Unanticipated discovery of two rare gastropod molluscs from recently located hydrothermally influenced areas in the Okinawa Trough

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Background. Deep-sea hydrothermal vent is one of the most 'extreme' environments in the marine realm. Few species are capable of inhabiting such ecosystems, despite extremely high productivity there supported by microbial chemosynthesis, leading to high biomass and low species richness. Although gastropod molluscs are one of the main constituents of megafaunal communities at vent ecosystems, most species belong to several typical families (e.g., Provannidae, Peltospiridae, Lepetodrilidae) specialised and adapted to life at vents.

Methods. During recent surveys of Okinawa Trough hydrothermal vent systems, two snails atypical of vent ecosystems were unexpectedly found in newly discovered hydrothermally influenced areas. Shell and radular characteristics were used to identify the gastropods morphologically.

Results. One species was a vetigastropod, the calliostomatid *Tristichotrochus ikukoae* (Sakurai, 1994); and the other was a caenogastropod, the muricid *Abyssotrophon soyoae* (Okutani, 1959). Both gastropods were previously only known from regular non-chemosynthetic deep-sea and very rare – only two published records exist for each species. The radula formula of *Tristichotrochus ikukoae* is accurately reported for the first time and based on that it is returned to genus *Otukaia*. For both species, barcode sequences of the cytochrome *c* oxidase I (COI) gene were obtained and deposited for future references.

Discussion. These new records represent the second record of calliostomatids from vents (third from chemosynthetic ecosystems) and the third record of muricids from vents (tenth from chemosynthetic ecosystems), and extend the distribution of both species to the southwest. Neither family has been recorded at chemosynthetic ecosystems in the western Pacific. Both were from weakly diffuse flow areas not subject to high temperature venting but were nevertheless associated with typical vent-reliant taxa such as *Lamellibrachia* tubeworms and *Bathymodiolus* mussels. These new records show that these species are capable of tolerating environmental stress associated with weak hydrothermally influenced areas, despite not being vent endemic species, adding to the list of known vent/non-vent species intersections. This signify that such weakly influenced areas may provide key habitats for them, and that such areas may play a role in the evolution of biological adaptations to 'extreme' chemosynthetic ecosystems.

1   *Research Article*

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3   **hydrothermally influenced areas in the Okinawa Trough**

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16   **Short title:** Two gastropods newly recorded from vents

17   **Abstract**

18   **Background.** Deep-sea hydrothermal vent is one of the most 'extreme' environments in the
19   marine realm. Few species are capable of inhabiting such ecosystems, despite extremely high
20   productivity there supported by microbial chemosynthesis, leading to high biomass and low
21   species richness. Although gastropod molluscs are one of the main constituents of megafaunal
22   communities at vent ecosystems, most species belong to several typical families (e.g.,
23   Provannidae, Peltospiridae, Lepetodrilidae) specialised and adapted to life at vents.

24   **Methods.** During recent surveys of Okinawa Trough hydrothermal vent systems, two snails
25   atypical of vent ecosystems were unexpectedly found in newly discovered hydrothermally
26   influenced areas. Shell and radular characteristics were used to identify the gastropods
27   morphologically.

28   **Results.** One species was a vetigastropod, the calliostomatid *Tristichotrochus ikukoae* (Sakurai,
29   1994); and the other was a caenogastropod, the muricid *Abyssotrophon soyoae* (Okutani, 1959).
30   Both gastropods were previously only known from regular non-chemosynthetic deep-sea and
31   very rare – only two published records exist for each species. The radula formula of
32   *Tristichotrochus ikukoae* is accurately reported for the first time and based on that it is returned to
33   genus *Otukaia*. For both species, barcode sequences of the cytochrome *c* oxidase I (COI) gene
34   were obtained and deposited for future references.

35   **Discussion.** These new records represent the second record of calliostomatids from vents (third
36   from chemosynthetic ecosystems) and the third record of muricids from vents (tenth from
37   chemosynthetic ecosystems), and extend the distribution of both species to the southwest. Neither
38   family has been recorded at chemosynthetic ecosystems in the western Pacific. Both were from
39   weakly diffuse flow areas not subject to high temperature venting but were nevertheless
40   associated with typical vent-reliant taxa such as *Lamellibrachia* tubeworms and *Bathymodiolus*
41   mussels. These new records show that these species are capable of tolerating environmental stress
42   associated with weak hydrothermally influenced areas, despite not being vent endemic species,
43   adding to the list of known vent/non-vent species intersections. This signify that such weakly
44   influenced areas may provide key habitats for them, and that such areas may play a role in the
45   evolution of biological adaptations to 'extreme' chemosynthetic ecosystems.

46   **Introduction**

47   Deep-sea hydrothermal vents exhibit increased water temperature, higher heavy metal
48   concentrations, and raised concentrations of substances acting as energy sources for
49   chemosynthesis (such as H_2, CH_4, H_2S) compared to the surrounding water (Van Dover, 2000;
50   Petersen *et al.*, 2011). Due to such 'extreme' environmental conditions, few species are capable
51   of inhabiting such ecosystems, despite extremely high productivity there for the deep-sea
52   supported by microbial chemosynthesis (Grassle, 1987). As a result, although vent ecosystems
53   support biomass comparable even to that of shallow water coral reefs, the macro- and megafaunal
54   communities there are characterised by a low species richness (Lutz & Kennish, 1993; Van Dover
55   & Trask, 2000).

56   Although gastropod molluscs are one of the main constituents of megafaunal communities at
57   hydrothermal vent ecosystems, species present mostly to belong to one of several typical families
58   (e.g., Provannidae, Peltospiridae, Lepetodrilidae) specialised and adapted to life at vent and other
59   chemosynthetic ecosystems such as hydrocarbon seeps and organic falls (Sasaki *et al.*, 2010).
60   Here, we report new records of two gastropod species, *Tristichotrochus ikukoae* (Sakurai, 1994)
61   (Vetigastropoda: Calliostomatidae) and *Abyssotrophon soyoae* (Okutani, 1959)
62   (Caenogastropoda: Muricidae), from two new diffuse flow hydrothermal sites discovered using
63   the multibeam echo-sounding (MBES) method (as outlined in Nakamura *et al.*, 2015). Both
64   species are exceedingly rare endemics of Japan, with only two published records each.

65   Calliostomatidae is an exclusively marine family of usually grazing gastropods which comprises
66   about 300 extant species (Marshall, 1995; Vilvens, 2014; Marshall, 2016). Only two species have
67   been recorded from chemosynthetic ecosystems, one from vent and one from seep (Sasaki *et al.*,
68   2010). Muricidae is a large family of carnivorous and generally predatory marine
69   caenogastropods comprising about 1600 living species (Merle *et al.*, 2011). A total of seven
70   muricids have been recorded from hydrocarbon seeps, but none from vents (Sasaki *et al.*, 2010)
71   and two species (*Enixotrophon carduelis* (Watson, 1882) and *E. obtusus* (Marshall & Houart,
72   2011) were recorded from Rumble V Volcano which is a hydrothermally influenced area on the
73   Kermadec Ridge (Marshall & Houart, 2011). The present study, therefore, presents the second
74   record of Calliostomatidae and the third record of Muricidae from hydrothermal vent ecosystems.

75   The two species newly recorded from vents herein are unlikely to be vent endemics but are
76   clearly interacting with vent environment and other vent endemic fauna. These records add to the
77   intersection of vent endemic and non-vent endemic species in the peripheral diffuse flow
78   environment, where non-endemic species have often been recorded due to the much less stressful

79   environment compared to focused flow venting (reviewed in Levin *et al.,* 2016).

Materials & Methods

81   Potential signals of hydrothermal activity were detected in the Okinawa Trough by detecting
82   acoustic water column anomalies, probably derived from CO_2 bubbles discharging from vents,
83   using an EM122 (Kongsberg Maritime) MBES system on R/V *Yokosuka* (cruises YK14-16 and
84   YK16-07), as outlined in Nakamura *et al.* (2015). The signals were then ground-truthed with
85   Remotely Operated Vehicle (ROV) *KAIKO* (with vehicle *Mk-IV*) dives on-board R/V *Kairei*
86   during cruises KR15-16 (Kawagucci *et al.*, 2015; Makabe *et al.*, 2016) and KR16-16 (Miyazaki
87   *et al.*, 2016; Makabe *et al.*, 2017). A suction sampler equipped on the ROV *KAIKO* was used to
88   collect the gastropods. A digital camera mounted on the same ROV was used to shoot the *in situ*
89   observation images.

90   Upon recovery on to the research vessel, the specimens were fixed and stored in 99% ethanol for
91   subsequent studies. Amplification and sequencing of the cytochrome *c* oxidase I (COI) barcode
92   gene was done using a piece of foot using the universal primer pair LCO1490 and HCO2198
93   (Folmer *et al.*, 1994). Molecular methods follow that outlined in Chen *et al* (2016). After taking
94   tissue snips, soft parts were extracted from the specimens after rehydrating in MilliQ. The radula
95   was dissected out from each specimen under a dissecting microscope (Olympus SZX1) and
96   cleaned by washing in diluted commercial bleach followed by two washes, in MilliQ water and
97   99% ethanol each. The cleaned radulae were mounted on SEM stubs with carbon tapes and
98   examined uncoated at 15kV with a table top scanning electron microscope (SEM; Hitachi TM-
99   3000).

Results and Discussion

101   ***Detection and discovery of hydrothermal sites***

102   One acoustic anomaly (28° 26.1' N, 128° 11.5' E; Fig. 1) was detected in the northern Okinawa
103   Trough near Tokara Islands, in the 'Higashi-Ensei' area (Makabe *et al.*, 2017) east-northeast of
104   Minami-Ensei Knoll (Hashimoto *et al.*, 1995). This site was explored on dive #724 of ROV
105   *Kaiko*, which discovered a weakly active hydrothermal vent site mostly with diffuse flow areas.
106   This site was 955–1180 m deep, dominated by dense *Lamellibrachia* tubeworm bushes grown

107 over by the barnacles *Ashinkailepas seepiophila* Yamaguchi, Newman & Hashimoto, 2004 and
108 *Leucolepas longa* Southward & Jones, 2003, and was named 'Fukai' (Fig. 2A; for details see
109 Miyazaki *et al*., 2016; Makabe *et al*., 2017). Other fauna typical of Okinawa Trough
110 hydrothermal vents (Watanabe & Kojima, 2015), such as the squat lobster *Shinkaia crosnieri*
111 Baba & Williams, 1998, the vesicomyid clam *Akebiconcha kawamurai* Kuroda, 1943, and the
112 mussel *Bathymodiolus platifrons* Hashimoto & Okutani, 1994 were also present in this site.
113 Another signal (25° 4.5' N, 124° 31.0' E; Fig. 1) was located about 1950–1990 m deep on the
114 northern slope of Tarama Hill in the southern Okinawa Trough, and was surveyed during ROV
115 *KAIKO* dive #669 (for details see Makabe *et al*., 2016). A small weakly hydrothermally
116 influenced site dominated by white branching Poecilosclerida sponges (*sensu* Ise, 2012), *Alaysia*
117 sp. and *Lamellibrachia* sp. tubeworms, and *Bathymodiolus aduloides* Hashimoto & Okutani,
118 1994 mussels was found and named 'Crane' (Fig. 2C; Makabe *et al*., 2017).

119 ### *Tristichotrochus ikukoae* (Sakurai, 1994)

120 One specimen of a calliostomatid gastropod was collected from Fukai site, Higashi-Ensei,
121 Okinawa Trough (28° 26.1064' N, 124° 11.4889' E, 962 m deep, R/V *Kairei* cruise KR16-16,
122 ROV *KAIKO* Dive #724, 2016/xii/04). Based on shell (diameter 22.6 mm, height 21.2 mm; Fig.
123 3A–D) and radular (Fig. 4A) characteristics this specimen was clearly identifiable as
124 *Tristichotrochus ikukoae* (Sakurai, 1994). To the knowledge of the authors, this is only the third
125 time it has been formally reported in the literature (Higo *et al.*, 1999; Hasegawa, 2009). A total of
126 three individuals were sighted at the same site (Fig. 2B), living attached on *Lamellibrachia* sp.
127 tubeworm tubes or hard surrounding substrata.

128 The taxonomic history of *Tristichotrochus ikukoae* is rather perplexing. It was initially described
129 from Kochi Prefecture, Japan as *Otukaia ikukoae* Sakurai, 1994. Sakurai compared it to *Otukaia*
130 *kiheiziebisu* (Otuka, 1939), commenting that *O. ikukoae* is 'smaller in size, and has obtuser ribs
131 with larger granules' as well as 'has only 21 basal ribs in comparison' to *O. kiheiziebisu* (Sakurai,
132 1994). Later, Okutani & Iwahori (1992) reported *O. kiheiziebisu* from Tosa Bay, Kochi
133 Prefecture, Japan which extended its distribution range from Sagami Bay. They reported that their
134 specimens were variable in spiral rib development, with some specimens exhibiting five equally
135 strong ribs. Radulae of such 'unusual' specimens were illustrated (Okutani & Iwahori, 1992: figs.
136 7, 10–12), and were actually virtually identical to the holotype of *O. ikukoae*. Following this,
137 Hasegawa & Saito (1995) considered *O. ikukoae* to be a junior synonym of *O. kiheiziebisu*, and
138 Hasegawa (2009) formally synonymised the two species. More recently, however, Marshall
139 (2016) figured the radula of a typical *O. kiheiziebisu* and found it to be clearly distinct from that

140   figured by Okutani & Iwahori (1992). *Otukaia ikukoae* was reinstated as a valid species and
141   transferred to *Tristichotrochus* based on its fewer lateral teeth compared to *Otukaia* (four to six
142   compared to nine in *O. kiheiziebisu*).

143   Though the radula of *T. ikukoae* was reported to have only four pairs of lateral teeth (Okutani &
144   Iwahori, 1992) compared to nine in *O. kiheiziebisu* (Marshall, 2016), this was questioned by
145   Marshall (2016) who reasoned that there may actually be as many as five to six pairs. The
146   specimen of *T. ikukoae* investigated in the present study has eight pairs of laterals (Fig. 4A),
147   which is more numerous than Marshall (2016) predicted and makes the radula formula
148   (n+8+1+8+n). Due to only a single specimen being collected it is not possible to comment on the
149   intraspecific variation in the radula of *T. ikukoae* at this point, but this is the most reliable account
150   of the radula characteristics of this rare calliostomatid. Having as many as eight pairs of laterals
151   means the radula of *T. ikukoae* actually fits well in genus *Otukaia*, and it is therefore returned to
152   that genus herein, as *Otukaia ikukoae* Sakurai, 1994.

153   It should be noted that *O. ikukoae* and *O. kiheiziebisu* are in fact easily distinguished by shell
154   characteristics as well, by the following two points: 1. *O. ikukoae* has five to six strong ribs on
155   the body whorl whereas *O. kiheiziebisu* only has two to three (including the base keel); 2. The
156   ribs of *O. ikukoae* are less sharply raised but more strongly beaded than those of *O. kiheiziebisu*.
157   To assist future identification of these two species based on molecular barcodes, a COI barcode
158   of the present specimen was sequenced and deposited in DNA Data Bank of Japan (DDBJ;
159   accession number LC333137). A sequence of the same gene is already available on GenBank for
160   *O. kiheiziebisu* (from Williams, 2010) under the accession number AB505274, the identity of the
161   individual sequenced has been ascertained and confirmed as *O. kiheiziebisu* (T. Nakano pers.
162   comm., in Marshall, 2016). Based on comparison between these two sequences, the pairwise
163   difference between the two species is 5.8%, which is higher than the average of 3–4% between
164   closely related species in marine gastropods (Meyer & Pauley, 2005).

165   The size of the present specimen (shell height 21.2 mm) is just slightly smaller than previous
166   reports as shell height of the holotype is 23.9 mm (Sakurai, 1994) and height of specimens
167   definitively referable to *O. ikukoae* reported by Okutani & Iwahori (1992) range between 23.5
168   mm–26.7 mm.

169   Since *O. ikukoae* has not been recorded from outside Kochi Prefecture (Higo *et al.*, 1999;
170   Hasegawa, 2009), the present new record extends its distribution range to the southwest by about
171   700 km. The collection depth of 962 m is deeper than the Tosa Bay record (700 m; Okutani &

172   Iwahori, 1992), and since the depth that the type specimen was collected from is not known

173   (Sakurai, 1994), this represents the deepest record for the species.

174   ***Abyssotrophon soyoae* (Okutani, 1959)**

175   Two specimens of trophonine muricids were collected from the Crane site, Tarama Hill, Okinawa

176   Trough (25° 4.5388' N, 124° 31.0103' E, 1973 m deep, R/V *Kairei* cruise KR15-16, ROV

177   *KAIKO* Dive #669, 2015/x/29). A total of five individuals was sighted at this site (Fig. 2D). The

178   morphology of the two specimens matched best, in terms of shell (specimen #1, height 22.2 mm,

179   width 11.0 mm, Fig. 3E–H; specimen #1, height 22.5 mm, width 10.5 mm, Fig. 3I, J) and radular

180   (specimen #1, Fig. 4B) characters, with *Abyssotrophon soyoae* (Okutani, 1959). Originally

181   described from off Miki-Saki, Kumano-nada, Kii Peninsula, Japan (33° 48.8' N, 136° 34.8' E) as

182   *Trophonopsis soyoae* Okutani, 1959, this is only the third time it has been recorded formally

183   (Okutani & Iwahori, 1992; Higo *et al*., 1999; Tsuchiya, 2017). Furthermore, three individuals that

184   appeared to belong to the same species were seen in Fukai site (Fig. 2B, the same location as *O.*

185   *ikukoae* collecting data above), but unfortunately none were collected.

186   Conchologically *A. soyoae* is easily recognised by two strong keels combined with very crowded

187   axial ribs (Tsuchiya, 2017). One significant difference between the present material and

188   previously reported specimens is that the axial ribs are less crowded (about 35 vs about 70;

189   Okutani & Iwahori, 1992). However, there appears to be some variability in rib frequency across

190   life stages (see Fig. 3J; Okutani & Iwahori, 1992: fig. 28) and the authors consider this difference

191   to be intraspecific, rather than interspecific, variability until further evidence becomes available

192   proving otherwise. The specimens examined herein also have higher spires than the holotype

193   (Okutani, 1959), but they match well with specimens previously reported from Tosa Bay, Kochi

194   Prefecture, Japan (Okutani & Iwahori, 1992). The shell height to width ratios were 2.02 and 2.14,

195   which gives an impression that they are more stout than the previous specimens which range

196   between 2.11 – 2.69 (2.11 in the holotype; Okutani & Iwahori, 1992) but only because the earlier

197   whorls are much more corroded in the present specimens, likely due to the acidic hydrothermal

198   environment. The radula is very much the same as imaged by Okutani & Iwahori (1992),

199   including the formula (1+1+1) and in having a single middle cusp on the central tooth. Although

200   the soft parts of the specimens investigated were not of good quality, eyes were present in both

201   individuals and the smaller individual (specimen #1, male) possessed a very large penis as

202   previously reported (Okutani & Iwahori, 1992). To facilitate future identification and genetic

203   comparison, the COI barcode sequence was obtained from specimen #1 and deposited in DDBJ

204   under accession number LC333138. The closest match available on GenBank is a sequence of

205   *Boreotrophon truncatus* (Strøm, 1768), accession number HQ919183 (from Layton *et al*., 2014)

206   with 9% pairwise difference.

207   Since this species has only been reported from the type locality and Tosa Bay (Higo *et al*., 1999;

208   Tsuchiya, 2017), the present record extends its distribution range over 1250 km to the southwest.

209   The collection depth of the present record (1973 m) is within the bathymetric range between the

210   type locality (2050 m; Okutani, 1959) and the Tosa Bay record (700 – 750 m; Okutani & Iwahori,

211   1992).

212   ***Significance of the new records from vent ecosystems***

213   Trochoid gastropods are commonly found in many hydrothermal vent sites around the globe (e.g.,

214   *Iheyaspira, Cantrainea*) but calliostomatids have only been recorded twice (Sasaki *et al*., 2010).

215   These include *Falsimargarita nauduri* Warén and Bouchet 2001 from the 17°S hydrothermal vent

216   site, East Pacific Rise (Warén & Bouchet, 2001) and *Maurea chilena* (Rehder, 1971) from

217   Concepción methane seep off Central Chile (Sellanes *et al*., 2008). Although Sellanes *et al*.

218   (2008) also reported *Otukaia crustulum* Vilvens & Sellanes, 2006 from the Concepción methane

219   seep, this species has been moved to Calliotropidae, as *Calliotropis crustulum* (Marshall, 2016).

220   The present report of *Otukaia ikukoae* is therefore the second record of calliostomatids from vent

221   ecosystems and the third record from chemosynthetic ecosystems.

222   Muricids are found from intertidal waters down to over 7000 m deep, and *Abyssotrophon* is one

223   of the deepest-living genera occurring even over 7200 m deep in the case of *Abyssotrophon*

224   *hadalis* (Sysoev, 1992) (Egorov, 1993). Five species of Muricidae are known from Concepción

225   methane seep off Central Chile (Sellanes *et al*., 2008) and two are known from methane seeps in

226   Barbados Prism (Warén & Bouchet, 2001), making a total of seven records from hydrocarbon

227   seeps. Except *Pagodula concepcionensis* Houart & Sellanes, 2006 which is in the subfamily

228   Pagodulinae (Houart & Sellanes, 2006), all other six species recorded are in the subfamily

229   Trophoninae (genera *Trophon* and *Coronium*; Sasaki *et al*., 2010). The present record of

230   *Abyssotrophon soyoae* is the third record of this family from vent ecosystems and the tenth from

231   chemosynthetic ecosystems. Both families were previously unrecorded from chemosynthetic

232   ecosystems of not only the Okinawa Trough but the western Pacific as a whole (Sasaki *et al*.,

233   2010; Watanabe & Kojima, 2015).

234   Although the two species reported herein were found in hydrothermally influenced settings, they

235   are also known from regular deep-sea bottom (Okutani & Iwahori, 1992) and thus not endemic to
236   chemosynthetic ecosystems. Neither new hydrothermally influenced site reported herein is
237   vigorously active, and is instead more like 'hydrothermal seeps' (Levin *et al*., 2012) than
238   conventional high-temperature fluid venting systems. The presence *O. ikukoae* and *A. soyoae* in
239   weakly diffuse flow areas indicate that they are able to tolerate some levels of environmental
240   stresses associated with hydrothermal ecosystems (e.g., increased acidity, concentration of toxic
241   substances and heavy metals), allowing them to invade and benefit from the high productivity of
242   hydrothermal vent ecosystems.

243   While feeding behaviour was not observed in the field, the ecological roles the two species play
244   may be inferred as follows. Deep-water calliostomatids are mostly carnivorous grazers feeding on
245   cnidarians or sponges (Marshall, 1995), and from the fact that they were observed living on
246   tubeworm tubes *O. ikukoae* in Fukai site probably graze on sponges and/or biofilm growing on
247   the tubes. Muricids are carnivores feeding on other marine invertebrates (Merle *et al*., 2011), and
248   *A. soyoae* is unlikely to be an exception. In both localities the *Abyssotrophon* individuals were
249   seen in close proximity to other molluscs (e.g., *Bathymodiolus aduloides* at Crane site) and
250   barnacles (e.g., *Ashinkailepas seepiophila* at Fukai site) which the *Abyssotrophon* likely prey
251   upon. In interacting with vent endemic species in such ways, *O. ikukoae* and *A. soyoae* may
252   contribute to trophic transfer of chemosynthetically derived production from vents to surrounding
253   ambient environment, which some other predators such as seastar, fish, and octopus have been
254   suggested to play a role in (Levin *et al*., 2016 and references therein). The apparent absence of
255   these two species from other Okinawa Trough vents is possibly an artefact caused by the
256   relatively little effort put into exploring the peripheral areas.

257   In fact, non-vent endemic deep-water predatory gastropods have been recorded from vent
258   periphery in several occasions, for example *Buccinum viridum* Dall, 1889 on the Juan de Fuca
259   Ridge (Tunnicliffe & Fontaine, 1987; Sarrazin *et al*., 1997), *Enixotrophon carduelis* and *E.*
260   *obtusus* on the Kermadec Ridge (Marshall & Houart, 2011), and *Neptunea robusta* Okutani, 1964
261   in the Okinawa Trough (Watanabe & Kojima, 2015). Their success may be a combined effect of
262   the ability to tolerate moderate environmental stress from vent periphery and the fact that few
263   vent endemic gastropod species are predators, meaning there is an available niche for these
264   predatory species given the high productivity. In the Okinawa Trough vents, for instance, only
265   one vent endemic predatory gastropod, *Thermosipho desbruyeresi* (Okutani & Ohta, 1993), is
266   known (Watanabe & Kojima, 2015).

267   The present records signify that outskirts of hydrothermally influenced areas likely provide

268 important habitats and energy resources for some non-endemic species (Levin *et al.*, 2016). These
269 peripheral areas may be important for the evolution of biological adaptations to chemosynthetic
270 ecosystems by providing the intermediate environment between 'extreme' vents and the regular
271 surrounding seafloor.

272 **Conclusions**

273 Exploration of two new hydrothermally influenced ecosystems in the Okinawa Trough led to the
274 collection of two gastropod snails previously unknown from chemosynthetic ecosystems. One
275 was *Otukaia ikukoae*, the second calliostomatid ever recorded from vents and the third record of
276 the family for chemosynthetic ecosystems overall. The other was the muricid *Abyssotrophon*
277 *soyoae*, marking the third time Muricidae has been found at hydrothermal vents and the tenth
278 record at chemosynthetic ecosystems. Both species are extremely rare and this report represents
279 the third record for each species in the literature as well as the first time they are seen in their
280 natural habitat. Neither family has been recorded at chemosynthetic ecosystems in the western
281 Pacific. Since both species are also known from regular non-chemosynthetic deep-sea and they
282 were recovered from areas with only weak diffuse flow venting, they have most likely invaded
283 vent environment from regular sea bottom and play a role in trophic transfer of chemosynthetic
284 production to the surrounding seafloor. The fact that such species are able to tolerate (some)
285 influence from hydrothermal vents signify that such 'outskirt' areas may provide key habitats for
286 them, and that these areas may play a role in the evolution of biological adaptations to 'extreme'
287 chemosynthetic ecosystems.

288 **Additional Information and Declarations**

289 *Data Availability*
290 Specimens studies in this study are deposited and accessioned at JAMSTEC marine biological
291 sample collection; *Otukaia ikukoae*: JAMSTEC No. 1160052681; *Abyssotrophon soyoae*:
292 JAMSTEC No. 1150046767. The newly generated COI barcode sequences are deposited in DNA
293 Data Bank of Japan with accession numbers LC333137 (*O. ikukoae*) and LC333138 (*A. soyoae*).

294 **Acknowledgements**

295   The authors would like to express their sincere gratitude to the Captain and crews of R/Vs *Kairei*
296   and *Yokosuka*, as well as the operation team of the ROV *KAIKO*, for their untiring support during
297   the relevant cruises (YK14-16, KR15-16, YK16-07, KR16-16). Kyoko Okino (AORI, the
298   University of Tokyo) is gratefully acknowledged for leading the YK14-16 cruise, and Kentaro
299   Nakamura (the University of Tokyo) for analysing the MBES data which led to the discovery of
300   the new hydrothermal sites. Roland Houart and Christopher Moe are thanked for their valuable
301   aid in identifying the *Abyssotrophon*. Kazuya Kitada (JAMSTEC) kindly provided the base map
302   for Fig. 1 used herein. Bruce Marshall (Museum of New Zealand Te Papa Tongarewa) and Verena
303   Tunnicliffe (University of Victoria) are thanked for providing commenting on an earlier version
304   which significantly improved the manuscript. The Fukai site was named in reference to a
305   poisonous jungle of the same name in the film "Nausicaä of the Valley of the Wind" (Studio
306   Ghibli, 1984); the director Hayao Miyazaki and Studio Ghibli are gratefully acknowledged for
307   being a great source of inspiration.

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Figure Captions

421

422 **Figure 1.** Map of the Okinawa Trough and surrounding areas showing locations of the two
423 relevant hydrothermally influenced sites. The blue star indicates Fukai site, Higashi-Ensei and the
424 red dot indicates Crane site, Tarama Hill. Black dots indicate known vent sites in the Okinawa
425 Trough (following Watanabe & Kojima, 2015). Site name abbreviations are as follows: Cr =
426 Crane Site, Ht = Hatoma Knoll, Ih = Iheya Ridge, Ik = Iheya North Knoll, Ir = Irabu Knoll, Iz =
427 Izena Hole, Me = Minami-Ensei Knoll, Yg = Daiyon-Yonaguni Knoll, Yr = Yoron Hole. Original
428 500 m mesh data originally obtained by Japan Coast Guard and publicly available from Japan
429 Oceanographic Data Center (http://jdoss1.jodc.go.jp/vpage/depth500_file_j.html).

430 **Figure 2.** *In situ* photographs of the two hydrothermally influenced sites. **A–B**: Fukai site,
431 Higashi-Ensei; **A**: Overview, **B**: Close-up; the black arrowhead indicates *Otukaia ikukoae* and the
432 white arrowhead indicates *Abyssotrophon* cf. *soyoae*. **C–D**: Crane site, Tarama Hill; **C**: Overview,
433 **D**: Close-up; the white arrowhead indicates *Abyssotrophon soyoae*. The cut-out photographs are
434 magnified images of gastropods seen at each site.

435 **Figure 3.** Specimens of the two gastropods collected from hydrothermally influenced areas. **A–**
436 **D**: *Otukaia ikukoae* from Fukai site, Higashi-Ensei. **E–J**: *Abyssotrophon soyoae* from Crane site,
437 Tarama Hill; **E–H** = Specimen #1, **I, J** = Specimen #2. Scale bars: **A–D** = 1 cm, **E–J** = 0.5 cm.

438 **Figure 4.** Scanning electron micrographs of radulae. **A**: *Otukaia ikukoae* from Fukai site,
439 Higashi-Ensei. **B**: *Abyssotrophon soyoae* from Crane site, Tarama Hill. Scale bars: **A** = 500 μm,
440 **B** = 50 μm.

Figure 1

Map of the Okinawa Trough and surrounding areas showing locations of the two relevant hydrothermally influenced site.

The blue star indicates Fukai site, Higashi-Ensei and the red dot indicates Crane site, Tarama Hill. Black dots indicate known vent sites in the Okinawa Trough (following Watanabe & Kojima, 2015). Site name abbreviations are as follows: Cr = Crane Site, Ht = Hatoma Knoll, Ih = Iheya Ridge, Ik = Iheya North Knoll, Ir = Irabu Knoll, Iz = Izena Hole, Me = Minami-Ensei Knoll, Yg = Daiyon-Yonaguni Knoll, Yr = Yoron Hole. Original 500 m mesh data originally obtained by Japan Coast Guard and publicly available from Japan Oceanographic Data Center (http://jdoss1.jodc.go.jp/vpage/depth500_file_j.html).
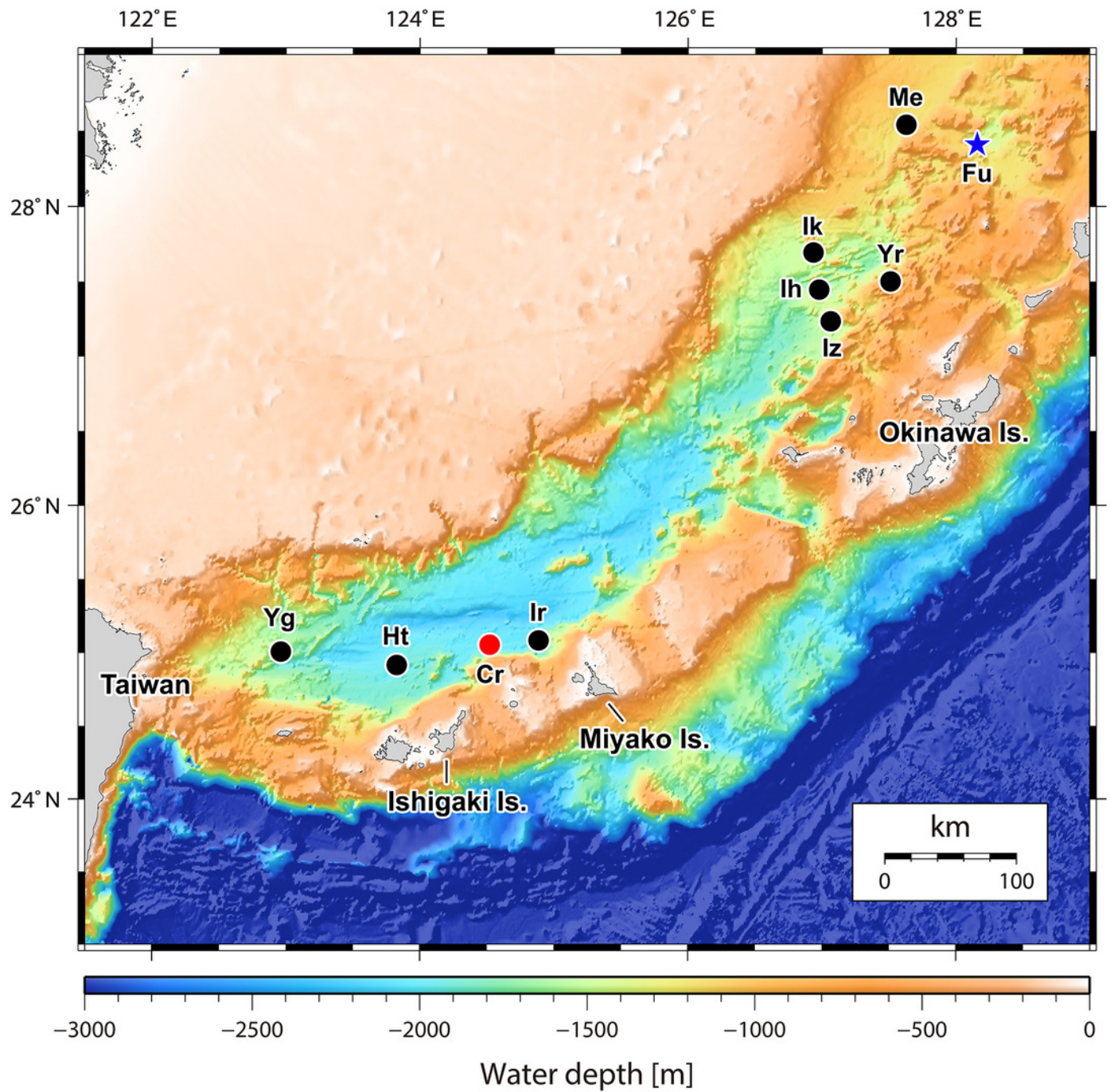
Figure 2

In situ photographs of the two hydrothermally influenced sites.

A-B: Fukai site, Higashi-Ensei; A: Overview, B: Close-up; the black arrowhead indicates *Tristichotrochus ikukoae* and the white arrowhead indicates *Abyssotrophon* cf. *soyoae*. C-D: Crane site, Tarama Hill; **C**: Overview, D: Close-up; the white arrowhead indicates *Abyssotrophon soyoae*. The cut-out photographs are magnified images of gastropods seen at each site.
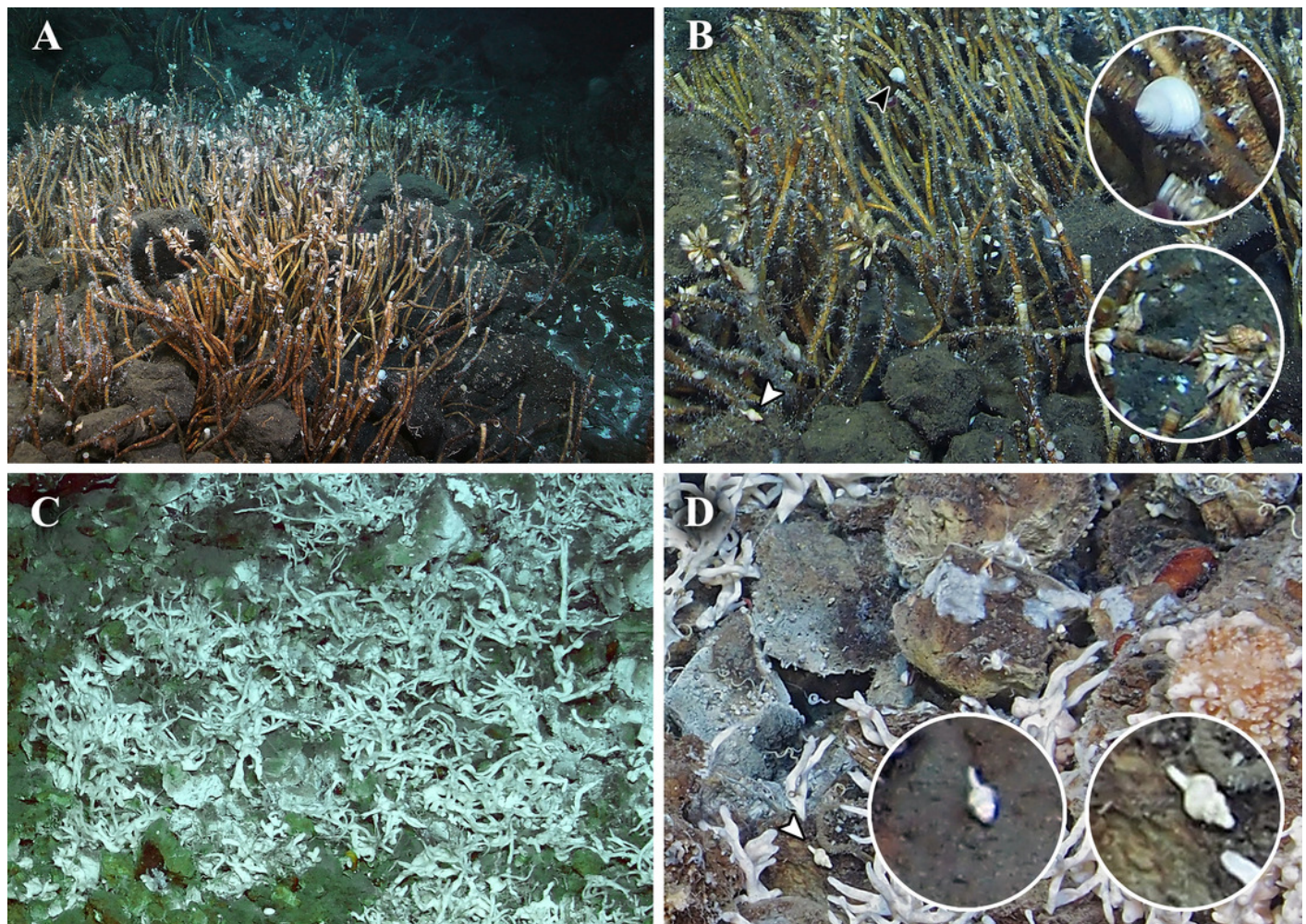
Figure 3

Specimens of the two gastropods collected from hydrothermally influenced areas.

A-D: *Tristichotrochus ikukoae* from Fukai site, Higashi-Ensei. **E-J**: *Abyssotrophon soyoae* from Crane site, Tarama Hill; **E-H** = Specimen #1, **I, J** = Specimen #2. Scale bars: **A-D** = 1 cm, **E-J** = 0.5 cm.
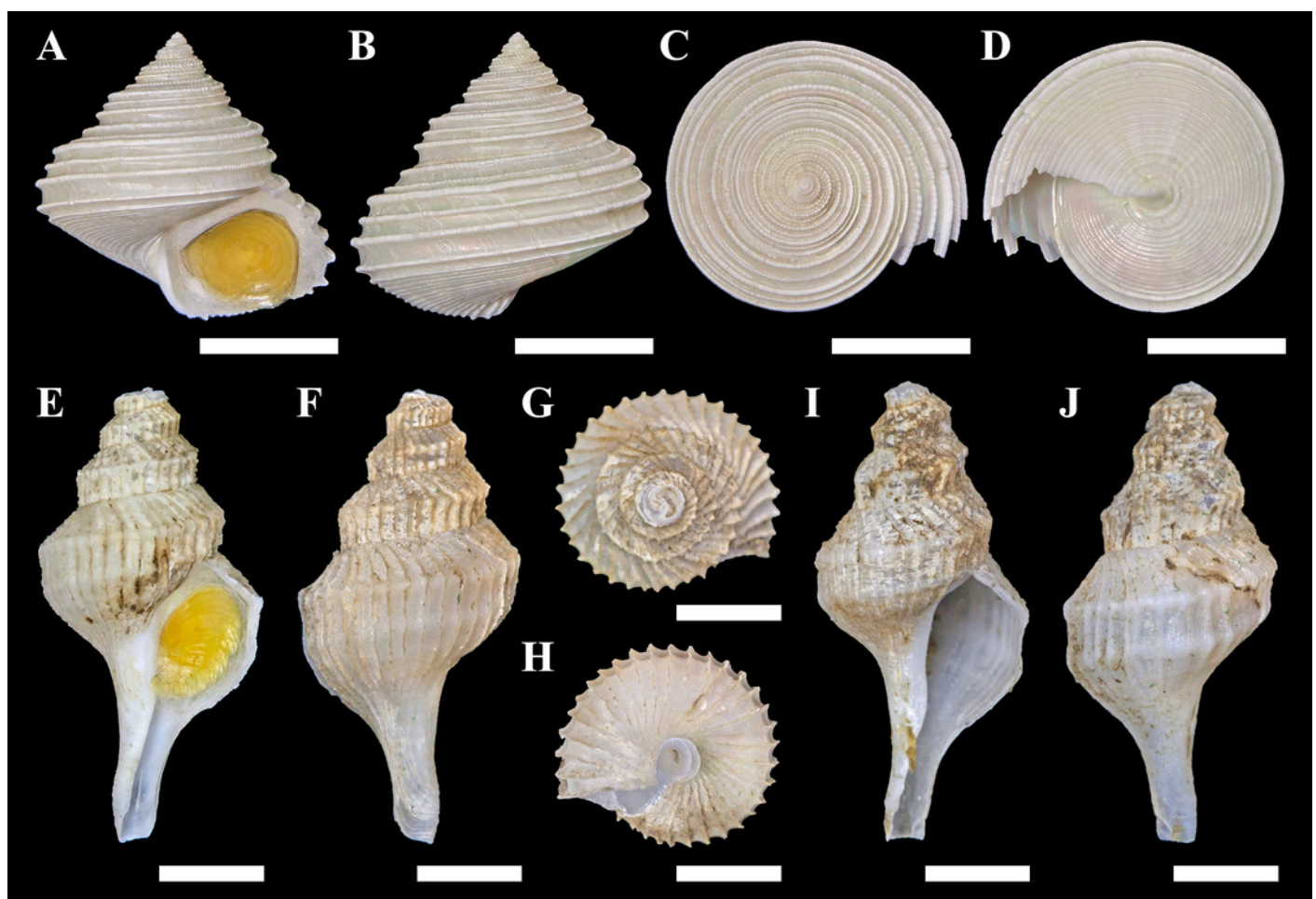
Figure 4

Scanning electron micrographs of radulae.

A: *Tristichotrochus ikukoae* from Fukai site, Higashi-Ensei. **B**: *Abyssotrophon soyoae* from Crane site, Tarama Hill. Scale bars: **A** = 500 μm, **B** = 50 μm.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.