

1 **Ichnological evidence for meiofaunal bilaterians from the Ediacaran–Cambrian**
2 **transition of Brazil**

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

55

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

60 clocks indicates a protracted Neoproterozoic history for the Metazoa, with the origin
61 of animals significantly pre-dating the base of the Cambrian³.

62 A range of biological phenomena typically associated with animals first
63 appears during the late Ediacaran interval (~580–541 Ma) including: skeletogenesis⁴,
64 reef-building⁵ and macroscopic predation⁶. Body fossils of late Ediacaran macro-
65 organisms include at least some early animals³, but crucially, most plausible claims
66 for metazoans lie within the diploblasts rather than the Bilateria³. *Kimberella*, which
67 is putatively a stem mollusc⁷, is a notable exception, but some authors suggest that it
68 can only be reliably considered as a member of total group Bilateria³.

69 Our understanding of early animal evolution is complemented by ichnological
70 investigations of latest Ediacaran to Ordovician strata^{1,2,8}. Diverse ichnofossil
71 assemblages in the earliest Cambrian place an important constraint on the tempo of
72 bilaterian origins, since they indicate that some groups, including total group
73 panarthropods and priapulid-like scalidophorans^{2,9}, were globally distributed and
74 abundant by this point. The major bilaterian divergences (i.e. the protostome-
75 deuterostome and ecdysozoan-lophotrochozoan divergences) must therefore pre-date
76 the Ediacaran–Cambrian boundary. To date, the Ediacaran trace fossil record has
77 provided limited insight into these early divergences. Most Ediacaran ichnofossils are
78 either surface traces or simple under-mat burrows, created either on or immediately
79 beneath matgrounds¹⁰. Such traces extend back to ~555 Ma¹¹, including: inferred
80 grazing traces (*Kimberichnus*¹²) associated with the body fossil *Kimberella*, vertical
81 adjustment structures in response to seafloor aggradation¹³, and in the latest
82 Ediacaran, shallow vertical burrows¹⁰, treptichnid-like burrows just below the
83 Ediacaran-Cambrian boundary¹⁴. Most Ediacaran ichnofossils are considered to have

84 been made by total group bilaterian¹⁵ or cnidarian^{13,16} eumetazoans. Notwithstanding
85 controversial claims for bioturbation and complex burrows at ~553 Ma¹⁷, widespread
86 substrate-penetrating burrows capable of significant sediment mixing do not appear
87 until close to the Precambrian-Cambrian boundary¹⁴.

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 Deuterostomia¹⁸. Palaeontological support for these suggestions is limited to
91 purported body fossils of sponges¹⁹, and demosponge biomarkers²⁰. 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 Eumetazoa³. Assuming that contemporary molecular clock analyses yield accurate, if
95 imprecise¹⁸, node ages for animal divergences, a small body size and concomitant
96 limited fossilisation potential²¹ could reconcile these discordant records of animal
97 evolution (though see reference²²).

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 trochozoans²⁴. 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 environments²⁵. Modern
107 meiofaunal communities include animals, foraminifera and some ciliates, and

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 sediments²⁶, 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.

129

130 **The Corumbá Group**

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

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 Craton³⁰ (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 Formation³¹ (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 wernerii* and *Paraconularia*⁴, along with *Cloudina*
147 *lucianoii*, in the upper Tamengo Formation, and possible vendotaenid algae
148 (*Eoholynia*) in the lowermost Guaicurus Formation³¹ (Fig. 1).

149

150

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 $^{206}\text{Pb}/^{238}\text{U}$ date of
157 $555.18 \pm 0.30/0.34/0.70$ 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 $^{206}\text{Pb}/^{238}\text{U}$ date of $541.85 \pm 0.75/0.77/0.97$ 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 $^{206}\text{Pb}/^{238}\text{U}$ date of $542.37 \pm 0.28/0.32/0.68$ 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 $^{206}\text{Pb}/^{238}\text{U}$ dates from sample 1.08 are considered to reflect Pb-loss based
171 upon the observations that: (i) they are non-overlapping, (ii) the $^{207}\text{Pb}/^{206}\text{Pb}$ 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 $542.37 \pm 0.28/0.32/0.68$ 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 Ma³² (level Y uncertainty, excluding the

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

181

182 *Trace fossils of the Guaicurus and Tamengo formations*

183

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 (μ CT; 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 ~ 7 mm of stratigraphic thickness, indicating
208 interstratal burrowing (Fig. 4g). Burrow diameters range from 45–573 μ m (Fig. 5g;
209 mean =193.2 μ m, 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 $< 541.85 \pm 0.77$ Ma, and is broadly
218 contemporaneous with the Ediacaran–Cambrian boundary³². 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 syneresis
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

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 felled³⁴. 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 herein³¹. 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 interval³⁵, 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 sporangia³¹. 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 lining³⁶ (Fig. 2e).

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 burial³⁶. Similar sized burrows of modern nematodes
257 possess a polysaccharide-rich mucous burrow lining³⁷, which would provide a locus
258 for the microbial reduction of sulphate from seawater within the burrows, causing
259 pyrite precipitation and consequently burrow preservation³⁸: 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, kinorhynchans, sipunculans); or 3) by a combination of the two (e.g.
273 priapulids)⁶⁰. These mechanisms compact sediment laterally at the burrow margins⁴⁰,
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 margins⁴¹. 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 endobenthic⁴². Annelids can be further excluded as potential
281 trace-makers since the smallest annelid eggs (50–70µm diameter⁴²) exceed the
282 diameter of the smallest traces.

283 Early spiralian may have been small bodied, with taxa such as gastrotrichs
284 and gnathiferans recovered as a paraphyletic grade in phylogenomic analyses²⁴. 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 platyhelminths⁴³ 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 spiralian, 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 size⁴⁴. Organisms

300 without body appendages possessing only longitudinal muscles, such as nematodes⁴⁵,
301 are restricted to sinusoidal locomotion since they lack the antagonistic circular
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 groups²⁸. 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 Ecdysozoa⁴⁸. 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 nematodes¹⁰.

324

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

326

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

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

348 corresponds well to similar latest Ediacaran fossil assemblages, some of which record
349 evidence for predation⁴⁹, a decline in Ediacaran soft-bodied macro-organisms⁵⁰, 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 section⁵¹.

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)**

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

376

377

378 **Figure 3.** Photographs **(a–d)** and CT volume renders **(e–g)** of *Multina minima*
379 burrows from the Ediacaran Tamengo Formation, Ladário, Mato Grosso do Sul State,
380 Brazil. **(a)** Representative hand specimen of Tamengo Formation samples, specimen
381 GPIE 11048b. **(b–d)** Oxidized burrows with sub-horizontal trajectories, viewed in
382 plan view on a bedding surface, **(b)** GPIE-11048b, **(c)** GPIE-11004b, **(d)** GPIE-
383 11005a. Note that these specimens have been heavily weathered. **(e–g)** Volume
384 renders of CT slice data through the burrows constructed using the programme
385 Drishti. The burrows show curved, sub-horizontal trajectories, and are mostly <100
386 μm in diameter.

387

388

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

396

397

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

406

407

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

415

416 **METHODS**

417 *U-Pb Geochronology*

418 U-Pb dates were obtained by the chemical abrasion isotope dilution thermal ionisation
419 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 from 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 from
428 samples 1.08 had aspect ratios ranging from 2 to 4 and long-dimension on the order of
429 100 to 200 µm 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 HNO₃ 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 HNO₃,
438 ultrasonically cleaned for an hour, and fluxed on a hotplate at 80°C for an hour. The
439 HNO₃ was removed and the grains were rinsed twice in ultrapure H₂O 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 ²³³U-²³⁵U-²⁰⁵Pb 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 HNO₃ at 220°C

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 H₃PO₄. Pb
447 and U were loaded on a single outgassed Re filament in 5 µl of a silica-gel/phosphoric
448 acid mixture⁵², 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 ²⁰²Pb/²⁰⁵Pb
453 (ET2535)-spiked samples analysed during the same experimental period. Transitory
454 isobaric interferences due to high-molecular weight organics, particularly on ²⁰⁴Pb,
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 UO₂⁺ ions in static Faraday mode on 10^{12} ohm resistors for 150 to 200
460 cycles, and corrected for isobaric interference of ²³³U¹⁸O¹⁶O on ²³⁵U¹⁶O¹⁶O with an
461 ¹⁸O/¹⁶O of 0.00206. Ionisation efficiency averaged 20 mV/ng of each U isotope. U
462 mass fractionation was corrected using the known ²³³U/²³⁵U 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 ²³⁸U/²³⁵U_{zircon} based
466 upon the work of ⁵⁶. ²⁰⁶Pb/²³⁸U ratios and dates were corrected for initial ²³⁰Th
467 disequilibrium using a Th/U[magma] = 3 ± 1 resulting in an increase in the ²⁰⁶Pb/²³⁸U

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 $^{206}\text{Pb}/^{238}\text{U}$
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 reliable gravimetric reference material. The Z uncertainty includes the above in
484 addition to uncertainty in the ^{238}U decay constant⁵⁵. This uncertainty level should be
485 used when comparing our dates with those derived from other decay schemes (e.g.
486 $^{40}\text{Ar}/^{39}\text{Ar}$, $^{187}\text{Re}-^{187}\text{Os}$).

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

492 in the supplementary information.

493 Following μ CT scanning, the data were imported into the programme Drishti⁹⁰
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 suite⁵⁸ following the methods of Garwood et al.⁵⁹, exported as STL meshes,
498 and then imported into the open source raytracer Blender⁶⁰. 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 ImageJ⁶¹. 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 μ CT 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 μ CT slices are approximately 2 pixels
512 ($\sim 40\mu\text{m}$) in diameter, and are thus at the limit of scan resolution. A Shapiro-Wilks test
513 and BIC analysis (using the R package mclust⁶²) were used to determine population
514 structure in the measurement data⁶³.

515

516

517 **DATA AVAILIBILITY**

518

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

524

525

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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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