

1 **Significance of the nudiform and tectiform modes of silica deposition, lorica assembly**  
2 **and cell division in choanoflagellates as exemplified by *Stephanoeca diplocostata* and**  
3 ***Enibas* spp.**

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17 **Highlights:**

18 1) Complete silicon starvation of tectiform *Stephanoeca diplocostata* results in  
19 diagonal (nudiform) division.

20 2) Silicon-replenished *S. diplocostata* cells accumulate costal strips in a nudiform  
21 manner

22 3) ‘Nudiform loricae’ assembled on silicon-replenished cells display variations with  
23 respect to the transverse costae

24 4) Newly identified nudiform genus *Enibas* possesses *Stephanoeca*-like loricae with  
25 similarities to nudiform loricae of *S. diplocostata*

26 5) Suggested that morphological similarities together with recent phylogenetic  
27 analyses could imply *Enibas* species had a *Stephanoeca*-like ancestor

28

29 **Keywords:** Acanthoecida, Choanoflagellata, *Stephanoeca diplocostata*, nudiform-tectiform  
30 relationship, *Enibas* lorica morphology, *Enibas* evolution

31

32 **Abstract**

33 The deposition of silicified costal strips and lorica assembly in choanoflagellates is precisely  
34 linked to the cell cycle. A minority of species undergo nudiform division whereby a loricate  
35 cell divides to produce a naked daughter cell that deposits a set of costal strips and then  
36 assembles a lorica. Most species undergo tectiform division whereby a parent loricate cell  
37 produces a set of costal strips, divides and passes on the stored strips to a daughter cell that  
38 immediately assembles a lorica. Many phylogenetic analyses recover nudiform and tectiform  
39 species as sister-clades giving the impression that they are distinct evolutionary lineages.  
40 However, the tectiform species *Stephanoeca diplocostata* is capable of undergoing nudiform  
41 division and depositing costal strips and assembling a lorica with certain modifications in a  
42 nudiform manner. The recent discovery of a new genus, *Enibas*, comprising species with  
43 *Stephanoeca*-like loricae that undergo nudiform cell division and on phylogenetic analysis  
44 occur as a sister group to other nudiform species has drawn attention to whether there are also  
45 unique features in lorica construction. A range of *Enibas* cells is illustrated and it appears that  
46 there are unique features which might be interpreted as being derived from a *Stephanoeca*-  
47 like ancestor.

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52 **Keywords:** Acanthoecida, Choanoflagellatea, *Stephanoeca diplocostata*, nudiform-tectiform  
53 relationship, *Enibas* lorica morphology, *Enibas* evolution

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57 **Introduction**

58 Lorica-bearing choanoflagellates, as their name implies, are distinguished by the possession  
59 of a 'basket-like' lorica composed of siliceous ribs known as costae. They are distributed  
60 universally in aquatic environments, mostly marine or brackish water, although there are at  
61 least two records of freshwater species (Nitsche 2014; Paul 2011). Taxonomically, they  
62 comprise one of two orders, Acanthoecida, within the class Choanoflagellata, the other  
63 equally important order being the non-loricate species taxonomically placed within the  
64 Craspedida.

65

66 Choanoflagellates are non-phototrophic protists readily distinguished by their possession of a  
67 single apical flagellum surrounded at its base by a collar of microvilli. They feed by  
68 entrapment of food particles, mainly bacteria and other prokaryotes, on the outer surface of  
69 the microvillous collar. Choanoflagellates are a well-established group of protists, the first  
70 complete record being that of Henry James-Clark in 1866. In the same publication James-  
71 Clark (1866) noted that the choanoflagellate cell resembled flagellated cells (choanocytes)  
72 within sponges and this observation established the generally accepted view that the  
73 choanoflagellates were the unicellular ancestors of sponges and therefore Metazoa.

74 Molecular phylogeny has now firmly established choanoflagellates and Metazoa as sister-  
75 groups (see Leadbeater 2015) although there is some doubt as to whether the coincidence in  
76 morphology between choanoflagellates and sponge choanocytes is homologous (Pozdnyakov  
77 2017).

78

79 Currently there are over 150 described species of loricate choanoflagellates (unpublished  
80 database) and since lorica form and the pattern of costae are used as taxonomic characters  
81 there are approximately the same number of variations in these characters as there are

82 species. Size can vary from a single costal ring in *Monocosta fennica* Thomsen 1979 to a  
83 relatively large lorica 60µm in length as illustrated by *Diaphanoeca multiannulata* Buck  
84 1981. Since choanoflagellates feed by generating water currents from which bacteria and  
85 other small particles are extracted, the lorica must provide for inhalant and exhalant currents  
86 of water. Cell division also requires sufficient space for a daughter protoplast resulting from  
87 division to be released from a lorica. In some species the protoplast is fully surrounded by the  
88 lorica whereas in others the protoplast is only partially covered at the posterior end. The  
89 possible functions of the lorica are much disputed but they are likely to be many, including  
90 protection of the protoplast, enhancement of the water currents and increasing the efficiency  
91 of prey capture (Asadzadeh et al. 2018).

92

93 Despite considerable variation in lorica form and architecture, the basic units of lorica  
94 construction, rod-shaped costal strips, are universal. Costal strips are produced within  
95 membrane-bounded vesicles and when mature are extruded either onto the surface of the cell  
96 or onto the inner surface of the microvillous collar. Costal strips are moved into position to  
97 form a lorica by extension of selected microvilli of the collar and a left-handed (clockwise)  
98 rotation of the developing lorica. These two movements can fundamentally account for all the  
99 various nuances of lorica construction (see Leadbeater 2015).

100

101 Whilst the development of siliceous costal strips and their assembly into a lorica is universal,  
102 there are two major variations based on the interaction between the sequence of events  
103 involved in lorica production and the cell cycle (Manton et al. 1981). One variation is  
104 displayed by ‘nudiform’ species and this is distinguished by diagonal cell division of a parent  
105 loricate cell to produce two daughter cells. One daughter cell remains with the parent lorica  
106 whilst the other, termed the juvenile, is ‘naked’ and swims away from the parent lorica.

107 Subsequently the juvenile deposits a complete set of costal strips on the protoplast surface  
108 after which it assembles a lorica within two to five minutes. The second variation,  
109 ‘tectiform’, is displayed by the majority of species and involves a parent cell with a lorica  
110 producing and accumulating a set of costal strips that are stored on the inward facing surface  
111 of the collar microvilli. When the accumulation of costal strips is complete, the parent cell  
112 undergoes an ‘inverted’ division whereby the upper daughter cell is rotated through 180° so  
113 the two daughter flagellar poles, one on each cell, face each other. The juvenile is then  
114 pushed backwards out of the parent lorica bearing the accumulation of costal strips and  
115 within 2-5 minutes a new lorica is assembled (see Leadbeater 2015). To date only nine  
116 species routinely undergo nudiform division (six species as discussed in Leadbeater (2015)  
117 and three recently described species of *Enibas* (Schiwitz et al. 2019; Schiwitza and Nitsche  
118 2021; Schiwitza and Thomsen 2022)).

119

120 Whilst the overall processes of costal strip manufacture and lorica assembly are standard for  
121 both nudiform and tectiform species, nevertheless the differences, which involve the timing  
122 of events within the cell cycle and the morphological differences of cell division, are also  
123 recognised in most molecular phylogenies. Nudiform species have been consistently  
124 recognised as monophyletic in molecular phylogenies (Carr et al. 2008; Nitsche et al. 2011;  
125 Schiwitza et al. 2019) but the situation is not so clear with regards to tectiform species, where  
126 some phylogenies have recovered tectiform species as being monophyletic (Carr et al. 2008;  
127 Nitsche et al. 2011) with the nudiform and tectiform taxa forming sister clades and thus  
128 justifying their separation into two taxonomic families, namely Acanthoecidae Norris 1965  
129 emend. Nitsche et al. 2011 (nudiform species) and Stephanoecidae Leadbeater in Nitsche et  
130 al. 2011 (tectiform species). However, other published trees have recovered tectiform

131 paraphyly which would invalidate Stephanoecidae as a family and require the description of  
132 multiple smaller families for groupings of tectiform species (see Carr and Leadbeater 2022).

133

134 Further insights into the interaction between costal strip deposition and lorica production  
135 within the cell cycle have come from work carried out on cultures of *Stephanoeca*  
136 *diplocostata* Ellis 1929. These have revealed that, whilst this species normally displays  
137 tectiform cell division, under a variety of experimental conditions it can also undergo  
138 diagonal division to produce a naked motile juvenile and subsequently deposit costal strips in  
139 a nudiform manner. Results contributing to this conclusion have accumulated piecemeal and  
140 it is only recently that this conclusion has been fully appreciated (Leadbeater 1989, 2015 loc.  
141 cit. Chapter 8).

142

143 Until three years ago conventional wisdom held that there were only six nudiform species  
144 distributed within four genera, namely *Acanthoeca* Ellis 1929, *Polyoeca* Kent 1881, *Savillea*  
145 Norris 1965 emend. Loeblich III 1967 and *Helgoeca* Leadbeater in Leadbeater et al. (2008).  
146 In 2019 Schiwitza et al. (2019) published details of a new nudiform genus *Enibas* Schiwitza,  
147 Arndt and Nitsche, 2019 that was to have a major impact on our appreciation of acanthoecid  
148 phylogeny. *Enibas tolerabilis* Schiwitza, Arndt and Nitsche 2019, the generic type species of  
149 *Enibas*, possessed a lorica that appeared to belong to the tectiform genus *Stephanoeca* Ellis  
150 1929. However, in a phylogenetic analysis including transcriptome data from five genes, *E.*  
151 *tolerabilis*, together with two environmental sequences, was recovered as a distinct sister  
152 clade to all other previously sequenced Acanthoecidae (nudiform taxa). This publication was  
153 followed almost immediately by establishment of a second species, *Enibas thessalia*  
154 Schiwitza and Nitsche 2021, this time the phylogenetic analysis was based on partial SSU  
155 rDNA (Schwitza and Nitsche 2021). In this publication the authors also included a sequence

156 of images that showed *E. tolerabilis* divided diagonally to produce a naked juvenile, a classic  
157 nudiform character (Schiwitz and Nitsche 2021 loc. cit. Fig. 4A-I). Finally, the long-  
158 established species *Stephanoeca urnula* Thomsen 1973, which morphologically resembles *E.*  
159 *thessalia*, was also shown in a molecular analysis of SSU and LSU rRNA to cluster within  
160 the nudiform family, Acanthoecidae, as a member of the genus *Enibas* (Schiwitz and  
161 Thomsen 2022).

162

163 The family Acanthoecidae now contains nine species comprising three closely related *Enibas*  
164 species and six belonging to four genera that show little morphological resemblance to each  
165 other or with *Enibas* and yet molecular phylogenetic analyses consistently recover the family  
166 Acanthoecidae as being monophyletic (Carr and Leadbeater 2022). The fact that *Enibas*  
167 species have loricae that would normally have been confused with those of *Stephanoeca* spp.  
168 and the fact that under certain circumstances *Stephanoeca diplocostata* can behave in a  
169 nudiform manner has prompted a more detailed investigation into the significance and  
170 possible interaction of these two phenomena. The results of these considerations are reported  
171 here.

172

## 173 **Results**

### 174 ***Stephanoeca diplocostata*: Tectiform mode of division and lorica production (Figs. 1-6)**

175

176 *Stephanoeca diplocostata* Ellis 1929 is a widely distributed common species typical of  
177 inshore waters including saltmarshes and brackish water. It has a wide tolerance of salinity  
178 and can be grown at 2 PSU or less. When grown in sterile natural seawater or synthetic  
179 medium with an adequate supply of silicon *S. diplocostata* displays the typical tectiform  
180 mode of cell division and lorica production. The robust behaviour and easy culture of *S.*

181 *diplocostata* has rendered this species ideal for laboratory experimentation (Leadbeater 2015  
182 loc. cit. Chapter 5).

183

184 The lorica of *S. diplocostata* is barrel-shaped, comprising two chambers separated by a waist  
185 which is coincident with the position at which the lorica is attached to the cell (Fig. 1). The  
186 anterior chamber is approximately twice the length (8–12  $\mu\text{m}$ ) of the posterior chamber (5–7  
187  $\mu\text{m}$ ) and about 1.5–2.0 times the width. The bottom two-thirds of the protoplast is located  
188 within the posterior chamber, while the anterior portion, including the flagellum and  
189 surrounding collar of microvilli, project into the anterior chamber (Figs 1, 2). Some loricae  
190 possess a posterior pedicel although this may be absent on sedentary cells. The lorica is two-  
191 layered, with the outer layer containing 15–24 (mode 19) longitudinal costae, each  
192 comprising five costal strips; three in the anterior chamber and two in the posterior. There are  
193 four transverse costae in the anterior chamber that are numbered 1-4 in Fig. 1 from the  
194 anterior backwards as follows: anterior ring (1); anterior intermediate transverse costa (2);  
195 posterior intermediate transverse costa (3); posterior transverse costa (waist) (4). All  
196 transverse costae except the anterior ring are within the longitudinal costae. The anterior ring  
197 is on the outside. The inner layer of the posterior chamber contains between 4 and 13 helical  
198 costae. The average total number of costal strips within a lorica is  $172 \pm \text{SD } 18.1$  (mode 163).  
199 Most of the costal strips in a *S. diplocostata* lorica are narrow crescentic rods that  
200 superficially appear similar although the strips comprising the longitudinal costae are slightly  
201 longer than those forming the transverse costae and have a longer radius of curvature. The  
202 transverse costa located at the waist (Fig. 1 arrow 4) contains between 6-8 slightly thicker  
203 crescentic strips with a shorter radius of curvature, better seen in Figs 7-9 arrows. These are  
204 easily identifiable and, on a tectiform cell, are produced early in the silicon deposition cycle



205 and are therefore useful as markers. An almost transparent organic investment lines the inner  
206 surface of the posterior chamber and extends up to the posterior intermediate transverse costa.

207

208 The cell illustrated in Fig. 1 is typical of early interphase; the protoplast is almost spherical,  
209 the flagellum and collar are visible and two pseudopods each containing a bacterium are on  
210 either side of the collar (p in Fig. 1). Fig. 2 illustrates a cell at late interphase; the protoplast is  
211 large and approximately pear-shaped and substantial accumulations of costal strips are  
212 positioned at the top of the collar microvilli (Fig. 2 asterisks). Fig. 3 illustrates a cell in which  
213 the protoplast has just undergone inverted division. The accumulation of costal strips is now  
214 positioned laterally on the upper daughter cell that is termed the juvenile (j in Fig. 3). The  
215 juvenile will subsequently be pushed backwards out of the parent lorica by two overlapping  
216 extensible threads containing actin.

217

218 A newly released juvenile has costal strips aligned with the lateral surface of the protoplast  
219 and a group of strips at the anterior end (Fig. 4 double-headed arrows). The latter have been  
220 moved from a lateral location as illustrated in Fig. 3 to their anterior alignment shown in Fig.  
221 4. A ring of lorica-assembling collar microvilli at the anterior end of the juvenile can just be  
222 seen (Fig. 4 arrowheads). These will be extended forwards, as shown in Fig. 5 arrowheads, to  
223 lengthen the developing longitudinal costae and to position the costal strips aligned with the  
224 anterior end into the developing transverse costae (Fig. 5 arrows 1-4). Fig. 6 shows an almost  
225 complete lorica with fully extended longitudinal costae and separated transverse costae (Fig.  
226 6 arrows 1-4). Accompanying this extension of the costal strips is a left-handed (clockwise)  
227 rotational movement of the developing lorica which results in the final organisation of costae.

228

229 Details of the order of costal strip production, apical extrusion from the protoplast, storage on  
230 the collar and assembly into a lorica throughout the tectiform cell cycle have been worked out  
231 in detail for *Didymoeca costata* (Valkanov 1970) Doweld 2003 (Leadbeater 2010, 2015).

232 What is apparent with this process is the precise order and positioning the developing costal  
233 strips display at each stage of the cell cycle so that despite the many strips involved the lorica  
234 is an accurately constructed entity, hence the value of lorica detail in a morphological based  
235 species taxonomy.

236

### 237 ***Stephanoeca diplocostata*: Nudiform mode of division and lorica production (Figs 7-18)**

238

239 The nudiform ‘behaviour’ of *Stephanoeca diplocostata* was only recognised twenty-five  
240 years after the first work was carried out. This was partly due to the tectiform mode of  
241 division and lorica production being so overwhelmingly obvious but also because clues  
242 relating to a nudiform mode of division were overlooked as being improbable. The first  
243 indication that modification of the cell cycle might be possible came from silicon depletion  
244 experiments (Leadbeater 1985, 1989). When cultures of *S. diplocostata* were repeatedly  
245 grown in medium with a total lack of silicon they eventually gave rise to fully functional  
246 ‘naked’ cells without loricae that were capable of division (Fig. 10). This result indicated that  
247 growth of *S. diplocostata* displayed a facultative requirement for silicon. From this it was  
248 anticipated that when naked cells were returned to a silicon-rich medium there would be a  
249 generation of cells that remained without loricae since the tectiform mode of lorica  
250 production required a supply of costal strips from a previous cell generation. However,  
251 surprisingly at the time, this was not the result, instead after a period of 24 hours over 80% of  
252 naked cells had produced their own loricae (Leadbeater 1989). Attention at the time was  
253 focussed on the extension of a lag period after silicon addition before growth increased again.

254 This was explained at the time: “to regain normal ‘tectiform’ synchrony between the cycle of  
255 silica uptake and costal strip deposition on the one hand and the cell cycle (interphase and  
256 division) on the other, each naked protoplast must produce two complete sets of strips within  
257 the equivalent of one cell cycle” (Leadbeater 1989).

258

259 Further consideration was given to this phenomenon when the relationship between nudiform  
260 and tectiform was considered from a physiological and evolutionary point of view. It was  
261 then appreciated that the production of costal strips and assembly of the lorica by a naked *S.*  
262 *diplocostata* cell must have required a sequence of events not dissimilar to that well known  
263 for nudiform species such as *Acanthoeca* and *Savillea* (Leadbeater 2015 see Chapter 8).

264

265 When observing fixed *S. diplocostata* cells prepared from a silicon-rich culture it was not  
266 uncommon to detect some cells with costal strip accumulations laterally around the protoplast  
267 as well as on the collar. These were largely ignored as ‘oddities’. However, when silicon-  
268 impoverished cells were re-supplied with silicon, cells with thin loricae were frequently seen  
269 to accumulate costal strips laterally around the protoplast (Figs 7-9). In the two specimens  
270 illustrated in Figs 7 and 8 there are also a few costal strips, including thicker crescentic strips,  
271 at the top of the collar microvilli (Figs 7 and 8 arrows). Since the thicker strips are among the  
272 first to be deposited in a full costal-strip deposition cycle, they may have been deposited  
273 either prior to the lateral bundles of strips located around the protoplast or at the beginning of  
274 a tectiform accumulation at the top of the collar following the lateral nudiform accumulation  
275 on the surface of the protoplast. The latter scenario would be in accord with the conclusion  
276 mentioned above “that two sets of strips must be deposited within the equivalent of one cell  
277 cycle”. The subdivision of the lateral accumulations of strips into subgroups is similar to that  
278 observed in tectiform cells (Fig. 2 and Leadbeater 2015 loc. cit. Figs 7.11- 7.13). In Fig. 9 a

279 complete set of strips has been accumulated around the protoplast and the thicker crescentic  
280 strips are indicated by arrows (Fig. 9 arrows).

281

282 Completely naked silicon-impooverished *S. diplocostata* cells (Fig. 10) present the best  
283 examples of the nudiform mode of lorica production. Costal strips are extruded through the  
284 side of the protoplast and accumulate in groupings on the surface (Fig. 12 asterisks). The best  
285 juvenile cells formalin preserved for electron microscopy from the experiments reported in  
286 Leadbeater (1989) show regular bundles of costal strips that are destined for future  
287 longitudinal costae and swirls of strips that comprise the combined bundles of strips destined  
288 for a transverse positioning on the developing lorica. In Fig. 13 a slightly flattened cell with a  
289 swirl of costal strips reveals the lorica-assembling collar microvilli (Fig. 13 arrows). In Fig.  
290 14 approximately 14 bundles of longitudinal strips can be observed (Fig. 14 arrowheads). The  
291 lorica in Fig. 15 is partially formed and shows approximately 17 longitudinal costae (Fig. 15  
292 arrowheads) and to the left of the protoplast groups of transversely located strips. The thicker  
293 crescentic strips are located towards the rear of the developing lorica (Fig. 15 white arrows).  
294 Note that the nudiform cells covered with accumulated costal strips have retained their  
295 flagella (f in Figs 13-15).

296

297 More completely formed *S. diplocostata* nudiform loricae illustrate several important features  
298 that should be noted (Figs 16-18). In general, the overall form of nudiform loricae comprising  
299 two chambers is reminiscent of equivalent tectiform loricae (compare Figs 17 and 1). In all  
300 nudiform loricae, continuous longitudinal costae can be recognised which is not surprising  
301 since the lorica is assembled involving the collar specific lorica-assembling microvilli.  
302 Occasionally unextended bundles of costal strips are apparent such as in the posterior  
303 chamber of the lorica illustrated in Fig. 16 arrowheads. Transverse elements can also be

304 observed but their appearance is less consistent. This is not surprising since they are not  
305 presented to the lorica-assembling microvilli in the same precise manner as in developing  
306 tectiform loricae (see Figs 4-6). In all nudiform loricae some of the transverse elements,  
307 particularly at the upper end of the anterior chamber are exterior to the longitudinal costae  
308 (Figs 16-18 short arrows) and those towards the bottom of the anterior chamber are internal  
309 (Fig. 18 arrowheads). The anterior ring is not clearly defined but, in most cases, the anterior  
310 opening is bounded by a series of overlapping strips (Fig. 17). In the majority of nudiform  
311 loricae the thicker crescentic transverse costal strips are located at the level of the waist as  
312 they are in tectiform loricae (Figs 16, 17 long arrows). This is even obvious for the partially  
313 developed lorica shown in Fig. 15 white arrows.

314

#### 315 **Diagonal division in *Stephanoeca diplocostata***

316

317 Growth of *S. diplocostata* in batch culture utilising silicon-free medium proceeded at an  
318 equivalent rate to fully silicified cells (Leadbeater 1985, 1989). Eventually the tectiform  
319 mode of cell division ceases and cells divide by a form of diagonal division (Leadbeater 2015  
320 loc. cit. Fig. 5.44).

321

322 In an experiment in which loricate cells grown in silica-rich medium were subjected to  
323 ultrasonic treatment that was powerful enough to dislodge tectiform accumulations of costal  
324 strips, damage loricae and render many cells naked, costal strips were subsequently  
325 accumulated either in the tectiform or nudiform manner according to the degree of damage  
326 (Leadbeater 1989). The choice may well depend on the response of the internal control  
327 mechanism of a cell to the loss of extracellular structures. Naked and partially damaged cells  
328 often exhibited diagonal division as shown in the partially loricate cell in Fig. 11. The two

329 ellipsoidal daughter cells, one slightly above the other, each with its flagellar pole directed  
330 forwards, is like the diagonal division seen under normal conditions in nudiform species  
331 *Acanthoecca spectabilis* (Leadbeater, 2015 loc. cit. Figs 6.37 – 6.41) and *Enibas tolerabilis*  
332 (Schiwitzka and Nitsche 2021 loc. cit. Fig. 4 E-I).

333

334 **Lorica construction in *Enibas urnula*, *Stephanoeca pyxidoides* and variants (Figs 19- 29)**

335

336 The recent discovery of two new loricate choanoflagellate species that resembled  
337 *Stephanoeca* species but which, based on molecular phylogenetic analysis, clustered within  
338 the Acanthoecidae, not only focussed attention on details of the mode of division and cell  
339 cycle of these species but also drew attention to the costal construction of their loricae  
340 (Schiwitzka et al. 2019; Schiwitzka and Nitsche 2021). A new genus *Enibas* was created to  
341 accommodate these species, namely *E. tolerabilis* and *E. thessalia*. In addition, *Stephanoeca*  
342 *urnula* Thomsen 1973, was also found to cluster with the two original *Enibas* species in the  
343 Acanthoecidae and has therefore now been transferred to *Enibas* (Schiwitzka and Thomsen  
344 2022). *Enibas urnula* loricae have for some time been recorded as showing morphological  
345 variation although, since this species was sufficiently distinctive, for many years it has  
346 remained as a single taxon (Thomsen 1979 loc.cit. Figs 6, 41, 42). The two new *Enibas*  
347 species have essentially extended this variation, particularly *E. thessalia* which almost forms  
348 a continuum with *E. urnula*. As Schiwitzka and Thomsen (2022) record, there are three  
349 important morphological features distinctive of *Enibas* loricae that separate this genus from  
350 *Stephanoeca*. These are equivalent to the three zones of the anterior lorica chamber noted in  
351 Fig. 3A of Schiwitzka and Thomsen (2022) and shown here as Zones 1-3 on the loricae of *E.*  
352 *urnula* in Figs 19 and 20 (double-headed arrows 1-3). The anterior zone (1) includes the  
353 distinctive pattern of oblique costae around the anterior lorica opening. The mid-zone (2)

354 contains several continuous transverse elements, and the posterior zone (3) is distinguished  
355 by another pattern of oblique costae. All three of these features are located outside the  
356 underlying longitudinal costae (Fig. 26).

357

358 These three features are explored here with respect to two sets of field collections, one made  
359 from a salt marsh at Freiston Shore, Lincolnshire, UK in 1973 and one made from seashore  
360 rock pools at Concarneau, France in 1976. Figs 19-24 display a selection of cells that show  
361 the range of lorica variation encountered. The feature that indisputably distinguishes all these  
362 loricae and the current *Enibas* species is the arrangement of costal strips that surrounds the  
363 anterior opening of the lorica. This is seen at its best in Figs 20 and 26, 21 and 27, 23 and 28  
364 and is illustrated diagrammatically with the aid of numbers and asterisks in Fig. 25 and with  
365 equivalent insertions in Fig. 28. This arrangement is equivalent to the anterior category of  
366 Schiwitza and Thomsen (2022). The anterior opening is bordered by a ring of external  
367 oblique costal strips (inclination lower right to upper left when viewed from the outside).  
368 Where these inclined strips surround the lorica opening they overlap each other in a  
369 consistent manner; a strip on the right overlaps the adjacent strip to its left (see Fig. 25) such  
370 that the direction of overlaps is consistent with a rising left-handed (clockwise) rotation (see  
371 Leadbeater 2015 Chapter 4). Each oblique strip is associated with the anterior tip of a single  
372 longitudinal costa (1 asterisk in Fig. 25) so there are the same number of longitudinal costae  
373 as there are oblique strips and this appears to be consistent for all *Enibas* loricae. The right-  
374 hand tip of each oblique strip abuts an anterior longitudinal costal strip at some distance from  
375 its upper end (asterisk between 3 and 2 in Fig. 25). The middle of the anterior longitudinal  
376 strip connects with the immediately posterior longitudinal strip (asterisk between 2 and 4 in  
377 Fig. 25) and its tip abuts the adjacent longitudinal costa on its right-hand side (asterisk 5 in  
378 Fig. 25). An important word of caution must be expressed when interpreting costal patterns

379 on specimens prepared as whole mounts for electron microscopy. Air drying causes severe  
380 deformation and collapse of structures because of forces present at the phase boundary as the  
381 liquid evaporates. Comparison of the specimens shown in Figs 20 and 24 illustrates the  
382 relationship between the angle of inclination of the anterior oblique costal strips and the  
383 width (diameter) of the anterior opening – the narrower the aperture the greater the angle of  
384 inclination from the horizontal (Fig. 20). A further problem that must also be taken into  
385 consideration is that costal strips are capable of sliding against each other and so costal  
386 patterns such as seen in Fig 20 may give a misleading impression of the undistorted state.  
387 However, the regularity of the costal strip arrangement and its universality in these loricae  
388 strongly suggests that it reflects the definitive state.

389

390 The transverse elements, and in some cases posterior oblique elements (the mid-chamber and  
391 basal categories of the anterior chamber in Schiwitza and Thomsen (2022)), show  
392 considerable variation and it is based on these characters that a morphological series is  
393 apparent, according to how far up the anterior chamber the elements rise, whether the  
394 transverse elements are inside or outside the longitudinal costae and the number of distinctive  
395 obliquely inclined costae. The lorica shown in Figs 20 and 26 most closely resembles the  
396 classic *Enibas (Stephanoeca) urnula* morphology (Thomsen 1973 loc. cit. Fig. 13). It exhibits  
397 a distinctive band of oblique costae on the basal section of the anterior lorica chamber and  
398 approximately 7 transverse elements in the mid-region both features being external to the  
399 longitudinal costae (Fig. 26). Below the basal band of oblique costae there is a substantial  
400 transverse band of costae that are located inside the longitudinal costae (Fig. 20 white double-  
401 headed arrow 4; Fig. 26 arrowheads). Fig. 19 would also be categorised as being *E. urnula*  
402 although it is less obvious. However, on close inspection of the anterior chamber of the lorica  
403 the three categories of external costae are present on the external surface of the longitudinal



404 costae. The intermediate band of transverse elements extends from the anterior to the basal  
405 bands of oblique costae. In Figs 19, 20, 26 the transverse elements are thinner and more  
406 flexuous than other costal strips comprising the lorica and they do not appear to have precise  
407 interactions with the longitudinal costae as they would normally have in most tectiform  
408 loricae (see Leadbeater 2015 loc. cit. Fig. 4.24).

409

410 The loricae in Figs 21–24 are obviously closely related to *Enibas* spp. but reveal characters  
411 that may require the generic diagnosis of *Enibas* to be broadened. Superficially, the lorica  
412 illustrated in Figs 21 and 27 might appear to belong to a cell of *Enibas thessalia*. Allowing for  
413 the difference in appearance that loricae can have when viewed as whole mounts in TEM and  
414 SEM, the transverse elements in Fig. 27 are not dissimilar to those of the type specimen of *E.*  
415 *thessalia* (Schiwitza and Nitsche 2021 loc. cit. 3B). However, on closer inspection the upper  
416 transverse elements on the mid-region of the anterior chamber are external to the longitudinal  
417 costae (Fig. 27 arrows) whereas the lower elements are internal (Fig. 27 arrowheads). The  
418 same difference can be observed in Fig. 22 where the two groupings of transverse elements  
419 are separated, the upper band being external to the longitudinal costae and the lower band  
420 being internal. The lorica illustrated in Figs 23 and 28 also reveals a single upper transverse  
421 element outside the longitudinal costae (Fig. 28 arrows) and a lower element within the  
422 longitudinal costae (Fig. 28 arrowheads). Figs 24 and 29 illustrate the loricae of two cells  
423 attributed to *Stephanoeca pyxidoides* Leadbeater 1980 (Leadbeater 1980). Both of these  
424 loricae have the typical *Enibas* arrangement of costal strips at the top of the anterior chamber  
425 but the single internal transverse costa is located at the junction between the intermediate and  
426 posterior strips of the longitudinal costae with which it has a more formal relationship (Fig.  
427 29 arrowheads).

428

429 The construction of the longitudinal costae in these loricae is also of significance. The  
430 interaction of the anterior tips of the longitudinal costae with the anterior oblique costal strips  
431 has already been described above and is illustrated in Figs 25 and 28. In the loricae illustrated  
432 in Figs 21-24 and 27-29 the longitudinal costae in the anterior chamber consist of three costal  
433 strips, namely the anterior, intermediate and posterior strips. The length and positioning of  
434 these three strips is particularly clear in Figs 23 and 28. It is at the junction between the  
435 intermediate and posterior strips that the change between external and internal transverse  
436 elements occurs in these loricae (see white rings in Fig. 27). The upper two longitudinal strips  
437 are inside the transverse elements whereas the posterior longitudinal strip is outside the  
438 transverse elements. In all these loricae an almost transparent organic investment extends  
439 from the top of the posterior longitudinal strip of the anterior chamber and lines the rest of the  
440 lorica below this level. In the posterior chamber there are two costal strips comprising each  
441 longitudinal costa and these extend to the base of the lorica outside the transverse or helical  
442 costae. The total number of costal strips in an individual longitudinal costa in these loricae is  
443 therefore five.

444

445 The situation in the three *Enibas* species is currently uncertain. On first inspection it appears  
446 that there are only two strips (equivalent to the anterior and intermediate strips of  
447 *Stephanoeca pyxidoides*) to each longitudinal costa in the anterior chamber (see Figs 19, 20  
448 and 26). Unfortunately, in virtually all published micrographs of *Enibas* loricae, including  
449 those that were illustrated as *Stephanoeca urnula*, the arrangement of the longitudinal costae  
450 below the upper two strips is confusing to say the least. There are four publications that give  
451 a partial insight into this situation, these are Thomsen (1979, loc. cit. Figs 41 and 42),  
452 Takahashi (1981 loc. cit. Fig 10), Vors (1992 loc. cit. Figs 16 B and C) and Bergesch et al.  
453 (2008 loc. cit. Fig. 26). The transverse band of costae, shown in Fig. 20 by the double-

454 headed white arrow (4) and Fig. 26 arrowheads, is internal to the longitudinal costae. This  
455 would suggest that there are only two costal strips to each longitudinal costa in the anterior  
456 chamber and that the changeover from the elements being outside the longitudinal costae to  
457 the band of costae being inside the longitudinal costae occurs in the waist region of the lorica.  
458 Thus, with the addition of two strips per longitudinal costa in the posterior chamber this  
459 would make a total of four throughout. However, the actual number remains uncertain and  
460 can only be resolved by analysis of well-preserved loricae.

461

## 462 **Discussion**

463 The recent discovery of two choanoflagellate species with loricae resembling *Stephanoeca*,  
464 which, when subjected to phylogenetic analysis, were found to cluster within the  
465 Acanthoecidae (nudiform species) was a great achievement and certainly challenged  
466 conventional wisdom. As discussed elsewhere the genus *Stephanoeca* Ellis 1929, although  
467 including a faultless original description of its tectiform reproduction (Ellis 1929), is now  
468 seriously in need of reform since it has accumulated an eclectic range of species that are  
469 small with a vase-like lorica completely enclosing the protoplast (Schiwitz and Thomsen  
470 2022). Nevertheless, using light microscopy *Enibas tolerabilis* and *E. thessalia* would have  
471 almost certainly been identified as species of *Stephanoeca*. Based on the similarity of lorica  
472 morphology and subsequent phylogenetic analysis the transfer of the well-established species  
473 *Stephanoeca urnula* to *Enibas* was inevitable. The fact that many published phylogenetic  
474 analyses recover nudiform and tectiform taxa as sister-clades has given the impression that  
475 the two families are distinct evolutionary lineages.

476

477 However, in tentative steps, it has become apparent that the familiar tectiform *Stephanoeca*  
478 *diplocostata* is capable of nudiform behaviour. As experimentation has shown, the overall

479 sequence of events involved in lorica production, including the deposition of silicified costal  
480 strips, their accumulation on the surface of the cell and subsequent assembly into a lorica are  
481 essentially similar processes in both nudiform and tectiform species. However, the  
482 relationship between the two conditions is complex. Apart from involving an interaction of  
483 the silica deposition cycle with the cell cycle, there are also important morphological  
484 differences that must be accounted for (see Leadbeater 2015 loc. cit. Chapter 8). The latter  
485 must include the orientation of the costal strips during deposition, the order in which the  
486 strips are produced, the location on the cell where strips are extruded and subsequently  
487 accumulated as well as the movements of strips that take place before and during lorica  
488 assembly.

489

490 Silicon deprivation experiments have cast some light on the mechanism controlling the  
491 interaction between the cell cycle and silicon metabolism. The cell cycle is not seriously  
492 disturbed by a lack of silicon. Minute traces of silicon will still be metabolised to produce  
493 fragmentary costal strips but once completely silicon limited, cells continue to undergo  
494 division (Leadbeater 1985). The return of silicon restarts the process of costal strip  
495 production but now the regulatory mechanism is capable of halting cell division until enough  
496 strips to produce a lorica in the nudiform mode is followed by deposition of a set of strips in  
497 the tectiform mode whereupon the cell can divide and complete the process of lorica  
498 production on the juvenile cell. Whether all tectiform species are capable of a similar  
499 response to silicon impoverishment followed by replenishment is not known. Experiments  
500 with *Diaphanoeca grandis* Ellis 1929, another tectiform species, have shown that severe  
501 silicon deprivation followed by replenishment results in some cells accumulating costal strips  
502 on the cell surface in a nudiform manner (Leadbeater 2015 loc. cit. Chapter 8).

503

504 Loricae resulting from the nudiform sequence of events in *S. diplocostata* approximately  
505 retain the form of the tectiform lorica. The accumulated costal strips make contact with the  
506 lorica-assembling collar microvilli and longitudinal costae are clearly identifiable. The  
507 situation regarding transverse elements is somewhat different. There is variation between  
508 individual cells but what can be gathered is that transverse elements and strips can be either  
509 external or internal to the longitudinal costae. External elements are usually towards the top  
510 of the anterior lorica chamber and internal elements towards the bottom. The readily  
511 recognisable thicker crescentic costal strips are usually located at the waist level in nudiform  
512 loricae. The anterior ring, instead of being a ring of costal strips, comprises an overlapping  
513 grouping of strips. A major difference between loricae assembled in the tectiform manner  
514 and those in the nudiform manner is that transverse costal strips are not presented in  
515 horizontal groupings to the lorica-assembling microvilli of the collar. This could explain why  
516 costal strips in many nudiform cells are not always precisely positioned in rings in the  
517 transverse plane.

518

519 The three current *Enibas* species share one morphological feature that is probably unique to  
520 this genus. This feature comprises the external oblique costal strips around the anterior lorica  
521 opening, and their interaction with the adjacent longitudinal costae. It is complex and is  
522 sufficiently distinctive and easily observed as to be of taxonomic significance. In this context  
523 it is now apparent that *Stephanoeca pyxidoides* should be transferred to *Enibas*. However,  
524 before this can be achieved it must be demonstrated that this species undergoes nudiform  
525 division and is phylogenetically closely related to other species of *Enibas*.

526

527 In *Enibas* species the overlap of the anterior oblique strips, right-hand strip over left-hand  
528 strip in a clockwise direction when viewed from above, is reminiscent of the way in which

529 the helical costae in *Savillea parva*, another nudiform species, overlap each other around the  
530 anterior opening (Leadbeater 2008, 2015). However, in *Savillea* spp the longitudinal costae  
531 are external to the helical costae whereas in the three *Enibas* species currently described the  
532 transverse and oblique elements on the anterior lorica chamber are all external to the  
533 longitudinal costae. This was one of the criteria that Schiwitza and Thomsen (2022) added to  
534 their emendation of the generic diagnosis of *Enibas*: “all anterior lorica chamber transverse  
535 costal strips are positioned exterior to the longitudinal strips”.

536

537 The variation in lorica construction of cells collected from Freiston shore, UK and  
538 Concarneau France (Figs 19-29) reveals a continuum of morphology that to some extent  
539 spans the gap between *Enibas urnula* and *Stephanoeca pyxidoides*. All the loricae illustrated  
540 have a similar pattern of costal strips to *Enibas* spp. around the anterior opening and the way  
541 in which the oblique strips interact with the longitudinal costae is similar (see Fig. 25). The  
542 apparent difference in the number of costal strips contributing to each longitudinal costa in  
543 the anterior lorica chamber of *Enibas* spp., with two strips, differs from *Stephanoeca*  
544 *pyxidoides* and the variants which have three strips. The importance of the higher number of  
545 costal strips in *Stephanoeca pyxidoides* and the variants is that it is just below the widest part  
546 of the anterior lorica chamber, at the junction between the intermediate and posterior costal  
547 strips of the longitudinal costae that the transverse elements change respectively from being  
548 external to being internal of the longitudinal costae (see Fig. 27 white rings). In *Enibas* spp.  
549 this change occurs nearer to the waist or actually at the waist. The important question that  
550 remains unanswered is whether there is third longitudinal costal strip between the lower end  
551 of the intermediate longitudinal strip and the longitudinal strips in the posterior chamber

552

553 It is now possible to list the characters that distinguish *Enibas* from *Stephanoeca* and other  
554 tectiform taxa (see also Table 1). The most important character must be that *Enibas* species  
555 undergo division in a nudiform manner, which has been indisputably demonstrated for  
556 *Enibas tolerabilis* (Schiwitza and Nitsche 2021). Morphologically, the costal arrangement  
557 around the anterior aperture and its relationship with adjacent longitudinal costae would  
558 appear to be unique to *Enibas* spp, *Stephanoeca pyxidoides* and variants. The pattern we  
559 observe may be distorted by flattening but, nevertheless, it is consistent and easily  
560 recognisable. The external helical pattern of costae comprising Zone 3 of the anterior lorica  
561 chamber of *Enibas urnula* is unusual. The nearest equivalents are the substantial helical  
562 arrangements of costae on the nudiform *Savillea parva* and *Acanthoeca* spp.

563

564 Two morphological features that appear to be similar and consistent to species of *Enibas*,  
565 *Stephanoeca diplocostata* and *S. norrisii* are that the longitudinal costal strips of the posterior  
566 chamber appear to be outside the transverse (or helical) costae. Secondly, the chirality of  
567 helical costae is left-handed (see especially *Enibas urnula* in Fig. 20), that is the helix rises  
568 from lower right to upper left when viewed from outside with the anterior end uppermost,  
569 equivalent to a clockwise direction when viewed from above (see Leadbeater 2015 loc. cit.  
570 page 68).

571

572 What is now apparent is that *Enibas* species, in addition to nudiform reproduction, display a  
573 suite of morphological characters that sets them apart from the considerable diversity  
574 exhibited by tectiform species. They also differ considerably from other nudiform taxa. Could  
575 it be that the ability of *Stephanoeca diplocostata*, a classic tectiform species, to behave in a  
576 nudiform manner provides some insight into how *Enibas* species have acquired their unusual  
577 status? If comparison is made between features of the ‘nudiform’ lorica of *S. diplocostata* and

578 the lorica of *Enibas urnula*, there are some interesting common features. In particular, the  
579 lack of well-defined transverse costae and the presence of transverse elements being exterior  
580 to the longitudinal costae. The latter is not an exclusive feature, for external transverse costae  
581 have been observed in tectiform taxa such as *Parvicorbicula* spp. (Thomsen et al. 2020) and  
582 *Pseudostephanoeca paucicostata* (Schiwitz et al. 2022). The approximate *Stephanoeca*  
583 lorica shape in both *Enibas* and nudiform *S. diplocostata* - could these features indicate that  
584 *Enibas* might have had a *Stephanoeca* (tectiform) like ancestor? Based on the results for *S.*  
585 *diplocostata* reported here, the morphological traits which we might use to reconstruct the  
586 last common ancestor of *Stephanoeca* and the nudiform species would include: an  
587 approximately similar lorica form and size; a similar number of costal strips in the two forms,  
588 as well as the equivalent positioning of longitudinal, transverse and obliquely inclined costal  
589 strips in both.

590

591 In phylogenetic analyses available at present, albeit with a limited range of species, the root of  
592 Acanthoecida has been disputed, with two alternative positions recovered in different studies. One  
593 placement recovers the nudiform and tectiform species as monophyletic sister-groups (Carr et al.  
594 2008; Nitsche et al. 2011; Schiwitz and Thomsen 2022). The second root placement lies within the  
595 tectiform species, which are recovered as paraphyletic with the monophyletic nudiform group nested  
596 within them (see Fig. 30) and reviewed in Carr and Leadbeater (2022). Tectiform monophyly appears  
597 to be an artefact caused by convergent phylogenetic signals in synonymous codons, with the  
598 paraphyletic tectiform species falling into three independent clades (Carr and Leadbeater 2022). In the  
599 latter analysis the two closest non-nudiform species to the Acanthoecidae are *S. diplocostata*  
600 and *S. norrisii* (Norris) Thomsen (Fig. 30). Of these two species, *Stephanoeca norrisii* would  
601 appear to possess the characters present in the direct ancestor of *Enibas urnula* and, by  
602 extension, all members of Acanthoecidae. Immediate comparison of two specimens of *Enibas*  
603 *urnula* (Figs 31 and 33) shown on either side of a lorica of *S. norrisii* (Fig. 32) show many



604 similar features (see also: Marchant et al. 1987; Norris 1965; Thomsen 1973). The different  
605 categories of costae are numbered to show their equivalence in *Enibas* and *Stephanoeca*  
606 *norrisii*.

607

608 It is interesting how identifications made by independent and impartial observers, without a  
609 knowledge of current taxonomy, can inadvertently confuse close similarities between species  
610 of *Enibas* and *Stephanoeca*. A good example of this phenomenon is demonstrated by  
611 Marchant and colleagues who published a SEM image of a loricate cell that they identified as  
612 *Stephanoeca complexa* Norris (Marchant and Perrin 1986 loc. cit. Fig. 2). On close  
613 examination and with current knowledge this species has the characters of *Enibas tolerabilis*,  
614 (compare with Schiwitza et al. 2019 loc. cit. Fig. 2C). A year later Marchant et al. (1987 loc.  
615 cit. Figs 7 and 8) published another SEM image of *Stephanoeca complexa* but this time the  
616 loricae illustrated are of a species of *Stephanoeca*. Note the longitudinal costae are outside  
617 the transverse elements and there is a suggestion of a costal strip accumulation at the top of a  
618 collar within the lower right lorica.

619

620

## 621 **Material and Methods**

622 For material collected from the field: sampling was from an extensive saltmarsh at Freiston  
623 Shore, Lincolnshire, UK (N 52° 57' 28" E 0° 05' 32") in June 1973 and from tide pools on a  
624 rocky shore outside Concarneau Marine Station, France (N 47° 54' 4.8" W 3° 55' 2.6")  
625 during August and September 1976. At both locations sampling was carried out by hand  
626 using sterile polypropylene sampling bottles. Samples were returned to the laboratory within  
627 two hours of collection for processing.

628

629 On all occasions sampled water was rough filtered through a 25 $\mu$ m mesh plankton net and  
630 allowed to stand in sterile glass bottles for one day at ambient temperature. Suspended  
631 nanoplankton within the clarified water was concentrated over a 3.0 $\mu$ m membrane filter and  
632 subsequently pelleted by centrifugation. A few drops of 2% osmium tetroxide in 0.1M  
633 cacodylate buffer at pH 7.0 were added to the resuspended pellet. Fixed cells were washed  
634 three times with distilled water and deposited onto Formvar coated copper grids. Grids were  
635 coated with gold/palladium and viewed on a Philips EM300 microscope. All figures  
636 depicting cells with loricae are shadowcast whole mounts. Those figures showing costal  
637 strips that appear white on a black background have been 'inverted' using Adobe Photoshop  
638 2022.

639

640 For silicon-depleted cultures of *Stephanoeca diplocostata* and for the reintroduction of  
641 silicon, full details are provided in Leadbeater (1989). Great care must be taken not to expose  
642 cultures to glassware, for dissolution of silica will occur making complete silicon deficiency  
643 impossible. Cells from silicon-controlled experiments were collected in a special culture  
644 apparatus, details of which were published in Leadbeater and Davies (1984). Cells were fixed  
645 in 5% formalin in 0.1M cacodylate buffer at pH 7.0. This fixative had to be used because it  
646 interfered minimally with the subsequent measurement of reactive silicate.

647

## 648 **Conclusion**

649 Silicon replenishment to completely silicon starved *Stephanoeca diplocostata* cells induces  
650 nudiform (diagonal) cell division and subsequently nudiform accumulation of costal strips on  
651 the lateral surface of the juvenile. Lorica assembly proceeds by means of lorica-assembling  
652 collar microvilli which accounts for the production of longitudinal costae in the normal  
653 manner but costae in the transverse plane are usually compound and inclined. They may be

654 inside or outside the longitudinal costae and do not form the usual junctions with longitudinal  
655 costae observed in tectiform cells. Extrusion of mature costal strips in nudiform cells is  
656 through the side of the cell in comparison with tectiform cells where strips are extruded  
657 within the apical collar and stored on the inner surface of the collar microvilli. The recently  
658 described genus *Enibas* contains three species with *Stephanoeca*-like loricae but cell division  
659 is nudiform (diagonal) as are the accumulation of costal strips and the assembly of the lorica.  
660 *Enibas* lorica construction reveals several unique features some in common with nudiform  
661 loricae of *S. diplocostata*. Phylogenetic analysis and the morphological distinctiveness of the  
662 lorica point to the possibility of species of *Enibas* being derived from a *Stephanoeca*-like  
663 ancestor.

664

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677

678

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680 The authors declare that they have no known financial or intellectual conflict of interest that  
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682

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687 **Barry Leadbeater:** Conceptualization, Specimen Collection, Electron Microscopy, Writing -  
688 Original Draft

689 **Martin Carr:** Conceptualization, Methodology, Formal analysis, Writing - Original Draft

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805 **Legends to Figures**

806 **Plate 1. Figures 1-6**

807 Figures 1-6. *Stephanoeca diplocostata*. Tectiform cells at selected stages in the cell cycle.

808 Shadowcast whole mounts. Scale bars 2 $\mu$ m.

809 Figure 1. Early interphase. The protoplast is relatively small and spherical, there are no stored  
810 costal strips at the top of the collar. Two pseudopodia (p) present on either side of the collar,  
811 the one on the right contains a bacterium. Arrows 1-4 point to the four transverse costae of  
812 the anterior chamber.

813 Figure 2. Late interphase. Protoplast with a substantial accumulation of costal strips  
814 (asterisks) at the top of the collar.

815 Figure 3. Two daughter protoplasts following division. The upper protoplast known as the  
816 juvenile (j) with a lateral covering of costal strips.

817 Figure 4. Newly released juvenile with lateral and anterior collections of costal strips (two  
818 headed arrows). Arrowheads indicate lorica assembling collar microvilli.

819 Figure 5. Lorica assembly on juvenile protoplast. Arrowheads indicate lorica-assembling  
820 collar microvilli. Arrows 1-4 show position of developing transverse costae.

821 Figure 6. Late stage in lorica assembly. Arrows and arrowheads as described in Fig. 5.

822 Figures 7-9. Three silicon-starved cells resupplied with silicon. All have 'nudiform'  
823 accumulations of costal strips around protoplast. Scale bars 2 $\mu$ m.

824 Figures 7 and 8. Lateral nudiform accumulations of costal strips around protoplast (n in Fig.  
825 7). Costal strips at top of collar are in the tectiform position. The presence of thicker  
826 crescentic costal strips (arrows) indicates that the accumulation is early in the silica  
827 deposition cycle.

828 Figure 9. Expanded nudiform accumulation of costal strips from surface of protoplast. Note  
829 subgroupings and six thicker crescentic strips (arrows).

830 **Plate 2 Figures 10-15**

831 Figures 10, 12-15. Silicon-starved cells before and after silica replenishment. Fig. 11  
832 'nudiform' dividing cell. Shadowcast whole mounts. Scale bars 2 $\mu$ m.

833 Figure 10. Naked, silicon-starved cell, showing apical flagellum (f), collar microvilli (c)  
834 pseudopodium containing a bacterium (p) and protoplast completely devoid of costal strips.

835 Figure 11. Ultrasonically treated cell with remaining lower half of a lorica, the upper half has  
836 been destroyed. Protoplast subsequently has divided in nudiform mode. Both ellipsoidal  
837 daughter protoplasts with forwardly directed flagella.

838 Figure 12. Previously naked protoplast surrounded laterally by subgroupings of costal strips  
839 (asterisks).

840 Figures 13 and 14. Two silicon replenished protoplasts with accumulations of costal strips.  
841 Strips that will give rise to longitudinal costae in subgroupings (Fig. 14 arrowheads). Swirl of  
842 strips in Fig 13 will form future transverse elements. Arrows (Fig. 13) point to lorica-  
843 assembling collar microvilli. (f) flagellum. Formalin fixed cells.

844 Figure 15. Partially completed nudiform lorica with longitudinal costae in place (arrowheads)  
845 but rotation of transverse elements partially complete. Thicker crescentic costal strips shown  
846 with white arrows. (f) flagellum.

847 **Plate 3 Figures 16-18**

848 Figures 16-18. Three 'nudiform' loricae to show general distribution of costae. On all loricae  
849 it is possible to distinguish longitudinal costae. The transverse 'elements' comprise circular  
850 overlapping groupings of costal strips sometimes with groups not fully extended. Portions of

851 transverse elements exterior to the longitudinal costae indicated by short arrows. Position of  
852 crescentic strips comprising transverse elements in loricae in Figs 16 and 17 indicated with  
853 longer arrows. Transverse costal strips internal to longitudinal costae indicated by arrowheads  
854 in Fig. 18. The cell in Fig. 18 had accumulated some thicker crescentic strips of the next  
855 tectiform generation indicated by asterisks. Shadowcast whole mounts. Fig. 18 reversed print.  
856 Scale bars 2 $\mu$ m.

857 **Plate 4 Figures 19-24**

858 Figures 19-24. A selection of loricate cells exhibiting *Enibas* like loricate features.  
859 Shadowcast whole mounts. Scale bars 2 $\mu$ m.

860 Figures 19 and 20. *Enibas urnula*. Double headed arrows indicate the three zones of costae  
861 outside the longitudinal costae. Zone 1 comprises the obliquely inclined strips around the  
862 anterior aperture. Zone 2 includes the continuous transverse elements. Zone 3 indicates a  
863 region of obliquely inclined costae. The lorica in Fig. 19 contains approximately 18  
864 longitudinal costae and that in Fig 20 contains 16 longitudinal costae (see also Fig. 26).

865 Figure 21. An *Enibas* like lorica with a mid-anterior chamber band of continuous transverse  
866 costae but without a basal band of obliquely inclined costae (see also Fig. 27).

867 Figure 22. *Enibas* like lorica with two bands of transverse elements in the mid- and basal  
868 regions of the anterior chamber. The band in the mid-region is external to the longitudinal  
869 costae. The band towards the base is inside the longitudinal costae.

870 Figure 23. An *Enibas* like lorica with a single band of costal strips outside the longitudinal  
871 costae in the mid-lorica region and another single band of costal strips at the level of the  
872 junction between the intermediate and posterior costal strips of the longitudinal costae inside  
873 the longitudinal costae towards the lorica chamber base (see also Fig. 28).

874 Figure 24. A cell of *Stephanoeca pyxidoides* with a single internal transverse band of costal  
875 strips at the level of the junction between the intermediate and posterior costal strips of the  
876 longitudinal costae (see also Fig. 29).

877 **Diagram Figure 25**

878 Figure 25. Diagram showing the organisation of the oblique and longitudinal costal strips  
879 around the anterior lorica opening of dried specimens of *Stephanoeca pyxidoides* and variants  
880 (modified after Leadbeater 1980). This pattern is also common, with occasional minor  
881 variation, to current species of *Enibas*. The numbering indicates: 1. An oblique costal strip  
882 contributing to the ring surrounding the anterior lorica aperture; 2. The curved anterior costal  
883 strip of a longitudinal costa; 3. A neighbouring oblique costal strip; 4. The intermediate costal  
884 strip of a longitudinal costa. 5. An intermediate longitudinal costal strip of an adjacent  
885 longitudinal costa. The asterisks denote the points of contact between the tip of a costal strip  
886 with the mid-portion of an adjacent strip. Equivalent numbers and asterisks are also shown on  
887 the lorica in Fig. 28.

888 **Plate 5 Figures 26-29**

889 Figures 26-29. Four *Enibas* loricae showing transverse bands of costal strips, those outside  
890 the longitudinal costae are indicated by arrows and those inside shown by arrowheads.  
891 Shadowcast whole mounts, reversed prints. Scale bars 2 $\mu$ m.

892 Figure 26 *Enibas urnula*. On the anterior chamber the three zones of external costae are  
893 clearly seen. The anterior Zone 1 comprising oblique costae around the anterior lorica  
894 aperture; Zone 2 comprises approximately seven flexuous transverse elements; Zone 3  
895 consists of a dense group of oblique costae. Below Zone 3 is a compact band of transverse  
896 costae inside the longitudinal costae at about waist height. (see also Fig. 20).

897 Figure 27. Lorica with two anterior external transverse elements outside longitudinal costae  
898 and two posterior internal transverse elements. The two circles are on a single longitudinal  
899 costa. The upper, smaller circle shows the intermediate costal strip of the longitudinal costa  
900 underneath the overlying transverse costa whereas the lower, larger circle shows the junction  
901 between the intermediate and the posterior costal strips of the longitudinal costa overlying the  
902 internal transverse costa. (see also Fig. 21).

903 Figure 28. Lorica with one mid-chamber external transverse costa and one lower chamber  
904 internal costa (see also Fig. 23).

905 Figure 29. *Stephanoeca pyxidoides* with a single internal transverse costa towards the base of  
906 the anterior chamber (see also Fig. 24). Scale bars 2 $\mu$ m.

#### 907 **Cladogram Figure 30**

908 Figure 30. Cladogram of Acanthoecida (amended from Carr and Leadbeater 2022). The  
909 choanoflagellate species have been rooted with a clade of six metazoans. Both the Metazoa and  
910 Craspedida clades have been collapsed. Asterisks denote strongly supported (maximum  
911 likelihood bootstrap percentage  $\geq 70\%$ , Bayesian inference posterior probability  $\geq 0.97$ ) nodes.

#### 912 **Plate 6 Figure 31-33**

913 Figures 31-33. Two images of *Enibas urnula* on either side of a lorica of *Stephanoeca*  
914 *norrisii*. Numbering on loricae shows approximately equivalent zones of costal strips in all  
915 three specimens. Shadowcast whole mounts. Scale bars 2 $\mu$ m.

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