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Palaeontology

Onychophoran-like musculature in a phosphatized Cambrian lobopodian

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The restricted, exclusively terrestrial distribution of modern Onychophora contrasts strikingly with the rich diversity of onychophoran-like fossils preserved in marine Cambrian *Lagerstätten*. The transition from these early forebears to the modern onychophoran body plan is poorly constrained, in part owing to the absence of fossils preserving details of the soft anatomy. Here, we report muscle tissue in a new early Cambrian (Stage 3) lobopodian, *Tritonychus phanerosarkus* gen. et sp. nov., preserved in the Orsten fashion by three-dimensional replication in phosphate. This first report of Palaeozoic onychophoran musculature establishes peripheral musculature as a characteristic of the ancestral panarthropod, but documents an unexpected muscular configuration. Phylogenetic analysis reconstructs *T. phanerosarkus* as one of a few members of the main onychophoran lineage—which was as rare and as cryptic in the Cambrian period as it is today.

1. Introduction

The Panarthropod phyla—Euarthropoda, Tardigrada and Onychophora—evolved from a paraphyletic grade of unsegmented leg-bearing worms, the lobopodians [1]. These organisms illuminate otherwise intractable details of early panarthropod evolution [2,3], even if the full significance of their fossils is difficult to evaluate.

Lobopodians from the onychophoran total group have a deep ancestry. First represented by occurrences of their dorsal armature in the Tommotian (Cambrian Stage 2) shelly fossil record [4,5], they rise to a more obvious prominence in the Chengjiang biota (Cambrian Stage 3), where their carbonaceous compression fossils display a rich array of morphologies [6]. Fine exterior structure, however, is only seen in three-dimensional microfossils of Orsten-type deposits [7]. *Orstenotubulus* [8], the single lobopodian preserved in this fashion, dates to the Guzhangian (latest mid-Cambrian), substantially after the burst of lobopodian disparity documented by the early Cambrian (Stage 3) Chengjiang biota [9].

This array of onychophoran-like lobopodians may straddle the onychophoran stem lineage [9], or may predominantly belong to an extinct sister group [3]. Some features of the onychophoran body plan are already evident in these Cambrian taxa [1], though other distinctive characteristics, such as slime papillae, necessarily arose after onychophorans colonized the land. New Cambrian fossils, and better resolution of their relationships, are key to elucidating the evolutionary trajectory that led to the specialized anatomy of modern onychophorans. We here present a new phosphatized lobopodian from early Cambrian Orsten-type deposits that provides a unique perspective on the cuticle and musculature of early onychophoran-like lobopodians.

2. Material and methods

The specimen was recovered by 5% acetic acid digestion of carbonate nodules from black shales, and is deposited at the Key Laboratory for Palaeobiology,

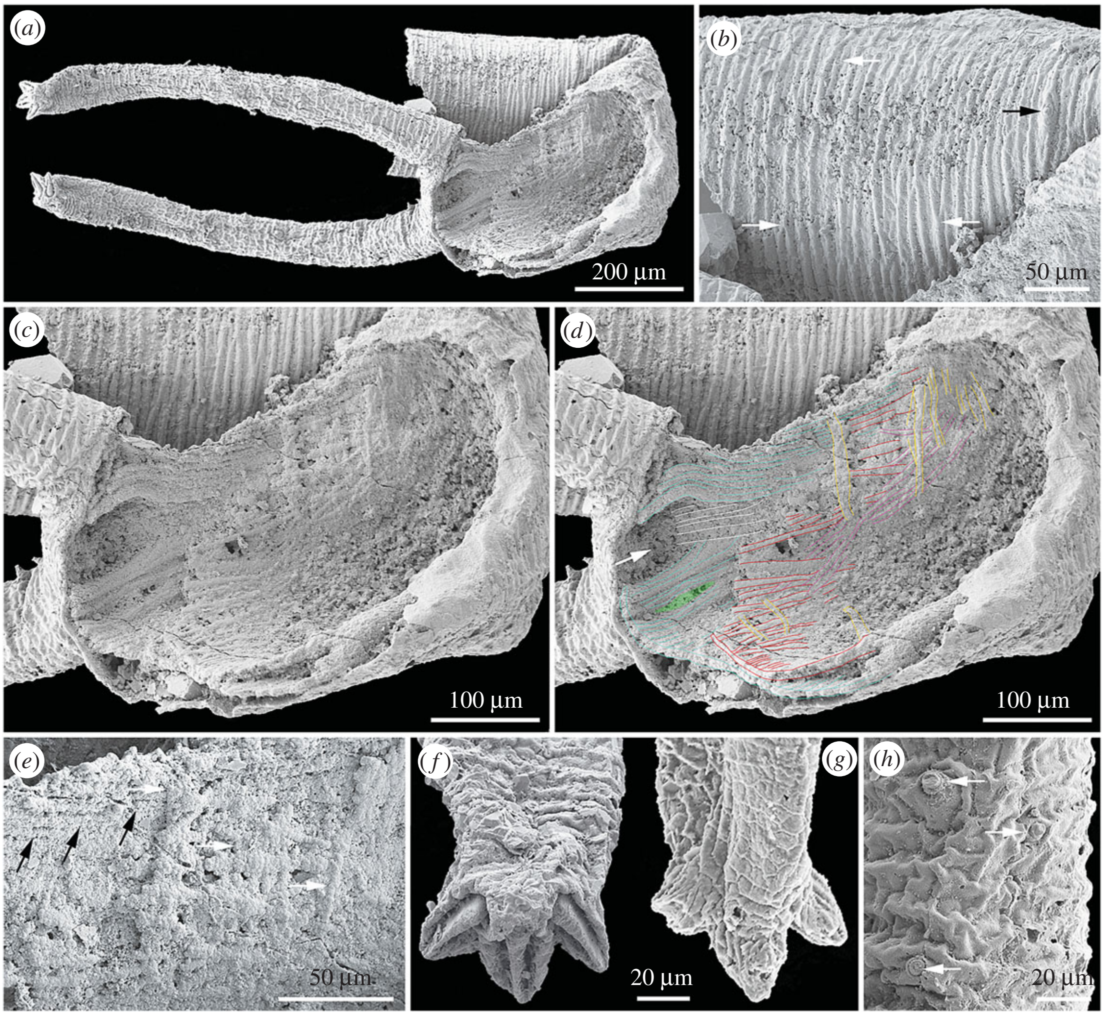

Figure 1. The Cambrian Stage 3 lobopodian *Tritonychus phanerosarkus* gen. et sp. nov. from China (YKLP 12335). (*a*) Overall morphology. (*b*) Surface ornament; circumferential wrinkles irregularly bifurcate and merge (white arrows) and bear papillae (black arrow). (*c*) Close up of musculature in panel (*a*). (*d*) Three layers of three-dimensionally preserved muscle fibres (myofibrils). Colours denote structures interpreted as: white, ventral longitudinal muscles; cyan, outer layer of longitudinal muscles, parting to leave gap (arrow); crimson/mauve, interwoven layer of oblique muscles; yellow, inner layer of circular muscles; green, point of leg levator insertion. (*e*) Details of linear fibres subparallel (black arrows) and perpendicular (white arrows) to the body axis. (*f*) Left appendage tip, bearing impressions of three claws. (*g*) Right appendage, showing the reverse side of claws. (*h*) Papillae of different sizes (arrows) on the surface of right appendage.

Yunnan University, Kunming, China (YKLP). Phylogenetic analysis was conducted in TNT [10], using the methods of Smith & Ortega Hernández [1], on a revised matrix of 49 taxa and 115 unordered characters, integrating data from recent lobopodian analyses [1,3,9,11] (data and scripts available at Dryad [12]). Parsimony analysis employed implied weights, with 99 values of Goloboff's concavity constant [13] picked from a lognormal distribution (range: 1.061–259.4; R function `qlnorm((1 : 99)/100, meanlog = log(4), sdlog = log(6)) + 1)`, and equal weights, with a consensus tree generated from all most parsimonious topologies [14]. Extended implied weighting [15] does not affect the consensus tree.

3. Results

(a) Systematic palaeontology

Superphylum Ecdysozoa, Aguinaldo *et al.* [16].

Stem-group Onychophora, Grube [17].

Genus *Tritonychus* Zhang et Smith, gen. nov.

LSID. urn:lsid:zoobank.org:act:959A47D4-3323-47CB-ADB2-B6A8F5B945A0.

Etymology. In reference to the third (τριτος, *tritos*) claw (ονυχος, *onychos*), a unique feature among lobopodians.

Diagnosis. Lobopodous appendages paired, 10 times longer than wide, four times narrower than trunk, each ending with three anteriorly directed claws. Trunk and appendages ornamented with bifurcating circumferential wrinkles and bearing dermal papillae. Two discrete layers of longitudinal fibres peripheral to body cavity.

Type species.

Tritonychus phanerosarkus Zhang et Smith gen. et sp. nov.

LSID. urn:lsid:zoobank.org:act:1715A32E-C258-4EC2-A44D-EC1A1E3DC23E.

Etymology. φανερος, *phaneros*, well-displayed, σαρκος, *sarkos*, muscle, flesh.

Holotype. YKLP 12335 (figure 1), the only known specimen.

Occurrence. Yu'anshan Formation (*Eoredlichia–Wutingaspis* Biozone, approximately late Atdabanian = Cambrian Series 2, Stage 3), Xiaotan section, Yongshan, Yunnan Province.

Diagnosis. As genus.

Description. The specimen is a millimetre-long section of lobopodian trunk that is folded at its midpoint and bears a pair of lobopods on its ventral surface near the presumed

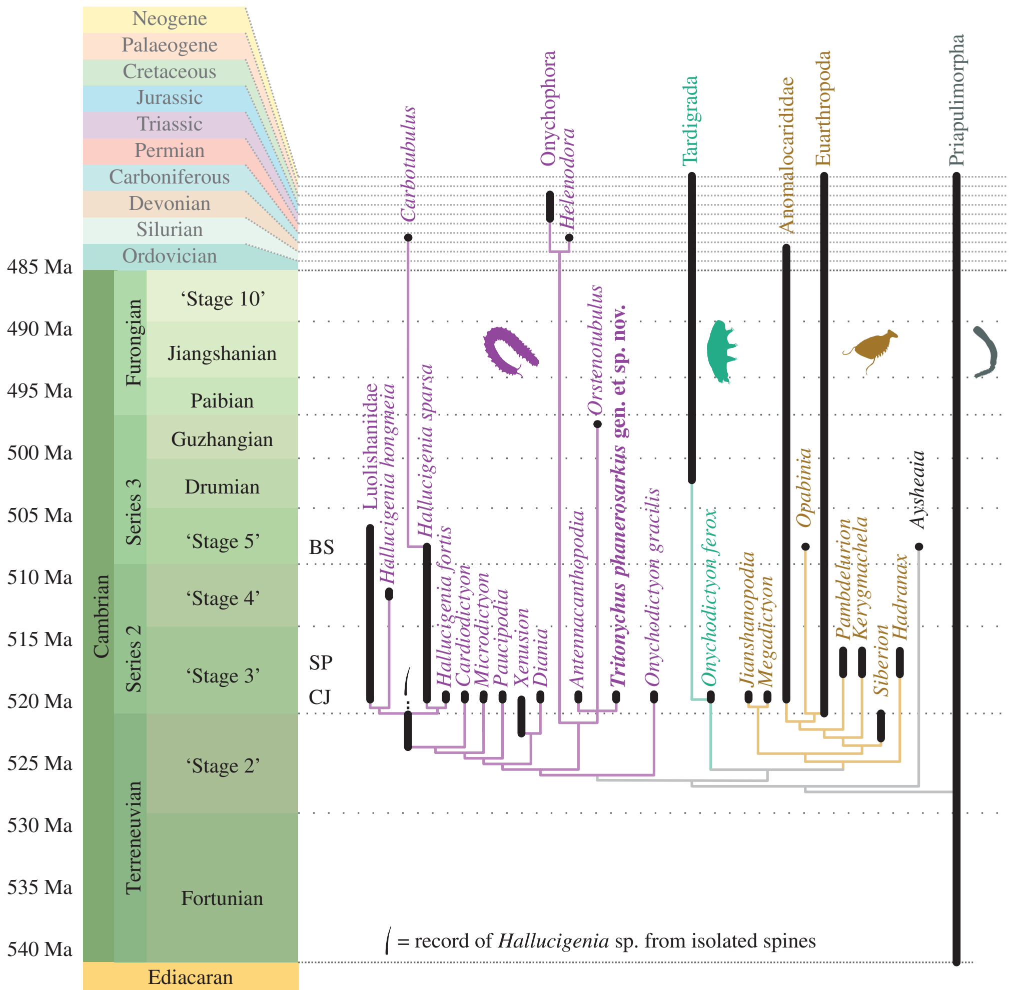

Figure 2. Phylogenetic results. Fossil occurrences marked as thick black lines, ghost ranges as thin lines. Divergence times are unconstrained, except in the case of hallucigeniids, where the record of isolated *Hallucigenia*-type spines [4] provides a minimum divergence time. BS, Burgess Shale, *ca* 508 Ma; SP, Sirius Passet, *ca* 518 Ma; CJ, Chengjiang, *ca* 520 Ma.

posterior margin (figure 1*a*). It is incomplete at each end, and lacks most of its dorsal surface.

The trunk is ornamented with circumferential wrinkles, spaced at 10 µm, which bifurcate and merge in an irregular fashion (figure 1*b*). Irregularly positioned conical projections, 5 µm in diameter and 7 µm in height and situated on a round cuticular base (figure 1*b*,*h*), presumably correspond to the dermal papillae of *Orstenotubulus* and extant Onychophora [8]. Moving distally along each appendage, the cuticle wrinkles give way to a reticulate pattern of polygonally arranged ridges that conceivably correspond to cell boundaries, and the papillae are less frequently expressed (figure 1*f*–*h*).

The trunk is lined with three layers of fibrous tissue, each around 10 µm thick, which we interpret as muscles. The outer layer (figure 1*c*–*e*, cyan) comprises 5–10 µm wide longitudinal fibres; it parts between the appendages to leave a 60 µm wide gap, through which a separate bundle of longitudinal fibres (white in figure 1*d*) passes. The fibres part again to the right of this point (green in figure 1*d*), perhaps reflecting the insertion of leg levator musculature. A second layer of interwoven oblique fibres (crimson and mauve in figure 1*d*) sits within the first, and within that layer lie further fibres oriented perpendicular to the body axis (yellow in figure 1*d*), presumably representing decayed remnants of an originally extensive layer of circular musculature.

Each of the two appendages is 800 µm long and a uniform 80 µm in diameter, with a circular cross section that is distorted in places by flattening. Each bears the impressions of three terminal claws, separated by 45°; the raised central bosses of these impressions denote an originally hollow claw (figure 1*f*,*g*). No distinct foot is present. Assuming the claws to be directed forwards (as in other lobopodians), the legs occupy the posterior limit of the fragment. The ventrolateral location of the appendages suggests that they served a conventional locomotory role, contrasting with the intriguing lateral position of appendages of *Orstenotubulus* [8].

4. Discussion

The preservation of muscular tissue is in some respects surprising, as body wall musculature is the first feature to decay when onychophorans are rotted in isotonic saline solution [18]. The absence of both labile (gonads, gut) and recalcitrant (claws) tissues in *T. phanerosarkus* indicates that the sequence of decay in salt water is a poor guide to the sequence of preservation in this fossil material. Here, early phosphatization initiated at the cuticle (evinced by the decreasing quality of preservation away from the body wall) clearly led to enhanced preservation of peripheral tissue (cf. [19]).

More generally, muscle tissue is atypical in phosphatized ('Orsten-type') microfossils (reference [20] provides a rare example), and though it may be concealed by overlying tissue layers in some cases [21], in most—including palaeoscolecid cuticles that occur alongside *T. phanerosarkus*—its absence is genuine. Muscle preservation is no less unusual in Burgess Shale-type settings [22]. The exception is Sirius Passet, where early diagenetic phosphatization records the evolution of muscle anatomy in stem-group Euarthropoda—documenting a conceived transition from peripheral musculature deep in the stem group (*Kerygmachela*), via peripheral + skeletal muscle (in *Pambdelurion*), to the skeletal muscle arrangement of crown group euarthropods (and tardigrades) [2]. The presence of peripheral musculature in *T. phanerosarkus* confirms that peripheral musculature was also ancestral within onychophorans, and thus for panarthropods as a whole.

Nevertheless, the derivation of three-layered musculature from the presumably ancestral twin layers observed in priapulids [23] is not straightforward. In priapulids and extant onychophorans, the outermost muscles are circular and the innermost longitudinal, with onychophorans incorporating an intermediate layer of interwoven oblique muscles [24,25]. *Tritonychus phanerosarkus* exhibits equivalent layers—though their order is reversed, leaving the homology of each layer with those in other panarthropods unclear, and revealing an unexpected diversity of muscle arrangement in early panarthropods.

A position within Onychophora is nonetheless robustly supported by phylogenetic analysis, which consistently places the new lobopodian in a clade comprising *Orstenotubulus*, *Antennacanthopodia, Helenodora* and crown group Onychophora (electronic supplementary material, summarized in figure 2). This 'onychophoran-like' clade is sister to all other Cambrian stem-group onychophorans, with the exception of *Onychodictyon gracilis*. It reflects cuticular similarities between *T. phanerosarkus*, *Orstenotubulus* [8] and extant onychophorans: bifurcating circumferential wrinkles, spinose projections mounted on with circular bases, and (in places) hexagonal patterning.

The new fossil extends the record of these features of the modern onychophoran cuticle into the lower Cambrian (Stage 3), along with other characteristics of the onychophoran body plan: the peripheral disposition of multiple muscular layers, the ventrolateral appendage location and conceivably a gonopore—one possible interpretation of the intra-appendicular gap in musculature, suggested by equivalent openings in *Orstenotubulus* and extant onychophorans [8].

Despite the early evolutionary origin of this suite of onychophoran features, it is striking that the 'onychophoran-like' clade is so poorly represented in the fossil record: only six specimens have yet been recovered, four of which are Orsten-type fragments. Whether or not the distinctively onychophoran-like features of the new fossil were also present in other Cambrian lobopodians, the lineage leading to modern Onychophora seems to have been as rare and depauperate during the formative stages of its evolution as it is today.

Data accessibility. Phylogenetic data: TreeBASE accession number S18871 [26]. Description of characters and detailed phylogenetic results: Dryad http://dx.doi.org/10.5061/dryad.7r10b [12]. This published work and the nomenclatural acts it contains have been registered in Zoobank: http://zoobank.org/References/D5C6C81C-2EEB-41CD-BE0C-48927554810D.

Authors' contributions. X.G.Z. and M.R.S. conceived the study. X.G.Z., J.Y. and J.B.H. collected the material. M.R.S. performed the phylogenetic analysis. J.Y. took the SEM photos. X.G.Z. and M.R.S. made the figures. M.R.S. drafted the manuscript with input from other authors. All authors have agreed to be held accountable for the content and approved the final version of the manuscript.

Competing interests. We have no competing interests.

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