1 **Ichnological evidence for meiofaunal bilaterians from the Ediacaran–Cambrian**

2 **transition of Brazil**

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The evolutionary events during the Ediacaran−Cambrian transition (~541 Ma) are unparalleled in Earth history. The fossil record suggests that most extant animal phyla appeared in a geologically brief interval, with the oldest unequivocal bilaterian body fossils found in early Cambrian. Molecular clocks and biomarkers provide independent estimates for the timing of animal origins, and both suggest a cryptic Neoproterozoic history for Metazoa that extends considerably beyond the Cambrian fossil record. We report an assemblage of ichnofossils from Ediacaran–Cambrian siltstones in Brazil, alongside U-Pb radioisotopic dates that constrain the age of the oldest specimens to 555–542 Ma. X-ray microtomography reveals three-dimensionally preserved traces ranging from 50–600μm in diameter, indicative of small-bodied, meiofaunal tracemakers. Burrow morphologies suggest they were created by a nematoid-like organism that utilised undulating locomotion to move through the sediment. This assemblage demonstrates animal-sediment interactions in the latest Ediacaran Period, and provides the oldest known fossil evidence for meiofaunal bilaterians. Our discovery highlights meiofaunal ichnofossils as a hitherto unexplored window for tracking animal evolution in deep time, and reveals that both meiofaunal and macrofaunal bilaterians began to explore infaunal niches during the late Ediacaran.

The lower Cambrian fossil record documents a major radiation of macroscopic animals (particularly bilaterian phyla), coupled with significant expansion of their behavioural interactions with substrates and other organisms[1,2]. However, a growing catalogue of evidence from body fossils, trace fossils, biomarkers and molecular

clocks indicates a protracted Neoproterozoic history for the Metazoa, with the origin of animals significantly pre-dating the base of the Cambrian[3].

A range of biological phenomena typically associated with animals first appears during the late Ediacaran interval (~580–541 Ma) including: skeletogenesis[4], reef-building[5] and macroscopic predation[6]. Body fossils of late Ediacaran macro-organisms include at least some early animals[3], but crucially, most plausible claims for metazoans lie within the diploblasts rather than the Bilateria[3]. *Kimberella*, which is putatively a stem mollusc[7], is a notable exception, but some authors suggest that it can only be reliably considered as a member of total group Bilateria[3].

Our understanding of early animal evolution is complemented by ichnological investigations of latest Ediacaran to Ordovician strata[1,2,8]. Diverse ichnofossil assemblages in the earliest Cambrian place an important constraint on the tempo of bilaterian origins, since they indicate that some groups, including total group panarthropods and priapulid-like scalidophorans[2,9], were globally distributed and abundant by this point. The major bilaterian divergences (i.e. the protostome-deuterostome and ecdysozoan-lophotrochozoan divergences) must therefore pre-date the Ediacaran–Cambrian boundary. To date, the Ediacaran trace fossil record has provided limited insight into these early divergences. Most Ediacaran ichnofossils are either surface traces or simple under-mat burrows, created either on or immediately beneath matgrounds[10]. Such traces extend back to ~555 Ma[11], including: inferred grazing traces (*Kimberichnus*[12]) associated with the body fossil *Kimberella*, vertical adjustment structures in response to seafloor aggradation[13], and in the latest Ediacaran, shallow vertical burrows[10], treptichnid-like burrows just below the Ediacaran-Cambrian boundary[14]. Most Ediacaran ichnofossils are considered to have

84   been made by total group bilaterian[15] or cnidarian[13,16] eumetazoans. Notwithstanding

85   controversial claims for bioturbation and complex burrows at ~553 Ma[17], widespread

86   substrate-penetrating burrows capable of significant sediment mixing do not appear

87   until close to the Precambrian-Cambrian boundary[14].

88   Molecular clock analyses predict an earlier, pre-Ediacaran origin for the

89   Metazoa and Eumetazoa, and an early Ediacaran origin of Bilateria, Protostomia and

90   Deuterostomia[18]. Palaeontological support for these suggestions is limited to

91   purported body fossils of sponges[19], and demosponge biomarkers[20]. A considerable

92   gap therefore remains between the fossil record of the late Ediacaran, and molecular

93   clock estimates for deep splits in the animal tree, e.g. between Metazoa and

94   Eumetazoa[3]. Assuming that contemporary molecular clock analyses yield accurate, if

95   imprecise[18], node ages for animal divergences, a small body size and concomitant

96   limited fossilisation potential[21] could reconcile these discordant records of animal

97   evolution (though see reference[22]).

98   The small body size of the ancestral bilaterian is supported by recent

99   phylogenomic analyses of deep animal relationships, with acoel flatworms and

100   xenoturbellids (Xenacoelomorpha) being a sister group to all remaining bilaterians

101   (Nephrozoa)[23], and small bodied spiralian taxa (the 'Platyzoa') recognised as a

102   paraphyletic grade with respect to macroscopic trochozoans[24]. This suggests that early

103   bilaterians and spiralians were small bodied, possibly meiofaunal, and moved using

104   ciliary gliding.

105   Meiofauna comprises all organisms between 32–1000μm in size, that inhabit

106   pore-water-rich sediments in freshwater to deep-marine environments[25]. Modern

107   meiofaunal communities include animals, foraminifera and some ciliates, and

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108   contribute significantly to sediment bioturbation and bioirrigation[26,27]. The meiofauna

109   can be divided into permanent members (i.e. animals with adults of a small size

110   adapted and restricted to the meiofaunal, interstitial realm), and temporary meiofauna

111   (e.g. the larvae of macrobiota)[25].

112   Despite its ecological and evolutionary importance, the deep-time record of

113   the meiofauna has received little discussion, principally due to the low preservation

114   potential of both meiofaunal body fossils and traces. Whilst meiofaunal burrows

115   (sometimes described as burrow mottling or cryptobioturbation) have occasionally

116   been reported from Cambrian to Recent sediments[26], they are rarely subjected to

117   detailed study. Body fossil discoveries also reveal organisms inhabiting meiofaunal

118   niches within early Cambrian communities, highlighting the potential for their

119   preservation within particular taphonomic windows[28,29].

120   Here we report a new assemblage of meiofaunal ichnofossils from siltstones of

121   the Ediacaran–Cambrian Tamengo and Guaicurus Formations, Corumbá Group,

122   central-western Brazil (Fig. 1). The age of the assemblage is constrained by U-Pb

123   (zircon) isotope dilution thermal ionisation mass spectrometry (ID-TIMS) dating of

124   inter-stratified ash beds. The dates indicate that the Tamengo Formation specimens

125   are late Ediacaran in age, and those in the Guaicurus Formation lie close to the

126   Ediacaran–Cambrian boundary. Our results constitute the oldest documented

127   meiofaunal burrows in the geological record, placing a constraint on the minimum age

128   of this key ecological innovation.

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130   **The Corumbá Group**

131   The Corumbá Group, part of the Southern Paraguay Belt, is a ~600m thick

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132 sedimentary unit comprising carbonate and siliciclastic facies deposited on a stable

133 continental margin following a late Neoproterozoic rift event[30,31] (Fig. 1). The

134 lowermost units of the Corumbá Group are the terrigenous Cadieus and Cerradinho

135 Formations, which are likely contemporaneous with the Puga Formation of the

136 Amazon Craton[30] (and thus possibly Marinoan-equivalent). Stromatolitic dolostones

137 and phosphorites of the Bocaina Formation lie above those siliciclastic units. The

138 lower Corumbá Group is unconformably overlain by the fossiliferous dark organic-

139 rich marls and limestones of the Tamengo Formation, and laminated siltstones of the

140 Guaicurus Formation[31] (Fig. 1). A breccia horizon marks the base of the Tamengo

141 Formation in several sections, and is concordantly overlain by interbedded mudstones

142 and grainstones deposited in a shallow platform setting. The laminated calcareous

143 siltstones of the Guaicurus Formation indicate deposition in a setting with low

144 hydrodynamic energy, probably below fair-weather wave-base. The sedimentary

145 succession has previously yielded macroscopic body fossils including the

146 scyphozoan–like *Corumbella werneri* and *Paraconularia*[4], along with *Cloudina*

147 *lucianoi*, in the upper Tamengo Formation, and possible vendotaenid algae

148 (*Eoholynia*) in the lowermost Guaicurus Formation[31] (Fig. 1).

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151 **Results**

152 *U-Pb Geochronology*

153 Three volcanic tuff horizons were sampled within the Corumbá Group (Fig. 1), and

154 zircons from these tuffs were dated using U-Pb chemical abrasion (CA) ID-TIMS

155 methods (see Methods for full methodology). An ash bed from the top of the Bocaina

156 Formation (from Porto Morrinhos; Fig. 1) yielded a weighted mean ^{206}Pb/^{238}U date of

157 555.18 ± 0.30/0.34/0.70 Ma (MSWD=1.6, *n*=8 out of 8) (Supp. Info. Fig. 6, Table

158 3,4), which we consider to approximate the age of the sample. This date provides a

159 maximum age for the overlying Tamengo Formation. Two further ash beds (samples

160 1.08 and 1.04) were collected from the top of the Tamengo Formation. Zircons from

161 sample 1.04 yielded U-Pb CA-ID-TIMS dates that ranged from 541.2 to 548 Ma, with

162 a cluster of the five youngest concordant analyses defining a weighted mean

163 ^{206}Pb/^{238}U date of 541.85 ± 0.75/0.77/0.97 Ma (MSWD=3.3, *n*=5 out of 11) ((Supp.

164 Info. Fig. 6, Table 3,4) that we consider approximates the age of the sample. Zircons

165 from sample 1.08 yielded U-Pb CA-ID-TIMS dates that ranged from 537 to 552 Ma,

166 with a coherent cluster of four concordant analyses (Fig. 2) defining a weighted mean

167 ^{206}Pb/^{238}U date of 542.37 ± 0.28/0.32/0.68 Ma (MSWD=0.68, *n*=4 out of 8) (Supp.

168 Info. Fig. 6, Table 3,4). We consider the single significantly older data point to result

169 from the incorporation of xenocrystic zircon, perhaps during eruption. The three

170 younger ^{206}Pb/^{238}U dates from sample 1.08 are considered to reflect Pb-loss based

171 upon the observations that: (i) they are non-overlapping, (ii) the ^{207}Pb/^{206}Pb dates are

172 similar to those that define the ~542 Ma population in both this sample and sample

173 1.04, and (iii) the derived dates from both upper Tamengo Formation samples are

174 consistent. Therefore 542.37 ± 0.28/0.32/0.68 Ma is taken to approximate the age of

175 sample 1.08. The data from samples 1.04 and 1.08 indicate an age of ~542 Ma for the

176 top of the Tamengo Formation, constraining the age of the upper Corumbá Group as

177 late Ediacaran (uppermost Bocaina–Tamengo formations, 555–542 Ma) to earliest

178 Cambrian (lower Guaicurus Formation, <542 Ma). The current accepted age for the

179 base of the Cambrian is 541.00 ± 0.29 Ma[32] (level Y uncertainty, excluding the

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180   systematic ^{238}U decay constant uncertainty).

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182   *Trace fossils of the Guaicurus and Tamengo formations*

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184   Three dimensionally mineralised fossils were collected from approximately 30–40m

185   above the base of the Tamengo Formation at two levels in the Ladário section (Fig.

186   1), and from a single horizon and loose material ~7 metres above the base of the

187   Guaicurus Formation from the Laginha Mine section (Fig. 1). The latter horizon is

188   <542.0 Ma in age based on the U-Pb CA-ID-TIMS data presented above.

189   Bi-lobed horizontal, iron oxide filled, ichnofossils occur in a single hand-

190   specimen, preserving part and counterpart, derived from float in the lower Guaicurus

191   Formation (Fig. 2c–d). The burrows are straight to curving, approximately 2 mm in

192   width, and exhibit dorsal and ventral median depressions, creating the bi-lobed

193   appearance typical of *Didymaulichnus lyelli*[33].

194   Small sub-horizontal structures occur in abundance in both the lower

195   Guaicurus (Fig. 2) and Tamengo Formation (Fig. 3). These consist of irregular multi-

196   tiered networks connected by short sub-vertical shafts. In bedding plane view, the

197   fossils are dark in colour relative to the matrix, forming dense assemblages comprised

198   of sinuous structures with rare dichotomous branches (Fig. 2a–b). The fossils are

199   filled with oxidized iron-rich minerals with framboidal morphologies, and authigenic

200   microcrystalline calcite (Fig. 2e–h). Framboids suggest that the fossils were originally

201   pyritized, and subsequently oxidized to iron oxides and oxyhydroxides (Supp. Fig. 1).

202   The presence of calcite and framboids throughout the infill suggests that the

203   framboids and calcite formed at a similar time.

204   The density contrast between the fossils and the host-rock allows them to be

205   visualized through X-ray microtomography (μCT; Figs 3–5), revealing a dense

206   ichnofabric (Figs 3e–g, 4e–f, 5f). Although many of the burrows are restricted to

207   single horizons, some cut across up to ~7mm of stratigraphic thickness, indicating

208   interstratal burrowing (Fig. 4g). Burrow diameters range from 45–573μm (Fig. 5g;

209   mean =193.2μm, n = 393). The Shapiro-Wilks test indicates these data are not drawn

210   from a normal distribution (p<0.01), and univariate BIC analysis supports either a

211   two- or three-component model. The lower limits of this distribution are likely

212   dictated by the voxel size of the scans, and so it is possible that the smallest size

213   fractions are omitted.

214

215   **Discussion**

216

217   The Guaicurus Formation assemblage is dated at <541.85 ± 0.77 Ma, and is broadly

218   contemporaneous with the Ediacaran–Cambrian boundary[32]. The Tamengo Formation

219   ichnofossils lie stratigraphically below our dated horizon of 542.37 ± 0.32 Ma, and

220   are thus between 542 and 555 Ma in age (late Ediacaran). The presence of different

221   size classes within the Corumbá Group data indicates different populations, further

222   supporting a biological, rather than abiological, mode of formation (see

223   supplementary information). As the structures are preserved as discrete, rounded

224   authigenically mineralised tubes they cannot be shrinkage features such as synaeresis

225   cracks.

226   A body fossil explanation for these structures is considered unlikely since

227   authigenically mineralized body fossils (e.g. algal filaments) would be expected to be

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228 confined to discrete horizons in finely laminated sediments rather than crossing

229 multiple horizons. Some Ediacaran body fossils, such as the simple conical

230 *Conotubus,* can grow through sedimentary laminae if felled[34]. In contrast, the

231 branching ichnofossils of the Guaicurus Formation are ~0.5 mm wide and cross up to

232 ~7 mm of stratigraphic thickness. The contemporaneous (Fig. 1) vendotaenid alga

233 *Eoholynia corumbensis* is superficially similar in size and morphology to the

234 ichnofossils described herein[31]. Two factors make algal origins for the fossils we

235 describe unlikely: mode of preservation, and morphology. First, in contrast to these

236 authigenically mineralised trace fossils, *Eoholynia* specimens in the Corumbá Group

237 are preserved as two-dimensional carbon films with some accessory oxides (possibly

238 after pyrite) (Supplementary Figure 5d-f). A comprehensive study of early Palaeozoic

239 non-biomineralized macroalgal taxa found that two dimensional compression (with

240 some accessory mineralisation) is the only taphonomic pathway through which

241 macroalgae fossilize during this time interval[35], consistent with the algal affinities of

242 *Eoholynia* and similar fossils. Although taphonomic mode should not be conflated

243 with affinity, the absence of three dimensionally pyritized algae from similar

244 localities of the same age renders an algal affinity for the proposed ichnofossils

245 unlikely. Secondly, *Eoholynia* have straight branches (rather than

246 undulating/sinusoidal) that taper after regular (dichotomous to polychotomous)

247 branching from a distinct main branch and have rounded terminal structures

248 interpreted as sporangia[31]. Polychotomous branching, tapering and rounded termini

249 are not present in the ichnofossils.

250   Iron oxides form a patina on the outer margin of some larger endichnial

251 burrows, possibly reflecting pyritization of a mucous burrow lining[36] (Fig. 2e).

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252 Three-dimensional preservation as authigenic pyrite and calcite suggests that the

253 burrows were open prior to burial and compaction, and were not backfilled by the

254 trace-maker. Preservation in almost undistorted full relief is uncommon in mudstones

255 in the absence of burrow fill, except where significant early diagenesis and

256 dewatering occurs before burial[36]. Similar sized burrows of modern nematodes

257 possess a polysaccharide-rich mucous burrow lining[37], which would provide a locus

258 for the microbial reduction of sulphate from seawater within the burrows, causing

259 pyrite precipitation and consequently burrow preservation[38]: a mechanism we

260 consider to have been responsible for preservation of the Corumbá Group structures.

261 The poorly organized, vertically stacked, network-like galleries connected by

262 short oblique shafts are typical of the ichnogenus *Multina*. A combination of size

263 range and irregularly sinuous gallery morphology allows attribution to *M. minima*[39].

264 The small burrow diameter, originally circular cross-sections, and lack of dorso-

265 ventral differentiation characteristic of the Corumbá Group *Multina* are consistent

266 with a narrow-bodied vermiform trace maker. It is unclear how many infaunalization

267 events are represented by the assemblages reconstructed in three dimensions (e.g. Fig.

268 5e), but the presence of continuous oblique shafts between levels suggests that the

269 burrows remained open throughout the life of the tracemaker.

270 Animal burrowing is typically achieved either by: 1) peristalsis (e.g. in

271 annelids like the Arenicolidae); 2) through the extension and retraction of an introvert

272 (e.g. loriciferans, kinorynchs, sipunculans); or 3) by a combination of the two (e.g.

273 priapulids)[60]. These mechanisms compact sediment laterally at the burrow margins[40],

274 but such compaction is absent in the Guaicurus traces (Fig. 2e–g). Compression

275 burrowing is similar, and involves the tracemaker forcing its way through the

276   sediment, compacting it at the margins[41]. Trochozoan taxa such as annelids, molluscs

277   and nemerteans can be excluded as potential trace-makers because the minimum

278   diameter *M. minima* resolved in the Guaicurus Formation is ~45μm; significantly

279   smaller than recently hatched trochophore larvae, which are approximately 100μm in

280   diameter and pelagic, not endobenthic[42]. Annelids can be further excluded as potential

281   trace-makers since the smallest annelid eggs (50–70μm diameter[42]) exceed the

282   diameter of the smallest traces.

283   Early spiralians may have been small bodied, with taxa such as gastrotrichs

284   and gnathiferans recovered as a paraphyletic grade in phylogenomic analyses[24]. Many

285   spiralian meiofaunal groups move using ciliary gliding, which is unlikely to have

286   formed continuous open burrows or achieved the sediment movement responsible for

287   interstratal burrowing. Mucociliary gliding by extant platyhelminths[43] creates traces

288   similar in gross morphology to horizontal Ediacaran trails, and so members of the

289   total groups of Bilateria, Xenacoelomorpha and Nephrozoa are candidate trace makers

290   for late Ediacaran surficial traces. Ciliary gliding has probably been independently

291   lost multiple times within Nephrozoa (e.g. Ecdysozoa, which lack external ciliation).

292   Ciliary gliding is retained in some macroscopic spiralians, including Nemertea,

293   Platyhelminthes and molluscan classes in which the foot is used in locomotion, such

294   as gastropods. Nevertheless, ciliary gliding was the likely locomotory mechanism for

295   the last common ancestor of both Bilateria and Nephrozoa. Ciliary gliding is unlikely

296   to produce open burrows in fine-grained sediments and in the meiofauna it is most

297   commonly utilised by organisms that live in interstitial spaces between sand grains.

298   Free-living nematodes use undulating motions to move through fine-grained

299   soft sediments, the low viscosity of which limits them to small body size[44]. Organisms

300 without body appendages possessing only longitudinal muscles, such as nematodes[45], are restricted to sinusoidal locomotion since they lack the antagonistic circular

301

302 muscles necessary for peristalsis. Nematodes are common bioturbators of modern

303 muddy sediments, and can create open mucus-lined burrows of a size range

304 comparable to that of the Brazilian *M. minima* (Fig. 5g). Similar but slightly larger *M.*

305 *minima*, potentially attributable to marine nematodes, have been described from the

306 Cambrian and Ordovician[46,47], but do not preserve the tiered networks of *M. minima*

307 we report. Burrow morphologies produced by extant or extinct nematomorphs are

308 unknown.

309   The size and morphology of the meiobenthic *Multina* is consistent with a

310 nematoid-like tracemaker that lacked body appendages and did not move by

311 peristalsis or ciliary gliding. As the ancestral bilaterian and nephrozoan moved using

312 ciliary gliding, this burrowing style suggests a trace maker that phylogenetically

313 postdates the nephrozoan crown node. These burrows may potentially provide an age

314 constraint for total group Nematoida (i.e. nematodes plus nematomorphs). This is

315 consistent with Early Cambrian body fossils, which include representatives of most

316 Ecdysozoan phyla, along with meiofaunal groups[28]. Total group nematoids are

317 therefore likely to have diverged from their closest living relatives by at least 520 Ma,

318 regardless of their controversial position within Ecdysozoa[48]. An alternative

319 interpretation is that these trace fossils were produced by a stem group ecdysozoan

320 that phylogenetically predates the evolution of an introvert but had already evolved a

321 chitinous cuticle and thus was unable to use ciliary gliding. A similar body plan is

322 present in larval insects, which produce freshwater and terrestrial *Cochlichnus*

323 burrows and move in a similar fashion to nematodes[10].

324

The Proterozoic–Phanerozoic biological radiation and the origin of the meiofauna

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The Corumbá Group trace fossils place an important latest Ediacaran (541–555 Ma) minimum constraint on the origin of meiofaunal animals and their interactions with soft substrates. Meiofauna are ubiquitous in both modern marine and freshwater environments, and their origin in deep time has been often discussed[21,22] but little explored from an evidential palaeontological perspective. Extant meioendobenthic organisms are particularly important contributors to biogeochemical cycling, microbial ecology, and ecosystem productivity, especially in muddy sediments[27,37]. Multiple studies discuss the trace fossil record of macrofaunal behaviour from the late Ediacaran onwards, its postulated impacts upon sediment geochemistry and benthic ecology, and its role in ecosystem engineering and ecological escalation[1,2,8]. Constraining the deep time origins of a meiofaunal mode of life may be equally important for understanding the biological and chemical evolution of marine sedimentary environments. It is unlikely that the meiofaunal burrowing described here had a substantial impact on substrate mixing, due to its small depth of penetration leaving sedimentary laminae largely undisturbed (Fig. 4e).

Our geochronological framework places temporal constraints on the first appearance of several biological and ecological innovations in the South American fossil record, and permits correlation of these events to other dated sections worldwide (Fig. 6). Biomineralizing macro-organisms (*Cloudina*), annulated tubular macrofossils (*Corumbella*) and meiofaunal burrowers all appear in the Corumbá sections after 555 Ma, but before 542 Ma. The temporal range for the macrofossils

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348 corresponds well to similar latest Ediacaran fossil assemblages, some of which record

349 evidence for predation[49], a decline in Ediacaran soft-bodied macro-organisms[50], and

350 the appearance of macroscopic burrows[10,17] in the interval immediately preceding the

351 Ediacaran-Cambrian boundary. Taken together, these records bear witness to several

352 major biological innovations amongst eumetazoans, indicating that this key interval

353 may offer significant scope for unravelling the intricacies surrounding the early stages

354 of bilaterian evolution.

355

356 **FIGURE CAPTIONS**

357

358 **Figure 1**. Stratigraphic column and locality map of the Ediacaran–early Cambrian

359 Corumbá Group: composite section compiled from logs in the Corumbá – Ladário

360 region, Mato Grosso do Sul State, Brazil. Dates are derived from this study. White

361 stars indicate localities from which samples for geochronology were obtained. Black

362 stars indicate ichnofossil localities described in this study: Laginha Mine (Guaicurus

363 Formation) S: 19° 07’ 09.8”, W: 057° 38’ 40.4”. Ladário (Tamengo Formation) S:

364 19° 0’ 04.0” W: 57° 36’ 00.7”. Carbon isotope stratigraphy comes from the Laginha

365 Mine section[51].

366

367 **Figure 2.** Hand specimens and SEM photomicrographs of *Multina minima* and

368 *Didymaulichnus lyelli* traces from the Guaicurus Formation, Laginha Mine, Mato

369 Grosso do Sul State, Brazil. **(a)** Hand specimen of small *Multina minima,* OUMNH

370 ÁU.4c. **(b)** Bedding plane view of *Multina minima* (inset of (a)). **(c–d)** Bedding plane

371 view of bilobed *Didymaulichnus lyelli* (part and counterpart, OUMNH ÁU.2). **(e–h)**

SEM photomicrographs of bedding-normal polished thin sections of samples containing *Multina minima*. Framboidal iron oxide (originally pyrite) burrows fills are clearly observed. Burrows in **(e–g)** are viewed in cross section through the burrow diameter. **(h)** A burrow in lateral view.

Figure 3. Photographs **(a–d)** and CT volume renders **(e–g)** of *Multina minima* burrows from the Ediacaran Tamengo Formation, Ladário, Mato Grosso do Sul State, Brazil. **(a)** Representative hand specimen of Tamengo Formation samples, specimen GPIE 11048b. **(b–d)** Oxidized burrows with sub-horizontal trajectories, viewed in plan view on a bedding surface, **(b)** GPIE-11048b, **(c)** GPIE-11004b, **(d)** GPIE-11005a. Note that these specimens have been heavily weathered. **(e–g)** Volume renders of CT slice data through the burrows constructed using the programme Drishti. The burrows show curved, sub-horizontal trajectories, and are mostly <100 μm in diameter.

Figure 4. CT slices and 3D reconstructions of a burrow assemblage in specimen OUMNH ÁU.3 from the early Cambrian/latest Ediacaran Guaicurus Formation. **(a–c)** Representative CT x-ray slices through the specimen in plan view, showing burrows in light grey against a dark grey rock matrix. **(d)** 3D render of the specimen produced using Blender, showing individual burrows in different colours. **(e)** The same CT data volume rendered in Drishti, with burrows in gold. (f) Drishti volume render normal to bedding, showing interstratal burrowing.

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Figure 5. Specimen OUMNH ÁU.4/p1 from the Guaicurus Formation from which burrow measurement data were obtained. **(a)** Hand specimen from the Laginha Mine section, plan view. **(b)** Drishti volume render of 3D CT scan data, plan view. **(c–d)** Individual CT slices in plan view, from which burrow measurements were obtained via comparison of 3D volume-render to determine the maximum diameter of each burrow. **(e)** The Drishti volume render in (b) in lateral view. **(f)** Individual burrow morphologies extracted from the volume render in (b). **(g)** Histogram plotting burrow width against frequency.

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Figure 6. Plot showing the temporal distribution of body and trace fossils from key Ediacaran and earliest Cambrian stratigraphic sections that are radio-isotopically constrained to a useful level of precision. Uncertainty in the temporal occurrence of a given fossil is constrained by dated ash layers that occur above or below the fossil type occurrence. The uncertainty in the placement of the first and last occurrence datum increases away from the dated levels. An asterisk (*) indicates data from this study.

415

METHODS

U-Pb Geochronology

U-Pb dates were obtained by the chemical abrasion isotope dilution thermal ionisation mass spectrometry (CA-ID-TIMS) method on selected single zircon grains (Table 1;

420 Supp. Info. Table 1), extracted from an aliquot of samples "Porto Morrinhos", "1.04"

421 and "1.08". Zircon grains were isolated from the rock samples using standard

422 magnetic and density separation techniques, and annealed in a muffle furnace at

423 900°C for 60 hours in quartz beakers. Zircon crystals from sample Porto Morrinhos

424 have aspect ratios varying from ~2 to ~5 and are typically 150 to 300 μm in their

425 longest dimension, and often contained a medial melt inclusion typical of volcanic

426 zircon. Zircon form samples 1.04 were smaller with the long-dimension on the order

427 of 50 to 100 μm with lower aspect ratios (~2) and doubly terminated. Zircon form

428 samples 1.08 had aspect ratios ranging from 2 to 4 and long-dimension on the order of

429 100 to 200 μm w and were doubly terminated. No cathodoluminescence imaging was

430 undertaken due to the small size of the zircon, and that the presence of medial melt

431 inclusions and the general external morphologies were indicative of inherited cores

432 not being present. Zircon selected for analyses based on external morphology, were

433 transferred to 3 ml Teflon PFA beakers, washed in dilute HNO_3 and water, and loaded

434 into 300 μl Teflon PFA microcapsules. Fifteen microcapsules were placed in a large-

435 capacity Parr vessel, and the crystals partially dissolved in 120 μl of 29 M HF for 12

436 hours at 180°C. The contents of each microcapsule were returned to 3 ml Teflon PFA

437 beakers, the HF removed and the residual grains immersed in 3.5 M HNO_3,

438 ultrasonically cleaned for an hour, and fluxed on a hotplate at 80°C for an hour. The

439 HNO_3 was removed and the grains were rinsed twice in ultrapure H_2O before being

440 reloaded into the same 300 μl Teflon PFA microcapsules (rinsed and fluxed in 6 M

441 HCl during crystal sonication and washing) and spiked with the EARTHTIME mixed

442 ^{233}U-^{235}U-^{205}Pb tracer solution (ET535). These chemically abraded grains were

443 dissolved in Parr vessels in 120 μl of 29 M HF with a trace of 3.5 M HNO_3 at 220°C

18

444 for 60 hours, dried to fluorides, and then re-dissolved in 6 M HCl at 180°C overnight.

445 U and Pb were separated from the zircon matrix using an HCl-based anion exchange

446 chromatographic procedure, eluted together and dried with 2 μl of 0.05N H_3PO_4. Pb

447 and U were loaded on a single outgassed Re filament in 5 μl of a silica-gel/phosphoric

448 acid mixture[52], and U and Pb isotopic measurements made on a Thermo Triton multi-

449 collector thermal ionisation mass spectrometer equipped with an ion-counting SEM

450 detector. Pb isotopes were measured by peak-jumping all isotopes on the SEM

451 detector for 100 to 150 cycles. Pb mass fractionation was externally corrected using a

452 mass bias factor of $0.14 \pm 0.03\%$/u determined via measurements of $^{202}Pb/^{205}Pb$

453 (ET2535)-spiked samples analysed during the same experimental period. Transitory

454 isobaric interferences due to high-molecular weight organics, particularly on ^{204}Pb,

455 disappeared within approximately 30 cycles or earlier, and ionisation efficiency

456 averaged 10^4 cps/pg of each Pb isotope. Linearity (to $\geq 1.4 \times 10^6$ cps) and the

457 associated deadtime correction of the SEM detector were monitored by repeated

458 analyses of NBS982, and have been constant since installation in 2006. Uranium was

459 analysed as UO_2+ ions in static Faraday mode on 10^{12} ohm resistors for 150 to 200

460 cycles, and corrected for isobaric interference of $^{233}U^{18}O^{16}O$ on $^{235}U^{16}O^{16}O$ with an

461 $^{18}O/^{16}O$ of 0.00206. Ionisation efficiency averaged 20 mV/ng of each U isotope. U

462 mass fractionation was corrected using the known $^{233}U/^{235}U$ ratio of the ET2535

463 tracer solution.

464   We used the ET535 tracer solution[53, 54] and U decay constants recommended

465 by Jaffey et al.[55]. A value of 137.818 ± 0.045 was used for the $^{238}U/^{235}U_{zircon}$ based

466 upon the work of [56]. $^{206}Pb/^{238}U$ ratios and dates were corrected for initial ^{230}Th

467 disequilibrium using a Th/U[magma] = 3 ±1 resulting in an increase in the $^{206}Pb/^{238}U$

468   dates of ~0.09 Myr. All common Pb in analyses was attributed to laboratory blank

469   and subtracted based on the measured laboratory Pb isotopic composition and

470   associated uncertainty. U blanks were estimated at 0.1 pg, based upon replicate total

471   procedural blanks.

472   In this manuscript the date uncertainties reporting is as X/Y/Z and reflect the

473   following sources: (X) analytical, (Y) analytical + tracer solution and (Z) analytical +

474   tracer solution + decay constants. The X uncertainty is the internal error based on

475   analytical uncertainties only, including counting statistics, subtraction of tracer

476   solution, and blank and initial common Pb subtraction. It is given at the 2σ confidence

477   interval. This error should be considered when comparing our dates with $^{206}Pb/^{238}U$

478   dates from other laboratories that used the same EARTHTIME tracer solution or a

479   tracer solution that was cross-calibrated using related gravimetric reference materials.

480   The Y uncertainty includes uncertainty in the tracer calibration and should be used

481   when comparing our dates with those derived from laboratories that did not use the

482   same EARTHTIME tracer solution or a tracer solution that was cross-calibrated using

483   relatable gravimetric reference material. The Z uncertainty includes the above in

484   addition to uncertainty in the ^{238}U decay constant[55]. This uncertainty level should be

485   used when comparing our dates with those derived from other decay schemes (e.g.

486    $^{40}Ar/^{39}Ar$, $^{187}Re-^{187}Os$).

487

488   *CT*

489   Four individual hand specimens were scanned using Nikon XTH-225 µCT scanners at

490   the Natural History Museum (London), and the Life Sciences Building, University of

491   Bristol. X-rays were generated using a tungsten target. Scan parameters are provided

20

492   in the supplementary information.

493   Following μCT scanning, the data were imported into the programme Drishti[90]

494   We used this programme to both volume render the data following the methods in

495   Hickman-Lewis et al.[57], and to reslice the volumes to create a TIFF stack of images

496   approximately parallel to bedding. The data were also segmented using the SPIERS

497   software suite[58] following the methods of Garwood et al.[59], exported as STL meshes,

498   and then imported into the open source raytracer Blender[60]. In Blender, the mesh of

499   the surface was rendered partially transparent, and the mesh encompassing all

500   burrows was split into its component islands, allowing them to be coloured separately.

501

502   *Burrow Measurements*

503   No statistical methods were used to predetermine sample size. Burrow measurements

504   were obtained using ImageJ[61]. Measurements of burrow diameter were taken from

505   individual slices from specimen OUMNH AU.3, in order to characterize the size

506   frequency distribution of the trace fossils (Fig. 5g). Burrows were measured from

507   approximately bedding-parallel μCT slices at maximum burrow width. This was

508   preferred over systematically measuring burrows from a sample of slices, as such a

509   method would not necessarily sample burrows at their maximum diameter, and

510   consequently would skew the size frequency distribution towards a smaller mean

511   diameter. The smallest burrows observed in μCT slices are approximately 2 pixels

512   (~40μm) in diameter, and are thus at the limit of scan resolution. A Shapiro-Wilks test

513   and BIC analysis (using the R package mclust[62]) were used to determine population

514   structure in the measurement data[63].

515

DATA AVAILIBILITY

U-Pb isotopic data used in this study is available in the supplementary information. CT data is stored on servers at the University of Bristol and will be made publically available upon acceptance and publication of this manuscript. All specimens analysed are held at the University of Sao Paulo and Oxford University Museum of Natural History.

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543

544

545 **AUTHOR CONTRIBUTIONS**

546 LP found and initially identified the *Multina* specimens in the Guaicurus Formation.

547 PCB, AGL, CQCD and JML found the *Multina* specimens in Tamengo Formation.

548 All authors collaborated in order to develop this research project. AGL and DC

549 secured funding for geochronological dating. LP, DC and RG conducted the analyses.

550 PCB, RT, JML, CQCD, MLAFP and GACC measured the stratigraphic section and

551 collected samples for dating. LP, DM, DC and AGL developed the manuscript, and

552 all the authors were involved in data interpretation and the final redrafting of the

553 manuscript.

554

555 **COMPETING INTERESTS**

556 The authors declare no competing financial interests.

557

558 **References**
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