Description of *Genesis vulcanoctopusi* gen. et sp. nov. (Copepoda: Tisbidae) parasitic on a hydrothermal vent octopod and a reinterpretation of the life cycle of cholidyinid harpacticoids

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Abstract: Juveniles and adult females of *Genesis vulcanoctopusi* gen. et sp. nov. (Copepoda: Harpacticoida) are described from the deepwater octopus *Vulcanoctopus hydrothermalis*, collected from a hydrothermal vent in the East Pacific Rise. The new genus is placed in the Tisbidae and is readily distinguishable from other Cholidyinae by the combination of body shape, structure of oral appendages and segmentation of legs 1-5. All stages were found embedded in the integument of the head and mantle of the cephalopod host, suggesting an endoparasitic copepodid phase. The generalized life cycle of cholidyinid harpacticoids is reinterpreted in the light of this discovery. Aspects of parasitism and pathological effects of *G. vulcanoctopusi* on the octopod host are discussed.

Résumé : *Description de* Genesis vulcanoctopusi gen. et sp. nov. *(Copepoda: Tisbidae), parasite d'un octopode des sources hydrothermales et réinterprétation du cycle biologique des Harpacticoides Cholidyinae.*
Les juvéniles et les femelles adultes de *Genesis vulcanoctopusi* gen. et sp. nov. (Copepoda : Harpacticoida) sont décrits sur l'octopode *Vulcanoctopus hydrothermalis*, récolté sur un site hydrothermal de la ride du Pacifique est. Le nouveau genre est placé dans les Tisbidae et se distingue nettement des autres Cholidyinae à la fois par la forme du corps, la structure des appendices oraux et la segmentation des pattes 1-5. Tous les stades ont été trouvés au sein du tégument de la tête et du manteau de l'hôte Céphalopode, ce qui suggère l'existence d'un stade copépodite endoparasite. Le cycle biologique des Harpacticoides Cholidyinae est réinterprété à la lumière de cette étude. Les aspects du parasitisme et des effets pathologiques de *G. vulcanoctopusi* sur l'hôte Octopode sont discutés.

Keywords : *Genesis vulcanoctopusi,* Copepoda, Harpacticoida, hydrothermal vents, *Vulcanoctopus hydrothermalis*, life cycle.

Introduction

Hot springs on the ocean floor in the East Pacific Rise were first suspected in 1976 (Londsdale, 1977). The discovery of deep-sea hydrothermal vents and their associated fauna was

one of the most exciting events in the history of oceanography. Black smokers are chimneys up to 10 m high where chemically changed sea water emerges from the sea-bed as a clear fluid, at approximately 350º C. The heated water immediately precipitates metal sulphide particles on contact with cold deep sea water, building the vent chimneys and forming the dense plume of black 'smoke' (Open University, 1991). The geological processes and chemical dynamics that occur in deep-sea hydrothermal vents create highly variable transient habitats. Megafaunal colonization of deep-sea hydrothermal vents is considered to be the consequence of an intimate interaction of the life-history strategies of individual species, physical oceanographic processes and the dynamic hydrothermal environment (Sank et al., 1998).

Studies on symbiotic copepods living in hydrothermal vents have been mainly centred on members of the family Dirivultidae (order Siphonostomatoida), which are often found in large numbers (Humes, 1997). While initial conjecture may have placed dirivultids as parasites of vestimentiferans, the diversity of potential hosts and habitats observed over the years suggests a variety of associations and strategies that may include grazing of bacteria (Dinet et al., 1988). One host category that has been poorly studied for associated copepods is the deepwater Cephalopoda. In this study we describe the endoparasitic stages of a remarkable harpacticoid copepod from three specimens of *Vulcanoctopus hydrothermalis* González & Guerra, 1998, an octopod recently described and collected in the vicinity of a high temperature hydrothermal vent in the East Pacific Rise (González et al., 1998). The possible life-cycle, mode of parasitism and pathology are discussed.

Material and methods

The study site
The three octopod hosts were collected near a high temperature hydrothermal vent (black smoker) with the aid of a suction sampler installed in the manned submersible Nautile during a biological programme of the French Research Vessel *Nadir* organized by the CNRS, University Paris VI and IFREMER-Brest (chief scientist: F. Gaill). The octopods were collected as part of the biological sampling programme carried out in the East Pacific Rise. The study area (12º48.43'-48.59'N 103º56.41-56.42'W; 2631-2647 m depth) is situated north of the site commonly known as *Genesis* which was first observed in March 1984. The temperature in *Genesis* ranges from 1-2º C in the surrounding sea water, 1.6-10º C around the *Riftia pachyptila* plumes, 7-91º C close to the alvinellid population, to 262-289º C in the fluid of the vent (Sarradin et al., 1998).

The hosts and their environment
The *Vulcanoctopus hydrotermalis* specimen collected during dive PL 1061 was an immature male of 21.2 mm dorsal mantle length (ML) (paratype). It was collected at 2647 m depth on 13/02/96, one metre away from the base of a small white smoker (old waning black smoker), north of *Genesis*. The substratum was a cliff of basaltic rocks covered by oxidate sulphides. The specimen caught in the dive PL 1067 was a fully mature male of 34.7 mm ML (holotype). It was collected on 19/02/96, two metres from the east wall of the main black smoker of *Genesis*. The collection sites of both specimens were situated 10 metres from each other (Segonzac, pers. comm). The substrate was a basaltic plateau and the associated fauna was similar at both sides, consisting of Vestimentifera (*Riftia pachyptila* Jones, 1981 and *Tevnia jerichonana* Jones, 1985), Polychaeta Alvinellidae (*Alvinella* spp.), turrid gastropods (*Phymorhynchus* spp.), caridean shrimps (*Alvinocaris lusca* Williams & Chace Jr., 1982), brachyuran crabs (*Bythograea thermydron* Williams, 1980), galatheids (*Munidopsis subsquamosa* Henderson, 1885), mussels (*Bathymodiolus thermophilus* Kenk & Wilson, 1985) and zoarcid fishes (*Thermarces cerberus* Rosenblatt & Cohen, 1986). These animals were identified from samples taken ashore and by means of video recording (Segonzac, pers. comm.).

Study methods in the laboratory
The host animals were fixed in formaldehyde (4% in sea water), then preserved in 70% ethanol. Only the octopod mature animal (holotype) was used for histopathological analysis. Pieces of parasitized fixed mantle and head of the octopus were dehydrated to 100% ethanol, embedded in paraffin wax, sectioned (4 mm), mounted, and stained with haematoxylin and eosin (H&E) and Wheathley's trichrome. Sections were examined to determine the site of infection, the pathology associated with the copepod, and reproductive stage of the copepod. The octopod symbiotype is deposited in the Muséum National d'Histoire Naturelle Paris, MNHN no. 2885.

For the taxonomic study, the copepods were removed from the hosts using a stereomicroscope, and transferred to 70% ethanol. All the stages studied were endoparasitic and found in the mantle wall of the octopod host. Description of females is based on copepodid V intermoult stage containing the adult female. Illustrations show adult female after removal of the cuticle of the preceding stage. Consequently setae and spines have not always been illustrated at their full length since many were not completely extended, and often considerably invaginated in segmental pockets. For the same reason no genital apertures or copulatory pores have been observed. For light microscopical investigations, specimens were stained with Chlorazol black E, dissected, mounted in lactophenol and

sealed with Entellan. All figures were drawn with the aid of a phase contrast microscope equipped with a camera lucida.

For scanning electron microscopy (SEM), selected specimens were carefully dissected, postfixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.3, then in 1% OsO_4 in the same buffer. After dehydration they were subsequently critical point dried, mounted on stubs, coated with gold-palladium and observed with a JEOL - JSM 840.

Results

Order Harpacticoida Sars, 1903
Family Tisbidae Stebbing, 1910
Subfamily Cholidyinae Boxshall, 1979
Genus *Genesis* López-González,
Bresciani & Huys, gen. nov.

Diagnosis
Female
Cholidyinae with indistinctly segmented body, but prosome laterally expanded, and pedigerous somites differentiated by lateral protruding margins. Antennule 4-segmented. Antenna with 1-segmented exopodite bearing one element; endopodite 2-segmented. Mandibular palp uniramous, 2-segmented. Praecoxal arthrite of maxillule with one spine and two setae, palp with 5 setae. Maxilla 2-segmented, of the clinging type, terminal segment claw-shaped. Maxilliped 3-segmented, terminal segment bearing a strong claw. Legs 1-4 biramous, exopodites of legs 1-4 and endopodite of leg 3 3-segmented, endopodites of leg 1, 3 and 4 2-segmented. Leg 5 like a lobe fused to the body, baseoendopod not differentiated.

Parasitic on octopods.

Type species. *Genesis vulcanoctopusi* López-González, Bresciani & Huys, gen. et sp. nov.

Etymology. The name *Genesis* refers to the hydrothermal vent area where the octopod hosts were collected. Gender feminine.

Genesis vulcanoctopusi López-González,
Bresciani & Huys, sp. nov.
(Figs 1-7)

Material examined
Twenty non-ovigerous females and three juveniles, from two specimens of the cephalopod *Vulcanoctopus hydrothermalis* González & Guerra, 1998 (in González et al., 1998) at East Pacific Rise, 12º48.43'N; 103º56.41'W, 2647 m depth, February 1996, and from an additional specimen of *V. hydrothermalis* collected at 12º48.59'; 103º56.42'W, 2631 m depth, 20 May 1999. The holotype has been deposited in the Muséum National d'Histoire Naturelle Paris (MNHN no. cp 1889). Paratypes have been deposited in the MNHN in Paris (2 specimens, MNHN no. cp 1890) and in the Natural History Museum in London (10 specimens, NHM no. 2000 1808-1817). The remaining specimens, most of them partially dissected, are deposited in the first author's collection.

External morphology of female.
Body (Fig. 1a, b) elongate, 2.5-2.8 mm in length and 1-1.1 mm in width (based on five specimens in lactophenol). In dorsal view the articulations between the pedigerous somites are not defined, however, their demarcation is indicated by swellings of the epimeral areas. Ratio of length to width of prosome 1.56:1. Ratio of length of prosome to that of urosome is 1.31:1. Minute spinules cover all the body surface (Fig. 4f), being more dense in determined areas like the legs (Figs 1e, 3, 4e) or caudal rami (Fig. 1d).

Cephalosome fused to leg 1 bearing somite forming cephalothorax. Cephalothorax broader than rest of prosome and urosome. Leg 5 bearing somite and genital somite completely fused, without sutures marking original segmentation; leg 5 fused with its somite. Two obscurely defined post-genital somites, one of them being the anal somite, bearing the caudal rami. Caudal rami (Fig. 1d) 163 x 69 µm, diverging laterally, each with three terminal setae, and one outer subterminal seta, all setae pinnate.

Rostrum produced beyond anterior margin of cephalothorax.

Antennule (Figs 2a, 4d) indistinctly 4-segmented (measured along the posterior margin): 37, 64, 128 and 42 µm. Armature formula: 2, 10, 11+ 1 aesthetasc, and 6. Most of the setae naked, only two setae on segment 3 minutely pinnate (arrowed in Fig. 2a).

Antenna (Fig. 2b) with separate coxa and basis. Exopod 1-segmented, with one apical element. Endopod 2-segmented, first segment unarmed, second segment with four stubby, spiniform setae bearing small denticles in distal part, and two normal setae.

Labrum and labium as shown in figure 1f. Gnathobase of mandible (Figs 1f, 2c) with group of short pointed teeth. Mandibular palp uniramous and 2-segmented, consisting of basis with one naked seta and 1-segmented endopod with five apical naked setae of unequal length and one lateral seta. Maxillule (Figs 1f, 2d) with reduced arthrite and 1-segmented palp; arthrite with a spine and two small setae, palp with five setae. Maxilla (Figs 1f, 2e) 2-segmented; syncoxa unarmed, with some minute spines and a projected process on the inner margin (rudimentary endite); allobasal claw acutely curved with a series of minute spinules along its inner edge. Maxilliped (Fig. 2f) 3-segmented with short syncoxa, long basis, and a claw on the endopod.

Legs 1-4 (Fig. 3a-d) biramous, exopodites of legs 1-4 and endopodite of leg 2 3-segmented, endopodites of legs 1, 3 and 4 2-segmented. Armature formula as follows:
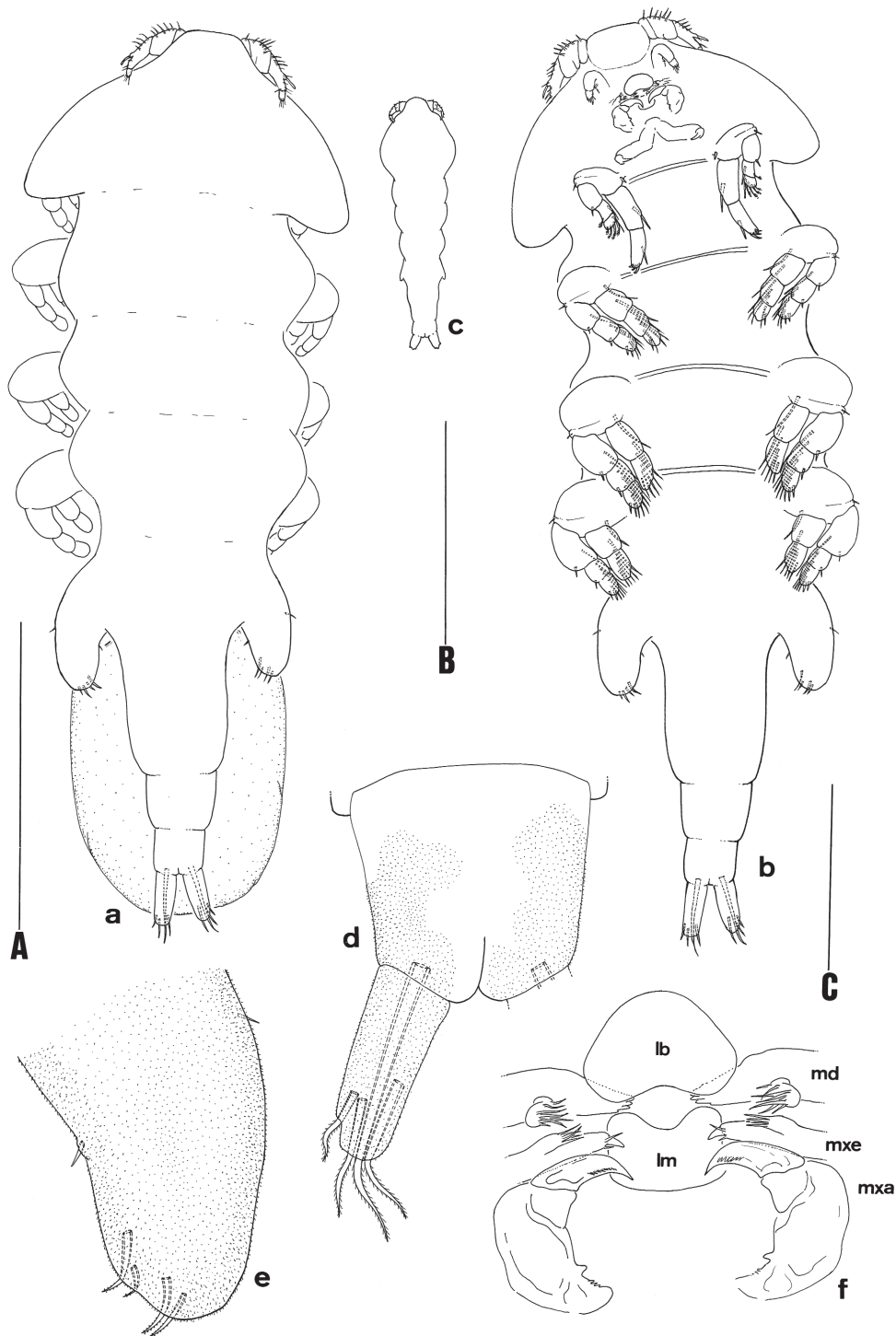
Figure 1. *Genesis vulcanoctopusi* gen. et sp. nov. Adult female: **a** habitus, dorsal; **b** habitus, ventral; **d** anal somite and left caudal ramus, dorsal; **e** leg 5, dorsal; **f** ventral view of oral appendages; **c** Copepodid: habitus, dorsal. (*lb*) labrum; (*lm*) labium; (*md*) mandible; (*mxa*) maxilla; (*mxe*) maxillule. Scale bars: A for a, b, c, 1 mm; B for d, e, 200 μm; C for f, 150 μm.

Figure 1. *Genesis vulcanoctopusi* gen. et sp. nov. Femelle adulte : **a** habitus, vue dorsale; **b** habitus, vue ventrale ; **d** somite anal et rame caudale gauche, vue dorsale ; **e** patte 5, vue dorsale ; **f** vue ventrale des appendices buccaux. **c** Copépodite, habitus, vue dorsale. (*lb*) labre ; (*lm*) labium ; (*md*) mandibule ; (*mxa*) maxille ; (*mxe*) maxillule. Echelles : A pour a, b, c, 1 mm ; B pour d, e, 200 μm ; C pour f, 150 μm.
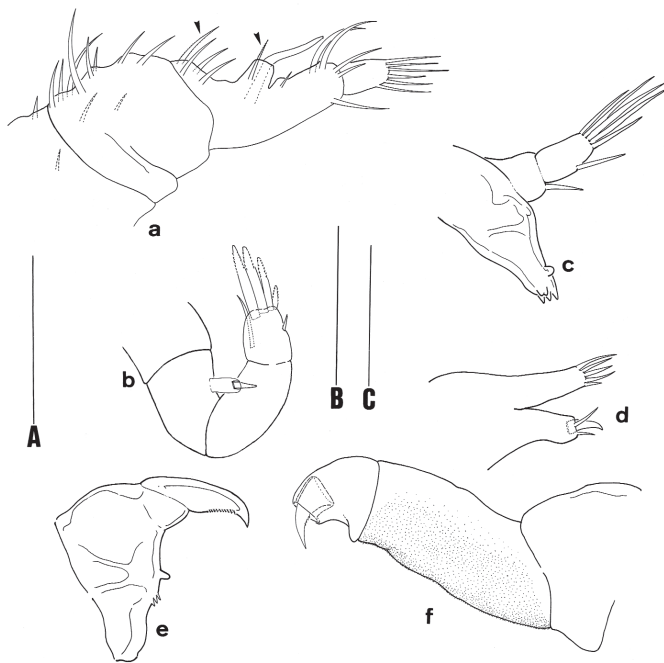
Figure 2. *Genesis vulcanoctopusi* gen. et sp. nov. Adult female: **a** antennule [pinnate setae arrowed]; **b** antenna; **c** mandible; **d** maxillule; **e** maxilla; **f** maxilliped. Scale bars: A for a, b, 150 μm; B for c, d, 100 μm; C for e, f, 100 μm.

Figure 2. *Genesis vulcanoctopusi* gen. et sp. nov. Femelle adulte: **a** antennule, [les flèches indiquent des soies pennées] ; **b** antenne ; **c** mandibule ; **d** maxillule; **e** maxille ; **f** maxillipède. Echelles : A pour a, b, 150 μm ; B pour c, d, 100 μm ; C pour e, f, 100 μm.

P1	protopod	I-1	exp	I-0; I-1; IV,2
			end	0-1; III,1
P2	protopod	I-0	exp	I-0; I-1; II,4
			end	0-1; 0-1; I,4
P3	protopod	I-0	exp	I-0; I-1; II,5
			end	0-1; 5
P4	protopod	I-0	exp	I-0; I-1; II,5
			end	0-1; 5

All leg setae pinnate.

Leg 5 (Figs 1a, b, e, 4e) lobate fused to the body, baseoendopod not differentiated, bearing four distal minutely pinnate setae, one outer proximal naked seta, and one inner proximal naked seta. Leg 6 and genital apertures not observed.

Male

None of the specimens examined showed any evidence of developing testes or geniculate antennules which could substantiate its male identity. However, the gross disparity in size between some copepodid III stages and the adult females (Fig. 1a, c) possibly reflects sexual dimorphism whereby the smaller specimens may represent developing

males. This is in agreement with previous reports of profound size dimorphism expressed in adult cholidyinids.

External morphology of juvenile

Body (Figs 1c, 5) elongate, 0.7-0.8 mm in length and 0.2-0.25 mm in width (based on two specimens in lactophenol). Very similar in appearance to the females, but more slender and with fewer traces of segmentation. All the oral and thoracic appendages described in the female are present, but simplified in structure as shown in Fig. 5. Comparison of the swimming leg segmentation and the state of development of leg 5 with those observed in free-living Tisbidae (e.g. Dahms, 1993) unequivocally identifies the specimen figured in Fig. 5 as a copepodid III.

Relation with the host

More than thirty copepods were found in the octopod hosts. Prevalence was 100% (N=3). Juveniles and part of the adult stages of *Genesis vulcanoctopusi* are endoparasitic, living in the inner connective sheath below the epithelium of the head and mantle of the octopus host, with a random distribution. The pear-shaped testis of one of the hosts examined was found to be infested by a single copepod, but no parasites were found in the gills.

Etymology. The name *vulcanoctopusi* refers to the generic name of the octopus host.

Anatomical and pathological observations

Only a few features can be illustrated of the internal anatomy of the copepod, due to the imperfect preservation of the parasites. The genital system is fully developed in females (Fig. 7a).

Infested host tissue showed loss of structural integrity (Fig. 7a). Compression, deformation and mechanical tissue disruption were apparent in the connective tissue inhabited by the parasite. Copepods were encased in a thin layer of fibrous tissue forming a capsule. There was a massive, localized, host tissue response around the site of the lesion. A large number of infiltrating cells were present in the connective tissue close to the antennae, and maxillipeds. A moderate inflammation was also observed beneath the parasite at the subepithelial region. One or two large areas (264-490 μm long) of chemical secretion from the parasites were found both close and at a distance from the large copepods. Cellular infiltration beneath this secretion is clearly discernible.

Discussion

Systematic remarks

In the Tisbidae, eleven species in seven genera are parasitic on octopod cephalopods (Humes & Voight, 1997). Two monotypic genera, *Yunona* Avdeev, 1983 (with *Y. marginata* Avdeev, 1983), and *Octopinella* Avdeev, 1986 (with
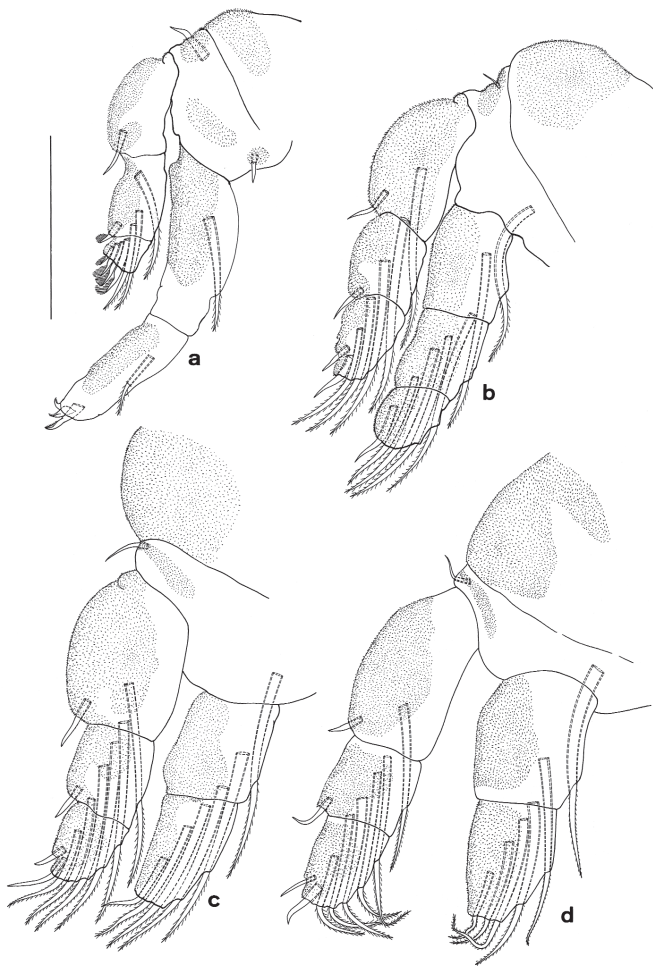
Figure 3. *Genesis vulcanoctopusi* gen. et sp. nov. Adult female, swimming legs: **a** leg 1, anterior; **b** leg 2, anterior; **c** leg 3, anterior; **d** leg 4, anterior. Scale bar: 200 µm.

Figure 3. *Genesis vulcanoctopusi* gen. et sp. nov. Femelle adulte, pattes natatoires : **a** patte 1, vue antérieure ; **b** patte 2, vue antérieure ; **c** patte 3, vue antérieure ; **d** patte 4, vue antérieure. Echelle : 200 µm.

O. tenacis Avdeev, 1986) belong to the subfamily Tisbinae (Avdeev, 1983, 1986). The remaining genera belong to the subfamily Cholidyinae created by Boxshall (1979): *Cholidya* Farran, 1914 (with *C. polypi* Farran, 1914), *Cholidyella* Avdeev, 1982 (with *C. incisa* Avdeev 1982, *C. intermedia* (Bresciani, 1970), *C. breviseta* Avdeev, 1986, and *C. nesisi* Avdeev, 1986), *Brescianiana* Avdeev, 1982 (with *B. rotundata* Avdeev, 1982), *Tripartisoma* Avdeev, 1983 (with *T. trapezoidalis* Avdeev, 1983, and *T. ovalis* Avdeev, 1983), and the most recently described genus *Avdeevia* Bresciani & Lützen, 1994 (with *A. antarctica* Bresciani & Lützen, 1994).

Among these genera, *Avdeevia* is the most modified member, being readily distinguished from *Genesis* by the possession of only the first two pairs of swimming legs. In addition, *Avdeevia* lacks leg 5, and its body shape is irregularly bean-shaped with the cephalothorax being clearly differentiated from the undivided trunk (Bresciani & Lützen, 1994).

Tripartisoma differs from *Genesis* in the presence of only three pairs of swimming legs, the division of the body into three recognizable tagmata, and leg 5 having a well developed baseoendopod which is completely absent in *Genesis* (see Avdeev, 1982).

Five other genera, *Cholidya*, *Yunona*, *Cholidyella*, *Brescianiana*, and *Octopinella* are characterized by the presence of four pairs of swimming legs and a well developed leg 5. Among them, *Cholidya polypi* has a very distinct body shape (see Farran, 1914), and legs 3 and 4 are rudimentary, being represented by minute segments (for more details about this species see Humes & Voight, 1997).

Brescianiana has a strongly inflated body shape lacking any defined segmentation, a 3-segmented endopod on leg 1 and a 2-segmented endopod on leg 2 (see Avdeev, 1982).

Genesis is most closely related to *Cholidyella* but differs in leg 5 position and morphology. In the former the fifth legs are laterally displaced and represented by lobate outgrowths bearing short elements, whereas in the latter they retain their original ventral position, are defined at the base and bear

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Figure 4. a Octopod host, *Vulcanoctopus hydrothermalis*, showing external marks produced by the subepithelial presence of three adult female copepods (*black arrows*), and two scars indicating previous infections (*white arrows*). **b - g** *Genesis vulcanoctopusi*, adult female, SEM photographs. **b** copepod embedded in the mantle tissues of the octopod, dorsal and lateral tissues of the host have been partly removed, showing part of the previous exuvium (*asterisk*); **c** detail of the dorsal region of the copepod cephalothorax (*arrow*) and integument of the host (upper); **d** antennule showing setae and part of the previous exuvium (*asterisk*); **e** detail of leg 5 (*arrow*) in dorsal view, showing also part of the previous exuvium (*asterisk*); **f** detail of the dorsal surface; **g** copepod musculature viewed through damaged cuticle. Scale bars: a, 1 cm; b, 1 mm; c, 50 µm; d, 60 µm; e, 20 µm; f, 5 µm; g, 4 µm.

Figure 4. a Manteau de l'hôte *Vulcanoctopus hydrothermalis* montrant les marques produites par la présence subépithéliale de trois femelles adultes du copépode parasite *Genesis vulcanoctopusi* (*flèches noires*), et deux cicatrices montrant des infections précédentes (*flèches blanches*). **b - g** *Genesis vulcanoctopusi*, photographies au microscope électronique à balayage des femelles adultes. **b** copépode femelle logée dans le tissu conjontif de l'hôte ; **c** détail du céphalothorax, vue dorsale (*flèche*), sous l'épiderme de l'hôte ; **d** antennule montrant les soies et une partie de l'éxuvie (*astérisque*) ; **e** détail de la patte 5 (*flèche*) en vue dorsale, montrant aussi une partie de l'éxuvie (*astérisque*) ; **f** détail de la surface dorsale ; **g** vue de la musculature sous le tégument endommagé. Echelles : a, 1 cm ; b, 1 mm ; c, 50 µm ; d, 60 µm ; e, 20 µm ; f, 5 µm ; g, 4 µm.
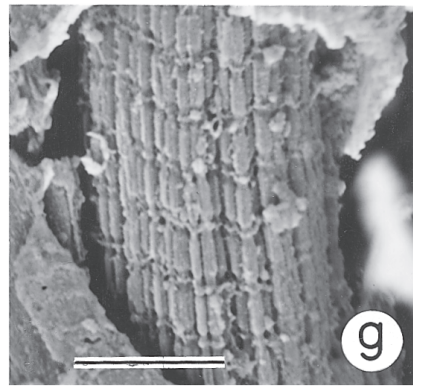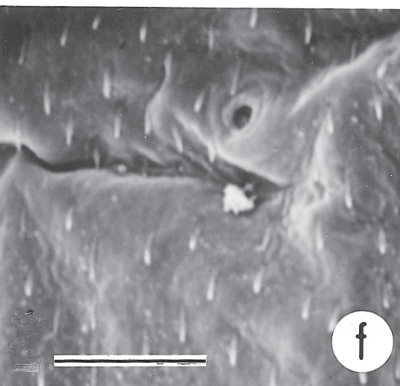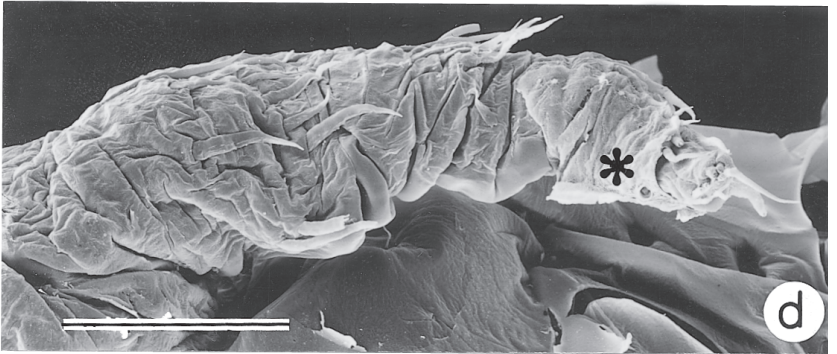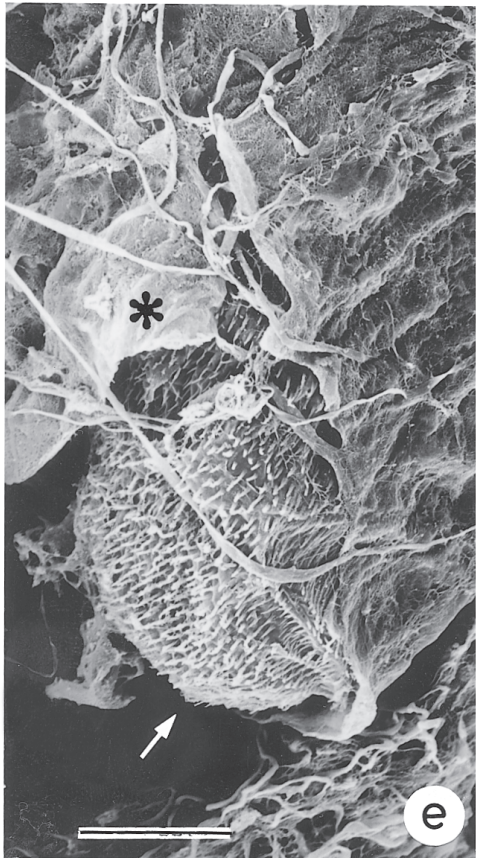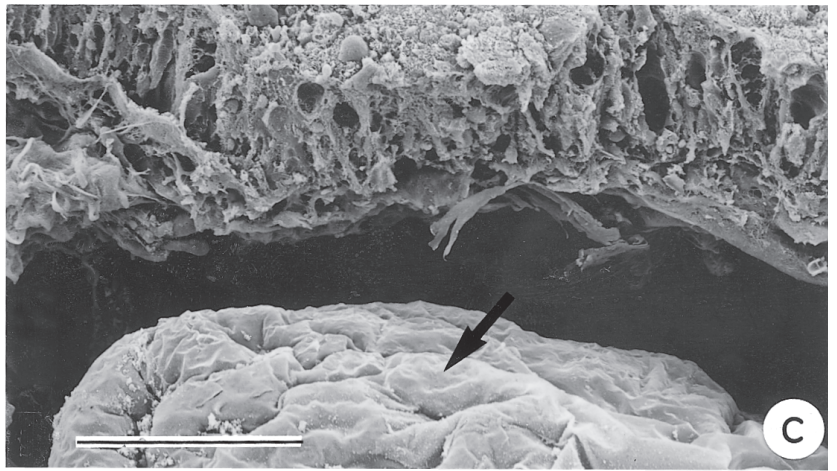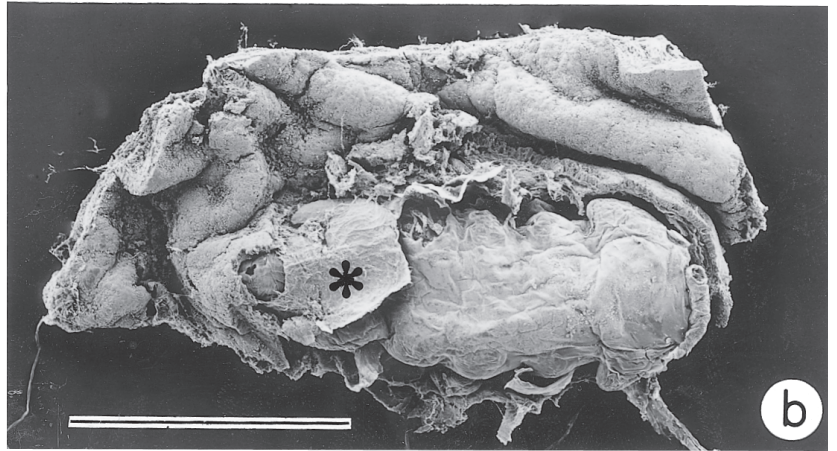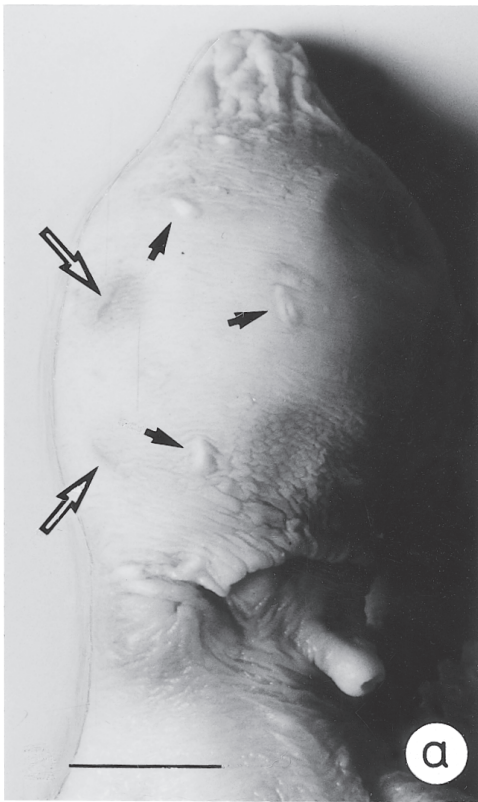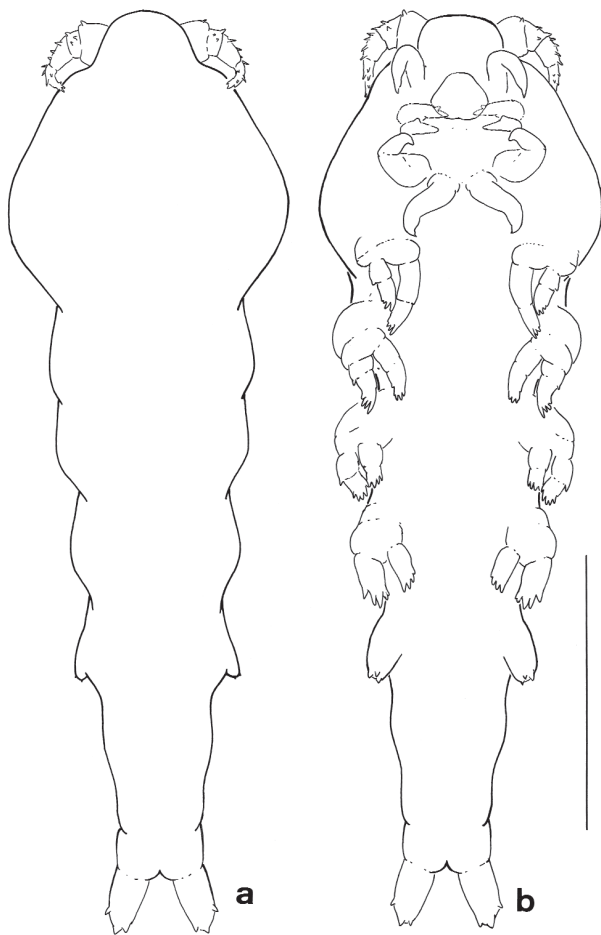
Figure 5. *Genesis vulcanoctopusi* gen. et sp. nov. Copepodid III. **a** dorsal view; **b** ventral view. Scale bar: 250 µm.

Figure 5. *Genesis vulcanoctopusi* gen. et sp. nov. Copépodite III. **a** vue dorsale ; **b** vue ventrale. Echelle : 250 µm.
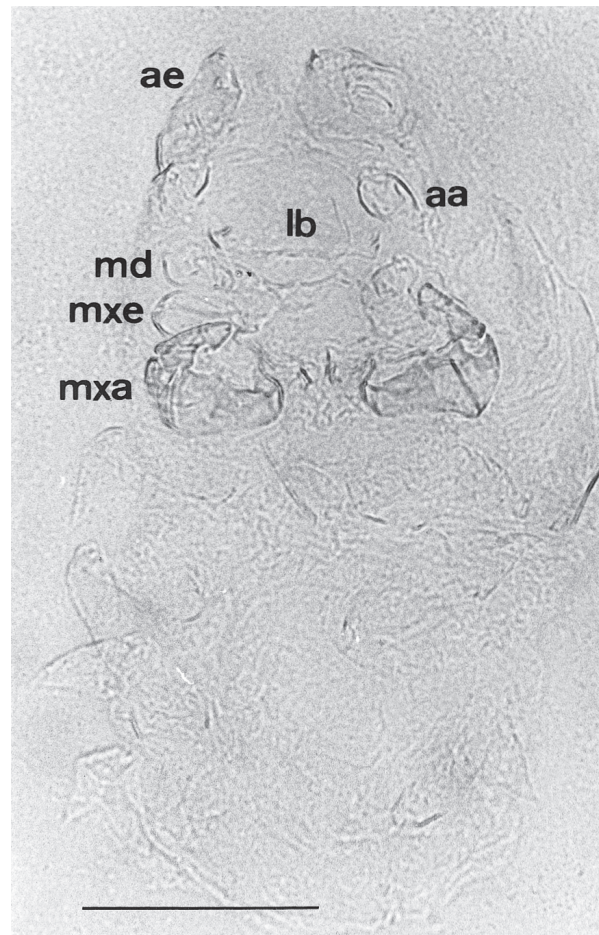
Figure 6. *Genesis vulcanoctopusi* gen. et sp. nov. Exuvial bodies showing remains of the cephalic appendages. (*aa*) antenna; (*ae*) antennule; (*lb*) labrum; (*md*) mandible; (*mxa*) maxilla; (*mxe*) maxillule. Scale bar: 100 µm.

Figure 6. *Genesis vulcanoctopusi* gen. et sp. nov. Exuvies montrant des restes d'appendices céphaliques. (*aa*) antenne; (*ae*) antennule ; (*lb*) labre ; (*md*) mandibule ; (*mxa*) maxille ; (*mxe*) maxillule. Echelle : 100 µm.

Figure 7. *Genesis vulcanoctopusi* gen. et sp. nov. Adult female in situ, longitudinal section. **a** frontal section (anterior end on the right side) showing a part of the degenerate midgut (*mg*), the median ovary (*ov*), fully developed lateral and coloured oviducts and the lesion of the host tissues (*ht*) caused by the copepod; **b-c** sagittal sections of the cephalothorax, and urosome respectively, showing different structures. (*gd*) dorsal ganglia; (*gv*) ventral ganglia; (*ht*) host tissues; (*lb*) labrum; (*lu*) lumen of the gut; (*mc*) mouth cavity; (*mg*) midgut; (*mxa*) maxilla; (*mxp*) maxilliped; (*oe*) oesophagus; (*ov*) ovary. Scale bars: a, 300 µm; b and c, 200 µm.

Figure 7. *Genesis vulcanoctopusi* gen. et sp. nov. Femelle adulte in situ, coupes longitudinales. **a** coupe frontale (extrémité antérieure à droite) montrant une partie de l'intestin moyen partiellement dégénéré (*mg*), l'ovaire (*ov*) médian, les deux oviductes latéraux bien développés et colorés, et les lésions dans les tissus de l'hôte (*ht*) causées par le copépode ; **b-c** coupes sagittales du céphalothorax et de l'urosome, respectivement, montrant différentes structures. (*gd*) ganglion dorsal ; (*gv*) ganglion ventral ; (*ht*) tissus de l'hôte ; (*lb*) labre ; (*lu*) lumière de l'intestin ; (*mc*) cavité buccale ; (*mg*) intestin moyen; (*mxa*) maxille ; (*mxp*) maxillipède ; (*oe*) oesophage ; (*ov*) ovaire. Echelles : a, 300 µm ; b et c, 200 µm.
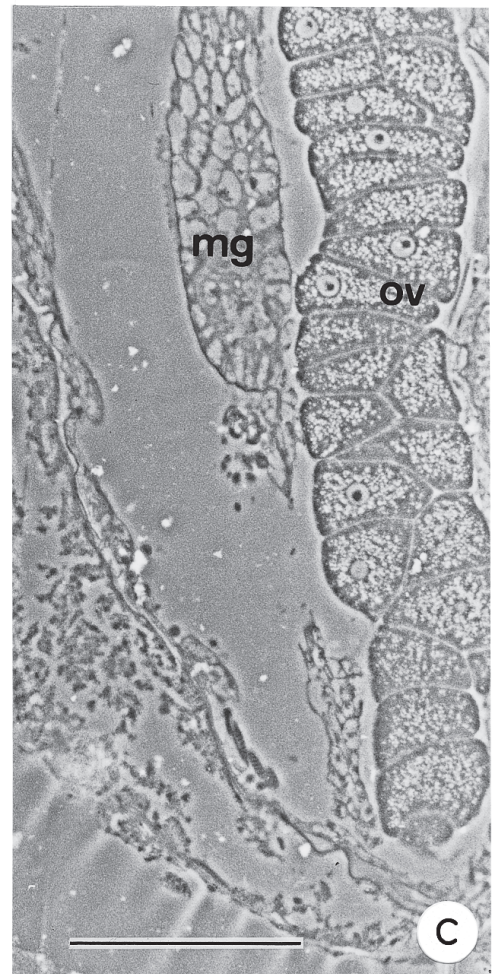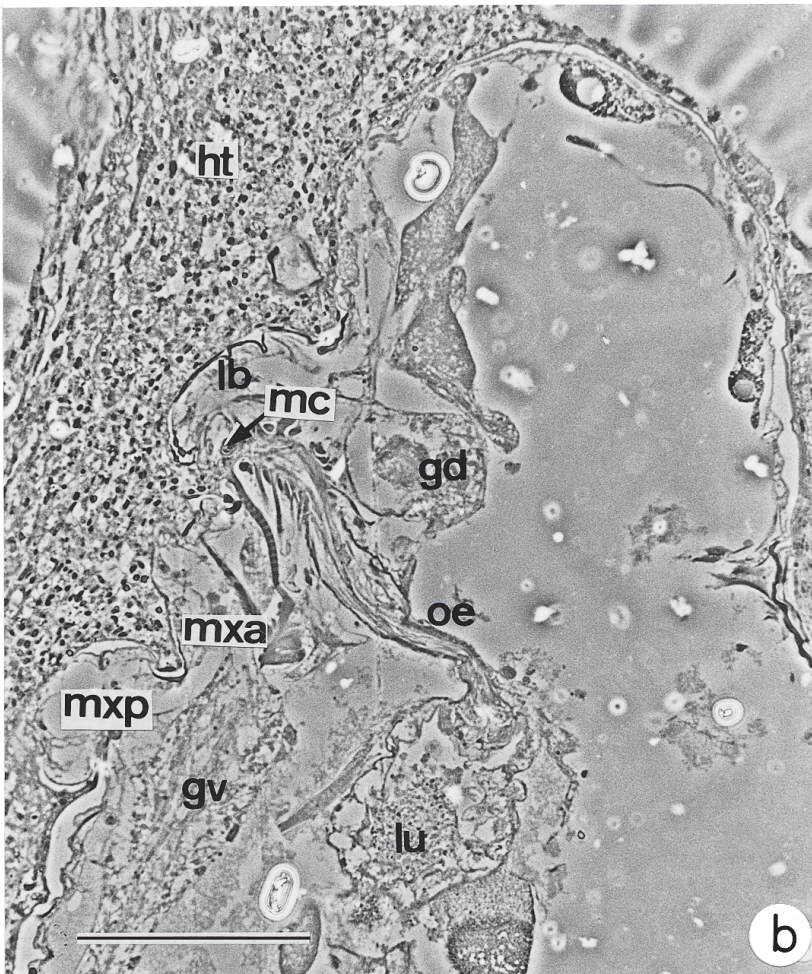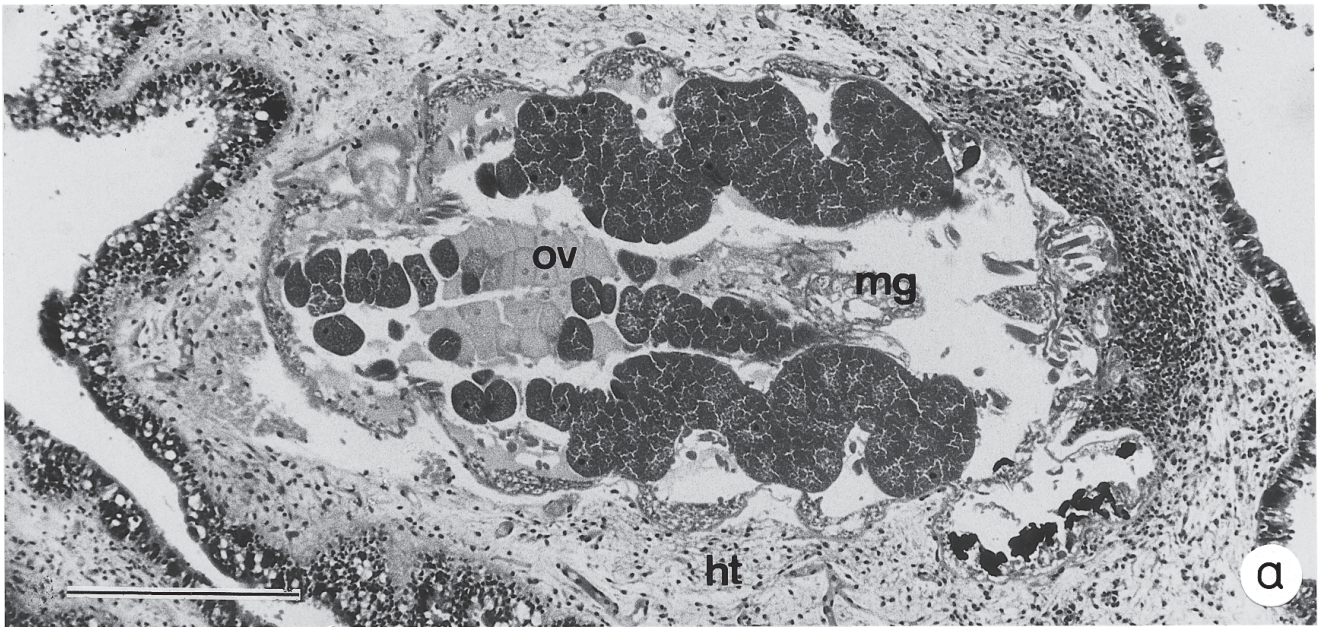
strongly developed setae which often extend to the posterior end of the body. An additional discrepancy is found in the endopod of leg 2 which is 3-segmented in *Genesis* but only 2-segmented in *Cholidyella* (see Avdeev, 1982; Bresciani, 1970).

Yunona and *Octopinella* were recently listed as members of the Cholidyinae (Bodin, 1997: 62-63). However, according to Humes & Voight (1997) these genera should be excluded from this subfamily, because they show a clearly segmented body, more than 2 elements on the exopod of the antenna (1 or 2 elements in the genera of Cholidyinae), a discrete baseoendopod and exopod in leg 5, and a biramous palp of the mandible (uniramous in the other genera, except for *Tripartisoma*, in which the exopod is represented by a short segment with a single seta). In addition, the structure of the maxillipeds in *Yunona* and *Octopinella* is also markedly different from that of *Genesis* (see Avdeev, 1983; 1986).

Habitat and life cycle

Various authors have compiled records of Tisbidae associated with octopods and summarized data on their host range, specificity and location in the host (Bresciani & Lützen, 1974; Humes & Voight, 1997). Data on the life cycle of Cholidyinae are virtually non-existent. No developmental stages have ever been observed and only four species are known of both sexes: *Cholidya polypi* (see Humes & Voight, 1997), *Cholidyella incisa*, *C. breviseta* and *C. nesisi* (see Avdeev, 1982, 1986). All the known male stages are presumably only loosely associated with their octopod hosts as temporary ectoparasites or commensals. They are typically less transformed than their respective females and, unlike many free-living Tisbidae, lack any distinct sexual dimorphism in the swimming legs or maxillipeds. Adult Cholidyinae are typically found attached to the gills, but other sites of infection such as the web, arms, pallial cavity and ventral surface of the head have occasionally been reported. Humes & Voight (1997) studied specimens of *Cholidya polypi* "partly buried in the skin of the arms at or near the base of the suckers", although "more frequently the copepods are found attached to the octopus by their heads, with the bodies extending free". Apart from this isolated observation, there is no other detailed information about host attachment in this group of copepods. Although infection rates can be considerable, it is remarkable that copepodid stages have as yet not been reported for any of the eleven tisbid species associated with cephalopods, not even in heavily infected hosts. This suggests that part of the life cycle is completed either in the water column, the benthic environment or elsewhere on the host.

The discovery of juveniles of *G. vulcanoctopusi* living inside the connective tissue of the octopod integument appears to corroborate this supposition and offers an explanation for their apparent absence in previous descriptions of cholidyinids which were primarily based on specimens recovered from the gills and other external sites. Since at least three different copepodid stages (III-V) were discovered in a single host individual, it is conceivable that the entire copepodid phase is completed inside the octopod. This previously unknown endoparasitic phase complements the ectoparasitic adult phase recorded for all other octopod associates, but which we were unable to demonstrate for *G. vulcanoctopusi*. The known life-history stages in the Cholidyinae now allow a more complete interpretation of the generalized life cycle in this lineage.

Host location and subsequent infection are probably accomplished by the first copepodids (or the final nauplius stages) which are the primary dispersal stages. However, the highly aggregated distribution of endoparasitic stages on the head of the octopod (Fig. 4a) may suggest that the host has encountered a swarm of infective stages, possibly the offspring of a single female, released *en masse*. In this scenario the ovigerous female, which retains a full complement of well developed swimming legs, may be involved in long-range host location. Upon successful settling on and penetration of the host's integument the first copepodid remains entirely embedded and undergoes subsequent moulting inside the host, probably completing the normal cycle of six copepodid stages. Within the subcutaneous cavity, the exuviae of successive moults accumulate at the posterior end of the urosome (see Fig. 1a), forming darkish-brown globular bodies that contain the chitinized remains of the antennae, maxillae and other appendages of previous stages. The later copepodid stages appear to remain in contact with the exterior via a transverse slit in the host's integument, situated at the frontal end of the copepod. This slit is used by the adult copepods to leave the host upon reaching sexual maturity and subsequently closes as a result of epithelial proliferation. The exuvial bodies remain inside the host for a substantial amount of time, marking previous infection sites (white arrows in Fig. 4a). Although we were unable to demonstrate the presence of males in the host tissues we suspect the life cycles of both sexes to be essentially alike, possibly differing in the timing of the final moult and sexual maturity. Mating presumably takes place on the host in places where adult Cholidyinae have previously been reported from, such as the gills, arms and mantle. Both sexes show the ability to maintain at least semipermanent contact with the host by means of their prehensile maxillae and maxillipeds, which are often found, hooked into the host's tissue. Presumably the adults continue to feed on the superficial tissues of the octopod for the remainder of the life cycle. The life cycle of Cholidyinae is clearly unique among harpacticoid copepods since the free-swimming period has been reduced to the naupliar
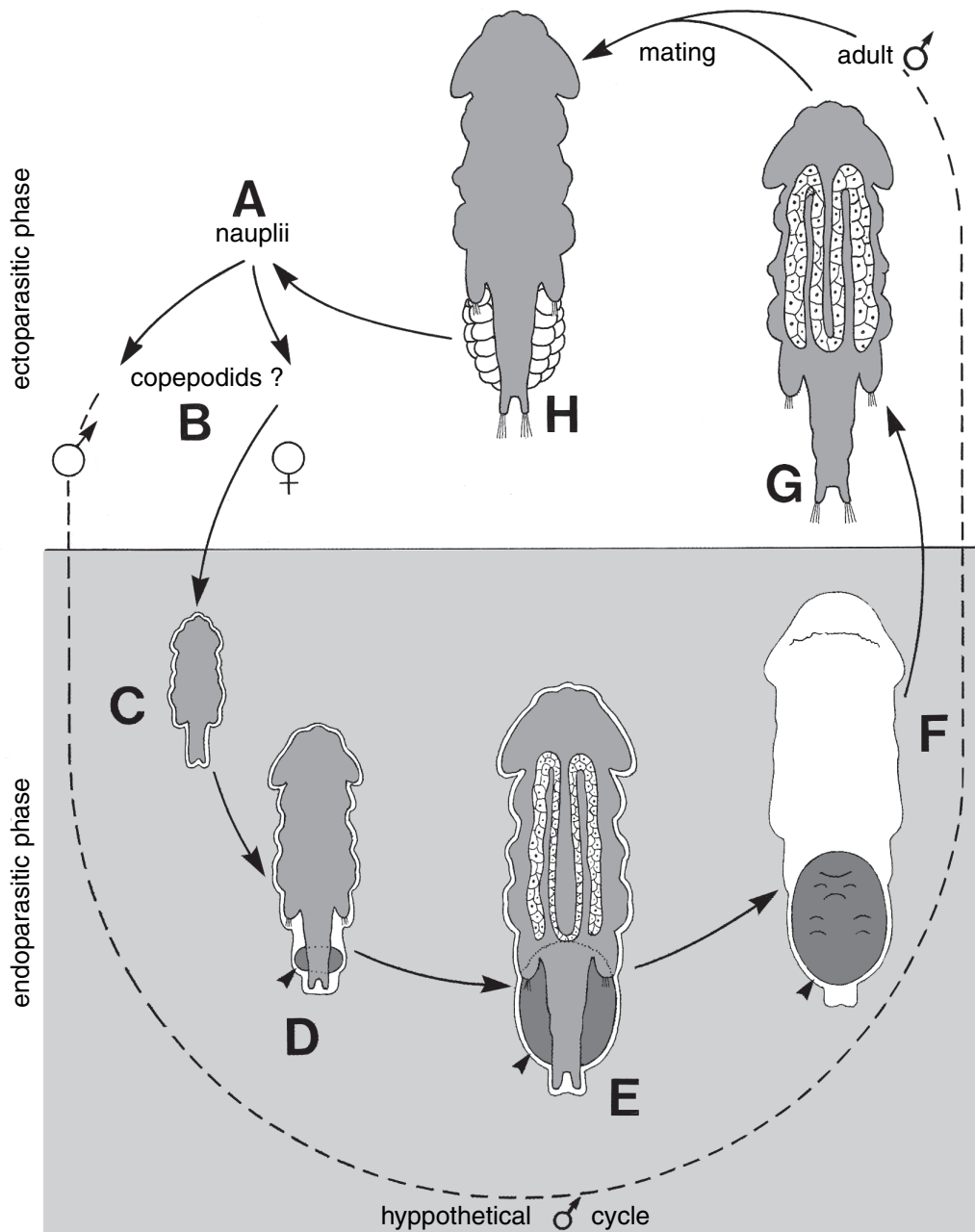
Figure 8. Hypothetical life cycle of *Genesis vulcanoctopusi* gen. et sp. nov., **A** naupliar phase; **B** infection by larval stage (copepodid?); **C** developing endoparasitic female in body wall of host; **D** young female with exuvial body (*arrow*) accumulating at posterior part of urosome; **E** copepodid V intermoult stage containing the adult female, note exuvial body (*arrow*); **F** escape of adult female through the host integument, leaving behind exuvial body (*arrow*); **G** free-swimming adult female; **H** free-swimming ovigerous female. [Drawn by B. Beyerholm].

Note that only the stages shown in **C**, **E**, and **F** have been observed in the present work. Male development presumably follows a similar endo-parasitic phase leading to the free-swimming ectoparasitic adult male. Mating between adult female and adult male presumably takes place during the ectoparasitic phase.

Figure 8. Cycle biologique hypothétique de *Genesis vulcanoctopusi* gen. et sp. nov., **A** phase nauplienne ; **B** infestation par le stade larvaire (copépodite ?) ; **C** femelle endoparasite en développement, dans le tégument de l'hôte; **D** femelle jeune avec restes d'éxuvies (*flèche*) accumulées à la partie postérieure de l'urosome ; **E** copépodite V, stade d'intermue renfermant la femelle adulte, remarquez les éxuvies (*flèche*) ; **F** la femel-le adulte a traversé le tégument de l'hôte et les exuvies (*flèche*) restent dans l'hôte; **G** phase femelle adulte libre ; **H** femelle libre, ovigère. [Dessiné par B. Beyerholm].

Remarquez que seuls les stades **C**, **E** et **F** ont été observés dans cette étude. Le développement des mâles présente probablement une phase endoparasite comparable à celle de la femelle, qui conduirait aussi à une phase adulte libre ectoparasite. L'accouplement entre les adultes se ferait durant la phase ectoparasite.

phase and the adults (Fig. 8). No other harpacticoids show alternating endoparasitic and ectoparasitic phases in their life cycle.

Geographical and depth distribution

Previous records of Tisbidae associated with octopods stem from considerable depths (up to 2500 m for *Cholidya polypi* at Cascadia Abyssal Plain) in the North Atlantic, the North Pacific and Antarctica. These records are summarized and profusely documented by Humes & Voight (1997). The discovery of *G. vulcanoctopusi* represents a further depth range extension (2647 m) and is the first record of Tisbidae infesting octopods at tropical latitudes (12°48.43'N; 103°56.41'W). Furthermore, all previous records indicate that octopod hosts appear to occur on soft substrata, especially on sand and fine mud. Conversely, the host *V. hydrothermalis* was collected on a cliff of basaltic rocks covered by oxidate sulphides. The new copepod species is also the first record of a Cholidyinae from hydrothermal vent environments and only the fifth harpacticoid to be described from this habitat (Huys & Conroy-Dalton, 1997; Conroy-Dalton & Huys, 1999; Lee & Huys, 1999).

Remarks on pathology

The pathological changes in *Vulcanoctopus hydrothermalis* infected by *G. vulcanoctopusi* were related to attachment, feeding and parasite size. Kabata (1970, 1981) considered the mechanical activity of attachment and feeding to be the primary cause of the inflammation associated with parasitic copepods. However, chemical factors have also been considered as major promoters of the severe inflammation associated with ergasilid copepods (Paperna & Zwerner, 1982) which are known to be external digestors (Kabata, 1970). The role of chemical factors in eliciting a tissue response in cephalopod tissues to *G. vulcanoctopusi* has yet to be determined, but the presence of infiltrates, comprising large areas of acellular deposits close and at a distance of the attachment site of the copepods, is indicative of digestive enzymes provoking a tissue response. Cephalopod amoebocytes, often in large numbers, were found in the connective tissue and intercellular spaces of certain organs (Wells, 1978). Amoebocytes form cellular clots and are responsible for clearing foreign substances from the circulatory system (Ford, 1992). It is not surprising that amoebocytes were found in *Vulcanoctopus hydrothermalis* (González et al., 1998; Figs 14 & 15), since cells phagocytizing bacteria, are probably present in high concentrations in the gills, gut and coelom of this animal (González et al., 1998). It is conceivable that amoebocytes are also associated with the tissue response of *V. hydrothermalis* to its mantle parasites. This could indicate a cell-mediated response, which may function in encapsulation and neutralization of the parasitic copepods. Encapsulation of metazoan parasites is a primary role of

molluscan amoebocytes (Ford, 1992). Proliferation of connective tissue was also considered by Kabata (1970) and occurs only when parasitic copepods penetrated the subepithelial region, as was found for *G. vulcanoctopusi*. The greater tissue response, but without foci of hyperplasia associated with the large parasite, implies a more sedentary habit resulting from frequent or prolonged feeding in a restricted area.

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