). Lateral teeth (Figure 2B, C) of similar size to central tooth, increasing in size outwards; with a long, rounded single central cusp, and an outer apical margin with several flanking denticles  $(>_7)$ ; dentition attenuates towards the cusp and is strongest on the outermost lateral. Marginal teeth (Figure 2B, D) exceed twenty in number on both sides; cutting plate concave, terminating in a single short cusp; apical margins oblique, each with about 10-14 denticles that are longer and finer than those on the lateral teeth. Outermost marginal teeth in a row are smaller, with weaker dentition and straighter shafts. Marginal rows overlap each other.

No jaws are present.

#### COMPARATIVE REMARKS

The shell of *Iheyaspira bathycodon* sp. nov. is superficially similar to those of several other skeneimorph taxa, but the radula pattern appears to be unique in both number of teeth and shape.

The new species is closest in morphology to Iheyaspira lequios Okutani, Sasaki & Tsuchida, 2000 (Turbinidae: Skeneinae), the type species of a monotypic genus. Affinities with I. lequios include conchological similarity and shared radula characters, most notably in the shape of the central tooth (see Okutuni et al., 2000: Figure 2, p. 269). The new species does however exhibit several important dissimilarities to *I. lequios*: (1) shell: small (maximum  $7.2 \times 6.1$  mm) rather than minute (maximum  $5.7 \times 5.4$  mm in *I. lequios*), umbilicate, teleoconch with more than two whorls; (2) radula: central tooth bell-shaped, not rhombic/arrow-shaped; only nine (not twelve) pairs of lateral teeth; (3) eyestalks: reduced, subequal in length and width to cephalic tentacles, as opposed to well-developed, thicker than cephalic tentacles; (4) neck lobes: right neck lobe composed of three (not two) tentacles; (5) epipodial tentacles: five (not four) on both sides; left ET1 and ET3 densely papillate (I. lequios ET1-3 lack papillae). In Iheyaspira bathycodon sp. nov., ET2 and ET<sub>3</sub> are very close together, arising as a pair from the same base; this is similar to I. lequios, and may be an epipodial sense organ.

The new species is also comparable with the turbinid *Fucaria mystax* Warén & Bouchet, 2001 (Skeneinae), based on similarities in shell and radula characters, especially the shape of the central tooth (see Warén & Bouchet, 2001: Figure 11C, p. 135). *Iheyaspira bathycodon* sp. nov. is differentiated from *F. mystax* by: (1) shell: umbilicus clearly visible in basal view, teleoconch with greater number of whorls (>2.5); (2) radula: central tooth bell-shaped, lacking drawn out and narrow anterior support; only nine (not eleven) pairs of lateral teeth; (3) eye stalks: do not encircle cephalic tentacles. Moreover, members of the genus *Fucaria* Warén & Bouchet, 1993 are equipped with a coat of sensory papillae on the snout, a feature not observed in the new species.

#### DISTRIBUTION AND HABITAT

Known only from the type locality, the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean in 2300 m depth. See Connelly *et al.* (2012) for a description of the geological, geochemical and biological setting of the Von Damm Vent Field. Accompanying fauna observed in close proximity to the new species included the alvinocaridid shrimps *Rimicaris hybisae* Nye, Copley & Plouviez, 2012 and *Alvinocaris* sp., the hippolytid shrimp *Lebbeus* sp., zoarcid fish and siboglinid polychaetes.

#### ETYMOLOGY

The species name *bathycodon* is derived from the Greek words for deep and bell, in reverence to the species' deep-sea habitat and bell-shaped rachidian tooth.

#### MOLECULAR PHYLOGENY

Partial sequences of the COI (549 bp), 16S (505 bp) and 18S (803 bp) region of *Iheyaspira bathycodon* sp. nov. were consistent amongst specimens. Fixed and unique mutations were observed in the partial sequences of the COI, 16S and 18S regions in comparison with other trochoid taxa. When compared with partial sequences of COI in the gastropod dataset of Suzanne Williams (NHMUK) the partial COI sequence of the new species was near other trochoidean skeneimorph taxa and unique amongst any of the species available (Suzanne Williams, personal communication). Based on a 425-bp alignment of partial 16S sequences, NJ and ML



Fig. 6. Neighbour-joining tree of turbinid gastropods based on a 425-bp alignment of partial nucleotide sequences from the mitochondrial 16S region with *Buccinum tenuissimum* (Caenogastropoda: Neogastropoda: Buccinoidea: Buccinidae) as outgroup. Evolutionary distances computed using the Jukes–Cantor method (Jukes & Cantor, 1969) are represented by branch length; scale bar is proportional to inferred nucleotide divergence. Bootstrap support calculated on 1000 re-sampling replicates is shown by the numbers along the branches (NJ, plain text; ML, italic text). GenBank accession numbers are given after species names.

phylogenetic trees have the same topologies and place the new species in the same clade (Turbinidae: Skeneinae) as *Dillwynella* cf. vitrea [AY163406.1], *Protolira valvatoides* [AY163405.1] and *Protolira* sp. [GQ160698.1], with 96% and 87% bootstrap support for NJ and ML methods respectively (Figure 6). Of the 16S partial sequences available in GenBank the new species is closest in evolutionary distance to *D.* cf. vitrea (14% divergence). Phylogenetic analyses on an 803-bp alignment of partial 18S sequences of turbinid species place the new species closest in evolutionary distance to *D. planorbis* [AB365310.1], with 39% and 42% bootstrap support for NJ and ML methods respectively. The new species exhibits 1.4% divergence from *D. planorbis* across an 803 bp of the 18S region.

#### DISCUSSION

The molecular and morphological analyses of specimens of this trochoid gastropod reveal the presence of a new species. The new species is similar in morphology to *Iheyaspira lequios* and *Fucaria mystax* (see above), both of which are known only from hydrothermal vents in the Pacific at water depths less than 1500 m (see Table 1). It is, however, excluded from both species by differences in the radula and appendage structure of the head-foot (see above). It is closest in morphology to *I. lequios* and, therefore, the most conservative approach is to give the new species the generic name *Iheyaspira* to indicate the similarity between the two species. Consistency between specimens of *I. bathycodon* sp. nov. in partial sequences of the COI, 16S and 18S regions confirm that they belong to a single species, but the presence of unique and fixed mutations in the sequences indicate that

they are genetically distinct from all other genera and species in the GenBank database.

The systematic position of both *Iheyaspira* and *Fucaria* is uncertain because there are no sequences available in GenBank for either genus; however both genera are classified currently within the family Turbinidae and subfamily Skeneinae (e.g. Bouchet, 2010 a, b; Sasaki *et al.*, 2010). After redefining the Turbinidae, Williams *et al.* (2008) remarked that it is hard to determine morphological characters that are typical of this family, and even suggested that the Skeneinae could be considered as a group distinct from (but most closely related to) Turbinidae (Williams *et al.*, 2008). The rank of the Skeneinae is still under discussion and further work will elucidate the systematic position of this taxon (Williams, in press).

Morphological features of the Skeneinae shared with the new species include the monopectinate ctenidium and absence of any visible nacre. In addition, the shell of the new species bears superficial resemblance to that of other members of the Skeneinae, such as *Protolira*. In the GenBank database sequences for the Skeneinae are available presently for a few species only. Despite this impediment, comparative 16S results presented herein suggest the proximity of the new species to members of the Skeneinae, with strong bootstrap support for inclusion of the new species within this clade. This is further supported by comparative 18S results, whereby NJ and ML methods both place the new species closest in evolutionary distance to *Dillwynella planorbis* [AB365310.1], although this is with weak bootstrap support (39% and 42% for NJ and ML methods respectively).

The first right neck lobe tentacle (RNL1) in the new species may be modified (see Figure 4 B, D). Warén & Bouchet (1989) described a modified neck lobe tentacle in *Bathymargarites*  *symplector* Warén & Bouchet 1989 and interpreted this modified appendage as a penis. Collection of further specimens will enable the reproductive anatomy of the new species to be characterized.

The recent discovery of hydrothermal vents and chemosynthetic assemblages on the Mid-Cayman Spreading Centre has provided an opportunity to enhance existing knowledge about biodiversity in the deep sea. *Iheyaspira bathycodon* sp. nov. is the second new species to be described from the Von Damm Vent Field, and the tenth turbinid gastropod to be described from a hydrothermal vent environment to date (Table 1). Description of species from Mid-Cayman Spreading Centre vents, and further characterization of their faunal assemblages by future collections has the potential to elucidate the factors determining vent biogeography of this region.

#### ACKNOWLEDGEMENTS

The authors extend their thanks to those on-board the 44th voyage of RRS 'James Cook', to P.A. Tyler and C.L. Van Dover for providing laboratory facilities at NOCS and DUML, and to A. Glover (NHMUK) and A. Page (Biomedical Imaging Unit, University of Southampton) for use of their microscopy facilities. A. Warén and S. Williams are also thanked for commenting on aspects of this work, which is supported by a UK NERC award (NE/F017774/1) to J.Copley and NASA ASTEP Grant (NNX09AB75G) to C.L. Van Dover. This paper benefitted from an anonymous referee who gave valuable comments for its improvement.

#### REFERENCES

- Bouchet P. (2010a) *Iheyaspira* Okutani, Sasaki & Tsuchida, 2000. In Bouchet P., Gofas S. and Rosenberg G. (eds) *World marine Mollusca database*. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=456431 on 17 December 2012.
- Bouchet P. (2010b) *Fucaria* Warén & Bouchet, 1993. In Bouchet P., Gofas S. and Rosenberg G. (eds) *World marine Mollusca database*. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=456416 on 17 December 2012.
- Bouchet P., Racroi J.P., Fryda J., Hausdorf B., Ponder W., Valdes A. and Warén A. (2005) Classification and nomenclature of gastropod families. *Malacologia* 47, 1–368.
- Clark W. (1851) On the classification of the British marine testaceous Mollusca. Annals and Magazine of Natural History 2, 469-482.
- Connelly D.P., Copley J.T., Murton B.J., Stansfield K., Tyler P.A., German C.R., Van Dover C.L., Amon D., Furlong M., Grindlay N., Hayman N., Hühnerbach V., Judge M., Le Bas T., McPhail S., Meier A., Nakamura K-I, Nye V., Pebody M., Pedersen R.B., Plouviez S., Sands C., Searle R.C., Taws S. and Wilcox S. (2012) Hydrothermal vent fields and chemosynthetic biota on the world's deepest seafloor spreading centre. *Nature Communications*. doi: 10.1038/ncomms1636.
- **Cossmann M.** (1917) In Cossman M. and Payrot A. (1917–1919). *Conchologie néogénique de l'Aquitaine. Tome 3. Gastropodes, Scaphopodes et Amphineures.* Bordeaux: Actes de la Société Linnéenne de Bordeaux, p. 354.

- Dall W.H. (1889) Reports on the results of dredgings, under the supervision of Alexander Agassiz, in the Gulf of Mexico (1877–78) and in the Caribbean Sea (1879–80), by the U.S. Coast Survey Steamer 'Blake'. Bulletin of the Museum of Comparative Zoology 18, 1–492.
- Desbruyères D., Alayse-Danet A.-M., Ohta S. and the Scientific Parties of BIOLAU and STARMER Cruises (1994) Deep-sea hydrothermal communities in south-western Pacific back-arc basins (the North Fiji and Lau Basins): composition, microdistribution and food web. *Marine Geology* 116, 227–242.
- **Doyle J.J. and Dickson E.** (1987) Preservation of plant samples from DNA restriction endonuclease analysis. *Taxon* 36, 715-722.
- Fabri M.-C., Bargain A., Briand P., Gebruk A., Fouquet Y., Morineaux M. and Desbruyères D. (2011) The hydrothermal vent community of a new deep-sea field, Ahasze-1, 12°58'N on the Mid-Atlantic Ridge. *Journal of the Marine Biological Association of the United Kingdom* 91, 1–13.
- Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294–299.
- Galkin S.V. (1997) Megafauna associated with hydrothermal vents in the Manus Back-Arc Basin (Bismarck Sea). *Marine Geology* 142, 197–206.
- Hickman C.S. and McLean J.H. (1990) Systematic revision and suprageneric classification of trochacean gastropods. *Natural History Museum of Los Angeles County Science Series* 35, 1–77.
- Jeffreys J.G. (1883) On the mollusca procured during the 'Lightning' and 'Porcupine' Expeditions, 1868–1870. Part 6. Proceedings of the Zoological Society of London 1883, 88–115.
- Jukes T.H. and Cantor C.R. (1969) Evolution of protein molecules. In Munro H.N. (ed.) *Mammalian protein metabolism*. New York: Academic Press, pp. 21–132.
- Kano Y. (2008) Vetigastropod phylogeny and a new concept of Seguenzioidea: independent evolution of copulatory organs in the deep-sea habitats. *Zoologica Scripta* 37, 1–21.
- **Kiel S.** (2004) Shell structures of selected gastropods from hydrothermal vents and seeps. *Malacologia* 46, 169–183.
- Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111–120.
- Kojima S., Segawa R., Fijiwara Y., Fujikura K., Ohta S. and Hashimoto J. (2001) Phylogeny of hydrothermal-vent endemic gastropods *Alvinoconcha* spp. from the western Pacific revealed by mitochondrial DNA sequences. *Biological Bulletin. Marine Biological Laboratory*, *Woods Hole* 200, 298–304.
- Nye V., Copley J. and Plouviez S. (2012) A new species of *Rimicaris* (Crustacea: Decapoda: Caridea: Alvinocarididae) from hydrothermal vent fields on the Mid-Cayman Spreading Centre, Caribbean. *Journal of the Marine Biological Association of the United Kingdom*. doi: 10.1017/S0025315411002001.
- Okutani T., Tsuchida E. and Fujikura K. (1992) Five bathyal gastropods living within or near the *Calyptogena* community of the Hatsushima Islet, Sagami Bay. *Venus* 51, 137–148.
- Okutani T., Sasaki T. and Tsuchida T. (2000) Two additional new species to gastropod fauna of chemosynthetic site on North Knoll of Iheya Ridge, Okinawa Trough. *Venus* 59, 267–275.
- **Okutani T., Hashimoto J. and Sasaki T.** (2004) New gastropod taxa from hydrothermal vent (Kairei Field) in the central Indian Ocean. *Venus* 63, 1–11.

- Palumbi S.R. (1996) Nucleic acids II: the polymerase chain reaction. In Hillis D.M., Moritz C. and Mable B.K. (eds) *Molecular systematics*. Sunderland, MA: Sinauer Associates, pp. 204–247.
- Rafinesque C.S. (1815) Analyse de la nature ou tableau de l'universe et des corps organisées. Palermo: L'Imprimerie de Jean Barravecchia.
- Saitou N. and Nei M. (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406–425.
- Salvini-Plawen L.v. (1980) A reconsideration of systematics in the Mollusca (phylogeny and higher classification). *Malacologica* 19, 249-278.
- Sasaki T., Okuntani T. and Fujikura K. (2005) Molluscs from hydrothermal vents and cold seeps in Japan: a review of taxa recorded in twenty recent years (1984–2004). *Venus* 64, 97–133.
- Sasaki T., Warén A., Kano Y., Okutani T. and Fujikura K. (2010) Gastropods from recent hot vents and cold seeps: systematics, diversity and life strategies. *Topics in Geobiology* 33, 169–254.
- Swainson W. (1840) A treatise on malacology; or the natural classification of shells and shellfish. London: Lardner's Cabinet Cyclopedia.
- Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*. doi:10.1093/ molbev/msr121.
- Tao C., Lin J., Guo S., Chen Y.J., Wu G., Han X., German C.R., Yoerger D.R., Zhou N., Li H., Su X. and the DY 115-19 (Legs 1-2) and DY115-20 (Legs 4-7) Science Parties (2012) First active hydrothermal vents on an ultraslow-spreading center: Southwest Indian Ridge. *Geology* 40, 47-50.
- Thiel J. (1924) *Handbuch der Zoologie 5, Mollusca.* Berlin: Walter de Gruyter.
- Van Dover C.L., Humphris S.E., Fornari D., Cavanaugh C.M., Collier R., Goffredi S.K., Hashimoto J., Lilley M.D., Reysenbach A.L., Shank T.M., Von Damm K.L., Banta A., Gallant R.M., Gotz D., Green D., Hall J., Harmer T.L., Hurtado L.A., Johnson P., McKiness Z.P., Meredith C., Olson E., Pan I.L., Turnipseed M., Won Y., Young C.R. and Vrijenhoek R.C. (2001) Biogeography

and ecological setting of Indian Ocean hydrothermal vents. *Science* 294, 818-823.

- Vilvens C. and Sellanes J. (2006) Descriptions of *Otukaia crustulum* new species (Gastropoda: Trochoidea: Calliostomatidae) and *Margarites huloti* new species (Gastropoda: Trochoidea: Trochidae) from a methane seep area off Chile. *Nautilus* 120, 15–20.
- Warén A. (1996) New and little known Mollusca from Iceland and Scandinavia, part 3. Sarsia 81, 197–245.
- Warén A. and Bouchet P. (1989) New gastropods from East Pacific hydrothermal vents. *Zoologica Scripta* 18, 67–102.
- Warén A. and Bouchet P. (1993) New records, species, genera and a new family of gastropods from hydrothermal vents and hydrocarbon cold seeps. *Zoologica Scripta* 22, 1–90.
- Warén A. and Bouchet P. (2001) Gastropoda and monoplacophora from hydrothermal vents and seeps: new taxa and records. *Veliger* 44, 116-231.
- Warén A., Bouchet P. and von Cosel R. (2006) Gastropoda. In Desbruyères D., Segonzac M. and Bright M. (eds) Handbook of deep-sea hydrothermal vent fauna. Vienna: Biologiezentrum der Oberosterreichische Landesmuseen, pp. 82–140.
- Williams S.T., Karube S. and Ozawa T. (2008) Molecular systematics of Vetigastropoda: Trochidae, Turbinidae and Trochoidea redefined. *Zoologica Scripta* 37, 483–506.

and

Williams S.T. (in press) Advances in systematics of the vestigastropod superfamily Trochoidea. Zoologica Scripta.

# Correspondence should be addressed to:

V. Nye Ocean & Earth Science National Oceanography Centre Southampton University of Southampton Waterfront Campus European Way, Southampton, SO14 3ZH, UK email: vn205@noc.soton.ac.uk