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1 **Do chitons have a brain? New evidence for diversity and complexity in the polyplacophoran**

2 **central nervous system**

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10 **Running title:** Do chitons have a brain?

11 **Abstract**

12 Molluscs demonstrate an astonishing degree of morphological diversity, and the relationships

13 among molluscan clades have been debated for more than a century. Molluscan nervous

14 systems range from simple 'ladder-like' arrangements of nerve cords to the complex brains

15 of cephalopods. Chitons (Polyplacophora) are assumed to retain many molluscan

16 plesiomorphies, lacking neural condensation and ganglionic structure, and therefore a brain.

17 We reconstructed three-dimensional anatomical models of the nervous system in eight

18 species of chitons in an attempt to clarify chiton neuroarchitecture and its variability. The

19 specimen material incorporated both new data and digitised historic slide material originally

20 used in the work of malacologist Johannes Thiele (1860-1935). Reconstructions of whole

21 nervous systems in *Acanthochitona fascicularis, Callochiton septemvalvis, Chiton olivaceus,*

22 *Hemiarthrum setulosum, Lepidochitona cinerea, Lepidopleurus cajetanus*, and *Leptochiton*

23 *asellus*, and the anterior nervous system of *Schizoplax brandtii*, demonstrated a consistent

24 and substantial anterior concentration of nervous tissue in the circumoesophageal nerve ring.

25 This neural mass is further organised into three concentric tracts, corresponding to the paired

26 lateral, ventral, and (putatively) cerebral nerve cords. These represent homologues to the

27 three main pairs of ganglia found in other molluscs. The relative size, shape and organisation

28 of these components is highly variable among the examined taxa, but consistent with

29 previous studies of select species, and we formulated a set of neuroanatomical characters for

30 chitons. These characters are parsimony-informative for reconstructing chiton phylogeny at

31 the ordinal and subordinal levels; the identification of robust detailed homologies in neural

32 architecture will be central to future comparisons among all molluscs, and more broadly in

33 Lophotrochozoa. Modern evolutionary thinking, and modern tomographic technology, bring

34 new light to an old problem. Contrary to almost all previous descriptions, the size and

35 structure of the chiton anterior nerve ring unambiguously qualify it as a true brain with cordal

36 substructure.

37 **Key words:** Molluscs, neuroanatomy, evolution, complexity.

38 **Research highlights**

39 3D reconstructions from historic histological slides reveal unappreciated complexity in chiton

40 nervous systems. The concentration and organisation of nervous tissue in the

41 circumoesophageal nerve ring in eight species unambiguously qualify it as a true brain.

Introduction

43   Chitons are benthic marine molluscs found from the intertidal to abyssal depths across the

44   globe. The class is characterised by eight articulated dorsal shell valves, which protect the

45   foot, viscera, and pallial cavity. Most species graze the substrata using a biomineralised radula

46   (Sigwart & Schwabe, 2017). They lack cephalic eyes and tentacles, but possess an extensive

47   network of sensory pores in the valves, of which some have evolved to form 'shell eyes'

48   capable of true image formation (Omelich, 1967; Speiser, Eernisse, & Johnsen, 2011). Their

49   simple body plan (dorsal shell, ventral foot; anterior mouth, posterior anus) has been

50   purported to reflect a plesiomorphic or 'primitive' state within molluscs (Hyman, 1967), with

51   many classical projections of a common ancestor to this incredibly diverse phylum resembling

52   living chitons (Haszprunar, 1992; Salvini-Plawen, 1981, 1985). This places chitons in a unique

53   position of interest to the evolution of animal body plans.

54   As a phylum, molluscs demonstrate some of the wildest morphological diversity and disparity

55   found in the animal kingdom, and conclusively resolving molluscan relationships remains a

56   fundamental challenge (Sigwart, 2017; Telford & Budd, 2011). Molecular studies provide

57   reasonably robust support for certain groupings, including a monophyletic Aplacophora and

58   its sister relationship with chitons (Aculifera; Kocot et al., 2011; Smith et al., 2011, 2013), and

59   recent fossil findings also support a chiton-like ancestor for the clade Aculifera (Caron,

60   Scheltema, Schander, & Rudkin, 2006; Sigwart & Sutton, 2007). But other nodes in molluscan

61   phylogeny remain uncertain, and recovered topologies vary with taxonomic coverage and

62   choice of outgroups (Kocot et al., 2011; Sigwart & Lindberg, 2015; Smith et al., 2011; Telford

63   & Budd, 2011). Another substantial problem with the interpretation of unstable phylogenies

64   is the interpreted polarity of key characters: among so much diversification, including radical

65 changes, convergence and reversals, which features are plesiomorphic and which are

66 derived?

67 One key aspect of the interpreted simplicity of polyplacophorans is their nervous system.

68 Chitons have no ganglia; the distinct cerebral, pedal and pleural ganglia that compose the

69 brain or circumoesophageal nerve ring in most other molluscan classes are entirely lacking

70 (Faller, Rothe, Todt, Schmidt-Rhaesa, & Loesel, 2012; Moroz, Nezlin, Elofsson, & Sakharov,

71 1994; Sigwart & Sumner-Rooney, 2015). Chiton nervous system architecture comprises two

72 pairs of medullary cords, lateral and ventral, running longitudinally through the body and

73 forming a visceral loop, and an anterior circumoesophageal nerve ring (Eernisse & Reynolds,

74 1994; Hyman, 1967; Moroz, 2009). The anterior part of this ring is thought to be homologous

75 to the cerebral ganglia in other molluscan classes (Sigwart & Sumner-Rooney, 2015;

76 Voronezhskaya, Tyurin, & Nezlin, 2002). Chitons are widely considered to lack a true brain

77 (Moroz, 2009; Sigwart & Sumner-Rooney, 2015), and their nervous system is invariably

78 described as "primitive" and "ladderlike" (Arbas, Levine, & Strausfeld, 2011a; Morton &

79 Yonge, 1964). Though they occupy a position of particular neuroevolutionary significance, the

80 assumption of a homogenously primitive neural architecture may explain why they have been

81 understudied neurobiologically, with the exception of their sensory aesthete and shell eye

82 structures.

83 Historical anatomical illustrations of different chiton species demonstrate reasonable

84 variation in key features, such as the shape of the circumoesophageal nerve ring and the size

85 and position of the buccal ganglia (Heath, 1904; Plate, 1899). In almost all schematic

86 depictions, the circumoesophageal nerve ring is slender, often no wider than the individual

87 lateral or ventral cords, with slight postero-lateral swellings at the origin of the cords (Bullock

88 & Horridge, 1965). The majority of previous works illustrate dissected material, which

89 naturally results in disruption to the specimen; it is not clear how much of the variation seen

90 in depicted nervous systems is *bona fide*. Despite this, objective comparisons of nervous

91 system architecture and neuroanatomy across the class are almost entirely absent.

92 The advent of three-dimensional anatomical imaging techniques has helped morphological

93 studies to blossom in the digital age (Sumner-Rooney & Sigwart, 2017). The ability to

94 accurately reconstruct internal structural characters of whole organisms has revolutionised

95 the field, and provides crucial insight to organ systems that have previously been observed

96 through potentially disruptive dissections or individual tissue sections, which can be

97 challenging to quantify. It is a testament to the astonishing skill and diligence of the classic

98 morphologists that many of their decades- or even centuries-old observations using these

99 methods still remain at the forefront of our knowledge today, but the application of new

100 technology to morphological studies is constantly improving accessibility, resolution and

101 efficiency in anatomy.

102 Morphology continues to play a crucial role in phylogenetics and systematics in the face of an

103 increasingly molecular future. Nervous system characters have already shed significant light

104 on arthropod phylogeny (Strausfeld & Andrew, 2011), and are also emerging as an important

105 tool in resolving molluscan relationships (Friedrich, Wanninger, Brückner, & Haszprunar,

106 2002; Shigeno, Parnaik, Albertin, & Ragsdale, 2015; Shigeno, Sasaki, & Haszprunar, 2007;

107 Sumner-Rooney et al., 2015; Wollesen, Rodríguez Monje, McDougall, Degnan, & Wanninger,

108 2015). Relationships within Polyplacophora are reasonably well understood, which provides

109 a phylogenetic backbone to test questions and models of character evolution; there is a well-

110 established divide between the two living orders, Lepidopleurida and Chitonida, and the

111   divisions of major subclades are increasingly robust (Eernisse, 2008; Okusu, Schwabe,

112   Eernisse, & Giribet, 2003; Sigwart, Stöger, & Schwabe, 2013). Remaining uncertainty is due in

113   part to the relatively low morphological variation found within the class, which can hamper

114   morphological phylogenetic analyses (Sigwart et al., 2013). New morphological character sets

115   that exhibit sufficient variation to be phylogenetically informative will contribute to the

116   resolution of long-standing questions at different taxonomic levels in molluscs, and

117   neurocladistics could potentially form a core part of future analyses (Faller et al., 2012;

118   Sumner-Rooney et al., 2015). Crucially, morphological data also provide insight to ancestral

119   conditions and characters, beyond pure relationships between taxa, which molecular data

120   often cannot. Such insights are critical to resolving high-level relationships as well as

121   evolutionary changes in body plan and organisation on a macroevolutionary scale.

122   Indeed, interest in molluscan nervous systems has already helped shed light on inter- and

123   intra-class molluscan evolutionary relationships (Faller et al., 2012; Friedrich et al., 2002;

124   Haszprunar, 1988; Shigeno et al., 2007, 2015; Sumner-Rooney et al., 2015; Wanninger &

125   Haszprunar, 2003). Among the resulting publications are several studies of chiton nervous

126   systems, of which some found greater than expected size or complexity of the

127   circumoesophageal nerve ring (Faller et al., 2012; Gantner, 1989; Sigwart et al., 2014);

128   however, capacity for diversity remains underappreciated as a fundamental characteristic of

129   the chiton nervous system. A departure from previous descriptions could undermine the

130   narrative consensus of polyplacophoran nervous systems as undifferentiated and primitive.

131   Here, we pursued two specific aims to move beyond the assumptions of "primitive" chitons

132   and provide a more objective identification and assessment of relevant characters. First, we

133   evaluated the available characters within the chiton nervous system and how these vary

134   among species in the class. Second, we examined neuroanatomical structures within the

135   chiton nervous system that represent homologies to standard features in other molluscan

136   classes, such as the main pairs of ganglia. A robust description of the chiton nervous system

137   and its range of morphological variation will be critical to understanding the relationships

138   between chitons and other molluscan classes and potentially between molluscs and other

139   phyla (Kocot, 2016).

140

141   **Materials and Methods**

142   *Specimen material*

143   Slides of serial histological sections, produced by Prof. Johannes Thiele between 1890-1910,

144   were drawn from the Malacology collection at the Museum für Naturkunde, Berlin (ZMB/Moll

145   230880-230999). Series of eight species were sufficiently complete and of sufficient quality

146   to reconstruct digital models of the nervous system: *Acanthochitona fascicularis* (Linnaeus

147   1767)*, Callochiton septemvalvis* (Montagu 1803)*, Chiton olivaceus* Spengler 1797*,

148   *Hemiarthrum setulosum* Carpenter 1876*, Lepidochitona cinerea* (Linnaeus 1767)*,

149   *Lepidopleurus cajetanus* (Poli 1791)*, Leptochiton asellus* (Gmelin 1791)*,* and *Schizoplax

150   *brandtii* (Middendorff 1847)*.* These species represent each of the five major clades of living

151   chitons (Sigwart et al., 2013); the order Lepidopleurida, and all four superfamilies in the order

152   Chitonida: Chitonoidea, Callochitonoidea, Mopalioidea and Cryptoplacoidea (Table 1).

153   Data on the anterior nervous system in *Leptochiton asellus* were taken from modern

154   histological sections, produced by Sigwart and colleagues (Sigwart et al., 2014), as the anterior

155   sections were missing from Thiele's collection.

156   *Tomographic modelling and digital analysis of the nervous system*

157   Slides were visualised on a Zeiss Axioskop stereomicroscope, using a Leica DFC490 mounted

158   camera and Leica LAS Core software for image capture. In total, 3699 histological sections

159   were digitised. Where sections or slides (sets of sections) were missing or damaged, preceding

160   or succeeding sections were duplicated to maintain voxel size during modelling. Sections of

161   *Leptochiton asellus* used for anterior nervous system modelling by Sigwart et al. (2014; slides

162   deposited to the Bavarian State Collection of Zoology, Munich) were also used in new

163   analyses: the original image stack was re-examined and re-modelled alongside the other

164   historic material.

165   Sampled images were processed and contrast-enhanced in Adobe Photoshop CS4 before

166   loading into AMIRA (v.5.3.3, FEI Visualisation Group). Image stack alignment, segmentation,

167   surface rendering, smoothing, and volume calculations were all performed in AMIRA.

168   All visible nervous tissue in the digitised images was segmented and included in

169   reconstructions. However, in some species, the identification of smaller transverse nerves or

170   commissures was hampered by section thickness, quality or stack sampling (where fine

171   nerves appeared in single sections only but the image stack sampled alternate sections). As

172   these are fine structures running parallel to the cutting plane, they often appear in only one

173   or two sections and thus are particularly vulnerable to these confounding factors. This

174   affected *Callochiton septemvalvis*, *Hemiarthrum setulosum* and *Lepidochitona cinerea*. These

175   minor nerves and their patterning are therefore not evaluated as potential characters herein,

176   for any taxa.

177   The degree of anterior concentration of nervous tissue was assessed by extracting nerve

178   volumes from the oesophageal nerve ring in AMIRA and comparing these with nerve volumes

179   taken from an equivalent section further posterior in the specimen; i.e. if the

180   circumoesophageal nerve ring was present in 25 images in the stack, a comparative nerve

181   volume was taken from a 25-image section at the anteroposterior midpoint of the animal. We

182   also calculated anterior nerve volumes as a proportion of total nervous system volumes, and

183   measured the relative lengths of the reconstructed oesophageal nerve ring and the whole

184   nervous system. Buccal ganglia were excluded from volume calculations as they do not form

185   part of the nerve ring and we were not able to reconstruct these in all taxa.

186   In order to document the variation in neural structures among the sampled taxa we

187   formulated 17 discrete morphological cladistic characters describing the chiton nervous

188   system from rendered models and literature (Table 2), and constructed a coded matrix for

189   the eight taxa examined here (Table 3).

190   **Results**

191   Nervous systems in all species examined were consistent with the expected general chiton

192   neural architecture: two pairs of medullary cords, the ventral and the lateral nerve cords, run

193   longitudinally through the foot and the roof of the pallial cavity respectively, and an anterior

194   circumoesophageal ring surrounds the oesophagus (Figures 1 and 2). Paired buccal ganglia,

195   linked posteriorly by the buccal nerves, and subradular ganglia, located posterior to the nerve

196   ring, were also identified in line with existing descriptions.

197   All species demonstrated concentration of nervous tissue in the anterior part of the animal in

198   the circumoesophageal nerve ring. The volume of the nerve ring ranged in various species

199   between 50–144% greater than the volume of an equivalent part at the midpoint of the

200   nervous system (Table 1). The nerve ring occupied between 22–28% of total nervous system

201   volume in different species, and extended along 13-18% of total nervous system length (Table

202   1). These figures exclude the volume of the buccal ganglia and minor nerves as these were

203   not reconstructed in all species (see Methods).

204   All examined species showed distinct mediolateral organisation of the circumoesophageal

205   nerve ring, giving the impression of three concentric tracts. The ring itself comprises central

206   neuropil and surrounding cell bodies, as described by many previous authors (reviewed in

207   Bullock & Horridge, 1965; Sigwart & Sumner-Rooney, 2015); however, the central neuropil is

208   incompletely subdivided by interjecting veins of cell bodies (Figure 3). This broadly divides the

209   anterior and lateral regions of the nerve ring into three concentric tracts, corresponding to

210   the lateral (outermost) and ventral (intermediate) nerve cords as well as a third central tract,

211   which we interpret to be homologous to the cerebral ganglia (Gantner, 1987).

212   From their origins in the circumoesophageal ring, the ventral nerve cords project posteriorly,

213   turning slightly medially prior to their entry to the foot, and then remaining roughly parallel

214   until slightly anterior of the anus, where they converge. A series of transverse nerves

215   (commissures) join the ventral cords at fairly regular intervals; these were not captured in all

216   subsampled image stacks and so do not appear in all reconstructions, but inspection of

217   complete slide series confirms they are a common feature across all eight species, as well as

218   many previous illustrations (Bullock & Horridge, 1965; Faller et al., 2012; Gantner, 1989;

219   Heath, 1904; Hyman, 1967). The lateral nerve cords extend posterolaterally from the nerve

220   ring and enter the roof of the pallial cavity, running very close to the surface within the gill

221   row, before joining posterior to the anus (sometimes referred to *in litt.* as the suprarectal

222   commissure, but in fact this appears to be a conjoining of the nerve cords, in agreement with

223   Faller et al. 2012). Smaller nerves connect the lateral nerve cords to the adjacent ventral

224   cords, but unlike the ventral commissures they do so at an angle, projecting posteriorly as

225   well as medially to the ventral cords. Again, these nerves were not captured in all

226   reconstructions but appeared in complete material for all taxa. The lateral and ventral nerve

227   cords are roughly evenly distributed mediolaterally. Distinct buccal ganglia and

228   medioposteriorly-projecting buccal nerves were visible dorsal to the circumoesophageal

229   nerve ring in all specimens except *Lepidochitona cinerea* and *Schizoplax brandtii*.

230   The descriptions below summarise the neuroanatomical features observed in each of the

231   eight species examined, with particular note of variations on the above generalised plan. A

232   summary of neuroanatomical characters and their occurrence is included in Tables 2 and 3.

233

234   *Descriptive neuroanatomy*

235   The circumoesophageal nerve ring in *Acanthochitona fascicularis* (Figure 1A-C) is large,

236   comprising 18% of the length of the nervous system and shows exaggerated posterolateral

237   expansion, with the posterior margin being three times the thickness of the anteriormost part

238   (sometimes referred to as the anterior commissure, Figure 1A,B). All three tracts are at their

239   widest in this region. The ventral (pedal) and cerebral tracts are much finer at the anterior

240   side of the ring, whereas the cerebral tract appears to be incomplete at the anterior side of

241   the nerve ring. The tracts of the circumoesophageal nerve ring are organised on a

242   mediolateral plane in the posterior two thirds, but the anterior part is slightly flexed so that

243   the cerebral and ventral tracts are ventral to the lateral tract, rather than medial to it. The

244 buccal ganglia are situated around three-quarters of the way up the circumoesophageal nerve

245 ring (Figure 1B). They are visible in the models as slight expansions of the buccal nerves, which

246 project posteriorly and appear to converge well posterior of the nerve ring; however, this was

247 not recovered in the available sections. Subradular ganglia are small and bean-shaped,

248 curving slightly laterally, and are located directly dorsal to the posterior edge of the cerebral

249 tract. Ventral-lateral commissures were not visible in the examined sections, but have been

250 illustrated in congeneric species (Pelseneer, 1898; Plate, 1899). The lateral and ventral nerve

251 cords originate in parallel (at the same point on the antero-posterior axis), at the posterior

252 edge of the nerve ring. The ventral cords are slightly thicker than the lateral cords, particularly

253 in the anterior half of the foot, and several ventral commissures were reconstructed, though

254 more may be present that were missed as a result of histological section thickness and stack

255 subsampling (Pelseneer, 1898; Plate, 1899).

256 *Callochiton septemvalvis* (Figure 1D–F) has a slightly shorter, ovoid circumoesophageal nerve

257 ring, occupying 14% of total nervous system length. The three tracts of the ring are organised

258 dorsoventrally as well as mediolaterally, and are of consistent thickness throughout, except

259 the anteriormost portion of the cerebral tract, which appears to be absent. The lateral and

260 ventral tracts are of similar thickness, and larger than the cerebral tract. The buccal ganglia

261 and nerves are situated quite anteriorly; the ganglia are dorsal to the anterior margin of the

262 nerve ring, and *C. septemvalvis* was the only species whose short, thick buccal nerves

263 converged within the circumoesophageal nerve ring, anterior to its posterior margin (Figure

264 1E). Subradular ganglia were small and triangular, located directly dorsal to the posterior edge

265 of the cerebral tract and joined posteriorly by a slim commissure that was free of cell bodies.

266 The ventral and lateral nerve cords are of similar thickness, and several ventral and ventral-

267   lateral commissures were reconstructed intermittently throughout the length of the body;

268   more were observed in complete section series but were not recovered in reconstruction.

269   *Chiton olivaceus* (Figure 1G–I) has a regularly organised circumoesophageal nerve ring

270   occupying 17% of the length of the nervous system. All three tracts are anteriorly joined and

271   of consistent thickness, and while the cerebral and ventral tracts are slightly ventral to the

272   lateral tract, the ring is mostly organised mediolaterally. The ventral tract is thicker than the

273   others, followed by the lateral tract. The buccal nerves and ganglia extend beyond the nerve

274   ring both anteriorly and posteriorly, with the centres of the buccal ganglia located dorsal to

275   the anteriormost edge of the nerve ring, and the slender buccal nerves conjoin posterior to

276   the base of the cerebral ring, in line with the subradular nerves. Paired triangular subradular

277   ganglia are situated ventral to the posterior margin of the nerve ring. The ventral and lateral

278   nerve cords are of similar thickness throughout the length of the body, and the ventral nerve

279   cords are slightly closer to each other than to the lateral nerve cords. Some ventral

280   commissures were reconstructed, though more were visible in the full series of histological

281   sections. We did not reconstruct any ventral-lateral commissures; however both types are

282   illustrated as numerous but very fine structures present along the whole length of the animal

283   in *Chiton olivaceus* (as *C. siculus*) by Haller (1882), so it is likely these were not captured by

284   thick sections and subsampling.

285   *Hemiarthrum setulosum* (Figure 1J–L) is the only species to show a potentially incomplete

286   lateral tract in the circumoesophageal nerve ring, with the ventral and cerebral tracts both

287   projecting further anterior than in other species (see Figure 1K). The nerve ring accounts for

288   14% of total nervous system length. The ventral tract of the circumoesophageal nerve ring is

289   again thicker than the lateral and cerebral tracts. The ring is flattened dorsoventrally, with

290   only a slight ventral projection of the medial regions. The lateral and ventral nerve cords

291   originate almost in parallel, with the ventral nerve cords being slightly further posterior. The

292   buccal nerves are quite short, with the buccal ganglia being positioned dorsal to the anterior

293   side of the circumoesophageal nerve ring, and the buccal nerves converging in line with the

294   posterior margin of the ring. The buccal ganglia are also innervated by two parallel nerves

295   projecting anterodorsally from the posterior side of the cerebral ring. Subradular ganglia are

296   clearly defined, and are located significantly posterior to the circumoesophageal nerve ring,

297   in line with the first ventral commissure. The ventral nerve cords are laterally distributed,

298   closer to the lateral nerve cords than to each other. The ventral and lateral nerve cords are

299   similar in diameter. A few ventral-lateral and ventral commissures were reconstructed, with

300   several more visible in the complete section series along the length of the specimen.

301   The condition of sections of *Lepidochitona cinerea* (Figure 2A–C) hampered the identification

302   of structures much beyond the basic architecture of the nervous system, but the

303   differentiation between the lateral, ventral and cerebral tracts of the circumoesophageal

304   nerve ring was still visible. The nerve ring is relatively short, occupying just 13% of the length

305   of the nervous system. The lateral tract is of roughly uniform thickness, but both the ventral

306   and cerebral tracts are narrower at the anterior side of the ring. The origins of the lateral

307   nerve cords are slightly anterior to those of the ventral nerve cords. No buccal or subradular

308   ganglia, or buccal nerves, were visible, but these have been described by Faller et al. (2012).

309   Lateral nerve cords appear to be slightly thicker than the ventral nerve cords. The ventral and

310   lateral nerve cords were both distributed laterally, with both pairs being wide-set within the

311   body cavity in comparison with the circumoesophageal nerve ring (Figure 2A). Only the

312   anterior and the two posteriormost ventral commissures were visible, but further

313   commissures have been observed along the length of the foot by other authors (Faller et al.,

314   2012; Gegenbaur, 1878). Ventral-lateral commissures are faintly visible (Faller et al., 2012) or

315   implied (Gegenbaur, 1878) in previous images of *L. cinerea*, but are presumably much finer

316   than the ventral commissures as they are not fully reconstructed in these, or the current,

317   studies.

318   *Lepidopleurus cajetanus* (Figure 2D–F) shows complete lateral, ventral and putative cerebral

319   nerve tracts in the circumoesophageal nerve ring, with the latter being greatly narrowed at

320   the anterior side. The nerve ring is quite short, accounting for 13% of the nervous system

321   length. The anterior and lateral sides of the ring are flexed slightly dorsally. The buccal nerves

322   converge posterior to the circumoesophageal nerve ring, but the buccal ganglia were not

323   visible. Triangular swellings are found at the lateral posterior margins of the putative cerebral

324   tract. The subradular ganglia are drop-shaped and curve slightly medially; they are located

325   dorsal and posterior to the circumoesophageal nerve ring (Figure 2E). The origins of the lateral

326   nerve cords are anterior to those of the ventral nerve cords, around three quarters of the way

327   to the posterior edge of the nerve ring. The ventral nerve cords were very closely associated

328   and connected by many visible commissures. The ventral nerve cords were thicker than the

329   lateral nerve cords, particularly at the anterior end of the foot. No ventrolateral commissures

330   are visible in the sections examined, but Plate described them as present in small numbers

331   and difficult to find (Plate, 1897).

332   *Leptochiton asellus* (Figure 2G–I) was reconstructed from two separate slide series: one for

333   the anterior nervous system (material from Sigwart et al. 2014) and one for the rest of the

334   body (from the Thiele collection, MfN), so nerve volumes were not comparable. Recently-

335   produced sections show the same concentric partitioning of the circumoesophageal nerve

336 ring, with all three tracts being joined anteriorly and of uniform thickness throughout. The

337 cerebral tract is much narrower than the ventral and lateral tracts, and the lateral tract is

338 slightly smaller than the ventral. The ring is flexed dorsally at the anterior edge. The lateral

339 nerve cords originate significantly anterior to the ventral nerve cords, with the latter

340 separating from the cerebral tract at the posterior side of the nerve ring. A pair of buccal

341 ganglia is located dorsal to the anterior edge of the nerve ring, turning anteromedially

342 towards each other. The buccal nerves briefly extend laterally before turning, projecting

343 posteromedially and converging in line with the posterior margin of the cerebral ring. The

344 large, distinct subradular ganglia are dorsal and posterior to the posterior margin of the

345 circumoesophageal ring. The lateral and ventral nerve cords are evenly distributed, though

346 the lateral nerve cords begin to turn medially anterior of the midpoint in the body. Both

347 ventral commissures and the ventrolateral connectives were prominent and could be

348 reconstructed throughout the body.

349 Only the anterior part of *Schizoplax brandtii* (Figure 2J, K) was available, but reconstruction

350 showed that the same concentric arrangement was discernible in the circumoesophageal

351 nerve ring. The ring is widest at the lateral sides, and flexes dorsally out the outer margins.

352 The cerebral tract is prominent and slightly wider at the lateral sides, with a slender anterior

353 completion. It was not possible to trace the posterior completion of the cerebral tract. By

354 contrast, the ventral tract appears to be incomplete anteriorly, and is broadest in the lateral

355 part of the ring even in comparison to the anterior part of the ventral cords. The lateral tract

356 is also widest at the postero-lateral edge of the circumoesophageal nerve ring. The origins of

357 the lateral nerve cords are situated definitively anterior to those of the ventral nerve cords.

358 We could not identify the buccal or subradular ganglia in the subsampled image stack.

359

360 **Discussion**

361 The chiton nervous system shows significant anterior concentration in terms of volume, and

362 the circumoesophageal nerve ring is in fact composed of three concentric regions that

363 correspond to the cerebral, ventral and lateral nerve cords, i.e. these tracts are likely

364 homologues of the ganglia found in other molluscan classes. This represents a far higher level

365 of neural structure than is reflected in the established literature, particularly in "textbook"

366 summaries of chiton biology. Further, we found that the architecture of the nervous system,

367 including the composition of the circumoesophageal nerve ring, is not constant between the

368 eight species studied herein, which includes representatives of all the major extant

369 polyplacophoran clades. Chiton neuroanatomy is demonstrably not homogeneous across

370 taxa. Our very simple cladistic analysis was intended only to test whether the variation

371 observed among the identified characters has any correlation with established phylogenetic

372 relationships. The details that characterise these neural structures correspond to known

373 divergences in chiton phylogeny. These structures are large, and can be characterised by

374 multiple morphological features. Based on the evidence of substructure within it, we propose

375 that the circumoesophageal nerve ring of chitons represents a true brain.

376

377 *The chiton anterior nerve ring as a brain*

378 Two main metrics can be used to assess and define central nervous systems and brains. The

379 first is simply size, relative to the rest of the nervous system. Richter et al. (2010), for example,

380 define a brain as 'the most prominent anterior condensation of neurons'; and Moroz (2009)

381   endorsed the classification of the brain as a 'concentration of neurons within a defined organ-

382   type structure'. The concentration of nerve tissue at the anterior end of the body in chitons

383   is clear and, in terms of the proportion of nervous system volume and length, relatively

384   consistent across multiple taxa (Table 1). The circumoesophageal nerve ring in chitons

385   therefore certainly meets this criterion to qualify as a brain.

386   The other potentially defining character of a brain is complexity; although this term can be

387   used ambiguously, size alone is not a robust indicator of complexity or 'advanced' brain

388   development (Chittka & Niven, 2009). This can be related to compartmentalisation and

389   processing of tasks or information (e.g. Riebli & Reichert, 2015). At its simplest, this implies a

390   subdivision of the nerve mass into distinct parts. In some cases this may be the division of the

391   cerebral ganglia into distinct neuropil compartments (e.g. Faller et al., 2012), or the

392   amalgamation of the cerebral, pleural ganglia and, in the case of cephalopods, the pedal

393   ganglia into clusters or even fused structures contributes to a multipartite brain structure

394   (Young, 1965). The oesophageal nerve ring also demonstrates a level of spatial organisation

395   unappreciated in much of the existing, and particularly the modern, literature. Gegenbaur

396   (1878: p.344) described the chiton anterior commissure as 'a nervous band formed of two

397   chords'. (The two cords are those identified herein as the lateral and the ventral tracts;

398   Gegenbaur did not identify the innermost tract as separate.) Plate (1897) later depicted the

399   medio-lateral partitioning of the circumoesophageal nerve ring into three distinct sections in

400   *Acanthopleura echinata* (Figs 104 and 105, plate 10). Gantner (1989) also observed

401   subdivisions of the neuropil in the nerve ring of *Lepidochitona monterosatoi* and identified

402   ventral, lateral and subcerebral parts in his thesis, and Faller et al. (2012) described

403   partitioning of the neuropil in at least the anterior part of the nerve ring in *Acanthochitona*

404   *crinita* and *Lepidochitona cinerea*. This is evidently the same organisational patterning we

405   have identified in representatives of all the major living clades of chitons. However, this

406   hugely significant feature is not reported in the majority of chiton literature, and the

407   consensus that chitons lack a brain has persisted (Arbas et al., 2011; Eernisse, 2007; Moroz,

408   2009; Sigwart & Sumner-Rooney, 2015). The circumoesophageal nerve ring in chitons is large,

409   and contains well described complex sub-structure (herein, and in previous studies), two

410   major criteria to qualify anterior neural mass as a *bona fide* brain.

411   In light of the classification of the nerve ring as a brain in the true anatomical sense, questions

412   immediately arise concerning its function and capacity. Almost nothing is known about

413   nervous system physiology in chitons. The extent of centralisation and processing in the

414   anterior nervous system has never been examined to our knowledge. The widespread belief

415   that such centralisation would be minimal based on the apparent absence of a brain would

416   limit motivation for such studies. Electrophysiological techniques have been deployed only

417   for studies of muscle physiology and pericardial innervation (Burnstock, Greenberg, Kirby, &

418   Willis, 1967; Matsumura & Kuwasawa, 1996), and no published recordings have ever been

419   taken directly from the nervous system itself. Exploratory recordings taken from the anterior

420   nervous system of *Leptochiton asellus* indicated potential concentration of nerve activity in

421   the circumoesophageal nerve ring compared to the lateral and ventral nerve cords (Sumner-

422   Rooney, 2015); spike frequency and amplitude were dramatically increased in the nerve ring.

423   This requires further investigation, but could represent the first physiological evidence of

424   potential cephalisation in chitons, beyond using volume as a proxy. Additionally, chiton

425   behaviour has traditionally been viewed as largely driven by localised reflexes (Arey & Crozier,

426   1919), but it is possible that the brain does play a more dominant role in centralised

427 processing, comparable to other molluscs. We also previously identified a putative vibration

428 stimulus response localised to a specific region in the anterolateral part of the ring, which was

429 consistent across subsequently tested animals (n=5) (Sumner-Rooney, 2015). Chitons lack

430 statocysts, and no specific vibration-sensitive organs are found in the immediate vicinity; it is

431 possible that this finding represents evidence of centralised processing of information from

432 mechanosensors elsewhere in the body (e.g. ciliary tufts in the pallial cavity), but we stress

433 the preliminary nature of these findings. It has been suggested that the shell eyes of some

434 species may also integrate information across multiple eyes (Speiser et al., 2011); however,

435 centralisation of this kind has never been physiologically observed.

436 *The chiton nervous system and phylogeny*

437 Neuroanatomical characters show promise in resolving longstanding questions of deep

438 molluscan phylogeny (Friedrich et al., 2002; Sumner-Rooney et al., 2015; Wanninger &

439 Haszprunar, 2003; Wollesen et al., 2015), but the perceived ambiguous arrangement of the

440 chiton nervous system has confounded comparisons with other classes (Sigwart & Sumner-

441 Rooney, 2015). The recognition of the cordal nature of the chiton brain is crucial to

442 comparisons across the phylum. As the chiton brain is clearly not an undifferentiated neural

443 mass, but shows a structure of concentric layers, the next question of interest is in identifying

444 homologies with the typical aspects of ganglionic nervous systems in other molluscan classes.

445 Species in most of the other molluscan classes show discrete or fused ganglionic organisation

446 (Sigwart & Sumner-Rooney, 2015), and we propose that the three nerve tracts identified here

447 are homologous, and therefore directly comparable, to the cerebral, lateral, and pedal ganglia

448 of other molluscs.

449   The development of the chiton nervous system has been studied in several species, and

450   previous findings also support our proposed model that concentrically-organised neural tracts

451   within the circumoesophageal nerve ring are homologues to the cerebral, lateral, and pedal

452   ganglia in other molluscs. Voronezhskaya et al. (2002) and Friedrich et al. (2002) showed that

453   the cerebral aspect of the anterior commissure originates in the larval apical cells and expands

454   laterally and posteriorly. The ventral nerve cords (or pedal system) appear several hours later,

455   followed by the lateral nerve cords, with both expanding bidirectionally from further

456   posterior in the developing animal (Friedrich et al., 2002; Voronezhskaya et al., 2002). Images

457   from Voronezhskaya et al. (2002) show the oesophageal nerve ring comprising discrete parts

458   as early as 42 hours post-fertilisation, when the ventral and lateral cords have made contact

459   with the cerebral region, with the "cerebral ganglia" labelled at the inner edge of the nerve

460   ring. Faller et al. (2012: p.166 figs. 9B, 10B) and Voronezhskaya et al. (2002: figs. 2F,G, 4F,G)

461   depict juvenile *Acanthochitona crinita*, *Lepidochitona cinerea* and *Ischnochiton hakodadensis*

462   with clear division in the circumoesophageal nerve ring that correspond to the three regions

463   we find here. Among all molluscs, cerebral ganglia originate from ectodermal invagination of

464   the apical region and this is considered a molluscan symplesiomorphy; additional ectodermal

465   invaginations give rise to lateral and pedal cords (Raven, 1959). The sequential and positional

466   development of the cerebral, ventral and lateral cords could support the formation of three

467   concentric tracts within the circumoesophageal nerve ring as indicated in a schematic

468   diagram (Figure 4).

469   The concentric ring arrangement of the chiton brain is not unique, and this architectural

470   configuration raises important questions regarding nervous system evolution in molluscs.

471   Shigeno et al. (2015) demonstrated that the brain of *Octopus bimaculoides* also develops as

472   a series of concentric cords, and not as discrete ganglionic structures. This is reminiscent of

473   the proposed model for the chiton nervous system, but significant differences between the

474   two discourage speculation about shared ancestral conditions. Developmental patterning in

475   the chiton and cephalopod nervous systems differ substantially (Fritsch, Wollesen, &

476   Wanninger, 2016; Fritsch, Wollesen, de Oliveira, & Wanninger, 2015), and the dominant

477   outermost region of the brain in cephalopods is homologous to the smaller inner cerebral

478   tract in chitons (Shigeno et al., 2015). Other complex brains follow this pattern of fusing

479   ganglionic structures into layers, including the vertebrate brain (Raven, 1959). The

480   appearance of two such systems among molluscs may reflect the relative ease of patterning

481   a concentric cordal brain in place of a ganglionic one as separately derived conditions.

482   Interestingly, there is evidence from within Gastropoda that molluscs exhibit a high degree of

483   neural plasticity, with varying levels of neural fusion in the brains of even relatively closely

484   related species (Haszprunar, 1988). However, without conclusive evidence regarding the

485   polarity of characters that describe molluscan nervous system architecture, we cannot

486   eliminate entirely the possibility that this cordal state is plesiomorphic.

487   The nature of the plesiomorphic state in molluscs requires careful, objective consideration.

488   The chiton nervous system has been compared to that of monoplacophorans (Lemche &

489   Wingstrand, 1959), and both monoplacophorans and chitons are colloquially considered

490   'primitive', but it is not clear to what extent that is informative. In contrast to chitons, the

491   monoplacophoran anterior nervous system is apparently composed of ganglia (Sigwart &

492   Sumner-Rooney, 2015). Indeed, in the original description of *Neopilina galatheae*, the

493   cerebral ganglia are explicitly described as "complex" and "tripartite, with swellings at the

494   base of the pedal cord, the lateral cord, and the cerebral commissure" (Lemche & Wingstrand,

495   1959), but whether this tripartite structure can be compared to the cordal structure seen in

496   the chiton circumoesophageal nerve ring is not clear from published histological sections

497   (Ruthensteiner, Schropel, & Haszprunar, 2010; Schaefer & Haszprunar, 1997).

498   The best-supported relationship among molluscan classes is the clade Aculifera, which

499   includes clades with ganglionic (aplacophoran) and non-ganglionic (chiton) nervous systems

500   as sister-taxa, and no fossils preserve the central nervous system, so there is a limited basis

501   to infer character polarity. The topology of the remaining molluscan classes is largely unclear,

502   but among these clades, most groups have a ganglionic arrangement (Lindberg & Sigwart,

503   2015), which speculatively suggests the cordal structure found in cepahlopods may be

504   derived. Chitons are popularly thought to be primitive, and cephalopods are perceived as

505   'advanced', but it is not entirely parsimonious to infer that a cordal brain structure is

506   plesiomorphic for aculiferans, yet derived in conchiferans. Nonetheless a convergent nervous

507   system evolution between chitons, cephalopods, and vertebrates would be highly

508   unexpected, given that the convergence between the two latter groups is attributed to their

509   similarly active lifestyles (e.g. Budelmann, 1996).

510   *Characters of the chiton nervous system*

511   In order to be useful in finer phylogenetic analyses, it is important to evaluate the consistency

512   of the nervous system characters we describe herein. Our findings can be closely compared

513   to descriptive results from other closely-related chiton species. In particular, our observations

514   of *Acanthochitona fascicularis* closely resemble those of *Acanthochitona crinita* made by

515   Faller et al. (2012): aligned origins of the ventral and lateral nerve cords at the posterior

516   margin of the nerve ring, lateral thickening of the circumoesophageal nerve ring and an

517   apparently anteriorly incomplete cerebral tract. *Hemiarthrum setulosum*, another member of

518   the Cryptoplacoidea, also exhibits origins of the two pairs of nerve cords almost in parallel,

519   but shows a complete cerebral ring. Despite some quality issues with the historic material of

520   *Lepidochitona cinerea*, we identified several features in line with the findings of Faller et al.

521   (2012) and Gegenbaur (1878) in the same species, and of Gantner (1989: Fig. 72) in

522   *Lepidochitona monterosatoi*, including a short circumoesophageal nerve ring, origins of the

523   lateral nerve cords anterior to those of the ventral nerve cords, and a prominent first ventral

524   commissure (both species), thicker lateral than ventral nerve cords, and anteriorly narrowed

525   ventral and cerebral tracts (*L. cinerea* only).

526   Chitons also possess notable diversity in sense organs, which have previously been used as

527   standard characters for differentiating major clades. The aesthetes are a system of innervated

528   shell pores that infuse the exposed dorsal shell layer in all chiton species (Eernisse & Reynolds,

529   1994; Sigwart & Sumner-Rooney, 2015). The proximal and distal termini of these nerve

530   channels are apparent as pores on the ventral and dorsal shell surface, which are used as

531   taxonomic characters. Fine differences in the arrangement of dorsal aesthete pores are used

532   in identifying lepidopleuran species and broader differences in the ventral patterning and the

533   points of penetration at slits in the shell insertion plates correspond to major clades (Sirenko,

534   1997, 2006). Another sense organ, the Schwabe organ, is an anatomical synapomorphy of

535   Lepidopleurida (Sigwart et al., 2014). The separate molluscan "osphradium" is a nonspecific

536   term for epithelial sense organs described from some chitons; in chitons this structure

537   represents a "posterior sense organ" that is not homologous to the osphradium *sensu stricto*

538   (Lindberg & Sigwart, 2015).

539   We proposed a set of identified neuroanatomical characters (Table 2; Table 3) for future use

540   in chiton phylogenetics analyses at the ordinal and subordinal level. Previous cladistic

541   analyses of chitons emphasised the need for input from diverse character sets (Sigwart, 2009).

542   These characters do not supplant other morphological or molecular data, but additional

543   independent evidence from neuroanatomical characters could provide useful additions to

544   larger analyses. In particular, there are features that separate Lepidopleurida from other

545   species, not only in the known features of the sense organs (Schwabe organ present and

546   posterior sense organ absent as likely apomorphies in Lepidopleurida; *vice versa* in Chitonida),

547   but also in features of the overall neural architecture. However, there are no clear

548   synapomorphies from the present data that would apparently support a monophyletic

549   Chitonida. The genus *Callochiton* is well known to be significantly different to other members

550   of the order Chitonida based on morphological and molecular data (Okusu, Schwabe,

551   Eernisse, & Giribet, 2003; Sigwart, Stöger, & Schwabe, 2013), and this is also reflected in its

552   neuroanatomy.

553   The limited taxon sampling and missing data available for both *Lepidochitona cinerea* and

554   *Schizoplax brandtii* may hinder the resolution of finer relationships. There are several specific

555   features that could represent synapomorphies of established groupings (Table 2), that

556   provide hypotheses to test with additional relevant taxa. The relative positions of the origins

557   of the lateral and ventral nerve cords (6), for example, are consistent within the major clades,

558   with the Cryptoplacoidea having origins in parallel (*Acanthochitona*) or near-parallel

559   (*Hemiarthrum setulosum*), the Mopalioidea having near-parallel origins, and the

560   Lepidopleurida and more plesiomorphic Chitonida having the origins of the lateral nerve cords

561   significantly anterior to those of the ventral nerve cords (this also holds true for *Leptochiton*

562   *rugatus* (Sigwart et al., 2014)). The major nerve cords are laterally broadly distributed in

563   *Lepidochitona cinerea*; this appears to also be the case in the anterior region of *Schizoplax*

564 *brandtii* but due to the incomplete nature of the specimen, this cannot be determined.

565 *Acanthochitona fascicularis* and *Hemiarthrum setulosum*, though both members of

566 Cryptoplacoidea, are strikingly different in their neuroanatomy, and they are not resolved as

567 sister taxa in our analysis. However, *A. fascicularis* shares several features with other

568 congeneric species studied by other authors. The distinctive overall shape of the oesophageal

569 nerve ring, which is heavily lateralised, is apparent in both illustrations and confocal images

570 (Faller et al., 2012; Pelseneer, 1898; Plate, 1899), so it is possible that this is a synapomorphy

571 of the genus. *Hemiarthrum* is also highly unusual morphologically (Sigwart et al., 2013), so it

572 may also be the case that it does not reflect the typical state of Cryptoplacoidea, or that this

573 group is characterised by a higher degree of variability in this regard than other chiton clades.

574 Of course, some neuroanatomical features may be the result of adaptation in body size or

575 form, such as the lateral distribution of the ventral nerve cords, which is, of course, heavily

576 dependent on the overall body plan and shape of the foot. But we recommend that the

577 characters identified here are suitable for inclusion in future phylogenetic analyses, and

578 suggest that further examination of nervous systems (central and peripheral) and increased

579 taxonomic coverage will contribute to resolving the longstanding questions surrounding

580 chiton relationships. Finally, similar studies in fresh material may of course cast light on the

581 robustness and phylogenetic utility of these characters, and comparative ultrastructural,

582 immunohistological and developmental studies will doubtless expand on this character set in

583 the future. The characters identified here are restricted to overall nervous architecture that

584 can be determined from historic slides more than a century old. However, the data we present

585 are a credit to the quality of both the original histological material and its subsequent

586 curation. Slide collections such as Thiele's offer an as yet underexploited resource for modern

587 morphological research through tomographic reconstruction; the technical expertise and

588   comprehensive taxonomic coverage of our predecessors provide a great asset and an efficient

589   starting point for comparative studies such as this, and we encourage further use of historic

590   slide collections in this way, in parallel with the increased recognition of wet material as a

591   resource for computed tomography (Sumner-Rooney & Sigwart, 2017).

592   *Conclusions*

593   The definitions of a brain as an anterior concentration of nervous tissue or a concentrated

594   multipartite neural mass are useful and (almost) universally applicable to identify brains in

595   individual taxa (Richter et al., 2010), and the chiton nervous system meets both of these

596   definitions. However, these structures may be difficult or inappropriate to compare among

597   distantly related groups. In this context, identifying homologous structures as 'brains' in

598   different taxa is much more valuable and informative from a both an evolutionary and a

599   functional perspective. This is a significant challenge in molluscs, as although the ganglia are

600   homologous, the brains may not be. In cephalopods, the brain comprises homologues of not

601   only the cerebral ganglia, but also the pedal and pleural ganglia, which together form the

602   circumoesophageal   ring.   However,   in   scaphopods,   bivalves,   caudofoveates   and

603   solenogastres, it is only the cerebral or fused cerebropleural ganglia that are interpreted to

604   form the brain, if they are attributed one at all (Faller et al., 2012; Sigwart & Sumner-Rooney,

605   2015). So, these are potentially competing definitions for what comprises the "brain" in

606   molluscs: one, or two, or three pairs of ganglia, which may or may not be fused. In chitons it

607   is impossible to delineate homologues of the three typically discrete pairs of ganglia. Chitons

608   evidentially have a brain, but its fused structure would appear to support the more expansive

609   definition of a brain as also seen in cephalopods. Importantly, this is not to imply any inherent

610   similarity between chiton and cephalopod brains, but is merely a test of the ontology of

611 "brain" among molluscs. Indeed, in most cases this would also complement Richter and

612 colleagues' definition (Richter et al., 2010), but in some taxa, such as scaphopods and bivalves,

613 an expansion of the "brain" to encompass the pedal, pleural, and cerebral ganglia would imply

614 that it is quite spatially disparate, with a distended nerve ring due to the displacement of the

615 foot (Sumner-Rooney et al., 2015). Conversely, if this is not the case, it implies either that

616 brain structures are highly plastic throughout the phylum, or that brains (not ganglia) have

617 evolved multiple times. There are distinctly different apomorphic brain characters in several

618 molluscan classes, such as the frontal swellings of the cerebral ganglia in caudofoveates and

619 solenogastres (Sigwart & Sumner-Rooney, 2015), which could be a result of multiple

620 independent origins of the brain. All of these possibilities remain to be investigated, but

621 objective comparisons and a clear ontology are a necessary first step. Thus, the combined

622 identification of the circumoesophageal nerve ring in chitons as both organised and

623 centralised (i.e., a brain) has substantial implications for the assessment of the central

624 nervous system and brain in other molluscs with recognised ganglionic organisation.

625 Three-dimensional visualisation of anatomy is a powerful tool to clarify the true extent and

626 variability of key structures. The interpretation of chiton anatomy may be historically stymied

627 by circular logic: if we assume that chitons are primitive, then we see their nervous system as

628 primitive, and the nervous systems is seen as "proof" that the animals retain plesiomorphic

629 features. Instead, the chiton nervous system shows an unappreciated level of complexity, and

630 a brain.

631

632 **Availability of data and materials**

633 The models produced during the current study are available online from the corresponding

634 author on request. The original histological sections from the collection of Johannes Thiele,

635 and a digitised set of these sections, remain the property of the Museum für Naturkunde,

636 Berlin; original slides of *Leptochiton asellus* are the property of the Bavarian State Collection

637 of Zoology, Munich; all are available on request from the relevant malacological collection.

638 **Competing interests**

639 The authors declare no competing interests.

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645 **Authors' contributions**

646 Both authors conceived and designed the project. LSR digitised slides, reconstructed

647 tomographic models, prepared figures and wrote the manuscript. JDS performed

648 phylogenetic analyses, prepared figures and wrote sections of the manuscript. Both authors

649 read and approved the final manuscript.

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822

823   Figure 1. Tomographic models of the body and nervous systems of chitons from the slide

824   collection of Johannes Thiele. **A–C**, *Acanthochitona fascicularis*. **D–F**, *Callochiton*

825   *septemvalvis*. **G–I**, *Chiton olivaceus*. **J–L**, *Hemiarthrum setulosum*. **A**, **D**, **G**, **J**: Whole body,

826   ventral view. Scale bar 500 μm. **B**, **E**, **H**, **K**: Circumoesophageal nerve ring, dorsal view. Scale

827   bar 250 μm. **C**, **F**, **I**, **L**: Animal *in vivo*. Purple: Lateral nerve cords and tracts. Green: Ventral

828   nerve cords and tracts. Pink: Cerebral tracts. Yellow: Buccal ganglia and nerves. Teal:

829   Subradular ganglia and nerves.

830

831   Figure 2. Tomographic models of the body and nervous systems of chitons from the slide

832   collection of Johannes Thiele. **A–C**, *Lepidochitona cinerea*. **D–F**, *Lepidopleurus cajetanus*. **G–I**,

833   *Leptochiton asellus*. **J–L**, *Schizoplax brandtii* (anterior only). **A**, **D**, **G**, **J**: Whole body, ventral

834   view. Scale bar 500 μm (**J**: 250 μm). **B**, **E**, **H**, **K**: Circumoesophageal nerve ring, dorsal view.

835   Scale bar 250 μm. **C**, **F**, **I**: Animal *in vivo*, dorsal view. **L**: Historic and updated views on the

836   chiton anterior nervous system, dorsal view. Left, *Lepidochitona monterosatoi* redrawn from

837   Gantner (1987); right, generalised plan combining aspects of the taxa used herein. Note the

838   difference in relative thicknesses of the nerve ring, lateral nerve cords (lnc) and ventral nerve

839   cords (vnc) between the original and updated figures, marked in black. Purple: Lateral nerve

840   cords and tracts. Green: Ventral nerve cords and tracts. Pink: Cerebral tracts. Yellow: Buccal

841   ganglia and nerves. Teal: Subradular ganglia and nerves.

842

843   Figure 3. Subdivision of the oesophageal nerve ring. The central neuropil of the nerve ring is

844   broadly divided into three distinct regions by interspersed veins of cell bodies. This was

845   originally illustrated by Plate (1895) in *Acanthopleura echinata* (left), and is clearly visible in

846   both historic (centre, *Hemiarthrum setulosum* from Thiele's material ZMB/Moll 230880-

847   230999) and recent semi-thin (right, *Leptochiton asellus*) histological sections. The three

848   regions correspond to the lateral nerve cords (lnc), ventral nerve cords (vnc) and a presumed

849   cerebral tract homologous to the cerebral ganglia (ct). All three images were taken from the

850   posterolateral part of the oesophageal nerve ring. Scales adjusted to facilitate comparison.

851

852   Figure 4. Proposed development of the chiton nervous system. Schematics drawn from

853   descriptions and data in Voronezhskaya et al. (2002), Friedrich et al. (2002) and the current

854   study. Ventral view, anterior at the top. The precursor to the cerebral cord appears at the

855   anterior of the developing larva, followed by the precursors to the ventral cords and then the

856   lateral cords, which extend posteriorly and then anteriorly in turn (c, cerebral region; l, lateral

857   region and nerve cord; v, ventral region and nerve cord).