Primitive soft-bodied cephalopods from the Cambrian

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The exquisite preservation of soft-bodied animals in Burgess Shale-type deposits provides important clues into the early evolution of body plans that emerged during the Cambrian explosion[1]. Until now, such deposits have remained silent regarding the early evolution of extant molluscan lineages – in particular the cephalopods. Nautiloids, traditionally considered basal within the cephalopods, are generally depicted as evolving from a creeping Cambrian ancestor whose dorsal shell afforded protection and buoyancy[2]. Whilst nautiloid-like shells occur from the Late Cambrian onwards, the fossil record provides little constraint on this model, or indeed on the early evolution of cephalopods. Here, we reinterpret the problematic Middle Cambrian animal *Nectocaris pteryx*[3, 4] as a primitive (i.e. stem-group), non-mineralized cephalopod, based on new material from the Burgess Shale. Together with *Nectocaris,* the problematic Lower Cambrian taxa *Petalilium*[5] and (probably) *Vetustovermis*[6, 7] form a distinctive clade, Nectocarididae, characterized by an open axial cavity with paired gills, wide lateral fins, a single pair of long, prehensile tentacles, a pair of non-faceted eyes on short stalks, and a large, flexible anterior funnel. This clade extends the cephalopods' fossil record by over 30 million years[2], and indicates that primitive cephalopods lacked a mineralized shell, were hyperbenthic, and were presumably

carnivorous. The presence of a funnel suggests that jet propulsion evolved in cephalopods before the acquisition of a shell. The explosive diversification of mineralized cephalopods in the Ordovician may have an understated Cambrian "fuse".

Phylum Mollusca

Stem-group Cephalopoda

Family Nectocarididae Conway Morris 1976

Nectocaris pteryx Conway Morris 1976[3]

Other genera. *Vetustovermis*[7], *Petalilium*[5] (=*Vetustovermis* in [6]) (Supplementary Discussion; Supplementary Fig. 1).

Revised diagnosis. Dorso-ventrally flattened, non-segmented body, lacking a mineralized shell; with a small head bearing a pair of eyes and tentacles, a hollow tubular element on the ventral surface of a short neck, a large, open axial cavity containing paired gills, and wide lateral fins.

Materials. 91 specimens (Royal Ontario Museum) plus holotype (National Museum of Natural History) from the Burgess Shale Formation, Fossil Ridge, British Columbia (Supplementary Table 1).

Description. *Nectocaris* is flat in lateral view (Fig. 1f; Supplementary Fig. 2) and rhomboid in dorso-ventral aspect; continuous lateral fins taper towards the posterior (Fig. 1c, d; Supplementary Fig. 3). The body comprises a torso, a tubular 'funnel' protruding from the ventral side of a short neck (Fig. 1a, b, f; Supplementary Figs. 2–4), and a small, eye-bearing head with a pair of non-segmented frontal projections ('tentacles' herein; Fig. 1a, b, f; Supplementary Figs. 2–6). The body length averages 37 mm (further measurements in Supplementary Table 2).

The flexible tentacles extend from the anterior of the head; abrupt variations in their width, due to rotations, indicate that they are flattened and presumably C-shaped in cross-section (Supplementary Figs. 3, 5). Each tentacle margin is delineated by a narrow dark line that sometimes bears small repeated lobate projections (Supplementary Figs. 5, 6). A squat triangular projection occurs at the base of each tentacle (Fig. 1b; Supplementary Figs. 5, 7). A symmetrical dark region is sometimes evident at the anterior end of the head, between the tentacles (Supplementary Figs. 5, 8); its position suggests a feeding apparatus.

Two large eyes are attached by short stalks to the latero-ventral surface of the head (Fig. 1a, b, f; Supplementary Figs. 2–8). Unlike probable compound eyes in the Burgess Shale, which preserve in the same fashion as body tissue (i.e. as carbon coated by diagenetic aluminosilicates[8, 9]), the eyes of *Nectocaris* preserve as a conspicuous carbon film that envelopes a thick layer of bedding-perpendicular muscovite crystals (Supplementary Fig. 8). These crystals evidence that a structure or a void within the eyes was replaced during diagenesis, consistent with a camera-type construction.

The external portion of the funnel widens away from the neck (Fig. 1a, b, f; Supplementary Figs. 2–4, 7) and is preserved in various orientations (e.g. Fig. 1a, b), indicating original flexibility. The internal portion widens laterally towards the posterior (Fig. 1b; Supplementary Fig. 7). A channel, which narrows in a constricted zone in the neck and often widens towards the funnel's aperture, follows the midline of the funnel (e.g. Fig. 1b; Supplementary Figs. 4, 7) and opens into an axial ovoid cavity that occupies the full length of the body. Sediment often fills the channel and the cavity, suggesting that the cavity opened to the environment via the funnel. Ovoid sediment-filled regions on either side of the funnel (Fig. 1b; Supplementary Fig. 7) may have been associated with openings of the axial cavity. The axial cavity's width does not correlate with body width ($r^2 = 0.00015$, $p = 0.952$), consistent with the former being variable during life.

A black structure comprising serially repeated blade-like elements lines each side of the axial cavity, tapering to the front and rear (e.g. Fig. 1a, Supplementary Figs. 4, 9). These structures, interpreted to represent gills, are connected to the cavity wall along their entire lateral length, as suggested by the homogeneous nature of the structures and the interleaving of sediment between gill-blades on the lumen side (Fig. 1e, Supplementary Fig. 9). Each gill-blade is connected to its neighbours along a lateral axis in a zig-zag fashion (Supplementary Fig. 9). The axis of some specimens bears a dark stain, narrower than the axial cavity, that tapers towards the rear of the body (Fig. 1c), suggestive of a digestive tube.

The animal's fins run from the base of the neck to the rear end of the animal, with their maximal width occurring towards the anterior of the body (Fig. 1a, d). Repeated bar-like elements cross the entire width of the fins at regular intervals. A longitudinal element extends along the base of fins, where they connect to the body (Fig. 1a, d; Supplementary Figs. 3, 9, 10). The bars cross this element and extend towards the edge of the axial cavity at an oblique angle; they are in phase with the gill-blades (Supplementary Fig. 9). The fins also bear a series of narrower lineations at regular intervals, oriented obliquely to the bars (Fig. 1d). We interpret the bars and lineations to represent connective tissues, which were presumably associated with musculature (Supplementary Fig. 11).

Nectocaris was buried by density mudflows[10], suggesting that it spent time close to the benthos and was neutrally or negatively buoyant. Sediment entered the axial cavity of some specimens via the funnel, around the time of burial – as suggested by the varying degree of infill and moulding of the sediment between gill blades, which precludes its interpretation as an infilled gut. A nektobenthic lifestyle can be inferred from the presence of latero-ventral eyes and flexible, muscular lateral fins; the large gills; and the absence of either limbs or a muscular sole (Fig. 2). The presence of a constriction within the base of the funnel suggests that water flow might have been controlled by a valve. Thus, during life, water entered the axial cavity through openings near the funnel's base and was expelled through the funnel (Supplementary Fig. 11), which could flex in order to direct any resultant motive force. This exhalent jet probably played a subsidiary role to the large fins in propulsion. Nectocaridids were likely predators or scavengers, feeding on small soft-bodied animals. The length, shape, flexibility and marginal projections of the tentacles probably facilitated the manipulation of small food items.

The unique nectocaridid body plan poses challenges for classification, but certain affinities nevertheless seem unlikely – for instance, segmented taxa such as arthropods and chordates. Some fossil taxa display characters that, whilst superficially similar to those observed in the nectocaridids, probably represent convergence. For example, the Burgess Shale *Amiskwia*[11] bears lateral fins and a pair of tentacles, but no eyes, gills or funnel (Supplementary Discussion). Both *Nectocaris* and the Carboniferous *Tullimonstrum*[12] bear stalked camera-type eyes, striated lateral fins, and serially repeated structures lateral to an axial cavity. However, taphonomic differences prohibit detailed consideration of these superficial similarities. Moreover, tentacles and gills are not preserved in *Tullimonstrum*, and its long, terminally-clawed, prehensile proboscis is fundamentally dissimilar in morphology and function to the hollow, clawless funnel of the nectocaridids. Among extant groups, we interpret the suite of characteristics seen in the cephalopods to be homologous to the nectocaridid character combination : an axial cavity containing paired gills (the mantle cavity of extant cephalopods); stalked, camera-type eyes posterior to cephalic tentacles and anterior to a distinct, funnel-bearing neck; and lateral fins with obliquely-intersecting connective tissues (Supplementary Fig. 12). The funnel's position and shape fits comfortably within the range of morphologies observed in extant cephalopods (Supplementary Fig. 13). All crown-group cephalopods also possess a horny beak, a radula, and at least eight tentacles[13]; further, a calcified shell is thought to be plesiomorphic to Cephalopoda[2]. In the absence of these synapomorphies, a position in the cephalopod stem- rather than crown-group is more conservative (Fig. 3). Nectocaridids' single pair of tentacles may originate via the fusion of multiple pairs[14], or represent the primitive state. Whilst no obvious beak or radula is evident, the presence of such structures cannot be excluded, owing to the poor preservation of the *Nectocaris* mouthparts. Alternatively, an apparent absence may result from a diminutive size, not conducive to fossilization; or a secondary loss : the radula, regarded as an important molluscan synapomorphy[15], is reduced or absent in some cephalopods[16].

The absence of a mineralized shell is particularly puzzling if the ancestral cephalopod resembled a monoplacophoran[2]. However, the "intermediate forms" supporting this proposition lack any diagnostic cephalopod character; some may not even be molluscs[17]. Stratigraphic evidence contradicts the possibility of secondary shell loss in *Nectocaris*: no obvious precursors have been identified in the rich Cambrian shelly fossil record. Whilst buoyancy obtained through gas-filled chambered shells was thought to be essential to the nektonic mobility of early cephalopods, the large muscular fins of *Nectocaris* suggest a more active pathway to the nektobenthonic realm. The chitinous organic framework within conchiferan shells[18] suggests that the primitive conchiferan (perhaps a creeping organism resembling *Kimberella*[19] or *Odontogriphus*[20]) possessed a non-mineralized chitinous integument, which was subsequently calcified[21], perhaps via the co-option of pre-existing chemical pathways (such as those used for calcium regulation)[22] . Given that the highly plastic molluscan secretome[23] has convergently produced similar shell microstructures in unrelated lineages[24], we suggest that nautiloids evolved from a non-mineralized, coleoid-like ancestor related to the nectocaridids.

The probable presence of carnivorous cephalopods in the Early to Middle Cambrian highlights the important role of nektonic links in Cambrian food chains[25]. Nectocaridids' lack of mineralization disputes the view that gas-filled shells preceded floatation in the Mollusca, and poses new challenges to the traditional explanation of cephalopod origins. The nectocaridids exemplify a lineage that diverged early and mineralized late. If this trend is widespread across bilaterians, the onset of mineralization may obscure the true phylogenetic signal of the Cambrian explosion.

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Figure 1 | *Nectocaris pteryx* from the Middle Cambrian Burgess Shale. Specimens **a–e** preserved dorso-ventrally, with head to top of image; specimen **f** preserved laterally. **a**, Royal Ontario Museum (ROM) 59656, ventral view of complete specimen, funnel oriented towards anterior. **b**, ROM 59660, ventral view of head and base of tentacles; the funnel is folded to the left of the image, and a channel connecting the funnel to the axial cavity is filled with sediment; lateral slit-like openings are present posterior to the neck. (Interpretative sketch: Supplementary Fig. 7.) **c**, ROM 59657, low-angle lighting highlights the relief of sediment within the axial cavity. A dark stain along the body axis, overlying the axial cavity, may represent digestive organs. **d**, ROM 59658, ventral view; bars are crossed by finer lineations. **e**, ROM 59659, sediment infilling the axial cavity interdigitates between gill-blades. **f**, ROM 59661, lateral specimen showing relative position of eyes, tentacles and funnel. Abbreviations: **ac**, axial cavity with sediment infill; **bars**, bar-like elements; **ch**, sediment-filled channel passing through funnel to axial cavity; **dig**, interdigitation of sediment between gill blades; **ds**, dark stain, interpreted as digestive organs; **eye**, stalked eye; **fin**, lateral fin; **fun**, funnel; **gill**, gills; **in-f**, internal portion of funnel; **le**, linear element at base of fin; **lin**, lineations on fins; **mou?**, inferred position of mouth; **op**, opening of axial cavity; **tent**, tentacle, **tp**, triangular projection. *Each scale bar represents 5 mm*.

Figure 2 | Reconstruction of *Nectocaris pteryx*. © 2010 Marianne Collins.

Figure 3 | Phylogenetic position of the nectocaridids. Arrows indicate the crown groups of **1**, molluscs; **2**, conchifera; **3**, cephalopods. Stars represent the earliest record of mineralization in each lineage (after ref. [23]). Clade divergence times (dotted lines) are unconstrained. Early branches follow previous phylogeny (after ref. [20]).