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NOTE

Occurrence of *Psorospermium haeckeli* in the stone crayfish *Austropotamobius torrentium* from a population naturally mixed with the noble crayfish *Astacus astacus*

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ABSTRACT: The crayfish parasite '*Psorospermium haeckeli*', which supposedly includes several species, is reported for the first time from the stone crayfish *Austropotamobius torrentium*. The infected specimens were from the Kammlach river in Bavaria (Germany), a place where *A. torrentium* and the noble crayfish *Astacus astacus* have coexisted for more than 20 yr. Screening of the mixed crayfish population for *P. haeckeli* revealed that both species were infected with the elongate morphotype of *P. haeckeli* which is typical of *A. astacus*. The distribution pattern of the parasite was similar in both species; most spores were in the collagenous wall of the blood vessels and the subepidermal connective tissue underneath the carapace. In the noble crayfish there was practically no immune response against *P. haeckeli*, whereas in the stone crayfish ca 10% of the spores were encapsulated by haemocytes, and approximately 1% were melanised. Since stone crayfish collected from other creeks were free of *P. haeckeli*, we presume that the parasite may have been transmitted to *A. torrentium* from *A. astacus*.

KEY WORDS: *Psorospermium haeckeli* · *Astacus astacus* · *Austropotamobius torrentium* · Crayfish Parasite

Psorospermium haeckeli is an organism of unknown taxonomic identity and life cycle which is frequently found in the connective tissue of freshwater crayfish (Alderman & Polglase 1988). Although it is not clear whether *P. haeckeli* causes damage to the host, it is generally referred to as a parasite. *P. haeckeli* seems to have been associated with crayfish from early times of radiation because it has been detected in geographically very distant locations in species of all families of the Astacidea, the Astacidae, Cambaridae and Parastacidae. Aside from crayfish, *P. haeckeli* has been found only once in tissues of the freshwater amphipod *Gammarus lacustris* from a lake in the Baikal region in Russia (Voronin 1975).

In Europe, *Psorospermium haeckeli* has been documented as occurring in native crayfish species *Astacus astacus* in Germany (Haeckel 1857, Scheer 1934), Poland (Grabda 1934), Sweden (Ljungberg & Monné 1968, Cerenius & Söderhäll 1993), Finland (Nylund & Westman 1978) and Norway (Taugbøl & Skurdal 1992), and *Astacus leptodactylus* in France (Vey 1978), Poland (Krucinska & Simon 1968) and Turkey (Fürst & Söderhäll 1987). It was detected also in American species *Pacifastacus leniusculus* introduced into France (Vey 1978), Spain (Diéguez-Uribeondo et al. 1993) and Sweden (Unestam 1973), and *Orconectes limosus* introduced into Poland (Krucinska & Simon 1968). In America, the parasite was found in the indigenous species *Orconectes virilis* in Alberta, Canada (Aiken 1989), *Orconectes immunis* and *Orconectes rusticus* in Minnesota and Wisconsin, USA (Henttonen et al. 1994), *Procambarus clarkii* and *Procambarus zonangulus* in Louisiana, USA (Henttonen et al. 1992), *Procambarus alleni* and *Procambarus fallax* in Florida, USA (Henttonen et al. 1994), *Procambarus clarkii* from Texas, USA (Lee et al. 1985) and in *Pacifastacus leniusculus* from western USA (Henttonen et al. 1994). In Australia, *P. haeckeli* was detected in *Cherax tenuimanus* and *Cherax quinquecarinatus* in Western Australia (Owens & Evans 1989) and *Cherax quadricarinatus* in Queensland (Herbert 1987).

Based on significant differences in size, morphology and mode of embedding into the connective tissue of the host, at least 4 different morphotypes of *Psorospermium haeckeli* can be distinguished, 2 in Europe, 1 in America and 1 in Australia. *Astacus astacus* can harbour both European types, an ovoid spore of ca 100 × 60 × 60 µm with lipid globules of very heterogenous diameter and a narrow envelope of connective tissue around the spore (Nylund et al. 1983, Henttonen et al. 1992, Vogt & Rug 1995) and a more elongate spore of ca 150 × 50 × 50 µm with small, uniform lipid globules and a broad envelope (Haeckel 1857, Grabda 1934, Rug & Vogt 1995). Both morphotypes develop

within the connective tissue of the crayfish from small, membrane-bound stages (Rug & Vogt 1994, 1995). The existence of separate developmental lines (Rug & Vogt 1995) and the abovementioned data on the variety of species infected and on the geographical distribution of the parasite suggest that the term '*Psorospermium haeckeli*' may include several species.

Thus far, the occurrence of *Psorospermium haeckeli* has been documented in Germany only in *Astacus astacus* (Haeckel 1857, Scheer 1934, Rug & Vogt 1995). A further paper by Scheer (1979) reported on *P. haeckeli* in *Orconectes limosus*, but the parasite was found only within the lumen of the digestive tract and not in tissues of the crayfish. Nothing was known of the presence or absence of *P. haeckeli* in the 2 other native German crayfish species (Troschel & Dehus 1993), the stone crayfish *Austropotamobius torrentium* and the white-clawed crayfish *Austropotamobius pallipes*, in Germany or in other countries.

Materials and methods. Specimens of the noble crayfish *Astacus astacus* (Linnaeus, 1758) and *Austropotamobius torrentium* (Schrank, 1803), both belonging to the family Astacidae (Crustacea, Decapoda), were collected in August 1995 from the Kammlach river north of Loppenhausen (District of Schwaben, Bavaria, Germany). The Kammlach is a tributary of the Danube. At the trapping area, about 500 m above sea level, the river meanders through cultivated meadows and is lined either by grass and weeds or by bushes and small trees. The river bed is approximately 5 m wide and 0.5 to 1.5 m deep with some deeper hollows. The earthen river banks are mostly steep and either bare or covered with grass, other plants or roots. The river bottom is gravel with smaller patches of sand and mud.

Sampling of both crayfish species was done at night with 80 'pirate traps' (Bock-Ås, Parainen, Finland) over a distance of ca 600 m. From this catch we removed 3 *Astacus astacus* and 3 *Austropotamobius torrentium* for screening for *Psorospermium haeckeli* Hilgendorf, 1883. This was done by scraping subepidermal connective tissue inclusive of blood vessels from the inner side of the carapace. These samples were examined either as fresh mounts or after treatment with 10% KOH. In addition, we examined the hepatopancreas, antennal gland, gills, musculature, testis, vas deferens, nerve cord and intestine. Photographic records of the results were made using a Leitz Aristoplan microscope. For comparison, we have included some micrographs (Fig. 1) of *P. haeckeli* from *A. astacus* collected in earlier years from ponds and streams in the District of Schwaben, where the Kammlach is located.

Results. In earlier samples of *Astacus astacus* from ponds and streams in the District of Schwaben we found both European morphotypes of *Psorospermium haeckeli* (Fig. 1a–c). The crayfish harboured either the elongate morphotype (Fig. 1b), the ovoid morphotype (Fig. 1c) or both of them (Fig. 1a). Most of the spores were embedded in the collagenous wall of the dorsal thoracic arteries (Fig. 1a) or in the subepidermal connective tissue underneath the carapace. Both morphotypes had an architecture characterised by a 3-layered shell with solid outer plates and many globules in the interior. They differed with respect to size, shape, embedding in the host connective tissue and appearance of the globules. The spore of the elongate morphotype had a size of roughly 150 × 50 × 50 µm, was enclosed by a broad envelope of connective tissue (Fig. 1b), and included many small lipid globules, ca 10 to 20 larger non-lipid globules, and 2 closely associated nuclei as revealed by histochemical investigations (Rug & Vogt 1995). The ovoid type measured ca 100 × 60 × 60 µm, had a less distinct and much narrower envelope of connective tissue, and also comprised 10 to 20 non-lipid globules of medial size and 2 closely associated nuclei (Vogt & Rug 1995) but had very heterogenous lipid globules (Fig. 1c).

In the August 1995 trapping we caught 403 crayfish in the Kammlach river with 80 traps on a stretch of about 600 m, 369 *Astacus astacus* (224 males, 145 females) and 34 *Austropotamobius torrentium* (14 males, 20 females). In 46 traps we found only *A. astacus*, in 5 traps only *A. torrentium*, in 21 traps both species, and 8 traps were empty. The smallest-sized *A. astacus* caught had a total length of 62 mm (14 g); the heaviest was almost 200 g. The smallest *A. torrentium* in the traps had a total length of 63 mm and a weight of 9 g. The maximal total length was 93 mm and the maximum weight 49 g.

The 3 male *Astacus astacus* examined for *Psorospermium haeckeli* had a total length of 91, 104 and 109 mm and a weight of 32, 46 and 60 g, respectively. All 3 crayfish were moderately infected with the elongate morphotype of *P. haeckeli* (Fig. 2a–f). Around 50 to 150 spores per crayfish were found in the connective tissue and blood vessels scraped from the inner side of the carapace, and only a few spores were distributed among other tissues. As usual in *A. astacus*, this elongate morphotype displayed considerable variation in shape and size. Approximately 70% had the typical elongate shape displayed in Fig. 2a. Others were somewhat broader (Fig. 2b), elliptic (Fig. 2c), ovoid, slightly curved or even heavily curved like a boomerang (Fig. 2d). Occasionally, we found developmental stages of the parasite (Fig. 2e–f). The youngest stage was limited by a membrane only but was already enclosed by the typical broad envelope (Fig. 2e). More mature stages showed various degrees of shell differentiation (Fig. 2f). Encapsulation of the spores by haemocytes or melanisation was not observed in the 3 noble crayfish examined.

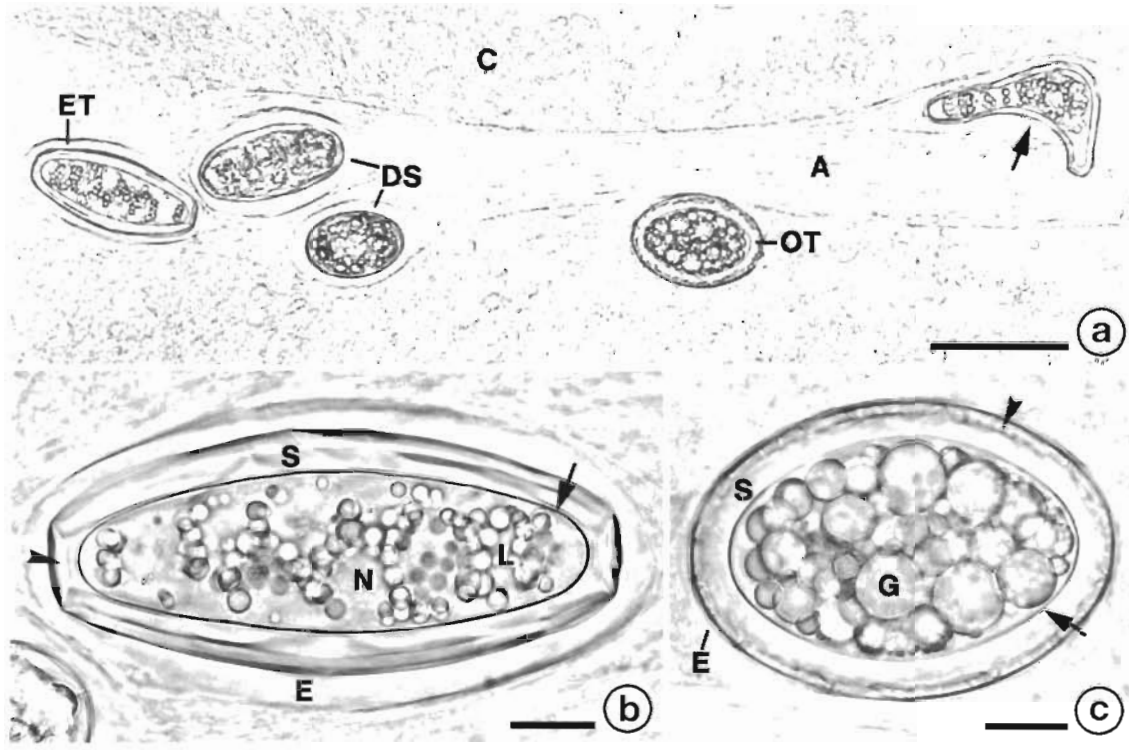

Fig. 1. Morphotypes of *Psorospermium haeckeli* in *Astacus astacus* from ponds and streams of the District of Schwaben. (a) Artery (A) with both elongate (ET) and ovoid type (OT) of *P. haeckeli*. C: connective tissue; DS: developmental stages of elongate type; arrow: aberrant variant of elongate type. (b) Mature spore of elongate morphotype with broad envelope (E) of connective tissue, many small and rather homogenous lipid globules (L) and a few, larger non-lipid globules (N). S: 3-layered shell; arrow: inner shell layer; arrowhead: outer shell layer of solid plates. (c) Mature spore of ovoid morphotype with narrow envelope and very heterogenous globules (G). Arrow: inner shell layer; arrowhead: shell plate. Fresh (a) and KOH-treated (b, c) mounts. Scale bars = 100 µm (a), 20 µm (b, c)

The 3 male stone crayfish investigated had a total length of 65, 92 and 93 mm and a weight of 11, 49 and 35 g, respectively. The 92 mm, 49 g specimen was totally free of *Psorospermium haeckeli*. The smallest individual (65 mm) had roughly 50 parasites of the elongate morphotype in the thoracic subepidermal connective tissue including blood vessels. The 93 mm crayfish was rather heavily infected. Around 150 spores of the elongate type were located in the connective tissue and blood vessels underneath the carapace. Some further 50 spores were detected in the connective tissue along the vas deferens and around the anterior part of the gut. Only a few spores were found in the antennal gland, hepatopancreas, gills and nerve cord. As in *Astacus astacus* most spores had the typical elongate shape (Fig. 3a), but others were spindle-shaped (Fig. 3b), banana-like (Fig. 3c), rhombus-like (Fig. 3d), elliptic (Fig. 3e) or ovoid (Fig. 3f). Developmental stages of the parasite were also present in smaller numbers (Fig. 3g), particularly in the walls of the blood vessels and around the anterior part of the gut. Around 10% of the spores were encapsulated by haemocytes, and around 1% were additionally melanised (Fig. 3h).

Discussion. For the stone crayfish *Austropotamobius torrentium* there are no data published thus far on infections with *Psorospermium haeckeli*. If the stone crayfish is susceptible to the parasite, one should most likely find it in specimens living sympatrically with a species which is almost regularly infected with *P. haeckeli* such as *Astacus astacus*. As suspected, 2 of the 3 stone crayfish examined from the mixed population of *A. torrentium* and *A. astacus* in the Kammlach harboured the same elongate morphotype of *P. haeckeli* as the cohabiting noble crayfish. One stone crayfish was free of the parasite. Since we have found no *P. haeckeli* at all in stone crayfish from 2 tributaries of the Rhine river, the Steinach and Steinbach, we presume that, in the Kammlach, *P. haeckeli* may have been transmitted to *A. torrentium* from *A. astacus*.

The distribution pattern of *Psorospermium haeckeli* in the body of *Austropotamobius torrentium* was like that in *Astacus astacus* (Vogt & Rug 1995). The highest number of spores was found in the collagenous walls of the blood vessels and the surrounding subepidermal connective tissue underneath the carapace. But the rate of encapsulation and melanisation of the parasite

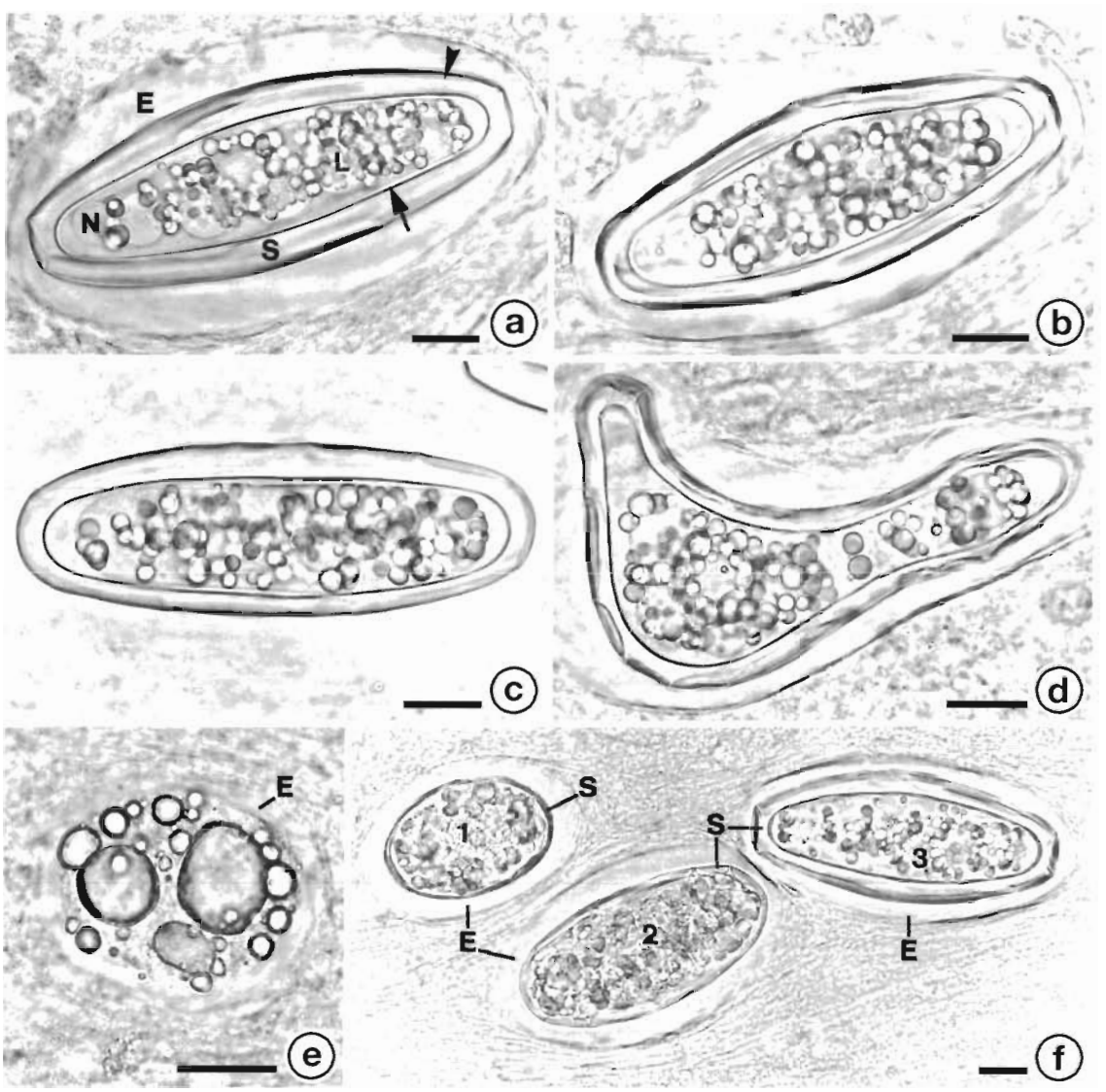

Fig. 2. Elongate morphotype of *Psorospermium haeckeli* in *Astacus astacus* from the Kammlach river. (a) Most frequent variant. (b) Shorter and somewhat broader variant. (c) Elliptic variant. (d) Boomerang variant. (e) Very young developing stage without shell. (f) Young (1), intermediate (2) and mature (3) stages displaying different degrees of shell formation. E: envelope of connective tissue; L: lipid globules; N: non-lipid globule; S: shell; arrow: inner shell layer; arrowhead: shell plate. Fresh (e, f) and KOH-treated (a–d) mounts. Scale bars = 20 µm

was much higher in *A. torrentium* than in *A. astacus*, which may indicate that the parasite is more adapted to the noble crayfish than to the stone crayfish. A rather intense immune response against the elongate morphotype was also reported for *Astacus leptodactylus* (Vranckx & Durliat 1981).

There is still controversy as to whether *Psorospermium haeckeli* can cause damage to crayfish populations. Söderhäll (1988) reported decreased catches in Swedish lakes but only when parasite numbers were high. Moreover, Cerenius et al. (1991) have shown that *P. haeckeli* has a measurable impact on the immune system of crayfish. They concluded that the parasite could contribute to failure of the immune system if the crayfish are subjected to other infections or stress (Söderhäll & Cerenius 1992). Because of these data and considerations, we strongly recommend against stocking stone crayfish in the wild without prior examination for *P. haeckeli* and other pathogens, particularly if autochthonous populations are present in the stocking area.

Currently, *Psorospermium haeckeli* has been clearly documented as occurring in 15 crayfish species, the 14 species listed in the 'Introduction' and in *Austropotamobius torrentium*. Data are lacking for some further 450 crayfish species distributed worldwide (Hobbs

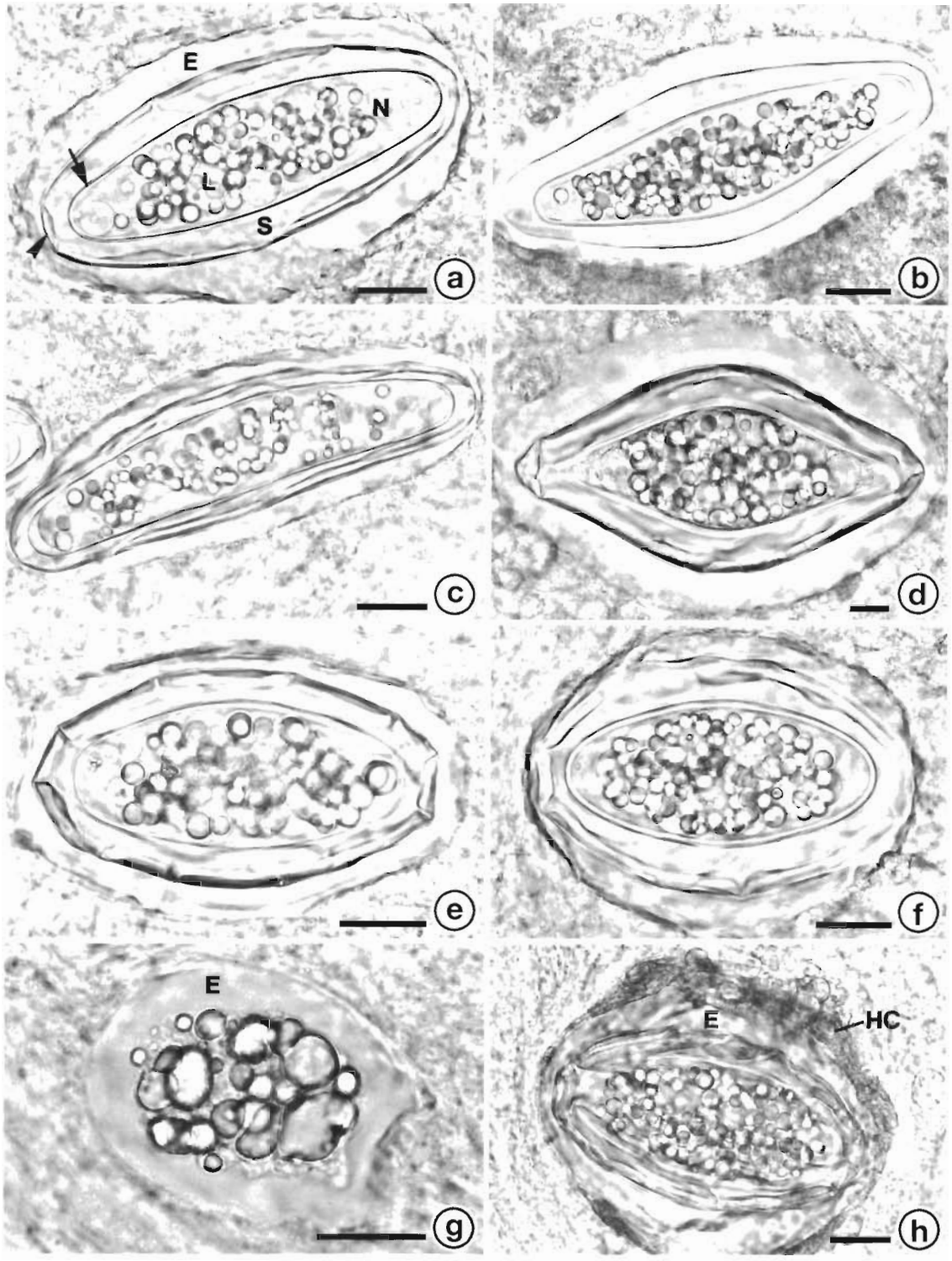

Fig. 3. Elongate morphotype of *Psorospermium haeckeli* in *Austropotamobius torrentium* from the Kammlach river. (a) Most frequent variant. (b) Spindle-shaped variant. (c) Banana-like variant. (d) Rhombus-shaped variant. (e) Elliptic variant. (f) Egg-shaped variant. (g) Very young developing stage. (h) Encapsulated and melanised spore. E: envelope of connective tissue; HC: haemocytic capsule; L: lipid globules; N: non-lipid globule; S: shell; arrow: inner shell layer; arrowhead: shell plate. Fresh (b, d, f–h) and KOH-treated (a, c, e) mounts. Scale bars = 20 µm

1988). A better database on the biogeographical distribution of the parasite, the range of species infected and possible interspecific transmissions could contribute significantly to the resolution of the century-old enigma called *'Psorospermium haeckeli'*.

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LITERATURE CITED

Aiken D (1989) Marron fungus? (Answer to a letter to the editor). Wld Aquaculture 20(2):6–7

Alderman DJ, Polglase JL (1988) Pathogens, parasites and commensals. In: Holdich DM, Lowery RS (eds) Freshwater crayfish. Biology, management and exploitation. Croom Helm, London, p 167–212

Cerenius L, Henttonen P, Lindqvist OV, Söderhäll K (1991) The crayfish pathogen *Psorospermium haeckeli* activates the prophenoloxidase activating system of freshwater crayfish in vitro. Aquaculture 99:225–233

Cerenius L, Söderhäll K (1993) The distribution of *Psorospermium haeckeli* in Sweden; a preliminary survey. Freshwat Crayfish 9:280–285

Diéguez-Uribeondo J, Pinedo-Ruiz J, Cerenius L, Söderhäll K (1993) Presence of *Psorospermium haeckeli* (Hilgendorf) in a *Pacifastacus leniusculus* (Dana) population of Spain. Freshwat Crayfish 9:286–288

Fürst M, Söderhäll K (1987) The crayfish *Astacus leptodactylus* in Turkey. Diseases and present distribution of the crayfish plague fungus, *Aphanomyces astaci*. FAO Report, Rome, Italy

Grabda E (1934) Recherches sur un parasite de l' écrevisse (*Potamobius fluviatilis* L.) connu sous le nom de *Psorospermium haeckeli* Hlgd. Mém Acad Pol Sci Lett, Cracovie (Sér B) 6:123–142

Haeckel E (1857) Ueber die Gewebe des Flusskrebses. Arch Anat Physiol Wiss Med (Müller's Archiv) 24:469–568

Henttonen P, Huner JV, Lindqvist O (1994) Occurrence of *Psorospermium* sp. in several North American crayfish species, with comparative notes on *Psorospermium haeckeli* in the European crayfish, *Astacus astacus*. Aquaculture 120:209–218

Henttonen P, Lindqvist OV, Huner JV (1992) Incidence of *Psorospermium* sp. in several cultivated populations of crayfishes, *Procambarus* spp. (Decapoda, Cambaridae), in Southern Louisiana. J Wld Aquacult Soc 23:31–37

Herbert B (1987) Notes on diseases and epibionts of *Cherax quadricarinatus* and *C. tenuimanus* (Decapoda: Parastacidae). Aquaculture 64:165–173

Hobbs HH (1988) Crayfish distribution, adaptive radiation and evolution. In: Holdich DM, Lowery RS (eds) Freshwater crayfish. Biology, management and exploitation. Croom Helm, London, p 52–82

Krucinska J, Simon E (1968) On the parasites and epibionts of the branchial cavity in crayfish at Wroclaw and vincinity. Przegl Zool 12:288–290 (in Polish)

Lee BJ, Sis RF, Lewis DH, Marks JE (1985) Histology of select organs of the crawfish *Procambarus clarkii* maintained at various temperatures and levels of calcium and ammonia. J Wld Maricult Soc 16:193–204

Ljungberg O, Monné L (1968) On the eggs of an enigmatic nematode parasite encapsulated in the connective tissue of the European crayfish, *Astacus astacus* in Sweden. Bull Off Int Epiz 69:1231–1235

Nylund V, Westman K (1978) *Psorospermium haeckeli*, a parasite on the European crayfish, *Astacus astacus*, found in Finland. Freshwat Crayfish 4:385–390

Nylund V, Westman K, Lounatmaa K (1983) Ultrastructure and taxonomic position of the crayfish parasite *Psorospermium haeckeli* Hilgendorf. Freshwat Crayfish 5:307–314

Owens L, Evans L (1989) Common diseases of freshwater prawns (*Macrobrachium*) and crayfish (marron and yabbies) relevant to Australia. In: Postgraduate Committee in Veterinary Science (ed) Invertebrates in aquaculture. Refresher course for veterinarians. University of Sydney, Australia. Proceedings 117:227–240

Rug M, Vogt G (1994) Developmental stages of the crayfish parasite *Psorospermium haeckeli* in thoracic arteries of *Astacus astacus*. J Invertebr Pathol 64:153–155

Rug M, Vogt G (1995) Histology and histochemistry of developing and mature spores of two morphotypes of *Psorospermium haeckeli*. Freshwat Crayfish 10:374–384

Scheer D (1934) Über einen mutmaßlichen Blutgefäßparasiten des Flußkrebses (*Potamobius astacus*). Z Parasitenkd 6:478–480

Scheer D (1979) *Psorospermium orconectis* n. sp., ein neuer Parasit in *Orconectes limosus*. Arch Protistenkd 121. 381–391

Söderhäll K (1988) Fungal parasites and other diseases on freshwater crayfish. In: Kovanen J, Lappalainen R (eds) Raputalous 2000, Keski-Suomen Kalastuspiin Kalastustoimisto, Vol 5. Tiedotus, Jyväskylä, Finland, p 23–46

Söderhäll K, Cerenius L (1992) Crustacean immunity. A Rev Fish Dis 2:3–23

Taugbøl T, Skurdal J (1992) Parasitten *Psorospermium haeckeli* påvist i norske krepsebestander. Fauna 45:32–39

Troschel HJ, Dehus P (1993) Distribution of crayfish species in the Federal Republic of Germany, with special reference to *Austropotamobius pallipes*. Freshwat Crayfish 9:390–398

Unestam T (1973) Significance of diseases on freshwater crayfish. Freshwat Crayfish 1:135–150

Vey A (1978) Recherches sur une maladie des écrevisses due au parasite *Psorospermium haeckeli* Hilgendorf. Freshwat Crayfish 4:411–418

Vogt G, Rug M (1995) Microscopic anatomy and histochemistry of the crayfish parasite *Psorospermium haeckeli*. Dis Aquat Org 21:79–90

Voronin VN (1975) On findings of *Psorospermium* sp. in *Gammarus lacustris*. Izv Gos Nauchno-Issled Inst Ozern Rechn Rybn Khoz (Leningrad) 93:125–126 (in Russian)

Vranckx R, Durliat M (1981) Encapsulation of *Psorospermium haeckeli* by the haemocytes of *Astacus leptodactylus*. Experientia 37:40–42

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