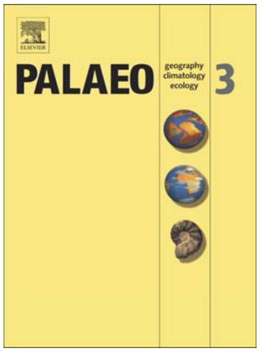
Olempska, E., & Wacey, D. (2016). Ambient inclusion trails in Palaeozoic crustaceans (Phosphatocopina and Ostracoda). Palaeogeography, Palaeoclimatology, Palaeoecology, 441(4), [949-958]. DOI: 10.1016/j.palaeo.2015.10.052



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PII: S0031-0182(15)00623-9
DOI: doi: 10.1016/j.palaeo.2015.10.052
Reference: PALAEO 7552

To appear in: *Palaeogeography, Palaeoclimatology, Palaeoecology*

Received date: 31 July 2015
Revised date: 27 October 2015
Accepted date: 29 October 2015

Please cite this article as: Olempska, Ewa, Wacey, David, Ambient Inclusion Trails in Palaeozoic crustaceans (Phosphatocopina and Ostracoda), *Palaeogeography, Palaeoclimatology, Palaeoecology* (2015), doi: 10.1016/j.palaeo.2015.10.052

Ambient Inclusion Trails in Palaeozoic crustaceans (Phosphatocopina and Ostracoda)

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Hollow microtubular structures with pyrite grains at their termini have been found in phosphatocopines (*Hesslandona toreborgensis* and *Hesslandona* cf. *angustata*) from the late Cambrian of northern Poland, and in the metacopine ostracod *Cytherellina submagna* from the Early Devonian of Podolia, Ukraine.

The Cambrian phosphatocopines are secondarily phosphatised and microtubes (5–20 µm in diameter) are preserved inside the phosphatized bivalved shield, visible on the shield surface as half-open microstructures with pyrite grains at their terminal end. They are visible only where an external coating layer has peeled off. In the Devonian ostracod *C. submagna*, microtubes (*c*. 1–8 µm in diameter) occur within the phosphatized internal mould/steinkern and are visible on the surface of the mould due to the dissolution of the calcite carapace during extraction of the limestone samples.

These microtubular structures are here interpreted as ambient inclusion trails (AITs) due to the presence of terminal pyrite crystals of equivalent diameter to the microtube, polygonal microtube cross-sections, plus longitudinal striations on the microtube walls that record the movement of the migrating angular pyrite crystal. AITs are thought to form when mineral crystals, typically pyrite, are impelled to migrate through a fine-grained mineral matrix under increased fluid/gas pressure.

Our new materials provide the first record of exceptionally preserved AITs occurring in crustaceans and only the third reported occurrence of AITs in the Palaeozoic. The intimate occurrence of AITs with phosphatized crustacean body fossils supports the hypothesis that AITs are likely driven by increases in gas pressure due the decomposition of soft tissue. In the case of *Cytherellina submagna*, our calculations suggest that as little as 20 μg of organic matter decaying within a volume of $0.06mm^3$, encased by an impermeable outer carapace, may have been sufficient to produce a large number (>100) of AITs.

Keywords: Ambient inclusion trails, Phosphatocopina, Ostracoda, Cambrian, Devonian

1. Introduction

Ambient inclusion trails (AITs) are microtubular structures that form during the migration of mineral crystals, often pyrite, through a lithified fine-grained substrate such as chert or apatite. AITs have a geological record extending back to the ~3500 Ma Warrawoona Group of Western Australia (Awramik et al., 1983). Most AITs have been reported from the Archean and Proterozoic from various localities in Australia and North America (Tyler and Barghoorn, 1963; Knoll and Barghoorn, 1974; Awramik et al., 1983; Grey, 1986; Buick, 1990; Xiao and Knoll, 1999; Brasier et al., 2006; Wacey et al., 2006; McLoughlin et al., 2007; Wacey et al., 2008a, b). Their frequent association with organic matter has led to AITs being investigated as possible indicators of early life on Earth (see Wacey et al., 2008b for an overview).

AIT structures have been studied up to now mostly in petrographic thin sections. Observations from thin sections show that most AITs are infilled by fine-grained minerals such as silica (e.g., 1900 Ma old Precambrian Gunflint Chert of Ontario) or iron carbonates (e.g., ~2000 Ma old Biwabik Formation of Minnesota; Tyler and Barghoorn, 1963). Alternatively AITs may remain empty preserving longitudinal striations on their walls, left behind by the migrating angular pyrite crystal, which itself is frequently preserved at the termination of the trail (Xiao and Knoll, 1999).

As currently documented, the fossil record of AITs is more or less continuous throughout the Archean and Proterozoic. The record of Palaeozoic AITs is very sparse, up to now they have been reported only from the Early Cambrian Soltanieh Formation of Iran and from the Middle Devonian of Northern Scotland, where they are preserved within phosphatic fish scales (McLoughlin et al., 2007; Wacey et al., 2008b). AITs remain unknown from rocks of Mesozoic and Cenozoic age.

Below, we describe AITs preserved in specimens of phosphatocopine *Hesslandona toreborgensis* Maas, Waloszek and Müller, 2003 and *Hesslandona* cf. *angustata* Maas, Waloszek and Müller, 2003 from limestone nodules of the uppermost mid-Cambrian to early Furongian (*c*. 502-495 Ma) of the western part of the Baltic Depression, located at the western slope of the East European Craton. The specimens described come from deposits sampled from drill cores of deep wells Hel IG-1, Żarnowiec IG-1 and Dębki 2 located in northern Poland (Fig. 1).

We also describe the phosphatized internal mould of the ostracod *Cytherellina submagna* (Krandijevsky, 1963) containing a high density of unusual exceptionally preserved AITs from ~416 Ma Lochkovian-earliest Pragian of the Ivanye Zolote outcrop in the Early Devonian of Podolia, Ukraine (Fig. 2). Such AITs preserved in crustacean carapaces from Cambrian of Poland and Devonian of Ukraine, are only the third reported finding of these structures in Palaeozoic rocks.

2. Material and methods

More than 2000 phosphatocopine specimens have been recovered from the relatively small Cambrian drill core samples. However, from 300 specimens investigated in SEM, only three of them show AIT structures. More than 40 specimens are preserved with soft-parts. The material of phosphatocopines is preserved as isolated valves and closed or half-open shields (‘carapaces’).

The record of AITs is also limited to a single specimen within the abundant Early Devonian ostracod fauna from Ukraine. The material of the metacopine ostracod *Cytherellina submagna* (Krandijevsky, 1963) is mostly comprised of isolated pyritized or phosphate-coated valves, plus numerous phosphatized internal moulds (steinkerns). Altogether more than 70 phosphatized internal moulds of *C. submagna* were collected. Of these, one specimen preserves soft-parts in the posterior part of the carapace (Olempska et al., 2012) and one additional specimen preserves AIT structures on the surface of the phosphatic internal mould.

The ostracods and phosphatocopines were extracted using 10% acetic acid. Specimens were photographed using a Philips XL 20 Scanning Electron Microscope (SEM) equipped with an energy spectrometer (EDAX-Dx4i, Genesis) in the Institute of Paleobiology, Polish Academy of Sciences, Warsaw. The material is housed in the Institute of Paleobiology, Polish Academy of Sciences, Warsaw (ZPAL Cr.11 for phosphatocopines; ZPAL O.60 for ostracods).

3. Geological setting

3.1. Cambrian

The Cambrian strata from the Polish Baltic region were encountered in deep boreholes drilled in the 1960-70s. The units of the uppermost middle Cambrian and Furongian in northern Poland are the Słowińska and Piaśnica formations (Bednarczyk and Turnau-Morawska, 1975). This succession is stratigraphically condensed and predominantly consists of dark gray to black alum-type shales with interbeds and lenses (stinkstones or *orsten*) of dark gray to black limestones. The total thickness of *Agnostus pisiformis*-*Acerocare* zones ranges from *c.* 4 m to 20 m (Bednarczyk and Turnau-Morawska, 1975; Lendzion, 1983a, b; Bednarczyk, 1979, 1984).

The biostratigraphy of the Polish Cambrian is based on trilobites and traditional biostratigraphy of Scandinavia (see Ahlberg, 2003; Nielsen et al., 2014). However, the samples available from cores are too small for the detailed correlation between Poland and more fossiliferous southernmost Swedish sequences exposed in quarries. However, some samples, e.g., from Dębki 2 borehole are extremely rich in trilobites, phosphatocopines and conodonts.

In dark grey to black mudstones with limestone lenses of the Słowińska Formation (up to *c.*13 m thick), a rich fauna of trilobites and conodonts indicate the *Agnostus pisiformis* (late middle Cambrian) through *Parabolbina spinulosa* Zone of the early Furongian (~ 502-495 Ma). The succeeding Piaśnica Formation (up to *c.*7 m thick) rest on the Słowińska Formation with an erosional hiatus (Fig. 1C). The conodont and trilobite fauna suggest an age of this formation corresponding to the Furongian *Peltura* and *Acerocare* zones (~490 Ma old; Bednarczyk and Turnau-Morawska, 1975; Lendzion, 1976a, 1983a, b; Lendzion and Gunia, 1991; Bednarczyk, 1984; Geyer et al., 2008). The upper part of the middle Cambrian and Furongian deposits represent shelf muds deposited in anoxic conditions (e.g., Modliński and Podhalańska, 2010).

The specimens of phosphatocopine *Hesslandona* described in this paper come from the borehole cores drilled at Hel IG 1 and Żarnowiec IG 1.The deposits of middle Cambrian *Agnostus pisiformis* Zone and the Furongian are 4.8 m thick in the Hel IG 1 borehole, and were drilled at the depths 3047.4 to 3042.6 m. The sequence of *A. pisiformis* Zone (3047.4-3046.8 m) consists of black mudstones with fragments of sandy limestones in its lower part and upwards of mudstones with intercalations of black limestones (Lendzion, 1986). The succeeding sequence of *Olenus*/*Homagnostus obesus* Zone (between depths 3046.8 and 3046.0 m) consists of alum-type shales with scattered lenses of black limestones (Lendzion, 1986). Specimens of *Hesslandona toreborgensis* from the black limestones of the *Olenus*/*H. obesus* Zone from the Hel IG 1 borehole show well-preserved AIT structures on the surface of valves.

The deposits of the *Agnostus pisiformis* Zone and Furongian zones occur in the borehole Żarnowiec IG 1 at depths 2731.4 - 2721.8 m (Lendzion, 1976b). This interval begins with 0.6 m thick conglomerate composed of fine-grained sandstone interbedded with claystones. Upwards, beginning at the depth of 2730.8 m, the dark-gray mudstones and claystones with intercalations of fine-grained sandstone occur. At their top, at the depth of 2727.7 m, there appear lenses of gray limestones with abundant specimens of *Agnostus pisiformis* (Lendzion, 1976b). AIT structures occur in specimens of *Hesslandona* cf. *angustata* from a limestone of *A. pisiformis* Zone of Żarnowiec IG 1 borehole. In the phosphatocopine specimen of ?*Cyclotron* sp. from the *Olenus*/*H. obesus* Zone of the Dębki 2 borehole, the microstructure of the shield has been observed.

3.2. Early Devonian

The specimen of *Cytherellina submagna* containing AITs described below comes from the uppermost part of the marine Lochkovian – earliest Pragian succession (~416 Ma) cropping out in the right escarpment of the River Dniester near the village of Ivanye Zolote, Podolia, Ukraine. The deposit represents the upper part of the Ivanye Formation of the Tyver Group (Małkowski et al., 2009; Voichyshyn, 2011; Drygant and Szaniawski, 2012) and is composed of shallow-marine argillites interbedded with argillaceous carbonates. Upwards these pass gradually into the Old Red Sandstone facies (Fig. 2C). The total thickness of the Formation is about 130 m. The sample of limestone was taken from a layer located about five meters below the Old Red Sandstone deposits.

4. Mode of preservation

4.1. Phosphatocopina

The valves of fossilized phosphatocopines are phosphatic, however, relatively little is known about the original chemical composition of their exoskeleton when alive. This style of preservation has been interpreted as primary phosphate (e.g., Müller, 1964, 1982; Hinz-Schallreuter and Schallreuter, 2009) or as a result of secondary diagenetic phosphatization (Kozur, 1974). Thin-sections normal to the surface of the shield (‘carapace’) of *Hesslandona* and *Falites* specimens from the Cambrian of Sweden show that the phosphatic coating layers are developed at both the outer and inner sides of valves, and probably also on both sides of the inner lamella (Müller, 1979). Between the coating layers there is a banded structure interpreted as representing the original outer lamella, composed of darker chitinous layers and lighter layers containing only a little organic matter (Müller, 1979, figs 7, 9).

The phosphatocopine specimen of ?*Cyclotron* sp. shows in transverse fracture section that the thin apatite layer, approximately 1.4 µm thick has developed at the external and internal surfaces of both valves. An empty space, approximately 2.75 µm in thickness, stretches between the coating layers (Fig. 3K-L). It seems likely that this space has been left by the etching of the poorly mineralized original cuticle of this specimen. The very thin coating layers are also developed at the inner and outer sides of the flexible inner lamella, and the empty space between these layers is less than 1 µm in thickness (Fig. 3L).

As observed with a stereo microscope, in most investigated phosphatocopine specimens from Poland, the external coating layer is slightly transparent, light grey in color and could easily be peeled off (Fig. 3A, B, E, G; indicated by white arrow). In contrast, the layer immediately below and probably representing the secondarily phosphatized original shield, is black. It seems more likely that the thin external layer represents a phosphatic coating, rather than replacement of the original external layer of the cuticle (i.e., epicuticle).

The phosphatic valves of many small Cambrian arthropods such as bradoriids are now interpreted as being unmineralized or poorly mineralized during life, and only phosphatized during early post-mortem diagenesis (Briggs et al., 1993, Wilby and Briggs, 1997; Vannier et al., 2005, Williams et al., 2007).

Similarly, it is most likely that the phosphatocopine shields described here were originally unmineralized or weakly mineralized; after death they were secondarily phosphatized by calcium phosphate. This seems especially likely for the specimens representing earlier growth stages, as is the case for most phosphatocopine specimens from Poland (Fig. 3A-J) and Sweden (Maas et al., 2003). In examples where this layer was not phosphatized, it could be dissolved by the etching process (Fig. 3K-L).

4.2. Ostracoda

The cuticle of the carapace in ostracods is mineralized with low magnesium calcium carbonate in the form of calcite (e.g., Kesling, 1951; Keyser and Walter, 2004). However, in the studied material of *Cytherellina submagna*, the original calcite shell material is not preserved, as it was dissolved during the dissolution of the limestone samples. As a result, only phosphatic internal moulds are preserved. The internal morphological features of the ostracod valves, e.g., well-preserved negative replicas of the muscle scars, pore exits and marginal structures are preserved on the external surface of the moulds. The phosphatic layer of the mould is relatively thin, ranging in thickness from 3–8 µm among 70 specimens. The phosphate layer is composed of very fine apatite crystals. Under an optical microscope this layer appears yellow-brown in color. The external surface of this mould shows fine details of AITs in specimen ZPAL O.60/65 (Figs 4-6).

5. Morphology of AITs

AITs are preserved three-dimensionally as half-open microtubes within phosphatized shields in phosphatocopines (Fig. 3) and within the phosphate layer of an internal mould of a single ostracod specimen (Figs 4-6). It is postulated that the half-open morphology of microtubes is due to the fact that they were formed at the boundary of two different layers. In the ostracod specimen they originate within the phosphatic inner layer at the boundary with the ostracod shell. In phosphatocopines, AITs originate within the phosphatized shield at the boundary with the external apatite coating layer. The ostracod shell and external coating layer in phosphatocopines appear to have been the ‘roofs’ of the microtubes (i.e. a zone through which AITs could not penetrate). After dissolving the ostracod shell and/or peeling off the phosphatocopine external coating layer, the microtubes became visible. The presence of a single pyrite crystal at the termination of each trail is the most characteristic feature of AITs. In the investigated specimens, the pyrite is represented mainly by euhedral crystals (e.g., Fig. 4E), while framboids rarely occur (e.g., Fig. 5I). The trails have random orientations. Inside, the microtubes are largely empty, however, in some of them there are small mineral particles. The diameter of each microtube is almost constant along its entire length, consistent with it being controlled by the size of the propelled pyrite crystal. The walls of microtubes are often covered by longitudinal striations, created by facets of the propelled pyrite crystals, consistent with other reported occurrences of AITs (see Wacey et al., 2008b).

5.1. Phosphatocopina

The majority of the AITs in *Hesslandona toreborgensis* and *Hesslandona* cf. *angustata* are gently curved or sinuous in shape (Fig. 3). They range in diameter from 5 to *c*. 20 µm and are up to 90 µm long. Euhedral pyrite crystals occur at the terminations of almost all microtubes, although some pyrite crystal edges appear to be eroded (Fig. 3G). The fine longitudinal wall striations are preserved in approximately three of the microtubes (Fig. 3G). Small grains of phosphatic material are preserved on the surface of some pyrite crystals (Fig. 3C-D). In some specimens the route of the AIT is marked by a row of perforations, however, towards the termination the trail becomes a half-open microtube (Fig. 3H-I).

5.2. Ostracoda

Microtubular structures are oriented parallel to the surfaces of the phosphatic layer (Figs 4-5). This orientation and the maximum diameter of the microtubes appears to have been controlled by the thickness of the phosphatic layer. The pyrite crystals located at the termination of the microtubes are mostly euhedral (hexahedrons and/or octahedrons and dodecahedrons), though frequently have rounded off edges (Fig. 4). The preservation of pyrite crystals at the terminations of almost all trails suggests that trails were restricted to the apatite layer. It is likely that the thick calcite shell of the ostracod was physically and/or chemically too resistant to be perforated by the pyrite crystals.

Most of the AITs are gently curved or almost straight, irregularly distributed and randomly oriented on the surface of the phosphatic internal mould of the ostracod carapace. Microtubes located within close proximity of each other are often oriented in either the same or in directly opposite directions (Fig. 5). They do not vary systematically in density across different parts of the valve surface (Fig. 4A).

AITs described herein range in diameter from less than 1 µm up to 7.7 µm (Figs 4-5) and are here split into two size classes. Microtubes with diameters of 4.4–5.6 µm and up to 35 µm in length dominate the mould surface (Fig. 4). The thickness of this dominant size class of microtubes is approximately equal to the thickness of the phosphate layer. The majority of them are almost straight or gently curved, but some have sharp turns (Fig. 4I). They have polygonal or rectangular cross sections, and their side walls are rather smooth compared to the Phosphatocopina examples.

The ventral surface of many microtubes is perforated into the ostracod domicilium (space occupied by the soft body and limbs) via several types of openings. A single perforation is situated at the originating end of the microtubes and is often somewhat polygonal or rectangular in shape (Figs 4-5). This perforation mimics the morphology of the terminal pyrite crystal and suggests it was the pyrite crystal’s original location. Sometimes these perforations are slightly rounded in shape. In many cases the walls at the originating ends of tubes are also replicas of the pyrite shape. This perforation suggests that pyrite crystals were initially formed at the internal surface of the phosphatic layer close to the decaying soft tissue of the animal that would have been housed in the domicilium.

Additional irregular, circular or elliptical perforations into the ostracod domicilium are developed along the ventral surface of many microtubes. Often, the ventral surface of a microtube is only fragmentarily preserved with ragged margins (Fig. 4H). These perforations occur in microtubes of larger size, with diameters comparable to the thickness of the phosphatic layer. In AIT where the diameter of the microtubes is rather smaller than the thickness of the phosphate layer, we observe that the pyrite crystal initially started to move perpendicularly to the phosphate layer, and then it changed its direction to move parallel with the ostracod shell (Figs 4K, 6).

The angular shape of microtube originating ends observed in *Cytherellina submagna* are similar to those illustrated by Wacey et al. (2008b, fig. 1b) from the Kangaroo Caves Formation and to those illustrated by Knoll and Barghoorn (1974, fig. 1A) from the Gunflint Formation.

AITs of the smaller size class are 1–1.2 µm wide and usually short. They often occur arranged in radiating or irregular dense clusters, however, single small AIT also occur (Fig. 5). Perforations of the ventral microtube wall, similar to that described above, can also occur at the originating ends of these small tubes. Additional perforations aligned along the ventral tube wall have not been observed, likely due to the narrowness of these microtubes, so they did not reach the lower surface of the phosphate coating layer. The pyrite crystals preserved in terminations of these microtubes are mostly octahedrons. In dense clusters, many of the pyrite grains are slightly displaced being preserved close to, in addition to within, the microtubes (e.g., Fig. 5D). The clusters of AITs are distributed irregularly on the surface of the ostracod mould. These AITs are somewhat similar to those with a ‘starburst’ distribution pattern described by Knoll and Barghoorn (1974, fig. 2) from the Gunflint Formation, Ontario, but differ in the occurrence of randomly oriented trails.

Microtubes with a diameter ranging from 4 to 7.7 µm, a maximum 10 µm length and terminated with framboidal rather than euhedral pyrite, occur only rarely. In some of them, longitudinal striations along the walls are well preserved (Fig. 5I).

6. Discussion

6.1. Association of AITs with phosphatization

The geological record of AITs preserved in apatite (calcium phosphate) dates back to the late Neoproterozoic Doushantuo Formation (550-580 Ma) of South China (Xiao and Knoll, 1999, fig. 8h-k). These occur on the surface of phosphatized globular microfossils and in surrounding phosphorites taking the form of half-open microtubes with continuous longitudinal striae on their walls. Furthermore, all AITs currently known from Palaeozoic rocks occur in phosphate or phosphatized fossils (see Wacey et al., 2008b).

Cambrian Orsten-type phosphatization associated with fossilized soft tissues is geographically widespread (see Müller and Walossek, 1985; Waloszek, 2003; Maas et al., 2003, 2006; Dong et al., 2005; Shen et al., 2013 for reviews). This type of phosphatization is usually restricted to fossils less than 2 mm in size. Therefore, most of the arthropods preserved in this way are likely representatives of larval ontogenetic stages. These small arthropods are preserved by early diagenetic encrustation/impregnation of the external layers of their weakly mineralized cuticle (Müller and Walossek, 1985). A protected anaerobic microenvironment is favourable for rapid phosphatization (Briggs et al., 1993; Briggs and Kear, 1993a, b; Briggs and Kear, 1994a, b; Briggs and Wilby, 1996; Briggs, 2003). The exact source of phosphorus in Orsten-type phosphatization remains unknown, however, decaying remains of animals, fecal pellets or precipitation of P from the water column have all been suggested (Briggs and Kear, 1994a, b; Maeda et al., 2011; Crevelling et al., 2014).

In contrast to the Cambrian, phosphatization is not a very common phenomenon during the Devonian (e.g., Trinajstic et al., 2007). However, the phosphatization of small microfossils with three-dimensional preservation of soft-tissue has previously been reported from the Lochkovian of Podolia (e.g., in the ostracod *Cytherellina submagna* (Olempska et al., 2012), and in boring ctenostome bryozoans (Olempska, 2012)).

It is not unexpected that pyrite crystals are associated with phosphatized material, since both may be bacterially induced in similar anaerobic microenvironments (e.g., Briggs et al., 1993). Pyrite nuclei can be produced within decaying soft tissues as a result of the reaction between dissolved iron, and sulphide produced from the metabolic activity of sulfate-reducing bacteria (SRB; see Briggs 2003 and references therein). Phosphatization is also favoured by low pH due to build up of CO_2 in enclosed spaces (Briggs and Kear, 1993b; Briggs et al., 1993); this same build up of CO_2 may have helped propel the newly formed pyrite crystals, forming the AIT.

In our phosphatocopine and ostracod specimens, most AITs contain euhedral pyrite crystals, with only rare examples of framboidal pyrite. In general, the morphology of the pyrite is thought to reflect the rate of supply of H_2S and hence the decay rate of the organic material being consumed by SRB (Brett and Baird, 1986; Butler and Rickard, 2000; Gabbott et al., 2004). Euhedral pyrite morphology indicates rather slow supply of H_2S from recalcitrant organic material (hence slow growth on fewer nucleation sites), whereas framboidal pyrite indicates more rapid supply of H_2S from more labile organic material (hence rapid growth on plentiful nucleation sites) (Gabbott et al., 2004).

The morphology of AITs in both the phosphatocopine shields and ostracod carapace are very similar. However, the phosphatization pathway is rather different in these two organisms. In the ostracod *C. submagna*, AITs occur inside the internal apatite coating, which had originally been covered by thick calcite carapace. In contrast, the phosphatization pathway in extinct phosphatocopines is more complicated and still not fully understood because the original composition of their shield remains unknown. Nonetheless, early and rapid mineralization of phosphatocopines is obvious from the exceptional preservation of their shields and soft tissues (Maas et al., 2003, 2006). It seems likely, that in phosphatocopines, AITs were formed inside the phosphatized shield.

6.2. Potential AIT formation mechanisms

AITs have previously been interpreted in terms of pyrite (or other crystal) being propelled by high gas/fluid pressure resulting from decay of organic matter (Knoll and Barghoorn, 1974; Xiao and Knoll, 1999). Gases produced from the decay of organic material are richer in methane, carbon dioxide and hydrogen sulfide than the atmosphere. Such a build up of gases creates upward-rising pressure within a decaying body (e.g., Dornbos et al., 2007). As the volume of these gases, probably combined with increasing temperatures, within the decaying body increases, fluids and gases search for a way to escape from orifices. If there are no natural orifices, it seems likely that high pressure may set the pyrite crystals in motion (Fig. 6).

In phosphatocopines, decaying gases often accumulate between the shield and the flexible inner lamella (Fig. 3J). Indeed, specimens with a strongly swollen inner lamellae are often preserved in the fossil record (e.g., Müller, 1979, fig. 15; Maas et al., 2003, pl.13:D). The exact timing of AIT formation remains difficult to deduce. The preservation of striations on most microtube walls, plus the perforations on ventral walls suggest that apatite was not in the form of a gel. However, it seems likely that the creation of AITs must at least slightly predate the final lithification of the apatite layer. The sharp turns in many AITs are also intriguing and we suggest that these may be caused by some sort of 'ballistic ricochet' at the moment when the pyrite collided with the uppermost more resistant external coating layer.

In *Cythrellina submagna*, the perforations of ventral walls of microtubes at their originating ends have been left by pyrite crystals when they started to move (Fig. 6). There may then have been some re-precipitation of dissolved calcium and phosphate ions around the edges of the microtubes, e.g., in the narrow pale area surrounding the microtubes on the surface of the phosphate coating (e.g., Figs 4F, 5A). EDAX analysis of this area confirms that it is comprised of calcium phosphate. It also seems possible that an efflux of dissolved calcium phosphate through the perforations to the ostracod domicilium may also occur. Re-precipitation of minerals in and around AIT appears to be a common occurrence, as for example reported by Knoll and Barghoorn (1974), in AITs developed in the 1878 Ma Gunflint chert, where dissolving silica was re-precipitated in trails, behind the moving pyrite grain.

In contrast to the other known AIT occurrences, the internal mould of *Cytherellina submagna* described herein, allows the possibility to estimate the approximate volume of its domicilial cavity and approximate weight of the soft body. The domicilial volume of *C. submagna* has been calculated using the formula for calculating the volume of the geometrically similar ellipsoid. The approximate calculated volume is $0.057 mm^3$, based on carapace length (1.14 mm), height (0.4 mm) and width (0.24 mm), measured using the dimensions of the phosphate coating.

The weight of the soft parts of *C. submagna* was calculated by comparing them with adult specimens of podocopan *Cyprideis torosa* (Jones), that have a similar size of carapace, measured by Herman and Heip (1982). The mean dry weight of soft parts for females of

Cyprideis torosa was determined as 19.81 µg, and 19.88 µg for males (Herman and Heip, 1982).

This suggests that a source of organic matter with an approximate dry weight of 20 µg, inside a closed space of an approximate volume of 0.06 mm^3, could produce sufficient gas pressure (and temperature increase?) to dissolve calcium phosphate and produce over 70 single AITs, plus several dense clusters of AITs, on the mould surface of the investigated single (right) ostracod valve. It seems likely that the number of AITs developed on the left valve would have been similar. However, we acknowledge that this is a very simplified calculation and requires further testing in the laboratory.

It is difficult to compare our Polish and Ukrainian material directly with those from other phosphatized fossils such as those from the ~570 Ma Doushantuo Formation (fig. 8 of Xiao and Knoll, 1999) and the ~390 Ma Achanarras Limestone (fig. 14 of Wacey et al., 2008b). The AITs from the Doushantuo Formation are preserved on both external surfaces and internally within globular embryo-like structures (Xiao and Knoll, 1999), while the Achanarras examples are preserved within fish scales. One thing that all examples likely had in common was the presence of a more resistant external coating (e.g., fig. 5.1-5.4 of Xiao and Knoll, 2000), or encasement by a less permeable mineral, allowing gas pressure to build to a sufficient state to enable pyrite crystal propulsion.

6.3. Interpretation of microtubular traces in the fossil record

Several microtubular structures discovered within Cambrian bradoriids, phosphatocopines and brachiopod valves have been interpreted as animal trace fossils (i.e., burrows; Hinz-Schallreuter, 1998; Stockfors and Peel, 2005; Zhang and Pratt, 2008), however, these may all be more parsimoniously interpreted as AITs.

Hinz-Schallreuter (1998) described hollow-tunnels on the surface of phosphatocopine *Vestrogothia longispinosa* and *Hesslandona abdominalis* from the Cambrian of Gotland, and interpreted them to have been produced by thallophytes. Stockfors and Peel (2005, fig. 2a-c, fig. 3h; fig. 4a) illustrated long striated tubules in phosphatic brachiopods from the late Middle Cambrian Holm Dal Formation of North Greenland, which they considered to be euendolithic borings produced by Cyanobacteria. These structures are almost identical to AITs described here, however, terminal pyrite crystals are not visible, probably due to their incomplete preservation.

Further structures similar to AITs preserved in bradoriid and phosphatocopine carapaces from the Early Cambrian Yu'anshan and Shuijingtuo formations of South China have been described by Zhang and Pratt (2008) as borings made by a heterotrophic organism and assigned to the ichnotaxon *Microptychoites fuquaensis* Dong (cf. Dong et al., 1984). However, the walls of these tunnels bear distinct longitudinal striations characteristic of AITs. Terminal pyrite crystals are rarely preserved in these structures, instead circular objects that terminate the ends of the microtubes are composed of calcium carbonate.

Microtubes described by Conway Morris and Bengston (1994, fig. 6:9-6.13) as predatory borings in phosphatized inarticulate brachiopod *Linnarssonia* sp. B from the Middle Cambrian of Sweden also have longitudinal striations on their walls and have been reinterpreted by Xiao and Knoll (1999) as non-biological in origin (AIT structures).

7. Conclusions

Ambient inclusion trails (AITs), comprising hollow microtubular structures with pyrite grains at their terminal ends, have been discovered for the first time in Palaeozoic crustaceans from Poland and Ukraine.

These AITs occur in the phosphatized shields of Cambrian phosphatocopines from Poland (*Hesslandona toreborgensis* Maas, Waloszek and Müller, 2003 and *Hesslandona* cf. *angustat*a Maas, Waloszek and Müller, 2003) and the phosphatic internal moulds of the Devonian ostracod *Cytherellina submagna* (Krandijevsky, 1963) from western Ukraine.

The occurrence of AITs in crustaceans, adjacent to their domicilial cavities where organic material would have decayed during diagenesis, supports the hypothesis that a biological component (e.g., decaying organic matter) is key to AIT formation. This builds on prior evidence from AITs in fish scales and fossil embryos suggesting organic decay produces sufficient fluid/gas pressure to propel small pyrite grains through a phosphate matrix. Our calculations suggest that as little as 20 μg of organic matter decaying within a volume of $0.06mm^3$, encased by an impermeable outer carapace, may have been sufficient to produce in excess of 100 AITs.

AITs are mostly a Precambrian phenomenon with only rare occurrences reported from Palaeozoic rocks. This is probably due to the fact that environments favouring AIT formation were more prevalent during significant portions of the Precambrian. Environmental drivers favouring AIT formation in the Precambrian included higher oceanic Si concentrations (Maliva et al., 2005) encouraging widespread and rapid chert formation, larger volumes of anoxic and ferruginous habitats (Holland 1984; Poulton and Canfield, 2011) encouraging the formation of pyrite via microbial sulphate reduction, plus periods of major phosphate deposition (Papineau, 2010).

Acknowledgements

E.O. thanks Hubert Szaniawski (Institute of Paleobiology Polish Academy of Sciences) for placing the samples from Hel IG 1 and Żarnowiec IG 1 at my disposal and Wiesław

Bednarczyk (Institute of Geological Sciences, PAS) for samples from Dębki 2 borehole. E.O. also expresses gratitude to Anna Żylińska from the Department of Geology, University of Warsaw, for giving information regarding Cambrian trilobite stratigraphy. D.W. acknowledges financial support from the Australian Research Council and the European Commission. We are especially grateful to the reviewer for constructive remarks.

References

Ahlberg, P. 2003. Trilobites and intercontinental tie points in the Upper Cambrian of Scandinavia. Geologica Acta 1, 127-134.

Awramik, S.M., Schopf, J.W., Walter, M.R. 1983. Filamentous fossil bacteria from the Archean of Western Australia. Precambrian Res. 20, 357-374.

Bednarczyk, W. 1979. On the occurrence of Bradorina Raymond 1935 ostracods in the Upper Cambrian of NW Poland. Bull. Acad. Pol. Sci.-Earth 26, 215-219.

Bednarczyk, W. 1984. Biostratigraphy of the Cambrian deposits in the Łeba area. Acta Geol. Pol. 34, 95-110.

Bednarczyk, W., Turnau-Morawska, M. 1975. Cambrian formations in the Łeba area (northern Poland). Acta Geol. Pol. 25, 537-566 (In Polish with English summary).

Brasier, M., McLoughlin, N., Green, O., Wacey, D. 2006. A fresh look at the fossil evidence for early Archaean cellular life. Philos. T. Roy. Soc. B 361, 887-902.

Brett, C.E., Baird, G.C. 1986. Comparative taphonomy: a key to paleoenvironmental interpretation based of fossil preservation. Palaios 1, 207-227.

Briggs, D.E.G. 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. Annu. Rev. Earth Pl. Sc. 31, 275-301.

Briggs, D.E.G., Kear, A.J. 1993a. Decay and preservation of polychaetes: taphonomic thresholds in soft-bodied organisms. Paleobiology 19, 107-135.

Briggs, D.E.G., Kear, A.J. 1993b. Fossilization of soft tissue in the laboratory. Science 259, 1439-1442.

Briggs, D.E.G., Kear, A.J. 1994a. Decay of *Branchiostoma*: implications for soft-tissue preservation in conodonts and other primitive chordates. Lethaia 26, 275-287.

Briggs, D.E.G., Kear, A.J. 1994b. Decay and mineralization of shrimps. Palaios 9, 431-456.

Briggs, D.E.G., Wilby, P.R. 1996. The role of the calcium carbonate-calcium phosphate switch in the mineralization of soft-bodied fossils. J. Geol. Soc. London 153, 665-668.

Briggs, D.E.G., Kear, A.J., Martill, D.M., Wilby, P.R. 1993. Phosphatization of soft-tissue in experiments and fossils. J. Geol. Soc. London. 150, 1035-1038.

Buick, R. 1990. Microfossil recognition in Archean rocks: an appraisal of spheroids and filaments from a 3500 M.Y. old chert-barite unit at North Pole, Western Australia. Palaios 5, 441-459.

Butler, I.B., Rickard, D. 2000. Framboidal pyrite formation via the oxidation of iron (II) monosulfide by hydrogen sulphide. Geochim. Cosmochim. Acta 64, 2665-2672.

Conway Morris, S., Bengston, S. 1994. Cambrian predators: possible evidence from boreholes. J. Paleontol. 68, 1-23.

Creveling, J.R., Johnston, D.T., Poulton, S.W., Kotrc, B., März, C., Schrag, D.P., Knoll, A.H. 2014. Phosphorus sources for phosphatic Cambrian carbonates. Geol. Soc. Am. Bull. 126, 145-163.

Dong, Y., Ji, Q., Wang, S. 1984. The discovery of microscopic trace fossils in the Sinian Doushantou Formation in southwestern China. Sci. Geol. Sin. 1984, 346-347.

Dong, X.P., Donoghue, P.C.J., Liu, Z., Liu, J., Peng. F. 2005. The fossils of Orsten-type preservation from Middle and Upper Cambrian in Hunan, China. Chinese Sci. Bull. 50, 1352-1357.

Dornbos, S.Q., Noffke, N., Hagadorn, J.W. 2007. Mat-decay features. In: Schieber, J., Bose, P.K., Eriksson, P.G., Banerjee, S., Sakar, S., Altermann, W., Catuneau, O. (Eds.), Atlas of microbial mat features within the clastic record. Elsevier, p.106-110.

Drygant, D., Szaniawski, H. 2012. Lochkovian conodonts from Podolia, Ukraine, and their stratigraphic significance. Acta Palaeontol. Pol. 57, 833-861.

Gabbott, S.E., Hou, X.-G., Norry, M.J., Siveter, David J. 2004. Preservation of Early Cambrian animals of the Chengjiang biota. Geology 32, 901-904.

Geyer, G., Elicki, O., Fatka, O., Żylińska, A. 2008. Cambrian. In: McCann, T. (Ed.), The Geology of Central Europe. Volume 1: Precambrian and Palaeozoic. Geol. Soc. Lond.,155-202.

Grey, K. 1986. Problematic microstructures in the Proterozoic Discovery Chert, Bangemall Group, Western Australia. Ambient grains or microfossils? Geol. Surv. Western Australia Prof. Papers for 1984, Perth, 22-31.

Herman, P.M.J., Heip, C. 1982. Growth and respiration of *Cyprideis torosa* Jones 1850 (Crustacea Ostracoda). Oecologia 54, 300-303.

Hinz-Schallreuter, I. 1998. Population structure, life strategies and systematic of phosphatocope ostracods from the Middle Cambrian of Bornholm. Mitt. Mus. Nat.kd. Berl. Geowiss. Reihe 1, 103-134.

Hinz-Schallreuter, I., Schallreuter, R. 2009. Phylogeny of Phosphatocopa. Mem. Assoc. Australas. Palaeontol. 37, 151-164.

Holland, H.D. 1984. The Chemical Evolution of the Atmosphere and Oceans. Princeton University Press, NJ, 582pp.

Kesling, R.V. 1951. Terminology of ostracod carapaces. Contr. Mus. Pal. Univ. Michigan 9, 93-171.

Keyser, D., Walter, R. 2004. Calcification in ostracodes. Rev. Esp. Micropal. 36, 1-11.

Knoll, A.H., Barghoorn, E.S. 1974. Ambient pyrite in Precambrian chert: new evidence and a theory. P. Nat. Acad. Sci. USA 71, 2329-2331.

Kozur, H. 1974. Die Bedeutung der Bradoriida als Vorlaeufer der postkambrischen Ostrakoden. Z. Geol. Wissenschaft. 2, 823-830.

Krandijevsky, V. S. 1963 The ostracod fauna of Silurian localities of Podolia. AN. URSR, Inst. Geol. Nauk, Kiev pp. 1-176 (In Ukrainian).

Lendzion, K. 1976a. Stratigraphy of the Cambrian in the western part of the Peri-Baltic Syneclise. Biul. Inst. Geol. 270, 59-84 (In Polish with English summary).

Lendzion, K. 1976b. Profil litologiczno-stratygraficzny. Wyniki badań stratygraficznych i litologicznych. Kambr. In: Witkowski, A. (Ed.), Żarnowiec IG 1. Prof. Głęb. Otw. Wiertn. Inst. Geol. 32, 36-42, 46-50 (In Polish).

Lendzion, K. 1983a. The development of the Cambrian platform deposits in Poland. Pr. Inst. Geol. 105, 1-55 (In Polish with English summary).

Lendzion, K. 1983b. Biostratigraphy of the Cambrian deposits in the Polish part of the East-European Platform. Kwart. Geol. 27, 669-694 (In Polish with English summary).

Lendzion, K. 1986. Profil litologiczno-stratygraficzny. Wyniki badań stratygraficznych i litologicznych. Kambr, wend górny. In: Witkowski, A. (Ed.), Hel IG 1. Prof. Głęb. Otw. Wiertn. Inst. Geol. 63, 44-49, 51-56 (In Polish).

Lendzion, K., Gunia, T. 1991. Biostratigraphic correlation of the Riphean, Vendian and Cambrian of Poland and other areas. In: Pajchlowa, M. (Ed.), Geology of Poland, vol. 3, Atlas of guide and characteristic fossils, part 1a Paleozoic (including Upper Proterozoic), 23-29. Publishing House Wydawnictwa Geologiczne, Warszawa.

Maeda, H., Tanaka, G., Shimobayashi, N., Ohno, T., Matsuoka, H. 2011. Cambrian Orsten Lagerstätte from the Alum Shale Formation: fecal pellets as a probable source of phosphorus preservation. Palaios 26, 225-231.

Maliva, R.G., Knoll, A.H., Simonson, B.M. 2005. Secular change in the Precambrian silica cycle: Insights from chert petrology. Bull. Geol. Soc. Am. 117, 835-845.

Małkowski, K., Racki, G., Drygant, D., Szaniawski, H. 2009. Carbon isotope stratigraphy across the Silurian-Devonian transition in Podolia, Ukraine: evidence for a global biogeochemical perturbation. Geol. Mag.146, 652-674.

Maas, A., Waloszek, D., Müller, K.J. 2003. Morphology, ontogeny and phylogeny of the Phosphatocopina (Crustacea) from the Upper Cambrian “Orsten” of Sweden. Fossils Strata 49, 1-238.

Maas, A., Braun, A., Dong, X.P., Donoghue, P.C.J., Müller, K.J., Olempska, E., Repetski, J.E., Siveter, David J., Stein, M., Waloszek, D. 2006. The ‘Orsten’ — more than a Cambrian Konservat-Lagerstätte yielding exceptional preservation. Palaeoworld 15, 266-282.

McLoughlin, N., Brasier,M.D., Wacey, D., Green, O.R., Perry, R.S. 2007. On biogenicity criteria for endolithic microborings on early Earth and beyond. Astrobiology 7, 10-26.

Modliński, Z., Podhalańska, T. 2010. Outline of the lithology and depositional features of the lower Paleozoic strata in the Polish part of the Baltic region. Geol. Quart. 54, 109-121.

Müller, K.J. 1964. Ostracoda (Bradorina) mit phosphatischen Gehäusen aus dem Oberkambrium von Schweden. N. Jb. Geol. Paläont. Abh. 121, 1-46.

Müller, K.J. 1979. Phosphatocopine ostracodes with preserved appendages from the Upper Cambrian of Sweden. Lethaia 12, 1-27.

Müller, K. J. 1982. *Hesslandona unisulcata* sp. nov. with phosphatised appendages from Upper Cambrian “Orsten” of Sweden. In: Bate, R.H., Robinson, E., Sheppard, L.M.

(Eds.), Fossil and Recent Ostracods. British Micro-Palaeontological Society. Ellis Horwood Lm. Chichester. 276-304.

Müller, K.J., Walossek, D. 1985. A remarkable arthropod fauna from the Upper Cambrian “Orsten” of Sweden. Trans. R. Soc. Edinburgh 76, 161-172.

Nielsen, A.T., Weidner, T., Terfelt, F., Høyberget, M. 2014. Upper Cambrian (Furongian) biostratigraphy in Scandinavia revisited: definition of superzones. GFF, doi: http://dx.doi.org/10.1080/11035897.2013.878748.

Olempska, E., 2012. Exceptional soft-tissue preservation in boring ctenostome bryozoans and associated “fungal” borings from the Early Devonian of Podolia, Ukraine. Acta Palaeontol. Pol. 57, 925-940.

Olempska, E., Horne, D.J., Szaniawski, H., 2012. First record of preserved soft parts in a Palaeozoic podocopid (Metacopina) ostracod, *Cyrherellina submagna*: phylogenetic implications. Proc. R. Soc. B 279, 564-570.

Papineau, D. 2010. Global biogeochemical changes at both ends of the Proterozoic: Insights from Phosphorites. Astrobiol. 10, 165-181.

Poulton, S.W., Canfield, D.E. 2011. Ferruginous conditions: a dominant feature of the ocean through Earth’s history. Elements 7, 107-112.

Schopf, J.W. 1993. A microfossils of the Early Archean Apex Chert: new evidence of the antiquity of life. Science 260, 640-646.

Shen, C., Pratt, B.R., Lan, T., Hou, J.B., Chen, L., Hao, B.Q., Zhang, X.G. 2013. The search for Orsten-type fossils in southern China. Palaeoworld 22, 1-9.

Stockfors, M., Peel, J.S. 2005. Euendoliths and cryptoendoliths within late Middle Cambrian brachiopod shells from North Greenland. GFF 127, 187-194.

Trinajstic, K. Marshall, C., Long, J., Bifield, K. 2007. Exceptional preservation of nerve and muscle tissues in Late Devonian placoderm fish and their evolutionary implications. Biology Letters 3, 197-200.

Tyler, S.A., Barghoorn, E.S. 1963. Ambient pyrite grains in Precambrian cherts. Am. J. Sci. 261, 424-432.

Vannier, J., Williams, M., Alvaro, J.J., Vizcaïno, D., Monceret, S., Monceret, E. 2005. New Early Cambrian bivalve arthropods from southern France. Geolog. Mag. 142, 751-763.

Voichyshyn, V. 2011. The Early Devonian armoured agnathans of Podolia, Ukraine. Palaeontol. Pol. 66, 1-211.

Wacey, D., McLoughlin, N., Green, O.R., Parnell, J., Stoakes, C.A., Brasier, M.D. 2006. The ~ 3.4 billion-year-old Strelley Pool Sandstone: a new window into early life on Earth. Int. J. Astrobiol. 5, 333-342.

Wacey, D., Kilburn, M.R., McLoughlin, N., Parnell, J., Stoakes, C.A., Grovenor, C.R.M., Brasier, M.D. 2008a. Use of NanoSIMS in the search for early life on Earth: ambient inclusion trails in a *c.* 3400 Ma sandstone. J. Geol. Soc. London. 165, 43-53.

Wacey, D., Kilburn, M., Stoakes, C., Aggleton, H., Brasier, M. 2008b. Ambient Inclusion Trails: their recognition, age range and applicability to early life on Earth. In: Dilek, Y. et al. (Eds.), Links Between Geological Processes, Microbial Activities & Evolution of Lve. Springer Science+Business Media B.V., 113-134.

Waloszek, D. 2003. The ‘Orsten’ window – a three-dimensionally preserved Upper Cambrian meiofauna and its contribution to our understanding of the evolution of Arthropoda. Paleontol. Res. 7, 71-88.

Wilby, P.R., Briggs, D.E.G. 1997. Taxonomic trends in the resolution of detail preserved in fossil phosphatised soft tissues. Geobios, M.S. 20, 493-502.

Williams, M., Siveter, David J., Popov, L.E., Vannier, J.M.C. 2007. Biogeography and affinities of the bradoriid arthropods: cosmopolitan microbenthos of the Cambrian seas. Palaeogeogr. Palaeoclimatol. Palaeoecol. 248, 202-232.

Xiao, S., Knoll, A.H. 1999. Fossil preservation in the Neoproterozoic Doushantuo phosphorite Lagerstätte, South China. Lethaia 32, 219-240.

Xiao, S., Knoll, A.H. 2000. Phosphatized animal embryos from the Neoproterozoic Doushantuo Formation at Weng'an: Guizhou: South China. J. Paleontol. 74, 767-788.

Zhang, X., Pratt, B.R. 2008. Microborings in Early Cambrian phosphatic and phosphatized fossils. Palaeogeogr. Palaeoclimatol. Palaeoecol. 267, 185-195.

Figure captions

Fig. 1. Geographic position of the studied Cambrian sections in Poland. (A), Map of the Polish part of the Baltic region: 1 - Caledonian deformation front; 2 - Post-Caledonian deformation front; 3 - Rift zones; 4 - Trans-European Suture Zone. (B), Map of northern Poland showing location of the sections described in this paper from Hel IG 1, Żarnowiec IG 1, and Dębki 2 boreholes. (C), General stratigraphy of the middle Cambrian and Furongian strata in northern Poland. Marked area indicates stratigraphic gap (erosion and/or no deposition).

Fig. 2. (A), Geographic position of the studied Devonian section in Podolia, Ukraine. (B), Location of Ivanye Zolote outcrop in the vicinity of Dniester valley. (C), General Lower Devonian stratigraphy of the Podolia region (modified from Drygant and Szaniawski, 2012).

Fig. 3. SEM photographs showing microtubular AIT structures from the Cambrian phosphatocopines of northern Poland. (A-E), *Hesslandona toreborgensis* Maas, Waloszek and

Müller, 2003, early Furongian, Hel IG 1 borehole section; (A-B), Left lateral and oblique ventral views with white arrows showing partly preserved external apatite layer; (C-E), Close up of external surface showing pyrite crystals at the termination of microtubes, with black arrow in (D) indicating remnants of calcium phosphate substance on the surface of pyrite, and with white arrow in (E) indicating partly preserved external coating layer, (specimen number ZPAL Cr. 11/ph014); (F-G), *Hesslandona toreborgensis* Maas, Waloszek and Müller, 2003, early Furongian, Hel IG 1 borehole section; (F), left lateral view; (G), close up showing AIT structures, black arrow indicate longitudinal striation on microtube walls, white arrow indicating partly preserved external coating layer (specimen number ZPAL Cr.11/ph034). (H-J), *Hesslandona* cf. *angustata* Maas, Waloszek and Müller, 2003, early Furongian, Żarnowiec IG 1 borehole section; (H), Dorsal view; (I), Close up of external surface showing AITs, (specimen number ZPAL Cr. 11/ph026); (J), Internal view showing inflation of the inner lamella by gas originating from decaying soft tissue (black arrows); (specimen number ZPAL Cr. 11/ph216). (K-L), ?*Cyclotron* sp. from the early Furongian of Dębki 2 borehole; (K), Transverse fracture view showing preservation of the shield; (L), Close up showing the external coating layers and the empty space between these layers which probably corresponds to the original shield (white arrow), black arrows indicate flexible inner lamella (specimen number ZPAL Cr. 11/ph139).

Fig. 4. SEM photographs of *Cytherellina submagna* (Krandijevsky, 1963) from the the Early Devonian (Lochkovian-early Pragian) of the Ivanye Zolote section, Podolia, Ukraine, showing microtubular AIT structures. (A-B), Phosphatised internal mould of the right valve in lateral and anterior views respectively. (C-K), AIT structures preserved on external surface of the mould, note the presence of a terminal pyrite crystal (e.g., white arrows in D) in almost every AIT. Other features to note are: the sharp turns of some trails (E, I); perforations through the phosphate layer marking the original position of the pyrite crystals at the

beginning of several microtubes (white arrows in F-I, K); perforations spaced along the ventral wall of a microtube (e.g., black arrows in F); and the missing ventral wall of a microtube (black arrow in H) (specimen number ZPAL O. 60/65).

Fig. 5. *Cytherellina submagna* (Krandijevsky, 1963), Ivanye Zolote section, Podolia, Ukraine, showing a second class of small microtubes, white arrows indicate perforations through the phosphate layer likely representing the originating end of several microtubes; (A-E, G-H), Clusters of AITs; (F), Single AIT with faint longitudinal striations on the microtube wall; (I), Single AIT with framboidal pyrite at the termination and longitudinal striations on the microtube wall (specimen number ZPAL O. 60/65).

Fig. 6. Model of AIT formation inside the phosphatized internal mould of the ostracod *Cytherellina submagna* (Krandijevsky, 1963).

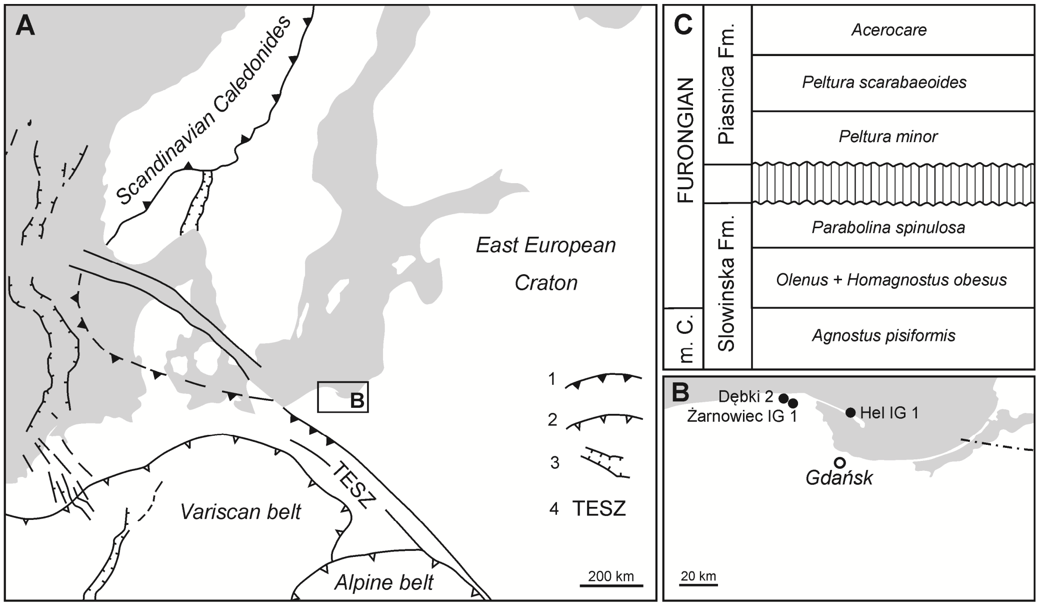

Fig. 1 [Olempska & Wacey]

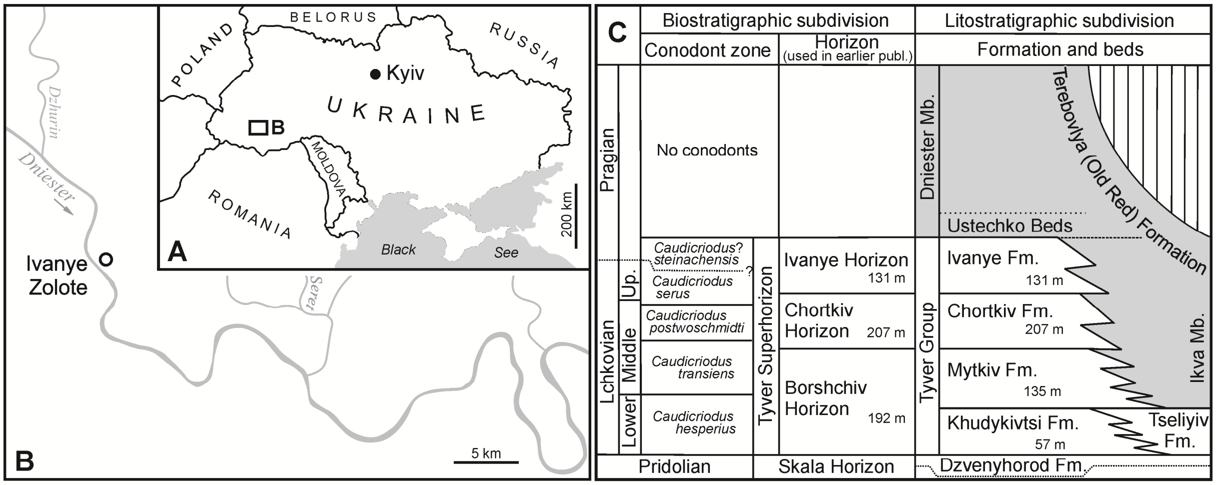

Fig. 2 [Olempska & Wacey]

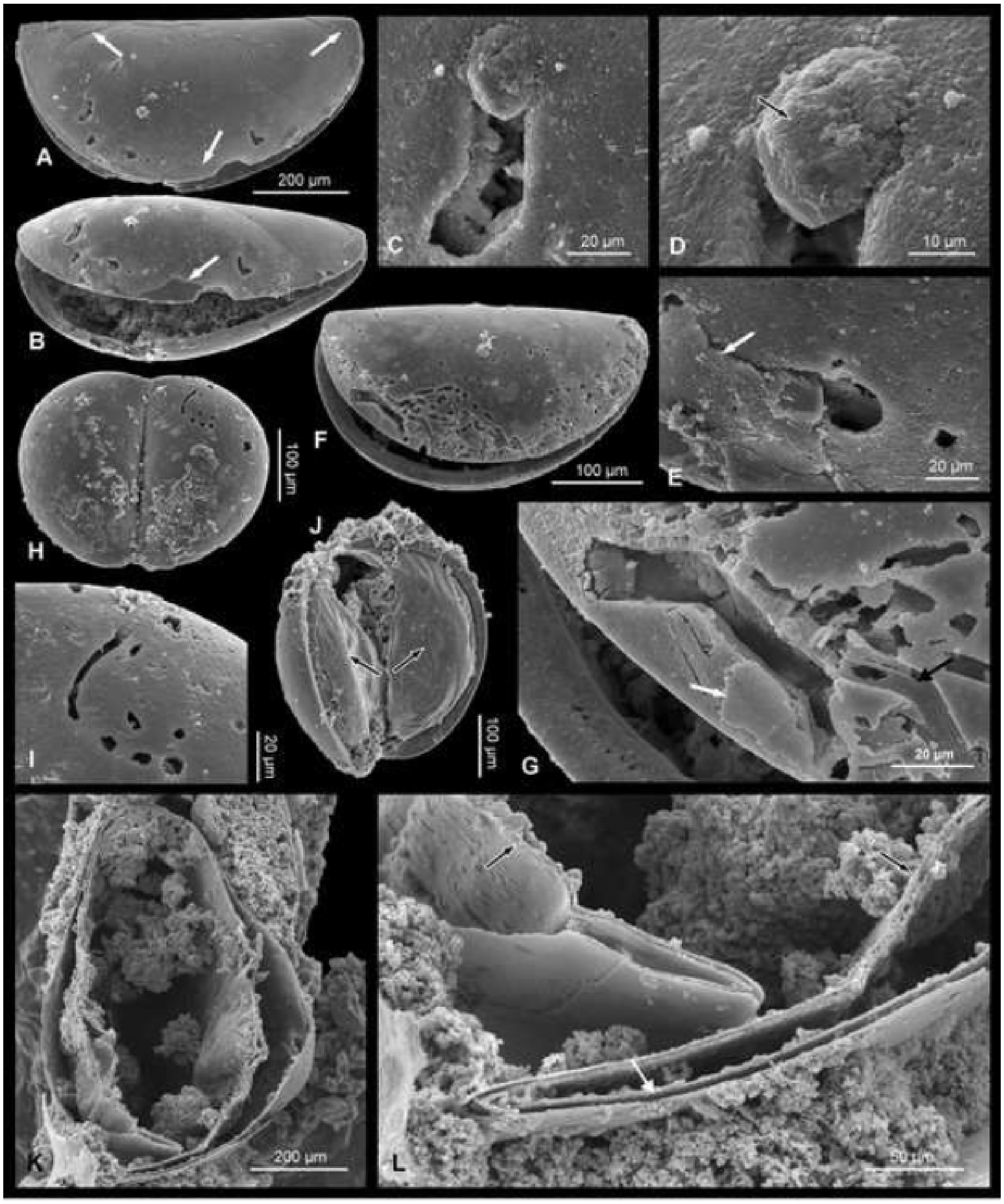

Fig. 3 [Olempska & Wacey]

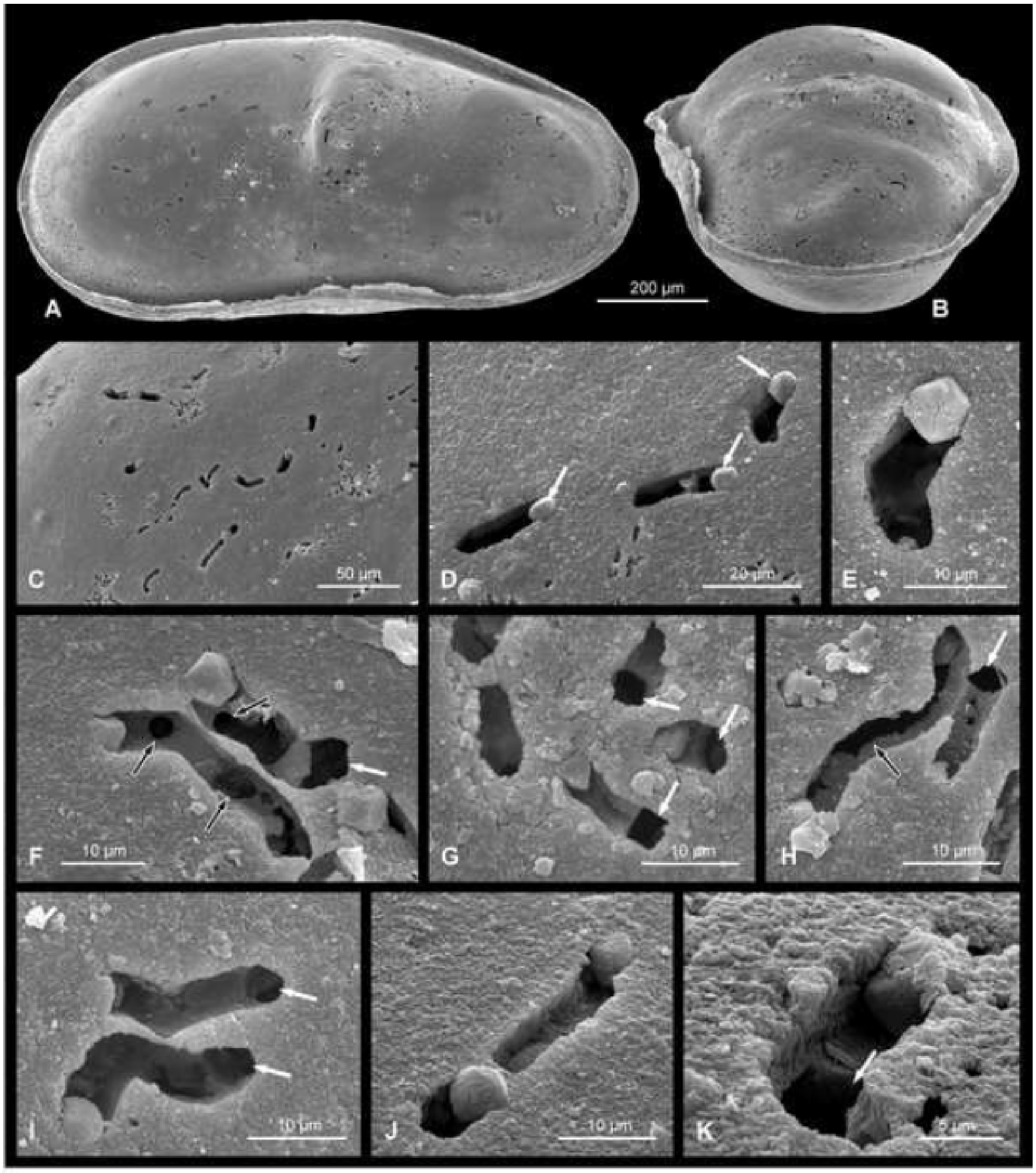

Fig. 4 [Olempska & Wacey]

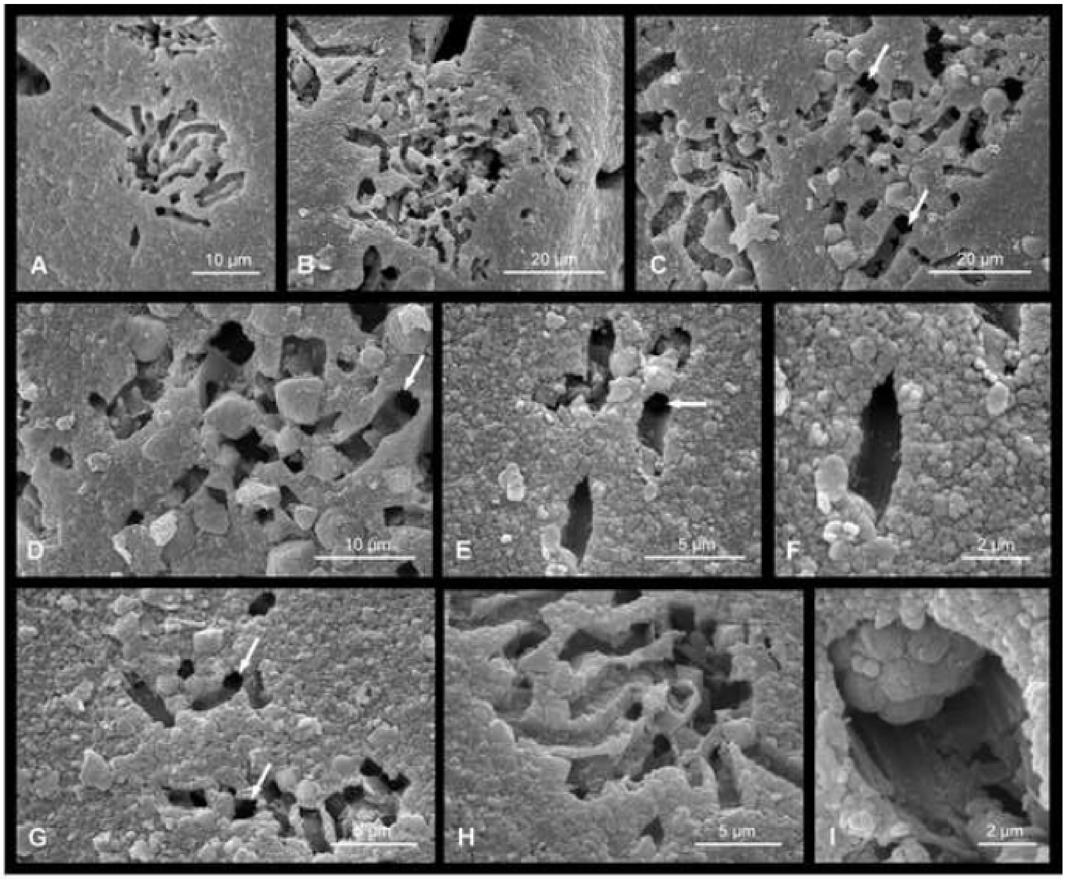

Fig. 5 [Olempska & Wacey]

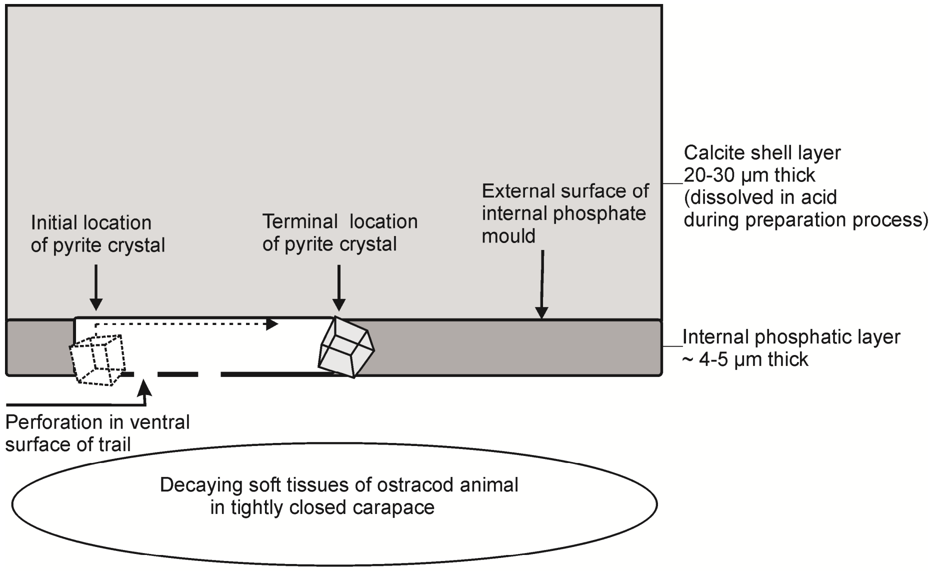

Fig. 6 [Olempska & Wacey]

Highlights

- We describe the first ambient inclusion trails (AITs) from Palaeozoic crustaceans
- AITs occur in phophatised shields and internal moulds of phosphatocopines and ostracods
- AITs occur adjacent to the locus of decaying organic matter
- Our data support a biological mediation of AIT formation
- Several other microtubular structures in Palaeozoic rocks may be reinterpreted as AITs