Journal of Wildlife Diseases, 38(2), 2002, pp. 473–477

First Report of an Endoparasitic Epicaridean Isopod Infecting Cephalopods

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ABSTRACT: This is the first report of an epicaridean isopod infecting cephalopods. Three cryptoniscus larvae (Isopoda, Epicaridea) were recognized externally and found entirely embedded in the oral bulb at the beginning of the esophagus of two female Patagonian squid (*Loligo gahi*) sampled in North Peruvian and South Chilean waters, respectively. There was an extremely low prevalence (<2%) and mean intensity (one) of infection which we believe indicated an accidental infection. However, the tissue location indicated successful penetration and colonization of the squid host.

Key words: Chilean waters, epicaridean cryptoniscus, Isopoda, *Loligo gahi,* Patagonian squid, Peruvian waters.

In the marine environment, ubiquitous Isopoda occur from littoral to abyssal zones with numerous parasitic representatives in crustaceans, fishes, and a few other animals. Cephalopods harbor a wide variety of micro- and macro-parasites which cause mild to severe systemic infections (Pascual et al., 1996a; Abollo et al., 1998; Gestal et al., 1999; López-González et al., 2000), and they have occasionally been reported to host ectoparasitic isopods in the mantle cavity and on their body surface (see review by Hochberg, 1990). There are few published reports on isopods associated with cephalopods; they have always been considered accidental or temporary infections. Though rare, cymothoid isopods have been found on squids of the species *Loligo* and *Sepioteuthis,* the bobtail squid *Sepiola,* the oceanic squid *Abraliopsis,* and cuttlefish of the genus *Sepia,* all from the Atlantic and Pacific Oceans and the Mediterranean Sea (Hochberg, 1990; Bello and Mariniello, 1998). This note reports the first epicaridean isopod truly infecting cephalopods.

A total of 234 specimens of the Patagonian squid *L. gahi* were examined. This sample comprised three subsamples: 82 (34 males, 47 females and 1 unsexed) specimens caught in Peru; 94 (78 males, 16 females) caught off Chile, and 58 (35 males, 23 females) caught off the Falkland Islands (UK). Sizes varied from 83 to 201 mm dorsal mantle length (ML). Individuals were taken from random samples obtained from commercial trawl landings at Paita (northern Peru) in October 1997, Talcahuano (southern Chile) in March 1998, and the Falkland Islands in April 1997. All individuals were frozen, transported to the laboratory, and preserved at −18 C. After thawing at room temperature, sex and maturity of each individual was determined according to Boyle and Ngoile (1993). The isopods were removed from host tissues with a dissecting microscope after removing the mandible and radula from the oral bulb. Parasites were preserved in 70% ethanol. A semi-permanent mount was made in lactophenol and sealed with a rapid embedding agent (Entellan, Merck KGaA, Darmstadt, Germany). For scanning electron microscopy (SEM), other specimens were postfixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7, then in 1% OsO_4 in the same buffer. After dehydration they were subsequently critical-point dried, mounted, coated with gold-palladium with a sputter coater (Polaron SC500, Quorum Technologies, Ltd., East Sussex, England), and observed with a Philips XC-30 electron microscope (Koninklijke Philips Electronics N. V., Eindhoven, The Netherlands).

A total of three isopods (specimens maintained at the Instituto de Investigaciones Marinas, Vigo, España) were found in two mature female squids (139 and 175 mm ML) from Paita (5°01′S; 81°05′W), and one was found in a mature male (128 mm ML) at Talcahuano (36°45′S; 73°05′W). All the isopods were discovered entirely embedded as minute opaque external marks in the oral bulb at the beginning of the esophagus surrounded by a membrane of host origin. Prevalence (2% at Paita; 1% at Talcahuano; 0% at the Falkland Islands) and mean intensity of infection (one at Paita and Talcahuano) were low.

The isopods were somewhat damaged in the process of collecting them from host tissue. Nevertheless, the general external morphologic features were characteristic of cryptoniscus larva of Epicaridea. The body was enlarged, 1.57–1.78 mm total length (measured from the anterior border of the head-segment to the base of the uropods) and 0.35–0.40 mm in breadth (Fig. 1a, b). They possessed an irregular double row of large, dark brown, diffuse chromatophores down the dorsum. The cephalon was larger than pereonites and these larger than pleonites. Pereonites were similar in size but pleonites unequal in width and length, decreasing towards the pleotelson. The cephalon (Fig. 1c) possessed a pair of antennules (Fig. 1d) comprised of a three-segmented basal peduncle and distal flagella (Fig. 1e). The cephalon also possessed a pair of larger antennae (Fig. 1c) formed by four articles and a segmented flagellum. The cephalic mouthpart was prognate (Fig. 1f) bordered anteriorly by the labrum. Each of the seven somites of the pereon possessed a pair of uniramous pereopods consisting of seven articles. Pereopods were stout, with strongly hooked dactylus, the form of the hook being nearly straight, with the tip bent down almost at right angles (Fig. 2a). The pleonal appendages took the form of five pairs of biramous pleopods (Fig. 2b) and one pair of subterminal biramous uropods (Fig. 2c). The endopodite and exopodite of pleopods bore four or five long setae. The pleotelson was markedly triangular. Uropods were long with a basal segment bearing exopodite and endopodites equal in shape and size with four terminal lobules and setae (Fig. 2d).

The suborder Epicaridea consists of isopods parasitic on crustaceans in both larval and adult stages. This suborder is divided into two superfamilies, the Bopyrina (Bopyridae, Dajidae, and Entoniscidae) with oostegites and the Cryptoniscina (Cryptoniscidae) without oostegites, both of which are parasitic on crustaceans (Naylor, 1972).

Epicarideans are particularly interesting because their life cycles involve two hosts and because sexes may be determined epigenetically. The first larval stage resembles the epicaridium, a small isopod, and possesses piercing and sucking mouth parts and claw-like appendages with which it attaches itself to the surface of free-swimming copepods. On its copepod host, it undergoes six successive molts (ecdyse) and changes progressively into two distinct larval stages known as the microniscus and the cryptoniscus stages. On reaching the latter, swimming-stage of development, the parasite leaves its copepod host, proceeds to the sandy or muddy sea bottom, and there seeks a final host, a free-living crab or shrimp into whose branchial chamber or brood pouch it enters. Within this second host, in the Bopyrina, the cryptoniscus stage develops into a bopyridium (Naylor, 1972).

Though accidental, the tissue location of crytoniscus specimens found in our material is not surprising and can be largely explained by the feeding pattern of the squid in the sampling area (Guerra et al., 1991; Portela and Rasero, 1998). The Patagonian squid is an active opportunistic predator on euphausids which may be infected (Table 1). It is likely that the cryptoniscus larvae thus infect the squid through infected crustacean prey. Guerra et al. (1991) reported that at least some of the euphausids eaten by *L. gahi* were mature specimens of 45–50 mm total length. Adult euphau-

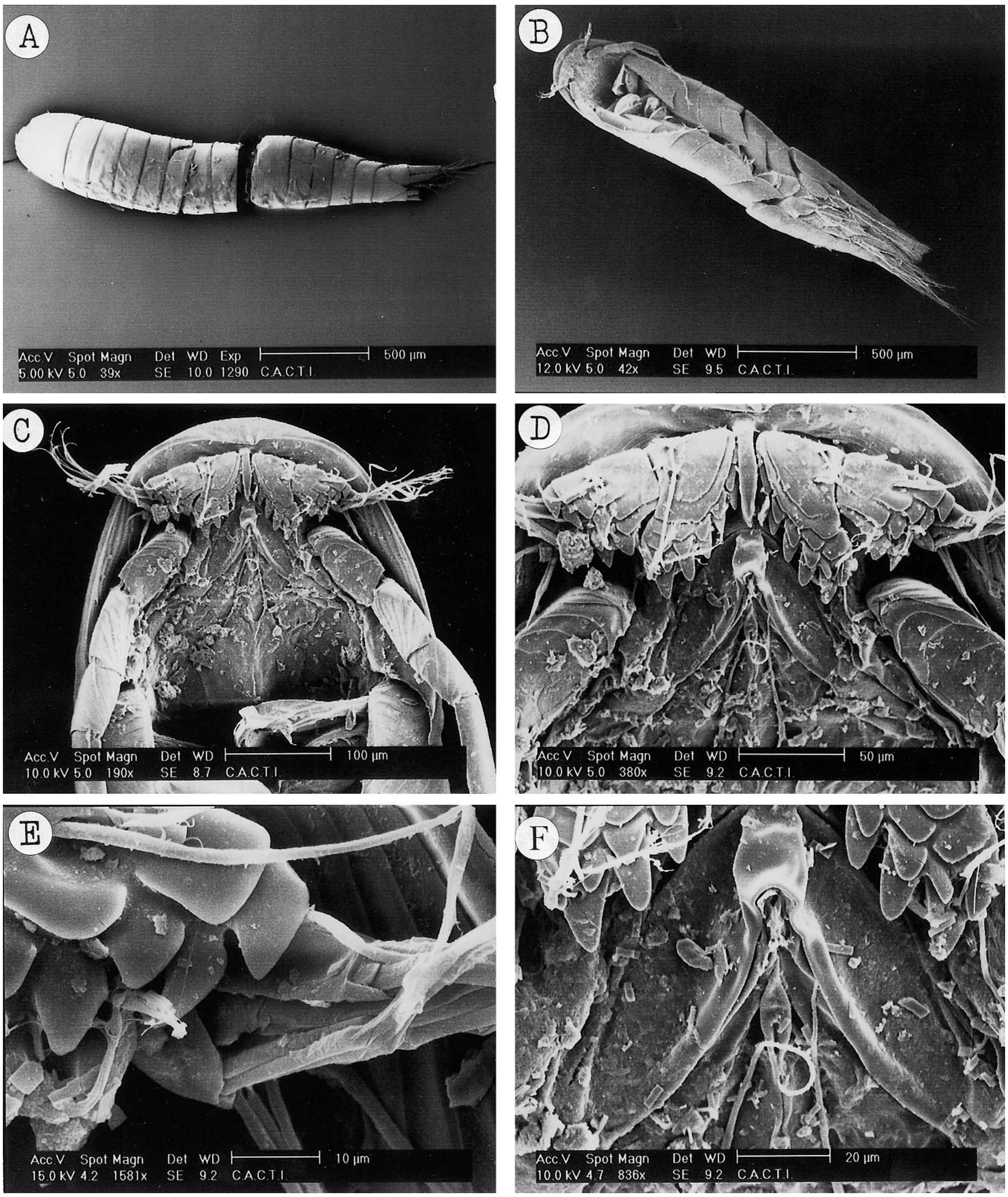

FIGURE 1. Cryptoniscus larvae. Dorsal (a) and ventral (b) view; cephalon (c) bearing a pair of antennules (d, e), antennae and mouthpart (f).

sids are bitten into fragments short enough to enter the buccal cavity and esophagus. Undamaged parasites could then enter the digestive tract (buccal mass) with the euphausid fragments. The cryptoniscus may then penetrate the buccal mass of the squid. The discovery of isopods living in host tissue (i.e., a previously unknown endoparasitic phase) suggest that they may have been deriving something nutritionally from the squid. Mechanical penetration and feeding activity of the isopods could produce host tissue damage. Though presence of the isopod in the oral bulb prob-

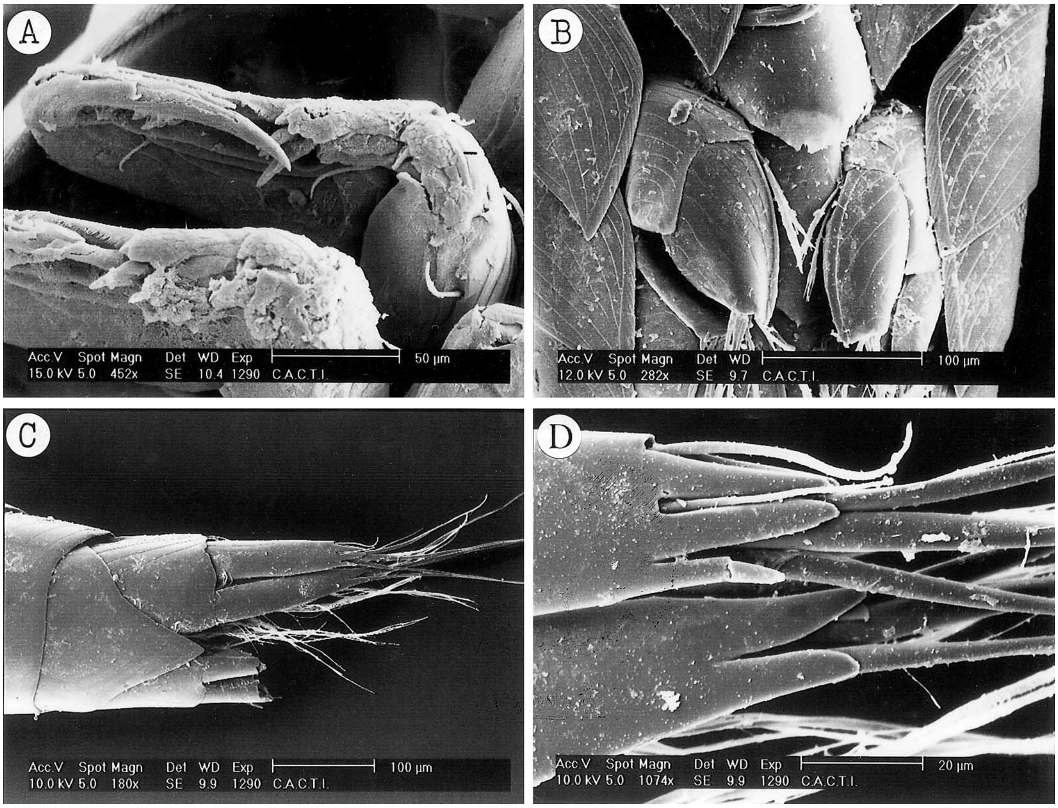

FIGURE 2. First pereopod (a); pleopods (b); pleotelson and uropods (c); terminal blobules and setae of exopod and endopod uropodites (d).

ably would not interfere with food intake, it could affect mastication by destroying the musculature that exert the mechanical activity of host mandible and radula (Mangold, 1983). This should be examined by histopathologic examination of infected squids.

Hanlon and Forsythe (unpubl. data cited in Hochberg, 1990) noted an unidentified isopod attached toward the distal end of the mouth of a mature male of the tropical nearshore squid *Sepioteuthis lessoniana* cultured for 175 days. The isopod was introduced into the culture tank along with live infected fish fed daily to the squid. The interchange of parasites between cephalopods and sympatric animals is common and occurs in wild and culture conditions (Leong and Holmes, 1981; Pascual et al., 1996b). The prolonged periods of time the isopod can attach to continuously swimming squids suggests such relationships may be common in nature (Hochberg, 1990).

TABLE 1. Euphausids associated with *Loligo gahi* in the sampling area.

	Sampling area		
	Peru	Chile	Falklands
Euphausia superba		X	X
Euphausia triacantha		X	X
Euphausia vallentini	X	X	X
Thysanoessa macrura		X	X

These isopods probably have been overlooked in squids due to their minute size and in many instances lack of color. The taxonomic status of more specimens of the parasite and determination of the host-parasite relationships should be studied.

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Received for publication 12 February 2001.