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Crystallography of craniid brachiopods by electron backscatter diffraction (EBSD)

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ABSTRACT: Electron backscatter diffraction (EBSD) is used to determine the detailed crystallographic orientation of calcite crystals of craniid brachiopods in the context of shell ultrastructure. Sections of shells of two Recent species, *Novocrania anomala* and *Novocrania huttoni*, are analysed to provide 3D crystallographic patterns at high spatial resolution. The *c*-axis of semi-nacre calcite crystals is oriented parallel to the laminae that define the ultrastructure of the secondary layer. This orientation differs from that of rhynchonelliform calcitic brachiopods where the *c*-axis is perpendicular to the length of morphological fibres and to the shell exterior.

KEY WORDS: Brachiopoda, calcite, *c*-axis, *Novocrania anomala*, *Novocrania huttoni*, secondary layer, semi-nacre

Williams & Wright (1970) conducted the most comprehensive study in understanding the shell ultrastructure of craniid brachiopods. Since the publication of this pioneering work, a few studies have dealt with this topic in the analysis of Recent species (e.g., Bassett 2000; Logan & Long 2001; Robinson & Lee 2007) and of fossil ones (e.g., Smirnova 1997; Halamski 2004). However, most of the research has been directed towards the knowledge of the chemico-structure of shells (e.g. Brown 1998; Williams *et al.* 1999; Cusack & Williams 2001). These studies have revealed the influence of organic matter in the process of biomineralisation and the control exerted by protein sheets in the arrangement of the shell fabric (Williams & Wright 1970; Williams *et al.* 1999; Cusack & Williams 2001). Yet, limited knowledge is available about how the biomineral crystallography can be related to the shell ultrastructure in craniid brachiopods. The main reason may be related to problems in acquiring *in situ* crystallographic information at high spatial resolution. X-ray diffraction, transmission electron microscopy (TEM) and the measurement of interfacial angles have been the principal methods for such purpose (e.g., Taylor & Weedon 2000; Cusack & Williams 2001; Hall *et al.* 2002), but mostly provide partial and indirect information. Electron backscatter diffraction (EBSD) is currently available to overcome these problems and its potential as a powerful analytical tool has been demonstrated for brachiopod shells (e.g., Schmahl *et al.* 2004; Cusack *et al.* 2008).

The aim of this present paper is to provide detailed crystallographic information of craniid brachiopod shells using EBSD. Data from sections of shells of two Recent species, *Novocrania anomala* and *N. huttoni*, are presented in this study. This information is used to correlate crystallographic orientation of calcite crystals and shell ultrastructure to obtain a better understanding of crystal nucleation and growth.

1. Brachiopod ultrastructure

Brachiopods are marine bivalved organisms that emerged in the Cambrian and are cosmopolitan in distribution and habitat in marine environments (Williams *et al.* 1997). They have been divided into three major subphyla based on shell mineralogy and ultrastructure (Williams *et al.* 1996). Two of these main groups, Craniiformea and Rhynchonelliformea, comprise taxa with calcite shells, but different ultrastructures that may reflect dissimilar specific biomineralisation processes. In Craniiformea, craniid brachiopods consist of shells with an outer organic periostracum, a primary layer with acicular calcite and an innermost non-fibrous secondary layer of calcite semi-nacre (Williams & Wright 1970). The secondary layer is composed of laminae bearing rhombohedral tablets that grow spirally from single or double screw dislocations (Williams & Wright 1970) (Fig. 1). The shell fabric of the secondary layer, which constitutes most of the shell, is regulated by the emplacement of proteins (Williams & Wright 1970; Cusack & Williams 2001). Besides the structure, craniid brachiopods grow their dorsal valves in a characteristic way from the location of the protegulum, which is the first-formed shell of periostracum and mineralised lining secreted by the mantle (Williams *et al.* 1997). Craniids grow by holoperipheral increase, all around the margins of the valve, from the centre of growth, coincident with the location of the protegulum (Fig. 1).

2. Material and methods

2.1. Samples

Specimens of *Novocrania anomala* (Müller, 1776) and *N. huttoni* (Thomson, 1916) were collected from the Fifth of Lorn (Oban), NW Scotland (56°24′N/5°38.4′W) and off the coast of Three Kings Islands in the South Pacific/Tasman Sea (34°13′S/172°11.5′W), respectively. Only dorsal valves of all taxa are used herein because ventral valves are more weakly developed and differentially mineralised (e.g. Williams & Wright 1970; Schumann 1970; Cusack & Williams 2001). Also, ventral valves of most craniid brachiopods only display primary layer fabric within the shell (Williams & Wright 1970; Schumann 1970).

2.2. Electron Backscatter Diffraction (EBSD)

EBSD is a technique conducted in a scanning electron microscope (SEM) that is used to determine the preferred crystallographic orientation of any crystalline or polycrystalline material. Its application has been traditionally focused on

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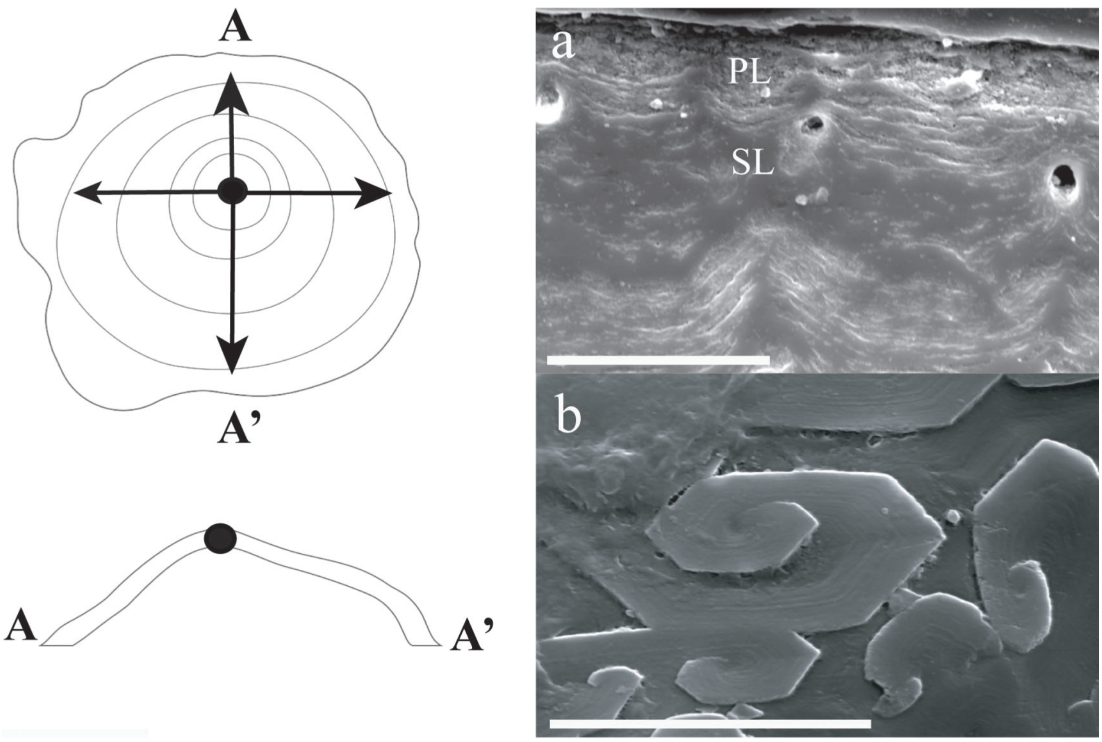

Figure 1 Diagrammatic illustration of *Novocrania anomala*, showing growth directions (arrows) from the location of the protegulum (dark circle) and section along the dorsal valve length from posterior to anterior regions (A–A′). Scanning electron micrographs (SEM) of *N. anomala:* (a) transition between the primary (Pl) and secondary (Sl) layers. Scale bar = 100 μm; (b) detail of the ultrastructure of the secondary layer, showing calcite tablets with screw-dislocation. Scale bar = 5 μm.

highly conductive materials (e.g. metals) in the fields of materials science and engineering. Carbonates are natural insulators and, therefore, *a priori* non suitable for the application of this technique, but its expedience has been already shown for biogenic calcite in molluscs (e.g., Checa *et al.* 2006; Dalbeck *et al.* 2006), brachiopods (e.g., Schmahl *et al.* 2004; Cusack *et al.* 2008) and avian eggshells (Dalbeck & Cusack 2006) after a specific sample preparation. Three specimens of each brachiopod species were analysed using EBSD. Shell sections from the posterior to the anterior margin along the shell length for each specimen (Fig. 1) and two sections polished down from outer shell surface at the location of the protegulum of *N. anomala* were used in this study. These sections were mounted in araldite resin blocks, polished and then coated with a thin layer of carbon (Cusack *et al.* 2008). Shell sections were surrounded by silver paint to avoid electron charging and then analysed in an SEM in high vacuum mode with an aperture and spot size of 4. The stage was tilted 70° and the electron beam diffracted by crystal planes in the shell sample. The diffracted beam interacts with a phosphor screen producing a series of Kikuchi bands that enable crystal identification and orientation to be determined. Data were analysed using the orientation imaging microscopy (OIM) software from EDAX and all data points below a confidence index [CI] of 0·1 were removed (Dalbeck *et al.* 2006).

3. Results

The brachiopod species *Novocrania anomala* is used here to analyse general crystallographic patterns while *Novocrania huttoni* is studied in greater detail to determine the specific relationship between the crystallographic orientation of calcite crystals and the laminae bearing tablets. In *N. anomala*, sections along the main direction of growth (posterior-anterior) (Fig. 1) have been indexed with EBSD to show the orientation of crystals at the centre of growth and posterior and anterior margins (Fig. 2), focusing on the secondary layer, which accounts for the majority of the shell thickness. At the location of the protegular node, crystals of calcite nucleate with the *c*-axis <0001> nearly 90° offset from the perpendicular direction to the shell surface (normal to the plane of view) (Fig. 2b). However, calcite crystals rotate along the shell length and their overall orientation is with the *c*-axis parallel to the shell laminae at the posterior and anterior margins (Fig. 2a–c). Two sections of *N. anomala* were also obtained by polishing parallel to the shell surface at the location of the protegulal node in order to determine the crystallographic orientation of calcite crystals perpendicular to the posterior–anterior sections (Fig. 3). EBSD crystallographic maps of these parallel sections show a crystallographic dominance of the $\{0\bar{1}01\}$ and $\{1\bar{1}00\}$ planes in contrast to the dominance of the $\{0001\}$ plane in the longitudinal section (Fig. 2b). The inverse pole figure of the $\{10\bar{1}4\}$ plane, the cleavage plane of calcite, also shows no preferential crystallographic orientation to prevent fracture by cleavage (Fig. 3).

In *N. huttoni*, the *c*-axis of calcite crystals is oriented parallel to the laminae that define the ultrastructure of the secondary layer (Fig. 4). As for *N. anomala*, this orientation results from the growth of individual calcite crystals by screw dislocation. The spread of poles (Fig. 4d) is due to the *c*-axis following the curvature of the laminae (Fig. 4c). There is also continuity in the crystallographic orientation of individual tablets across laminae (Figs. 4b, c) similar to that observed in aragonite nacre (Feng *et al.* 2000; Hou & Feng 2003; Dalbeck *et al.* 2006).

4. Conclusions

In conclusion, the detailed crystallography of craniid brachiopod shells using electron backscatter diffraction (EBSD) has been determined in 3D at high spatial resolution. Here, we

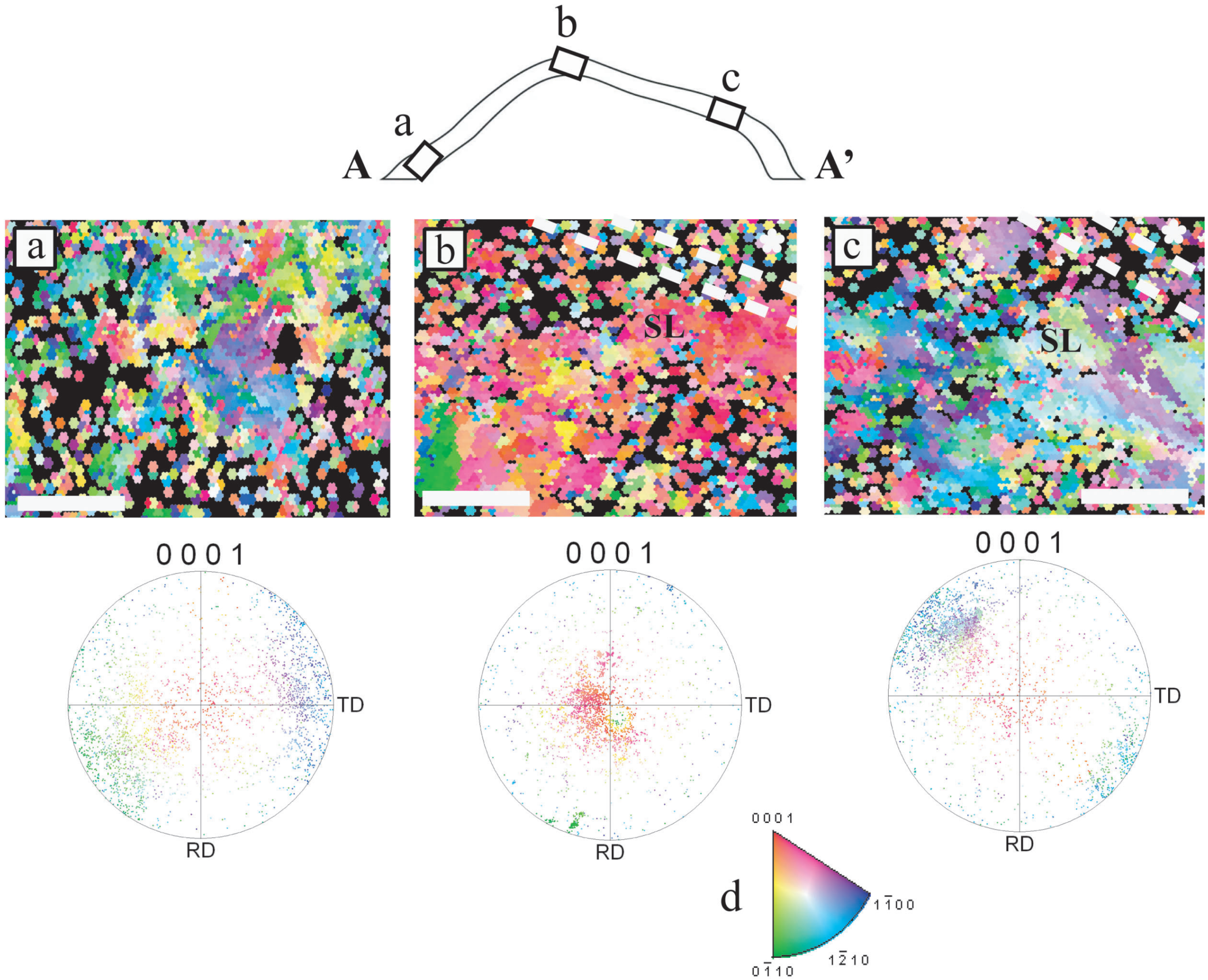

Figure 2 Electron backscatter diffraction (EBSD) maps and pole figures [in normal direction view (ND) to the sample surface in a three axes reference system with indication of the reference (RD) and transverse (TD) directions], indicating crystallographic orientation of calcite crystals in reference to the {0001} plane in a section along the dorsal valve length from posterior to anterior regions (A–A′) of *Novocrania anomala*: (a) posterior region; (b) central region; (c) anterior region; (d) crystallographic key indicating colour coding of crystallographic planes [*indicates shell exterior; primary layer (PL) between dashed lines; secondary layer (SL). Scale bars = 50 µm.

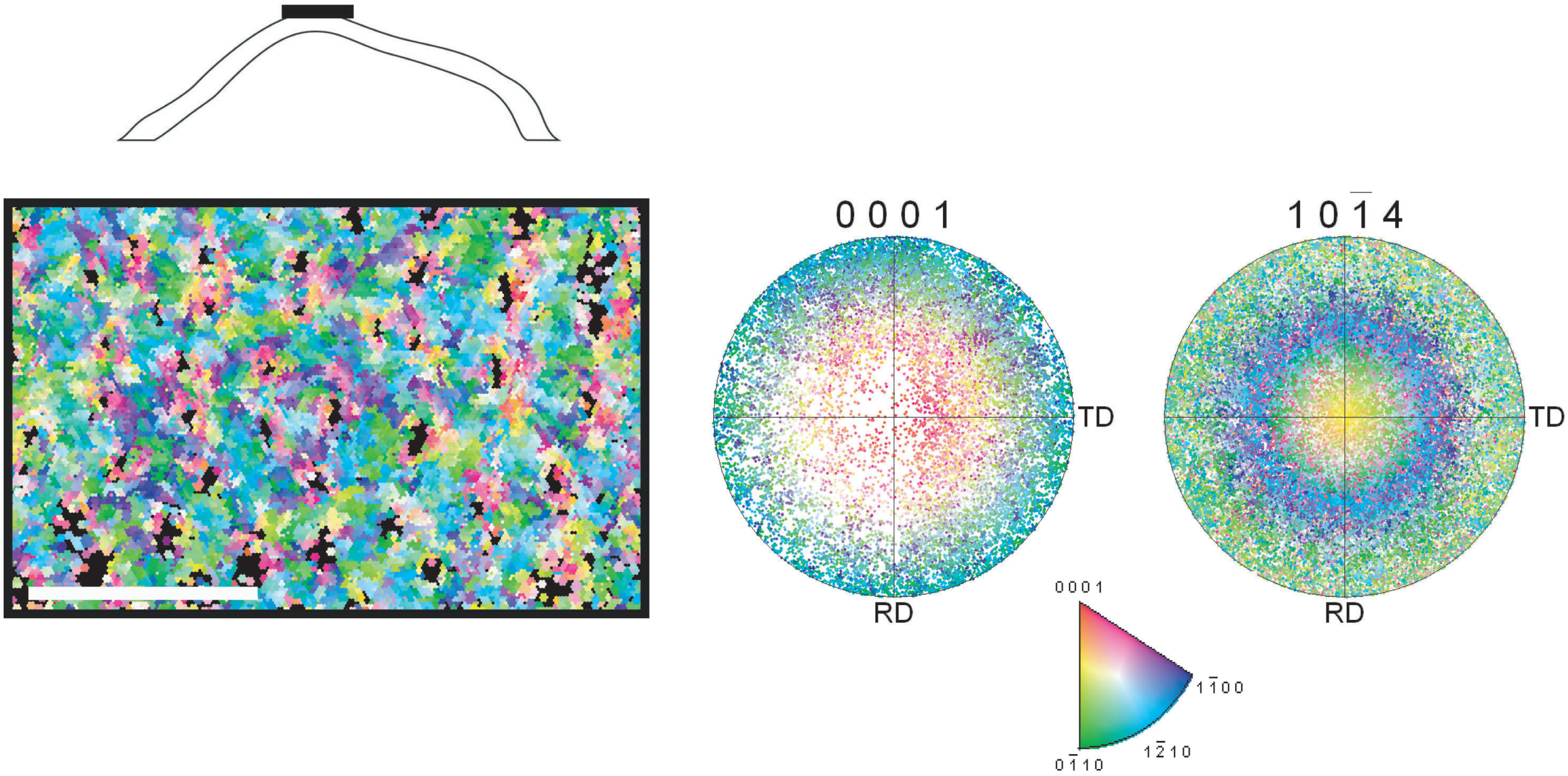

Figure 3 Electron backscatter diffraction (EBSD) map and pole figures [in normal direction view (ND) to the sample surface in a three axes reference system with indication of the reference (RD) and transverse (TD) directions], indicating crystallographic orientation of calcite crystals in reference to the {0001} and $\{10\bar{1}4\}$ planes (see crystallographic key indicating colour coding of crystallographic planes), in a section parallel to the shell surface at the location of the protegulal node of *Novocrania anomala*. Scale bar=100 µm.

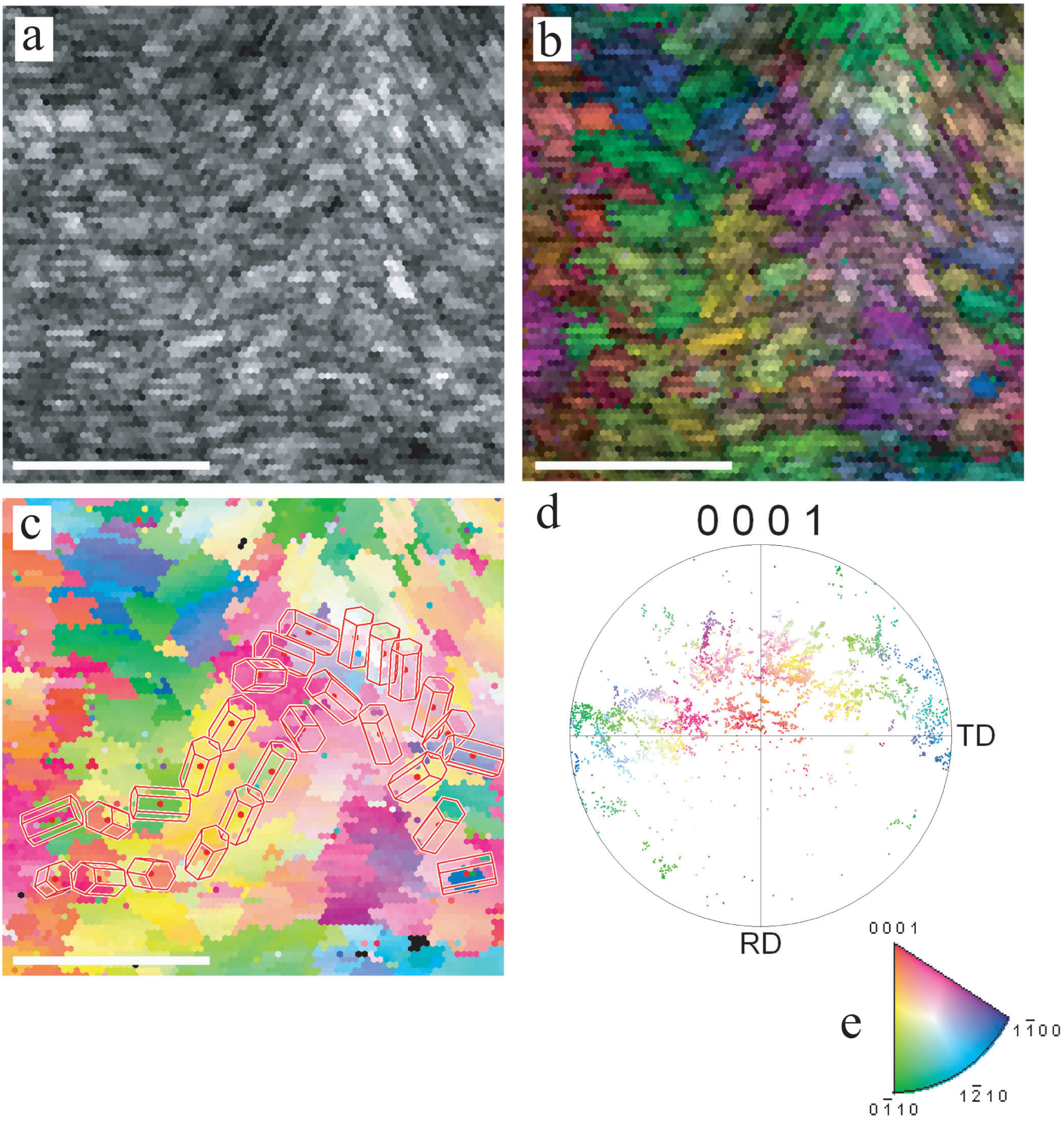

Figure 4 Detailed crystallography by electron backscatter diffraction (EBSD) of the secondary layer in *Novocrania huttoni*: (a) index intensity map, showing detail of the laminae; (b) combination of the diffraction intensity map and the crystallographic orientation maps; (c) crystallographic orientation map, showing the change in orientation of calcite crystals (wire frames) with the laminae; (d) pole figure [in normal direction view (ND) to the sample surface in a three axes reference system with indication of the reference (RD) and transverse (TD) directions] indicating crystallographic orientation of calcite crystals in reference to the {0001} plane; (e) crystallographic key indicating colour coding of crystallographic planes. Scale bars = 30 µm.

show that electron backscatter diffraction (EBSD) is a powerful analytical tool to obtain crystallographic information in the context of shell ultrastructure without having to isolate components. The *c*-axis of calcite crystals in *N. anomala* and *N. huttoni* is oriented parallel to the laminae that define the ultrastructure of the secondary layer. This orientation differs from that of other calcitic brachiopod shells, such as *Mergelia truncata* and *Terebratulina retusa*, which belong to the subphylum Rhynchonelliformea. In the anterior section of adult specimens of these brachiopods, the *c*-axis of calcite crystals is perpendicular to the length of each morphological fibre (Schmahl *et al.* 2004; Cusack *et al.* 2008). It has been suggested that this crystallographic orientation prevents fracture by cleavage along the $\{10\bar{1}4\}$ plane of calcite crystals (Schmahl *et al.* 2004). In contrast, craniid brachiopods do not show a preferential orientation within the $\{10\bar{1}4\}$]plane (Fig. 4) suggesting that fracture by cleavage would be confined to localised crystals.

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6. References

Bassett, M. G. 2000. Craniida. *In* Kaesler, R. L. (ed.) *Treatise on Invertebrate Paleontology Part H Brachiopoda, Revised* **2**: *Linguliformea, Craniiformea and Rhnchonelliformea (part)*, 169–83. Boulder, Colorado and Lawrence, Kansas: Geological Society of America and University of Kansas Press.

Brown, K. 1998. Biomineralisation of the calcitic-shelled inarticulate brachiopod *Neocrania anomala*. Unpublished Ph.D.Thesis, University of Glasgow.

Checa, A. G., Okamoto, T. & Ramírez, J. 2006. Organization pattern of nacre in Pteriidae (Bivalvia: Mollusca) explained by crystal competition. *Proceedings of the Royal Society B* **273**, 1329–37.

Cusack, M., England, J., Parkinson, D., Dalbeck, P., Lee, M., Curry, G. B. & Fallick, A. E. 2008. Oxygen isotope composition, magnesium distribution and crystallography of *Terebratulina retusa*. *In* Harper, D. A. T., Long, S. L. & Nielsen, C. (eds) *Brachiopoda: Fossil and Recent. Fossils and Strata* **54**, 259–67. Chichester: Wiley-Blackwell.

Cusack, M. & Williams, A. 2001. Evolutionary and diagenetic changes in the chemico-structure of the shell of the cranioid brachiopods. *Palaeontology* **44**, 875–903.

Dalbeck, P., England, J., Cusack, M., Lee, M. & Fallick, A. E. 2006. Crystallography and chemistry of the calcium carbonate polymorph switch in *M. edulis* shells. *European Journal of Mineralogy* **18**, 601–9.

Dalbeck, P. & Cusack, M. 2006. Crystallography (electron backscatter diffraction) and chemistry (electron probe microanalysis) of the avian eggshell. *Crystal Growth & Design* **6**, 2558–62.

Feng, Q. L., Cui, F. Z., Pu, G., Wang, R. Z. & Li, H. D. 2000. Crystal orientation, toughening mechanisms and a mimic of nacre. *Materials Science Engineering C* **11**, 19–25.

Halamski, A. T. 2004. *Deliella*, a new Devonian craniid brachiopod. *Neus Jahrbuch für Geologie und Palaeontologie* **3**, 181–92.

Hall, S. R., Taylor, P. D., Davis, S. A. & Mann, S. 2002. Electron diffraction studies of the calcareous skeletons of bryozoans. *Journal of Inorganic Biochemistry* **88**, 410–19.

Hou, W. T. & Feng, Q. L. 2003. Crystal orientation preference and formation mechanism of nacreous layer in mussel. *Journal of Crystal Growth* **258**, 402–8.

Logan, A. & Long, S. L. 2001. Shell morphology and geographical distribution of *Neocrania* (Brachiopoda, Recent) in the eastern North Atlantic and Mediterranean Sea. *In* Brunton C. H. C., Cocks, L. R. M. & Long, S. L. (eds) *Brachiopods Past and Present. The Systematics Association, Special Volume Series* **63**, 71–9.

Müller, O. F. 1776. *Zoologiae Danicae Prodomus, Seu Animalium Daniae et Norvegiae Indigenarum Characteres, namira, et synonmya imprimis popurarium*. xxxii+282 pp. Havinae [=Copenhagen].

Robinson, J. H. & Lee, D. E. 2007. The Recent and Paleogene craniid brachiopod, *Valdiviathyris quenstedti* Helmcke, 1940. *Systematics and Biodiversity* **5**, 123–31.

Schmahl, W. W., Griesshaber, E., Neuser, R., Lenze, A., Job, R. & Brand, U. 2004. The microstructure of the fibrous layer of terebratulide brachiopod shell calcite. *European Journal of Mineralogy* **16**, 693–7.

Schumann, D. 1970. Inäquivalver Schalenbau bei *Crania anomala*. *Lethaia* **3**, 413–21.

Smirnova, T. N. 1997. Shell structure of Upper Jurassic and Lower Cretaceous craniids (Brachiopoda) and its systematic significance. *Journal of Paleontology* **71**, 998–1007.

Taylor, P. D. & Weedon, M. J. 2000. Skeletal ultrastructure and phylogeny of cyclostome bryozoans. *Zoological Journal of the Linnean Society* **128**, 337–99.

Thomson, J. A. 1916. Additions to the knowledge of the Recent and Tertiary Brachiopoda of New Zealand and Australia. *Transactions of the New Zealand National Institute* **48**, 41–7.

Williams, A., Carlson, S. J., Brunton, C. H. C., Holmer, L. E. & Popov, L. E. 1996. A supra-ordinal classification of the Brachiopoda. *Philosophical Transactions of the Royal Society of London B* **351**, 1171–93.

Williams, A., Brunton, C. H. C. & MacKinnon, D. I. 1997. Morphology. *In* Kaesler, R. L. (ed.) *Treatise on Invertebrate Paleontology Part H Brachiopoda, Revised* **1**: *Introduction*, 321–422. Boulder, Colorado and Lawrence, Kansas: Geological Society of America and University of Kansas Press.

Williams, A., Cusack, M. & Brown, K. 1999. Growth of protein-doped rhombohedra in the calcite shell of craniid brachiopods. *Proceedings of the Royal Society of London* B **266**, 1601–7.

Williams, A. & Wright, A. D. 1970. Shell structure of the Craniacea and other calcareous inarticulate Brachiopoda. *Special Papers in Palaeontology* **7**. London: The Palaeontological Association Press.

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