150 million years old isopods on fishes: a possible case of palaeo-parasitism

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Parasites are found in all habitats and within all groups of animals, and their influence on food webs, ecosystems and evolutionary development is significant. However, the fossil record of direct parasitism is very scarce. We present here probable examples of parasitic isopods on fishes from the Upper Jurassic Solnhofen Lithographic Limestones (150 million years old, southern Germany). Individual fishes appear to be infested with one to three isopods each. All specimens were documented with up-to-date imaging methods (macrophotography, stereo-photography, composite imaging). Position, orientation and other aspects clearly indicate that the isopods were already attached to the fishes before they died and hence do not represent scavengers but (more or less permanently) attached parasites. While the morphology of the specimens is somewhat uninformative about the systematic position of the isopods, their specific type of parasitism is an indicator for a position in the early lineage towards Cymothoidae. This would represent the first fossil record of this group of obligate fish parasites. • Key words: palaeo-parasitology, fossil Cymothoida, *Nerocila*, host-parasite interaction.

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Parasites influence food webs and are ecologically and evolutionary significant, as parasitism is an important driver of co-evolutionary host-parasite patterns. Parasites are ubiquitous and abundant in all ecosystems and in all groups of living forms (*e.g.* Klompmaker *et al.* 2014).

One example of a group with abundant parasites in marine habitats is the peracarid crustacean ingroup Isopoda (Bunckley-Williams *et al.* 2006). Parasitic isopods represent an important factor in fisheries and aquaculture, because they reduce host fitness by influencing physiological, behavioural and morphological aspects (Yesmin & Khanum 2011, Roche *et al.* 2013). For understanding the evolution of specific groups, including the evolution towards a parasitic lifestyle, fossils are an important source of information (*e.g.* Donoghue *et al.* 1989; Rust 2007; Edgecombe 2010; Haug *et al.* 2010, 2013; Klompmaker *et al.* 2013; Bracken-Grissom *et al.* 2014). The fossil record of metazoan parasites dates back even further than the Late Ordovician (450 million years old) records stated by Baumiller & Gahn (2002), but at least to 495 million years (Late Cambrian; Walossek & Müller 1994, Walossek *et al.* 1994, recent review in Castellani *et al.* 2011). Yet, fossil parasitic isopods are still a rarity.

This may be coupled to the fact that the identification of a fossil parasite as such is not always easy. There are different criteria for identifying a parasite in the fossil record; some are less direct than others (for a longer discussion see Nagler & Haug 2015). Identification can be made possible by:

1) Pathological deformations as reactions of the host's body to the parasite (Conway Morris 1981, Wilson *et al.* 2011) such as pearls in molluscs (*e.g.* De Baets *et al.* 2011), galls (*e.g.* Brett 1978, 1985; Radwanska & Radwanski 2005; Radwanska & Poirot 2010), and asymmetric growth patterns. Such cases are also known for parasitic isopods of the group Bopyridae (*e.g.* Klompmaker *et al.* 2014, Klompmaker & Boxshall 2015).

2) Comparative functional morphology, and/or assignment to a specific monophyletic group, when the parasite is found isolated, such as fossil parasitic copepods (*e.g.* Cressey & Boxshall 1989), pentastomids (*e.g.* Maas & Waloszek 2001, Castellani *et al.* 2011), or different insects (*e.g.* Gao *et al.* 2012). Also a possible case for an infective

stage of a parasitic isopod has been described (Serrano-Sánchez *et al*. in review).

3) Finding the parasite directly on or in the host, as the most direct evidence for palaeo-parasitology. Examples are mainly found in amber such as mites or lice on insects (Poinar 1985, Arillo 2007, Perrichot *et al*. 2012).

We report here a possible type 3 for isopods on fishes. The finds are 150 million years old and possibly represent the oldest example for such a direct parasite-host interaction.

Material and methods

Material. – We investigated five slabs of lithographic limestones of the Solnhofen Formation (Upper Jurassic, Tithonian, southern Germany) found in Wegscheid near Eichstätt with fishes and associated isopods:

(1) *Pholidophorus* sp. Agassiz, 1843 (total length/TL = 130 mm), with one isopod (16 mm) on the ventral side of the pelvic fin formerly from the private collection of Udo Resch, Eichstätt (Germany), now reposited in the collections of the Palaeontological Institute of the University of Vienna (IPUW 7404) (Fig. 1A).

(2) *Amblysemius belicianus* Thiollière, 1852 (TL = 121 mm), with two isopods (15 mm, on the fin; 8 mm, on the pleon, straight behind the first one) on the ventral side of the pelvic fin from the private collection of Dr. Felthaus, Minden (Germany) (Fig. 1B).

(3) *Caturus* sp. Agassiz, 1843 (TL = 146 mm), with three isopods on the ventral side of the fish formerly from the private collection of Udo Resch, Eichstätt (Germany), now reposited in the collections of the Palaeontological Institute of the University of Vienna (IPUW 7405). One isopod (21 mm) on the pelvic fin and one isopod (15 mm) directly posterior to the fin close to the first one. The third isopod (11 mm) is on the lateral body side (Fig. 1C).

(4) *Anaethalion angustus* Münster, 1832 (TL = 138 mm), with one isopod (19 mm) on the lower lobe of the caudal fin from the private collection of Udo Resch (Fig. 1D).

(5) *Leptolepides sprattiformis* De Blainville, 1818 (TL = 66 mm), with a ball-like crystallisation (6 mm) on the ventral side of the pelvic fin; formerly from the private collection of Udo Resch, now State Museum of Natural History Stuttgart (SMNS 96920) (Fig. 1E).

Additionally, we investigated two species of extant parasitic marine isopods that were collected by students of the Ludwig Maximilians University of Munich (Munich, Germany) during a sampling campaign organized by the Zoologische Staatssammlung München in May 2014 in the Mediterranean Sea in Croatia Cross Bay (45°7.06´ N, 13°3.99´ E):

6) Caudal fin of a representative of Mugilidae, extant, with one parasitic isopod, *Nerocila acuminata* (Leach, 1818) (ZSMA 20159001) (Fig. 6A, C, D).

7) *Symphodus cinereus* Nordmann, 1840, extant, with one parasitic isopod, *Nerocila* sp. (Leach, 1818) and three parasitic copepod larvae (*Caligus* sp.) (ZSMA 20159002) (Fig. 6B, E, F).

Documentation methods. – Stereo-photography and macro-photography combined with composite imaging were performed (following *e.g*. Haug *et al*. 2011, 2012a, 2013), both under cross-polarized light. We used a Canon EOS Rebel T3i camera, either with the Canon EF-S (18–55 mm) lens (for overview images) or the Canon MP-E (65 mm) macro lens (for detail images). Illumination was provided by the Canon Macro Twin Lite MT-24EX flash from the two opposing sites. The single images were edited with Photoshop CS4 (Adobe). Image stacks were processed with the freeware CombineZP (Alan Hadley) and ImageAnalyzer (Meesoft). Stratigraphy of the Solnhofen limestone follows Schweigert (2007).

Presentation method. – We present colour-marked versions of the images directly alongside stereo images for better recognizing the interpreted structures. For this purpose the stereo image was simply copied. Then one colour channel of the stereo image was deleted, hence leaving only one half image of the stereo pair. This half image was desaturated; then structures apparent in the stereo images were marked with the lasso tool in Photoshop CS5 (Adobe) on the desaturated half image (Haug *et al*. 2010, 2012b).

We marked all visible structures of the isopods as follows: For two adjacent segments we used nearby shades of colouring. The functional head (h) is marked brown. The head appendages (antenna = a, unidentifiable mouthparts = mp, maxilliped = mxp, mandible = md) are marked in yellow, orange, brownish and red. The posterior seven thorax segments (t) are marked in cyan and blue. The thoracopods (tp) are marked in light blue and dark blue. The spines (s) on these appendages are marked in bright green. The free pleon segments (pl) are marked in red and pink. The pleopods (plp) are marked in magenta. The uropods (u) are marked in orange. The pleotelson (pt) is marked in dark red.

Terminology. – A neutral descriptive terminology is employed allowing the non-specialist to follow our interpretations. For example "functional head" is used instead of "cephalothorax". Also terms, which might be misleading in comparison to other groups are avoided. For example, we use the term "posterior thorax" instead of "pereion", "pereon" or "peraeon" as the latter terms have quite a different meaning, for example in decapods.

Also the term "thoracopod" is used instead of "pereio-

Figure 1. Fishes with associated isopods from the Solnhofen limestones; associated isopods marked in red and are indicated by arrows. • A – *Pholidophorus* sp. Agassiz, 1843 (IPUW 7404). • B – *Amblysemius belicianus* Thiollière, 1852. • C – *Caturus* sp. Agassiz, 1843 (IPUW 7405). • D – *Anaethalion angustus* Muenster, 1832. • E – *Leptolepides sprattiformis* De Blainville, 1818 (SMNS 96920).

pod", again as the latter term has quite different meaning in different groups of Malacostraca, while thoracopod offers a neutral alternative directly comparable to other malacostracan groups. Specialists' terminology is given in brackets.

Institutional abbreviations. – IPUW – Institute of Palaeontology, University of Vienna, Austria; SMNS – State Museum of Natural History Stuttgart, Germany; ZSM – Zoological State Collection Munich, Germany.

Results

Description of specimen 1. – *Pholidophorus* sp. (early derivative of the teleost lineage) with one isopod specimen that is curled on its pelvic fin (Figs 1A, 2A). The isopod is twisted around the fin, *i.e*. the posterior part of the pleon and the pleotelson is seen in a ventral view, while the head is seen in lateral view.

This isopod shows the most details of all specimens

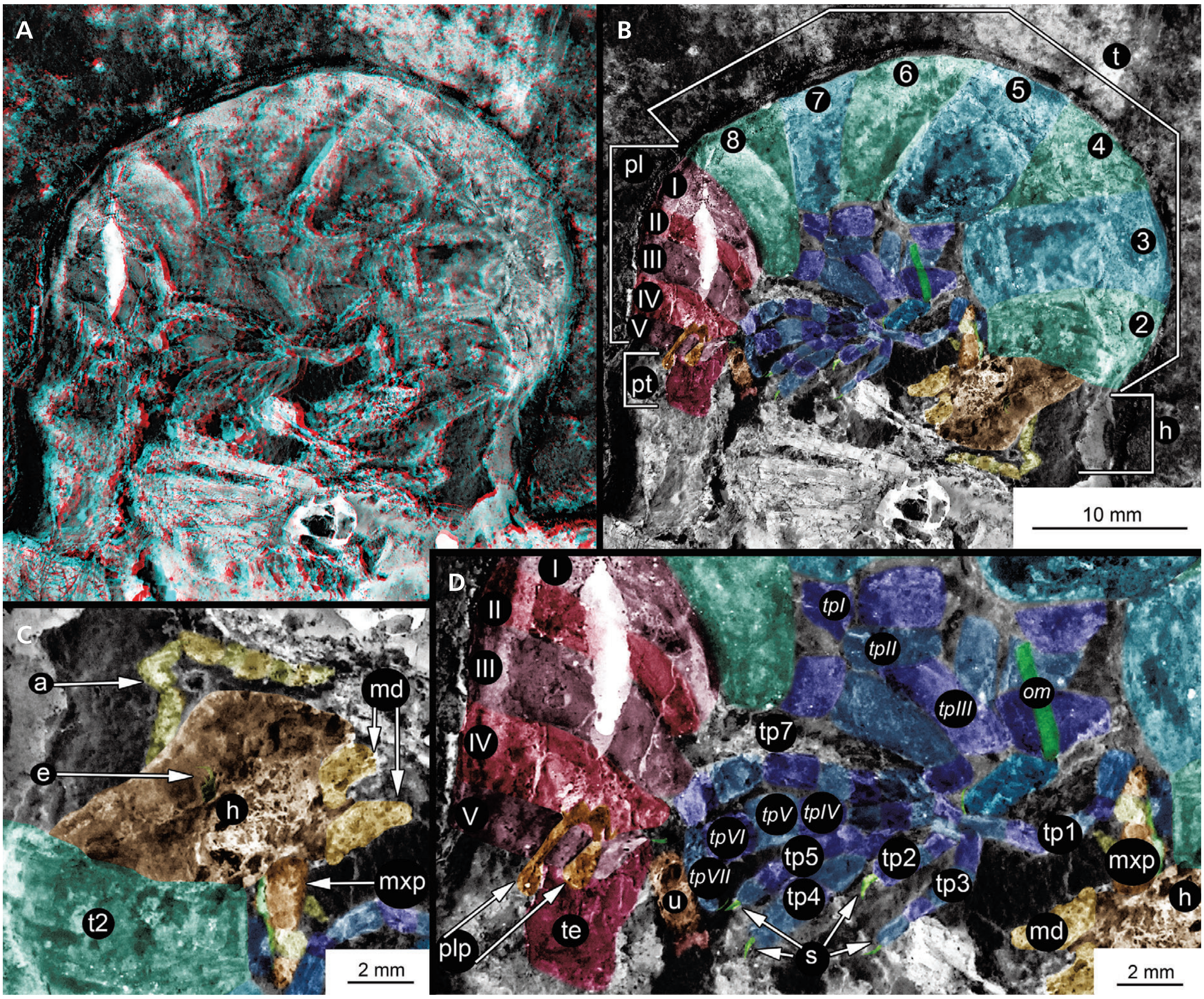

Figure 2. Details of the isopod associated with *Pholidophorus* sp. (IPUW 7404). • A – stereo image of the curved isopod on the pelvic fin, please use red-cyan anaglyphs to view. • B – colour-marked version of A. Head (h), thorax (t) with seven free segments (t2–t8), pleon (pl) with 5 segments (pl I–pl V) and pleotelson (pt). • C – detail of the head (h) with one antenna (a), two mandibles (md), one maxilliped (mxp) and one not further identifiable endopod (e) with spines and setae. • D – detail of the posterior part of the isopod. All elements of the thoracopods (tpI – tpVII) are visible and marked for one thoracopod tp6. On all dactyli of the thoracopods (tp1 – tp7) spines are visible (s). Pleon (pl) with five segments (pl I–pl V) and two pleopods (plp). Pleotelson (te) with one uropod (u) with endopods; om = organic matter.

investigated. We were able to identify the functional head (apparently eucrustacean head plus first thorax segment), the remaining thorax (pereion) with seven segments, the anterior pleon with five free segments and the pleotelson (last pleon segment plus telson; Fig. 2B). The thorax is 4.5 times as long as the functional head. The pleon length, including the pleotelson, is about 1.3 times the head length. The head (Fig. 2C) bears several appendages. The anterior-most one is interpreted here as the antenna with eleven elements. Two mouthparts, located more posteriorly, may represent mandibles. Next, from anterior to posterior, an endite-like structure of a mouthpart is visible. It remains unclear to which appendage it originally belonged, but it possibly represents the reduced maxilla (see Discussion). Posterior-most on the head is an appendage with three solid elements. It bears a spine on the first element and possibly represents the maxilliped. The middle and posterior parts of the isopod (Fig. 2D) show all seven thoracopods with the corresponding elements (exemplified by the sixth thoracopod) and seven dactyli that are claw-like and appear to be prehensile (on various thoracopods). The coxal plates and the proximal elements of the thoracopods appear similar in all seven thoracopods. A single, straight tube is present in the fossil specimen, which is interpreted here as organic matter. The pleon with its five free segments, bears on the fourth segment two interconnected pleopods. Finally, also the uropod with an exopod and the pleotelson are visible.

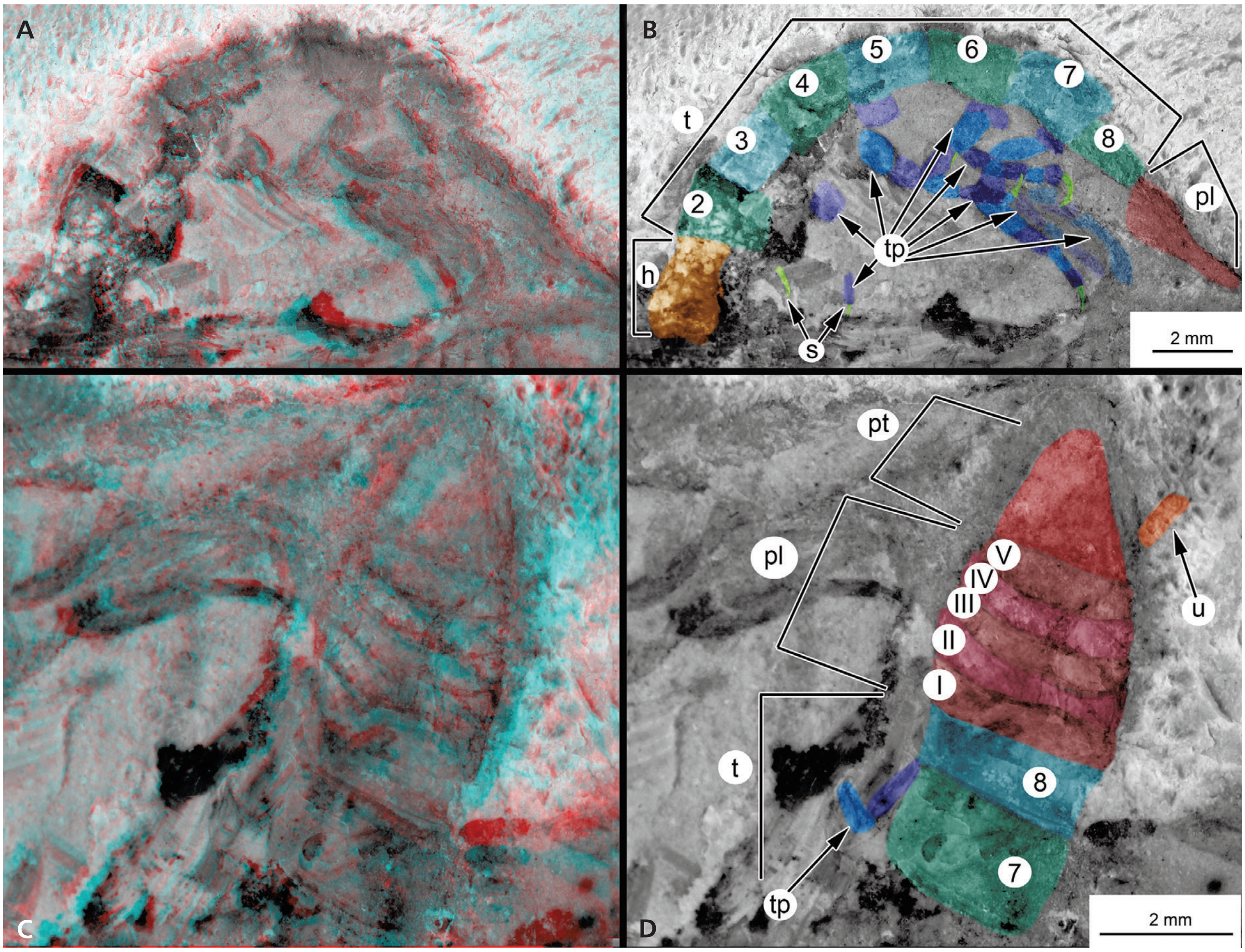

Figure 3. Details of the two isopods on *Amblysemius belicianus*. • A – isopod curled on pelvic fin, stereo image. • B – colour-marked version of A. Head (h) without any visible mouthparts, thorax (t) with seven free segments, 28 elements of endopods of thoracopods (tp) and six spines (s). Pleon (pl) and pleotelson could not be identified in a more specific way. • C – smaller isopod straight behind the first one, stereo image. • D – colour-marked version of C. Thorax (t) with the two posterior segments (t7, t8) and two elements of one thoracopod (tp). Pleon (pl) with five segments (pl I–pl V) and pleotelson (pt) with one uropod element (u).

Description of specimen 2. – *Amblysemius belicianus* (representative of the extinct group Caturoidea within Amiiformes) with two isopod specimens (Fig. 1B). One isopod is curled and twisted around the pelvic fin with the anterior part of the body (functional head and thorax) being visible in dorsal view and the posterior part (remains of the pleon and pleotelson) in lateral view (Fig. 3A).

It was possible to identify the functional head, the thorax with seven free segments and remains of the pleon and the pleotelson, the latter without any structural details. The thorax length is 4.5 times the head length, the pleon plus the pleotelson length is 1.3 times the head length. Four more or less complete thoracopods and four isolated elements of additional thoracopods are apparent. Six spines (on various thoracopods) are visible; four of the spines are directly associated with the almost complete thoracopods, one is associated with an isolated element of one thoracopod, and one is entirely isolated. The proximal elements of the thoracopods and especially the coxal plates are similar in the four visible thoracopods. The distal parts appear to be prehensile (Fig. 3B).

The second isopod is located directly posterior to the first one at an angle of 45° in relation to the pelvic fin (in lateral view; Fig. 3C). The isopod is completely exposed in dorsal view. We identified the last two thorax segments (t7 and t8), the anterior five free pleon segments and the pleotelson. The pleon plus the pleotelson is 1.2 times longer than the last two thorax segments together. The pleotelson is as long as the last thorax segments. Two elements of one thoracopod, possibly representing the appendage of one of the last two thorax segments, are visible. Posterior to the pleotelson, an imprint of an element of the uropods is visible (Fig. 3D).

Description of specimen 3. – *Caturus* sp. (also a representative of the extinct group Caturoidea; Fig. 1C) with three isopod specimens. One is twisted on the vertical fin (Fig. 4A).

The head and the thorax are seen in lateral view, while the free pleon segments and the pleotelson are seen in dorsal view. We could identify the functional head, the thorax with seven free segments, the pleon with five free segments, and the last segment of the pleon and the telson are continuous and form a pleotelson. The thorax length is 4 times the head length; the pleon plus the pleotelson length is 1.5 times the head length. The pleotelson is as long as the head. The head bears the antenna with eleven elements. Two mouthparts, further posterior, might represent the mandibles. Next, from anterior to posterior, a possible structure of a mouthpart can be seen; it is not clear which one and not if the part represents an exopod or endopod. It could represent the reduced maxilla (see Discussion). Located posteriorly is an appendage with two solid, almost rectangular elements. It bears a spine on the first element and probably represents the maxilliped. The mid-body area (thorax) has eight non-connected elements of the thoracopods and one more or less complete thoracopod with three elements, probably representing the first thoracopod. Additionally, three spines are visible, two of them connected to elements of the thoracopods. The pleon bears the rounded endopod of the fifth pleopod and two flattened uropods (Fig. 4B).

The second isopod is located directly above the lateral line organ (Fig. 4C). The isopod is visible in dorso-lateral view. The functional head and the first two free thorax segments are not visible. It was possible to identify segments 4–8 of the thorax, the five free pleon segments and the pleotelson. The five free pleon segments combined are as long as the last two thorax segments combined; the pleotelson is as long as the last free pleon segment. The pleon bears three rounded pleopods and three parts of the pleopods. The left flattened uropod is visible (Fig. 4D).

The third isopod is located posterior to the first one at an angle of 45° in relation to the pelvic fin (Fig. 4E). All visible parts of the isopod are exposed in dorsal view. The head is, unfortunately, not present in this specimen. The thorax appears completely re-crystallized. The pleon has five free segments and the pleotelson is visible. The free part of the pleon is as long as the thorax and the pleotelson is as long as two pleon segments if the recrystallized pattern comprises the whole thorax (Fig. 4F). Another isopod specimen located on the left side of the fish got lost during preparation (pers. obs. UR).

Description of specimen 4. – *Anaethalion angustus* (early representative of Teleostoi, more precisely of Elopiformes), comprises a single curled isopod on the pelvic fin (Fig. 1D). We were able to identify the functional head (cephalothorax) and seven free thorax segments. The pleon and the pleotelson probably are located under the pelvic fin and are not detectable (Fig. 5A). The thorax length is 5 times the head length (Fig. 5B). The antenna with eleven elements and three mouthparts are preserved. The first mouthpart with one element and the second mouthpart with two elements and a spine at the distal end most likely represent the mandibles. The following mouthpart consists of three elements and bears two spines, one on the second element and one on the third element. This appendage probably represents the maxilliped (Fig. 5C, E). Two thoracopods are visible. The anterior one has two elements preserved and a claw-like spine on the distal end, the other one comprises three elements with two spines or setae on the second element (Fig. 5D). One claw-like spine lies in the thorax segment 1 without any connection probably due to the process of fossilization.

Description of the crystalline ball (specimen 5). – *Leptolepides sprattiformis* (representative of Euteleostei, more precisely of the extinct group Leptolepididae, Fig. 1E) with a "crystalline ball" on the pelvic fin. This elongated, oval "ball" is 1/10 as long as the host fish and is located around the pelvic fin. This structure might represent a parasitising isopod that became entirely recrystallized, based on size, shape, and position.

Description of extant material (specimens 6+7). – Extant material studied for comparison includes a representative of mugilid teleosteans, which displays one parasitic isopod ascribed to *Nerocila acuminata* on its pelvic fin (Fig. 6A, C, D). A specimen of *Symphodus cinereus* with one parasitic isopod ascribed to *Nerocila* sp. located on the right lateral side posterior-ventral to the orbit on the gill cover and three parasitic copepod larvae (*Caligus*) (Fig. 6B, E, F).

Discussion

Coarse systematic interpretation of the specimens

The overall habitus of all specimens clearly identifies them as arthropods, more precisely, the trunk subdivision is strongly indicative of eumalacostracan crustaceans. The lack of a posteriorly extending shield in all specimens argues for an isopod affinity. Amphipods or syncarids have a similar general body organization. Yet, the cephalothorax and pleotelson together with the general shape are more compatible with an isopod affinity, because all specimens show seven thorax segments (*vs* eight in many syncarids) and five free pleon segments (*vs* six in syncarids and amphipods).

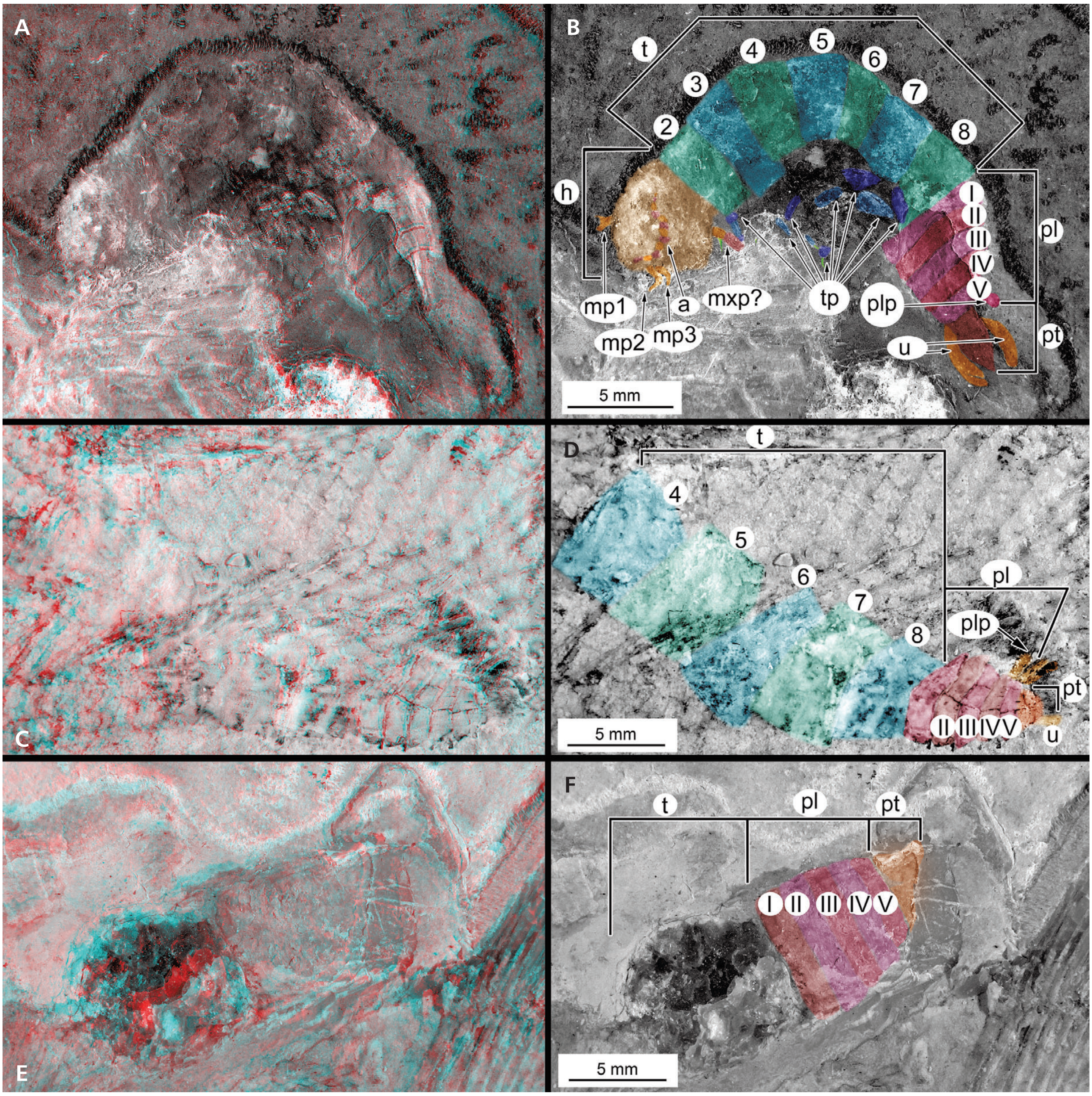

Figure 4. Details of the three associated isopods on *Caturus* sp. (IPUW 7405). • A – detail of one isopod curved around the pelvic fin, stereo image. • B – colour-marked version of A. h = head with antenna (a), mouthparts (mp1–mp3) and the maxilliped (mxp). Thorax (t) with seven free segments (t2–t8) and eleven elements of the thoracopods (tp) with three spines (s). The pleon with five free segments (pl I–pl V), one pleopod (plp), and the pleotelson (pt) with two uropods (u). • C – detail of one isopod straight on the lateral line, stereo image. • D – colour-marked version of C. Thorax (t) with the posterior segments (t4–t8), pleon (pl) with five free segments, with one pleopod (plp) and the pleotelson (pt) with one uropod (u). • E – detail of the isopod straight behind the ventral fin, stereo image. • F – colour-marked version of E. Thorax (t) is completely re-crystallized. Pleon (pl) with five free segments and pleotelson (pt).

We consider all investigated fossil isopods as conspecific as we cannot find significant differences justifying separation. Due to their comparatively large head and the long thorax, the isopods roughly resemble the famous “very hungry caterpillar” (Carle 1969) in appearance.

The general tagmosis is derived from the eumalacostracan ground pattern, as the first thorax segment is continuous with the head and the last pleon segment with the telson (forming a pleotelson), leading to seven observable free thorax tergites and five free pleon tergites.

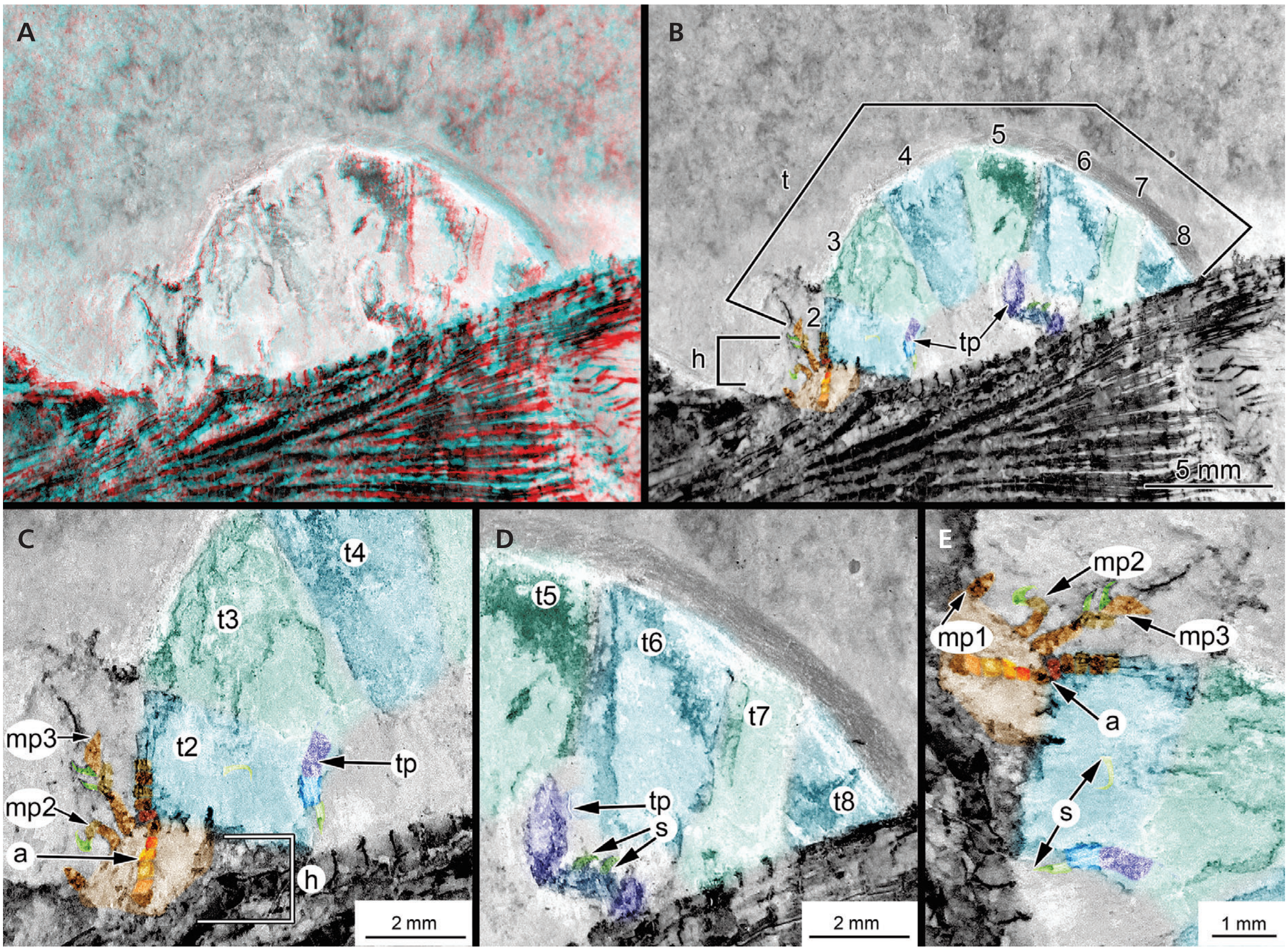

Figure 5. Detail of the associated isopod on *Anaethalion angustus*. • A – stereo-image. • B – colour-marked version of A. Head (h), seven free segments of the thorax (t), and five elements of the thoracopods (tp). • C – detail of the head (brown) with some mouthparts (mp1–mp3) and one antenna (a). Three thorax segments (t2–t4) and two elements of a thoracopod (tp). • D – detail of the posterior thorax segments (t5–t8), with three elements of a thoracopod (tp) and two spines (s) on it. • E – detail of the head with antenna (a), mouthparts (mp), and two spines (s).

Ecological interpretation

Based on the position of the isopods on the fishes they could either be interpreted as scavengers or parasites. In other words: the isopods could have been attached to the fishes before these died, or to an already dead carcass.

The following points clearly argue for a parasitic nature of the specimens:

1) There are only a relatively few specimens on the fishes. In cases of scavenging we should expect quite higher numbers of isopods (examples and comparably discussions in Frickhinger 1999, Polz 2004, Wilson *et al*. 2011).

2) All specimens are in a non-random orientation. They are all in a head-forward orientation. Such an orientation is most compatible with the assumption that the specimens were already on the fishes when these were alive. This very position leads to a maximally stream-lined position of the isopod specimens, and also reduces the danger for the possible parasites to be removed by cleaner fish. If the isopods did represent scavengers we should expect a random orientation of the specimens.

3) All specimens are in a non-random position on the fishes. Most of them are positioned on the fins, only in specimens where these positions are already occupied by other parasites, they occur in other positions. Also here, if the isopods represented scavengers we should expect a significantly more random pattern. Moreover, we should expect scavengers in more "fleshy" regions.

4) The isopods that are curved around the fins appear to have a twisted body (Figs 2A, 3A, 4A, 5A). This is a typical result of a growth response caused by the permanent position on the host fish (Brusca 1981, Strong 2012, Smit *et al*. 2014). This also indicates that the curved isopods are adult females and the smaller straight isopods are males or juveniles. Among modern parasites the latter only transform into females when no isopod has parasitized the fish before (Bowman 1960, Lincoln 1971, Williams & Williams 1985, Tsai *et al*. 2000, Lester 2005, Ravichandran *et al*. 2009).

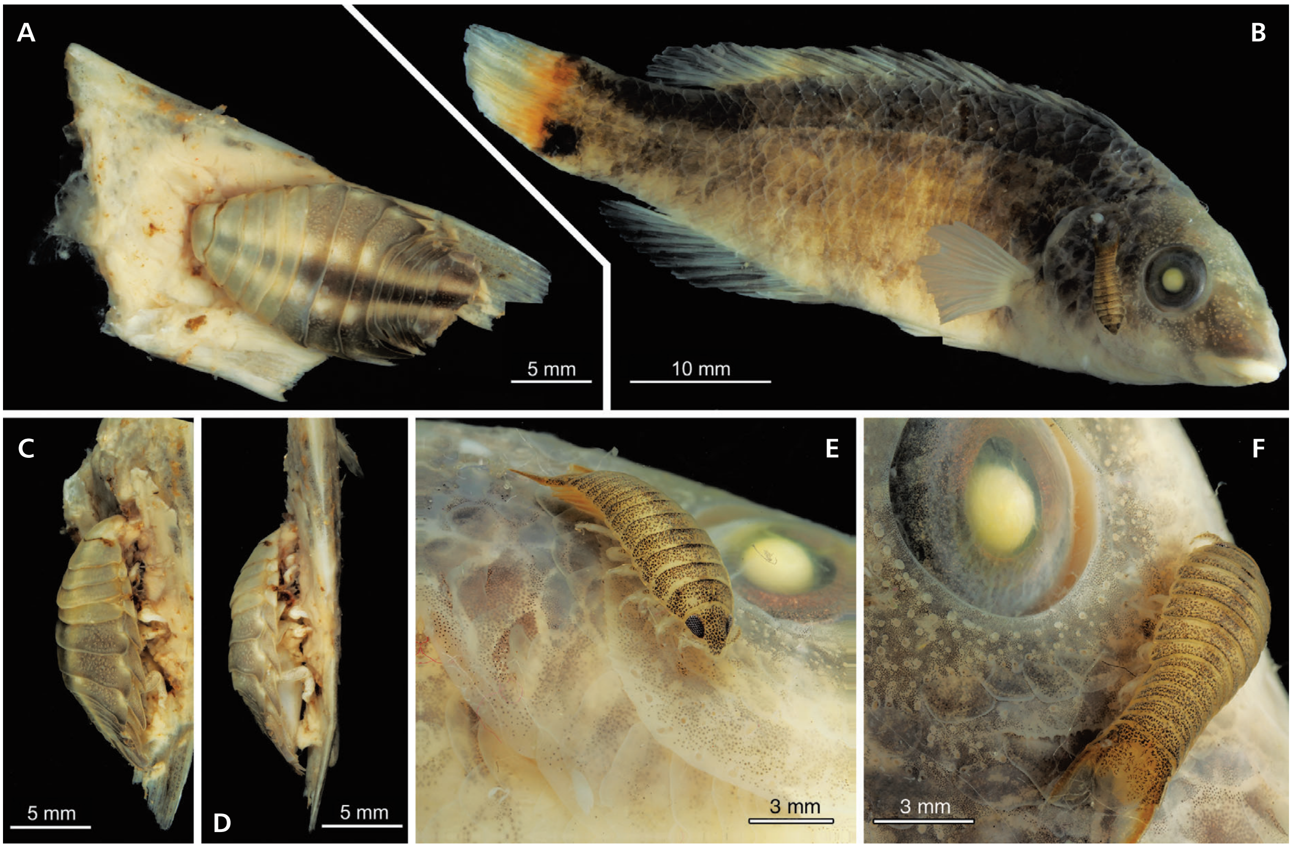

Figure 6. Macrophotographic images of two extant parasitic isopods. • A – dorsal view of *Nerocila acuminata* on the pelvic fin of a representative of Mugilidae (ZSMA 20159001). The pleotelson is absent. • B – *Symphodus cinereus* with one specimen of *Nerocila* sp. (ZSMA 20159002). • C – antero-lateral view of *Nerocila acuminata*. • D – postero-lateral view of *Nerocila acuminata*. • E – antero-lateral view of *Nerocila* sp. • F – detail of the posterior part of *Nerocila* sp. and one parasitic copepod behind pleotelson.

5) The pleon region is rather short and the pleopods do not appear to be prominent. Also the thoracopods do not appear to be adapted for swimming. Hence, it seems possible that the isopods were attached to the fish for an extended period of time

Given these indications, we see it as likely that the isopods do not represent scavengers, but parasites. We furthermore suppose that these parasites were attached to the host fish for an extended period of time during life.

Broader systematic interpretations

The preservation of the isopod specimens is rather poor and makes a clear systematic discussion, based on morphology alone, difficult. Yet, the type of parasitism indicated by the fossils can provide some hints for a slightly clearer systematic interpretation of the specimens.

Most parasitic isopods that are ectoparasites occur within five major groups: Cymothoidae, Aegidae, Bopyroidea, Cryptoniscoidea and Gnathiidae (Williams & Boyko 2012, Boyko *et al.* 2013), which are all ingroups of Cymothoida. Representatives of Cymothoidae and Gnathiidae prefer fishes as hosts, while species of Bopyridea and Cryptoniscoidea are generally found on decapod crustaceans. Species of Gnathiidae only parasitise fishes as larvae, whereas those of Cymothoidae and Aegidae infest fishes in larval and adult stages (Romestand *et al.* 1982, Lester 2005, McKiernan *et al.* 2005, Ravichandran *et al.* 2009, Wilson *et al.* 2011, Roche *et al.* 2013). Representatives of Cymothoidae are obligate parasites of both marine and freshwater fishes and are more or less permanently attached to their host fish, while representatives of Aegidae visit a host only for a short blood meal (*e.g.* Bowman 1981; like a "marine mosquito").

Based on the indication that the specimens described herein have been attached to their possible host fish more permanently, we see it as likely that they are early representatives of Cymothoidae. The principle tagmosis of the fossils is consistent with affinities to the isopod subgroup Cymothoida (Brandt & Poore 2003). Yet, many important morphological characters that could support a cymothoid affinity are not available in the fossils.

In many aspects the fossils more resemble modern representatives of Cirolanidae (non-parasitic forms, closely realted to Aegidae and Cymothoidae), including:

1) an elongated pereopodal basis (Figs 2, 3B) in contrast to a short and rather robust pereopodal basis in modern cymothoids,

2) the way pleomere 5 is laterally overlapped by pleomere 4 (Fig. 3D), in contrast to free lateral margins on all pleomeres in modern cymothoids,

3) the rather strongly vaulted body shape seen in lateral view in contrast to a rather flat dorsum of modern cymothoids,

4) the rather short dactyli of the posterior thoracopods, while these are longer and more scimitar-like in modern cymothoids.

Yet, as the fossil isopods indicate a parasitic lifestyle, including body deformations of some specimens in reaction to the host, we still see it as likely that the fossils are early representatives of the cymothoid lineage. It has been suggested that cymothoids evolved from cirolanid-like ancestors (Brusca 1981). Hence these fossils may represent the very first steps into the cymothoid lineage.

Within modern Cymothoidae, *Nerocila* is the sister group to all others. *Nerocila* is characterized by a functional head that is not "immersed in pereonite 1" (Brusca *et al*. 2001, p. 12), which would probably mean that the tergite of thorax segment 2 covers the head. In the fossils the functional head also appears to be free and not covered. Brusca (1981) suggested that the more derived forms of Cymothoidae evolved from a "*Nerocila*-like" ancestor. Hence, *Nerocila* retains numerous plesiomorphic characters and is therefore the best comparison for supposedly early cymothoids.

The morphology of our fossils can be roughly compared to that of representatives of *Nerocila*, yet the fossils lack at least the elongated dactyli and are less dorso-ventrally compressed. Due to the lack of these characters it is unlikely that our fossils are representatives of *Nerocila*. The most likely interpretation is therefore that the fossils represent offshoots of the lineage towards Cymothoidae below the node where *Nerocila* branches off.

Evolutionary history of Cymothoidae

The stem species (more or less equivalent to "last common ancestor") of Cymothoidae, which was specialised as a fish parasite with prehensile appendages, is considered to have evolved from a cirolanid-like ancestor *ca* 290 million years ago during the Permian (Brusca 1981). Aegiid-like forms, only temporarily attaching themselves to fishes, should have been present 250 million years ago, during the Permian-Triassic radiation in the Tethyan Sea (Schram 1977, Brusca 1981), although the fossil record only dates back to the Jurassic (Polz *et al*. 2006, Hansen & Hansen 2010). So far, no fossil evidence has been found for parasitising aegiids, but due to the temporary attachment such a find seems quite unlikely.

Brusca (1981) speculated that the evolution of cymothoids should have started after the Tethyan Sea radiation, with a *Nerocila*-like form with an obligatory parasitic lifestyle, permanently attached to the host and having lost the swimming ability. The specimens presented here are in concordance with such a suggestion and provide a more direct evidence for a form distantly reminding of *Nerocila* in the Jurassic some 150 million years ago.

Brusca (1981) and Smit *et al*. (2014) stated that Cymothoidae is one of the least understood and most troublesome groups within Isopoda. Smit *et al*. (2014, p. 192) stated that there is no fossil record for Cymothoidae, because "it is not possible to place fossil isopods without appendages into an extant family with any degree of confidence". Due to the special preservation of relevant morphological structures of the examined specimens, we interpret these isopod fossils as probable early representatives of Cymothoidae. This would represent the oldest known occurrence of this group. The fossil isopods described herein appear to be rare, but we assume that parasitic isopods might have been overlooked quite often during the preparation process, especially when considering that the isopods can be re-crystallized and appear like a simple crystalline blob (Fig. 1E).

Conclusions

The fossils described here are interpreted to represent:

– early cymothoid isopods, parasitising on fishes,

– the oldest fossil record for this group,

– the oldest direct evidence for isopods parasitising on vertebrates.

Our findings provide evidence that fossil parasitic isopods might be found in direct association with their hosts. Hopefully, fossil fishes recovered in the future will be carefully inspected for possible parasites before preparation so that they will be available for research.

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References

AGASSIZ, L. 1837–1843. *Recherches sur les poissons fossiles. 3. Contenant l'histoire de l'ordre des Placoïdes*. 422 pp. Petitpierre, Neuchâtel.

ARILLO, A. 2007. Paleoethology: fossilized behaviours in amber. *Geologica Acta 5*, 159–166.

BAUMILLER, T.K. & GAHN, F.J. 2002. Fossil record of parasitism on marine invertebrates with special emphasis on the platyceratid-crinoid interaction. *Paleontological Society Papers 8*, 195–210.

BLAINVILLE, H. DE 1818. Mémoire sur la classe des Sétipodes, partie des Vers à sang rouge de M. Cuvier, et des Annélides de M. de Lamarck. *Bulletin des Sciences, par la Société Philomatique de Paris 1818*, 78–85.

BOWMAN, T.E. 1960. Description and notes on the biology of *Lironeca puhi* n. sp. (Isopoda: Cymothoidae) parasite of the Hawaiian Moray Eel, *Gymnothorax eurostus* (Abbott). *Crustaceana 1*, 82–91. DOI 10.1163/156854060X00131

BOWMAN, T.E. 1981. *Thermosphaeroma milleri* and *T. smithi*, new sphaeromatid isopod crustaceans from hot springs in Chihuahua, Mexico, with a review of the genus. *Journal of Crustacean Biology 1*, 105–122. DOI 10.2307/1548208

BOYKO, C.B., MOSS, J., WILLIAMS, J.D. & SHIELDS, J.D. 2013. A molecular phylogeny of Bopyroidea and Cryptoniscoidea (Crustacea: Isopoda), *Systematics and Biodiversity 11*, 495–506. DOI 10.1080/14772000.2013.865679

BRACKEN-GRISSOM, H.D., AHYONG, S.T., WILKINSON, R.D., FELDMANN, R.M., SCHWEITZER, C.E., BREINHOLT, J.W., BENDALL, M., PALERO, F., CHAN, T.Y., FELDER, D.L., ROBLES, R., CHU, K.H., TSANG, L.M., KIM, D., MARTIN, J.W. & CRANDALL, K.A. 2014. Emergence of lobsters: phylogenetic relationships, morphological evolution and divergence time comparisons of an ancient group (Decapoda: Achelata, Astacidea, Glypheidea, Polychelida). *Systematic Biology 63(4)*, 1–23. DOI 10.1093/sysbio/syu008

BRANDT, A. & POORE, G.C.B. 2003. Higher classification of the flabelliferan and related Isopoda based on a reappraisal of relationships. *Invertebrate Systematics 17*, 893–923. DOI 10.1071/IS02032

BRETT, C.E. 1978. Hostspecific pitforming epizoans on Silurian crinoids. *Lethaia 11*, 217–231. DOI 10.1111/j.1502-3931.1978.tb01229.x

BRETT, C.E. 1985. *Tremichnus*: A new ichnogenus of circular parabolic pits in fossil echinoderms. *Journal of Paleontology 59(3)*, 625–635.

BRUSCA, R.C. 1981. A monograph on the Isopoda Cymothoidae (Crustacea) of the eastern Pacific. *Zoological Journal of the Linnean Society 73*, 117–199. DOI 10.1111/j.1096-3642.1981.tb01592.x

BRUSCA, R.C., COELHO, V. & TAITI, S. 2001. A Guide to the Coastal Isopods of California. *http://tolweb.org/notes/?note_id=3004*, downloaded November 2014.

BUNCKLEY-WILLIAMS, L., WILLIAMS, E.H. & BASHIRULLAH, A.K.M. 2006. Isopods (Isopoda: Aegidae, Cymothoidae, Gnathiidae) associated with Venezuelan marine fishes (Elasmobranchii, Actinopterygii). *Revista de Biological Tropical 54*, 175–188.

CARLE, E. 1969. *The very hungry caterpillar*. 22 pp. World Publishing Company, Cleveland.

CASTELLANI, C., MAAS, A., WALOSZEK, D. & HAUG, J.T. 2011. New pentastomids from the Late Cambrian of Sweden – deeper insight of the ontogeny of fossil tongue worms. *Palaeontographica, Abteilung A: Palaeozoology-Stratigraphy 293(4–6)*, 95–145.

CONWAY MORRIS, S. 1981. Parasites and the fossil record. *Parasitology 82*, 489–509. DOI 10.1017/S0031182000067020

CRESSEY, R. & BOXSHALL, G. 1989. *Kabatarina pattersoni*, a fossil parasitic copepod (Dichelesthiidae) from a lower Cretaceous fish. *Micropaleontology 35(2)*, 150–167. DOI 10.2307/1485466

DE BAETS, K., KLUG, C. & KORN, D. 2011. Devonian pearls and ammonoid-endoparasite coevolution. *Acta Palaeontologica Polonica 56*, 159–180. DOI 10.4202/app.2010.0044

DONOGHUE, M.J., DOYLE, J.A., GAUTHIER, J., KLUGE, A.G. & ROWE, T. 1989. The importance of fossils in phylogeny reconstruction. *Annual Review of Ecology and Systematics 20*, 431–460. DOI 10.1146/annurev.es.20.110189.002243

EDGECOMBE, G.D. 2010. Palaeomorphology: fossils and the inference of cladistic relationships. *Acta Zoologica 91*, 72–80. DOI 10.1111/j.1463-6395.2009.00426.x

FRICKHINGER, K.A. 1999. *Die Fossilien von Solnhofen. Dokumentation der aus den Plattenkalken bekannten Tiere und Pflanzen 2*. 333 pp. Goldschneck, Wiebelsheim.

GAO, T.P., SHIH, C.K., XU, X., WANG, S. & REN, D. 2012. Mid-Mesozoic flea-like ectoparasites of feathered or haired vertebrates. *Current Biology 22*, 732–735. DOI 10.1016/j.cub.2012.03.012

HANSEN, T. & HANSEN, J. 2010. First fossils of the isopod genus *Aega* Leach, 1815. *Journal of Paleontology 84*, 141–147. DOI 10.1666/08-083.1

HAUG, C., KUTSCHERA, V., AHYONG, S.T., VEGA, F.V., MAAS, A., WALOSZEK, D. & HAUG, J.T. 2013. Re-evaluation of the Mesozoic mantis shrimp *Ursquilla yehoachi* based on new material and the virtual peel technique. *Palaeontologia Electronica, http://palaeo-electronica.org/content/2013/495-ursquilla-and-virtual-peel*, 16.2.5T.

HAUG, C., MAYER, G., KUTSCHERA, V., WALOSZEK, D., MAAS, A. & HAUG, J.T. 2011. Imaging and documenting gammarideans. *International Journal of Zoology*, 380829. DOI 10.1155/2011/380829

HAUG, C., VAN ROY, P., LEIPNER, A., FUNCH, P., RUDKIN, D.M., SCHÖLLMANN, L.& HAUG, J.T. 2012a. A holomorph approach to xiphosuran evolution – a case study on the ontogeny of *Euproops*. *Development Genes and Evolution 222*, 253–268. DOI 10.1007/s00427-012-0407-7

HAUG, J.T., HAUG, C., MAAS, A., KUTSCHERA, V. & WALOSZEK, D. 2010. Evolution of mantis shrimps (Stomatopoda, Malcostraca) in the light of new Mesozoic fossils. *BMC Evolutionary Biology 10*, 290. DOI 10.1186/1471-2148-10-290

HAUG, J.T., MAYER, G., HAUG, C. & BRIGGS, D.E.G. 2012b. A Carboniferous non-onychophoran lobopodian reveals long-term survival of a Cambrian morphotype. *Current Biology 22*, 1673–1675. DOI 10.1016/j.cub.2012.06.066

KLOMPMAKER, A.A., ARTAL, P., VAN BAKEL, B.W.M., FRAAIJE, R.H.B. & Jagt, J.W.M. 2014. Parasites in the fossil record: a Cretaceous fauna with isopod-infested decapod crustaceans, infestation patterns through time, and a new ichnotaxon. *Plos One 9*, e92551. DOI 10.1371/journal.pone.0092551

Klompmaker, A.A. & Boxshall, G. 2015. Fossil Crustaceans as Parasites and Hosts. *In* De Baets, K. & Littlewood, T. (eds) *Advances in Parasitology*. Elsevier, Amsterdam. DOI 10.1016/bs.apar.2015.06.001

Klompmaker, A.A., Schweitzer, C.E., Feldmann, R.M. & Kowalewski, M. 2013. The influence of reefs on the rise of Mesozoic marine crustaceans. *Geology 41*, 1179–1182. DOI 10.1130/G34768.1

Leach, W.E. 1818. Cymothoadées, 338–354. *In* Cuvier, F. (ed.) *Dictionnaire des sciences naturelles, Vol. 12*. F.G. Levrault & Le Normant, Paris & Strasbourg.

Lester, R.J.G. 2005. Isopoda, 138–144. *In* Rhode, K. (ed.) *Marine Parasitology*. CSIRO Publishing, Clayton South.

Lincoln, R.J. 1971. Isopod fish parasites. *Marine Observations 41*, 184–186.

Maas, A. & Waloszek, D. 2001. Cambrian Derivates of the early arthropod stem lineage, Pentastomids, Tardigrades and Lobopodians – an "Orsten" perspective. *Zoologischer Anzeiger 240*, 451–459. DOI 10.1078/0044-5231-00053

McKiernan, J.P., Grutter, A.S. & Davies, A.J. 2005. Reproductive feeding ecology of parasitic gnathiid isopods of epaulette sharks (*Hemiscyllium ocellatum*) with consideration of their role in the transmission of a haemogregarine. *International Journal for Parasitology 35*, 19–27. DOI 10.1016/j.ijpara.2004.10.016

Muenster, G. Graf zu 1832. *Ueber die Planuliten und Gonititen im Uebergangs-Kalk des Fichtelgebirges*. 38 pp. Birner, Bayreuth.

Nagler, C. & Haug, J.T. 2015 (accepted). From fossil parasitoids to vectors: Insects as parasites and hosts. *In* De Baets, K. & Littlewood, T. (eds) *Advances in Parasitology*. Elsevier, Amsterdam.

Perrichot, V., Beaucournu, J.C. & Velten, J. 2012. First extinct gens of a flea (Siphonaptera: Pulicidae) in Miocene amber from the Dominican Republic. *Zootaxa 3438*, 54–61.

Poinar, G.P. 1985. Fossil evidence of insect parasitism by mites. *International Journal of Acarology 11*, 37–38. DOI 10.1080/01647958508683393

Polz, H. 2004. Asselansammlung auf einer Wasserwanze aus den Solnhofener Plattenkalken. *Archaeopteryx 22*, 51–60.

Polz, H., Schweigert, G. & Maisch, M.W. 2006. Two new species of *Palaega* (Isopoda: Cymothoida: Cirolanidae) from the Upper Jurassic of Swabian Alb, South Germany. *Stuttgarter Beiträge zur Naturkunde 362*, 1–17.

Radwanska, U. & Poirot, E. 2010. Copepod-infest Bathonian (Middle Jurassic) echinoids from northern France. *Acta Geologica Polonica 60*, 549–555.

Radwanska, U. & Radwanski, A. 2005. Myzostomid and copepod infestation of Jurassic echinoderms: A general approach, some new occurrences, and/or re-interpretation of previous reports. *Acta Geologica Polonica 55*, 109–130.

Ravichandran, S., Rameshkumar, G. & Kumaravel, K. 2009. Variation in the morphological features of isopod fish parasites. *World Journal of Fish and Marine Sciences 1*, 137–140.

Roche, D.G., Strong, L.E. & Binning, S.A. 2013. Prevalence of the parasitic cymothoid isopod *Anilocra nemipteri* on its fish host at Lizard Island, Great Barrier Reef. *Australian Journal of Zoology 60*, 330–333. DOI 10.1071/ZO12130

Romestand, B., Thuet, P. & Trilles, J.P. 1982. Some aspects of nutritional mechanisms in the isopod *Ceratothoa oestroides* Risso, 1826 parasite of fishes. *Annales de Parasitologie Humaine et Comparee 57*, 79–89.

Rust, J. 2007. Die Bedeutung von Fossilien für phylogenetische Rekonstruktionen. *Species, Phylogeny and Evolution 1*, 73–87.

Schram, F.R. 1977. Paleozoography of late Paleozoic and Triassic Malacostraca. *Systematic Biology 26*, 367–379.

Schweigert, G. 2007. Ammonite biostratigraphy as a tool for dating Upper Jurassic lithographic limestones from South Germany – first results and open questions. *Neues Jahrbuch der geologischen paläontologischen Abhandlungen 245*, 117–125.

Serrano-Sánchez, M.D.L., Nagler, C., Haug, C., Haug, J.T., Centeno-García, E. & Vega, F.J. in review. The first fossil record of larval stages of parasitic isopods: cryptoniscus larvae preserved in Miocene amber. *Neues Jahrbuch für Geologie und Paläontologie*.

Smit, J.N., Bruce, N.L. & Hadfield, K.A. 2014. Global diversity of fish parasitic isopod crustaceans of the family Cymotoidae. *International Journal for Parasitology: Parasites and Wildlife 3*, 188–197.

Strong, L. 2012. Effects of a cymothoid ectoparasite on the turning behavior (lateralization) of the bridled monocle bream *Scolopsis bilineata*. *Independent Study Project (ISP) Collection 1267*.

Thiollière, V. 1852. Troisième notice sur les gisements à poissons fossiles situes dans le Jura du department de l'Ain. *Annales (Extrait des Proces-Verbaux), des Societe royale d'Agriculture, d'Histoire naturelle et Arts utiles de Lyon 4*, 352–446.

Tsai, M.L., Li, J.J. & Dai, C.F. 1999. Why selection favors protandrous sex change for the parasitic isopod, *Ichthyoxenus fushanensis* (Isopoda: Cymothoidae). *Evolutionary Ecology 13*, 327–338. DOI 10.1023/A:1006784330895

Walossek, D. & Müller, K.J. 1994. Pentastomid parasites from the Lower Palaeozoic of Sweden. *Transactions of the Royal Society of Edinburgh: Earth Sciences 85(01)*, 1–37. DOI 10.1017/S0263593300006295

Walossek, D., Repetski, J.E. & Müller, K.J. 1994. An exceptionally preserved parasitic arthropod, *Heymonsicambria taylori* n.sp. (Arthropoda incertae sedis: Pentastomida), from Cambrian–Ordovician boundary beds of Newfoundland, Canada. *Canadian Journal of Earth Sciences 31(11)*, 1664–1671. DOI 10.1139/e94-149

Williams, E.H. Jr. & Williams, L.B. 1985. *Cuna insularis* n. gen. and n. sp. (Isopoda: Cymothoidae) from the gill chamber of the sergeant major, *Abudefduf saxatilis* Linnaeus (Osteichthyes) in the West Indies. *Journal of Parasitology 71*, 209–214. DOI 10.2307/3281904

Williams, J.D. & Boyko, C.B. 2012. The global diversity of parasitic isopods associated with crustacean hosts (Isopoda: Bopyroidea and Cryptoniscoidea). *PLoS ONE 7*, e35350. DOI 10.1371/journal.pone.0035350

Wilson, G.D.F, Paterson, J.R. & Kear, B.P. 2011. Fossil isopod associated with a fish skeleton from the Lower Cretaceous of Queensland, Australia – direct evidence of a scavenging lifestyle in Mesozoic Cymothoida. *Palaeontology 54*, 1053–1068. DOI 10.1111/j.1475-4983.2011.01095.x

Yesmin, S. & Khanum, H. 2011. On the studies of parasites infestation in *Clarias batrachus* (Linneaeus) and *Clarias gariepinus* (Burchell). *Proceedings of 22nd National Congress on Parasitology, 30.10.2010–01.11.2010, West Bengal, India*, 252–259.