Methods of detection, collection and preservation of parasitic isopods (Isopoda: Epicaridea)

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Abstract.—Epicarideans, parasitic isopods found in and on other crustaceans, are cryptic in nature. Detecting and collecting these parasites can be difficult and requires special techniques. The present paper reviews these techniques, providing helpful information on locating the isopods and isolating them from their hosts. In addition, information on preservation of these animals is provided. Using these methods, ecologists and other researchers could provide critical material for future studies on the life histories and evolutionary relationships of these parasites.

Keywords: Bopyroidea, Cryptoniscoidea, ectoparasite, endoparasite

All species of isopods found parasitic on other crustaceans are members of the superfamilies Bopyroidea and Cryptoniscoidea, that together form the Epicaridea within the suborder Cymothoida (Boyko et al. 2013). Bopyroidea is monophyletic and contains Bopyridae (at ∼600 spp. one of the most speciose of all isopod families, all ectoparasitic on decapod hosts), Ionidae (8 spp., ectoparasitic on ghost shrimps) and Entoniscidae (∼40 spp., endoparasitic in decapods) (Williams & Boyko 2012). In species of Bopyroidea, a female develops as a moderately (Bopyridae, Ionidae) to extremely (Entoniscidae) modified form with a large marsupium for maximizing brood size, whereas males are little modified from the isopod *bauplan* (Figs. 1–3). Cryptoniscoidea contains 9 families with ∼150 spp. of parasites on a wide variety of crustacean hosts: amphipods, isopods, mysids, decapods, barnacles (both free living and parasitic), and ostracods (Williams & Boyko 2012). An additional 16 species are described but unplaced as to family. A few of these species are from identified hosts (nebaliaceans, cumaceans, ascothoracican barnacles), but most are known only from cryptoniscus larval stages obtained from plankton samples. In most Cryptoniscoidea, these larvae either develop into females, which are usually sac-like forms devoid of nearly all isopod characters and superficially resembling rhizocephalan barnacles (Fig. 1A), or become sexually functional males while retaining their larval appearance (neoteny; Fig. 2F). However, in Dajidae (∼50 spp., ectoparasitic on shrimp, mysid, and euphausiid hosts) and Entophilidae (2 spp., endoparasitic in squat lobsters and ghost shrimps), the females and males more closely resemble those in Bopyroidea and the cryptoniscus larvae metamorphose into morphologically distinct males (Fig.
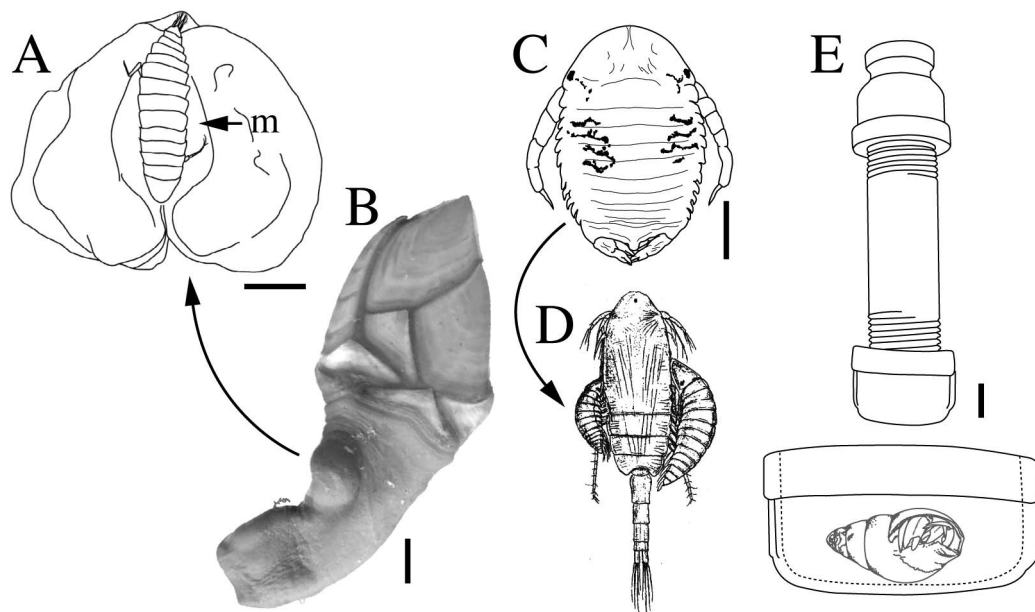
Fig. 1. Representative bopyroid and cryptoniscoid isopods and the mortar and pestle apparatus. A) The cryptoniscoid *Scalpelloniscus vomicus* Hosie, 2008 (Hemioniscidae), female and male pair; m denotes male parasite. B) The host barnacle *Smilium zancleanum* (Seguenza, 1876) with gall formed in peduncle by female *S. vomicus*. C) The epicaridium larva of the athelgine *Athelges takanoshimensis* Ishii, 1914. D) A copepod intermediate host parasitized by early and late microniscus larvae. E) Mortar and pestle constructed of galvanized steel for cracking shells and extracting hermit crabs to examine for presence of parasites. (A, B from Hosie 2008; C from Cericola & Williams 2015; D from Sars 1899). Scale bars: A, E = 1 cm; B = 0.5 mm; C = 0.05 mm; rest not to scale.

3G; see also Williams & Boyko 2012). The systematics of Cryptoniscoidea is poorly understood and, at present, the families are primarily defined on the basis of host taxa, rather than intrinsic characters of parasite morphology. Some cryptoniscoids are primary parasites, whereas others (e.g., Cabiropidae, Fig. 2A–C) are hyperparasitic, mostly on bopyrids (Boyko 2013).

In both superfamilies, the parasite life cycle requires two hosts: an intermediate calanoid copepod host and another, non-copepod, crustacean definitive host. Eggs in the female marsupium hatch into epicaridium larvae (Fig. 1C) that seek out the intermediate hosts. Upon contact with a copepod host, the larva metamorphoses into a microniscus stage that feeds on hemolymph (Fig. 1D). After this feeding period, the microniscus larva detaches from the copepod and transforms into a free-swimming cryptoniscus larva (compare to Fig. 2F). The cryptoniscus larva seeks out a definitive host whose identity depends on the species of parasite involved. Overviews of morphology and biodiversity in these groups are given by Trilles (1999) and Williams & Boyko (2012); discussions regarding phylogeny can be found in Boyko & Williams (2009) and Boyko et al. (2013).

Habitats

The habitats of these parasitic isopods are as diverse as those of their hosts. Most species are known from relatively shallow waters, although a few records of bopyrids are known from 4100–5210 m (Boyko et al. 2012). Both benthic and pelagic hosts are known, with distribution patterns of the parasites influenced by a combination
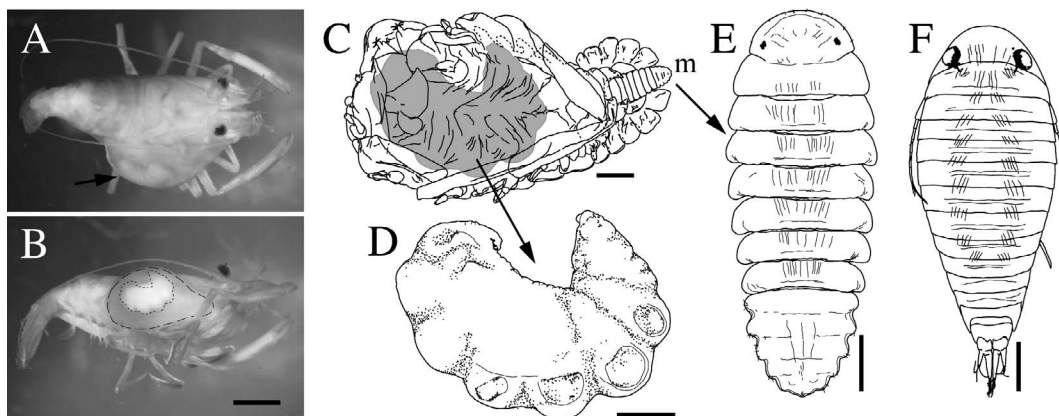
Fig. 2. Representative bopyroid and cryptoniscoid isopods. A) The bumblebee shrimp *Gnathophyllum americanum* Guérin-Méneville, 1855 harboring the branchial bopyrid *Schizobopyrina bombyliaster* Williams & Boyko, 2004 (Bopyrinae) that is in turn hyperparasitized with the cryptoniscoid *Cabirops bombyliophyla* Williams & Boyko, 2004 (Cabiropidae), dorsal view; arrow shows swollen branchial chamber and opaque outline of hyperparasite. B) *G. americanum* with *S. bombyliaster* (outline shown with long dashed line) and *C. bombyliophyla* (shown with short dashed line). C) Female *S. bombyliaster* removed from host, ventral view with position of female hyperparasite *C. bombyliophyla*, shown in grey. D) Female *C. bombyliophyla*. E) Male of *S. bombyliaster*, dorsal view. F) Male of *C. bombyliophyla*, dorsal view; this stage is nearly morphologically indistinguishable from the cryptoniscus larva. (A–F from Williams & Boyko 2004. Scale bars: D, C = 0.5 mm; A, B = 1 mm; E, F = 0.15 mm.

of definitive host and intermediate host (copepod) distributions. It is unclear how many species of crustaceans are infected by these parasites, but there are many undescribed epicaridean species, especially in Cryptoniscoidea.

Collecting

Collecting these parasitic isopods is a two or three-step process. First, the host must be procured, and the methods for this depend on the habitats of the host. In many cases, bopyrids infect only a small percentage of the hosts (generally less than 5%), but much higher prevalences are known (Cericola & Williams 2015); cryptoniscoids can be more variable in their prevalence based on host species, spatial distribution of hosts, and season (Blower & Roughgarden 1988). Ecological studies typically require large number of hosts to adequately sample for the parasites in a given area. Care must therefore be taken to look for developmental stages of the parasite on the definitive hosts, as newly settled cryptoniscus larvae and even juvenile females are easily overlooked. For paguroids inhabiting gastropod shells, the hosts must also be extracted from their shells prior to examination. Various methods, such as inserting a small wire or piece of fishing line through a drilled hole in the shell and lightly prodding the crab's abdomen (e.g., Brooks 1988, Damiani 2003, Pechenik et al. 2015), are known to induce live hermit crabs into moving out of their shells; however, processing large, previously preserved samples requires a faster method. A mortar and pestle constructed of galvanized steel (Fig. 1E) can be used to crack the shells; either by hand in the case of weak shells or with a hammer for strong shells. Whereas a vise can often damage the hermit crab occupying the shell, the mortar and pestle typically causes the shells to crack along weak points of the shell sutures and leaves the host undamaged. In addition, the mortar
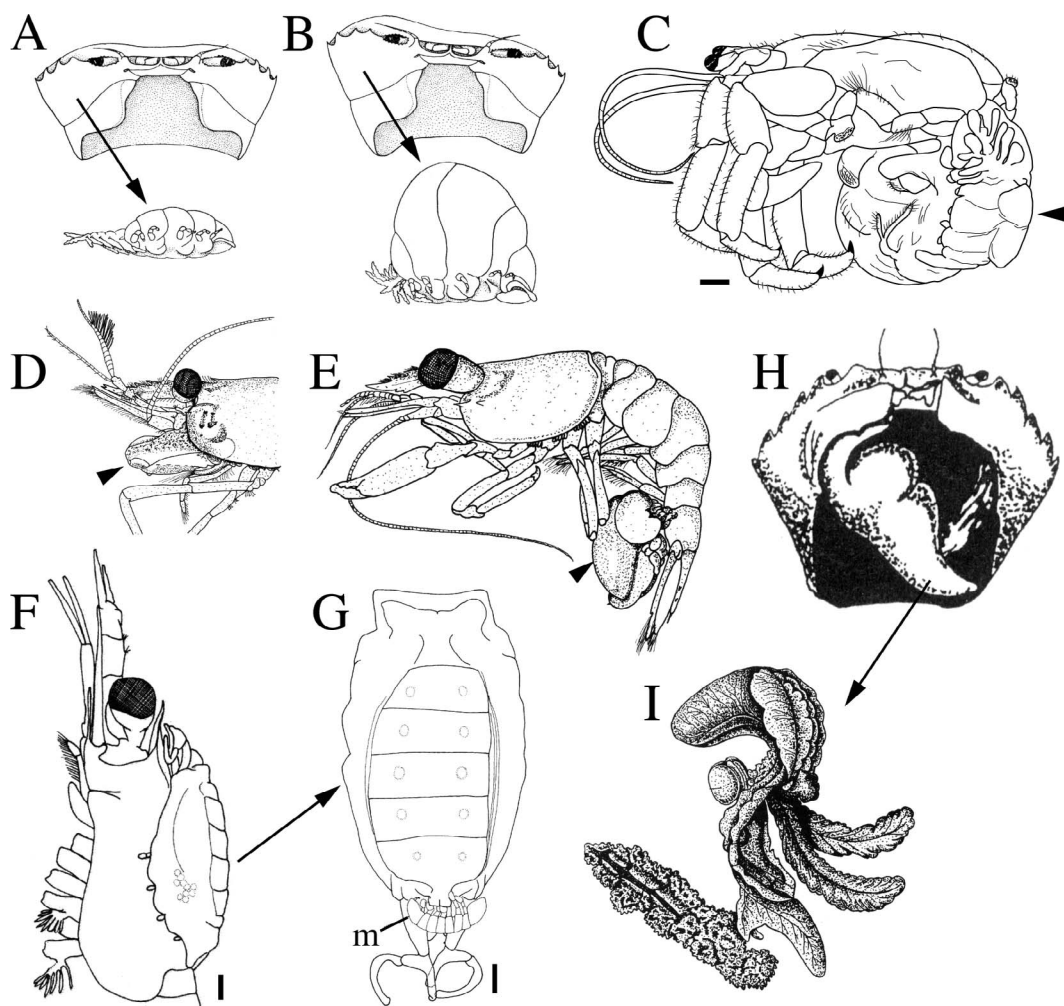
Fig. 3.   Representative bopyroid and cryptoniscoid isopods. A) The carapace of the host crab *Pilumnus* sp. with immature female bopyrid *Cancricepon elegans* Giard & Bonnier, 1887 (Keponinae) removed. B) The carapace of the host crab *Pilumnus* sp. with mature female bopyrid *C. elegans* removed. C) The hermit crab *Calcinus minutus* Buitendijk, 1937 extracted from its shell with the abdominal bopyrid *Athelges takanoshimensis* attached, shown by arrowhead. D) The hemiarthrine *Orophryxus shiinoi* Bruce, 1972 attached to host shrimp, *Cuapetes grandis* (Stimpson, 1860), shown by arrowhead. E) The hemiarthrine *Mesophryxus ventralis* Bruce, 1973 attached to host shrimp, *Harpiliopsis beaupresii* (Audouin, 1826), shown by arrowhead. F) The dajid *Heterophryxus appendiculatus* G. O. Sars, 1885 attached to its host euphausid *Euphasia recurva* Hansen, 1905. G) *H. appendiculatus*, dorsal view of female and male pair removed from host; m denotes male parasite. H) The carapace of the host crab *Carcinus maenas* (Linnaeus, 1758) with the entoniscid *Portunion maenadis* (Giard, 1886) shown *in situ*. I) Female *P. maenadis* removed from host and sheath. (A, B from Bourdon 1968; C from Cericola & Williams 2015); D, E, H, I from Trilles 1999; F, G modified from Shimomura & Ohtsuka 2008). Scale bars: C = 1 mm; F, G = 0.3 mm; rest not to scale.

acts as a receptacle that will contain the small parasites that can be easily lost; removal of the threading allows the contents of the mortar to be rinsed directly into a glass dish for examination.

The larval stages of parasitic isopods found on copepod intermediate hosts (Fig. 1D) are poorly known but can be observed in plankton samples; unfortunately, identification to species is not feasible in most

cases due to the lack of knowledge on the microniscus and cryptoniscus stages (but see Owens & Rothlisberg 1995). Female epicarideans attract cryptoniscus larvae (presumably through pheromones), and live hosts harboring females could be used to collect larvae for life cycle studies.

Most bopyrid isopods of the subfamilies Bopyrinae, Pseudioninae, Keponinae, Argeiinae and Orbioninae, as well as those in Ionidae, cause a swelling in the branchial chamber of the host organism; however, these parasites can be overlooked early in development unless the carapace of the host is examined carefully (Figs. 2A, B, 3A, B). In contrast, species in the bopyrid subfamilies Hemiarthrinae, Phyllodurinae and Athelginae are mostly abdominal parasites on caridean shrimps, mud shrimps, and anomuran crabs and, although easily visible, cause no swellings of the host cuticle (Fig. 3C–E). Fortunately these abdominal parasites tend to remain *in situ* on the hosts, either because they are protected by the gastropod shell and have reflexed dactyli that grip the soft cuticle of the hermit crab abdomen (most athelgines) or have minute sucker-like discs on the first oostegites (hemiarthrines) that presumably function in keeping the parasite attached to its shrimp host (Boyko 2012). Dajids, adults of which are very similar to bopyrids in many aspects despite belonging to a different superfamily, are the taxon for which we have the poorest knowledge of the hosts for many species because they occur mainly on the dorsal surface of the cephalothorax of their pelagic hosts (Boyko & Williams 2012) and are easily dislodged when the hosts are caught, usually via trawl net. Some (e.g., species of *Heterophryxus*) have modifications to their posterior pereopods that allow them to firmly grip the antennae of their hosts; these species are less likely to be dislodged upon host capture (Fig. 3F, G). Entoniscids and entophilids can sometimes be detected externally via color changes in the abdominal wall, swelling of abdominal somites, and the atrophied hepatopancreas of their hosts (Markham & Dworschak 2005); but for shrimp and crab hosts, removal of the carapace is usually necessary to determine the presence of even the largest parasites (Fig. 3H, I). Entophilids, entoniscids, and some other bopyrids are known to cause parasitic castration of female hosts and to influence the secondary sexual characteristics of male hosts (O'Brien & Van Wyk 1985, Markham & Dworschak 2005), thus findings of feminized males or intersexuality in the form of broadened abdomens or reduced chelae of males may be useful in detecting these parasites.

Most cryptoniscoid isopods can be difficult to detect, as the females are degenerate and most males are essentially sexually mature cryptoniscus larvae. This is especially true if the taxon is hyperparasitic and the female may be mistaken for the brood inside the marsupium of the host (Fig. 2C, D). Most of the cryptoniscoids are brood-pouch parasites, being found in the marsupia of many peracarids, ostracods and mysids, as well as in the mantle cavities of sessile, pedunculate, and rhizocephalan barnacles (Williams & Boyko 2012). Cryptoniscoids are known to cause parasitic castration of hosts; within hermaphroditic barnacles they sterilize the female gonad and thus leave them functionally male (Blower & Roughgarden 1988). Although many cryptoniscoids produce no evidence of their presence in the host externally, some are known to produce bulges or galls in the host (Fig. 1A, B). These brood and mantle parasites have no specialized attachment structures and are found free inside the occupied space. In contrast, cryptoniscoids attaching to the external surface of their hosts (most often rhizocephalans and decapods) have anchoring adaptations, either in the forms of hook-like processes (*Danalia* spp.) or by inserting their bodies through the cuticle of the host with half the parasite inside and half outside (*Liriopsis* spp.). Some female cryptoniscoids are so reduced that they resemble rhizocephalans, and have been confused with them in the literature.

The fossil record of epicarideans is mostly limited to carapace swellings seen on hosts, mostly squat lobsters and brachyurans, usually attributed to epicaridean infestation and dating back to the Jurassic (Klompmaker et al. 2014). The most likely source of these swellings is bopyrids, although some extant entoniscids also produce carapace swellings. Klompmaker et al. (2014) erected an ichnotaxon, *Kanthyloma crusta*, to accommodate all such carapace swellings in fossil hosts, although this act has been somewhat controversial (see Donovan 2015, Klompmaker & Boxshall 2015). The first body fossils of epicarideans were recently described from Miocene amber, but they are cryptoniscus larvae whose superfamilial affinities are indeterminate (Serrano-Sánchez et al., 2016).

Preservation

Today, most newly collected samples of organisms have at least a part of their anatomy preserved for genetic analysis. Isopods are no exception to this, and numerous publications on the genetics of isopods have resulted from this line of research. Although many of these papers deal with genetics at the population level (e.g., Wares et al. 2007), some have attempted to address long-standing questions about phylogeny within isopod families, the major isopod lineages, or the placement of the isopods within peracarids (e.g., Held 2000, Brandt & Poore 2003, Spears et al. 2005). The epicaridean isopods have not been well represented until recently (Boyko et al. 2013) and even now only a small fraction of described species have corresponding molecular sequences, almost all of which are from 18S rDNA. Examination of isopods in museum collections shows that many are still being fixed in formalin and transferred to alcohol for preservation, usually accompanied by their hosts. It is unclear why this is still such a prevalent method of treatment for these parasitic isopods, rather than the preferable method of placing specimens immediately in 70% ethanol (for gross morphological study) or 100% ethanol (for molecular study). One possible explanation is a perception that, as parasites, these are organisms that will preserve poorly without first being fixed. This is not correct, as the chitinous exoskeleton stabilizes the morphology of the specimens, even in the case of the extremely modified (entoniscid) or reduced (cryptoniscoid) females. The gross morphology of the external structures is well defined even if fixative is omitted. If histological work is needed, then a small portion of the organism can be removed for genetic work before fixation, so long as it is unambiguously labeled as being associated with the specimen from which it was extracted. If eggs and/or larvae are present in the marsupium of a female, they may also be preserved in 100% ethanol for genetic work, although care must be taken to ensure that larvae are those of the parent and not larvae of a hyperparasitic species! Additionally, due to their small sizes, male bopyroids and cryptoniscoids should always be placed into separate glass vials, as they are susceptible to being lost. However, it should be noted that when a female bopyrid is hyperparasitized by cryptoniscoids, usually no male bopyrid is present.

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