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Source: Zoological Science, 38(3) : 223-230

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zs200153>

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Distribution and Genetic Divergence of Deep-sea Hydrothermal Vent Copepods (Dirivultidae: Siphonostomatoida: Copepoda) in the Northwestern Pacific

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Copepods in the family Dirivultidae are one of the most successful meiofauna in deep-sea hydrothermal vent fields and are abundant near venting fluid. Although vents are spatially limited ocean habitats, they are distributed widely in the Atlantic, Pacific, and Indian Oceans. However, knowledge of dirivultid biogeography and phylogeography remains limited, especially in the northwestern Pacific. Here, we obtained partial mitochondrial COI gene sequences of three dirivultids from the northwestern Pacific—*Stygiopontius senokuchiae* and an unidentified *Chasmatoptentius* species from vent fields in the Izu–Bonin Arc and *Stygiopontius senckenbergi* associated with the squat lobster *Shinkaia crosnieri* in the Okinawa Trough—and analyzed them in comparison with existing data. The among-species sequence diversity exceeded 80 out of 560 bp (14% or 0.166 in Kimura 2-parameter distance), whereas the within-species diversity was less than 10 bp (2% or 0.018 in Kimura 2-parameter distance), with no genetic saturation. Each species formed a monophyletic clade and the genetic region targeted is deemed reliable for identifying species and populations for these copepods. Among the three genera targeted, only *Chasmatoptentius* formed a monophyletic cluster, while *Aphotopontius* and *Stygiopontius* did not. Species delimitation analyses suggested the existence of cryptic species in *Chasmatoptentius*. Subdivision among local populations was observed in *Aphotopontius*, but not in *Stygiopontius* in the same distribution, implying potential differences in dispersal ability among different genera of dirivultids. Further sampling is required, to fill the spatial gaps to elucidate the biogeography and evolution of dirivultids in the global deep ocean.

Key words: chemosynthesis-based community, DNA barcoding, Izu–Bonin–Mariana Arc, mitochondrial COI, Okinawa Trough

INTRODUCTION

Copepods are abundant animals in marine ecosystems, both as plankton and benthos. Their community dynamics are thought to be correlated with environmental variables, even in remote environments such as the deep-sea floor, and they have been used to predict how the ecosystem might shift with climate change (Zeppilli et al., 2015). However, little is known about how much the ecology of different species varies, particularly those inhabiting the deep ocean, which

comprises over 90% of the marine ecosystem in volume.

Hydrothermal vent fields are island-like habitats distributed along seafloor spreading centers. The mixing zone between hot venting fluid and cold ambient water hosts a high animal biomass supported by chemosynthetic primary production by microbes, forming chemosynthesis-based biological communities (Van Dover, 2000). Vent communities mostly comprise species specific to this environment, and the biomass can reach 40 kg/m² (Tunnicliffe et al., 1998; Podowski et al., 2009). Since their discovery in the 1970s the ecology of vent animals has been a topic of intensive research, but most of it has targeted conspicuous, iconic macro- and megafauna such as giant tubeworms and chemosymbiotic bivalves. Much

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doi:10.2108/zs200153

less is known about meiofauna, including copepods, which have been found to account for more than 40% of the species richness in vents (Gollner et al., 2007). Recently, vent fields are targeted by deep-sea mining for massive sulfides, and environmental regulations based on scientific knowledge of vent ecosystems are urgently required (Van Dover et al., 2018). The sensitivity of copepods to fine environmental changes make them especially useful for assessing ecosystem resilience (Gieryn, 2009; Zeppilli et al., 2015). Knowledge of their ecology and biogeography is therefore important in evaluating resilience of biological assemblages in vent fields to prevent the loss of biodiversity that may be caused by upcoming deep-sea mining.

Copepods of the family Dirivultidae (Copepoda: Siphonostomatoidea) are abundant meiofauna in vent fields of the Atlantic and Pacific Oceans (Ivanenko et al., 2011). Although not all vent copepods are endemic to hydrothermal ecosystems, dirivultids are almost only known from deep-sea hydrothermal vents with three exceptions: on a *Lamellibrachia* Webb, 1969 tubeworm in a hydrocarbon seep in southern California, on a *Lamellibrachia* tubeworm off Kagoshima, Japan, and at a methane seep site in the South China Sea (Gollner et al., 2010; Ma et al., 2020; Uyeno et al., 2020). The family contains 62 species in 13 genera, of which five (*Fissuricola* Humes, 1987, *Humesipontius* Ivanenko & Ferrari, 2003, *Nilva* Humes, 1987, *Rimipontius* Humes, 1996, and *Scotoecetes* Humes, 1987) are monospecific. *Stygiopontius* Humes, 1987 is the largest genus and contains 30 species endemic to deep-sea vent fields and hydrocarbon seeps sites (Ma et al., 2020; Uyeno et al., 2020; World Register of Marine Species at <http://www.marinespecies.org/>, accessed on 8 October, 2020). Most *Stygiopontius* species were described from vent fields in the eastern Pacific (including Explorer Ridge, East Pacific Rise [EPR] and Guaymas Basin), and a few from the TAG field on the Mid-Atlantic Ridge. Some species are associated with macro- or megafauna, e.g., *Stygiopontius flexus* Humes, 1987 with siboglinid tubeworms (although also found in bacterial mats), *Stygiopontius hispidulus* Humes, 1987 with alvinellid polychaetes, *Stygiopontius lauensis* Humes, 1991 with the gastropod *Iremeria nautilei* Bouchet & Warén, 1991, and *Stygiopontius pectinatus* Humes, 1987 with the swarming vent shrimp *Rimicaris exoculata* Williams & Rona, 1986 (Humes, 1987; Gollner et al., 2016). Only three *Stygiopontius* species

have been reported from the northwestern Pacific in two regions: *S. pectinatus* and *Stygiopontius stabilitus* Humes, 1990 from the Alice Springs field in the Mariana Trough and *Stygiopontius senokuchiae* Uyeno, Watanabe & Shimanaga, 2018 from the Izu–Bonin–Mariana (IBM) Arc (Humes, 1990; Humes and Segonzac, 1998; Senokuchi et al., 2018). *Stygiopontius senokuchiae* is the most abundant meiofauna (on average they make up 79% of copepods collected) associated with active venting on the Myojin-sho Caldera and Bayonnaise Knoll on the IBM Arc (Uejima et al., 2017; Senokuchi et al., 2018; Uyeno et al., 2018). This copepod species exhibits red coloration in its body when alive, a character also known from another dirivultid copepod, *Benthoxynus spiculifer* Humes, 1984, which is red in color due to the presence of hemoglobin (Hourdez et al., 2000). *Stygiopontius senokuchiae* lives among dense assemblages of nest-building polychaetes in the genus *Paralvinella* Desbruyères & Laubier, 1982 on active chimney surfaces, receiving most of its nutrition from the bacterial mat on the chimney (Nomaki et al., 2019). *Stygiopontius* copepods are known to have a planktonic larval period and may be more widely distributed than other genera without it (Uyeno et al., 2018). Biogeography of the northwestern Pacific *Stygiopontius* species based on genetic data has been lacking, as only ecological and morphological studies have been carried out in the past.

As dirivultids exhibit both sexual dimorphism and developmental changes in morphology, genetic data helps us to correctly identify species in order to understand their diversity and ecology. The barcoding region of the mitochondrial

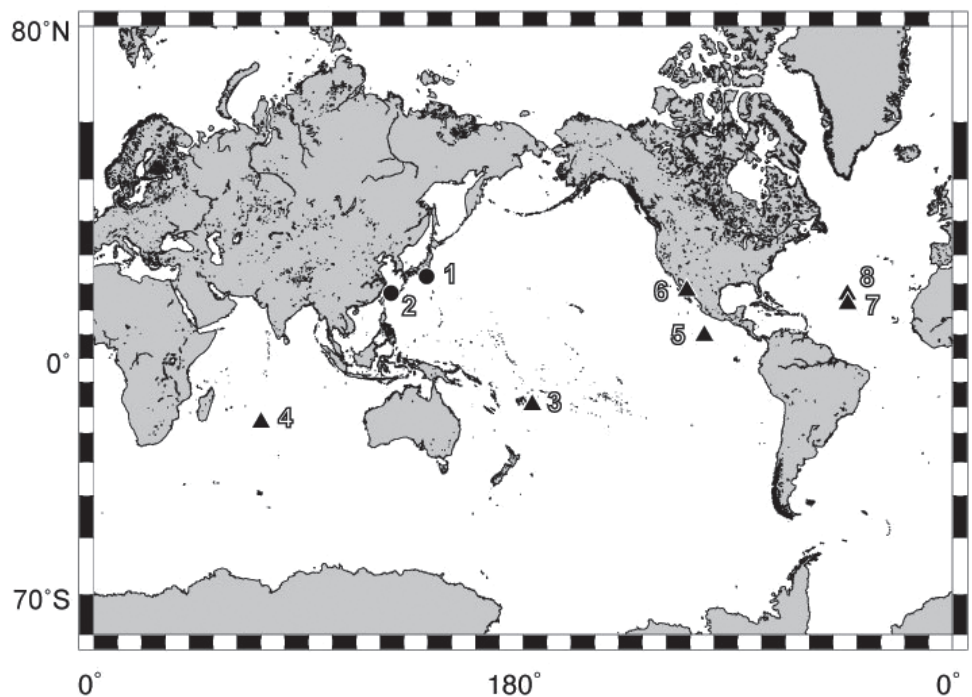


Fig. 1. Distribution of the dirivultid copepods studied. Circles, sampling sites in this study; triangles, sampling sites in other studies. 1) Myojin-sho Caldera and Bayonnaise Knoll, Izu Arc, 2) Sakai field, Okinawa Trough, 3) ABE, Kilo Moana, and Tu'i Marina fields, Lau Basin, 4) Kairei field, Central Indian Ridge, 5) Bio9, Marker 28, V-Vent, Tica and East Wall fields, East Pacific Rise, 6) Rebecca's Roost field, Guaymas Basin, 7) Snake Pit field, and 8) TAG field, Mid-Atlantic Ridge.

COI gene, the most widely used DNA barcoding region for metazoans, has been confirmed as a useful region for identifying species in copepods (Gollner et al., 2016). For example, comparisons using the COI gene enabled the differentiation of the males of *S. hispidulus* from those of *Stygiopontius quadrispinosus* Humes, 1987, which were indistinguishable morphologically (Gollner et al., 2011). Here, we obtained the partial COI sequence of vent copepods from the northwestern Pacific to examine 1) the consistency of species identification and delimitation based on morphological and molecular methods, 2) the spatial distribution of dirivultids in the western Pacific, and 3) the population subdivisions or phylogeography of these dirivultids.

MATERIALS AND METHODS

Three morphologically identified species of dirivultid copepods were collected from two vent fields on the IBM Arc and one vent field in the Okinawa Trough. *Stygiopontius senokuchiae*, the most abundant meiofauna in hydrothermal vent fields on the Myojin-sho

Caldera (791–853 m in depth) and Bayonnaise Knoll (742–778 m in depth), was collected with other animals from the chimney surface using a suction sampler on the Remotely Operated Vehicle (ROV) *Hyper-Dolphin* during dives #1518 (Myojin-sho Caldera) and #1647 (Bayonnaise Knoll) during two separate cruises (NT13-09 and NT14-06, respectively; PI: Motohiro Shimanaga) of *R/V Natsushima*, as previously reported (Fig. 1; Senokuchi et al., 2018; Nomaki et al., 2019). Specimens were preserved with 99.5% ethanol upon recovery on board. Individual copepods were picked from the bulk detrital sample under a binocular microscope in the laboratory, and three females from each sample were prepared for DNA extraction. An unidentified species in the genus *Chasmatopontius* Humes, 1990, which was the second most abundant species in the Myojin-sho Caldera (Senokuchi et al., 2018), was prepared in the same way. *Stygiopontius senckenbergi* Ivanenko & Ferrari, 2013, a very abundant dirivultid associating with the hydrothermal vent squat lobster (*Shinkaia crosnieri* Baba & Williams, 1998) in the Okinawa Trough was also collected (Uyeno et al., 2020). Many *Sh. crosnieri* were collected with a suction sampler by the ROV *Hyper-Dolphin* during dive #1860 during the cruise NT15-13 (PI: Ken Takai) in the Sakai field (1600 m deep), Okinawa Trough, and

Table 1. Between-sequence divergence of the different dirivultid species. Upper right, Kimura 2-parameter distance; lower left, mean sequence difference in 560 bp.

	<i>A. limatus</i>	<i>A. mammillatus</i>	<i>Aphotopontius</i> sp. 1	<i>Chasmatopontius</i> sp. 1	<i>S. brevispina</i>	<i>S. hispidulus</i>	<i>S. lauensis</i>
<i>A. limatus</i>		0.281	0.331	0.419	0.320	0.326	0.301
<i>A. mammillatus</i>	130.600		0.313	0.409	0.383	0.381	0.351
<i>Aphotopontius</i> sp. 1	149.300	142.083		0.369	0.312	0.288	0.321
<i>Chasmatopontius</i> sp. 1	178.233	175.069	161.375		0.336	0.326	0.339
<i>S. brevispina</i>	144.578	166.407	141.278	150.630		0.269	0.166
<i>S. hispidulus</i>	146.754	165.173	132.923	146.333	124.607		0.244
<i>S. lauensis</i>	137.756	156.204	145.139	151.370	82.407	114.709	
<i>S. pectinatus</i>	164.550	168.417	162.250	141.042	145.500	152.365	145.278
<i>S. senckenberghi</i>	169.400	170.917	159.500	185.500	168.333	170.692	164.556
<i>S. senokuchiae</i>	157.767	168.500	155.333	149.167	142.889	141.974	135.611
<i>Stygiopontius</i> sp. 1	135.733	153.833	125.833	143.000	110.889	100.051	105.778
<i>Stygiopontius</i> sp. 2	164.300	165.042	154.750	125.500	136.833	134.423	124.722
<i>Stygiopontius</i> S73	154.400	174.083	152.500	147.667	123.222	104.692	123.444
<i>M. helgolandica</i>	228.800	235.917	224.750	218.167	219.000	214.231	222.556

	<i>S. pectinatus</i>	<i>S. senckenbergi</i>	<i>S. senokuchiae</i>	<i>Stygiopontius</i> sp. 1	<i>Stygiopontius</i> sp. 2	<i>Stygiopontius</i> S73	<i>M. helgolandica</i>
<i>A. limatus</i>	0.376	0.393	0.357	0.294	0.378	0.348	0.429
<i>A. mammillatus</i>	0.388	0.398	0.389	0.344	0.378	0.408	0.436
<i>Aphotopontius</i> sp. 1	0.373	0.366	0.349	0.268	0.348	0.343	0.442
<i>Chasmatopontius</i> sp. 1	0.312	0.451	0.332	0.315	0.269	0.332	0.406
<i>S. brevispina</i>	0.324	0.389	0.315	0.232	0.300	0.265	0.412
<i>S. hispidulus</i>	0.349	0.398	0.313	0.207	0.296	0.217	0.409
<i>S. lauensis</i>	0.325	0.377	0.294	0.220	0.267	0.265	0.383
<i>S. pectinatus</i>		0.433	0.383	0.282	0.249	0.353	0.331
<i>S. senckenberghi</i>	180.000		0.437	0.381	0.441	0.386	0.501
<i>S. senokuchiae</i>	165.250	182.333		0.286	0.356	0.300	0.434
<i>Stygiopontius</i> sp. 1	130.333	165.667	131.833		0.267	0.256	0.393
<i>Stygiopontius</i> sp. 2	117.750	183.000	156.333	124.500		0.304	0.352
<i>Stygiopontius</i> S73	154.500	167.000	137.000	120.000	137.000		0.434
<i>M. helgolandica</i>	232.250	251.000	227.833	218.667	227.000	231.000	

preserved in 99.5% ethanol. *Stygiopontius senckenbergi* were found from washings of *Sh. crosnieri* in ethanol, and transferred into fresh ethanol before DNA extraction.

DNA of the three species was extracted using InstaGene matrix to obtain 30 μ L of extracts, and a partial mitochondrial COI gene sequence was amplified using the methods detailed in Watanabe et al. (2016). The obtained sequences were registered in GenBank with accession numbers from MT934831 to MT934840.

The DNA sequences were compared with those of other dirivultids available in GenBank (the accession numbers are listed in Fig. 3). The GenBank sequences varied in length and we selected sequences longer than 560 bp from each local population of species, as each local population appeared to be genetically homogeneous (Gollner et al., 2016). We call the 'haplotype S73' in Gollner et al. (2011) "*Stygiopontius* sp. S73" herein, because it was regarded as a "new species" in that study.

The dirivultid sequences were aligned with three sequences of the monstrellid copepod *Monstrilla helgolandica* Claus, 1863 (GenBank accessions: KT209301, KT209330, and KT209371) using CLUSTALW in MEGA 7.0.26 (Kumar et al., 2016), resulting in a dataset consisting of 560 bp from 69 operational taxonomic units (OTUs). Mean pairwise sequence differences within and among species were calculated using the same software. Relationships between transitions or transversions and genetic distance were visualized using DAMBE ver. 7.2.136 (Xia, 2018) to test for substitution saturation, which leads to the loss of phylogenetic informativeness. Phylogenetic relationship among the OTUs was reconstructed using both maximum likelihood and Bayesian inference. GTR+G was selected by the model selection test in MEGA 7.0.26 to reconstruct the maximum likelihood tree, and the significance of the branching was tested with 2000 bootstrap replicates. The same dataset was used to estimate the Bayesian tree using MrBayes (Ronquist et al., 2012) with 500,000 Markov chain Monte Carlo generations; the first 10,000 generations were removed. For species delimitation analyses in each cluster, Bayesian implementation of the Poisson Tree Process model (bPTP; Zhang et al., 2013) and Automatic Barcoding Gap Delimitation (ABGD; Puillandre et al., 2012) were performed for the genus *Chasmatoptontius* (the COI gene sequences of *Stygiopontius* were too divergent for these analyses; see details in Results). The bPTP was carried out with 100,000 MCMC generations with the first 1000 generations removed using its web interface (<https://species.h-its.org/ptp/>) and $P_{min} = 0.001$ and $P_{max} = 0.1$ were set for ABGD analyses using its web interface (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>).

RESULTS

The COI barcoding sequences were highly divergent in dirivultids, with between 82 and 186 bp (15–33%) variable sites in the 560 bp fragment examined (Table 1); the within-species variability was mostly less than 10 bp (2%; Table 2). The sequence divergence from the out-group was higher (225 bp or 40%) than the intra-familial divergence. As the genetic distance increased, the numbers of transitions and transversions both increased in the same manner, which suggests that no substitution saturation occurred

in the sequence examined (Fig. 2). The recently described species *S. senokuchiae* was clearly divergent from the other *Stygiopontius* with known sequences, and the most closely related species was *Stygiopontius* sp. 1 from Central Indian Ridge in Gollner et al. (2011), with about 130 bp (23%) divergence; the within-species variability among the sequences of *S. senokuchiae* was very low (only 1 bp or < 1%). *Stygiopontius senckenbergi* in the Okinawa Trough was recovered as being most closely related to *Aphotopontius* sp. 1 from Central Indian Ridge in Gollner et al. (2011), which was about 160 bp (29%). No difference was detected among all sequences obtained for *S. senckenbergi* in the Okinawa Trough. The unidentified species of the genus *Chasmatoptontius* in the Myojin-sho Caldera fell within the

Table 2. Average sequence divergence between sequences pairs within species.

	Mean sequence difference	Kimura 2-parameter distance
<i>A. limatus</i>	5.200	0.009
<i>A. mammillatus</i>	12.530	0.024
<i>Aphotopontius</i> sp. 1	4.333	0.008
<i>Chasmatoptontius</i> sp. 1	33.800	0.065
<i>S. brevispina</i>	3.333	0.006
<i>S. hispidulus</i>	4.641	0.008
<i>S. lauensis</i>	9.889	0.018
<i>S. pectinatus</i>	3.500	0.006
<i>S. senckenbergi</i>	0.000	0.000
<i>S. senokuchiae</i>	1.000	0.002
<i>Stygiopontius</i> sp. 1	2.000	0.004
<i>Stygiopontius</i> sp. 2	4.000	0.007
<i>M. helgolandica</i>	0.000	0.000

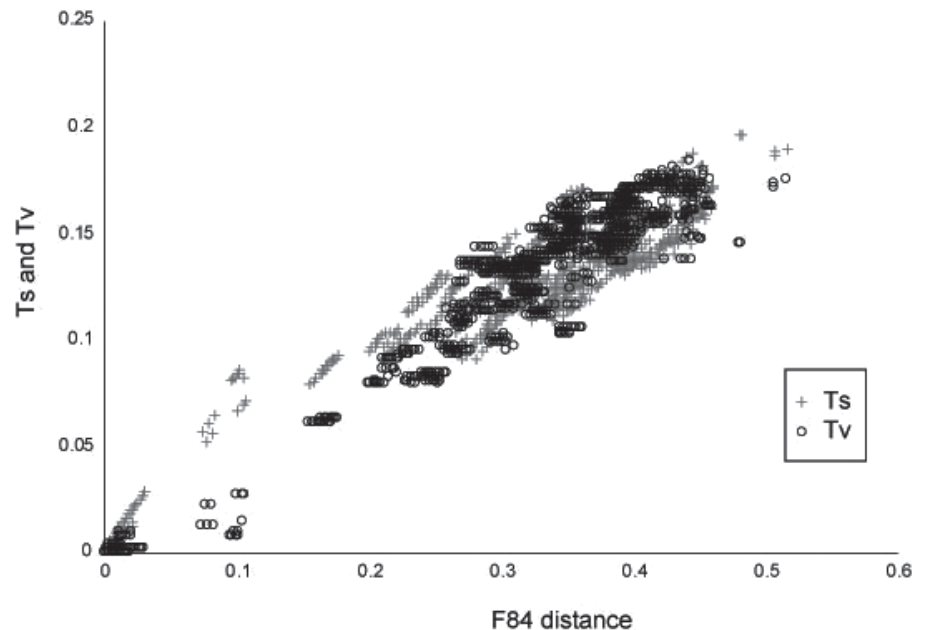


Fig. 2. Relationships between transitions (Ts) or transversions (Tv) and genetic distance (Felsenstein 84; F84) in the sequence variability of the examined dirivultids.

variation of *Chasmatopontius* sp. 1 in Gollner et al. (2011), reported from the Lau Basin in the south-western Pacific, which is more than 7000 km distant from the Myojin-sho Caldera.

Phylogenetic reconstruction showed that each species, including *S. senokuchiae* and *S. senckenbergi*, formed monophyletic clades, whereas the unidentified *Chasmatopontius* from the Myojin-sho Caldera was nested within *Chasmatopontius* sp. 1 from the Lau Basin (Fig. 3). Sequences identified as *Chasmatopontius* formed a monophyletic clade, whereas monophyly of both *Aphotopontius* and *Stygiopontius* was not supported. The analyses supported two sister-relationships among the *Stygiopontius* species: “*S. brevispina* and *S. lauensis*” and “*Stygiopontius* sp. S73 and *S. hispidulus*”. Four species, namely *S. pectinatus*, *S. senokuchiae*, as well as *Stygiopontius* sp. 1 and 2 sensu Gollner et al. (2016), were recovered as monophyletic clades separate from all other species included. Therefore, species delimitation analyses were performed only for *Chasmatopontius*, which was recovered as monophyletic.

Both bPTP and ABGD resulted in the same partitioning patterns: three OTUs in *Chasmatopontius* (Fig. 3). The clade of *Chasmatopontius* sp. 1 in Gollner et al. (2011) could be subdivided into two monophyletic groups, consistent with results from Gollner et al. (2011; one clade consisted of individuals collected only at ABE field [labeled “a” in Fig. 3], and the other was collected at both the ABE and Tu’i Manila fields, both in the Lau Basin [labeled “b” in Fig. 3]). The within-group sequence variability was less than 10 bp (2%), and there were about 30 base (5%) substitutions between the groups. The unidentified *Chasmatopontius* from the Myojin-sho Caldera had a sequence similar to that of group “a” (Fig. 3), with about 40 base

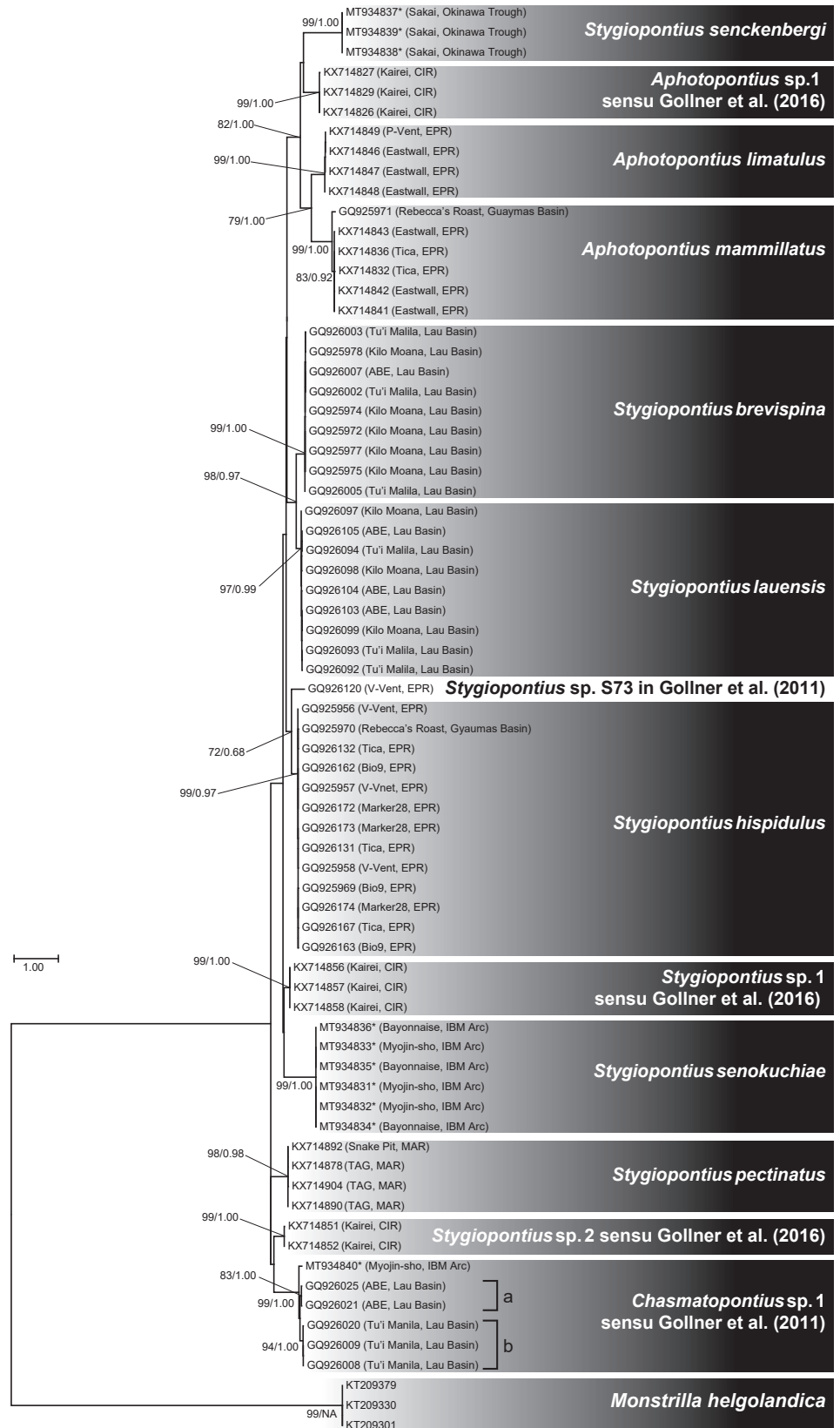


Fig. 3. Maximum likelihood tree based on 560 bp of mitochondrial COI of dirivultid copepods (Bayesian and ML methods gave identical topologies). Node values are ML bootstrap values/Bayesian posterior probabilities. Operational taxonomic units are indicated by GenBank accession numbers, with the sampling sites in parentheses. The partitions in the *Chasmatopontius* clades indicate the result from ABGD and bPTP.

(7%) substitutions between the two. In the *Aphotopontius mammillatus* Humes, 1987 clade, a haplotype collected at Rebecca's Roost field in the Guaymas Basin exhibited more than 40 bp sequence divergence compared to the haplotypes of *A. mammillatus* collected at the Tica field in the EPR, which is about 2000 km south of Rebecca's Roost field (Gollner et al., 2016).

DISCUSSION

Our genetic comparison showed that *S. senokuchiae* and *S. senckenbergi* each constitute a monophyletic group. Due to the high variability of the barcode sequence in the genus *Stygiopontius*, species delimitation analyses could not be performed for this genus. However, the mitochondrial COI barcoding region is not saturated in substitution and therefore can be considered reliable for identifying the described species (Fig. 2 and Table 2; Gollner et al., 2016). Indeed, there was a clear barcoding gap between the intra- (< 33 bp or 6% differences out of 560 bp) and inter- (> 80 bp or 14% differences; Fig. 3 and Table 1) species sequence variability. DNA sequences of other loci in dirivultids will be necessary to reconstruct the phylogenetic relationships of the family Dirivultidae, especially in the genus *Stygiopontius*. However, the extraction of high-quality DNA from copepods is often difficult due to their small sizes, especially from sediment-contaminated samples. This is even more so in male dirivultids, which are much smaller than conspecific females. Careful sample preparation and technical improvement may be required for further elucidation and application to dirivultids, which are highly abundant and yet so far much neglected in vent ecosystems.

Of the analyzed dirivultids, only the genus *Chasmatopontius* formed a monophyletic cluster with high support (Fig. 3). *Stygiopontius senckenbergi* was included in the *Aphotopontius* clade. The species delimitation analyses, i.e., bPTP and ABGD, suggested that *S. senckenbergi* is a separate species from the currently analyzed dirivultids with sequence data available. In *A. mammillatus*, a single specimen collected at Rebecca's Roost field was separated from the other individuals from the Tica field by the species delimitation analyses (Fig. 3), due to the sequence divergence of up to 40 bp. As all the *A. mammillatus* specimens were morphologically identical, the sequence divergence may be due to genetic subdivision by physical population separation. In the phylogenetic analyses, the unidentified *Chasmatopontius* from the Myojin-sho Caldera was closely related to *Chasmatopontius* sp. 1 in the Lau Basin with high support; however, the species delimitation analyses suggested that the clade could be separated into three potentially species-level groups: *Chasmatopontius* sp. 1 group "a" and "b", and the unidentified *Chasmatopontius* from the Myojin-sho Caldera (Fig. 3). Detailed morphological observation of *Chasmatopontius* in these lineages is required; however, it is unlikely that two lineages of the same species co-occur without gene flow.

Genetic analyses often provide evidence of cryptic species in animal communities. In deep-sea hydrothermal vent fields, for example, some cryptic species of snails of *Alviniconcha* Okutani & Ohta, 1988 (Kojima et al., 2001; Suzuki et al., 2006) and *Lamellibrachia* tubeworms (Kojima et al., 2012) were initially identified by molecular analyses,

and subsequent detailed observations confirmed the morphological characteristics of each lineage (Kobayashi et al., 2015; Laming et al., 2020). The genus *Chasmatopontius* currently consists of two described species: *Chasmatopontius thescalus* Humes, 1990 from hydrothermal vent fields in the Mariana Trough off Guam and *Chasmatopontius inflatus* Kim & Kim, 2013 from a hydrothermal vent field on the Tofua Arc, east of the Lau Basin. The two species are distinguished by morphological characters, such as the caudal ramus and female maxilliped (Kim and Kim, 2013). Careful morphological observation of *Chasmatopontius* sp. 1 is required to elucidate the true species-level diversity contained under this name.

The present analyses combined with a review of previous data (Gollner et al., 2011, 2016) showed variation in the dispersal capabilities of dirivultids. The distributions of *Aphotopontius limatulus* Humes, 1987 and *Aphotopontius* sp. 1 are limited to a single segment of a seafloor spreading center, 9°50'N in the EPR and Eastern Lau Spreading Center, respectively. *Aphotopontius mammillatus* from two different areas (9°50'N in EPR and Rebecca's Roost, Guaymas Basin in the Gulf of California) exhibited genetic differences of approximately 40 out of 560 bp (7%) between the two localities, which is a marked variation compared to that between the two vents in the EPR (10 out of 560 bp or 2%; Fig. 3). Together with previous results from Gollner et al. (2016) that showed that populations within each EPR population do not exhibit over 10 bp of variation in the COI barcoding region, this suggests that the *Aphotopontius* species examined may not be able to disperse long distances. However, this is not applicable to *Stygiopontius hispidulus* because the haplotype obtained at the Rebecca's Roost field (S137 in Gollner et al., 2016) showed only 6 bp (1%) difference, and this falls within the range of genetic variation in populations on the EPR (Fig. 3). Some dirivultids occur in more than one biogeographical region: *A. limatulus* is distributed in both the northeastern Pacific and Central Indian Ridge; *Aphotopontius forcipatus* Humes, 1987 and *Stygiopontius mirus* Humes, 1996 in both the northeastern Pacific and Mid-Atlantic Ridge; *S. pectinatus* in both the Mid-Atlantic Ridge and Mariana Trough; *S. stabilitus* in both the northeastern Pacific and Mariana Trough; and *S. senckenbergi* in both the New Ireland Forearc and Okinawa Trough (Humes and Segonzac, 1998; Lee et al., 2020; Uyeno et al., 2020).

Recently, *Stygiopontius liui* Ma, Wang & Li, 2020 was described based on the morphology of females associated with the squat lobster *Sh. crosnieri* from a methane seep site in the South China Sea (Ma et al., 2020), but they did not have access to male specimens. The original description of *S. senckenbergi* was only based on male specimens from the Edison Seamount, New Ireland Forearc (Ivanenko and Ferrari, 2013). By examining specimens of both sexes, Uyeno et al. (2020) was able to identify the *Stygiopontius* from the same squat lobster species in the Okinawa Trough as *S. senckenbergi*. There is no significant morphological difference between the original description of *S. liui* and the female of *S. senckenbergi*, only first reported by Uyeno et al. (2020), and therefore *S. liui* is to be considered a junior synonym of *S. senckenbergi*. The abundance of male *S. senckenbergi* individuals may be lower than that of the

females, or the male may be too small to be collected by suction sampler. Molecular data from the South China Sea population will be of great interest in the future to compare with the Okinawa Trough population in a phylogeographic context.

Connectivity among distant populations of widely distributed vent copepods, which is important for managing the ecosystem in terms anthropogenic disturbance (i.e., Boschen et al., 2015), remains little examined. The genetic divergence of *S. hispidulus* in the Guaymas Basin was within the variation of *S. hispidulus* in the EPR, as described above (Fig. 3; Gollner et al., 2016); but in *A. mammillatus* a higher divergence was found between the two areas. This result suggests a high variability of larval dispersal capability among dirivultid genera.

In summary, our comparison of partial mitochondrial COI barcoding sequences confirmed the monophyly of *Chasmatopontius* from the currently available genetic data, with a clear barcoding gap between the intra- and interspecies sequence variability. However, the phylogenetic relationships in the genus *Stygiopontius*, the most species-rich genus in the family Dirivultidae, were complex and as far as COI sequences suggest, it appears to be paraphyletic. Multi-locus phylogenetic analyses combined with morphological observation or identification will help our understanding of the genus-level taxonomic relationships of dirivultids to determine their evolutionary history associated with global ocean spreading. Future population level genetic analyses will be useful to reveal potential population structure among different localities, which likely reflects potential differences in the planktonic period in different copepods. Such studies will allow us to gain a comprehensive understanding of the diversity, dispersal, and reproductive strategies of these vent-specific copepods, which mediate nutrient flow from bacteria to macro- and megafauna in vent ecosystems but have been much overlooked in studies of vent ecosystems until recently.

ACKNOWLEDGMENTS

We appreciate the support of the team operating *ROV Hyper-Dolphin* and the captain and crew of the *R/V Natsushima*, which enabled us to obtain the copepod samples. We also thank Ken Takai for collecting the samples and Chong Chen for helping to revise the English language in addition to the sample preparation. This study was partly funded by a grant from the Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI) program (grants JP26440246, JP18K06401, and JP19H03305).

COMPETING INTERESTS

There are no competing interests to be declared.

AUTHOR CONTRIBUTIONS

HKW and MS conceived and designed the study. HKW, RS, HN, and MS collected and prepared the specimens. HKW obtained and analyzed the DNA sequence dataset. HKW, HN, TK, DU, and MS prepared the figures and tables and wrote the manuscript.

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(Received October 12, 2020 / Accepted January 17, 2021 /
Published online April 7, 2021)