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THE GIANTS OF THE PHYLUM BRACHIOPODA: A MATTER OF DIET?

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Abstract: The species of the brachiopod *Gigantoproductus* are giants within the Palaeozoic sedentary benthos. This presents a dilemma as living brachiopods have low-energy lifestyles. Although brachiopod metabolic rates were probably higher during the Palaeozoic than today, the massive size reached by species of *Gigantoproductus* is nevertheless unusual. By examining the diet of *Gigantoproductus* species from the Visean (Mississippian, Carboniferous) of Derbyshire (UK), we seek to understand the

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3 mechanisms that enabled those low-metabolism brachiopod species to become giants.
4 Were they suspension feeders, similar to all other brachiopods or did endosymbiosis
5 provide a lifestyle that allowed them to have higher metabolic rates and become giants?
6 We suggest that the answer to this conundrum may be solved by the identification of the
7 biogeochemical signatures of symbionts, through combined analyses of the carbon- and
8 nitrogen-isotopic compositions of the occluded organic matrix within their calcite shells.
9 The shells are formed of remarkably long, and a few hundreds of micrometres wide,
10 substructured columnar units deemed to be mostly pristine based on multiple analyses
11 [petrography, cathodoluminescence (CL), Scanning Electron Microscopy (SEM), Electron
12 Backscatter Diffraction (EBSD), Transmission Electron Microscopy (TEM)]; they contain
13 occluded organic fractions detected by TEM, Nuclear Magnetic Resonance (NMR), and
14 Gas Chromatography Mass Spectrometry (GC-MS) analyses. We conclude that the
15 gigantic size reached by the species of *Gigantoproductus* is probably the result of a
16 mixotroph lifestyle, by which they could rely on the energy and nutrients derived both from
17 photosymbiotic microbes and from filtered particulate food.
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30 **Key words:** *Gigantoproductus*, gigantism, brachiopods, diet, endosymbiosis,
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36 The species of *Gigantoproductus* are notable giants within the Brachiopoda, attaining
37 sizes of more than an order of magnitude higher than all other members of this marine
38 phylum of lophotrochozoans. They reach over 30 cm in width and over 1 cm in shell
39 thickness (e.g., Muir-Wood & Cooper 1960; Angiolini *et al.* 2012; Qiao & Shen 2015;
40 Vermeij 2016). Living brachiopods have a low-energy lifestyle (low metabolism, low
41 growth, feeding and reproduction rates) (e.g., Curry *et al.* 1989; Peck 1996; Vermeij 2016),
42 and so the size of *Gigantoproductus* is puzzling, though the reason for this has never been
43 investigated in detail nor satisfactorily explained. Brachiopod metabolic rates were
44 probably higher and more variable during the Palaeozoic (Payne *et al.* 2014; Vermeij
45 2016) and brachiopods had a trend towards larger size (Novack-Gottshall & Lanier 2008;
46 Zhang *et al.* 2015). The size reached by species of *Gigantoproductus* is, nonetheless, still
47 dramatically larger compared with the average size of other Palaeozoic brachiopods.
48 The size increase during the Palaeozoic has been attributed to increases in available
49 oxygen, primary productivity, and predation pressure (e.g., Zhang *et al.* 2015); although
50 other recent studies (e.g., Vermeij 2016) emphasized that the gigantism of fossil and
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3 recent plants and animals remains poorly understood. Gigantism has been interpreted as
4 an evolutionary strategy for protection against predators, because the cost of killing and
5 consuming prey is higher than the benefit (Palmer 1999; Vermeij 2012). The early growth
6 stages of giant prey, when the individual is more vulnerable, are characterized by rapid
7 growth (Vermeij 2012). However, competitive interactions, more than predation, may have
8 been the main reason for gigantism (Vermeij 2016).

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10 One of the key features of gigantism is a high metabolic rate (Vermeij 2016), apparent also
11 in the case of marine ectotherms such as normal suspension feeders, photosymbiotic and
12 chemosymbiotic animals. In the latter, the larger the size, the higher the activity levels and
13 metabolic rates, and symbiotic associations are known to have frequently led to gigantism
14 (Vermeij 2016). Through removal of CO₂, algal symbiosis also promotes calcification
15 leading to skeletal gigantism (Cowen 1983; Key *et al.* 2005). This has been demonstrated
16 in Recent and fossil foraminifera, corals and bivalves (e.g., Vogel 1975; Lee *et al.* 1979;
17 Cowen 1988). Similarly, the large size of the Permian lyttonioid and richthofenioid
18 brachiopods is thought to have been related to their tropical distributions and possible
19 association with zooxanthellae algae (Cowen 1970); an attractive hypothesis established
20 on the basis of their large size and comparison with the giant bivalve *Tridacna* and its
21 symbionts. Other brachiopods from deep time have been hypothesized to have reached a
22 very large size through methano- or thiotrophic diets, such as the Lower Cretaceous
23 rhynchonellid *Peregrinella* (e.g., Campbell & Bottjer 1995; Posenato & Morsilli 1999; Kiel *et*
24 *al.* 2014).

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26 Gigantism is also reported in species recording the 'gentle giant syndrome'. These species
27 live under conditions with poor resources – such as those in the polar regions and the
28 deep sea - and are characterized by low metabolic rates, slow-growth, minimal
29 maintenance costs, and low juvenile mortality (e.g., Antarctic marine animals and island
30 tortoises; Arnaud 1974; Rosa & Seibel 2010; Vermeij 2016).

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32 Here, we investigate and seek to understand the mechanisms that enabled ectothermic,
33 low-metabolism and physiologically unbuffered brachiopod species with a primitive
34 lophophore to become giants. We examine their feeding strategy and whether they were
35 normal suspension feeders as typical for all other brachiopods, or influenced by an
36 endosymbiotic lifestyle that possibly enabled them to have higher metabolic rates and
37 become giants.

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39 One of the best ways to evaluate these causes is to look for the biogeochemical
40 signatures that identify symbioses (e.g., Levin & Michener 2002; O'Donnell *et al.* 2003;

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3 Mae *et al.* 2007; Dreier *et al.* 2012, 2014), such as the carbon and nitrogen isotope
4 composition ($\delta^{13}\text{C}_{\text{org}}$, $\delta^{15}\text{N}_{\text{org}}$) of the primary organic fraction occluded in the shell, after the
5 evaluation of its degree of preservation. To discriminate between a suspension-feeding
6 lifestyle versus a symbiotic lifestyle, we compared different species of *Gigantoproductus*
7 occurring together in different palaeoenvironmental settings (Nolan *et al.* 2017).
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15 MATERIAL AND METHODS

16 Fifty specimens of *Gigantoproductus elongatus* (Pattison, 1981), *Gigantoproductus inflatus*
17 (Sarytcheva, 1928) and *Gigantoproductus okensis* (Sarytcheva, 1928), from the
18 Derbyshire Platform, Visean (Mississippian, Carboniferous) Monsal Dale Limestone and
19 Eyam Limestone formations of Ible (specimens id “IB”), Wensley Dale (specimens id “WI”),
20 Once-a-Week Quarry (specimen id “OAW”) and Ricklow Quarry (specimens id “RCK”) in
21 Derbyshire, UK (Fig. 1, Table 1A-B), were measured to quantify their size [for details on
22 their provenance and depositional environment see Angiolini *et al.* (2012) and Nolan *et al.*
23 (2017)]. Sixteen specimens from the Once-a-Week and Ricklow quarries were used for
24 petrographic and cathodoluminescence analyses, and ten specimens were selected for
25 ultrastructural (SEM, TEM, EBSD), oxygen and carbon isotope analyses of calcite, and
26 carbon and nitrogen isotope analyses of occluded organic material. Nuclear magnetic
27 resonance and GC-MS analyses were performed on the shell organic matrix of specimen
28 RCK16. The specimens are permanently curated in the Palaeontological Museum of the
29 Department of Earth Sciences ‘A. Desio’, University of Milan, Italy and registered with a
30 prefix MPUM (11800-11815).
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33 In addition, 69 specimens of species belonging to genera other than *Gigantoproductus* ,
34 co-occurring with *Gigantoproductus* in the same formations at Ible, Wensley Dale, Once-a-
35 Week Quarry and Ricklow Quarry were also measured for size (Table 1B).
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38 *Petrographic and CL analyses*

39 Sixteen brachiopod shells of *G. okensis*, *G. inflatus* and *G. elongatus* from the Eyam
40 Limestone Formation at Once-a-Week Quarry (OAW3, OAW8, OAW50, OAW203,
41 OAW205, OAW209, OAW212) and Ricklow Quarry (RCK11, RCK16, RCK33, RCK35,
42 RCK36, RCK41, RCK100, RCK221, RCK300) were embedded in resin, cut longitudinally
43 and thin sectioned. Petrographic analysis was performed with a polarized light optical
44 microscope. Cathodoluminescence (CL) was performed using a cold stage cathode
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3 luminoscope (CITL Cambridge Image Technology Limited, Cambridge, UK, model MK 5-2)
4 operating at 10-14 kV accelerating voltage with a beam current of 200-300 μ A at the
5 Department of Earth Sciences 'A. Desio', University of Milan, Italy. The CL is a screening
6 technique widely used for the preliminary assessment of preservation of brachiopod shells
7 (e.g., Angiolini *et al.* 2009; Garbelli *et al.* 2012, 2014).
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10 11 12 13 *SEM, TEM and EBSD analyses*

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15 To further assess shell preservation and the reliability of the isotope data, several
16 screening techniques were applied to ten selected specimens (OAW3, OAW203,
17 OAW212, RCK16, RCK33, RCK35, RCK36, RCK41, RCK221, and RCK300). Their
18 ultrastructure was examined by Scanning Electron Microscope (SEM), Transmission
19 Electron Microscope (TEM), and Electron Backscatter Diffraction (EBSD). The sectioned
20 specimens were etched with 5% HCl for 20 s before coating with gold to examine the
21 preservation of their shell fabric under SEM following the methodology described in
22 Angiolini *et al.* (2008, 2009).
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26 TEM mounts were prepared from epoxy embedded samples and doubly-polished
27 petrographic thin sections (30 μ m thick) were prepared. Electron transparency was
28 achieved by ion milling 3 mm wide discs cut out from the petrographic thin sections using a
29 Gatan PIPS (Department of Earth Sciences 'A. Desio', University of Milan, Italy). The TEM
30 mounts were then carbon coated to avoid electrostatic charging during observation. TEM
31 observations were performed with a Jeol JEM 2010 operating at 200 kV and equipped with
32 an Oxford Link energy dispersive spectrometer (EDS) and with an Olympus Tengra 2.3k x
33 2.3k x 14 bit slow-scan CCD camera (Department of Physics, Earth and Environment,
34 University of Siena, Italy).
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38 For Electron Backscatter Diffraction (EBSD) analyses, 5 x 5 mm thick pieces were excised
39 from the shell and embedded in epoxy resin. The surface of the embedded samples was
40 subjected to several sequential mechanical grinding and polishing steps down to a grain
41 size of 1 μ m. The final step was etch-polishing with colloidal alumina (particle size \sim 0.06
42 μ m) in a vibratory polisher. For EBSD analysis, the samples were coated with 4-6 nm of
43 carbon. EBSD measurements were carried out on Hitachi SU5000 field emission SEM,
44 equipped with a Nordlys-II EBSD detector. The SEM was operated at 20 kV and
45 measurements were indexed with the CHANNEL 5 HKL software. Information obtained from
46 EBSD measurements is presented as band contrast images, and as colour-coded crystal
47 orientation maps with corresponding pole figures, the latter displaying in one set of pole
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3 figures the data points and, in a second set, calculated calcite c- and a*-axes data density
4 distributions. The EBSD band contrast is the signal strength of the EBSD-Kikuchi diffraction
5 pattern and is displayed as a grey-scale component in EBSD maps. The strength of the
6 EBSD signal is high when a crystal is detected (bright in the map), while it is weak or absent
7 when a polymer such as an organic fraction is scanned (dark/black in the map).
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11 The brachiopod specimens OAW3, OAW203, OAW212, RCK16, RCK33, RCK35, RCK36,
12 RCK41, RCK221, and RCK300 were sampled for geochemical and stable isotope
13 analyses using a hand-held microdrill at low speed. Several grams (10-20 g) were taken
14 from the middle part of the very thick columnar shell layer of the ventral valve using a
15 diamond drill bit carefully avoiding to sample secondary fractures filled by burial diagenesis
16 calcite and marginal shell portions affected by silicification. When sufficient powder was
17 obtained, it was split into two parts, one for carbon and oxygen isotope analysis on shell
18 calcite (10 specimens), and the other one for nitrogen and carbon isotope measurements
19 on organic fraction isolated from the shell (six specimens). RCK16 was also sampled for
20 Nuclear Magnetic Resonance (NMR) and Gas Chromatography Mass Spectrometry (GC-
21 MS) analyses on shell organic matrix.
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30 *O and C stable isotopes from shell calcite and O isotopes of silica*

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32 The stable isotope ratios of shell calcite were measured on shells OAW3, OAW203,
33 OAW212, RCK16, RCK33, RCK35, RCK36, RCK41, RCK221 and RCK300 at the British
34 Geological Survey, Nottingham, UK. Approximately 50-100 μg of calcite was used, and
35 analysed for C and O isotope ratios using an Isoprime dual inlet mass spectrometer plus
36 Multiprep device. Samples were loaded into glass vials and sealed with septa. The
37 automated system evacuates vials and delivers anhydrous phosphoric acid to the
38 carbonate at 90°C. The evolved CO_2 is cryogenically cleaned and passed to the mass
39 spectrometer. Isotope values ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) are reported as per mil (‰) deviations of the
40 isotopic ratios ($^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$) calculated to the VPDB scale using a within-run laboratory
41 standard calibrated against NBS-19. The Craig correction is also applied to account for ^{17}O
42 (Craig 1957). Analytical reproducibility for the standard calcite (KCM) and sample material
43 was $< 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ [(both 1 standard deviation (SD))].
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53 Classical fluorination with the step wise approach is employed at the British Geological
54 Survey for the oxygen isotope analysis of silica. The method involves a three-stage
55 process. Stage one involves 'outgassing' to remove surficial and loosely bound water;
56 stage two involves a prefluorination step involving a stoichiometric deficiency of the
57 reagent chlorine trifluoride (ClF_3) at low temperature; and the third stage is a full reaction
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3 at high temperature for an extended period with an excess of reagent. The oxygen
4 liberated is then converted to CO₂ by exposure to graphite. Oxygen yields were monitored
5 by comparison with the calculated theoretical yield for SiO₂. Isotope values ($\delta^{18}\text{O}$) are
6 reported as per mil (‰) deviations of the isotopic ratio ($^{18}\text{O}/^{16}\text{O}$) calculated to the VSMOW
7 scale using a within-run laboratory standard calibrated against NBS-28. Analytical
8 reproducibility of the standard silica (BFC) and sample material was < 0.3‰ for $\delta^{18}\text{O}$
9 (1SD).
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17 *C and N isotopes from shell organic fraction*

18 Carbon and nitrogen isotope ratios of the organic fraction were measured on shells
19 OAW212, RCK16, RCK33, RCK36, RCK41 and RCK300 at Memorial University of
20 Newfoundland, St. John's, Canada. Several grams of powder were obtained from six
21 brachiopod specimens and weighed into 150 mL glass beakers. A total of 100 mL of 6M
22 distilled HCl diluted to 20% was added gradually in 10 mL increments over a period of
23 several days. The samples were stirred after each addition of HCl until there was no visible
24 reaction. After the carbonate reaction was complete, the samples were transferred to
25 centrifuge tubes to remove the acid and retain the organics and silicates. This residue was
26 rinsed into 15 mL Teflon jars using deionised water and left to evaporate. Approximately 2
27 mL HF and 2 mL 6.2N HCl was added to the sample, covered and left on a hot plate for 4-
28 5 days; heating was not vigorous (around 50°C, to prevent alteration of the organic matrix).
29 The cover was removed, rinsed and the sample left to evaporate. Two mL of 6.2N HCl
30 were added and evaporated twice. The sample was rinsed into microcentrifuge tubes
31 using deionised water and left to evaporate.
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43 Organic carbon ($\delta^{13}\text{C}_{\text{org}}$) and nitrogen ($\delta^{15}\text{N}_{\text{org}}$) isotope ratios were measured on isolated
44 kerogen after repeated treatment with pure concentrated hydrochloric acid, using a Carlo
45 Erba Elemental Analyzer coupled to a Thermo Fisher DELTA V plus isotope ratio mass
46 spectrometer in a stream of helium, where the gas was ionized and measured for isotope
47 ratios. The results were normalized to the standards IAEA-CH-6 ($\delta^{13}\text{C} = -10.4$ ‰ VPDB),
48 NBS-18 ($\delta^{13}\text{C} = -5.0$ ‰ VPDB), USGS-24 ($\delta^{13}\text{C} = -16.0$ ‰ VPDB), IAEA-N-1 ($\delta^{15}\text{N} = +0.4$
49 ‰ air), and IAEA-N-2 ($\delta^{15}\text{N} = +20.3$ ‰ air). Based on repeated measurements of $\delta^{13}\text{C}$ and
50 $\delta^{15}\text{N}$ standards (organic), uncertainty was ~ 0.2 ‰.
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58 *GC-MS and ¹H-NMR analyses on shell organic fraction*

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3 The pulverized RCK16 samples (around 10 g) were dried in an oven 2 h at 90°C and then
4 were dewaxed by washing three times with diethyl ether (100 mL) under magnetic stirring
5 and sonication. The powders were recovered by paper filtration. The dewaxed fossil
6 samples were hydrolyzed with 200 mL of 2 M HCl under vigorous magnetic stirring and at
7 the end of the reaction, the pH of the suspensions was adjusted at around 7 by the
8 addition of 5 M NaOH dropwise. The suspensions were then lyophilized overnight in order
9 to obtain a white powder.

15 *¹H-NMR analysis.* The white powder was extracted with 200 mL of a solution 20:80 v/v of
16 chloroform:methanol under magnetic stirring and sonication. The organic phase was
17 recovered by filtration and then the solvent was distilled under vacuum. The residue was
18 suspended in CDCl₃ and submitted to ¹H-NMR analysis by Bruker 600 MHz.

22 *GC-MS analysis.* Two methods were followed: A) the white powder was suspended in 150
23 mL of dry methanol and then 0.5 mL of 98% H₂SO₄ were added in order to promote the
24 methylation of carboxylic acids. The suspension was refluxed 5 h and then, after cooling, it
25 was diluted with 200 mL of distilled water and neutralized with NaHCO₃ until pH 9. The
26 suspension was then extracted by diethyl ether (3 times with 100 mL). The organic phase
27 was treated with anhydrous NaSO₄ and then dried in rotavapor. The residue was dissolved
28 in CH₂Cl₂ and then submitted to GC-MS analysis. B) the white powder (1 g) was
29 suspended in 1 mL of acetonitrile/water and then it was derivatized by the addition of 500
30 μL of isobutanol, 300 μL of pyridine and 250 μL of isobutylchloroformiate. After 30 minutes,
31 1 mL of chloroform was added and the vial was shaken. After phase separation, the
32 organic layer (2 μL) was submitted to GC-MS analysis.

42 RESULTS

44 *Size of the species of Gigantoproductus from the Visean of Derbyshire*

46 To quantify the size of *Gigantoproductus* species in comparison with the average size of
47 co-occurring Palaeozoic taxa, we measured the width and length of 50 specimens of *G.*
48 *elongatus*, *G. inflatus* and *G. okensis* and of 69 specimens of species of brachiopod
49 genera other than *Gigantoproductus* belonging to four different orders (Productida,
50 Rhynchonellida, Spiriferida, Terebratulida) from the Visean Monsal Dale Limestone and
51 Eyam Limestone formations from the Ible, Wensley Dale, Once-a-Week and Ricklow
52 quarries (Table 1A-B). All the size measurements are in mm and shell areas in millimetres
53 squared (mm²). Following the method of Zhang *et al.* (2015), we calculated the log-
54 transformed shell area, where the shell area is the product of measured width and length
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3 (in mm). The average log-transformed shell area of the species of *Gigantoproductus* is 4.0,
4 and the maximum recorded value is 4.3. By comparison, the log-transformed shell area of
5 the other non-gigantoproductid brachiopod taxa associated with the species of
6 *Gigantoproductus* is lower, around 2.4. In other words, the average shell area of species of
7 *Gigantoproductus* is 11393 mm², whereas that of species of associated genera is 679
8 mm².
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15 *Shell preservation*

16 Based on a combination of petrographic (Fig. 2; Angiolini *et al.* 2019, fig. S1), CL (Fig. 3;
17 Angiolini *et al.* 2019, fig. S2), SEM (Fig. 4; Angiolini *et al.* 2019, fig. S4), TEM (Figs 5-6),
18 and EBSD examinations (Fig. 7), ten specimens of *G. okensis*, *G. inflatus* and *G.*
19 *elongatus* were found to be the best preserved (Table 2) and were thus selected for stable
20 isotope analyses. The ventral valve of most of the specimens has a pseudopunctate
21 laminar secondary layer, occasionally preserved, and a well-preserved thick columnar
22 tertiary layer (Fig. 2A-B).
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29 Petrographic investigation shows that the analysed shells are locally altered by authigenic
30 silica replacement (Fig. 2C-F). This occurs as a brownish, fibrous chalcedony (mostly
31 length-slow quartzine followed by length-fast chalcedonite) and anhedral to subhedral
32 microquartz forming concentrically laminated spherulites with undulose extinction (from 0.5
33 mm to several millimetres in size elongated parallel to shell boundary), and euhedral
34 megaquartz crystals with hexagonal basal section, sometimes with undulose extinction
35 (from 0.2 mm up to several millimetres in size), which embed calcite crystals and growth
36 lines of the brachiopod shells. The observed types of silicification are described in previous
37 studies (Folk & Pittman 1971; Maliva & Siever 1988; Daley & Boyd 1996) and resemble
38 those classified by Schmitt & Boyd (1981) as pattern II and V for the silicification of
39 Permian bivalves and brachiopods.
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48 Chalcedony spherulites may be the only type of silica replacement present or they can be
49 adjacent to hexagonal quartz crystal clusters or overgrown by euhedral quartz (Fig. 2C),
50 as also observed through SEM analysis (Fig. 4). In the ventral valves, chalcedony
51 spherulites occur along the outer margin of the columnar layer aligned parallel to it, or at or
52 close to the boundary between the columnar tertiary and laminar secondary layer when
53 present, especially at the boundaries between individual columnar crystals. Less
54 frequently, chalcedony spherulites and megaquartz are observed along the internal margin
55 of the columnar layer and very rarely in the middle portion of the ventral valve. The dorsal
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3 valves appear more affected by silicification, especially on the laminar layer and on the
4 inner and outer margins of the columnar layer (Fig. 2D), but also across the entire valve
5 thickness, in particular towards the umbonal region and anterior margin (Angiolini *et al.*
6 2019, fig. S1). Chalcedony spherulites are affected by stylolites and cut by fractures filled
7 by burial calcite cement (Fig. 2E-F) suggesting that they formed relatively early during
8 burial diagenesis before mechanical compaction, pressure solution and brittle fracturing
9 occurred. Euhedral megaquartz crystals followed the formation of chalcedony spherulites,
10 overgrowing them but they also formed independently, in particular on the laminar
11 secondary layer.
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13 X Ray Powder Diffraction (XRPD) analyses of powders extracted from the
14 gigantoproductids confirm the presence of quartz with a variable crystallinity index (from
15 5.43 to 7.26 out of a maximum of 10) as calculated following Marinoni & Broekmans
16 (2013), which revised that of Murata & Norman (1976) (Angiolini *et al.* 2019, fig. S3). This
17 method has the advantage of being rather simple allowing crystallinity index determination
18 by inspecting the height of the quartz (212) reflection. The low crystallinity of some
19 analysed samples might be related to the chalcedony spherulites mixed with the quartz
20 crystals because the two phases could not be separated during the extraction of the
21 sample powders. $\delta^{18}\text{O}$ in the quartz phases of four gigantoproductid shells provide an
22 average $\delta^{18}\text{O}$ of +29.5 ‰ (V-SMOW) indicative of quartz precipitation at low temperature
23 (up to 45-50°C calculated based on the equations of Clayton *et al.* 1972 and Murata *et al.*
24 1977). This suggests that silica replacement of the gigantoproductid outer shell occurred
25 during early diagenetic phases rather than during deep burial, supporting the petrographic
26 observation that chalcedony spherulites formed before compaction (stylolite) and fracture
27 formation. Orme (1973) evaluated the variety of chert and sphaerulitic chalcedony to
28 quartz observed in the Visean Derbyshire limestones suggesting that replacement of the
29 host limestone appeared to have occurred prior to its complete consolidation. According to
30 Hollis (1998), silicification of the Derbyshire platform limestones occurred in various
31 phases during burial diagenesis at temperatures of 60-100°C and burial depths of 1-2 km
32 during the Serpukhovian-Bashkirian (Namurian).
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34 The analysed shells displayed variable responses to cathodoluminescence (Fig. 3;
35 Angiolini *et al.* 2019, fig. S2) ranging from non-luminescent to partially- or fully-
36 luminescent, indicative of variable degrees of diagenetic alteration (e.g., calcite
37 recrystallization and replacement during burial diagenesis, silicification, calcite and
38 dolomite precipitation in fractures). The ventral valve appeared better preserved than the
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3 dorsal valve (Fig. 3A-B; Angiolini *et al.* 2019, fig. S2A-D) and in particular, the columnar
4 layer of the ventral valve was non-luminescent, except locally along a few secondary
5 fractures, boundaries between columnar crystals and the outer and inner margins of the
6 tertiary layer where it is silicified (Table 2). In the ventral valve, the secondary laminar
7 layer, when present, and the outer and inner margins of the tertiary columnar layer appear
8 bright luminescent with evidence of calcite recrystallization, associated with silicification
9 (Fig. 3A-D), in particular towards the umbonal region and anterior margin. Growth lines
10 show a weak luminescence, but this may be a primary feature (Angiolini *et al.* 2008, 2009).
11 Fractures (from a few microns up to 1 mm wide) might occur filled either by bright
12 luminescent calcite or dull luminescent calcite followed by dolomite euhedral rhombs (Fig.
13 3E-F). The dorsal valves often exhibit bright luminescence with evidence of calcite crystal
14 replacement, silicification and fracture-filling bright luminescent sparite (Fig. 3A-B; Angiolini
15 *et al.* 2019, fig. S2D-F).

16 Thus, the central portion of the columnar tertiary layer in the middle part of the ventral
17 valve, away from fractures cutting the shell, appears to be the best preserved part of the
18 investigated gigantoproductid valves. These parts were therefore sampled for stable
19 isotope analyses (Table 2).

20 The Derbyshire platform limestones were affected by diagenesis during burial with
21 precipitation of several calcite phases and dolomite within fractures (e.g., Hollis 1998).
22 Burial history curves constructed by Hollis (1998) and Breislin (2018) suggest a maximum
23 burial temperature for the Brigantian on the Derbyshire Platform of 110-120°C (assuming a
24 geothermal gradient of 30°C/km) and a maximum depth of burial of 3-4 km during the
25 Serpukhovian to Moscovian post-rift thermal subsidence before the Variscan Orogeny
26 basin inversion. Burial diagenesis and the flow of burial brines were controlled by
27 Caledonian-Variscan faults and fractures (Hollis & Walkden 1996; Hollis 1998; Hollis &
28 Walkden 2002), but our sample localities are not affected by these faults and fractures.
29 SEM analysis confirms that shell fabric is pristine (Fig. 4). The microstructure is a three-
30 layered rhynchonelliformean architecture (Williams & Cusack 2007), with thin cross-bladed
31 laminar secondary and a thick columnar tertiary layers, but the primary layer is absent due
32 to dissolution. There is no sign of calcite crystals with a rhombohedral morphology in the
33 columnar layer, which argues against alteration (Casella *et al.* 2018a). The columnar
34 microstructure (Fig. 4 C-D) is very similar to that of Recent brachiopods (see figs 1-2 in Ye
35 *et al.* 2018a; and plate 4A-C in Ye *et al.* 2018b) suggesting that the preservation of the
36 tertiary layer of the selected specimens is very good, except for the outer margins around
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3 chalcedony spherulites and megaquartz. In any case, these outer regions were avoided
4 when sampling for geochemistry. Altered specimens that were not selected for study have
5 lost the columnar fabric and are often highly silicified over the entire shell thickness (see
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7 Angiolini *et al.* 2019, fig. S4).

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10 TEM analyses of shell RCK16 (*G. okensis*) (Figs 5-6) show that the columnar layer is
11 formed by nanoscopic, biocomposite mesocrystal calcite (*sensu* Casella *et al.* 2018a), that
12 is built up by grains approximately co-oriented and with the c-axis lying on the plane of
13 observation (the longitudinal section of the brachiopod shell; Fig. 5A), as observed in
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15 Recent taxa (Ye *et al.* 2018a).

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18 EBSD analyses show that the original shell microstructures are well preserved, with the
19 shells still showing the presence of large columnar units with crystallites being highly co-
20 oriented (Figs 7A-C). The MUD values of the three samples (OAW203, RCK16, RCK 221)
21 are 71, 48 and 41, respectively. According to Casella *et al.* (2018a, b), MUD values of well-
22 preserved brachiopods range between 40 and 80 (exceptionally being over 100); moderately
23 to fairly well-preserved brachiopod shells have MUD values between 20 and 40, and
24 overprinted brachiopod shells have MUD values lower than 20. This suggests the
25
26 *Gigantoproductus* shells used for geochemical analyses are indeed well preserved.
27 Occasionally the shell margins are overprinted (Fig. 7B), but this is readily seen, as randomly
28 oriented calcite crystallites completely destroy the original microstructure, and have low
29 MUD values of around 14. These materials were not used for analyses.

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32 Finally, oxygen and carbon isotope data from the shell calcite are within the range of
33 values (respectively -4.7‰ to -3.4‰ and $+0.8\text{‰}$ to $+2.6\text{‰}$; Table 2) previously reported
34 for pristine shells of Visean species of *Gigantoproductus* (Popp *et al.* 1986; Armendáriz *et al.*
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36 2008; Angiolini *et al.* 2012). The mean $\delta^{18}\text{O}$ values of the Carboniferous ($\text{mean} \pm 1\sigma$) in
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38 Veizer *et al.* (1999) vary from $-5 \pm 3 \text{‰}$ (Mississippian) to $-2.5 \pm 1 \text{‰}$ (Pennsylvanian) and our
39 results are within this range. Similar values were also reported for the Visean by
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41 Bruckschen *et al.* (1999) with $\delta^{18}\text{O}$ between -7‰ and -2‰ and $\delta^{13}\text{C}$ between $+1\text{‰}$ and $+6$
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43 ‰ , and by Grossman *et al.* (2008) with $\delta^{18}\text{O}$ between -5‰ and -2‰ and $\delta^{13}\text{C}$ between
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45 $+1\text{‰}$ and $+4\text{‰}$.

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48 The Sr, Mn and Fe contents of the brachiopod shells are given in Angiolini *et al.* (2019, fig.
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50 S5) and the data fit within the range of trace elements values reported for modern
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52 brachiopods and for well-preserved Palaeozoic brachiopods (Angiolini *et al.* 2009 and
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54 references therein).

Organic fraction occluded in the calcite of the columnar layer of the brachiopod shell

TEM analyses of the columnar shell layer RCK16 (*G. okensis*) show that it consists of nanoscopic, biocomposite mesocrystalline calcite. The calcite grains contain a large number of dispersed, intragranular, oval inclusions, with dimensions ranging from less than 10 nm to ~100 nm in diameter. From the contrast in the bright field images, the largest inclusions are polyphasic and contain: 1) a void, possibly filled by a gas; 2) an amorphous material (which is either a fluid, a glass, a gel, or unrecognizable) with a darker tone due to a mass-thickness contrast; and 3) sometimes a solid, possibly a crystalline precipitate, with a euhedral habit and characterized by a very dark tone due to high diffraction contrast (Fig. 5B-C). The largest inclusions are often connected by dislocations (Fig. 6A), which are especially visible close to the $\langle 1-1\ 0 \rangle$ zone, and may form trails along dislocation lines (Fig. 6B). Finally, we found a different type of inclusion, a few nanometres in size, with a dark contrast, forming trails between calcite grain borders (Fig. 6C) similar to those observed by Schmahl *et al.* (2012) in Recent brachiopod shells.

These organic inclusions within the shell were analysed in the preserved columnar layer of one of the specimens (RCK16) by nuclear magnetic resonance and GC-MS. The $^1\text{H-NMR}$ analysis shows clusters at 1.90, 1.70, 1.45 ppm typical of amino acid aliphatic chains such as alanine, leucine or isoleucine (Fig. 8A). A cluster observed at 2.90-2.80 ppm may be explained as being a lateral chain of aspartate/asparagine (Fig. 8B).

In order to confirm these preliminary results, GC-MS analysis was performed using two different kinds of derivatization. First, a Fisher derivatization was performed to transform the carboxylic function of amino acids in a methyl ester derivatives. More than 15 different acids with aliphatic chains were detected, not necessary amino acids, but all with relatively high molecular weight; so that the low molecular weight amino acids such as glycine or alanine were impossible to detect. In order to overcome this masking problem, the sample was derivatized with isobutylchloroformiate before running the GC-MS to derive the amines and eventually the amide function. The chromatogram of GC-MS analysis shows more than 50 different peaks corresponding to organic compounds and at least 10 with the typical fragmentation of aliphatic amino acids. At this level it is not possible to attribute the type of amino acid by mass fragmentation in GC-MS, but according to the results of $^1\text{H-NMR}$ and GC-MS, it is possible to ascertain that there are several aliphatic amino acids in RCK16, particularly alanine, leucine and serine.

Isotope geochemistry of shell organic fraction

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3 $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{org}}$ were measured in organic fractions isolated from the nanoscopic
4 biocomposite mesocrystalline calcite of the preserved part of the columnar layer of six
5 specimens of two species of *Gigantoproductus* (*G. inflatus* and *G. okensis*; Table 3). They
6 all reach a gigantic size and have the same lifestyle, but in two different shallow-water
7 settings: high-energy crinoidal shoals in inner ramp settings at Once-a-Week Quarry and
8 within the shelter provided by relict mud mounds in middle ramp settings at Ricklow Quarry
9 (Nolan *et al.* 2017).

10 The $\delta^{13}\text{C}_{\text{org}}$ values are typically low and range from -25.9 to -29.0 ‰, while $\delta^{15}\text{N}_{\text{org}}$ varies
11 between -2.0 to $+4.1$ ‰ at both localities (Table 3) and are apparently unrelated to a
12 taxon-specific effect or any palaeoecological constraints. In particular, $\delta^{13}\text{C}_{\text{org}}$ is rather
13 uniform, with only one specimen of *G. okensis* from Ricklow Quarry having a slightly
14 higher value. Unlike $\delta^{13}\text{C}_{\text{org}}$ values, more variation occurs within the $\delta^{15}\text{N}_{\text{org}}$ data. Three
15 specimens of *G. inflatus* and *G. okensis* from Ricklow Quarry have positive values,
16 whereas a specimen each of *G. inflatus* from Ricklow and Once-a-Week Quarry have
17 slightly negative values. There is no relationship between each species, their
18 palaeoenvironmental setting, and the isotope data. The correlation between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
19 is poor ($R^2=0.24$), and the data are scattered around the regression line and not tightly
20 clustered which does not support the absolute p value (significance level). This is
21 consistent with the low R^2 values of $\delta^{15}\text{N}_{\text{org}}$ vs TOC (0.17) and $\delta^{13}\text{C}_{\text{org}}$ vs TOC (0.002).
22 These correlations do not support significant or consistent changes in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
23 values with their corresponding TOC values (Azmy *et al.* 2015). This argues against
24 covarying post-depositional diagenetic alteration.

25 Finally, measurements of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in low concentration samples are more accurate
26 than low abundance C and N concentration measurements. We assume that the C/N ratio
27 data are inaccurate because of the low %N. The C/N is high which could be due to
28 preferential loss of N. There are no studies known by the authors on how N loss affects
29 $\delta^{15}\text{N}_{\text{org}}$ in marine fauna, but studies of C loss in plants show that the $\delta^{13}\text{C}_{\text{org}}$ change is
30 small (Schleser *et al.* 1999; Smith *et al.* 2017).

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 **DISCUSSION**

56 *Are Gigantoproductus species true giants?*

57 Our results show that the size reached by *Gigantoproductus* species is significantly larger
58 than the average size of all other Palaeozoic brachiopods. The mean body size of all
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3 Carboniferous brachiopods measured by Zhang *et al.* (2015) as log-transformed shell area
4 (shell areas measured in mm²) is around 2.6, comparable with the value of 2.5 obtained
5 for non-gigantoproductid brachiopods from the Visean of Derbyshire. The same parameter
6 measured for *Gigantoproductus* species is up to 4.3, their average shell area being nearly
7 the double of that of the other Visean taxa. This is consistent with the survey of giants
8 throughout the Phanerozoic by Vermeij (2016) who considered *Gigantoproductus* as one
9 of the largest sedentary bottom-dwellers of the Palaeozoic.

10 Growth rates in *Gigantoproductus* were high, especially at the juvenile stages, as shown
11 by growth line spacing and by the growth curve published by Angiolini *et al.* (2012, fig. 6).
12 Based on sclerochronology and on spiral deviation analysis, Angiolini *et al.* (2012) showed
13 that the lifespan of a large *G. okensis* was 20 years, in agreement with the survival rates of
14 extant brachiopods, and in no way comparable to the Recent longest-living bivalve *Arctica*
15 *islandica* with a lifespan of at least 500 years (e.g., Butler *et al.* 2013). So extreme
16 longevity cannot be the cause of *Gigantoproductus* gigantism. There are Recent bivalves
17 that reach a large size through rapid growth [as species of *Crassostrea*, (e.g.
18 Chávez-Villalba *et al.* 2005)], but they are efficient suspension-feeder capable of
19 qualitative particle selection (Decottignies *et al.*, 2007).

20 The largest Recent brachiopod species is the terebratulide *Magellania venosa* (Dixon,
21 1789) which has an average length between 30-45 mm, and a maximum length of 70 mm
22 (McCammon 1973; Baumgartner *et al.* 2014). This translates into an average log-
23 transformed shell area of 3.2 and a maximum log-transformed shell area of 3.6, which are
24 lower than the values calculated for our *Gigantoproductus* species. Furthermore,
25 *Magellania venosa* is usually very thin-shelled, its shell thickness being 1-2 mm (e.g.,
26 Cohen *et al.*, 2011; Ye *et al.* 2019), even though an unpublished specimen from Huinay
27 Station is 5 mm-thick in section (Elizabeth Harper, pers. comm.). In comparison,
28 *Gigantoproductus* is a very thick-shelled brachiopod (c. 10 mm), generating substantial
29 amounts of calcium carbonate. In contrast with its giant size, the lophophore of
30 *Gigantoproductus* is quite simple, as shown by the brachial ridges in the dorsal valve (Fig.
31 9; Muir-Wood & Cooper 1960; Angiolini *et al.* 2012), accompanied by a pair of conical pits
32 (brachial cones) deeply impressed in the shell substance of the ventral valve (Fig. 9; Muir-
33 Wood & Cooper 1960; Angiolini *et al.* 2012). These features suggest the presence of a
34 schizolophe lophophore similar to that reconstructed by Brunton (1982, fig. 13) for the
35 Visean productid *Levitusia*, where the direction of current inflow and outflow has been
36 recently interpreted as consisting of lateral inhalant currents and a median exhalant
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3 current (Shiino & Suzuki 2011). Brunton himself (1982, p. 161-162) was surprised by the
4 contrast between the low efficiency of the small and simple reconstructed lophophore and
5 the size and thickness of the shell of *Levitusia*, so that he suggested that nutrition could
6 have been sustained by absorption of nutrients by the epithelium.
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10 On the other hand, *Levitusia* (with a log-transformed shell area of about 3.3) is smaller
11 than *Gigantoproductus*, with a thinner shell.
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13 A comparison with the lophophore type of the largest Recent brachiopod *M. venosa*
14 emphasizes even more the disparity between the size of the species of *Gigantoproductus*
15 and their simple type of lophophore. *M. venosa* has a plectolophe lophophore, which is the
16 most complex of the coiled feeding organs (MacKinnon & Lee 2006) and allows the animal
17 to acquire and assimilate more food resources than other invertebrates (McCammon 1973,
18 p. 271). The plectolophe type is developed at the adult stage after transition through
19 different stages which are much simpler [trocholophe – schizolophe - zygolophe (Emig
20 1992)]; the schizolophe in particular (i.e. the *Gigantoproductus* type of lophophore) is
21 typical of the larval and juvenile stages of *M. venosa*. This is also explicitly demonstrated
22 in the diagrams of LaBarbera (1986, figs 1-2, p. 317) which scale the total lophophore area
23 to the ash-free dry masses (AFDM) of the soft tissues of two terebratulide species with
24 plectolophe lophophore: they have a schizolophe lophophore at small body masses (0.05
25 to 0.4 mg AFDM), and a plectolophe lophophore at body masses greater than 0.5 mg up to
26 900 mg.
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29 Alternative explanations are clearly required to explain how the large size of
30 *Gigantoproductus* species was achieved with a simple schizolophe lophophore. In
31 addition, most *Gigantoproductus* species lack a median sulcus, which, as shown by Emig
32 (1992) and Shiino & Kuwazuru (2011), has a very important role in increasing the
33 efficiency of the lophophore by creating water currents flowing over the tentacles and
34 enhancing food acquisition.
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37 An hypothesis is that of a symbiotic lifestyle which may be tested through the analysis of
38 the $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{org}}$ of the preserved organic matrix of the shell (cf. O'Donnell *et al.*
39 2003; Mae *et al.* 2007; Dreier *et al.* 2012, 2014; Dreier & Hoppert 2014).
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48 *Is the shell-occluded organic fraction preserved?*

49 Recent rhynchonelliformean brachiopods have a shell composed of calcite containing a
50 minor organic fraction (less than 1% of the dry powder weight) that is involved in
51 calcification and becomes occluded in the shell microstructure when formed (e.g. Immel *et*
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3 *al.* 2015). TEM investigation during the current study has proved the occurrence of
4 numerous nano-sized oval inclusions containing an amorphous material (fluid, gel or
5 glass), a void (possibly filled by a gas) and sometimes a solid. It is worth noting that such
6 oval inclusions do not occur in non-biogenic calcite prepared with ion milling techniques,
7 and they cannot be related to calcite decomposition by electron beam damage, as they
8 were present in the first observations. In addition, calcite decomposition in air commences
9 at $T > 600\text{ }^{\circ}\text{C}$ (Rodríguez-Navarro *et al.* 2009), a temperature too high to be reached
10 during ion milling. According to Park *et al.* (2007), the local temperature in a thin sample
11 (200 nm) during ion-milling under typical conditions (6 KeV, beam incidence 10° ,
12 comparable to those employed here) depends on many factors, but especially on sample
13 conductivity. The highest temperature value recorded after testing several materials is 330
14 $^{\circ}\text{C}$ for silica glass, therefore much lower than that required to initiate calcite decomposition.
15 Moreover, calcite decomposition would result in the formation of CaO nanorods
16 (Rodríguez-Navarro *et al.* 2009) in crystallographic relationship with the calcite single
17 crystals, which we did not observe.

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19 Skeletal carbonates, unlike non-biogenic carbonates, contain organics and comparatively
20 large amounts (up to 3% weight) of water (Gaffey 1988; Casella *et al.* 2018a, b) which
21 form an intrinsic part of the carbonate skeleton incorporated during growth. Organic
22 material (as biopolymer membranes and network of fibrils) and water are intimately
23 associated and are disseminated throughout skeletons in minute inter- and intra-crystalline
24 cavities. The ovoidal inclusions we detected by TEM may represent evidence of these
25 organic materials and water. These inclusions may have been trapped when solid or
26 precipitated from the original fluid after its entrapment.

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28 Microstructures very similar to those we observe have been reported by Ye *et al.* (2018a)
29 from the tertiary layer of the Recent brachiopod *Liothyrella neozelanica*. They have also
30 been reported in the secondary layer of both *L. neozelanica* and *L. uva* by Goetz *et al.*
31 (2011), who described “small somatoid (cigar-like) areas of lower density” in the primary
32 layer of the Recent brachiopod *Gryphus vitreus*. Kelm *et al.* (2012) described similar
33 inclusions in the spines of *Holopneustes porissisimus*.

34
35 Lécuyer & O’Neil (1994), in a study of stable isotopes in fluid inclusions within biogenic
36 calcite, concluded that trapped fluids are the remnants of metabolic fluids and could
37 provide information about metabolic activity recorded in well-preserved fossils. The key
38 question is whether the inclusions are pristine or altered by diagenesis or any post-
39 diagenetic events.
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3 The samples selected for this study show fabrics consistent with primary growth
4 mechanisms, free of signs of recrystallization, and show a microstructure very similar to
5 those of modern brachiopods for which their optimal preservation state has been assessed
6 (i.e., Goetz *et al.* 2011; Ye *et al.* 2018a, b). Almost certainly, intense deformation would
7 breach fluid inclusions and cause exchanges with external fluids (Goldstein 1986). In the
8 selected samples we used here, however, calcite grains are free of microstructures typical
9 of deformation, such as deformation twins, undulose extinction, and healed fractures. On
10 the other hand, since fluid entrapment occurred at ambient temperatures, some degree of
11 natural heating during diagenesis may be expected. Heating causes internal overpressure
12 that may be released by decrepitation, a leakage in the fluid inclusion content that can
13 reequilibrate with the ambient pore fluid (Goldstein 2001). We explain the dislocations
14 detected in some of our samples this way. However, whereas some degree of thermal
15 reequilibration cannot be ruled out, thermal reequilibration did not necessarily affect every
16 fluid inclusion in the mineral phase. In fact, not all the fluid inclusions are connected with
17 dislocations, and it is generally observed that minute inclusions, such as those observed
18 here, are more resistant to thermal reequilibration - a physical effect related to the very
19 small size of the inclusions (Goldstein 2001). Moreover, whereas overpressure can cause
20 fluid content to leak out of the inclusion, the reverse, i.e. the refill of the inclusion with
21 foreign material through dislocations, is improbable. Therefore, if organic molecules were
22 entrapped during crystal growth, it is likely that at least some of them could be preserved
23 in the inclusions. Finally, the TEM investigation in the current study has proved the
24 occurrence of nanometre-sized dark inclusions similar to those observed by Schmahl *et al.*
25 (2012) and Casella *et al.* (2018a) in modern brachiopods and attributed to “intercrystalline
26 organics”. Thus, at least some of the numerous inclusions revealed by TEM analyses are
27 likely to be the remnants of occluded organic biopolymers within the columnar tertiary layer
28 of calcite in the *Gigantoproductus* species shell.

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48 The occurrence of organic matrix in the nanoscopic, biocomposite mesocrystal calcite of
49 the columnar layer is also proven by NMR and GC-MS analyses, which show the presence
50 of alanine, leucine/isoleucine, serine, and asparagine that occur in Recent
51 rhynchonelliformean brachiopods (Immel *et al.* 2015). According to Cusack & Williams
52 (2007), serine and asparagine occur in high concentrations also in the shells of
53 *Novocrania*.

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58 Although it is uncommon, the preservation of organic components inside fossil biominerals
59 has been already documented by earlier studies (e.g., Cuif 1972; Sorauf 1999; O’Donnel
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3 *et al.* 2003; Muscatine *et al.* 2005; Vandenbroucke & Largeau 2007 and references
4 therein; Frankowiak *et al.* 2013; Yamazaki *et al.* 2013; Tornabene *et al.* 2017). Simple and
5 robust amino acids (aspartic acid/asparagine, serine and glycine) have been extracted
6 from Carboniferous cranioid and linguloid brachiopods, possibly preserved by interaction
7 with the carbonate and apatitic matrices (Cusack & Williams 2007).
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13 *What diet enabled these species to become giants?*

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15 The biosignature associated with symbioses was investigated through the measurement of
16 $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{org}}$ in the organic matrix extracted from the columnar tertiary layer calcite of
17 *Gigantoproductus* species shells. These proxies are considered more robust for the
18 detection of ancient symbioses than the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of the shell calcite. MacLeod &
19 Hoppe (1992) interpreted enriched calcite $\delta^{13}\text{C}$ values from bivalve inoceramid shells as
20 an indicator of a chemosymbiotic life-style. However, Grossman in Grossman *et al.* (1993)
21 argued against this interpretation, as modern chemosymbiont-harboring bivalves are
22 depleted in ^{13}C relative to equilibrium. In their reply, MacLeod & Hoppe in Grossman *et al.*
23 (1993) emphasized that “both enriched and depleted calcite ^{13}C values are found in the
24 shells of chemosynthetic bivalves” and that more data from Recent shells are needed to
25 discriminate between symbiotic and non-symbiotic life-styles based on shell carbonate
26 stable isotopes. Stanley & Swart (1995) observed the wide range in the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$
27 values of modern scleractinian corals and considerable overlap between symbiotic and
28 non-symbiotic species; however, skeletal samples from non-symbiotic corals always show
29 a strong positive correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, which is not recorded in symbiotic
30 corals. This was confirmed more recently by Rollion-Bard *et al.* (2003), who analysed $\delta^{13}\text{C}$
31 and $\delta^{18}\text{O}$ of recent azooxanthellate and zooxanthellate corals.
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34 While the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of shell calcite from our *Gigantoproductus* species are within the
35 range of values previously reported for Viséan brachiopods (Veizer *et al.*, 1999), there is
36 no strong positive correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Table 2).
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39 Jakubowicz *et al.* (2015) suggested that the isotopic signatures of Palaeozoic corals
40 cannot be interpreted based on data from modern Scleractinia and so the absence of
41 $\delta^{13}\text{C}$ - $\delta^{18}\text{O}$ co-variance in Palaeozoic cnidarians cannot be interpreted as indicative of a
42 photosymbiotic lifestyle. Others have also shown that the $\delta^{13}\text{C}$ - $\delta^{18}\text{O}$ coral skeleton proxy
43 can be used only in very few cases, because of the impact of diagenesis (Tornabene *et al.*
44 2017). It is beginning to be recognized that the $\delta^{15}\text{N}$ (and $\delta^{13}\text{C}$) of occluded organic
45 material is much more robust and applicable, as the skeleton-bound organic matrix
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3 remains preserved and protected from diagenesis by the skeletal structure itself
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5 (Tornabene *et al.* 2017).

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7 It has been reported that soft tissues of filter-feeding bivalves in normal salinity ocean
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9 waters have a $\delta^{13}\text{C}_{\text{org}}$ ranging from -24‰ to -17‰ VPDB (Rau & Hedges 1979; Fry
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11 1988; Kwak & Zedler 1997). Generally speaking, each increase in trophic level
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13 corresponds to a 1‰ soft tissue $\delta^{13}\text{C}_{\text{org}}$ enrichment relative to dietary carbon (e.g.,
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15 O'Donnel *et al.* 2003). In contrast, soft tissues of chemosymbiotic organisms show a large
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17 $\delta^{13}\text{C}_{\text{org}}$ range from -70‰ to -15‰ (Nelson & Fisher 1995; Van Dover 2000; Mizota &
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19 Yamanaka 2003; Mae *et al.* 2007). In particular, chemosymbiotic bivalves show $\delta^{13}\text{C}_{\text{org}}$
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21 values ranging from -35‰ to -27‰ (Childress & Fisher 1992; Robinson & Cavanaugh
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23 1995; Cavanaugh *et al.* 2006).

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25 The $\delta^{15}\text{N}_{\text{org}}$ values depend on the sources of the original nitrogen, but generally increase
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27 by 3.4‰ per trophic level (Minagawa & Wada 1984; Peirera *et al.* 2010). In marine primary
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29 consumers, the $\delta^{15}\text{N}_{\text{org}}$ values are between $+8.4\text{‰}$ and $+9.5\text{‰}$ and around $+16.2\text{‰}$ in
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31 tertiary consumers (Minagawa & Wada 1984). Marine filter-feeding bivalves have a range
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33 of $\delta^{15}\text{N}_{\text{org}}$ values from $+6$ to $+14\text{‰}$ (Minagawa & Wada 1984; Lorrain *et al.* 2002; Jennings
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35 & Warr 2003; O'Donnel *et al.* 2003). The soft tissues of chemosymbiotic animals have a
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37 wider $\delta^{15}\text{N}_{\text{org}}$ range, from -20‰ to $+7\text{‰}$, but the values are negative in most cases
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39 (Nelson & Fisher 1995; Van Dover 2000; Mizota & Yamanaka 2003; Mae *et al.* 2007).

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41 In a recent study, Dreier *et al.* (2014) suggested that bulk organic shell matrices in Recent
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43 phototrophic and thiotrophic bivalves have lower $\delta^{13}\text{C}_{\text{org}}$ values (ranging from -31.0‰ to
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45 -25.5‰) than those of filter-feeding non-symbiotic bivalves where the latter have $\delta^{13}\text{C}_{\text{org}}$
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47 values from -22.0‰ to -17.8‰ . Comparable $\delta^{15}\text{N}_{\text{org}}$ values are -2.2‰ to $+0.1\text{‰}$ for
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49 thiotrophic bivalves, $+2.6\text{‰}$ to $+2.9\text{‰}$ for phototrophic bivalves and $+3.1\text{‰}$ to $+4.3\text{‰}$ for
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51 filter-feeders. Correlation of the $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{org}}$ values (Dreier *et al.* 2014, fig. 1)
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53 suggests that the chemosymbiotic lifestyle is characterized by low $\delta^{13}\text{C}_{\text{org}}$ /low $\delta^{15}\text{N}_{\text{org}}$, the
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55 non-symbiotic lifestyle by high $\delta^{13}\text{C}_{\text{org}}$ /high $\delta^{15}\text{N}_{\text{org}}$ and the phototrophic lifestyle by low
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57 $\delta^{13}\text{C}_{\text{org}}$ /intermediate $\delta^{15}\text{N}_{\text{org}}$. This defines a fingerprint for distinguishing symbiotic from
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59 non-symbiotic lifestyles in living invertebrates. Muscatine *et al.* (2005), however, observed
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a highly significant difference in $\delta^{15}\text{N}_{\text{org}}$ between the coral skeletal organic matrix of Recent
symbiotic and non-symbiotic corals, but not in $\delta^{13}\text{C}_{\text{org}}$; consequently, they considered
 $\delta^{15}\text{N}_{\text{org}}$ as a potential proxy for photosymbiosis. In particular, they found that $\delta^{15}\text{N}_{\text{org}}$ of the
skeletal organic matrix of symbiotic corals is about 8‰ lower with respect to non-symbiotic
corals. Tornabene *et al.* (2017) demonstrated the nitrogen isotopic ratio offset between

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3 zooxanthellate- and azooxanthellate-corals and emphasized the robustness of the organic
4 matrix $\delta^{15}\text{N}$ proxy for photosymbiosis.

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6 According to Kirkendale & Paulay (2017), photosymbiotic metazoans do not rely entirely
7 on symbiont-derived nutrition. In fact, some bivalves depend both on symbiont-fixed
8 carbon and nitrogen together with filter feeding (e.g. Duplessis *et al.* 2004; Rossi *et al.*
9 2013 and references therein), so that their soft tissues record variable isotopic
10 compositions ($\delta^{13}\text{C}_{\text{org}} = -62\text{‰}$ to -26‰ ; $\delta^{15}\text{N}_{\text{org}} = -16\text{‰}$ to $+5\text{‰}$; Pile & Young 1999;
11 Dattagupta *et al.* 2004; McKiness *et al.* 2005; Mae *et al.* 2007). However, they apparently
12 subsist more on symbiont-derived metabolites than on filter-feeding (e.g., Duperron *et al.*
13 2007; Dreier *et al.* 2014). Species of the same genus may show variation of $\sim 5\text{‰}$ in
14 $\delta^{13}\text{C}_{\text{org}}$ and 1‰ in $\delta^{15}\text{N}_{\text{org}}$ (Dreier *et al.* 2014) depending on the proportion between the two
15 nutrient sources (symbiosis vs. filter feeding). Rossi *et al.* (2013) reported variations of \sim
16 1‰ in $\delta^{13}\text{C}_{\text{org}}$ and 3‰ in $\delta^{15}\text{N}_{\text{org}}$ for individuals of a population of the mixotroph bivalve
17 *Loripes lacteus* having different sizes and living in different positions of eelgrass meadows.
18 A recent study on *Tridacna maxima* (Dreier *et al.* 2014) suggested that the proportion
19 between the two lifestyles (symbiosis vs. filter feeding) may also change during ontogeny.
20 Some studies of Triassic, Miocene and Pleistocene fossil shells (e.g. O'Donnell *et al.*
21 2003; Muscatine *et al.* 2005; Mae *et al.* 2007; Dreier *et al.* 2012; Tornabene *et al.* 2017)
22 indicated that, under certain circumstances, it is possible to detect ancient symbioses
23 using shell organic matrix carbon and nitrogen isotope signatures. Muscatine *et al.* (2005)
24 were able to use this proxy on Upper Triassic corals from Turkey, confirming that
25 *Pachytheccalis major* (with a skeletal organic matrix $\delta^{15}\text{N}_{\text{org}}$ of 4.65‰) was most likely
26 photosymbiotic, as already suggested by Stanley & Swart (1995). Tornabene *et al.* (2017)
27 demonstrated a $\sim 7\text{‰}$ nitrogen isotopic ratio offset between zooxanthellate- and
28 azooxanthellate-corals also in Triassic and Miocene fossil Scleractinia with different levels
29 of diagenetic alteration. We have thus used these proxies to detect if *Gigantoproductus*
30 species may have hosted symbionts.

31
32 One of the important achievements of the work of Mae *et al.* (2007) is the measurement of
33 the differences between the isotopic values of the shell organic matrix (conchioline) and
34 the soft tissues; the latter have lower $\delta^{13}\text{C}_{\text{org}}$ (about -3‰) and higher $\delta^{15}\text{N}_{\text{org}}$ (variable, but
35 on average $+1\text{‰}$), which is consistent with the results of a later study by Dreier *et al.*
36 (2012). As based on mollusc data, soft tissues have different isotopic values from shell
37 organic matrix, to aid our discussion, we consider a simplified model of $\Delta^{13}\text{C}_{\text{org}}$ and $\Delta^{15}\text{N}_{\text{org}}$
38 between shell organic material and soft tissues as that calculated for bivalves (Mae *et al.*
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2007; Dreier *et al.* 2012) and we extend it to brachiopods. We thus suggest that estimated soft tissue $\delta^{13}\text{C}_{\text{org}}$ values of our *Gigantoproductus* species might have varied from -32.0‰ to -28.9‰ and those of $\delta^{15}\text{N}_{\text{org}}$ from -1.0‰ to $+5.1\text{‰}$ (Fig. 10). These low values, and their wide range of variation, are within the range expected by organisms that host symbionts and that may have combined this strategy with suspension feeding (Mae *et al.* 2007; Dreier *et al.* 2012; Rossi *et al.* 2013). In fact, in the case of a strict suspension feeding strategy, we would have expected $\delta^{13}\text{C}_{\text{org}}$ soft tissue values at least higher than -27‰ (yellow field in Fig. 10), as $\delta^{13}\text{C}_{\text{org}}$ values documented for the Visean marine and terrestrial organic matter are respectively around -28‰ and -23‰ , (Lewan 1980; Strauss & Peters-Kottig 2003), and there is an increase of 1‰ at each step in the trophic chain and there is at least one step from dietary carbon to suspension feeders. This is also supported by the slightly negative to slightly positive soft tissue $\delta^{15}\text{N}_{\text{org}}$ values, which are intermediate between known values of symbiotic animals and filter feeders. In particular, the intraspecific range of variation of $\delta^{15}\text{N}_{\text{org}}$ recorded by specimens of *G. inflatus* at Ricklow Quarry is similar to that reported by Rossi *et al.* (2013) for the mixotroph bivalve *Loripes lacteus* living at the edge rather than the inner parts of eelgrass meadows.

A comprehensive, detailed analysis of the possible explanations for gigantism in the Late Palaeozoic is beyond the scope of this study, but our data seem to suggest that the gigantic size reached by the species of *Gigantoproductus* could be the result of a mixotroph lifestyle, by which they could rely on the energy and nutrients derived both from endosymbiotic microbes and from filtered particulate food. These results, coupled with the high growth rates, exclude the 'gentle giant syndrome' for *Gigantoproductus* species.

Photosymbionts or chemosymbionts?

It is very difficult to speculate further if these large brachiopods hosted phototrophic or chemotrophic symbionts. However, there are several lines of evidence that make the first alternative more plausible: 1) the low $\delta^{13}\text{C}_{\text{org}}$ /intermediate $\delta^{15}\text{N}_{\text{org}}$; 2) the massive calcite skeleton produced by the animal; and 3) the depositional environment.

The isotope values measured in the shell's organic matrix, and in particular the $\delta^{15}\text{N}_{\text{org}}$, which is considered the most robust proxy for the detection of ancient symbioses (see discussion above), seem to be more compatible with photosymbiosis than with chemosymbiosis. $\delta^{15}\text{N}_{\text{org}}$ is lower than expected for a filter-feeding strategy, but higher than for chemosymbiotic animals which usually show negative values (Nelson & Fisher

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3 1995; Van Dover 2000; Mizota & Yamanaka 2003; Mae *et al.* 2007), although this may
4 also be explained by a mixotroph strategy.

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6 The biomineralization of the heavy exoskeleton of these *Gigantoproductus* species –
7 which are not only large, but also very thick shelled - could have been aided by algal
8 symbioses which led to removal of CO₂, facilitating the conversion from bicarbonate to
9 carbonate and the growth of a thick columnar layer.

10
11 Finally, as shown in detail by Nolan *et al.* (2017), the Once-a-Week Quarry
12 gigantoproductids were deposited in a high-energy setting. The gigantoproductid bed
13 overlies cross-bedded crinoidal grainstone-rudstone accumulated in an inner ramp swept
14 by currents. There is no clear evidence of palaeo-water depths, but the crinoidal
15 grainstone-rudstone beds contain sparse specimens of putative red algae (*Ungdarella*,
16 *Stacheia* and *Stacheoides*) and rare possible paleoberesellid fragments. The Ricklow
17 Quarry gigantoproductid bed accumulated in a lower energy environment, below effective
18 wave and current action within the shelter provided by relict mud mounds in middle ramp
19 settings. The skeletal packstone deposits associated with the gigantoproductids contain
20 sparse red algae (*Ungdarella*, *Fasciella* and *Stacheia*), abundant calcispheres, sparse to
21 rare fragments of paleoberesellid algae and dasycladacean algae (*Koninkopora* and
22 *Velebitella*, cf. Mamet & Roux 1975; Mamet 1991). The presence of algal fragments
23 suggests that the water depths of deposition were within the photic zone in both settings,
24 where the *Gigantoproductus* species may have exposed their photosymbionts to the light.
25 There is no evidence of deviation from normal oxygen levels and the occurrence of a
26 diverse fauna of associated crinoids, echinoids, benthic foraminifera (palaeotextularids,
27 archaeidiscids, endothryids, *Omphalotis*, *Globoendothyra*, *Pseudoendothyra*, *Tetrataxis*,
28 *Tuberitina*, *Earlandia vulgaris*, *Bradyina*, *Valvulinella*, *Monotaxinoides*), fenestellid and
29 fistuliporid bryozoans and corals indicates normal salinities.

30
31 The question is: Where could these animals have hosted their symbionts? We exclude the
32 presence of symbionts in the lophophore arms, not only because there they could not have
33 been exposed to the light, but mainly because the lophophore structure is simple and
34 rudimentary, as discussed above. We may hypothesize that the symbionts were hosted in
35 the mantle, as in Recent Tridacninae (Kirkendale & Paulay 2017). This may be supported
36 by the life position of *Gigantoproductus* species, which are semi-infaunal and concave-
37 convex, but have long trails raised well above the water-sediment interface, with the
38 commissural plane more or less perpendicular to the plane of the substrate (Ferguson
39 1978; Angiolini *et al.* 2012). They were thus orthothetic as *Tridacna* and they may have
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3 exposed their mantle with symbionts to light along the trail's edge. Furthermore, the
4 anterior margin of the valves of the analysed species (and particularly of *G. inflatus*) is
5 plicated by flutings (Pattison 1981), which lengthen the anterior commissure increasing the
6 amount of mantle surface that can be exposed, as observed in some Tridacninae.
7
8 Gigantoproductids may not have been alone in adopting alternative feeding strategies to
9 promote large size and rapid growth. Apart from the lyttonioids and richthofenioids of the
10 Permian (Cowen 1970), the large trimerellid brachiopods of the Ordovician and Silurian
11 possibly possessed similar properties. These latter brachiopods probably grew aragonitic
12 shells, since replaced by calcite. Nevertheless, in the giant *Gasconsia*, from the Katian
13 rocks of the Oslo Region, 'warts' within the shell fabric were illustrated that were possibly
14 generated around organic inclusions within the original shell (Hanken & Harper 1985).
15 In the geological record, some brachiopods have large shells (up to 10 cm) possibly
16 obtained through methano- or thiotrophic diets, such as those of the superfamily
17 Dimerelloidea, among which the Lower Cretaceous (late Hauterivian – Barremian)
18 *Peregrinella* is the largest known Mesozoic rhynchonellid. It forms nearly monospecific
19 shell concentrations in deep-water environments (e.g., Kiel *et al.* 2014). *Peregrinella* has a
20 cosmopolitan distribution, but it was interpreted to be confined to ancient methane seep
21 sites based on the geochemistry of the shell and the limestone host rock (e.g., Campbell &
22 Bottjer 1995; Posenato & Morsilli 1999; Kiel *et al.* 2014). Isotope data suggest the
23 occurrence of methanotrophic bacteria at seafloor seepage; however, it is not clear if
24 *Peregrinella* was filter-feeding on bacterioplankton or had a symbiotic relationship
25 (methanotrophy) with the microbes (Kiel *et al.* 2014). Other Palaeozoic and Mesozoic
26 dimerelloids have been interpreted as having been associated with cold seeps and
27 chemosynthetic environments, such as *Dzieduszyckia* (Devonian; Peckmann *et al.* 2007),
28 *Ibergirhynchia* (Early Carboniferous; Gischler *et al.* 2003), *Halorella* (Late Triassic;
29 Peckmann *et al.* 2011), and *Sulcirostra* (Early Jurassic; Peckmann *et al.* 2013). Among
30 these, only the shell of *Dzieduszyckia* reached a large size similar to that of *Peregrinella*,
31 but the shell size of the other dimerelloids is, however, in the range of a few centimetres.
32 Also the Lower Jurassic *Anarhynchia* cf. *gabbi* has been related to hydrothermal vent
33 deposits, with primary producers represented by chemoautotrophic bacteria that oxidize
34 sulphides (Little *et al.* 1999).
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58 CONCLUSIONS

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3 A multidisciplinary approach, including palaeontology, size analysis, petrography, CL,
4 SEM, EBSD, TEM, inorganic and organic stable isotope geochemistry, has been utilized to
5 study species of *Gigantoproductus* from the Visean of Derbyshire, UK. We conclude that:
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8 - a calcite-occluded organic fraction is preserved in the fossil shells, and its
9 preserved amino acid composition is comparable with that observed in Recent
10 brachiopod taxa;
11
- 12 - one of the possible explanation for the gigantic size and thick carbonate skeleton of
13 these Palaeozoic benthic brachiopods could be a mixotroph lifestyle (indicated by
14 the presence of amino acid), by which they could rely on the energy and nutrients
15 derived both from photosymbiotic microbes and from filtered particulate food;
16
- 17 - $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{org}}$ of the organic fraction can possibly be used to reconstruct
18 models for feeding strategies in well preserved Palaeozoic brachiopods.
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26
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33 British Geological Survey.
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41 **DATA ARCHIVING STATEMENT**

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44 Additional photomicrographs and supplementary figures for this study are available in the Dryad
45 Digital Repository: <https://datadryad.org/review?doi=doi:10.5061/dryad.m42t6tm> **[please note**
46 **that the data for this paper are not yet published and this temporary link should not be shared**
47 **without the express permission of the author]**
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FIGURE CAPTION

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3 Fig. 1. Geological sketch map (from Nolan *et al.* 2017). A, The location of the Derbyshire
4 carbonate platform (DCP) within mainland Great Britain. B, Outline of the platform with
5 outcrop of Mississippian formations indicated. Sites: Ricklow Quarry (53.192N, 1.755W),
6 Once-a-Week Quarry (53.209N, 1.766W), Ible (53.111N, 1.630W), Wensley Dale
7 (53.146N, 1.608W). On the right: Derbyshire carbonate platform stratigraphy of the Peak
8 Limestone Group: on-shelf (right) and off-shelf (left) provinces with regional
9 chronostratigraphy. Key: M, carbonate mud-mounds; SB, Stanton Basin with eastern
10 margin marked with the dashed line along with the Taddington–Bakewell Anticline and the
11 Cronkston-Bonsall Fault; SS, Stanton syncline.

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19 Fig. 2. A, and B, Photomicrographs under parallel and crossed polarizers of the ventral
20 valve showing patches of silicification as spherulitic chalcedony only at the inner (top) and
21 outer (bottom) margins of the columnar tertiary layer (specimen OAW203; scale bar 2
22 mm). C, Close-up view of the two types of silica replacement: spherulitic fibrous
23 chalcedony on the upper right and euhedral quartz on the bottom left following the fibrous
24 chalcedony (image in crossed polarizers, specimen OAW105b; scale bar 2 mm). D, The
25 dorsal valve (in the centre) is often affected by silica replacement in both the inner and
26 outer margins whereas the ventral valve (left side of image) appears pristine (specimen
27 OAW8; scale bar 3 mm). E, Silicification on the outer margin of the dorsal valve affected
28 by a stylolite suggesting that silica replacement took place early, during diagenesis before
29 pressure solution (specimen RCK11; scale bar 1 mm). F, Silicification on the outer margin
30 of the dorsal valve crossed by a fracture filled by burial diagenesis calcite (specimen
31 OAW105; scale bar 1 mm).

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41 Fig. 3. A, and B, Photomicrographs under parallel polarizers (A) and cathodoluminescence
42 (B) of the specimen *G. inflatus* show that the ventral valve (below) is largely non
43 luminescent except the outer margin of the columnar layer at the boundary with the
44 laminar layer (often not present) where chalcedony spherulites occur and along some
45 growth lines. The dorsal valve (above) shows evidence of skeletal calcite recrystallization
46 substituted by luminescent equant sparite. The peloidal skeletal packstone-wackestone
47 sediment in between the valves displays a blotchy luminescence (specimen RCK41
48 oriented with the outer margin of ventral valve at the bottom of the image; scale bar 1 mm).
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C, and D, Photomicrographs under parallel polarizers (C) and cathodoluminescence (D) of
the outer margin of the columnar layer of the ventral valve showing non luminescent
chalcedony spherulites surrounded by luminescent calcite that represents the altered outer
portion of the tertiary layer adjacent to non altered columnar crystals towards the middle

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3 portion of the valve (towards the right), except along the crystal boundaries (specimen
4 OAW209; scale bar 1 mm). E, and F, Photomicrographs under parallel polarizers (E) and
5 cathodoluminescence (F) of the columnar tertiary layer cut by a fracture filled by dull
6 luminescent calcite followed by euhedral rhombic dolomite crystals which have an
7 euhedral non luminescent nucleus overgrown by a luminescent growth phase (specimen
8 RCK35; scale bar 1 mm).
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13 Fig. 4. SEM photomicrographs showing shell microstructure. A, laminar secondary layer
14 with pseudopunctae, *G. inflatus* (specimen OAW3; scale bar 200 μm). B, intercalation of
15 laminar secondary layer and columnar tertiary layer; the transition between the fabrics is
16 well preserved, *G. inflatus* (specimen RCK300; scale bar 100 μm). C, well preserved
17 columnar tertiary layer with stepped growth lines inside the columns, *G. inflatus* (specimen
18 OAW3; scale bar 200 μm). D, details of stepped growth lines inside the columns, *G.*
19 *inflatus* (specimen RCK35; scale bar 20 μm). E, silicification as spherulitic chalcedony in
20 the outer part of the shell, *G. okensis* (specimen RCK36; scale bar 500 μm). F, Detail of
21 the growth of euhedral megaquartz crystals overgrowing chalcedony spherulites within the
22 columnar tertiary layer, *G. inflatus* (specimen OAW212; scale bar 100 μm). These outer
23 parts of the columnar layer were not sampled for geochemical analyses.
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33 Fig. 5. TEM images of specimen RCK16. A, selected area diffraction pattern of calcite
34 along the $\langle 1-1\ 0 \rangle$ direction showing the c-axis oriented parallel to the section plane. B,
35 bright field image showing an ovoidal inclusion where a crystalline precipitate, an
36 amorphous material (fluid, gel, glass) and a void, possibly filled by a gas, are visible (scale
37 bar 50 nm). C, ovoidal inclusions with euhedral crystalline precipitates (scale bar 100 nm).
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41 Fig. 6. TEM bright field images of specimen RCK16. A, inclusions interconnected by
42 dislocations (arrows; scale bar 50 nm). B, inclusion trail along a dislocation (scale bar 100
43 nm). C, nanosized inclusions along calcite grain boundary (intergranular organics; scale
44 bar 50 nm).
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48 Fig. 7. EBSD images. A, EBSD band contrast (1) and orientation (2) measurement images
49 with corresponding pole figures of a shell portion of *G. elongatus* (OAW203). Large
50 columnar units of the tertiary layer form the shell, with calcite crystallite being highly co-
51 oriented, thus, not altered by diagenetic overprint. B, EBSD band contrast (1) and
52 orientation (2) measurement images with corresponding pole figures of a shell portion of
53 *G. inflatus* (RCK221). The specimen is not altered as calcite co-orientation is high (see
54 pole figures for calcite c- and a-axes) and the columnar microstructure characteristic of the
55 tertiary layer is well preserved. C, EBSD band contrast (1) and orientation (2)
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3 measurement images with corresponding pole figures of a shell portion of *G. okensis*
4 (RCK16). According to differences in microstructure (Figure B, compare shell portions
5 highlighted with black and blue rectangle) we can clearly distinguish between overprinted
6 and pristine or very little disturbed shell portions. Unaltered or slight altered portions of the
7 tertiary layer show the original biogenic microstructure (blue rectangle in 2) as well as a
8 tight coherence of calcite crystallite c- and a*axes (3). In contrast, in the overprinted
9 secondary layer (black rectangle in 2), the biogenic microstructure is entirely erased by
10 newly formed calcite, the latter crystallized with a random orientation pattern (4).

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13 Fig. 8. $^1\text{H-NMR}$ analysis. A, cluster at 1.90, 1.70, 1.45 ppm typical of amino acid aliphatic
14 chain such as alanine, leucine or isoleucine. B, cluster at 2.9 e 2.80 ppm referring to lateral
15 chain of aspartate/asparagine.

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18 Fig. 9. Internal characters of *Gigantoproductus*. The specimen on the right is a composite
19 fossil showing the ventral valve thickness, part of the ventral valve internal mould (with
20 mould of brachial cones) and part of the interior of the dorsal valve with brachial ridges
21 (modified from Angiolini *et al.* 2012).

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24 Fig. 10. $\delta^{13}\text{C}_{\text{org}} - \delta^{15}\text{N}_{\text{org}}$ plot showing the measured isotope values for the shell's organic
25 matrix (orange and grey dots) and the reconstructed isotopic composition of soft tissues
26 (arrows to red and blue dots) of the investigated species of *Gigantoproductus*, based on a
27 bivalve model. $\delta^{13}\text{C}_{\text{org}}$ values for Visean organic and terrestrial matter are reported from
28 the literature (their $\delta^{15}\text{N}_{\text{org}}$ values are not known) (Lewan 1980; Strauss & Peters-Kottig
29 2003). The yellow field represents theoretical values of $\delta^{13}\text{C}_{\text{org}}$ for the soft tissues of
30 species pursuing a strict suspension feeding strategy and is calculated considering an
31 increase of 1‰ relative to dietary carbon at each step-wise increase in trophic level
32 (O'Donnel *et al.* 2003). See text for discussion.

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34
35 Table 1. Width, length (mm) and log-transformed shell area of 50 specimens of *G.*
36 *elongatus*, *G. inflatus* and *G. okensis* (1A) and of 69 specimens of species of other
37 brachiopod genera (1B) from the Visean Monsal Dale Limestone and Eyam Limestone
38 formations of Ible, Wensley Dale, Once-a-week and Ricklow quarries.

39
40
41 Table 2. SEM and cathodoluminescence screening and stable oxygen and carbon isotope
42 composition of the calcite of the shell of *Gigantoproductus* species.

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44
45 Table 3. Carbon and nitrogen isotopes from the organic fractions isolated from the shell
46 calcite.

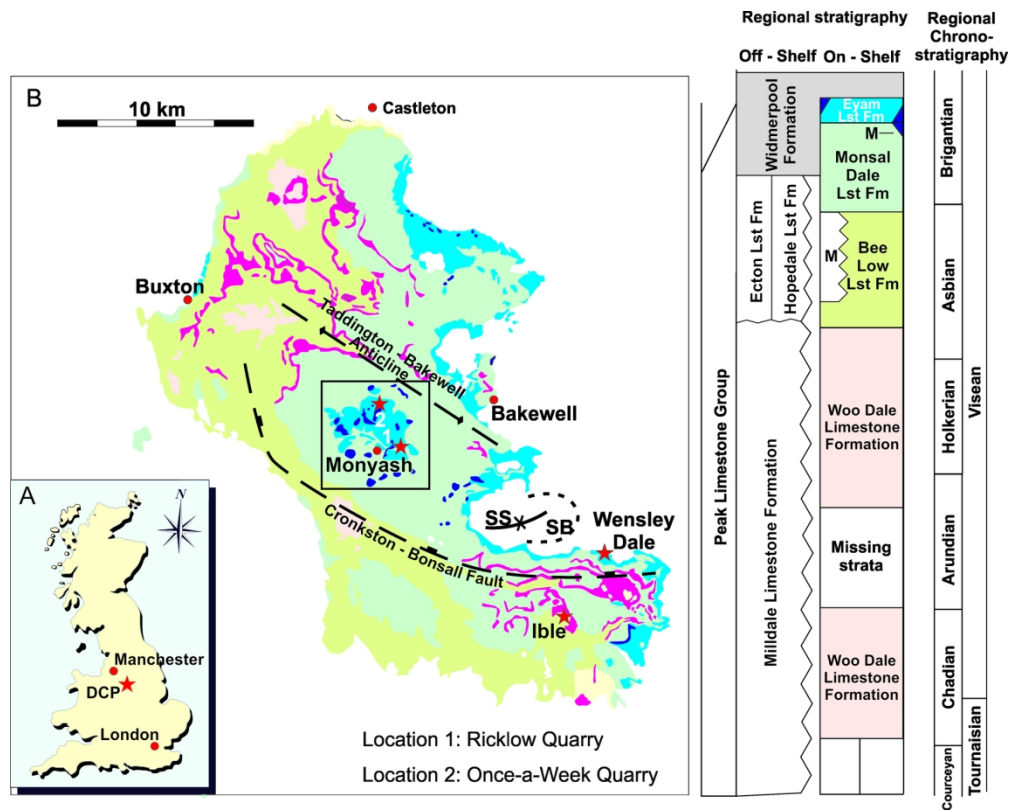


Figure 1

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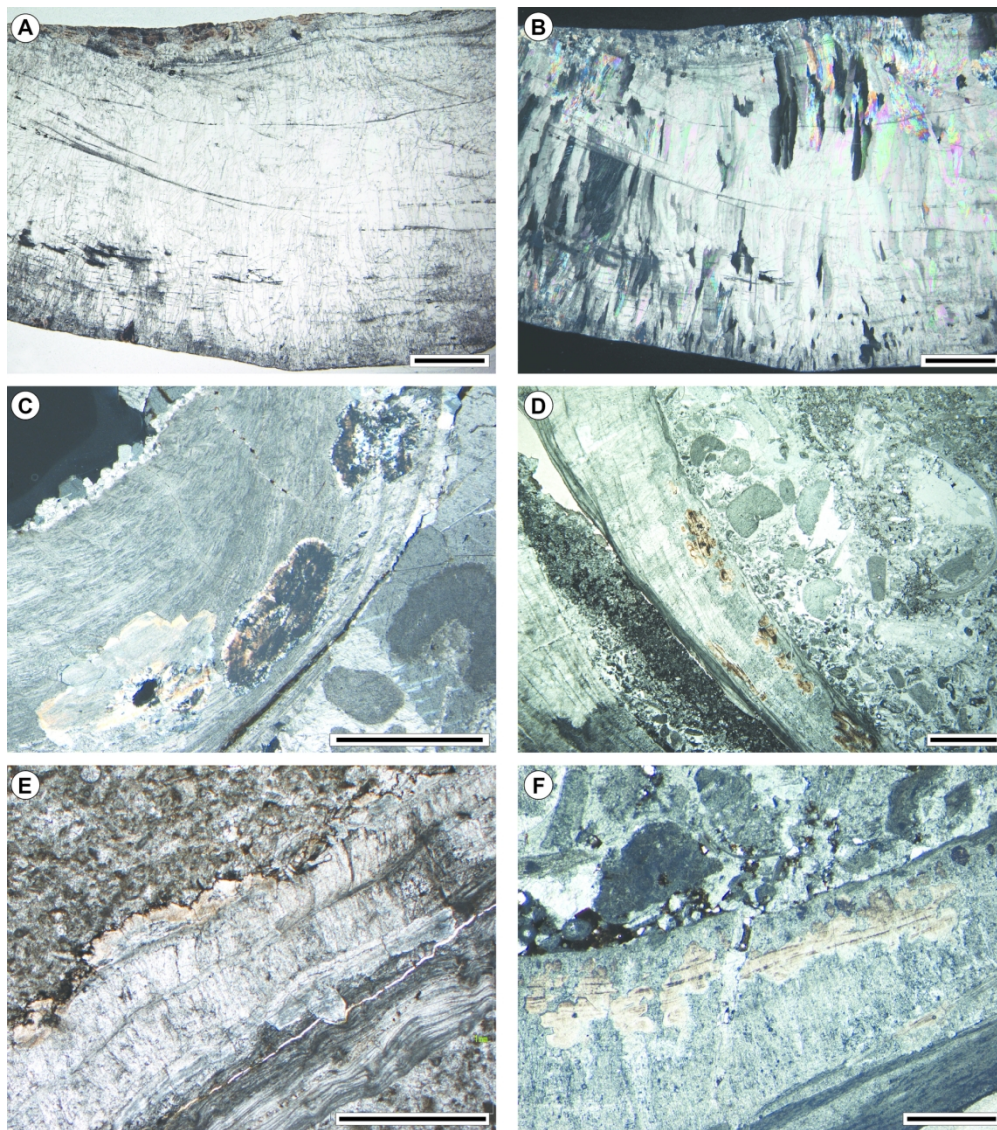


Figure 2

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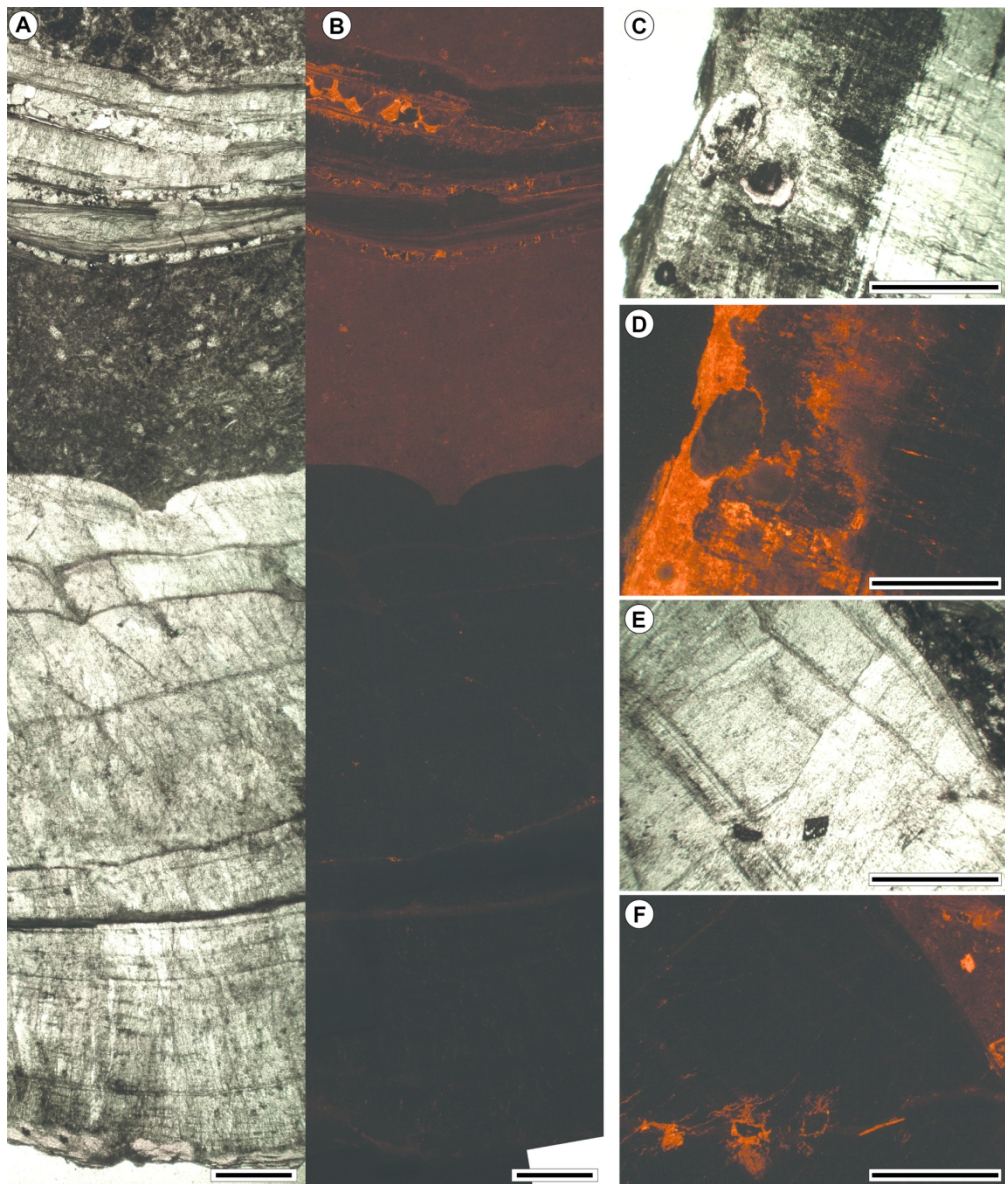


Figure 3

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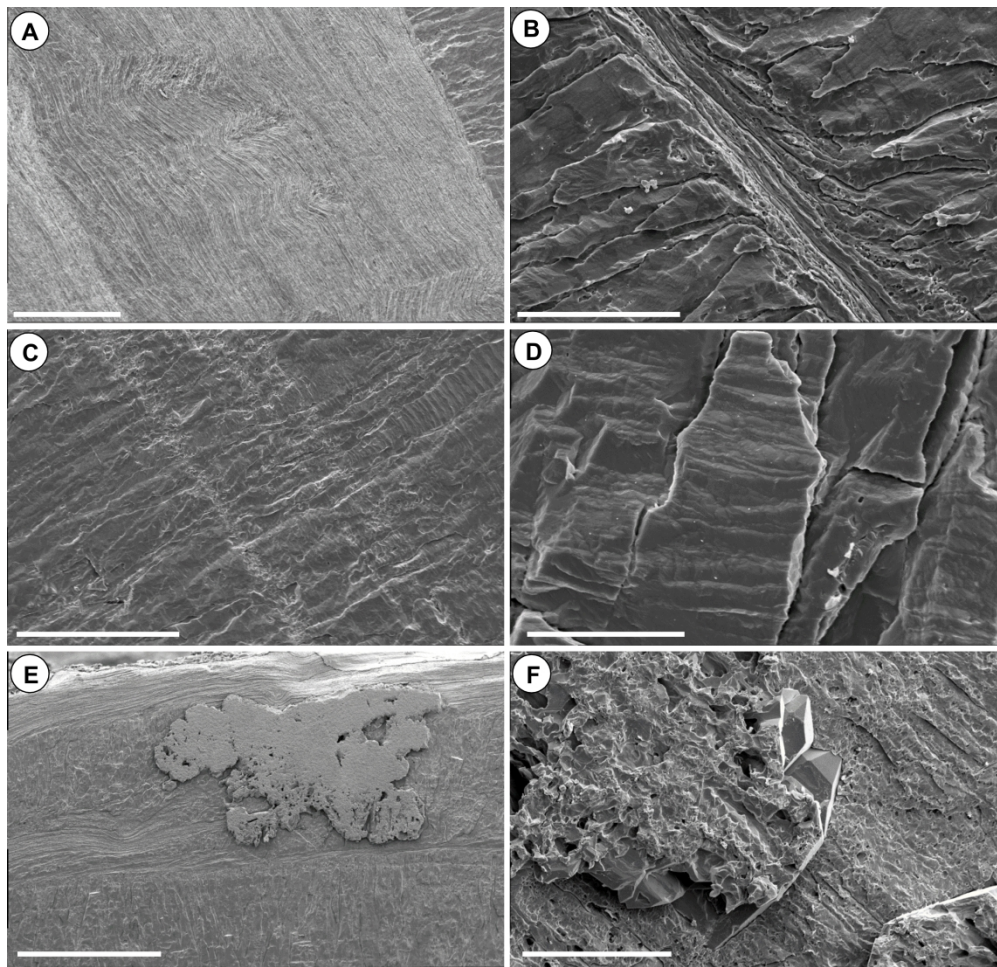


Figure 4

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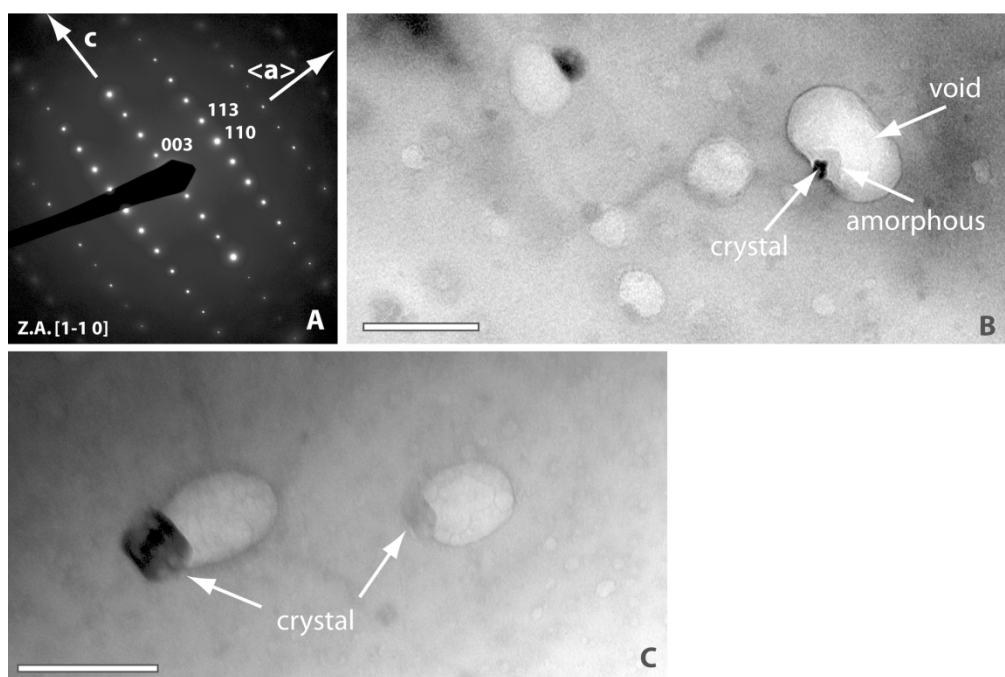


Figure 5

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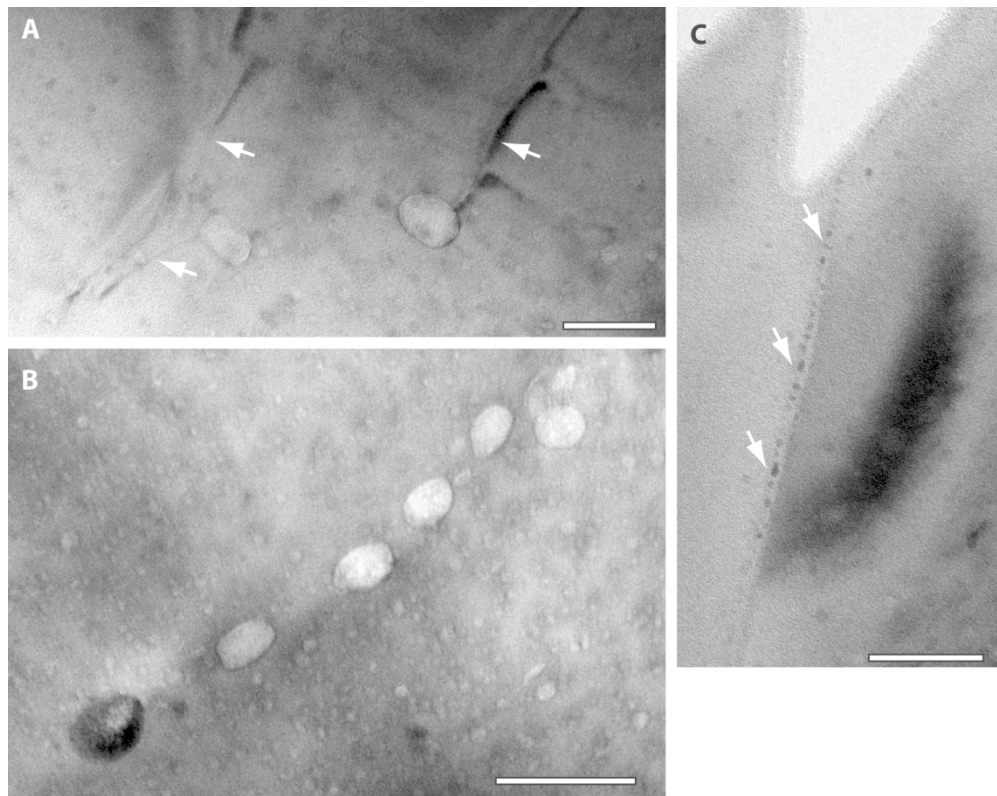
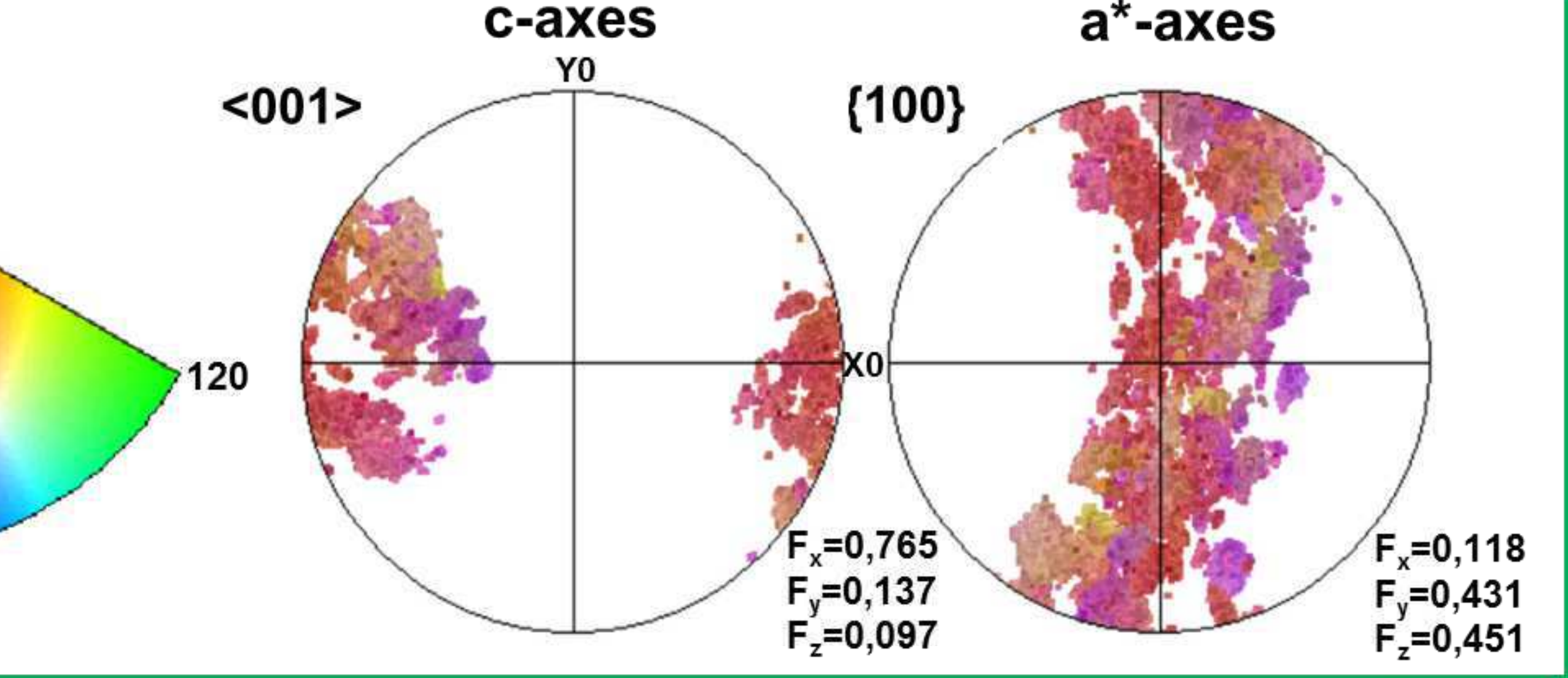
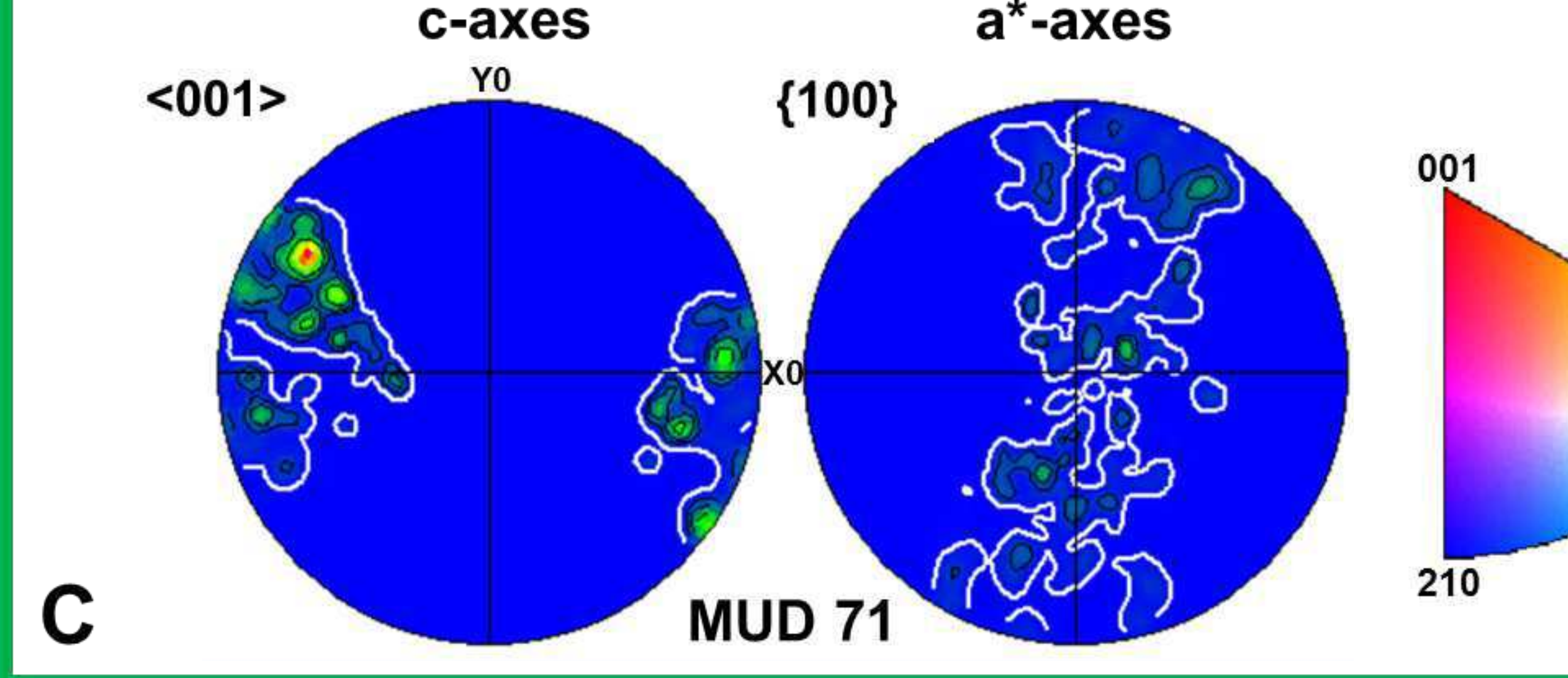
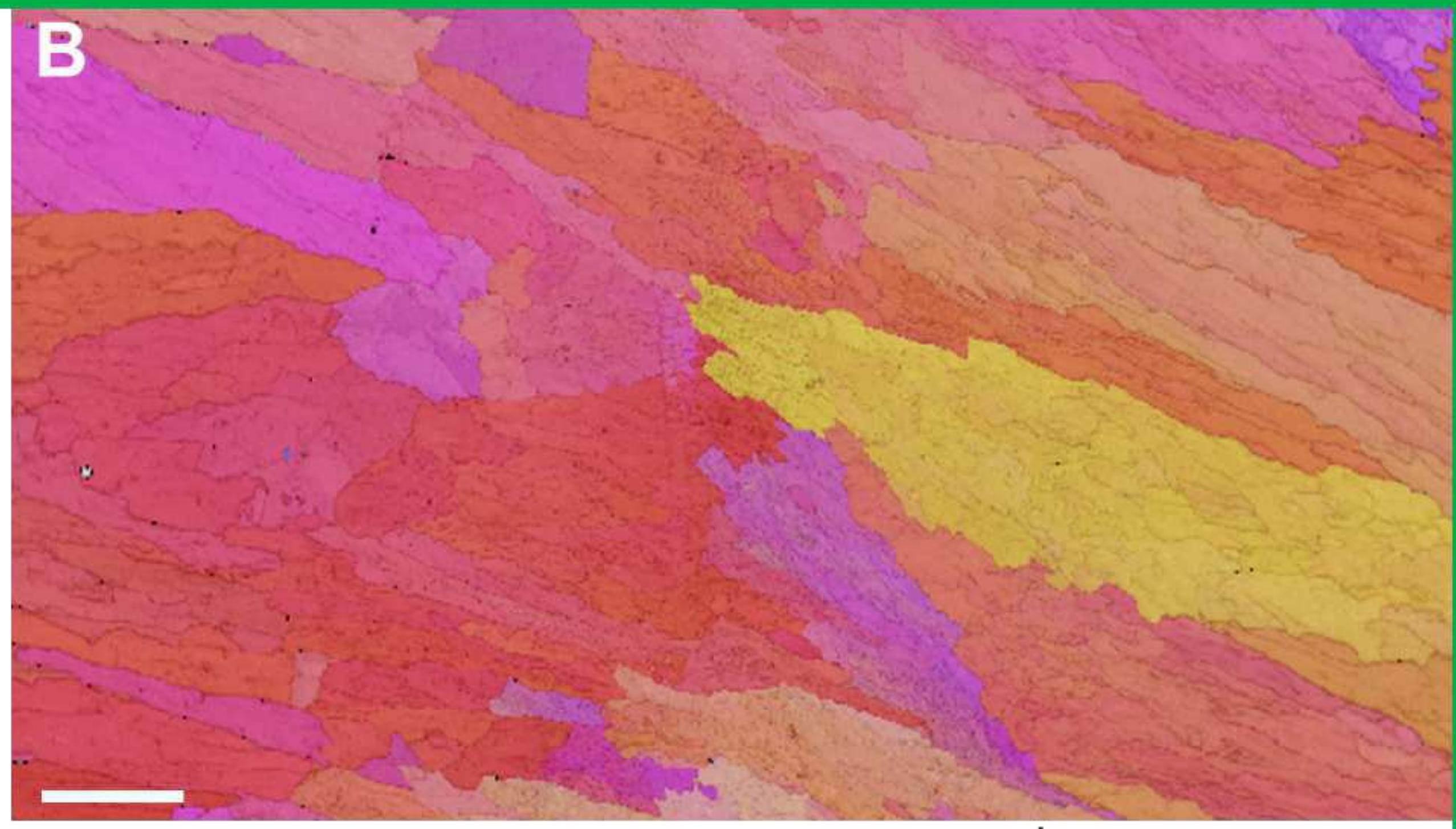
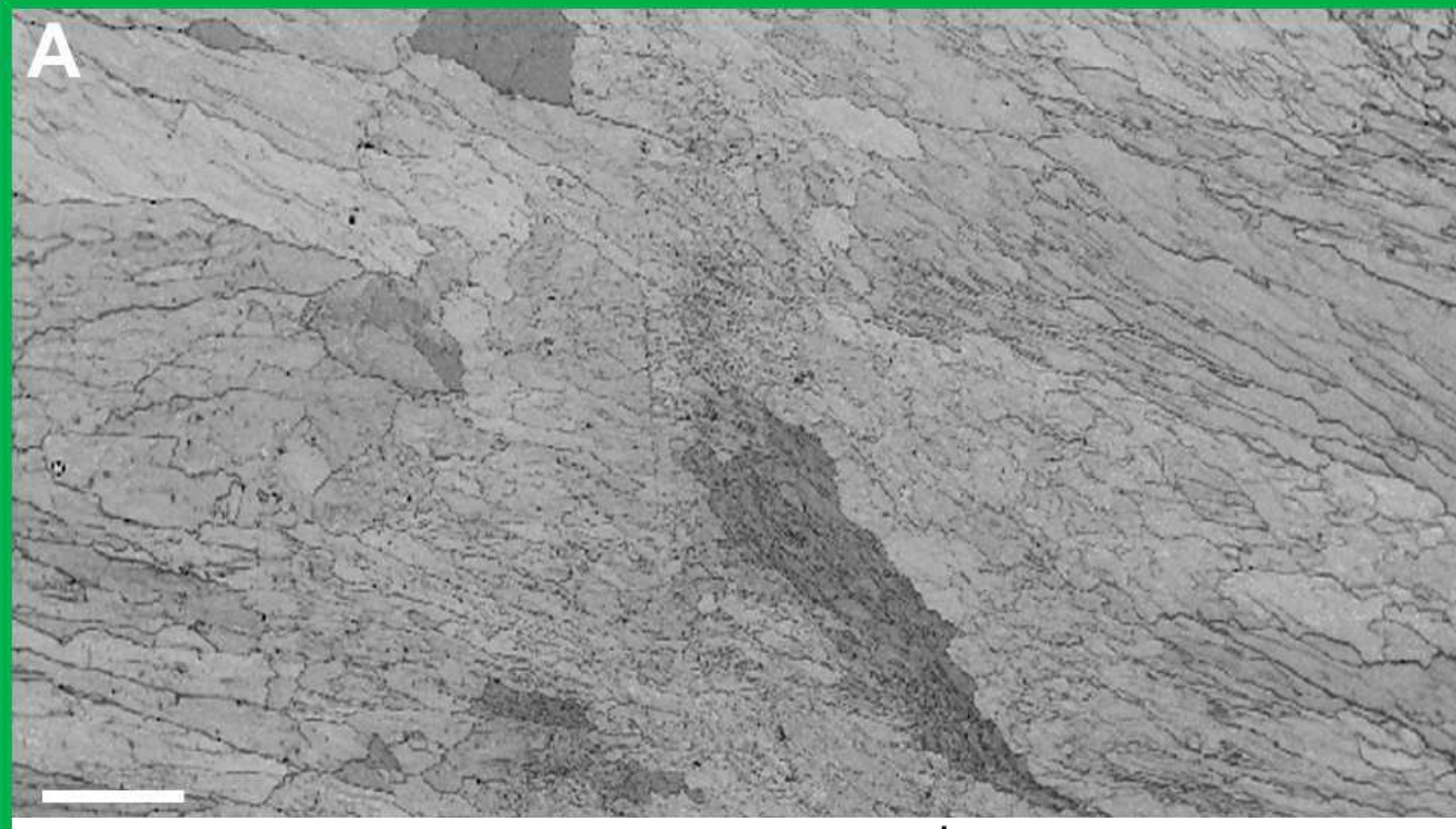


Figure 6

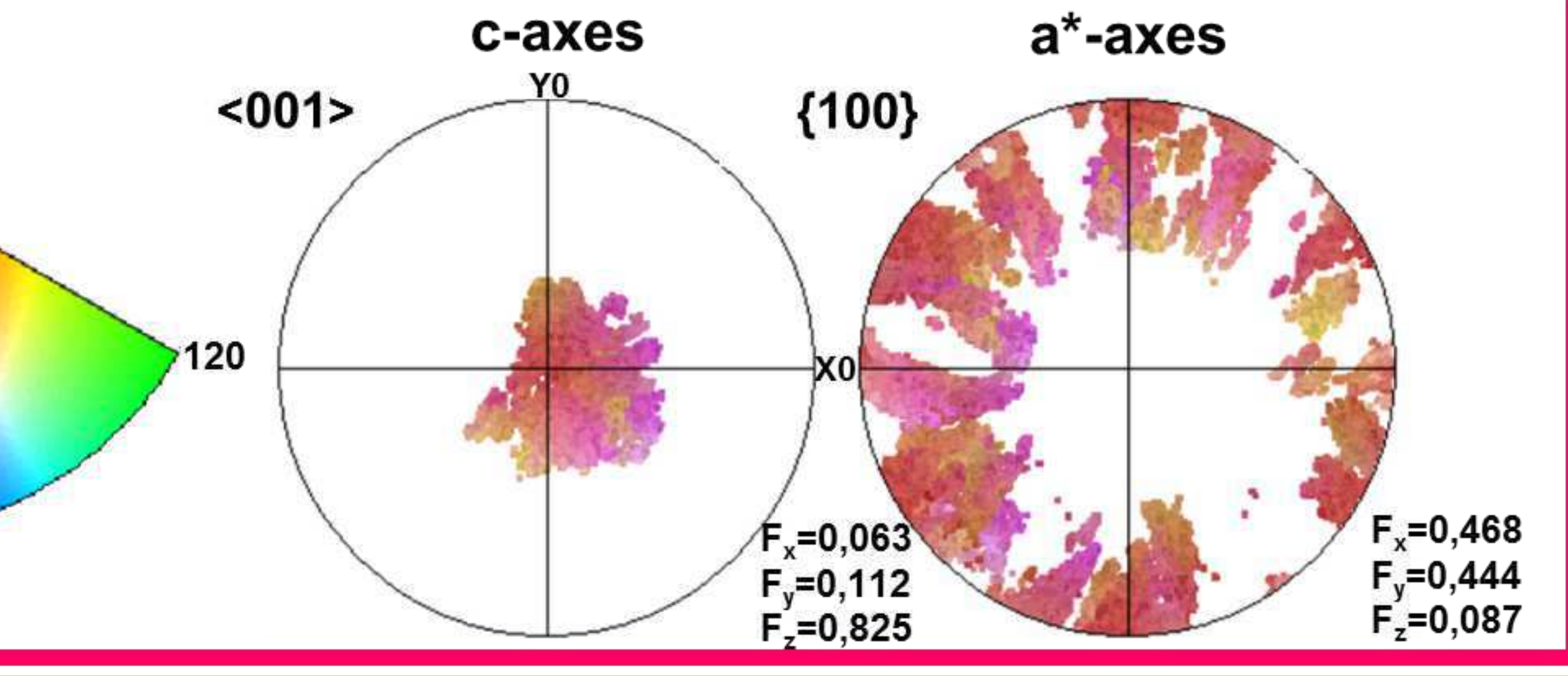
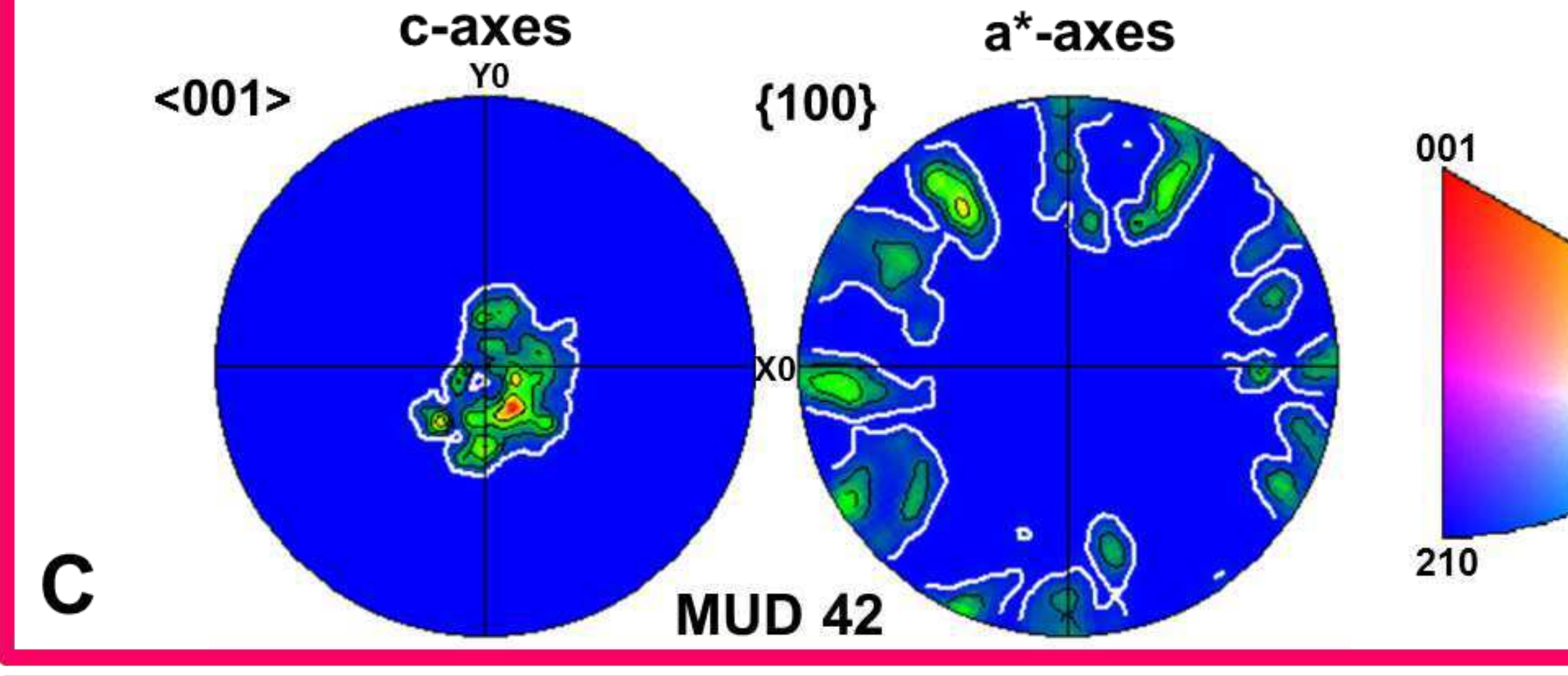
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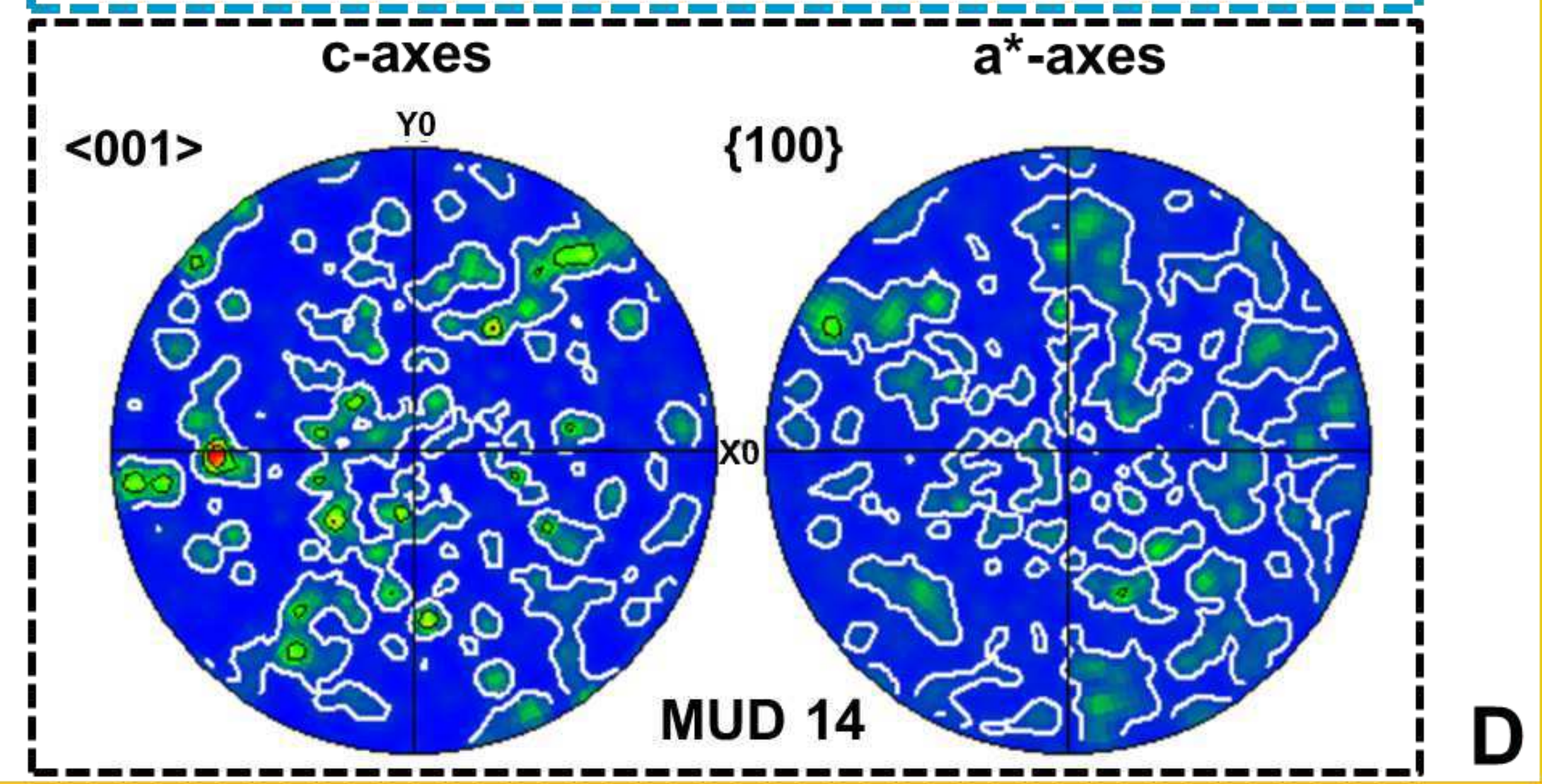
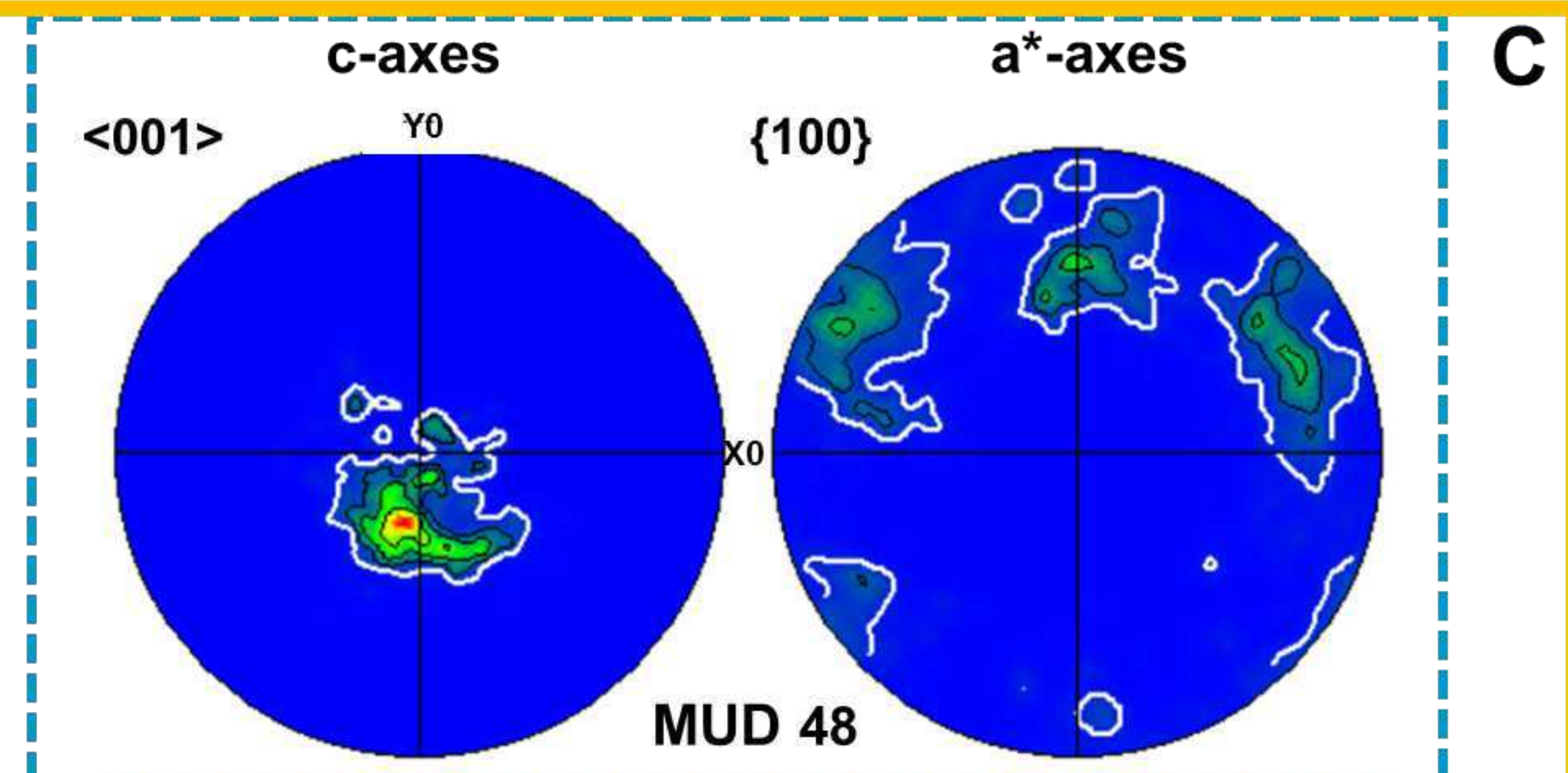
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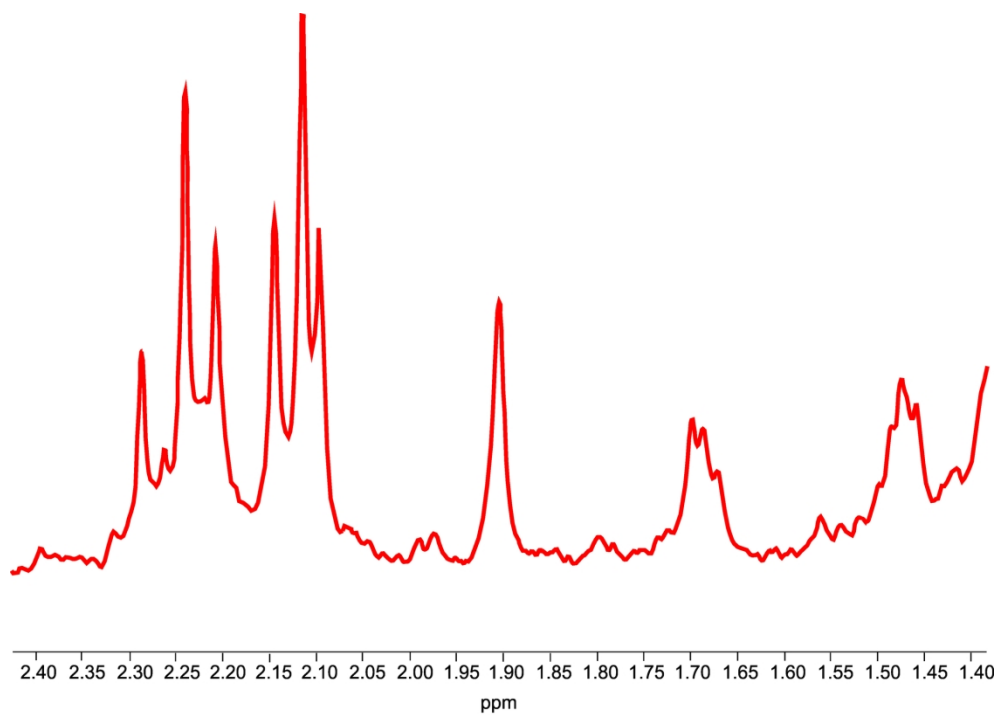


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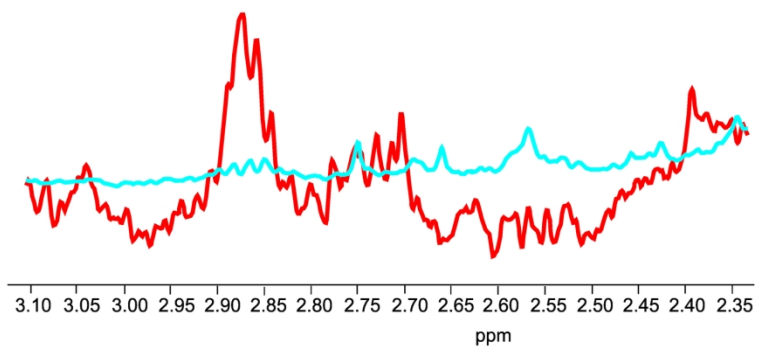


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Figure 8

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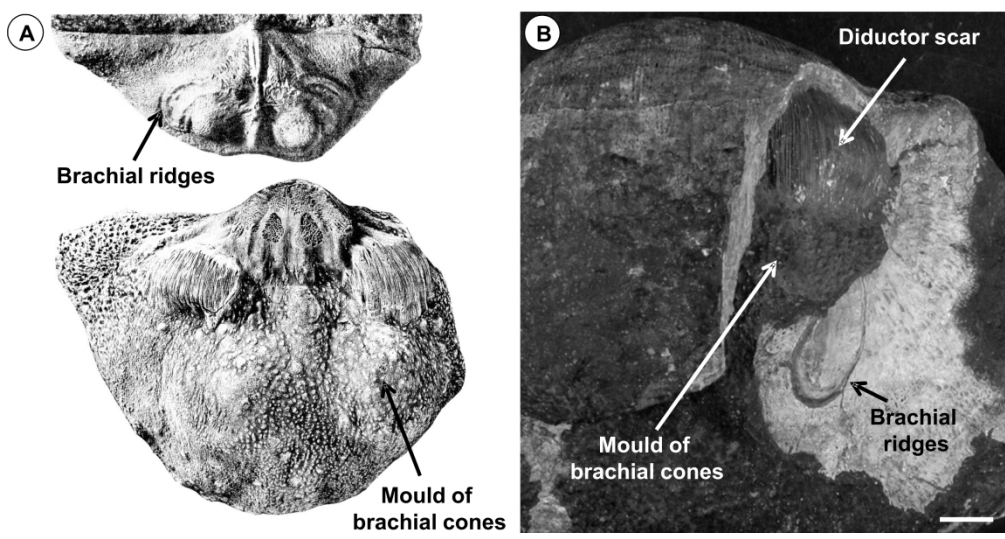
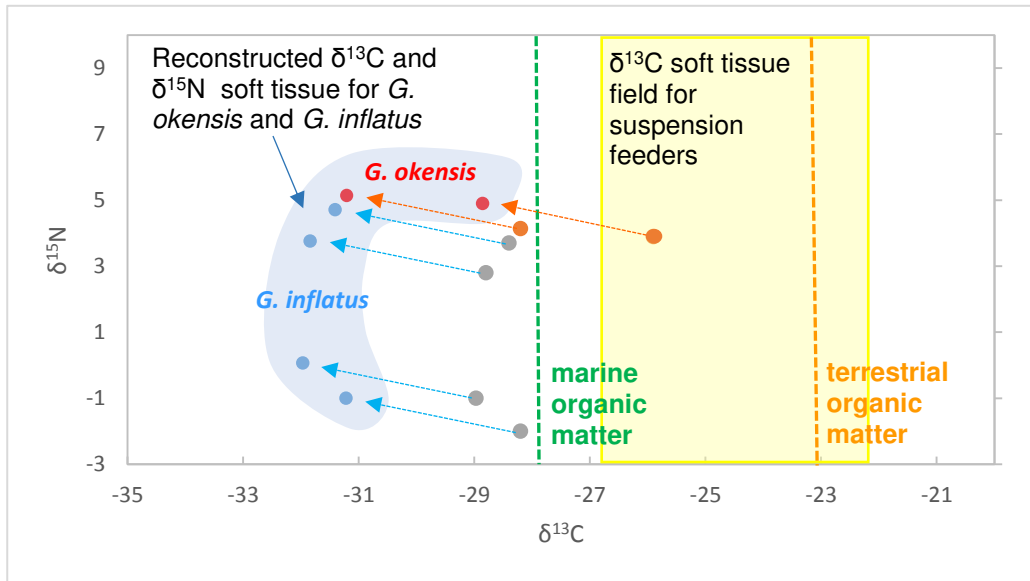


Figure 9



Species of <i>Gigantoproductus</i>					
Specimen	Taxon	Width (mm)	Length (mm)	Area	Log
RCK 5	<i>Gigantoproductus</i> sp.	153.2	59.1	9054.1	4
RCK 40	<i>G. okensis</i>	134.8	60.1	8101.4	3.9
RCK 25	<i>G. okensis</i>	120.2	59.3	7127.9	3.9
RCK 22	<i>Gigantoproductus</i> sp.	152.8	69.8	10665.4	4
RCK 9	<i>G. okensis</i>	120.2	65.2	7837.0	3.9
RCK 42 A	<i>G. okensis</i>	142.4	87.7	12488.5	4.1
RCK 16	<i>G. okensis</i>	122.8	88.3	10843.2	4
RCK 36 B	<i>G. okensis</i>	188.2	72.1	13569.2	4.1
RCK 13	<i>G. okensis</i>	145.2	81.8	11877.4	4.1
RCK 15	<i>G. okensis</i>	122.8	94.6	11616.9	4.1
RCK 203	<i>G. inflatus</i>	171.8	87.1	14963.8	4.2
OAW 224	<i>G. elongatus</i>	120.2	86.9	10445.4	4
OAW 236	<i>G. elongatus</i>	116.4	84.3	9812.5	4
OAW 207	<i>G. elongatus</i>	102.2	68.4	6990.5	3.8
OAW 218	<i>Gigantoproductus</i> sp.	138.2	81.7	11290.9	4.1
OAW 202	<i>G. elongatus</i>	118.2	71.2	8415.8	3.9
OAW 229	<i>Gigantoproductus</i> sp.	112.6	74.1	8343.7	3.9
OAW 227	<i>G. elongatus</i>	147	103.8	15258.6	4.2
RCK 225	<i>G. elongatus</i>	244.6	77.3	18907.6	4.3
OAW 7	<i>G. elongatus</i>	126.2	71.2	8985.4	4
OAW 25	<i>G. elongatus</i>	119.8	75.1	8997	4
OAW 21	<i>G. inflatus</i>	145.2	86.1	12501.7	4.1
OAW 12	<i>G. elongatus</i>	152.2	79.3	12069.5	4.1
OAW 10	<i>Gigantoproductus</i> sp.	168.6	100.1	16876.9	4.2
OAW 17	<i>Gigantoproductus</i> sp.	148.2	97.3	14419.9	4.2
RCK 228	<i>G. okensis</i>	94.4	74.2	7004.5	3.8
IBL 122	<i>G. okensis</i>	123.6	94.4	11667.8	4.1
IBL 123	<i>Gigantoproductus</i> sp.	93.2	68.4	6374.9	3.8
RCK 200	<i>G. inflatus</i>	163.2	126.2	20595.8	4.3
RCK 201	<i>G. inflatus</i>	133.2	78.6	10469.5	4
OAW 242	<i>G. okensis</i>	131.6	63.4	8343.4	3.9
OAW 241	<i>G. elongatus</i>	179.8	68.4	12298.3	4.1
OAW 226	<i>G. okensis</i>	116.4	100.6	11709.8	4.1
IBL 120	<i>G. okensis</i>	74.4	62.4	4642.6	3.7
OAW 216	<i>G. okensis</i>	118.2	67.5	7978.5	3.9
OAW 27	<i>G. elongatus</i>	152.4	89.4	13624.6	4.1
OAW 104	<i>G. inflatus</i>	112.4	87.4	9823.8	4
OAW 500	<i>Gigantoproductus</i> sp.	114.4	82.6	9449.4	4
OAW 2	<i>G. elongatus</i>	110.4	70.4	7772.2	3.9
OAW 3	<i>G. inflatus</i>	132.4	85.7	11346.7	4.1
OAW 8	<i>G. inflatus</i>	173.2	124.4	21546.1	4.3
OAW 50	<i>G. inflatus</i>	121.4	96.9	11763.7	4.1
OAW 6	<i>G. inflatus</i>	94.8	62.6	5934.5	3.8
OAW 5	<i>G. inflatus</i>	129.6	93.5	12117.6	4.1
OAW501	<i>G. elongatus</i>	132.5	102.5	13581	4.1
OAW502	<i>G. elongatus</i>	131.1	80.7	10580	4
W11	<i>Gigantoproductus</i> sp.	167.6	122.6	20547.8	4.3

RCK 9.A	<i>G. okensis</i>	115.2	78.6	9054.7	4
WI 3	<i>G. inflatus</i>	184.4	102.6	18919.4	4.3
WI 2A	<i>Gigantoproductus</i> sp.	146.4	75.4	11038.6	4
				total	201.7
Average log-transformed shell area 201.7/50 = 4					

Species of genera other than <i>Gigantoproductus</i>					
Specimen	Taxon	Width (mm)	Length (mm)	Area	Log
RCK 42 C	<i>Latibrachythyris</i>	36.8	19.3	710.2	2.9
IBL 108	<i>Antiquatonia</i>	37.6	16.8	631.7	2.8
RCK 217	Linoproductinae	39.6	17.1	677.2	2.8
RCK 218	<i>Latibrachythyris</i>	24.8	23.2	575.4	2.8
OAW 210	Linoproductinae	30.4	15.4	468.2	2.7
OAW 243	<i>Latibrachythyris</i>	31.6	17.1	540.4	2.7
OAW 247	Tolmatchoffiini	18.8	11.4	214.3	2.3
RCK 211	<i>Antiquatonia</i>	43.6	23.2	1011.5	3
RCK 17 - 1	<i>Girtyella</i>	6.6	9.9	65.3	1.8
RCK 17 - 2	Tolmatchoffiini	10.8	14.8	159.8	2.2
RCK 15 - 1	<i>Latibrachythyris</i>	9.2	10.6	97.5	2
RCK 15 - 2	<i>Girtyella</i>	9.4	12.1	113.7	2.1
RCK 15 - 4	<i>Antiquatonia</i>	25.6	15.6	399.4	2.6
RCK 15 - 5	<i>Latibrachythyris</i>	4.6	5.2	23.9	1.4
RCK 15 - 6	<i>Hartella</i>	8.2	8.6	70.5	1.8
RCK 15 - 7	<i>Antiquatonia</i>	14.8	22.2	328.6	2.5
RCK 15 - 8	Girtyellinae	5.6	7.2	40.3	1.6
RCK 15 - 9	<i>Phricodothyris</i>	12.2	6.8	83	1.9
RCK15-10	<i>Krotovia</i>	19.2	15.8	303.4	2.5
RCK 15 - 11	<i>Antiquatonia</i>	24.8	15.7	389.4	2.6
RCK 15 - 12	Tolmatchoffiini	11.6	17.2	199.5	2.3
RCK 15 - 14	<i>Hartella</i>	9.6	8.4	80.6	1.9
RCK 15 - 18	<i>Girtyella</i>	9.2	12.4	114.1	2.1
RCK 15 - 19	<i>Pleuropugnoides</i>	10.8	11.4	123.1	2.1
RCK 15 - 26	<i>Girtyella</i>	6.4	7.6	48.6	1.7
RCK 15 - 27	Girtyellinae	6.2	6.8	42.2	1.6
RCK 15 - 29	<i>Pleuropugnoides</i>	19.8	11.4	225.7	2.4
RCK 15 - 33	<i>Phricodothyris</i>	8.6	7.4	63.6	1.8
WI - 1	Girtyellinae	23.8	21.4	509.3	2.7
WI - 2	<i>Antiquatonia</i>	42.4	39.2	1662.1	3.2
WI - 3	Girtyellinae	6.1	5.1	31.1	1.5
WI - 4	<i>Echinoconchus</i>	48.4	23.6	1142.2	3.1
WI - 6	<i>Latibrachythyris</i>	34.2	27.4	937.1	3
WI - 8	<i>Antiquatonia</i>	41.2	34.9	1437.9	3.2
WI - 10	<i>Phricodothyris</i>	21.1	16.8	354.5	2.5
WI - 14	<i>Antiquatonia</i>	42.8	34.9	1493.7	3.2
WI - 15	<i>Echinoconchus</i>	46.8	24.8	1160.6	3.1
WI - 16	<i>Pleuropugnoides</i>	21.4	18.4	393.8	2.6
WI - 17	<i>Antiquatonia</i>	45.2	35.4	1600.1	3.2
3OCCM -OAW	<i>Phricodothyris</i>	24.8	20.4	505.9	2.7
WI 1-1	<i>Echinoconchus</i>	52.4	38.8	2033.1	3.3

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2	WI 1-2	<i>Antiquatonia</i>	46.4	28.1	1303.8	3.1
3	WI 1-3	<i>Antiquatonia</i>	45.2	29.2	1319.8	3.1
4	WI 1-4	Girtyellinae	9.8	12.4	121.5	2.1
5	WI 1-5	<i>Antiquatonia</i>	21.2	11.4	241.7	2.4
6	WI 1-6	<i>Antiquatonia</i>	12.6	11.8	148.7	2.2
7	WI 1-7	<i>Echinoconchus</i>	46.6	49.4	2302	3.4
8	WI 1-8	<i>Echinoconchus</i>	53	48.2	2554.6	3.4
9	WI 1-9	<i>Antiquatonia</i>	21.6	13.6	293.8	2.5
10	WI 1-10	<i>Latibrachythyris</i>	23.8	20.2	480.8	2.7
11	WI 1-11	<i>Latibrachythyris</i>	21.6	16.4	354.2	2.5
12	WI 1-12	<i>Phricodothyris</i>	20.8	15.6	324.5	2.5
13	WI 1-13	<i>Latibrachythyris</i>	34.4	23.8	818.7	2.9
14	WI 1-14	<i>Antiquatonia</i>	48.4	31.8	1539.1	3.2
15	WI 1-15	<i>Antiquatonia</i>	10.2	13.2	134.6	2.1
16	WI 1-22	<i>Echinoconchus</i>	45.8	35.1	1607.6	3.2
17	WI 1-25	<i>Antiquatonia</i>	38.3	26.9	1030.3	3.0
18	WI 1-28	<i>Echinoconchus</i>	48.2	46.5	2229.2	3.3
19	WI 1-33	<i>Pleuropugnoides</i>	20.5	17.5	358.7	2.5
20	WI 1-36	<i>Latibrachythyris</i>	28.2	18.7	537.3	2.7
21	WI 2-1	<i>Latibrachythyris</i>	42.4	28.3	1199.9	3.1
22	WI 2-2	<i>Antiquatonia</i>	43.6	28.2	1229.5	3.1
23	WI 2-3	<i>Antiquatonia</i>	38.4	29.2	1121.3	3
24	WI 2-4	<i>Latibrachythyris</i>	43.2	25.8	1114.6	3
25	WI 2-5	<i>Antiquatonia</i>	35.6	23.6	840.2	2.9
26	WI 2-6	Girtyellinae	5.1	3.8	19.4	1.3
27	WI 2-7	<i>Echinoconchus</i>	21.4	17.6	376.6	2.6
28	WI 2-8	<i>Echinoconchus</i>	46.8	46.6	2180.9	3.3
29	WI 3-2	Girtyellinae	3.96	2.34	92664	1
30	WI 4-6	Girtyellinae	2.76	2.42	66792	0.8
31						165.5
32	Average log-transformed shell area $165.5/69= 2.4$					
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Table 2 (NL Non luminescent; BL Bright luminescent; WL Weak luminescent; DL Dull Luminescent)

Sample #	Species Name	SEM analysis	Cathodoluminescence analyses	Weight (g)	$\delta^{13}\text{C}$ (‰ VPDB)	$\delta^{18}\text{O}$ (‰ VPDB)
OAW3	<i>G. inflatus</i>	preserved laminar layer; well preserved columnar layer; localized silicification at the outer margin of the columnar layer	NL, except BL at the outer margin of the columnar layer where silicification occurs (1 mm wide belt parallel to margin), along some growth lines close to the outer and inner margins and in microfractures (a few μm wide) parallel to intercrystalline boundaries	1.3	+0.8	-4.6
OAW203	<i>G. elongatus</i>	well preserved columnar layer; limited silicification at the outer margin of the columnar layer	NL, except BL at the outer margin of the columnar layer where silicified; along some growth lines and intercrystalline boundaries close to the outer margin	1.5	+1.1	-4.3
OAW212	<i>G. inflatus</i>	preserved laminar layer; well preserved columnar layer; localized silicification at the outer margin of the columnar layer	NL, except localized BL along growth lines and intercrystalline boundaries	1.4	+0.6	-4.7
RCK16	<i>G. okensis</i>	poorly preserved laminar layer; well preserved columnar layer; localized silicification at the outer margin	NL, except localized BL along growth lines	1.2	+2.3	-4.1
RCK33	<i>G. inflatus</i>	preserved laminar layer; well preserved columnar layer; localized silicification at the boundary between columnar and laminar layer	NL, except BL at laminar layer and boundary with columnar layer where silicification occurs; along some growth lines close to inner and outer margins, at intercrystalline boundaries and fractures crossing the valve perpendicularly	1.7	+2.0	-4.3
RCK35	<i>G. inflatus</i>	poorly preserved laminar layer; well preserved columnar layer; localized silicification at the outer and inner margin of the columnar layer and at the boundary with the laminar layer where present	NL in the intermediate portion of ventral valve; BL at inner and outer margin of columnar and laminar layers, along growth lines. One fracture crossing the valve with DL sparite followed by euhedral rhombic dolomite with a NL nucleus followed by BL growth phase	2.1	+1.6	-4.7
RCK36	<i>G. okensis</i>	preserved laminar layer; well preserved columnar layer; localized	NL, except BL at laminar layer, outer margin of columnar layer where silicified, along marginal growth lines and in a microfracture (μm wide)	1.3	+1.7	-3.9

		silicification at the outer margin	crossing the valve parallel to intercrystalline boundaries of the columnar layer			
RCK41	<i>G. inflatus</i>	no laminar layer; well preserved columnar layer; localized silicification at the outer margin	Largely N, except at the outer margin of columnar layer where silicified (0.5-1 mm wide zone parallel to margin); WL along 4 growth lines towards the inner margin and in the central part of columnar layer; fractures crossing the ventral valve perpendicularly, filled by BL sparite	0.5	+2.4	-3.5
RCK221	<i>G. inflatus</i>	well preserved columnar layer; localized silicification at the outer and inner shell margin	NL, except WL at growth lines close to the margins	2.6	+1.9	-3.8
RCK300	<i>G. inflatus</i>	poorly preserved laminar layer; well preserved columnar layer; localized silicification at the outer margin	NL, except BL along growth lines and intercrystalline boundaries	2.2	+2.6	-3.4

OurLabID	Sample ID	Species	$\delta^{15}\text{N}_{\text{air}}$ of Peak	%N of Sample Analysis	$\delta^{13}\text{C}_{\text{VPDB}}$ of Peak	%C of Sample Analysis
G-14044	1 - RCK16	<i>G. okensis</i>	+3.9	0.6	-28.2	71.6
G-14044	1 - RCK16	<i>G. okensis</i>	+4.4	0.7		
G-14046	3 - RCK33	<i>G. inflatus</i>	-1.8	2.9	-28.2	65.3
G-14046	3 - RCK33	<i>G. inflatus</i>	-2.2	2.9		
G-14047	4 - RCK36	<i>G. okensis</i>	+3.9	1.3	-25.9	70.2
G-14048	5 - RCK41	<i>G. inflatus</i>	+3.7	1	-28.4	83.3
G-14049	6 - RCK300	<i>G. inflatus</i>	+2.7	0.7	-28.8	80.1
G-14049	6 - RCK300	<i>G. inflatus</i>	+2.8	0.8		
G-14052	9 - OAW212	<i>G. inflatus</i>	-0.9	2.4	-29	66.6