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Supplemental Material

Detailed description and discussion of the armature pattern of the fossil, Figures S1–S4, and Micro-CT Videos S1–S3.

SUPPLEMENTARY MATERIAL

Exceptional preservation of a marine tapeworm tentacle in Cretaceous amber

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Video S3. Higher resolution micro-tomographic reconstruction of anteriormost part of the fossil tapeworm (NIGP203253). (separately uploaded).

Text S1. Detailed method of the X-ray microcomputed tomography.

For 3-dimensional reconstruction we scanned the fossil at the micro-CT laboratory of NIGPAS, using a 3D X-ray microscope (3D-XRM), Zeiss Xradia 620 versa. Unlike conventional micro-CT, which relies on maximum geometric magnification and a flat panel detector to achieve high resolution, 3D-XRM uses a charge-coupled device (CCD)-based objective to achieve higher spatial resolution. We conducted three different scans to achieve best results. For scanning the whole tentacle, two independent scans with the same parameter were conducted, a CCD-based 4× objective was used, providing isotropic voxel sizes of 3.655 μm with the help of geometric magnification. During these two scans, the acceleration voltage for the X-ray source was 40 kV (power 3 W), a thin filter (Air) being used to avoid beam hardening artefacts, the exposure time for each projection was 1.5 s, and the number of TIFF images was 1002 (Video S1 and S2). For scanning the apical part of the tentacle, a CCD-based 20× objective was used, providing isotropic voxel sizes of 0.7263 μm with the help of geometric magnification; during this scan, the acceleration voltage for the X-ray source was 80 kV (power 10 W), a thin filter (LE1) being used to avoid beam hardening artefacts; the exposure time for each projection was 2.5 s, and the number of TIFF images was 976 (Video S3).

The datasets generated and/or analyzed during the current study including the full-resolution image stack and the final 3D models are available in the Zenodo repository (https://doi.org/10.5281/zenodo.10693594).

Text S2. Detailed description of the fossil.

Remarks. Because the fossil consists of one tentacle only and other body parts were not preserved, concise naming according to regular procedures in trypanorhynch taxonomy was not possible. Designation of the tentacle surfaces follows the description of recent *Dollfusiella* spp. (Platyhelminthes, Cestoda, Trypanorhyncha) based on its very long tentacles and similar armature types and hook patterns.

Description. Tentacle long and slender, 9.81 mm long and 0.16 mm wide, estimating a total scolex length of about 20–30 mm (based on ratio of 2–3:1 scolex to tentacle length in recent trypanorhynch tapeworms); basal swelling absent (Fig. 1A and 1B, Video S1 and S2). Hooks numerous, tentacle with approximately 180 rows of hooks, divided into approximately 60 rows of hooks each in basal, metabasal and apical armature, partly invaginated. Metabasal and apical tentacular armature heteromorphous (Fig. 1 A,B,D–F; Fig. S2; Video S3), with paired hooks 1–2 (1′–2′) on antibothrial surface deviating (Fig. S2A), hooks 7 (7′) abutting to form inverted V′s (Fig. S2B and S2C), heteroacanthous typical. Basal armature present, in rotational symmetry, homeoacanthous homeomorphous (Fig. 1G). Hooks hollow (Fig. 2A). Half-spiral row (hsr) apical=7, hsr metabasal=8–9, hsr basal=11–12. Hook shape changing along tentacle. Basal hooks smallest, triangular shaped with broad base, total hook length (L)=13–20 µm, total basal length (B)=9–13 µm, strongly recurved at tip, arranged in quincunxes (Fig. 1G). Towards metabasal and apical armature, files deviate on antibothrial surface, creating a glide reflection symmetry. Hooks increasing

in size along row, antibothrial hooks 1–2 (1′–2′) in pairs, uncinate, with robust blade, with anterior extension of base, 1(1′) L=70–80 µm, B=60–70 µm, 2(2′) L=60–70 µm, B=30–35 µm (Fig. S2A). On internal surface, hooks 3 (3′) robust and falciform with broad base, L=40–45 µm, B=30–35 µm. Hooks 4–6 (4′–6′) falciform, L=30–35 µm, B=15–20 µm (Fig. S2B). On bothrial surface, hooks 7 (7′) abut, forming inverted V′s, appearing more triangular with broad base, L=20–25 µm, B=15–20 µm, as observed apically (Fig. S2C). Other body structures not preserved.

Text S2. Detailed discussion of the armature pattern of the fossil and its implication for the evolution of trypanorhynch tapeworms.

At present, the Trypanorhyncha include two suborders, Trypanobatoida (mainly parasitizing rays) and Trypanoselachoida (mainly parasitizing sharks) (Olson et al., 2010), in around 15 families (Palm, 2004). Armature patterns have been described to characterize certain trypanorhynch families and especially genera in the most recent classification (Palm, 2004). The exact armature pattern as described for our fossil is not known from any recent trypanorhynch because it combines two very different armature patterns showing rotational and glide reflection symmetry on the same tentacle. The armature of the fossil, in brief, can be described as a homeoacanthous homeomorphous basal and a transition between a homeoacanthous heteromorphous and a heteroacanthous typical heteromorphous in the metabasal/apical armature, with a very long tentacle that lacks a basal swelling. The number of hook rows is very high compared with extant species; also, the size of the hollow hooks with a length of 80 µm is known in recent forms. The estimated scolex length of our fossil (2–3 cm) is also reached in some extant trypanorhynch species known to date, such as *Dasyrhynchus* (Beveridge and Campbell, 1993).

Palm (1995, 2004) suggested that the 'modern' trypanorhynchs developed either from homeoacanthous- or heteroacanthous-type armature patterns. A change between a homeoacanthous-homeomorphous-basal and a homeoacanthous-heteromorphous-metabasal armature has been described in *Mixonybelinia* Palm, 1999 (Trypanobatoida, Fig. 1C) but with solid hooks (Palm, 1999). The enormous length of

the tentacle (160 rows), combined with a heteroacanthous typical armature and homeomorphous or heteromorphous hollow hooks, is well known in *Dollfusiella* Campbell & Beveridge, 1994 (Trypanobatoida, Fig. 2B), however, with a basal swelling (Campbell and Beveridge, 1994). The scolex lengths of *Dollfusiella taminii* Menoret & Ivanov, 2014 and *Dollfusiella vooremi* (Sao Clemente & Gomes, 1989) reach 0.93 and 5.7 mm, with 0.47 and 1.9 mm long tentacles (Menoret and Ivanov, 2014), a ratio of 2–3 tentacle to scolex lengths. *D. taminii* has a heteroacanthous homeomorphous metabasal armature with 7–9 falcate hooks per principal row (Menoret and Ivanov, 2014), alike our fossil in the metabasal and apical armature. Another similar genus with very long tentacles (3 mm) and without a basal swelling is *Eutetrarhynchus* Pintner, 1913 (Trypanobatoida) (Schaeffner, 2014).

According to a molecular clock analysis of trypanorhynchs and their hosts, the common ancestors of *Dollfusiella* (with a close relationship to *Eutetrarhynchus* from triakid sharks) and *Nybelinia* (closely related to *Mixonybelinia*) separated in the Cretaceous (Olson et al., 2010). Both genera, *Dollfusiella* and *Nybelinia*, co-infect Rajiidae, Ginglystomatidae and Triakidae (Palm, 2004), with the earliest known fossil records of these elasmobranchs also dating back about 100 million years ago (Underwood, 2006). We conclude that the fossil probably represents an ancient trypanobatoid trypanorhynch with a scolex length of about 2–3 cm, a size that is also reached in some extant trypanorhynch species known to date. However, the armature pattern is a combination of two different armature types (with rotational basal and glide reflection symmetry metabasal and apical), probably representing a intermediate

form between homeoacanthous and heteroacanthous armatures of modern trypanorhynchs (Palm, 1995, 2004).

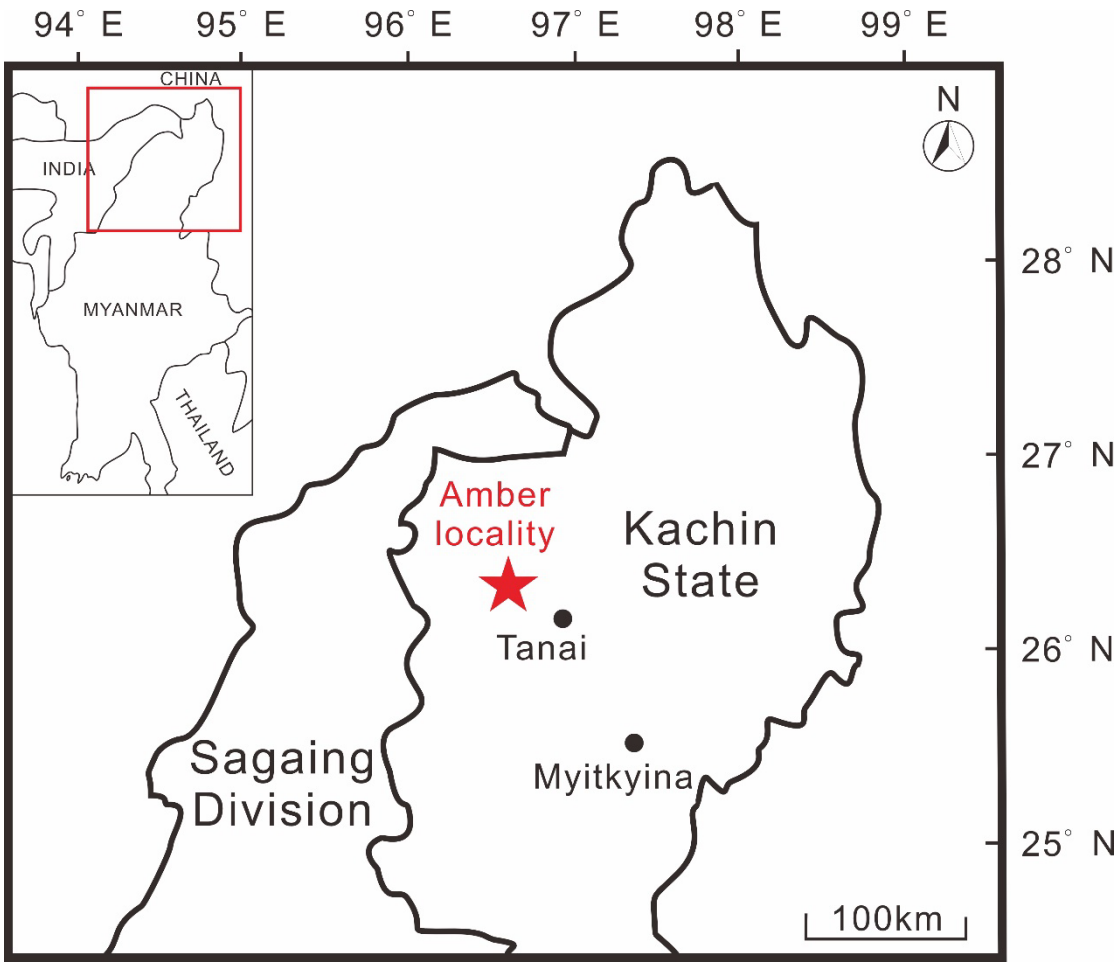

Fig. S1. Map of the amber locality near Tanai Village in the Hukawng Valley.

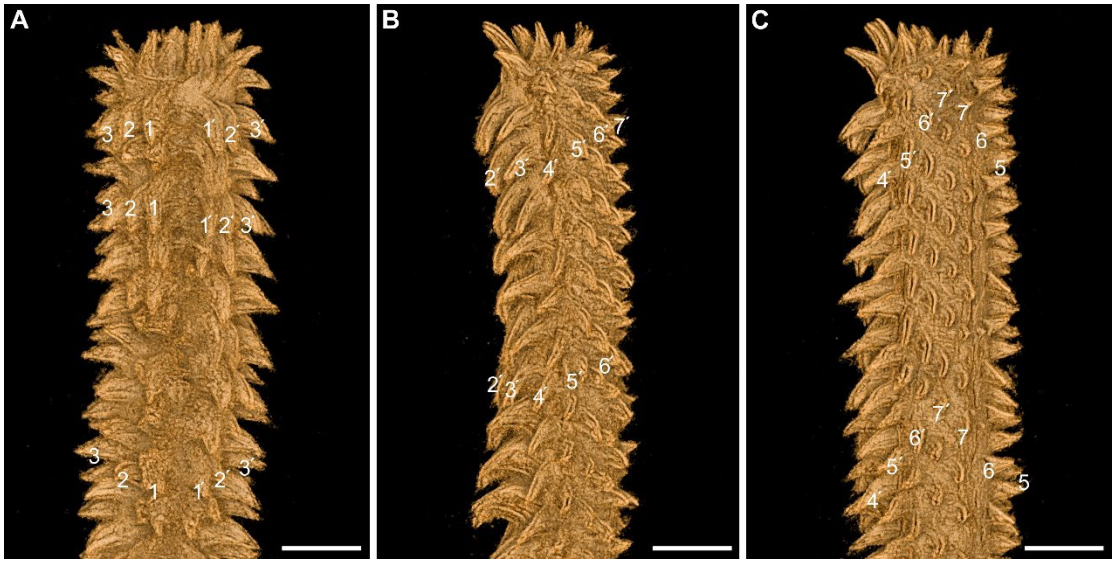

Fig. S2. The metabasal and apical tentacular armature pattern of the trypanorhynch fossil (NIGP203253). (A) Antibothrial surface with paired hooks 1–2(1′–2′). (B) Internal surface with half spiral rows. (C) Bothrial surface. Scale bars = 0.1 mm.

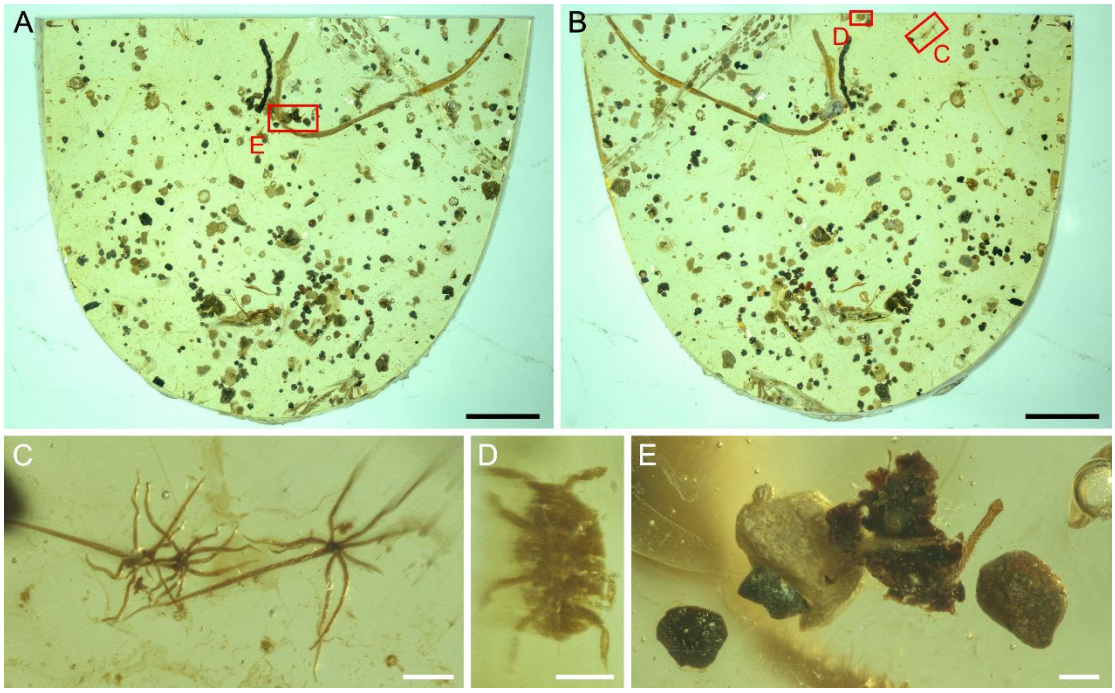

Fig. S3. The amber piece containing the fossil tapeworm and other inclusions preserved together with the fossil (NIGP203253). (A–B) The overall view of the amber piece. (C) Gleicheniacean trichomes. (D) Scale insect nymph. (E) Sand grains. Scale bars = 2.0 mm (A,B), 0.1 mm (C–E).

Fig. S4. A hypothetical ecological reconstruction of the fossil trypanorhynch tapeworm. The fossil tapeworm was lodged in the intestine of an elasmobranch and the dead host was possibly scavenged by a dinosaur on a strandline with resin extruding nearby.

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