

1 **Loricifera inhabiting spherical agglutinated structures in the abyssal eastern equatorial**  
2 **Pacific nodule fields**

3  
4 **Reinhardt M. Kristensen<sup>1</sup>, Andrew J Gooday<sup>2</sup>, Aurélie Goineau<sup>2</sup>**

5  
6  
7 <sup>1</sup>Natural History Museum of Denmark, Section for Biosystematics, Universitetsparken 15,  
8 DK-2100 Copenhagen Ø, Denmark<sup>1</sup>

9  
10  
11 <sup>2</sup>National Oceanography Centre, Southampton, University of Southampton Waterfront  
12 Campus, European Way, Southampton SO14 3ZH, UK

13  
14  
15  
16  
17 Corresponding author. Andrew Gooday: email: [ang@noc.ac.uk](mailto:ang@noc.ac.uk); tel: +44 (0)23 80596353 fax:  
18 +44 (0)23 80596247 (A.J. Gooday)

19  
20  
21 **Acknowledgements**

22 We thank Craig Smith for his inviting two of us to participate in the ABYSSLINE project and  
23 for his management of both the project and the two cruises during which the samples were  
24 collected. We also thank Ivan Voltski and Alexandra Weber who supported our work at sea.  
25 Support for a visit by the senior author to the National Oceanography Centre, Southampton,  
26 was provided by Carlsberg Foundation grant CF-16-0236. Laura Pavesi (NHMD) is  
27 acknowledged for the registration of the slides. We thank 4 anonymous Reviewers for their  
28 detailed comments that helped to improve the manuscript. The ABYSSLINE project is  
29 funded through a commercial arrangement with UK Seabed Resources Ltd, whose support  
30 we gratefully acknowledge.

31  
32

33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55

**Abstract**

Loriciferans are known to survive in extreme environments, most notably in the case of a recently described *Spinoloricus* species from a hypersaline anoxic Mediterranean basin. Our new discovery of members of the genus *Rugiloricus* inside spherical agglutinated structures from sediment samples collected in the manganese nodules fields of the eastern Clarion-Clipperton Zone (CCZ, abyssal equatorial Pacific) demonstrates that these tiny animals are able to spring fresh surprises. Nearly all developmental stages of an undescribed *Rugiloricus* species were found inside the spheres, from the first instar larva and large free larva to the two stages of the postlarva inside the larval exuvium. Only the adults were missing. The spheres themselves were almost certainly not created by the loriciferans. Their origin is unclear, although similar agglutinated structures from the same study area contain cells that resemble monothalamous foraminifera, suggesting that they are possibly made by foraminifera. One of our CCZ samples also yielded a single free specimen of the loriciferan genus *Pliciloricus* that was not hidden inside an agglutinated structure. This specimen is particularly interesting because, like nested Russian dolls, it has all stages inside the larval exuvium: first the larval exuvium itself with the two toes, then a very thin postlarval exuvium, and finally the adult male with two testes filled with mature spermatozoa.

**Keywords** Metazoan meiofauna. loriciferans. *Rugiloricus*. *Pliciloricus*. monothalamous foraminifera. polymetallic nodules.

## 56 Introduction

57

58 The phylum Loricifera was described in 1983 from coarse sand at low water in Roscoff,  
59 France (Kristensen, 1983). Since then, 36 loriciferan species have been established (Neves et  
60 al., 2018). However, there have been relatively few records of loriciferans from the deep  
61 Pacific Ocean. Kristensen and Shirayama (1988), Gad (2004) and Neves and Kristensen  
62 (2014) described new genera and species from the western Pacific. In the eastern Pacific, a  
63 new genus (*Spinoloricus* Heiner and Neuhaus, 2007) was described by Heiner and Neuhaus  
64 (2007) and a new species of this genus by Neves and Kristensen (2016), in both cases from  
65 the equatorial Galapagos Spreading Center. Recently, Neves et al. (2018) established two  
66 new species of *Rugiloricus* Higgins and Kristensen, 1986 and *Urnaloricus* Heiner and  
67 Kristensen, 2009 from the Oregon and Californian margins. However, little attention has  
68 been paid to these meiofaunal metazoans in the Clarion-Clipperton Zone (CCZ). This  
69 extensive tract of the equatorial Pacific hosts vast deposits of polymetallic nodules of  
70 considerable potential economic importance. Because it lies in international waters,  
71 commercial activities in the CCZ are regulated by the International Seabed Authority, a UN  
72 body that enters into contracts with companies and governments having interests in seafloor  
73 mining (Lodge et al., 2014; Lodge and Verlaan, 2018). The CCZ is currently the focus of a  
74 large international research effort to characterise the benthic fauna prior to any future  
75 exploitation of this valuable mineral resource (Jones et al., 2018). Radziejewska et al. (2001)  
76 and Radziejewska (2002) mention loriciferans (larvae and adults) among the ‘less abundant’  
77 meiofaunal taxa present in some samples from the Interoceanmetal Joint Organisation (IOM)  
78 contract area in the eastern CCZ (~11° 40’N, 119° 40’W, ~4400 m depth). Similarly,  
79 Mahatma (2009) records loriciferans among the ‘rare’ or ‘very rare’ meiofaunal taxa in the  
80 French contract area in the more central part of the CCZ (14°N, 130°W, ~5000 m depth),  
81 where they occur in densities of 0 - 0.05 indiv.core<sup>-1</sup>. Nothing further has been published,  
82 however, about the loriciferans from these two areas.

83 In 2013 and 2015, we (AJG and AG) took part in two cruises as part of the  
84 ABYSSLINE project, a baseline study of benthic fauna in the UK-1 and Ocean Minerals  
85 Singapore (OMS) contract areas in the eastern part of the CCZ (Glover et al., 2016). Our  
86 main interest was in benthic foraminifera (Goineau and Gooday, 2017; Gooday and Goineau,  
87 2019). However, during the course of this work, we encountered five loriciferans, four of  
88 which, all larvae and postlarvae of the genus *Rugiloricus*, were hidden inside spherical  
89 agglutinated structures. The fifth specimen, which was not enclosed in such a structure,  
90 belonged to the genus *Pliciloricus* Higgins and Kristensen, 1986. The purpose of the present  
91 note is to 1) report the totally novel occurrence of loriciferans inside agglutinated structures  
92 and 2) briefly describe the five loriciferans.

93 Both *Rugiloricus* and *Pliciloricus* are members of the family Pliciloricidae. Fig. 1  
94 (from figure 19 of Neves et al., 2016) illustrates the life-cycle of the Pliciloricidae in order to  
95 provide a context for the observations reported here. For a full description of the life-cycle  
96 see Neves et al. (2016).

97

98

## 99 Materials and Methods

100

101 Detailed descriptions of ship-board and laboratory methods are given by Goineau and  
102 Gooday, 2017). In brief, samples were collected using a Megacorer (BCMEGA OSIL Bowers  
103 and Connelly) equipped with 10-cm-diameter core tubes during ABYSSLINE cruises AB01  
104 (R/V *Melville* cruise MV1313, 3 to 27 October 2013; Smith et al., 2013) and AB02 (R/V  
105 *Thomas G Thompson* cruise TN319, 12 February to 25 March, 2015) in the UK-1 and OMS

106 contract areas. Station details are summarised in Table 1. The sediment was a fine-grained,  
107 brown, siliceous (radiolarian) ooze with some diatom frustules and micronodules.

108 The samples were sliced into horizontal layers (0.5 cm thick to 2 cm depth and  
109 thereafter in 1-cm-thick layers down to 10 cm depth) using a cutting plate, and each sediment  
110 slice stored separately in 10% borax-buffered seawater formalin. In the laboratory, preserved  
111 slices were split into 1/8th fractions using a wet sample splitter (Jensen, 1982). The present  
112 observations were made serendipitously during a series of wider studies of foraminifera based  
113 on these core slices. All samples yielding loriciferans were sieved on 300 and 150  $\mu\text{m}$  mesh  
114 screens. For the upper two layers (0-0.5 and 0.5-1.0 cm) either three or four 1/8<sup>th</sup> splits were  
115 analysed (Table 2). Single splits from two samples (MC09, MC11) were also sieved on 125  
116 and 63  $\mu\text{m}$  screens in order to examine foraminifera in these finer fractions. In the case of the  
117 MC09 sample, a single split from each of the deeper layers down to 5 cm was sieved on 300  
118 and 150  $\mu\text{m}$  screens. All sieve residues were stained overnight in Rose Bengal and sorted in  
119 water for foraminifera and metazoan meiofauna under a stereo-microscope. Loricifera and  
120 other metazoans were stored in glycerol in open cavity slides. Specimens were photographed  
121 using either an SLR digital camera (Canon EOS 350D) fitted to an Olympus BH2 compound  
122 microscope (Southampton) or an Olympus DP27 digital camera fitted to an Olympus BX51  
123 compound microscope (Copenhagen). Finally, all the loriciferans were removed from the  
124 open slides in glycerol to permanent slides and the cover slips sealed with Glyceel. This  
125 unique material, a total of six slides, is deposited in the loriciferan collection at the Natural  
126 History Museum of Denmark under the numbers NHMD 287289 to 287294.

127

128

## 129 **Results**

130

131 Five loriciferans were sorted from 150-300  $\mu\text{m}$  sediment fractions; none were found in the  
132  $>300 \mu\text{m}$ , 125-150  $\mu\text{m}$  or 63-125  $\mu\text{m}$  sieve fractions. Two originated from the 0-0.5 cm  
133 sediment layer (MC11, MC25), two from the 0.5-1.0 cm layer (both MC25), and the fifth  
134 from the 3-4 cm layer (MC09) (Table 2). Four specimens (from MC09, MC11 and MC22),  
135 were found inside more or less spherical agglutinated structures (Fig. 2a,b,e; Fig. 3a,b,e)  
136 measuring 230 - 275  $\mu\text{m}$  in diameter (Table 3). The spheres had a wall composed of finely  
137 agglutinated grains through which the loriciferans were visible when viewed in transmitted  
138 light. The wall sometimes incorporated a radiolarian test. The spheres were flexible and so  
139 presumably included some organic matrix. In three cases, the wall could be pulled apart with  
140 a pair of dissecting needles fairly easily to release the loriciferans (Fig. 3b). However, the  
141 sphere from sample MC09, although soft and easily distorted (Fig. 2b,c), proved to be  
142 resistant to tearing, making it impossible to extract the inhabitant.

143 The lengths of all specimens are given in Table 3. The two specimens from samples  
144 MC09 and MC11 were larvae (Fig. 2b,d,f) while sample MC22 yielded two larval moults  
145 containing developing post-larvae (Fig. 3c,d,f). All are considered to represent an  
146 undescribed species of the genus *Rugiloricus*. The fifth specimen from sample MC20, which  
147 unlike the other four was not associated with an agglutinated sphere, was a last instar Higgins  
148 larva containing a postlarval form moulting to an adult male of the genus *Pliciloricus* (Figs 4  
149 and 5). Unfortunately we could not establish new species based on this small collection. In  
150 the case of *Rugiloricus*, the adult stage was lacking. In the case of the new *Pliciloricus*, many  
151 of the morphological characters, such as all the scalids of the introvert and the lorica type of  
152 the adult male, could not be observed inside the larva/postlarva exuvium. Therefore we only  
153 give a short description of the five loriciferans.

154 1) *Rugiloricus*-larva (sample MC09, Figs 2a-d; corresponding to stage ‘C’ in Fig. 1). This  
155 is the first instar Higgins larva of the genus *Rugiloricus*. The pharyngeal bulb is filled with  
156 glandular tissue and two giant nuclei (nu) are seen in the middle of the pharyngeal bulb (ph).  
157 The abdomen (ab) is filled with absorptive and glandular cells, which would only be present  
158 if substantial amounts of food material had been ingested. The larva could not be removed  
159 from the agglutinated sphere, which was relatively cohesive and deformed when attempts  
160 were made to tear it open.

161 2) *Rugiloricus*-larva (sample MC11, Figs 2 e, f; corresponding to stage ‘D’ in Fig. 1).  
162 This specimen is clearly the last instar of the loriferan Higgins larva, just before moulting to  
163 the postlarva (Higgins and Kristensen, 1986). Both the pharyngeal bulb (ph) and the midgut  
164 (mg) are clearly visible. The two toes (to) are of the *Rugiloricus carolinensis* Higgins and  
165 Kristensen, 1986 type (Heiner, 2008). The larva could easily be removed from the  
166 agglutinated sphere.

167 3) Late *Rugiloricus*-postlarva inside the Higgins larval exuvium (sample MC22, Figs  
168 3a,c,d; corresponding to stage ‘N’ in Fig. 1). The postlarva was clearly visible when the  
169 loriferan was first observed within the agglutinated sphere (Fig. 3a). However, in addition  
170 to the late developing postlarva, the sphere also contained the very thin larval exuvium,  
171 which became obvious when the loriferan was removed (Fig. 3c,d). The larval exuvium has  
172 all the characters of a *Rugiloricus* species; the combination of short toes (to), long  
173 unbranched anterior lateroventral seta (alv) and the moulted larval scalids (sc<sub>1</sub>) seen anterior  
174 to the postlarva (po) is found only in this genus. The new scalids can be seen protruding from  
175 the introvert (head) in Fig.3c The postlarva is only 98 µm long

176 4) Early *Rugiloricus*-postlarva inside the Higgins larva exuvium (sample MC22; Figs 3e-  
177 f, corresponding to stage ‘N’ in Fig. 1). Only the postlarva was clearly visible within the  
178 agglutinated sphere containing the loriferan when it was first observed (Fig. 3e). The larval  
179 exuvium, as well as the early developed postlarva, were obvious when they were removed  
180 from the structure. The larval exuvium displays many of the same characters as the specimen  
181 in Fig. 3a-d and the posterolateral seta (se<sub>2</sub>) can also be seen. However, this postlarva lacks  
182 obvious scalids. The postlarva is also only 98 µm long, making the two specimens from  
183 MC22 the smallest recorded loriferan postlarvae.

184 5) *Pliciloricus* (sample MC25; Figs 4 and 5, corresponding to stage ‘O’ in Fig. 1). This  
185 specimen encompasses three stages in one specimen: the Higgins larval exuvium with toes  
186 (to), a very thin postlarval exuvium, and the adult male with two testes (te) containing mature  
187 spermatozoa (sz). Fig 4a,b illustrate the larval structures: the toes and the larval setae. In Fig  
188 4c,d all adult characters are seen, revealing that the animal is a male with two testes. The  
189 spermatozoa (sz) are of the *Pliciloricus*-type (see Kristensen, 1991a). Finally, Fig.5 shows the  
190 moulted larval internal armature (ia) and the toes (to), clearly indicating that the specimen is a  
191 *Pliciloricus*.

192

193

## 194 Discussion

195

196 Except in the hypersaline, permanently anoxic, sulphidic environment of the l’Atalante Basin  
197 in the deep Mediterranean (3363-3600 m water depth), where they are the only living  
198 metazoans (Danovaro et al., 2010, Neves et al., 2014), loriferans are usually an occasional  
199 element of deep-sea meiofaunal assemblages, for example, representing only 0.10% of  
200 meiofaunal animals in the case of abyssal samples from the Angola Basin (SW Atlantic)  
201 (Gad, 2005a). Shallow-water loriferans typically live interstitially in sandy substrates and  
202 shell gravel, whereas lower bathyal and abyssal species usually inhabit fine-grained, often  
203 muddy sediments (Neves et al., 2016; Neves et al., 2018). Little is known about their biology,

204 although like their relatives in coastal waters (Kristensen, 1991b), deep-sea species are  
205 probably bacterivores.

206 Our samples from the UK-1 and OMS contract areas yielded four larvae and  
207 postlarvae of the loriciferan genus *Rugiloricus* found inside agglutinated spheres, in addition  
208 to a single free Higgins larva of *Pliciloricus*. Both of these genera have been reported  
209 previously from deep-sea settings; *Rugiloricus* from the Indian (3400 m depth) and Arctic  
210 (1210 m depth) Oceans (Kristensen et al., 2013; Gad and Martinez Arbizu, 2005),  
211 *Pliciloricus* from the upper bathyal NW Atlantic (293-439 m), the abyssal SE Atlantic (5388-  
212 5448 m), and at hadal depths (8260 m) in the NW Pacific (Higgins and Kristensen, 1986;  
213 Kristensen and Shirayama, 1988; Gad, 2004, 2009), and both genera from the Galapagos  
214 Spreading Centre (Heiner and Neuhaus, 2007) in the bathyal eastern equatorial Pacific (2121-  
215 2776 m). However, this is the first time that either genus has been recorded in samples from  
216 the CCZ or indeed in any area of seafloor hosting polymetallic nodules.

217 We are also unaware of any previous records of loriciferans residing inside  
218 agglutinated structures, perhaps because they are difficult to spot when hidden within these  
219 microhabitats. Figure 6a shows a group of agglutinated structures from the 150-300  $\mu\text{m}$   
220 fraction of the M22 sample. It includes one of those that contained a loriciferan, as well as  
221 three radiolarian tests inhabited by benthic foraminifera, and an agglutinated structure  
222 containing a cell-like body. Radiolarian tests occupied by foraminifera are very common in  
223 our samples (Goineau and Gooday, 2015). Spheres with loriciferans may be difficult to  
224 distinguish from them at first sight, particularly under a stereo-microscope, although the  
225 radial spines of radiolarians usually become obvious when specimens are viewed under a  
226 compound microscope. Our serendipitous observations, although limited, suggest that  
227 loriciferans may be more common in this part of the Pacific than is apparent from current  
228 records (Mahatma, 2009).

229 It is intriguing that all four *Rugiloricus* Higgins larvae/postlarvae were living inside  
230 spheres while the single *Pliciloricus* specimen, comprising the larval exuvium and postlarval  
231 exuvium with the mature male inside, was found free in the sieved sediment residue. It would  
232 be interesting to know whether this is a coincidence or a consistent relationship, although a  
233 much larger number of specimens would be required to resolve this question. The nature of  
234 the agglutinated structures containing the loriciferans is also unclear. It seems highly unlikely  
235 that they are created by the loriciferans themselves, while the absence of a skeletal  
236 framework confirms that they are not spherical radiolarian tests. The structures are most  
237 similar to tests of the spherical, single-chambered agglutinated foraminifera (monothalamids)  
238 that are particularly abundant in the fine sieve fractions of abyssal Pacific samples (Nozawa  
239 et al., 2006; Ohkawara et al., 2009). In Fig. 6c,d we show two examples of spherical  
240 monothalamids with dark stercomata (waste pellets) that are very common in our samples.  
241 However, the spheres containing loriciferans were larger (230-275  $\mu\text{m}$ ) than most of the  
242 monothalamid tests present in our samples (generally  $<150 \mu\text{m}$ ) and their walls were also  
243 finer-grained and rather thicker. Except for the specimen from MC09, they could be torn  
244 open but still retain their basic shape (Fig. 3a,b) whereas monothalamid spheres tend to  
245 disintegrate. Finally, there is no trace of stercomata within the loriciferan spheres. The  
246 identity of these microhabitats is therefore unresolved, although a foraminiferal origin still  
247 remains the most likely possibility. Indeed, the two spherical structures containing cell bodies  
248 illustrated in Figs 6e,f are very similar to those hosting the loriciferans. These are possibly  
249 allogromiid foraminifera inside resting or feeding cysts (Heinz et al., 2005).

250 Why the loriciferans occupy agglutinated spheres is equally unclear. Possibly, these  
251 microhabitats resemble the interstitial spaces that loriciferans inhabit in much coarser-grained  
252 shallow-water sediments, although this does not explain how the *Rugiloricus*-larva can obtain  
253 enough food to support growth and moulting to a postlarva inside them. Another possibility,

254 therefore, is that these structures may host enhanced bacterial populations that the larva  
255 (although not the postlarva contained within the moult) can use as a food source. This could  
256 explain why they contain nearly the whole life-cycle of the loriciferans. However, since only  
257 larvae and postlarvae were found within the spheres, there is no evidence that the adults  
258 obtain any benefit from the occupancy of this microhabitat.

259 The *Pliciloricus* specimen comprised the larval exuvium with its two toes enclosing a  
260 very thin postlarval exuvium, and finally the mature adult male. Higgins and Kristensen  
261 (1986, Fig. 3) illustrated the paratypic larva of *Pliciloricus enigmaticus* Higgins and  
262 Kristensen, 1986 moulting into an adult male. In addition, Gad (2005b) illustrated an adult  
263 female and an immature adult of *P. peduncularis* Gad, 2005 nested within postlarval and  
264 larval exuvia (see figures 3b and 7 in Gad, 2005b). However, our specimen is really unique  
265 because the three stages are very condensed, so that the adult completely fills the postlarval  
266 exuvium, which in turn completely fills the larva exuvium, so that only the adult male  
267 structures are visible.

268

### 269 **Concluding remarks**

270 Very little is known about loriciferans within the CCZ, part of the equatorial Pacific that  
271 hosts commercially significant deposits of polymetallic nodules. In samples from the eastern  
272 CCZ, we observed undescribed larvae and postlarvae of the genus *Rugiloricus* and an adult  
273 *Pliciloricus* (also undescribed) nested like a Russian doll inside a postlarval exuvium that  
274 itself was nested inside a Higgins larval exuvium. Most interestingly, the *Rugiloricus*  
275 specimens occupied agglutinated spheres of uncertain origin, the first time such an  
276 association has been reported. The loriciferans were difficult to see inside these microhabitats  
277 and could be easily overlooked. What benefit they derived from this mode of life is unknown  
278 but a future investigation of potential food sources (e.g., bacteria and other micro-organisms)  
279 associated with the structures might prove fruitful. There is clearly much to be learnt about  
280 the ecology, as well as the distribution, of these remarkable animals in the CCZ, and  
281 elsewhere in the deep ocean.

282

283

284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331

**Funding** RMK received funding from the Carlsberg Foundation grant CF-16-0236. The wider ABYSSLINE project, including the salary of AG, part of the salary of AJG, and costs associated with the two cruises, were funded by UK Seabed Resources Ltd under contract number SRD100300.

## Compliance with ethical standards

**Conflict of interest.** The authors declare no conflicts of interest.

**Ethical approval.** This article does not contain any studies with animals performed by any of the authors.

**Sampling and field studies.** The sampling was conducted in international waters within areas covered by contracts for seabed exploration issued by the International Seabed Authority.

## Data availability statement

All data generated or analysed during this study are included in this published article.

## References

- Danovaro D, Dell'Anno A, Pusceddu A, Gambi C, Heiner I, Kristensen RM (2010) The first metazoa living in permanently anoxic conditions. *BMC Biol* 8: 30.  
<https://doi.org/10.1186/1741-7007-8-30>
- Gad G (2004) A new genus of Nanaloricidae (Loricifera) from deep-sea sediments of volcanic origin in the Kilinailau Trench north of Papua New Guinea. *Helgol Mar Res* 58: 40-53.
- Gad G (2005a) Giant Higgins-larvae with paedogenetic reproduction from the deep sea of the Angola Basin - evidence for a new life cycle and for abyssal gigantism in Loricifera? *Org Divers Evol* 5 (suppl. 1): 44-59.
- Gad G (2005b) A parthenogenetic, simplified adult in the life cycle of *Pliciloricus pedicularis* sp. n. (Loricifera) from the deep sea of the Angola Basin (Atlantic). *Org Divers Evol* 5: 77-103
- Gad G (2009) A clearly identifiable postlarva in the life cycle of a new species of *Pliciloricus* (Loricifera) from the deep sea of the Angola Basin. *Zootaxa* 2096: 50-81
- Gad G, Martínez Arbizu P (2005) First report of Arctic Loricifera—a new *Rugiloricus*-species from the Laptev Sea. *Mar Biol Res* 1: 313-325.
- Glover AG, Dahlgren T, Wiklund H, Mohrbeck I, Smith C R (2016) An end-to-end DNA taxonomy methodology for benthic biodiversity survey in the Clarion-Clipperton Zone, central Pacific Abyss. *J Mar Sci Engineering*, 4: 2.  
<http://doi.org/10.3390/jmse4010002>
- Goineau A, Gooday AJ (2015) Radiolarian tests as microhabitats for novel benthic foraminifera: observations from the abyssal eastern equatorial Pacific (Clarion-Clipperton Fracture Zone). *Deep-Sea Res I* 103: 73-85.  
<http://doi.org/10.1016/j.dsr.2015.04.011>



332 Goineau A, Gooday AJ (2017) Novel benthic foraminifera are abundant and diverse in an  
333 area of the abyssal equatorial Pacific licensed for polymetallic nodule exploration. Sci  
334 Rep 7: 45288. <http://doi.org/10.1038/srep45288>.

335 Gooday AJ, Goineau A (2019) The contribution of fine sieve fractions (63–150 µm) to  
336 foraminiferal abundance and diversity in an area of the eastern Pacific Ocean licensed  
337 for polymetallic nodule exploration. Front Mar Sci.  
338 <http://doi:10.3389/fmars.2019.00114>

339 Heiner, I. (2008) *Rugiloricus bacatus* sp. nov. (Loricifera-Pliciloricidae) and a ghost larva  
340 with paedogenetic reproduction. Syst Biodiv 6: 225-247.

341 Heiner I, Kristensen RM (2009) *Urnaloricus gadi* nov. gen. et nov. sp. (Loricifera,  
342 Urnaloricidae nov. fam.), an aberrant Loricifera with a viviparous pedogenetic life  
343 cycle. J Morphol 270: 129-153

344 Heiner I, Neuhaus B (2007) Loricifera from the deep sea at the Galápagos Spreading Center,  
345 with a description of *Spinoloricus turbatio* gen. et sp. nov. (Nanaloricidae). Helgol  
346 Mar Res 61: 167-182.

347 Heinz P, Geslin E, Hemleben C, 2005. Laboratory observations of benthic foraminiferal  
348 cysts. Mar Biol Res 1: 149-159. <http://dx.doi.org/10.1080/17451000510019114>

349 Higgins RP, Kristensen RM (1986) New Loricifera from South-eastern United States Coastal  
350 Waters. Smithson Contrib Zool 438: 1-70.

351 Jensen P (1982). A new meiofaunal splitter. Ann. Zool. Fennici 19: 233-236.

352 Jones DOB, Amon DJ, Chapman ASA (2017) Mining deep-ocean deposits: what are the  
353 ecological risks. Elements 14: 325-330.

354 Kristensen RM (1983) Loricifera, a new phylum with Aschelminthes characters from the  
355 meiobenthos. Z Zool Syst Evolutionsforsch 21: 163-180.

356 Kristensen RM (1991a) Loricifera. In: Harrison FW, Ruppert EE (eds). Microscopic  
357 Anatomy of Invertebrates, Vol 4, Aschelminthes. Wiley-Liss, New York, pp 351-375.

358 Kristensen RM (1991b) Loricifera—a general biological and phylogenetic overview. Verh.  
359 Dtsch Zool Ges 84: 231-246.

360 Kristensen RM, Shirayama Y (1988) *Pliciloricus hadalis* (Pliciloricidae), a new Loriciferan  
361 species collected from the Izu- Ogasawara Trench, Western Pacific. Zool Sci 5: 875-  
362 881.

363 Kristensen RM, Neves RC, Gad G (2013) First report of Loricifera from the Indian Ocean: a  
364 new *Rugiloricus* species represented by a hermaphrodite. Cah Biol Mar 54: 161-171.

365 Lodge M, Johnson D, Le Gurun G, Wengler M, Weaver P, Gunn V (2014) Seabed mining:  
366 International Seabed Authority environmental management plan for the Clarion-  
367 Clipperton Zone. Mar Policy 49: 66-72.

368 Lodge M, Verlaan PA (2018) Deep-Sea mining: international regulatory challenges and  
369 responses. Elements 14: 331-336

370 Mahatma R (2009) Meiofauna Communities of the Pacific Nodule Province: abundance,  
371 diversity and community structure. PhD Thesis, University of Oldenburg, 143 pp.  
372 Available at <http://oops.uni-oldenburg.de/1009/1/mahmei09.pdf>.

373 Neves RC, Kristensen RM (2014) A new type of loriciferan larva (Shira larva) from the deep  
374 sea of Shatsky Rise, Pacific Ocean. Org Divers Evol 14: 163-171.  
375 <http://doi.org/10.1007/s13127-013-0160-4>

376 Neves RC, Kristensen RM (2016) *Spinoloricus neuhausi* (Loricifera, Nanaloricidae), a new  
377 deep sea species from Galápagos Spreading Center. Zool Anz 265: 171-177,  
378 <http://dx.doi.org/10.1016/j.jcz.2016.01.001>.

379 Neves RC, Gambi C, Danovaro R, Kristensen, RM (2014) *Spinoloricus cinziae* (Phylum  
380 Loricifera) a new species from a hypersaline anoxic deep basin in the Mediterranean  
381 Sea. Syst Biodivers 12: 489-502.

- 382 Neves RC, Reichert H, Sørensen MV, Kristensen RM (2016) Systematics of phylum  
383 Loricifera: identification keys of families, genera and species. *Zool Anz* 265:141-170
- 384 Neves RC, Kristensen RM, Rohal M, Thistle D, Sørensen MV (2018) First report of  
385 Loricifera from the North East Pacific Region, with the description of two new  
386 species. *Mar Biodiv.* <https://doi.org/10.1007/s12526-018-0898-1>
- 387 Nozawa F, Kitazato H, Tsuchiya M, Gooday AJ (2006) ‘Live’ benthic foraminifera at an  
388 abyssal site in the equatorial Pacific nodule province: abundance, diversity and  
389 taxonomic composition. *Deep-Sea Res I* 51: 1406-1422.
- 390 Ohkawara N, Kitazato H, Uematsu K, Gooday AJ (2009) A minute new genus and species of  
391 monothalamous Foraminifera (Protista) from the abyssal Pacific. *J Micropalaeontol*  
392 20: 143-151.
- 393 Radziejewska T (2002) Responses of deep-sea meiobenthic communities to sediment  
394 disturbance simulating effects of polymetallic nodule mining. *Int Rev Hydrobiol* 87:  
395 457-477.
- 396 Radziejewska T, Rokicka-Praxmajer J, Stoyanova V (2001) IOM BIE revisited: meiobenthos  
397 at the IOM BIE site 5 years after the experimental disturbance. *Proceedings of the*  
398 *fourth (2001) Ocean Mining Symposium, Szczecin, Poland, September 23-27, 2001,*  
399 *p. 63-68.*
- 400 Smith CR, Dahlgren TG, Drazen J, Gooday AJ, Glover AG, Kurras G, Martinez AP, Shulse  
401 C, Spickermann R, Sweetman AK, Vetter E. (2013) Abyssal Baseline Study  
402 (ABYSSLINE) Cruise Report. *Seafloor Investigations Report 2013-1304-051J-*  
403 *SRDL-AB01.*
- 404

405

406 **Figure captions**

407 **Fig. 1** Life cycle of the family Pliciloricidae (after figure 19 in Neves et al., 2016). The  
408 loriciferans described here correspond to C (first instar of Higgins larva; Fig. 2a-d), D (late  
409 instar of Higgins larva; Fig. 2e,f), N (last instar of Higgins larva with postlarva inside; Fig.  
410 3c,d,f), and O (last instar of Higgins larva with postlarva and the young male inside; Figs  
411 3,4). Reprinted from Zoologischer Anzeiger, vol. 265, R.C. Neves, H. Reichert, M.V.  
412 Sørensen, R.M. Kristensen, Systematics of phylum Loricifera: Identification keys of families,  
413 genera and species, pp. 141-170, 2016, with permission from Elsevier.

414

415 **Fig. 2** Light micrographs of undescribed *Rugiloricus* Higgins larvae inhabiting agglutinated  
416 structures. **a-d** Specimen from MC09 (3-4 cm layer). **a** 1st instar Higgins larva as first found  
417 inside agglutinated sphere. **b** Agglutinated sphere distorted after attempts to extract larva. **c, d**  
418 Same larva viewed using differential interference contrast. **e, f** Sample MC11 (0-0.5 cm  
419 layer). **e** Late instar Higgins larva as first found inside agglutinated sphere. **f** Same larva after  
420 removal from sphere. Abbreviations: ab = abdomen; in = introvert; mg = midgut; ph =  
421 pharyngeal bulb; se<sub>1</sub> = posterodorsal seta; to = toes. Scale bars = 100 µm (**a-c, e**), 50 µm (**d,**  
422 **f**)

423

424 **Fig. 3** Light micrographs of undescribed *Rugiloricus* larval moult containing postlarvae and  
425 associated agglutinated structure from sample MC22 (0-0.5 cm layer). **a-d** Specimen 1. **a**  
426 Agglutinated structure containing loriciferan as first found. **b** Remains of agglutinated  
427 structure after removal of loriciferan. **c, d** Specimen removed from sphere, viewed using  
428 differential interference contrast (DIC). **e, f** Specimen 2. **e** Agglutinated structure containing  
429 loriciferan as first found. **f** Specimen removed from sphere, viewed using DIC.  
430 Abbreviations: alv = anterior lateroventral seta; po = postlarva; sc = moulted larval scalids;  
431 se<sub>2</sub> = posterolateral seta; to = larval toes. Scale bars = 100 µm

432

433 **Fig. 4** Light micrographs of undescribed *Pliciloricus* (last instar Higgins larva with very thin  
434 cuticle of postlarva and young male inside) from Sample MC25 (0-0.5 cm layer). **a** Specimen  
435 as first found, stained with Rose Bengal. **b-d**. Specimen viewed using differential  
436 interference contrast. **b** View with one toe clearly shown. **c, d** Photographs with  
437 morphological features labelled. Abbreviations: alv = anterior lateroventral seta (Higgins  
438 larva); br = brain (adult); ca = cuticle (adult); cs = clavoscalids (adult); le = exuvium (Higgins  
439 larva); mc = mouth cone (adult); mg = midgut (adult); mt = mouth tube (adult); ple =  
440 postlarval exuvium; ru = ruff (adult); sc<sub>a</sub> = spinoscalids (adult); sc<sub>1</sub> = scalids of moulted  
441 Higgins larva; se<sub>2</sub> = posterolateral seta (Higgins larva); sz = spermatozoa (adult); te = testis  
442 (adult); to = larval toes (Higgins larva). Scale bars = 50 µm.

443

444 **Fig. 5** Differential interference contrast micrographs of undescribed *Pliciloricus* from sample  
445 MC25 (0-0.5 cm layer). This is the same animal as in Fig. 4. Abbreviations: alv = anterior  
446 lateroventral seta (moulted Higgins larva); br = brain (adult); ia = internal (prepharyngeal)  
447 armature (Higgins larva); in = introvert (adult); ru = ruff (adult); sc<sub>1</sub> = scalids (Higgins larva);  
448 se<sub>2</sub> = posterolateral setae (Higgins larva); to = larval toe (Higgins larva). Scale bars = 20 µm  
449 (**a-c**), 50 µm

450

451 **Fig. 6** Light micrographs of various spherical structures from ABYSSLINE samples. **a** Group  
452 from sample MC22 (0.5-1.0 cm, 150-300 µm fraction) including: sphere with *Rugiloricus*  
453 larval moult containing postlarvae (specimen 1; largest sphere on left), three radiolarian tests  
454 hosting foraminiferal inhabitants (3 spherical structures on right-hand side), and an

455 agglutinated structure containing a cell-like body, possibly an organic-walled monothalamid  
456 foraminiferan (top centre). **b** Radiolarian test hosting 4 spherical monothalamous  
457 foraminifera; from sample MC20 (0.5-1.0 cm, 150-300  $\mu\text{m}$  fraction). **c.d** Two different kinds  
458 of spherical monothalamids containing dark stercomata (waste material); from sample MC11  
459 (0-0.5 cm, 63-125  $\mu\text{m}$  fraction). **e** Agglutinated sphere containing cell with prominent  
460 nucleus; this is from the same sample as the loriciferan (MC22) and probably the same as the  
461 structure in Fig 6a, top centre. **f** Similar sphere containing cell body stained with Rose  
462 Bengal; from sample MC05 (0-0.5 cm, 63-125  $\mu\text{m}$  fraction). Scale bars = 200  $\mu\text{m}$  (**a**), 50  $\mu\text{m}$   
463 (**b-f**)  
464

1  
2  
3  
4**Table 1.** Station details. MC = Megacorer

<b>Cruise</b>	<b>Site</b>	<b>Sample</b>	<b>Latitude °N</b>	<b>Longitude °W</b>	<b>Water depth (m)</b>
AB01	H	MC09	13°53.300'	116°41.399'	4150
AB01	J	MC11	13°54.104'	116°35.402'	4166
AB02	S08	MC22	12°11.409'	117°22.384'	4179
AB02	U15	MC25	12°34.953'	116°39.058'	4224

5

1

2

3 **Table 2.** Sediment layers and sieve fractions analysed for each of the samples in which loriciferans were found. The figures in columns 3-6  
 4 indicate the numbers of eighth splits sorted in each sieve residue.

5

Sample	Sediment layer	Sieve fraction ( $\mu\text{m}$ )				Reference
		>300	150-300	125-150	63-125	
MC09	0-0.5 cm	3/8	3/8	1/8	1/8	Goineau & Gooday (2017) Goineau & Gooday (unpublished) Gooday and Goineau (2019)
	0.5-1.0 cm	3/8	3/8	1/8	1/8	
	1.0-1.5 cm	1/8	1/8			
	1.5-2.0 cm	1/8	1/8			
	2.0-3.0 cm	1/8	1/8			
	3.0-4.0 cm	1/8	1/8			
	4.0-5.0 cm	1/8	1/8			
MC11	0-0.5 cm	3/8	3/8	1/8	1/8	Goineau & Gooday (2017) Goineau & Gooday (submitted) Gooday and Goineau (2019)
	0.5-1.0 cm	3/8	3/8	1/8	1/8	
MC22	0-0.5 cm	3/8	3/8			Goineau & Gooday (2017) Goineau & Gooday (submitted)
	0.5-1.0 cm	3/8	3/8			
MC25	0-0.5 cm	4/8	4/8			Goineau & Gooday (2017) Goineau & Gooday (submitted)
	0.5-1.0 cm	4/8	4/8			

6

1

2 **Table 3.** Identity and dimensions of Loricifera and associated agglutinated spheres

3

<b>Sample</b>	<b>Sediment layer</b>	<b>Genus</b>	<b>Sphere diameter</b>	<b>Length of larva</b>	<b>Length of postlarva</b>	<b>Registration number</b>
MC09	3-4 cm	<i>Rugiloricus</i>	230 µm	171 µm	-	NHMD 287289
MC11	0-0.5 cm	<i>Rugiloricus</i>	248 µm	173 µm	-	NHMD 287290
MC22-1	0.5-1.0 cm	<i>Rugiloricus</i>	275 µm	212 µm	98 µm	NHMD 287291
MC22-2	0.5-1.0 cm	<i>Rugiloricus</i>	263 µm	218 µm	98 µm	NHMD 287292
MC25	0-0.5 cm	<i>Pliciloricus</i>	-	209 µm	-	NHMD 287293

4

paedogenetic cycle

sexual cycle













