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Molecular phylogenetic analysis of a known and a new hydrothermal vent octopod: their relationships with the genus *Benthoctopus* (Cephalopoda: Octopodidae)

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Abstract

The resolution of evolutionary relationships among deep-sea incirrate octopuses has been hindered by the paucity of individuals available for morphological studies and by the lack of tissue samples preserved using fixatives compatible with simple DNA extraction techniques. Evolutionary relationships from 11 species of deep-sea incirrate octopuses were investigated using 2392 base pairs (bp) of DNA from four mitochondrial genes (12S rDNA, 16S rDNA, cytochrome c oxidase subunit III, and cytochrome b) and the nuclear gene, *rhodopsin*. Morphological examination of these species was also undertaken. Molecular analyses distinguish a species of octopus from hydrothermal vents at Manus Basin from the vent octopodid *Vulcanoctopus hydrothermalis* known from vents on the East Pacific Rise. Both are herein considered members of the clade currently assigned the name *Benthoctopus*, although taxonomic implications preclude formally naming *Vulcanoctopus* as a junior synonym. Morphological investigations led to the conclusion that *Benthoctopus macrophallus* is a junior synonym of *Benthoctopus yaquinae.* An amended diagnosis of *Benthoctopus* is provided with additional information on male reproductive characteristics.

Key words: Octopodiformes, *Vulcanoctopus, Enteroctopus*

Introduction

The family Octopodidae Orbigny comprises the familiar benthic octopuses and contains most genera and species within the suborder Incirrata Grimpe (order Octopoda Leach). Deep-sea octopodids comprise a significant proportion of the family's known diversity. Of the 37 genera currently recognised (Norman & Hochberg 2005), 11 contain at least one species known from depths beyond 1000 m. These octopodids are found in diverse deep-sea habitats and have been recorded from the Arctic to the Antarctic, and from abyssal plains to hydrothermal vents (Voss 1988b; González *et al.* 1998).

Deep-sea octopodids are unsurprisingly characterized by features thought to be adaptations to the deep sea, such as the absence of an ink sac, reduction in gills, radula and crop, large eggs and spermatophores (Robson 1924; Voss 1967; 1988a; Muus 2002). Confusion of the evolutionary relationships of deep-sea octopodid taxa has arisen because many of these features also serve as a basis for traditional systematics.

Voss (1988a,b) split the deep-sea octopodids into two subfamilies, depending on whether sucker seriation

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was uniserial (Graneledoninae Voss) or biserial (Bathypolypodinae Robson). The genera *Bathypolypus* Grimpe, *Benthoctopus* Grimpe, *Teretoctopus* Robson and *Grimpella* Robson were assigned to Bathypolypodinae. *Grimpella* and *Teretoctopus* contain only one and two species respectively and *Bathypolypus* has recently been reviewed and revised in depth by Muus (2002). *Benthoctopus* contains at least 20 named species with representatives from nearly every ocean basin. Authors have argued that this genus may be polyphyletic (e.g. Norman *et al.* 1997) and that it is in need of investigation (Muus 2002; Allcock *et al.* 2006).

An additional genus with biserial suckers has been described since Voss (1988a) erected his new subfamily. *Vulcanoctopus* González & Guerra was described based on two male octopodids from a hydrothermal vent on the East Pacific Rise. The genus remains monospecific and *Vulcanoctopus hydrothermalis* González & Guerra, in González *et al.* remains the only octopodid species considered to be endemic to vent sites. González *et al*. (1998) suggested that this new species possessed a number of new characters, which may warrant the erection of a new subfamily, the Vulcanoctopodinae. These included an eye with no trace of an iris, the absence of optic chiasma, a multilobate digestive gland, a dark swelling in the proximal intestine and the lack of any type of chromatic elements in the skin. However, *V. hydrothermalis* is also known to share a number of morphological characteristics with *Benthoctopus*, including biserial suckers, a deep-sea habit, a multicuspid rhachidian tooth and the lack of an ink sac (González *et al*. 1998; González *et al.* 2008). Its phylogenetic position within the Octopodidae is uncertain.

Benthoctopus and *Vulcanoctopus* are less than ideal genera for molecular phylogenetic studies. Their deep-water to abyssal habitat means that many species are known from only a few individuals, most of which were fixed using formalin or other preservatives, which are not compatible with simple DNA extraction methods. As a result, no published studies contain molecular sequence data of *V. hydrothermalis*. Species of *Benthoctopus* have been included in very few molecular phylogenetic studies, the majority of which have included a single individual of an unknown species (Carlini *et al.* 2001; Lindgren *et al.* 2004; Strugnell *et al.* 2004; 2005).

Allcock *et al.* (2006) is unique in having examined relationships among four species of *Benthoctopus* using molecular phylogenetics. Using maximum likelihood and Bayesian analysis of four mitochondrial genes (12S rDNA, 16S rDNA, cytochrome oxidase I, cytochrome oxidase III) and the nuclear gene *rhodopsin*, a highly supported sister taxa relationship was discovered between *Enteroctopus* Rochebrune and Mabille and *Benthoctopus* (to the exclusion of *Octopus vulgaris* Cuvier and *Bathypolypus*). In addition, *Benthoctopus normani* (Massy) was the sister taxon to the remaining *Benthoctopus* species included in the study. Highly supported sister taxa relationships were also reported between *B. johnsoniana* Allcock, Strugnell, Ruggiero & Collins and *B.* cf. *levis* (Hoyle) and between these two species and *B. eureka* (Robson).

The increasing availability of remotely operated vehicles, human occupied vehicles, scientific research cruises and the expansion of commercial fishing efforts into deeper waters has led to increased captures of deep-sea octopodids. The present study investigates the molecular phylogenetic relationships of a newly discovered octopus captured from an active hydrothermal vent in the Manus Basin off Papua New Guinea, several little known species of *Benthoctopus* and of *Vulcanoctopus hydrothermalis*. Most of the species included in this study have been described or redescribed in recent years and the descriptions are relatively comprehensive. We aim therefore not to redescribe them, but to identify distinguishing characters that may further morphological studies and be useful in elucidating evolutionary relationships. Short diagnoses only are therefore given for each species in the systematics section

Methods

Sampling

This study included tissue samples from the DNA bank and the preserved specimens at the Field Museum of Natural History (FMNH), Chicago, U.S.A, in addition to a vent associated octopodid captured during a baseline survey of an active vent in the Manus Basin off Papua New Guinea for Nautilus Minerals (Figure 1). Full details of capture locations (and catalogue numbers of relevant reference specimens if available) are detailed in Table 1. Normally, tissue samples were taken prior to the fixation of the whole animals and preserved in 70–100% ethanol for subsequent DNA extraction. The exception is the Manus Basin specimen, which was fixed in formalin overnight prior to removal of a small piece of tissue to 95% ethanol for DNA extraction.

TABLE 1. Cephalopod tissue samples used for molecular analyses in this study.

Species	Station	Date	Depth (m)	Latitude and longitude	Museum catalogue
Octopus vulgaris	Banyuls, France				
Bathypolypus sponsalis	RV *Discovery* Stn 14170#1	1 Sep 2001	775–842	51° 36.4' N 11° 53.3' W	NMSZ 2002126.002
Enteroctopus dofleini	Living Elements Research, North Vancouver, Canada				
Benthoctopus normani	RV *Discovery* Stn 14163#1	29 Aug 2001	1340–1397	49° 27' N 12° 41' W	NMSZ 2002126.001
Benthoctopus rigbyae	61/048-1	16 Mar 2002	343.2	61°10'S 54°34'W	NMSZ 2002037.032
Benthoctopus yaquinae	*Alvin* Dive 4045	02 Sep 2004	2213	47° 56' 52" N 129° 05' 51" W	FMNH 308673
Vulcanoctopus hydrothermalis	East Pacific Rise, *Alvin* Dive 3926,	07 Nov 2003	2541	08° 38' 15" N 104° 12' 54" W	FMNH 307184
Benthoctopus sp A	Sta. 3, off the coast of Oregon	17 Apr 1997	2850	44° 45' 57" N 125° 31' 44" W to 44° 36' 54" N 125° 37' 24" W	FMNH 278117
Benthoctopus sp A	*Alvin* Dive 4046	03 Sep 2004	2658	47° 47' 11" N 127° 41' 53" W	FMNH 308674
Benthoctopus sp B (embryo)	*Tiburon* Dive 884, Gorda Ridge, GR14	23 Aug 2005	2751.7	42° 45' 18" N 126° 42' 35" W	FMNH 309724
Benthoctopus cf. *profundorum*	*Alvin* Dive 3934	15 Nov 2003	2492	11° 24' 54" N 103° 47' 12" W	FMNH 307179
Benthoctopus oregonensis	Sta. 15, off the coast of Oregon	21 Apr 1997	2750	44° 45' 47" N 125° 31' 14" W to 44° 37' 06" N 125° 36' 00" W	FMNH 278314
Benthoctopus johnsonianus	RV *Discovery* Stn 14309	16 Mar 2002	2011–2218	49° 44.2' N 13° 10.4' W	-
Benthoctopus eureka	Falkland Islands	07 Oct 1992	230		SBMNH 423134
Benthoctopus Manus Vent	Manus Basin, Papua New Guinea	04 Apr 2007	1500	3° 43' 30" S 151° 40' E	FMNH 310455

Systematics

Wherever possible, octopodids included in the analysis were identified to species level. The Manus Vent octopus (Figure 1) is only known from a single specimen (FMNH 310455) collected from near a hydrothermal vent in Manus Basin. It is in such poor condition that initially it could not be placed to genus. *Benthoctopus* sp. A is only known from females. *Benthoctopus* sp. B is merely an embryo. *Benthoctopus* cf. *profundorum* is known from the single female sequenced herein. The poor condition of the Manus Vent octopus, lack of male specimens of *Benthoctopus* sp. A and of *Benthoctopus* cf. *profundorum* and the lack of adults of *Benthoctopus* sp. B prevent us from describing these taxa at this time. A new species *B. rigbyae*, has just been described (Vecchione *et al.* 2009).

Other specimens were identified with reference to keys (e.g., Voss & Pearcy 1990) and then compared with primary type material examined at the Natural History Museum, London (BMNH), the National Museum of Scotland, Edinburgh (NMSZ), the National Museums and Galleries of Wales (NMGWZ) and the Smithsonian Institution, Washington DC (USNM). Under material examined, an asterisk (*) identifies each specimen used in the molecular analyses.

Abbreviations: Stn, station; leg., legit; det., determined; ML, mantle length; LLI, ligula length index; SpLI, spermatophoric length index; AL(L3), length of 3rd left arm; AL(R3), length of 3rd right arm; ASC(L3), number of suckers on 3rd left arm; ASC(R3), number of suckers on 3rd right arm; SDI, sucker diameter index; WDI, web depth index.

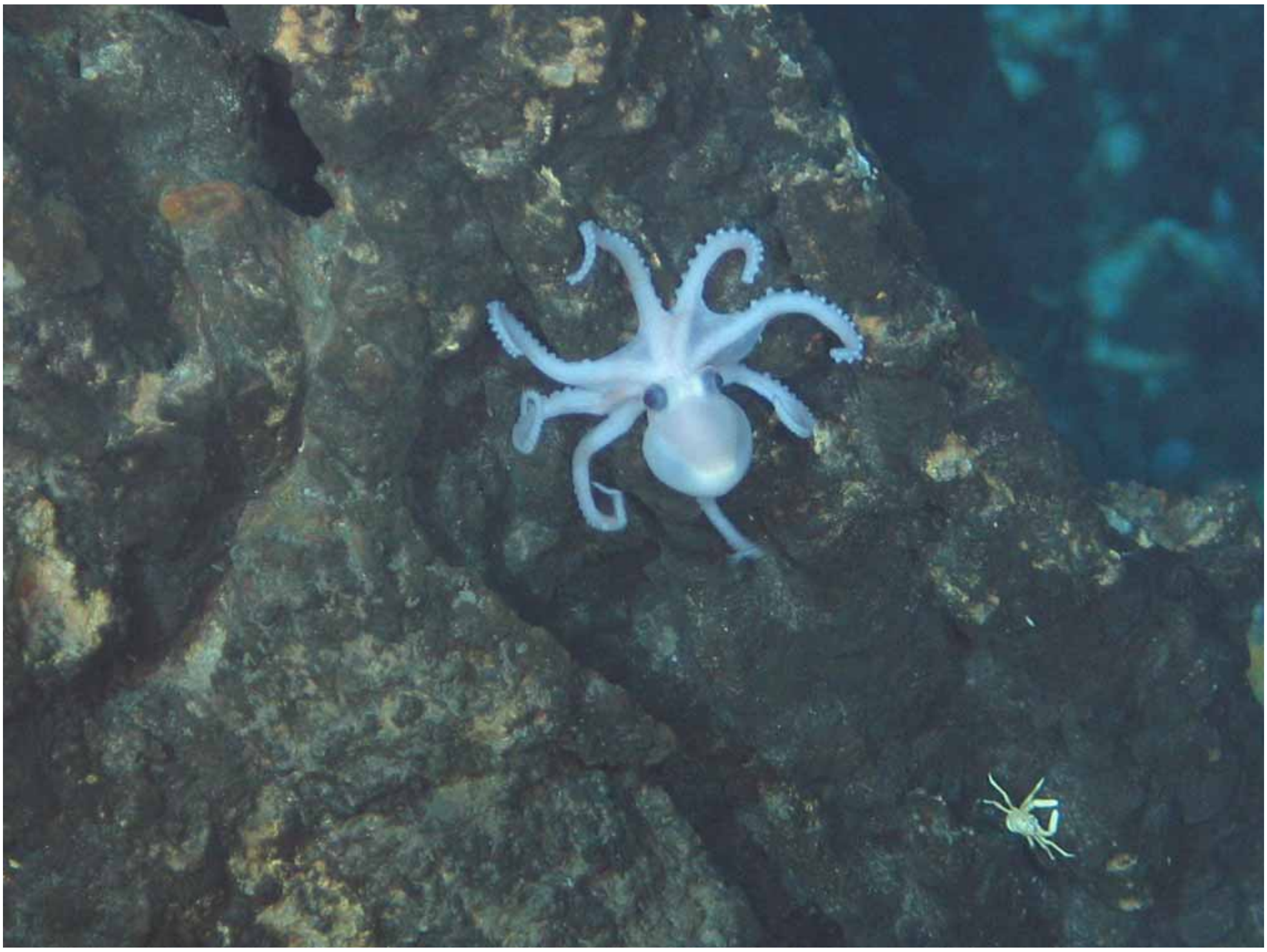

FIGURE 1. The Manus Vent octopus specimen (FMNH 310455) *in situ* near a hydrothermal vent in the Manus Basin off Papua New Guinea, 1500 m depth.

Molecular analyses

DNA extraction and PCR amplification followed the methods outlined in Allcock *et al.* (2006). Primers for fragments of three mitochondrial genes (12S rDNA, 16S rDNA, cytochrome c oxidase subunit III [COIII]) and the nuclear gene *rhodopsin* are detailed in Allcock *et al.* (2008). Primers for the mitochondrial gene cytochrome b [cyt b] are detailed in Guzik *et al.* (2005). In addition to these genes, the barcoding gene cytochrome c oxidase subunit I (COI) was targeted, but unfortunately it could not be amplified for most specimens included in the study.

Difficulties were encountered in amplifying DNA from the Manus Vent octopodid. To obtain a 12S rDNA sequence from this individual, a nested PCR approach was employed. The starting template for a second PCR was 0.5 µl of the 12S rDNA PCR product. The forward primer for this PCR was designed from octopodid sequences (and is available on request from the first author) to complement the sequence just inside the position of the initial 12S rDNA forward primer. The same 12S rDNA reverse primer was used as in the first round of the PCR.

DNA sequences were aligned by eye in Se-Al v2.0a11 Carbon (Rambaut 2002).

Concordance of the mitochondrial and nuclear gene datasets was evaluated with the partition-homogeneity test implemented in PAUP v4.0b10 (Swofford 1998) using 1000 random repartitions.

PAUP v4.0b10 (Swofford 1998) was used to perform full heuristic searches. Starting trees were generated by the neighbor-joining (NJ) method (Saitou & Nei 1987). A GTR+Γ+I likelihood model incorporating four rate categories was used. Branch swapping was performed using tree-bisection-reconnection (TBR). Parameters were then re-estimated and branch swapping was then performed using nearest-neighbor-interchange (NNI). Substitution model parameter values for each data set are included in the supplementary information. Maximum likelihood (ML) bootstrap values of clade support were generated using the above parameters, with starting trees obtained using NJ.

MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) was used to calculate marginal posterior probabilities using the GTR+I+Γ model of nucleotide substitution for each partition. Model parameter values were treated as unknown and were estimated in each analysis. Random starting trees were used and analyses were run 1000000 generations, sampling the Markov chain every 100 generations. The analysis was performed twice, in each case starting from a different random tree to ensure the analyses were not trapped in local optima. Stationarity was deemed to be reached when the average standard deviation of split frequencies, shown in MrBayes 3.1.2, was less than 0.01 (Ronquist & Huelsenbeck 2003).

The program Tracer v 1.3 (Rambaut & Drummond 2003) was used to determine the correct "burn-in" for the analysis (i.e. the number of initial generations that must be discarded before stationarity is reached).

Systematics

Benthoctopus Grimpe

Emended diagnosis. Deepwater incirrate octopodids. Arms 1.5–4 times ML. Suckers biserial. Ligula slightly to moderately excavated with indistinct midrib, smooth or bearing low, often indistinct rugae, never laminate. Crop present, usually with diverticulum. Ink sac absent, anal flaps absent. Radula with multicuspid rachidian.

Type species: Octopus piscatorum Verrill, 1879. By original designation.

Remarks. *Octopus piscatorum* was designated a junior synonym of *Bathypolypus bairdii* by Muus (2002) and the genus *Benthoctopus* is currently invalid. We continue to use the name as an interim measure because this large group of rarely acquired and potentially highly diverse species requires further attention before nomenclatural acts are made, to ensure that such acts provide nomenclatural stability rather than create additional uncertainty.

Benthoctopus normani (Massy, 1907)

Synonymy: Polypus normani Massy, 1907: 377–384.

Type material examined: Benthoctopus normani (Massy, 1907) neotype, NMSZ 1999158.186, RV *Challenger*, 49°38'N 11°49'W, Stn C134/16, 14 August 1997, 985 m, male.

Additional material examined: Benthoctopus normani: *NMSZ 2002126.001, RV *Discovery*, 49°27'N 12°41'W (North East Atlantic), Stn 14163#1, 29 August 2001, 1340–1397m, female, leg. & det. AL Allcock. An additional 16 specimens detailed in Allcock *et al.* (2006): BMNH 2006170-7, NMSZ 1999158.027/.030/.032/.292/.298, NMSZ 1994128.46511.

Diagnosis. Suckers widely spaced. Arms approximately 4 times ML. Arm formula 1>2>3>4. Funnel organ VV-shaped. Gills with 7–10 lamellae per outer demibranch, 6–9 lamellae per inner demibranch. LLI 6–11. Maximum spermatophore length 119 mm, maximum SpLI 121, maximum number of spermatophores 25. Mature males without enlarged suckers. Integument smooth.

Benthoctopus yaquinae Voss & Pearcy, 1990

Synonymy: Benthoctopus macrophallus Voss & Pearcy, 1990: 82, fig 17.

Type material examined: Benthoctopus yaquinae Voss & Pearcy holotype, USNM 729992, RV *Yaquina*, 44°49'N 125°59'W (off Oregon coast), Stn 6501-49, 12 January 1965, 2800 m, male. *Benthoctopus* *macrophallus* Voss & Pearcy holotype, USNM 730713, RV *Yaquina*, 44°59'N 126°32'W (off Oregon coast), Stn 7102B-267, 19 February 1971, 2795 m, male.

Additional material examined: Benthoctopus yaquinae: FMNH 278119, RV *Wecoma*, 44°46'N 125°32'W (off Oregon coast), Stn 5, 17 April 1997, 2750 m, male, leg. & det. JR Voight. *FMNH 308673, RV *Atlantis* HOV *Alvin*, 47°57'N 129°06'W (Juan de Fuca Ridge), *Alvin* Dive 4045, 2 September 2004, 2213 m, leg. & det. JR Voight. FMNH 278315, RV *Wecoma*, 44°46'N 125°32'W (off Oregon coast), Stn 5, 17 April 1997 2750 m, female, leg. & det. JR Voight. FMNH 278118, RV *Wecoma*, 44°46'N 125°32'W (off Oregon coast), Stn 1, 16 April 1997, 2850 m, female, leg. & det. JR Voight.

Diagnosis. Suckers erect, widely spaced. Ink sac and anal flaps absent. Arms approximately 3 times ML. Arm formula 1≥2≥3≥4. Funnel organ W-shaped. Gills with 7–9 lamellae per demibranch. LLI 8–12. Maximum spermatophore length approximately 110 mm, maximum SpLI 136, maximum number of spermatophores unknown – holotype contained remains of approximately 4. Mature males without enlarged suckers. Integument smooth. Strong reverse counter-shading apparent.

Remarks. Benthoctopus macrophallus is herein designated a junior synonym of *Benthoctopus yaquinae*. There are no discernable differences between the holotypes of these nominal species. Voss and Pearcy (1990) separated *B. macrophallus* ‘from all other *Benthoctopus* by the unusual characters of the radula, the beaks, the very large spermatophores, and the unusually long, separate crop diverticulum’ and states that the name ‘refers to the exceptionally long penial apparatus’. Both holotypes are large mature male specimens in excellent condition. The holotype of *B. macrophallus* has mantle length approximately 70 mm, ligula length 22 mm, ligula groove length 17 mm, calamus length 6 mm, AL(L3) 260 mm, AL(R3) 190 mm, ASC(L3) 119, ASC(R3) 55. The holotype of *B. yaquinae* has mantle length 75 mm, ligula length 21 mm, ligula groove length 17 mm, calamus 6 mm, AL(L3) 250 mm, AL(R3)170 mm, ASC(L3) 113, ASC(R3) 52. The beak of *macrophallus* was clearly damaged on extraction but it is not exceptional. The radula is missing. Spermatophores of both holotypes were fragmented. We are puzzled by the description of the crop diverticulum because the digestive system had not been dissected. Furthermore, *B. macrophallus* is known only from the holotype. The penial apparatus of *B. macrophallus* is long (58 mm) but it is also long in the holotype of *B. yaquinae* (47 mm); a difference we argue lies within the bounds of intraspecific variation.

***Benthoctopus* sp. A**

Material examined: Benthoctopus sp. A: *FMNH 278117, RV *Wecoma*, 42°45'N 126°43'W (off Oregon coast), Stn 3, 17 April 1997, 2850 m, female, leg. & det. JR Voight. *FMNH 308674, RV *Atlantis* HOV *Alvin*, (near Wuzza Bear Mount), *Alvin* Dive 4046, 3 September 2004, 2656 m, female, leg. TA Haney, det. JR Voight.

Diagnosis. Arms 1.5–2.5 times ML. Arm formula typically 1≥2≥3≥4 but differences in arms lengths only 5 mm. Funnel organ W-shaped. Gills with 6^+ lamellae per demibranch. Male characters unknown. Integument smooth.

Remarks. Known from Cascadia Abyssal Plain. Strong reverse counter-shading apparent. The only other putative species of *Benthoctopus* known to have 6 gill lamellae per demibranch is *B. profundorum* Robson but this has arms 3–4 times ML and can therefore be discounted.

***Benthoctopus* sp. B (embryo) FMNH 309724**

Material examined: Benthoctopus sp.: *FMNH 309724, RV *Western Flyer* ROV *Tiburon*, 42°45'N 126°43'W (near Sea Cliff Hydrothermal vent field Gorda Ridge), ROV *Tiburon Lowering* 884, 23 August 2005, 2752 m, embryo, leg. R. A. Zierenberg and D. Clague, det. JR Voight.

Remarks. A diagnosis of this species is not possible at this time; the only known specimen is an embryo with a mantle length of 7.5 mm. The hectocotylus carries a differentiated ligula and 47 suckers (Voight, 2008). Outer gill with 11 lamellae. Funnel organ W-shaped. The skin has a few, scattered chromatophore organs. Whether the high gill lamellae count relates to the age of the specimen, or to its species membership is debatable. The embryo was collected from what appears to be an established area in which females brood eggs near the Sea Cliff, or GR-14 hydrothermal vent site. The aggregation has been seen twice since 2002; the high density of octopodids in this area was noted by Rona *et al.* (1990). Brooding by these octopodids has been suggested to occur within hydrothermal fluid flow which is unusually low in vent toxins (R. Zierenberg, pers. comm.). Unfortunately, to our knowledge, no mature specimen has been collected. Measurements of the embryo are reported by Voight (2008).

Although this collection was made at a hydrothermal vent, the species cannot be held to be vent endemic for two reasons. First, the brooding females in situ appear to have normal skin with normal pigmentation; species we consider here to be more closely associated with vents, *V. hydrothermalis* and the Manus Vent specimen (Figure 1), generally have translucent skin that lacks colour. Second, outside of a small area in which they brood eggs, octopodids are not often encountered at vents on Gorda Ridge. On the East Pacific Rise, individuals of *V. hydrothermalis* are encountered across broad areas of hydrothermally active mid-ocean ridge from at least 21°S to 13°N. Too few data are available from Manus Basin to allow comparisons. At Gorda Ridge, geologists speculate that brooding females congregate at this site as the warm hydrothermal fluid, or perhaps the warm rocks to which the eggs are attached, speed embryonic development.

***Benthoctopus* cf. *profundorum* Robson, 1932**

Material examined: Benthoctopus cf. *profundorum:* *FMNH 307179, RV *Atlantis* HOV *Alvin*, 11°25'N 103°47'W (East Pacific Rise), Dive 3934, 15 November 2003, 2492 m, female, leg. & det. JR Voight.

Diagnosis. Arms 3–4 times ML. Arm formula 1>2>3=4. Funnel organ W-shaped. Gills 6 lamellae per demibranch. Male characters unknown. Integument smooth.

Remarks. This species shows some affinity with *B. profundorum* as described by Robson in his 1932 monograph. It has a similar W-shaped funnel organ, the same number of gill lamellae per demibranch (6–7 for the type of *profundorum*) and similarly sized suckers (SDI 5.9 reported for the type of *profundorum* versus 5.8 in our specimen). The arms appear to be very slightly shorter (83% of the total length in *B. profundorum* versus 78% in our specimen) and the web is slightly shallower (WDI 22.6% versus 34% in *profundorum*). Despite the similarities, we hesitate to place our specimen firmly in *profundorum* given the very great distance between the catch location and the type locality (34°37'N 140°32'E) of *profundorum*. Although other specimens have previously been attributed to *profundorum*, these need to be treated with caution. It is doubtful that any specimens other than the type can be referred to this species (Voss & Pearcy 1990).

***Benthoctopus oregonensis* Voss & Pearcy, 1990**

Type material examined: Benthoctopus oregonensis Voss & Pearcy, 1990 holotype, USNM 729991, RV *Yaquina*, 44°37'N 125°01'W (off Oregon Coast), Stn 6606-123, 6 August 1966, 1260 m, male.

Additional material examined: Benthoctopus oregonensis: *FMNH 278314, RV *Wecoma*, 44°46'N 125°31'W (off Oregon coast), Stn 15, 21 April 1997, 2750 m, female, leg. & det. JR Voight.

Diagnosis. Arms approximately 3–4 times ML. Arm formula usually 1>2>3>4. Funnel organ W-shaped. Gills with 11 lamellae per outer demibranch. LLI approximately 6 (holotype). Maximum spermatophore length 70, maximum SpLI 80, maximum number of spermatophores 27. Mature males without enlarged suckers. Integument smooth.

Remarks. Medial element of the funnel organ narrowly incised.

***Benthoctopus johnsonianus* Allcock, Strugnell, Ruggeiro & Collins, 2006**

Synonymy: Benthoctopus johnsoniana Allcock, Strugnell, Ruggeiro & Collins, 2006: 379–386, figs 6–9.

Type material examined: Benthoctopus johnsoniana Allcock, Strugnell, Ruggeiro & Collins, 2006 holotype: BMNH 20060178, RV *Discovery*, Stn 50518, 49°27'N 13°21'W, 7 June 1979, 2045–2110 m, male.

Additional material examined: Benthoctopus johnsonianus: *RV *Discovery*, 49°44'N 13°10'W (NE Atlantic), Stn 14309, 16 March 2002, 2011–2218 m, female, leg. & det. MA Collins. 8 additional specimens detailed in Allcock *et al.*, 2006: BMNH 20060179-82, NMSZ 1999158.300, NMSZ 1994128.46501/.46503/.46506.

Diagnosis. Suckers closely set. Arms approximately 3.5 times ML. Arm formula 2>1>3>4. Funnel organ W-shaped. Gills with 8–11 lamellae per demibranch. LLI 10–11. Maximum spermatophore length 104, maximum SpLI 92, maximum number of spermatophores 19. Mature males without enlarged suckers. Integument smooth.

Remarks. The ending of the specific name has been corrected herein to agree with the male gender of *Benthoctopus*.

Benthoctopus eureka **(Robson, 1929)**

Type material examined: Enteroctopus eureka Robson, 1929 syntype NMSZ 1921143.558a, RV *Scotia*, 51°49'S 57°51'W (Falkland Islands), Stn 118, 6 February 1904, shore, male, leg. & det. WS Bruce; syntype NMSZ 1921143.558b, RV *Scotia*, 51°45'S 57°56'W (Falkland Islands), February 1904, female, leg. & det. WS Bruce.

Additional material examined: *SBMNH 423134, RV Falkland Islands Protector, Falkland Islands, Stn 366, 7 October 1992, 230 m, female, leg. FI Fisheries Department, det. AL Allcock. NMGWZ 78.14.364, RV Scotia, Port Stanley, Falkland Islands, Stn 118, 18 January 1903. USNM 817395, 53°S 70°30'W (Punta Arenas, Chile), 22 January 1968.

Diagnosis. Arms approximately 3.7 times ML. Arm formula 1=2=3=4. Funnel organ W-shaped. Gills with 8–10 lamellae per demibranch. LLI 8–10. Maximum spermatophore length 60 mm, maximum SpLI 86, maximum number of spermatophores 115. Mature males without enlarged suckers. Integument smooth.

Benthoctopus rigbyae **Vecchione, Allcock, Piatkowski, Strugnell, 2009**

Type material examined: Benthoctopus rigbyae Vecchione, Allcock, Piatkowski, Strugnell, 2009: 18 holotype USNM 1117765, *RV* Polarstern, 61° 14'S 56° 25.8' W (Antarctic Peninsula), Stn 42/022, 21 November 1996, 394-412 m, male. *Octopus levis* Hoyle, 1885: 220 holotype BMNH 1889.4.24.43, *RV* Challenger, 52°59'S 73°33'E (Heard Island), Stn 151, 7 February 1874, 75 fathoms, male.

Additional material examined: Benthoctopus rigbyae: *NMSZ 2002037.032, RV *Polarstern*, 61°10'S 54°34'W (Antarctic Peninsula), Stn 61/048-1, 16 March 2002, 343 m, male, leg. & det. AL Allcock. 10 additional specimens from the Antarctic Peninsula detailed in Vecchione *et al.* (2009) preserved under catalogue numbers NMSZ 2002037.030-031 and NMSZ 2002037.033-034.

Diagnosis. Arms approximately 3–4 times ML. Arm formula variable. Funnel organ W-shaped. Gills with 5–8 lamellae per inner demibranch and 6–8 lamellae per outer demibranch. LLI 6–16 (mean 10). Maximum spermatophore length 102, maximum SpLI 110, maximum number of spermatophores 28. Mature males without enlarged suckers. Integument smooth.

Remarks. This species differs from *Benthoctopus levis* by the funnel organ (VV in *B. levis*) and arm length (shorter in *B. levis*).

Manus Vent octopus

Material examined: FMNH 310455, 3° 47' 16" S 152° 5' 41" E Papua New Guinea Manus Basin Solwara I project: Ad:LB Dive 15, 4 April 2007, 1517 m, male, leg. P Collins, det. JR Voight

Remarks. The single specimen of this species is a mature male in poor condition. The mantle is separate from the viscera but estimated mantle length is approximately 29 mm. Ink sac and anal flaps absent. Funnel organ large VV-shaped. The suckers are damaged: parts of the arms lack suckers, in other places only a single series remains, but close to the buccal mass the suckers are biserial and well-spaced; we assume this to be their true arrangement. The oesophageal dilation is well formed. The posterior salivary glands are fairly large with prominent tubules. The internal reproductive organs are missing and the gills are in poor condition. The hectocotylised arm is shorter than its counterpart (OAI approximately 63). The ligula is simple without transverse ridges and has a well-defined central groove. LLI is approximately 7.5. The calamus is well defined and unusually long and strong; however, the aboral surface of the ligula lacks skin, and the base of the calamus is 5 mm from the most distal sucker. This damage, likely due to a suction sampler, precludes any definitive statements concerning unusual morphology. The radula has a classic multicuspid rachidian with asymetric seriation. The full set of lateral teeth and marginal plates are present. These characters lead us to suggest that the species is best referred to the genus *Benthoctopus*. The skin carries chromatophore organs, in contrast to the skin of *V. hydrothermalis*, but in situ the animal appears to be nearly colourless (Figure 1). Other octopodids that appear to be overwhelmed by sulphides at cold seeps are known to dilate their chromatophore organs, to produce vivid colour (C. Fisher, pers. comm. to JRV). The specimen was taken from an active site, although not directly from the hot fluids. We presume that the species may feed on the large numbers of squat lobsters (mostly *Munidopsis lauensis* but a few *Munida magniantennulata* and *Munidopsis starmer*), and shrimp (*Chorocaris, Alvinocaris, Lebbeus, Opaepele*) observed around the vents. Other fauna sampled at the site included *Alviniconcha* and *Ifremeria* snails as well as *Eochionelasmus* barnacles, all of which house symbionts. Five other octopodid individuals, presumed by observers to be the same species, were photographed in the region. The locations and depths at which these individuals were observed are 3° 43' 40" S 151° 40' 52"E, 1708 m (2 individuals), 3° 47' 26" S 152° 5' 30"E, 1530 m, 3° 47' 24" S 152° 5' 46"E, 1485 m and 3° 47' 26" S 152° 5' 28"E, 1562 m. A series of photographs is held by Nautilus Minerals.

Vulcanoctopus **González & Guerra, 1998**

Vulcanoctopus hydrothermalis **González & Guerra, 1998**

Type material examined: Vulcanoctopus hydrothermalis González & Guerra, 1998 in González, Guerra, Pascual & Briand, holotype, MNHN 2885, HOT 96 East Pacific Rise, North of Genesis, 12°48'N 103°56'W, 19 February 1996, 2647 m, male.

Additional material examined: Vulcanoctopus hydrothermalis: FMNH 307183, RV Atlantis HOV *Alvin*, 10°46'N 103°39'W (East Pacific Rise), *Alvin* Dive 3936, 17 November 2003, 2574 m, male, coll & det JR Voight. *FMNH 307184, RV *Atlantis* HOV *Alvin*, 08°38'N 104°13'W (East Pacific Rise), *Alvin* Dive 3926, 7 November 2003, 2541 m, male, coll & det JR Voight. FMNH 278064, RV *Atlantis* HOV *Alvin*, 9°50.333'N 104°17.482'W (East Pacific Rise), Dive 2846, 24 Oct. 1994, 2512 m, male, coll R. Lutz, det JR Voight. FMNH 287365, RV *Atlantis* HOV *Alvin*, 9°49.136'N 104°17.332'W (East Pacific Rise), Dive 3217, 17 May 1998, 2495 m, male, coll C. Fisher, det JR Voight. FMNH 287366, RV *Atlantis* HOV *Alvin*, 9°50.624'N 104°17.48'W (East Pacific Rise), Dive 3212, 13 May 1998, 2509 m, male, coll C. Fisher, det JR Voight. FMNH 307185, RV *Atlantis* HOV *Alvin*, 12°49'N 103°56'W (East Pacific Rise), Dive 3938, 19 November 2003, 2619 m, male, coll & det JR Voight. FMNH 306500, RV *Atlantis* HOV *Alvin*, 12°48.673'N 103°56.408'W (East Pacific Rise), Dive 3939, 20 November 2003, 2620 m, male, coll A. Berry & J. Voight, det JR Voight.

Diagnosis. Incirrate octopod. Biserial Suckers. Ink sac and anal flaps absent. Arms 2–4.5 times ML. Arm formula 1>2>3>4; arm length somewhat variable among 8 specimens with seemingly intact (or fully regenerated arms): arm I at least 10 mm longer than II in 5, subequal in 3; arms II at least 10 mm longer than III in 4 of 8; arms III and IV subequal in 5 of 8. Arm I with up to 166 suckers, other arms with fewer; hectocotylus carries between 58 and 83 suckers. Funnel organ W-shaped, lateral limbs shorter than medial limbs. Gills with 8 to 10 lamellae per outer demibranch. Ligula of males without strong transverse ridges LLI 8–10. Digestive gland multilobed. Maximum spermatophore length 62, maximum SpLI 125, maximum number of spermatophores 114. Mature males without enlarged suckers. Rachidian multicuspid. Integument of holotype shows evidence of some sculpture in the form of widely spaced small papillae on the dorsal and ventral mantle, but most conspicuous are the low wrinkles that cover the animal, including the oral web. These are present in both fresh and preserved specimens, not as the description stated “wrinkled in preservation” (González *et al.* 1998 p. 173).

Remarks. A female of this species was recently described (González *et al.* 2008) as lacking the dark swelling described in the intestine of males (González *et al.* 1998). Neither has the dark swelling been seen in 13 additional specimens, 8 from the type locality, in Field Museum collections. The condition of the optic chiasma is nearly unexplored in other deep-sea octopodids. Information on spermatophores given in diagnoses was extracted from González *et al.* (2002), which details variation in male specimens from the type locality. The holotype has been heavily dissected; the reproductive tract, for example, is in eight parts. Only 69 spermatophores remain in the holotype; this is similar to the next highest counts we found in 30 male specimens (70, 72, 74). The funnel organ shape is herein described for the first time based on specimens from the type locality.

Results

New nucleotide sequences generated in this study were deposited in GenBank under Accession no. FJ603515–FJ603553. Alignment of COIII and cyt b required no insertion/deletion events (indels). Indels were introduced into aligned sequences of 12S rDNA, 16S rDNA and the 3’ end of *rhodopsin*. Highly variable loop regions within 12S rDNA (44 in total) and 16S rDNA (83 in total) that were unalignable were removed prior to analysis.

Of the five genes used in the analysis, COIII was the most variable (35.9% variable characters) and *rhodopsin* was the least variable (16.3% variable characters) (Table 2).

TABLE 2. Sequence variability of the genes used in this study. *data corresponds to alignment after unalignable portions have been removed.

Gene	Sequence length	Number of variable characters	Percentage of variable characters
12S rDNA*	376	89	23.7%
16S rDNA*	476	88	18.5%
COIII	529	190	35.9%
cyt b	390	130	33.3%
rhodopsin	621	101	16.3%

Results of the partition homogeneity test (p = 0.212) indicated that there was no significant conflict between the partitions. Therefore the mitochondrial and nuclear genes were concatenated into a single dataset for analysis.

Unfortunately only the gene 12S rDNA could be sequenced for the Manus Vent octopus. Therefore the 12S rDNA sequences of 15 taxa were also analysed as a single dataset.

Concatenated dataset

The phylogenetic tree resulting from the analysis of all five genes concatenated is rooted using *Octopus vulgaris* (Figure 2) as previous studies have shown *Octopus* to be a suitable outgroup to *Benthoctopus* (Strugnell *et al.* 2004; 2005; Allcock *et al.* 2006).

Enteroctopus is the sister taxa to a clade containing *Benthoctopus* and *V. hydrothermalis. V. hydrothermalis* falls within the clade of *Benthoctopus* species (bootstrap [BS] = 100, posterior probability [PP] = 100) .

Benthoctopus normani is the sister taxa to a clade (BS = 74, PP = 100) containing the remaining *Benthoctopus* species and *V. hydrothermalis*; within this clade, *B. yaquinae* FMNH 308673 is basal to the remaining *Benthoctopus* species and *V. hydrothermalis* (Figure 2).

Benthoctopus sp. A and *B.* cf. *profundorum* are sister taxa (BS = 81, PP = 99) (Figure 2); together these species form a monophyletic group with *Benthoctopus* sp. B (embryo) FMNH 309724 (BS = 74, PP = 83).

Benthoctopus eureka and *Benthoctopus rigbyae* are sister taxa in a highly supported relationship (BS = 95, PP= 100).

Uncorrected pairwise distances of the concatenated dataset varied from 2.1–7.4% between *Benthoctopus* species and *V. hydrothermalis*.

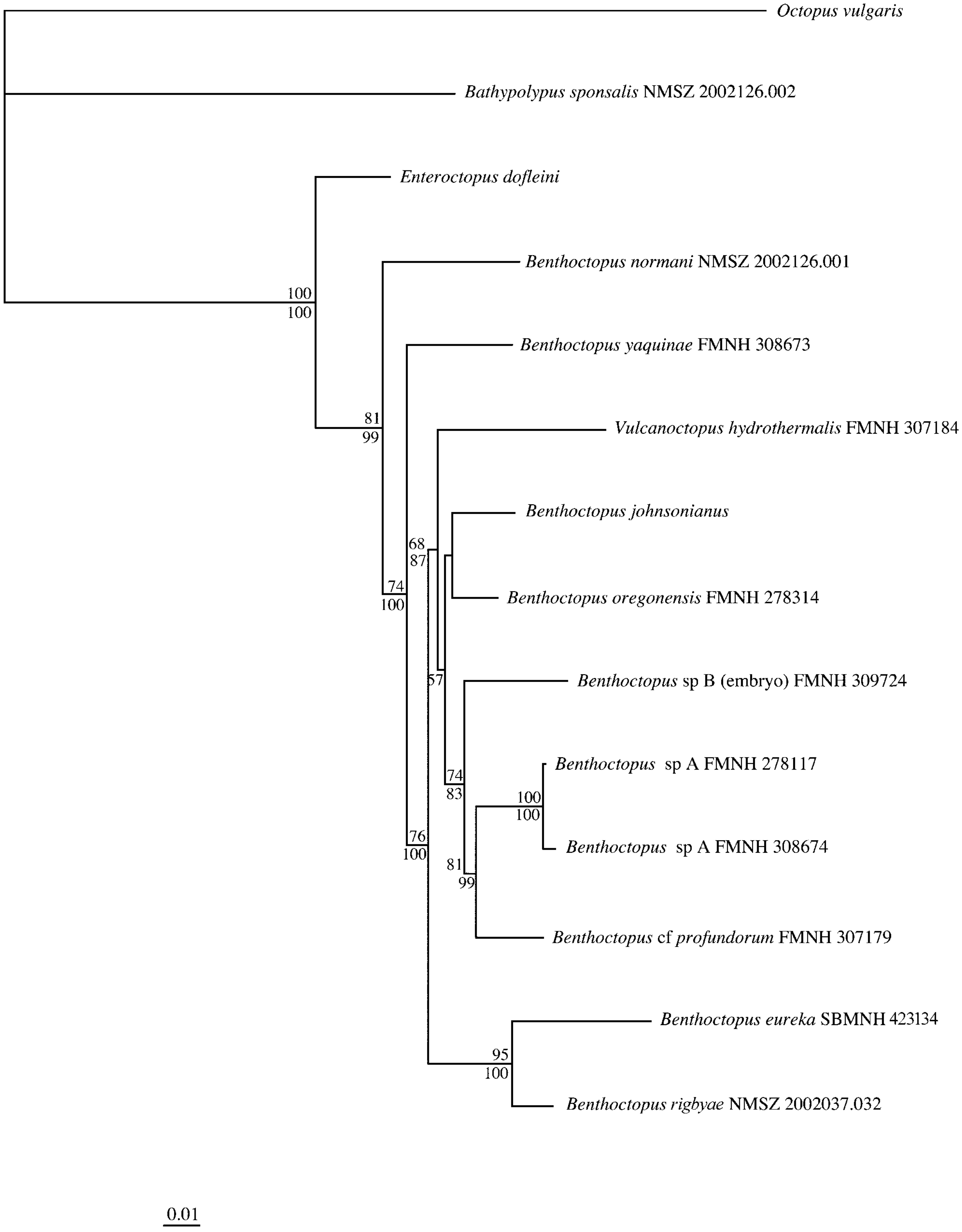

FIGURE 2. Maximum likelihood tree depicting the phylogenetic relationship of 13 species (14 individuals) of Octopodidae. The analysis employed a portion of four mitochondrial genes (12S rDNA, 16S rDNA, COIII, cyt b) and a portion of the nuclear gene *rhodopsin*. Bayesian posterior probability support values are indicated below the nodes and maximum likelihood bootstrap values with 50% support or greater are indicated above the nodes.

12S rDNA dataset

The 12S rDNA sequence of the Manus Vent octopus differs from *V. hydrothermalis* and each of the other *Benthoctopus* individuals sequenced. Uncorrected pairwise distances show the Manus Basin octopus 12S rDNA sequence to differ from each of the other *Benthoctopus* sequences by 1.8–3.1%.

The Manus Basin octopus 12S rDNA sequence differs from *V. hydrothermalis* by 3.1%, with 17 nucleotide differences evident between the two species. Furthermore the sequence of the Manus Basin octopus possesses six more base pairs in the loop regions of the 12S rDNA fragment than does *V. hydrothermalis* and thus indels were required to align them.

Little structure is evident in the phylogenetic tree constructed using 12S rDNA only (Figure 3). The Manus Vent octopus is contained within a clade formed of the *Benthoctopus* species and *V. hydrothermalis*, however its position within this clade is not supported. A sister taxa relationship is supported between the two *Benthoctopus* sp. A individuals (PP = 98, BS = 85) (Figure 3).

A clade containing *B.* cf. *profundorum*, *Benthoctopus* sp. A, *Benthoctopus* sp. B and *B. eureka* received low but significant posterior probability support (PP = 58) (Figure 3).

Discussion

The type species of *Benthoctopus* is *Octopus piscatorum* Verrill, 1879. Voss, in his paper with Pearcy describing several species of North Pacific *Benthoctopus* (Voss & Pearcy 1990), first noted that the type specimen of *Octopus piscatorum* is probably synonymous with a species of *Bathypolypus*. This fact was confirmed by Muus (2002) and subsequent authors. Although this observation renders the genus invalid, Voss suggested that the name *Benthoctopus* be conserved to maintain nomenclatural stability in this group. Most authors have concurred and, whilst no submission has been made to the ICZN, the name *Benthoctopus* remains in common usage. However, Gleadall (2004) erected a new genus *Muusoctopus* into which he placed *Benthoctopus januarii* (Hoyle, 1885). He did not include any other species, stating that it was not clear which other species of *Benthoctopus* had sufficient affinities to be included. Although there have been several diagnoses of the genus, much conjecture also exists as to whether the genus is monophyletic. Most authors suspect not, yet suitable morphological analyses are not undertaken because of a paucity of specimens. Because we have not yet obtained suitable tissue samples of *januarii*, and therefore lack molecular evidence to identify which, if any, of our specimens are particularly closely aligned with the type species of *Muusoctopus*, we continue to use the genus name *Benthoctopus*, rather than introduce further complications and synonymies.

The *Benthoctopus* species considered here group into a well-supported clade (which includes *Vulcanoctopus hydrothermalis*) to which *Enteroctopus* and *Bathypolypus* are basal. This, at least for the species considered here, refutes the suggestion that *Benthoctopus* is a ‘hold-all’ genus for species sharing adaptations to the deep sea, and pleisiomorphic characters that are unable to reveal common ancestry (e.g. Gleadall 2004; Norman *et al.* 1997). The level of sequence divergence between species within this clade for all genes concatenated, ranges from between 2.1 and 7.4%. This is slightly greater than is seen within the octopodid genus *Pareledone* (1–2%; Allcock *et al.* 2007) and comparable with *Thaumeledone* (2–3.3%; Strugnell *et al.* 2008). Furthermore this is consistent with levels of divergence seen in taxa such as moths and birds (Moore 1995; Hebert *et al.* 2004).

The placement of the hydrothermal vent species *V. hydrothermalis* within the clade containing other species of *Benthoctopus* is strongly supported by both maximum likelihood and Bayesian analyses. Although *V. hydrothermalis* is known to possess many of the characteristics traditionally used to define *Benthoctopus*, González *et al.* (1998) suggested that it might warrant its own subfamily. However, in their recent description of the female of this species, González *et al.* (2008) suggest that *Benthoctopus* with its ‘smooth skin, moderate size ligula and lack of laminae’ might be the most likely ancestor of this species. This statement implies that they believe the integument of *Vulcanoctopus* to be smooth also. Allcock (this study), on examination of the

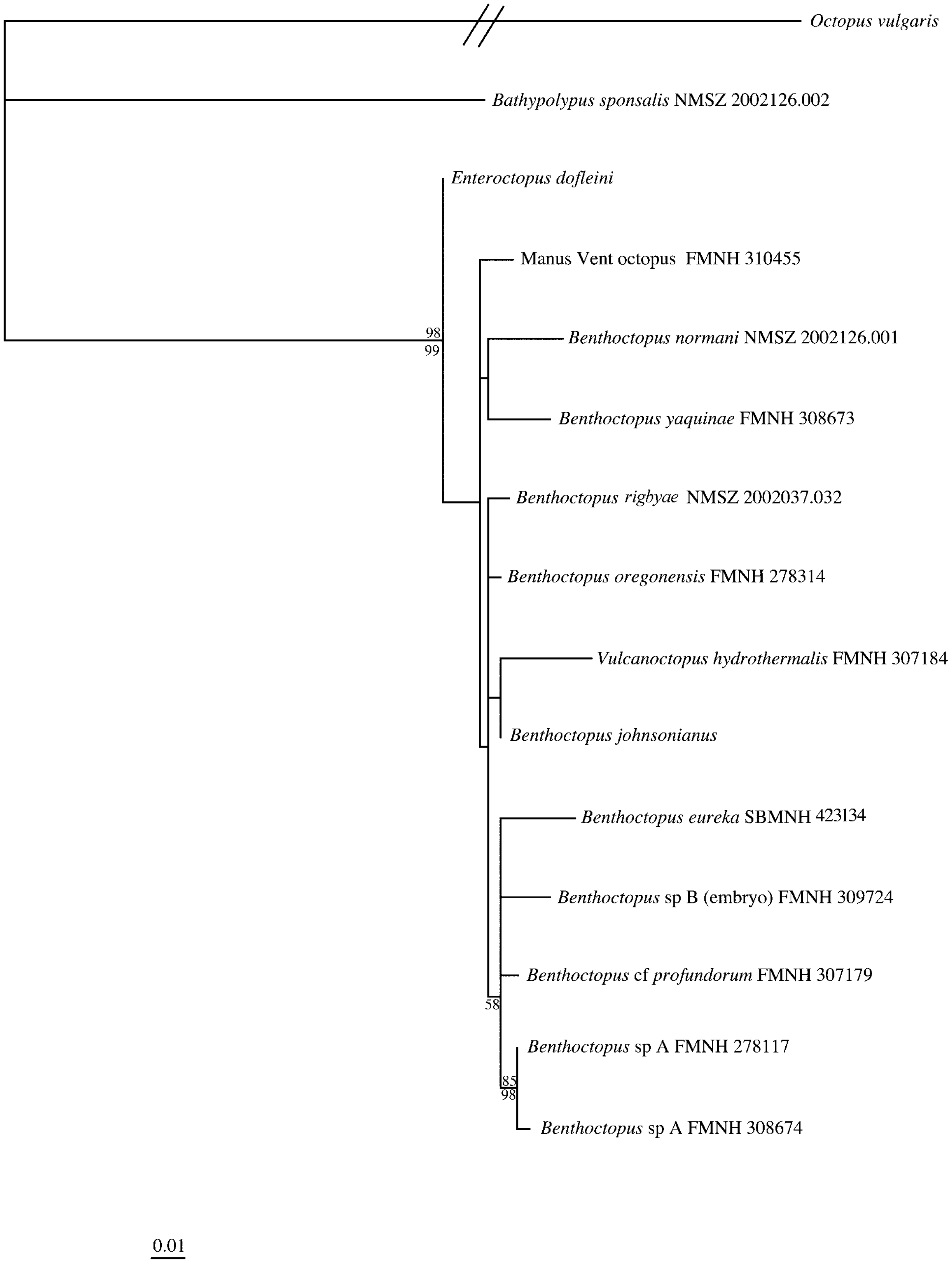

FIGURE 3. Maximum likelihood tree depicting the phylogenetic relationship of 14 species (15 individuals) of Octopodidae. The analysis employed a portion of the mitochondrial gene, 12S rDNA. Bayesian posterior probability support values are indicated below the nodes and maximum likelihood bootstrap values with 50% support or greater are indicated above the nodes.

holotype, felt there was evidence of a few very widely spaced papillae on the mantle surface. These may also be apparent in Figures 3, 4 and 9 (photographs of the intact animal) of the original description, although it is possible that these are artefacts of preservation. Interestingly, no mention of these papillae has been made during behavioural observations (Rocha *et al*. 2002; Voight 2005), nor do they appear to be present on specimens deposited at FMNH; their status therefore remains unclear. At least one species of *Benthoctopus*, *B. karubar* Norman *et al*., 1997 has been reported to have 'dorsal head and some of mantle scattered with small low rounded papillae' (Norman *et al*. 1997) but we have not yet obtained tissue samples from this species therefore cannot include it in our study. In addition, although noting that its warty skin was unusual for the genus *Benthoctopus*, Muus (2002) moved the species *salebrosus* from *Bathypolypus* to *Benthoctopus*. However, a new genus is being erected for *salebrosus* (E. Jorgensen pers. comm.). Robson (1930) notes the presence of wart-like features in *B. magellanicus* Robson, although he also suggests that in the male, at least, these might just be the product of wrinkling. Norman and Hochberg consider *B. magellanicus* to be synonymus with *B. eureka* and our studies of this species suggest that wrinkling is the correct interpretation.

Our investigations found no other morphological characters that would warrant the distinction of *Vulcanoctopus* from species of *Benthoctopus* studied herein, although the heavy dissection of the holotype of *Vulcanoctopus* complicated study of many characters. The *Vulcanoctopus* holotype had a high number of spermatophores (64) compared with most species we examined herein, but was similar to that of *B. eureka*. Maximum spermatophore count ranged between 19 and 27 in *johnsonianus*, *rigbyae*, *oregonensis* and *normani*, but was considerably lower in *yaquinae* (4). The grouping of these species (Figure 2) in the phylogenetic tree produced using multiple gene sequences suggests that this character is not phylogenetically informative.

Many characters of *V. hydrothermalis* that led to the erection of this genus, and suggestion of a new subfamily, are likely adaptations to the deep sea and/or its hydrothermal vent habitat. These include an eye with no trace of an iris and the absence of optic chiasma (González *et al.* 1998). The other characters unique to *V. hydrothermalis* include a multilobate digestive gland and the report of a dark swelling in the intestine. Although the function of the former feature is not clear, and the second feature appears to be more often absent than present, these may be adaptations to an existence associated with hydrothermal vents which emit fluids with high concentrations of metal sulphides and other toxins.

Our data provide evidence that the species *hydrothermalis* should be placed in the genus *Benthoctopus*, but the invalidity of this latter genus leaves us unable to propose nomenclatural changes at this time.

Characters that are common to all species falling within our *Benthoctopus* clade include radula with multicuspid rachidian tooth, ligula without laminae, no ink sac or anal flaps and biserial suckers. With the exception of absence of anal flaps (which appear always to be absent when the ink sac is absent) all these characters have been previously used to define *Benthoctopus* (Robson 1924; Voss 1988a, b). Other characters that we have examined but which are clearly not applicable include 'hectocotylus *Octopus*-like' and 'normal *Octopus*-like appearance' (Voss 1988a, b). Species within *Octopus sensu strictu* (as defined by Norman and Hochberg 2005) have a much smaller ligula, perhaps due to its unique construction (Thompson & Voight 2003), although the shape is not dissimilar, and they have patch and groove patterning on their integument and multiple enlarged papillae, including superocular papillae. Robson (1924) stated that the hectocotylus was 'small'. Presumably he was referring to the ligula rather than the hectocotylised arm, however, the term 'small' is not particularly informative. Based on the diagnoses of the species in our *Benthoctopus* clade we suggest that the ligula of *Benthoctopus* is defined by an LLI of between 6 and 16.

The species within our *Benthoctopus* clade also appear to share the possession of long spermatophores (SpLI > 80), however, information on males is not available for all species. Many species also appear to have an unusually large number of spermatophores considering their size, but *Benthoctopus yaquinae* has only 4 so this character is not uniform across the clade (and data are also not available for some species). Voss (1988a) reported that few, large spermatophores characterized deep-sea octopods. Other characters that are inconsistent across the clade include the funnel organ, which is W-shaped in most species but VV-shaped in *B. normani*, the most basal species of the *Benthoctopus* clade and in the Manus Vent specimen, and gill lamellae count, which varies from 6–11, and to a certain extent refutes the hypothesis of uniformly reduced gill lamellae in deep-sea species (see also Voss, 1988a). None of these characters appear to be phylogenetically informative at this level. A summary of the characters that vary across the clade is provided in Table 3. These characters were used to construct the diagnoses but they are not sufficient to distinguish all species from one another. They successfully separate species that occur within a given ocean basin, hence they are useful diagnostic features. However, as the number of species known and the number of specimens within a given species increase to allow character variation to be better understood, additional diagnostic features will be required.

TABLE 3. Phylogenetic characters which vary across the clade of interest.

	Ocean basin	Arms X ML	Arm formula	Funnel organ	Gills per outer demibranch	LLI	Max sp. L	Max SpLI	Max No Sp
B. sp. A	North Pacific	1.5-2.5	1≥2≥3≥4	w	6^+	-	-	-	-
B. cf. *profundorum*	North Pacific	3-4	1>2>3=4	w	6	-	-	-	-
B. yaquinae	North Pacific	3	1>2>3>4	w	7-9	8-12	110	136	4
V. hydrothermalis	North Pacific	2-4.5	1>2>3>4	w	7-8	8-10	62	125	114
B. oregonensis	North Pacific	3-4	1>2>3>4	w	11	6	70	80	27
Manus Vent *Benthoctopus*	South Pacific	-	-	vv	-	7.5	-	-	-
B. johnsonianus	North Atlantic	3.5	2>1>3>4	w	8-11	10-11	104	92	19
B. rigbyae	Southern Ocean	3-4	variable	w	6-8	6-16	102	110	28
B. normani	North Atlantic	4	1>2>3>4	vv	7-10	6-11	119	121	24
B. eureka	South Atlantic	3.7	1=2=3=4	w	8-10	8-10	60	86	115

One character that is consistent across the clade is that mature males lack enlarged suckers. We have not included this in the generic diagnosis of *Benthoctopus* at this stage because exceptions may become known. Currently, females of *B. canthylus* are known to have enlarged suckers on extraordinary flared arms, but males of the species remain unknown. To our knowledge, only two putative species of *Benthoctopus* (as defined by Norman and Hochberg 2005) have enlarged suckers in the male. These are *B. abruptus* and *B. sibiricus*. Allcock has studied the holotypes of both specimens, which appear to have much thinner ligulae than the *Benthoctopus* species included in our molecular investigations. These species may indeed not pertain to this clade. This requires further investigation and is dependent on the acquisition of additional specimens from the type localities of *B. abruptus* and *B. sibiricus*.

Unfortunately difficulties encountered in amplifying and sequencing the targeted genes for the Manus Vent octopus meant it could not be included in the analysis using all five genes concatenated. 12S rDNA was the only gene that we were able to sequence for this species and this was possible only after a nested approach. Initial fixation of the individual in formalin before a tissue sample was taken for DNA analysis likely created these issues.

Although many relationships are unsupported in the phylogenetic tree constructed using only 12S rDNA sequences, the *Benthoctopus* species collected from the hydrothermal vent in the Manus Basin is clearly distinct from all others considered. The 12S rDNA sequence obtained from this individual was unique, differing between 1.8–3.1% from the other *Benthoctopus* species and from *V. hydrothermalis*. As new and more effective methods become available for successfully extracting DNA from formalin-fixed tissues it is likely that more genes could be extracted from this individual; this would greatly aid in our understanding of the evolutionary history of this species. We know, at least, that *V. hydrothermalis* is no longer the only vent associated octopodid species and that these vent species belong in a clade comprising species currently placed in *Benthoctopus*. When we are able to include additional taxa in these analyses, and when additional specimens facilitate full descriptions of taxa that are currently known only from one or two specimens, natural subdivisions within this clade are likely to emerge. None, however, are currently apparent.

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Supplementary information

TABLE 1. Substitution model parameters for all five genes concatenated and for 12S rDNA.

Substitution model parameters	All genes concatenated	12S rDNA
A	0.31479	0.42533
C	0.15928	0.16784
G	0.15176	0.07187
T	0.37417	0.33496
A↔C	0.882450	3.1517e-10
A↔G	7.915280	7.1700e+07
A↔T	2.203430	7.6155e+07
C↔G	1.435150	9.7700e-05
C↔T	11.541400	6.3574e+08
G↔T	1	1
I	0.48493	0.229935
Γ	0.49074	0.295725

I = assumed proportion of invariable sites, Γ = shape parameter (alpha)