

Questa (Annelida, Polychaeta, Orbiniidae) from Pacific regions — new species and reassessment of the genus *Periquesta*

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Abstract

On the basis of new material from various Pacific regions we critically evaluate the characters for the taxonomy of the genus *Questa* and the former Questidae, a taxon now concluded to be a lineage within the family Orbiniidae. Two new species, *Questa retrospermatika* sp. n. from Hawaii (with similar forms also from New Caledonia and China), and *Questa fijiensis* sp. n. from Fiji, are described, and the morphological character patterns and phylogeny of the genus are revisited on the basis of a cladistic analysis. The finding of Hawaiian material of a new species, *Levinsenia hawaiiensis* sp. n., similar to *Periquesta canariensis* Brito & Nunez, 2002 (= *Levinsenia canariensis* comb. n.), challenges the view that *Periquesta* Brito & Nunez, 2002 is closely related to *Questa*; rather, it belongs to Paraonidae as a junior synonym of *Levinsenia* Mesnil, 1897.

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Keywords: *Questa*; Orbiniidae; Paraonidae; Polychaeta; Pacific ocean; Reassessment

Introduction

The polychaete taxon previously referred to as the family Questidae Hartman, 1966 consists of only a few species and is recognized mainly by aberrant internal features. The arrangement of genital organs in this group partly resembles that of some oligochaetes (Giere and Riser 1981, Giere and Erséus 1998). Recently, Almeida et al. (2003) even proposed a new taxon name (Apoclitellata) for Questidae and Clitellata; their paper is an elaboration of the conclusion also published by Garraffoni and Amorim (2003) that these two are sister

groups. Many of the external characteristics of questids, however, suggest a closer affinity to Orbiniidae or Paraonidae (see e.g. Rouse and Fauchald 1997). Molecular genetic analyses have confirmed the close phylogenetic relationship between Questidae and Orbiniidae and refuted the link with clitellates (Erséus et al. 2000, Rota et al. 2001, Struck et al. 2002, Erséus and Källersjö 2004, Bleidorn 2005); with extended taxon sampling it is now clear that *Questa* is merely a derived group within Orbiniidae (Rousset et al. 2007), making Questidae a junior synonym of the latter. The phylogenetic position of Paraonidae vis-a-vis *Questa*/Orbiniidae is less certain (Bleidorn 2005; Rousset et al. 2007). Since earlier studies did not give any family definitions, a first formal diagnosis of Questidae referring to external as well as internal organs was compiled by Giere and

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Erséus (1998) in a study comprising eight species assigned to *Questa* Hartman, 1966. Brito and Núñez (2002) later established a second genus of Questidae, *Periquesta*, for a single species, *Periquesta canariensis*, but they restricted their diagnosis of questid worms to external features and did not consider several important internal characters.

The aim of the present study is to describe new material of *Questa* from various Pacific regions, including a new species from Hawai'i with a previously unknown arrangement of genital organs, and a second new species from Fiji. During the course of our investigations, the genus *Periquesta* Brito & Núñez, 2002 was found not to conform with the former family diagnosis of Questidae (Giere and Erséus 1998), but rather to belong to Paraonidae based on several external and internal characters. Further, the species *Periquesta canariensis* Brito & Núñez, 2002 is regarded as a member the paraonid genus *Levinsenia* Mesnil, 1867. A second new species from Hawai'i, quite similar to *P. canariensis*, also turned out to belong to *Levinsenia*. On this extended basis the circumscription of *Questa* is revised and supplemented by a suggestion of the phylogeny of all its species known to date.

Material and methods

Material for microscopical study

Questa material was collected by C.E. in coastal sediments in Hainan (China) and various parts of the South Pacific Ocean: Fiji, Great Barrier Reef (Queensland, Australia), Lord Howe Island (New South Wales, Australia), and New Caledonia. The New Caledonian material was collected during two workshops: one in the area of Touho on the main island (Atelier Biodiversité Récifale, Expédition Montrouzier, 1993), the other on Lifou in the Loyalty Islands (Atelier Biodiversité LIFOU, 2000). Polychaete material collected in Hawai'i in 1987 and 1997–2001 was sent to us by Mr. Dale Davis, and Dr. Julie Bailey-Brock/Mrs. Jennifer Dreyer, respectively. For comparison, specimens of *P. canariensis* Brito & Núñez from the Canary Islands were placed at our disposal by the original authors and the Museo de la Naturaleza in Santa Cruz de Tenerife.

The specimens collected by C.E. were sorted from decanted, sieved fractions of shallow subtidal samples, fixed in Bouin's fluid, and subsequently transferred into 70–80% ethanol. The Hawai'ian material was collected with a van Veen grab at depths of 5–70 m and fixed in buffered aqueous formaldehyde solution (4%) or (1987 sample) by hand in shallow water and fixed in Bouin's fluid.

A majority of the worms were stained in alcoholic paracarmine, cleared in xylene or BioClear (CIAB, Chemical Instruments AB, Lidingö, Sweden), and whole-mounted in Canada Balsam. Others were embedded in Paraplast, sectioned (4–6 µm), mounted on microscope slides, and stained (in either toluidine-blue or nucleic red/light green/orange). A few additional specimens were freeze-dried and gold-sputtered for scanning electron microscopy (SEM). Details of the material are given in association with the descriptions of the various species below.

Types and other reference material are deposited in the Swedish Museum of Natural History (SMNH), Stockholm, Sweden; Zoologisches Museum der Universität Hamburg (ZMUH), Hamburg, Germany; the United States National Museum of Natural History (USNM), Washington, D.C., USA; and the University of Hawai'i, Zoology Department, The Wormlab, Honolulu, USA.

In the text, Arabic numerals are used to denote individual segments, the peristomium (buccal region) being considered the first segment (referred to as 1), the first chaetiger in the species described here being segment 2, and terms such as 7/8 denoting the corresponding intersegmental region.

Phylogenetic analysis

A character matrix developed by Giere and Erséus (1998) is expanded and reassessed here. Morphological characters and character states (listed below) for ten species of *Questa* (ingroup taxa) and four species of Orbiniidae (outgroup taxa) were scored (Table 1) and

Table 1. Data matrix for parsimony analysis of *Questa*

Species	Character		
	00000 12345	00001 67890	11111 12345
<i>Naineris quadricuspida</i> (F., 1780)	10001	10300	00000
<i>Scoloplos armiger</i> (Müller, 1776)	10001	00300	01000
<i>Orbinia norvegica</i> (M. Sars, 1783)	10001	00300	00000
<i>Naineris laevigata</i> (Grube, 1855)	10001	10300	00??0
<i>Questa retrospermatice</i> sp. n.	11111	00111	11111
<i>Questa ersei</i>	0?111	10211	11111
<i>Questa paucibranchiata</i>	0?111	10211	11111
<i>Questa caudicirra</i>	0?111	10111	11111
<i>Questa mediterranea</i>	0?111	11211	11111
<i>Questa bicirrata</i>	0?111	00211	11111
<i>Questa riseri</i>	11010	11111	11111
<i>Questa media</i>	11110	11?11	11111
<i>Questa trifurcata</i>	111??	?1211	11111
<i>Questa fijiensis</i> sp. n.	0?111	01011	1111?

For characters and character states, see text. The top four species are outgroups. ? = unknown or not applicable. Authors of *Questa* species are given in the text.

analysed using PAUP, version 4.0 b10 (Swofford 2003). The branch-and-bound option (addition sequence: furthest) was selected to find the most parsimonious tree, which was arbitrarily rooted among the outgroup taxa. Branches were collapsed (creating polytomies) if minimum branch length was zero (option “amb-”); otherwise, default settings were used. Non-applicable character states were treated as “?”, i.e. as unknown. Bootstrap values were calculated to show branch support, using branch-and-bound searches on 500 replicates.

Characters and character states used

- (1) Forks: absent (0); present (1).
- (2) Median dents in fork: numerous (0); one or two (1).
- (3) Secondary annuli: none or few (0); numerous (1).
- (4) Branchial distribution: anterior and posterior (0); posterior only (1).
- (5) Branchial cilia: absent (0); present (1).
- (6) Pygidial cirri: two (0); four (1).
- (7) Dents in crenulations: ten or more (0); less than ten (1).
- (8) Origin of caeca in males: in segment 6 (0); in segment 7 (1); in segment 8 (2); further back (3).
- (9) Crotchets with ligaments: absent (0); present (1).
- (10) Dorsal crotchets: absent (0); present (1).
- (11) Dorsal fold (glandular, opening of spermioducts) in males: absent (0); present (1).
- (12) Pygidial end: rounded (0); bilobed (1).
- (13) Oocytes: diffuse (0); concentrated (1).
- (14) Sperm receptacles: absent (0); present (1).
- (15) Complete ventral buccal pad: absent (0); present (1).

Taxonomic descriptions

Family ORBINIIDAE

Now including taxa previously referred to as ‘Questidae’; see the introduction.

Genus *Questa* Hartman, 1966

Description (modified from Giere and Erséus 1998)

External morphology. Small and slender worms up to 10 mm long with 45–65 segments. Segments with secondary annulation, mostly 2–3 annuli in anterior segments and 7–8 annuli in median body region, number decreasing posteriorly, some species little annulated; annulation depending also upon state of contraction and fixation. Prostomium subtriangular, lacking appendages, with a pair of slit-like nuchal organs in latero-dorsal position near border line to peristomium. Peristomium as wide as subsequent segments, achaetous. Parapodia vestigial (tiny humps); notopodial and

neuropodial bundles (rami) of chaetae in lateral position and well separate (chaetation below). Nephridiopores slightly ventral to notopodial bundle. Pairs of dorsal appendages (1 pair per segment), mostly assigned as branchiae, in varying number of posterior segments, but totally absent in *Q. trifurcata*. In some species their outer side (facing the lateral chaetae) with row of cilia. Pygidium vertically bilobed with 1 pair (dorsal) or 2 pairs (dorsal and ventral) of caudal (or anal) cirri [in *Q. trifurcata* caudal cirri absent]; terminal anus in dorso-ventral cleft.

Chaetation. All species of *Questa* with at least two kinds of chaetae: (1) long, serrated capillary or hair chaetae, serrated (crenulated) on one side, except at basis; (2) short and stout jointed bifid crotchets (hooks) with sigmoid shafts. The lower prong with subdental ligament towards shaft. In some *Questa* sp. a third type of chaeta in dorsal anterior bundles: furcate chaeta on slender, smooth shaft with 1–2 shorter median dents. Chaetation subject to some intraspecific variation.

External genital structures. In mature males segments 13–14 (sometimes also 15) with a cup-like or slit-like ‘dorsal fold’, its elevation depending on species, stage of maturity, and degree of retraction at fixation. Mature females of almost all species with more or less papillated glandular epidermis in anterior segments. Extension and shape of this papillation species-specific, but also depending on stage of maturity. Egg region (mostly segments 12–13) of mature females slightly swollen due to formation of 2–3 large eggs. Ovipores not visible.

Internal morphology (light microscopy). With antecerebral vascular loop, supraoesophageal ganglion in prostomium, pharynx with complete ventral buccal pad. Subsequent alimentary tract tortuous and, except for pharynx, strongly ciliated. At transition from oesophagus to intestine, two latero-ventral caeca extending anteriorly; caeca set off from intestine by sphincters. Their extension and origin varies between and within species, but also between the sexes. Mature males with paired massive sperm sacs extending mostly from 10 or 11 to 12 and containing sperm cells in all stages of maturation. These sacs in 12 or 13 connected by a tortuous duct with a pair of globular to oval seminal reservoirs. After passing a region of large and lobed mucus glands, gonoducts open in inner lateral wall of the ‘dorsal fold’. Dorsal parts of segments 14–15 filled with large, lobed glands.

Mature females with one or two unpaired sperm receptacles located in ventro-lateral position, mostly in segment 5 or 5–6, their ectal duct bending dorsally and opening mostly in latero-dorsal position on left side. [Receptacles paired in *Questa fijiensis* and *Questa retrospermatika*, but in the latter located in segments 12–13.] Two to three developing oocytes in 12–13, if fully mature extending into 14. Unique sperm ultrastructure (Jamieson and Webb 1984).

Species included

Type species: *Questa caudicirra* Hartman, 1966.

Other species: *Questa trifurcata* (Hobson, 1970); *Q. media* Westheide, 1981; *Q. ersei* Jamieson & Webb, 1984; *Q. bicirrata* Giere & Erséus, 1998; *Q. mediterranea* Giere & Erséus, 1998; *Q. paucibranchiata* Giere & Erséus, 1998; *Q. riseri* Giere & Erséus, 1998; *Q. fijiensis* sp. n.; *Q. retrospermatice* sp. n.

Questa retrospermatice sp. n.

(Fig. 1A–H)

Etymology

Species with sperm receptacles located further back than in all other *Questa* species, in ovarian segments (12–14). The species epithet is adjectival for the purposes of nomenclature.

Material examined

Holotype. ZMUH P-24693, whole-mounted female; Mamala Regional Station 6, O'ahu, Hawai'i, USA, 21°16.56'N, 157°04.26'W, 14.6 m, 11 August 2001.

Paratypes (all from Hawai'i). ZMUH P-24694a–b, 2 whole-mounted specimens on separate slides: male, Puako Beach, Big Island, 19°58.24'N, 155°50.27'W, 5.0 m, 3 September 2001; male (6 postgenital segments removed for EM work), station SI B5R1, 21°17.00'N, 157°54.03'W, 70.1 m, 21 August 1997. ZMUH P-24695a–b, 2 whole-mounted specimens on separate slides: female, station SI B4R2, 21°17.01'N, 157°54.24'W, 58.5 m, 10 August 1998; female, Puako Beach, Big Island, 19°58.24'N, 155°50.27'W, 5.0 m, 3 September 2001. ZMUH P-24696, longitudinal sections mounted on 6 slides: male, Mamala Regional Station 32, O'ahu, 21°16.51'N, 157°53.47'W, 53.0 m, 14 August 2001. ZMUH P-24697, longitudinal sections mounted on 6 slides: female, from type locality. ZMUH P-24698, SEM preparation on stub: male, Mamala Regional Station 32, O'ahu, 21°16.51'N, 157°53.47'W, 53.0 m, 14 August 2001. ZMUH P-24699, SEM preparation on stub: male, station SI B4R2, O'ahu, 21°17.01'N, 157°54.24'W, 58.5 m, 10 August 1998. ZMUH P-24700, longitudinal sections mounted on 5 slides: male, Mamala Regional Station 32, O'ahu, 21°16.51'N, 157°53.47'W, 53.0 m, 14 August 2001. USNM 1104646, male, and USNM 1104647, female, both whole-mounted, shallow-water flat at Beach Park, Maunaloa Bay, near Honolulu, O'ahu, Hawai'i, 21°16.52'N, 157°42.57'W, 2 April 1987, coll. D. Davis. University of Hawai'i, Zoology Department P 0281, whole-mounted male, from type locality, 11 August 2001; and P 0282, whole-mounted female, Paiko Beach, O'ahu, Hawai'i, 21°16.53'N, 157°43.40'W, 2 April 1987, coll. D. Davis.

Other material. ZMUH P-24700, longitudinal sections mounted on 5 slides: anterior part of male, Mamala

Regional Station 32, O'ahu, Hawai'i, 21°16.51'N, 157°53.47'W, 53.0 m, 14 August 2001. ZMUH P-24701, SEM preparation on stub: male, Mamala Regional Station 6, O'ahu, 21°16.56'N, 157°04.26'W, 14.6 m, 11 August 2001. ZMUH P-24702, SEM preparation on stub: male, station SI B5R1, O'ahu, Hawai'i, 21°17.00'N, 157°54.03'W, 70.1 m, 21 August 1997. ZMUH P-24703, incomplete male, and ZMUH P-24704, female, both whole-mounted: Ximaozhou (West Coral Islet), southern coast of Hainan, China, 18°14.56'N, 109°21.97'E, 0.3 m, coarse sand, 17–19 March 2000. SMNH 93174, whole-mounted female (hind end lacking): Wuzhi Island, southern coast of Hainan, China, 18°18.97'N, 109°45.85'E, intertidal station, rocky sand, 3 March 2000. First author's collection: 6 whole-mounted immature or incomplete specimens, and sections (on 1 slide) of incomplete male, all from Hawai'i; and whole-mounted submature specimen, from Ximaozhou, Hainan (see above), 17 March 2000.

Differential diagnosis

Questa retrospermatice is distinct from other questiid species by the presence of sperm receptacles in the egg segments 12–13 rather than in the oesophageal region (segments 5–6), by the paired arrangement of sperm receptacles (two pairs as opposed to one or two unpaired receptacles in other questids), and the presence of a duplicate median tine in the furcate chaetae.

Description of type material (from Hawai'i)

Body size and segments. Length (fixed) about 3.5–4.8 mm, width in segment 8 about 0.2 mm, in segment 20 about 0.15 mm; 30–47 segments.

External morphology. Anterior end conical with typical ventral mouth opening and large pharyngeal pad (Fig. 1A). In median body region mostly three annuli per segment, decreasing in number towards anterior and posterior body end. Last 11–22 segments each with one pair of dorsal branchial appendages. One dorsal pair plus one ventral pair of pygidial cirri, these cirri longer than pygidium and with sparse tuft of short cilia (Fig. 1B, G). In mature males, segment 14 ventrally shortened (body often bent here); small 'dorsal fold' (Fig. 1C) extending over 14 only, often just slightly elevated, with honey-combed internal surface. In females, a region of glandular epidermal papillae in segments 7–11, beginning with a few small dotted glands, developing into conspicuous warty glands in 8–11; extension of glandular girdle probably depending on stage of maturity. Pygidium bilobed with vertical median cleft and terminal anus (Fig. 1B).

Chaetation. Segment 1 achaetous. Three types of chaetae present in other segments: (1) 'serrated' ('crenulate') hair chaetae (Fig. 1D), in pre-genital region dorsally three to four per bundle, ventrally two to three per bundle, in post-genital region dorsally two or three,

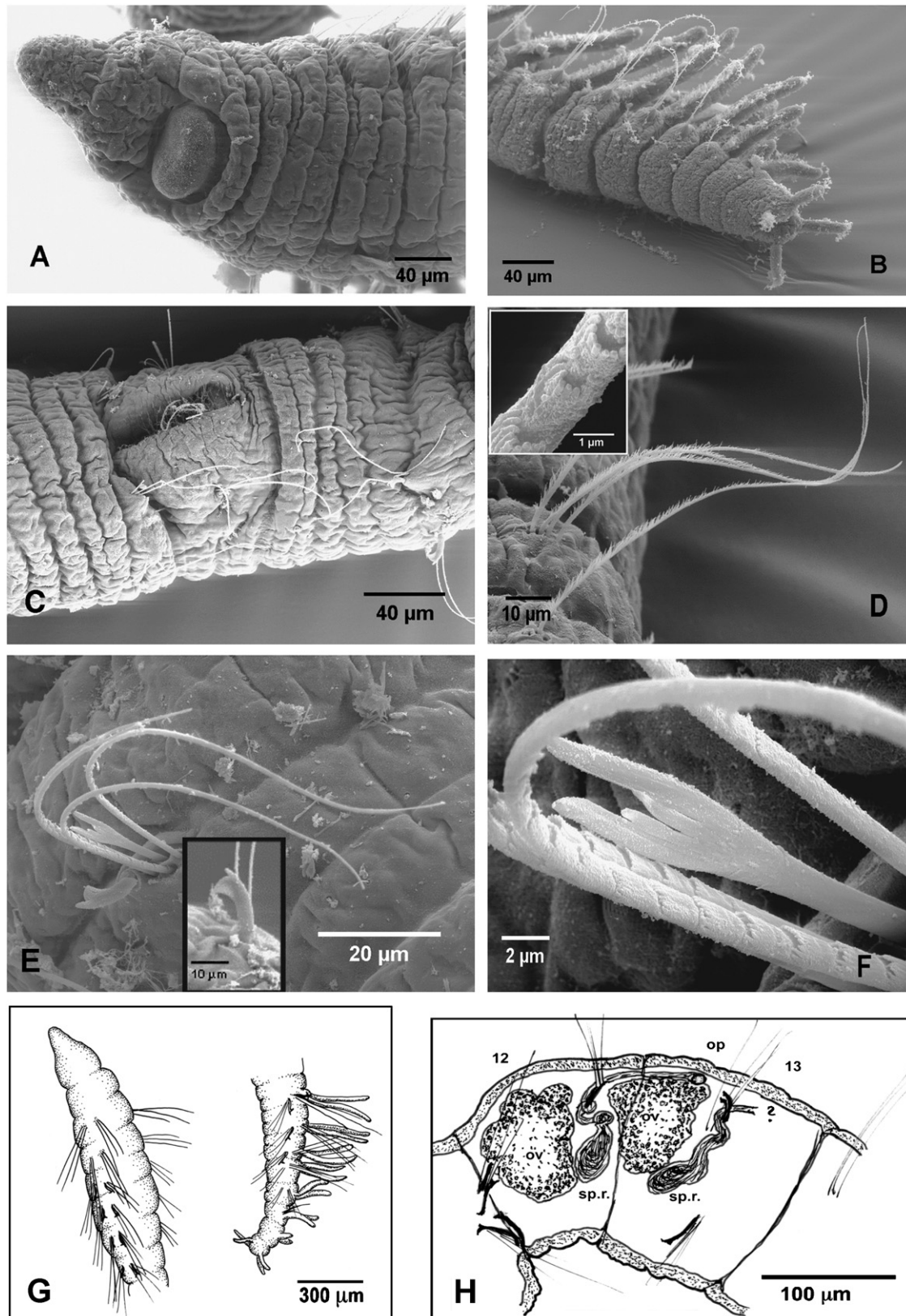


Fig. 1. *Questa retrospermatica* sp. n.; A–F: SEM micrographs, G–H: camera lucida drawings. (A) Anterior end, ventrally with buccal pad. (B) Posterior end, ventrolateral view. (C) Dorsal fold. (D) Capillary chaetae; inset: gallery of 8–10 long dents. (E) Chaetal fascicle with capillary chaetae and one furcate chaeta and bifid crotchet each; inset: crotchet with subdental ligament. (F) Furcate chaeta with double median dent beside crenulated hair chaetae. (G) Left: anterior end, laterodorsal view; right: lateral view of posterior end of paratype P-24694 (ZMUH). (H) Lateral view of genital region of holotype female: one sperm receptacle (sp.r.) with dorsal opening (op) and one egg in segments 12 and 13, respectively.

ventrally one to two per bundle; (2) stout bifid crotchets with subdental ligaments, dorsally and ventrally one per bundle (Fig. 1E and inset); (3) furcate chaetae with double median tine (dent) (Fig. 1F) dorsally in segments 3–6(7), two (rarely three) per bundle, sometimes only one; fold region (segment 14 in males) dorsally without chaetae, ventrally with normal chaetation, but sometimes number of ventral chaetae also reduced; chaetae in egg-bearing segments in females with normal number, only sometimes number reduced. In anterior region of body, chaetal bundles in mid-segmental position, in 14–15 somewhat shifted to the anterior, in post-genital segments in posterior position, close to septum.

Chaetal structure. About 22–25 transverse serrations (crenulate galleries) on each hair chaeta, each gallery formed as a series of about 8–10 long dents (Fig. 1D, inset). Bifid crotchets correspond to general pattern in questids (see Giere and Riser 1981; Giere and Erséus 1998). Median ‘double-tine’ of furca at least half the length of lateral dents (Fig. 1F).

Internal morphology. With complete ventral buccal pad (for details see fig. 2 in Giere and Riser 1981). In males and females, transition of oesophagus to intestine in middle to posterior part of 7 marked by origin of paired caeca extending forward into anterior part of 6, sometimes into mid 5. In males, paired sperm sacs extend from 11 (or 12) to 13; a pair of globular seminal

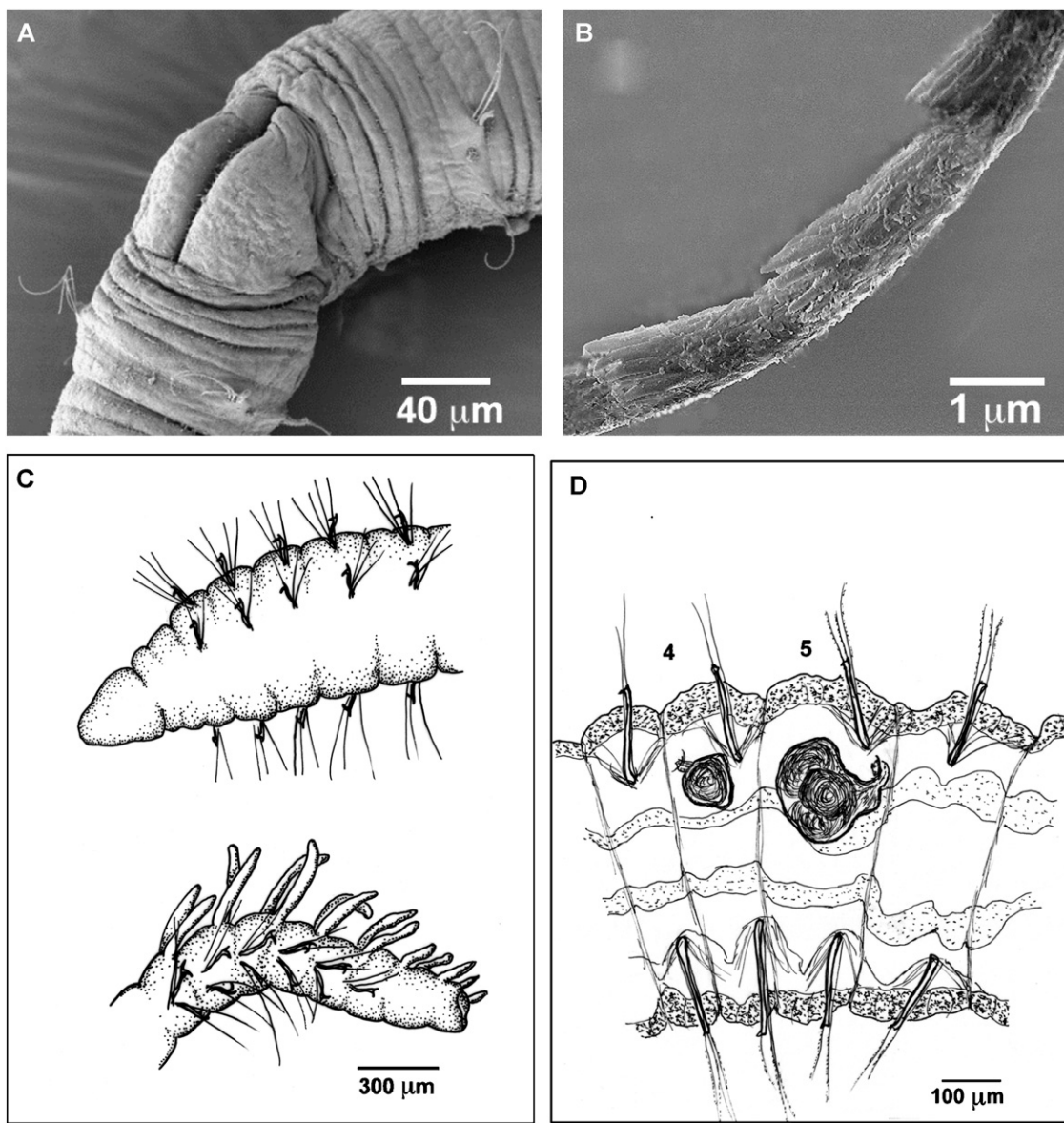


Fig. 2. *Questa fijiensis* sp. n.; A–B: SEM micrographs, C–D: camera lucida drawings. (A) Dorsal fold. (B) Capillary chaeta with galleries of long dents. (C) Top: anterior end, lateral view; bottom: ventrolateral view of posterior end of paratype P-24707 (ZMUH). (D) Holotype female with paired sperm receptacles in segments 4 and 5.

reservoirs present laterally in 13. Mucous glands fill dorsal fold and dorsal body parts of 14–15. In mature females, two pairs of sperm receptacles, located laterally behind eggs in posterior 12 to anterior 13, and in posterior 13 to anterior 14, respectively (Fig. 1H). Receptacles filled with loose and irregular bundles of sperm. From receptacles, coiled ducts lead dorsally to open in laterodorsal position in both 13 and 14 (Fig. 1H). (1)2(3) developing oocytes dorsally in 12–13. Septa in 12–13 often bulged out obliquely, distorted by eggs.

Description of Chinese material

The single mature, but incomplete, male had a large, albeit not much elevated dorsal fold extending from 14 to 17. The single complete female attained a length of 5–6 mm. In all other features these specimens correspond well with the above description of *Q. retrospermatika*.

Distribution and habitat

Hawai'i and southern China, but see also the possibly identical species reported as *Q. cf. retrospermatika* below. Subtidal sediments, mostly calcareous medium sand, 0–70 m depth.

Questa cf. retrospermatika (from New Caledonia)

Material examined

First author's collection, 11 whole-mounted specimens from around Touho, main island of New Caledonia: 3 females and 1 male, S of Ilot Ain, S end of Grand Récif Mengalia, 20°45.1'S, 165°16.0'E, lower intertidal, greyish coarse sand, 14 September 1993 (CE station NC93-13); submature specimen, E of Baie de Touho, S side of large intertidal bank N of Kombounou, 20°46.0'S, 165°14.1'E, barely subtidal, greyish medium to coarse sand, 16 September 1993 (NC93-27); male, N of Touho, SE corner of Ilot Ouao, 20°43.3'S, 165°08.7'E, lower intertidal, greyish sand with sparse seagrass, 17 September 1993 (NC93-31); female, N of Touho, reef platform NE of Ilot Ouao, 20°42.9'S, 165°09.1'E, lower intertidal, coarse, poorly oxygenated sand, 17 September 1993 (NC93-33); female, N of Touho, back reef E of Ilot Ouao, 20°43.0'S, 165°09.0'E, 0.5 m, small pool in reef flat, with coarse, poorly oxygenated sand, 17 September 1993 (NC93-35); and 2 females and 1 male, W of S end of Grand Récif Mengalia, 20°44.4'S, 165°15.8'E, 3 m, coarse sand and coral gravel, 23 September 1993 (NC93-83).

Remarks

Identical to the situation in *Q. retrospermatika* sp. n. from Hawai'i and China, these questids from New Caledonia have sperm receptacles occupying the segments of the egg region. However, in six out of the seven mature females studied, additional loose sperm aggregations were found irregularly scattered in segments 5–6,

sometimes occurring as far back as in 9. Walls and ducts confining these additional sperm aggregations were found only in two cases, where ectal ducts extended dorsally from the sperm receptacles and opened laterodorsally in 7/8. On average, these *Questa* specimens from New Caledonia are larger than those from Hawai'i: they are up to 6.5 mm long and 0.4 mm wide (segment 9). In all other structural features they correspond to *Q. retrospermatika* from Hawai'i.

Questa fijiensis sp. n.

(Fig. 2A–D)

Etymology

Named for the Fiji Islands. The species epithet is adjectival for the purposes of nomenclature.

Material examined

Holotype. ZMUH P-24705, whole-mounted female specimen; south beach, Mana Island (W of resort), West of Lautoka, Viti Levu, Fiji Islands, 0.5 m, sand, 5 December 1982.

Paratypes. ZMUH P-24706, mature male, and ZMUH P-24707, mature female: whole-mounted on one slide each, from type locality.

Other material. First author's collection: whole-mounted immature specimen without hind end, and incomplete specimen (sex not identifiable) on stub for SEM; both from type locality.

Differential diagnosis

Among the previously known questids, *Q. bicirrata* Giere & Erséus, 1998 from the Philippines is the only other *Questa* species that has a single (dorsal) pair of pygidial cirri; although the most parsimonious tree suggested by the phylogenetic analysis (see Fig. 5 and text below) does not place *Q. bicirrata* and *Q. fijiensis* as sister taxa. *Q. fijiensis* is distinct from this species mainly by the presence of two pairs of sperm receptacles (in segments 4–5) rather than just one pair in segment 4, by the lower number of segments and branchiae, and the smaller number of dents in the crenulate galleries of the capillary chaetae (about 10 as opposed to 12–14 in *Q. bicirrata*). The absence of a glandular papillary region in mature females of *Q. fijiensis* adds to the differences from *Q. bicirrata*.

Description of type material

Body size and segments. Length about 3.8–5.3 mm (fixed), width pre-genitally about 0.2–0.3 mm, post-genitally about 0.1–0.15 mm; 35–48 segments.

External morphology. In median body region six to seven small annuli per segment, decreasing in number towards anterior and posterior ends. Last 10–17 segments each with one pair of dorsal branchial appendages (Fig. 2C); branchial appendages with external and internal row of cilia. One dorsal pair of pygidial cirri,

with short and sparse cilia. In mature males, small ‘dorsal fold’ (Fig. 2A) extending over segments 13–14, only slightly elevated. Ventral part of 13–14 shortened, causing body to bend in this region. In females, a region of glandular epidermal papillae, typical for other questids, not discernible. Pygidium bilobed with vertical median cleft and terminal anus (Fig. 2C).

Chaetation. Segment 1 achaetous. Two types of chaetae present in other segments: (1) serrated (‘crenulate’) hair chaetae, in pre-genital region two to three per bundle, in post-genital region only one per bundle; (2) stout bifid crotchets with subdental ligaments, dorsally and ventrally one per bundle. Furcate chaetae absent. Fold region of males (segments 13–14) and egg-bearing region of females (12–13/14) with normal chaetation, but sometimes chaetae reduced in number. Chaetal bundles in segments 2–4 in mid-segmental position, in following segments in posterior position; especially in branchial segments bundles close to posterior septa.

Chaetal structure. About 20 transverse crenulate galleries on each hair chaeta formed as a series of about 8–10 long dents (Fig. 2B). Bifid crotchets correspond to general pattern in questids (see Giere and Riser 1981, Giere and Erséus 1998).

Internal morphology. In males and females, transition of oesophagus to intestine in anterior part of segment 7 marked by origin of paired caeca extending forward into anterior part of 6, sometimes reaching into mid 5. In males, paired sperm sacs extend through 12–13; a pair of globular seminal reservoirs present laterally in 12. Mucous glands fill dorsal fold and dorsal body parts of 13–14. In mature females, two pairs of sperm receptacles in mid 4 and mid 5, respectively (receptacles in 4 being smaller). Receptacles filled with coiled sperm. Short ducts of sperm receptacles lead laterodorsally to open near posterior septum of their respective segment (Fig. 2D). Two to three developing oocytes in 12–13, sometimes in anterior part of 14. Septa in 12–13 often distorted by eggs.

Distribution and habitat

Fiji Islands. Fine to medium coralline sand with scattered sea grass, barely subtidal (0.5 m depth).

Questa ersei Jamieson & Webb, 1984

Questa ersei Jamieson & Webb, 1984: 23–29, figs. 1–28, 33. – Giere and Erséus (1998, p. 352).

New material examined

ZMUH P-24708, male, and P-24709, female, both whole-mounted: Lord Howe Island, East of New South Wales, Australia, about 31°33'S 159°05'E, subtidal sediment, 0.5–3 m, 13 and 15 February 1988. ZMUH P-24710, male, and P-24711, female (incomplete), both whole-mounted: in front of Heron Island Research

Station, Heron Island, Great Barrier Reef, Queensland, Australia (CE station Q91-21), lower intertidal, fine to medium sand with some gravel, 7 Jan. 1991. ZMUH P-24712, female, longitudinal sections mounted on 1 slide: back reef of S part of Carter Reef, outer barrier off Lizard Island, Great Barrier Reef, Queensland, Australia (CE station Q82-31), 3 m, small patch of fine sand and coral rubble, 16 November 1982. ZMUH P-24713a–m, 13 whole-mounted specimens (some incomplete) from New Caledonia. 1 male (P-24713a), NW of Ilot Ain, inside S end of Grand Récif Mengalia, off Touho on main island of New Caledonia, 20°44.5'S, 165°15.7'E, 8 m, fine sand, 14 September 1993 (CE station NC93-17); female (P-24713b) and male (P-24713c), S side of large intertidal bank N of Kombounou, E of Baie de Touho, off Touho, 20°46.0'S, 165°14.2'E, 0.5 m, medium to coarse sand under dead coral clump, 16 September 1993 (CE station NC93-28); female (P-24713d) and male (P-24713e), W of S end of Grand Récif Mengalia, off Touho, 20°44.7'S, 165°16.0'E, 3.5 m, medium to fine sand, 23 September 1993 (CE station NC93-86); female (P-24713f) and male (P-24713g), Easo Village, Baie du Santal, Lifou, Loyalty Islands, 20°47'S, 167°08'E, 0.5 m, seagrass with coarse sand and some corals, 14 November 2000 (CE station NC00-3); 1 female (P-24713h) and 2 males (P-24713i–j), N of Cape Aimé Martin, W side of Lifou, Loyalty Islands, 20°46'S, 167°02'E, 31 m, sand, 21 November 2000 (CE station NC00-22); 2 males (P-24713k–l), off Easo Village, Baie du Santal, Lifou, Loyalty Islands, 20°47'S, 167°08'E, 3 m, fine to medium, poorly sorted sand, with some seagrass roots, 22 November 2000 (CE station NC00-26); and female (P-24713m), E of Cape Aimé Martin, Baie du Santal, Lifou, Loyalty Islands, 20°46.8'S, 167°02.7'E, 15–18 m, poorly sorted, largely medium sand, 27 November 2000 (CE station NC00-44). ZMUH P-24714: male without hind end, sectioned, mounted on 3 slides, (CE station NC00-26) (see above). ZMUH P-24715: female without hind end, sectioned, mounted on 2 slides, (CE station NC00-22) (see above). ZMUH P-24779–24780, 2 whole-mounted males (1 incomplete): Shark Bay, Heron Island, Great Barrier Reef, Queensland, Australia (CE station Q91-2), intertidal, heterogeneous sand, 4 January 1991. ZMUH P-24781, whole-mounted male: Heron Island (CE station Q91-21; see above). SMNH 93175–93185, 11 whole-mounted specimens (some incomplete) from New Caledonia: submature worm (93175), CE station NC93-17 (see above); female (93176), about 200 m SW of school at Kombounou, Touho, 20°46.7'S, 165°13.9'E, lower intertidal, heterogeneous sand, 15 September 1993 (CE station NC93-21); 2 males (93177–93178), CE station NC93-28 (see above); male (93179), SE corner of Ilot Ouao, N of Touho, 20°43.3'S, 165°08.7'E, lower intertidal, greyish sand with seagrass, 17 September 1993 (CE station NC93-31); female (93180), W of Pte de

Easo, Baie d'Hunete, Baie du Santal, Lifou, 20°47'S, 167°07'E, 4–5 m, poorly sorted sand, 17 November 2000 (NC00-12); male (93181), station NC00-22 (see above); submature worm (93182), CE station NC00-26 (see above); male (93183), CE station NC93-86 (see above); male (93184), NE part of Baie du Santal (at level of Cila), Lifou, 20°49'S, 167°11'E, 5–7 m, sand, 26 November 2000 (CE station NC00-39); and female (93185), E of (islet) Huca Uthigé, Baie du Santal, Lifou, 20°52.6'S, 167°08.3'E, 4 m, coarse sand, 26 November 2000 (CE station NC00-40).

Remarks

The individuals from New Caledonia, Lord Howe Island and Heron Island conform in all relevant details with Jamieson and Webb's (1984) original description of *Questa ersei*, which was based on material from Lizard Island (Great Barrier Reef). They are therefore assigned to this species. Additional records of *Q. ersei*, from other parts of the Great Barrier Reef as well as the Houtman Abrolhos in Western Australia (Indian Ocean), were given by Giere and Erséus (1998).

Distribution and habitat

New Caledonia, Lord Howe Island (both new records), and Great Barrier Reef in SW Pacific Ocean. Intertidal and subtidal sandy sediments, to at least 31 m depth.

Family PARAONIDAE

Genus *Levinsonia* Mesnil, 1897

Periqueta Brito & Núñez, 2002 syn. n.

Levinsonia canariensis (Brito & Núñez, 2002) comb. n. (Fig. 3A–C)

Periqueta canariensis Brito & Núñez, 2002: 284–285, fig. 2.

Material examined

Received on loan from the Museo de Ciencias Naturales de Tenerife, Canary Islands, Spain: paratype AN/000203, from type locality, unstained subadult worm, mounted on slide. Provided by Dr. Jorge Núñez, Departamento de Biología Animal (Zoología), Universidad de La Laguna, Tenerife, Canary Islands, Spain: 2 unstained paratypes, ZMUH P-23285 and P-23286,

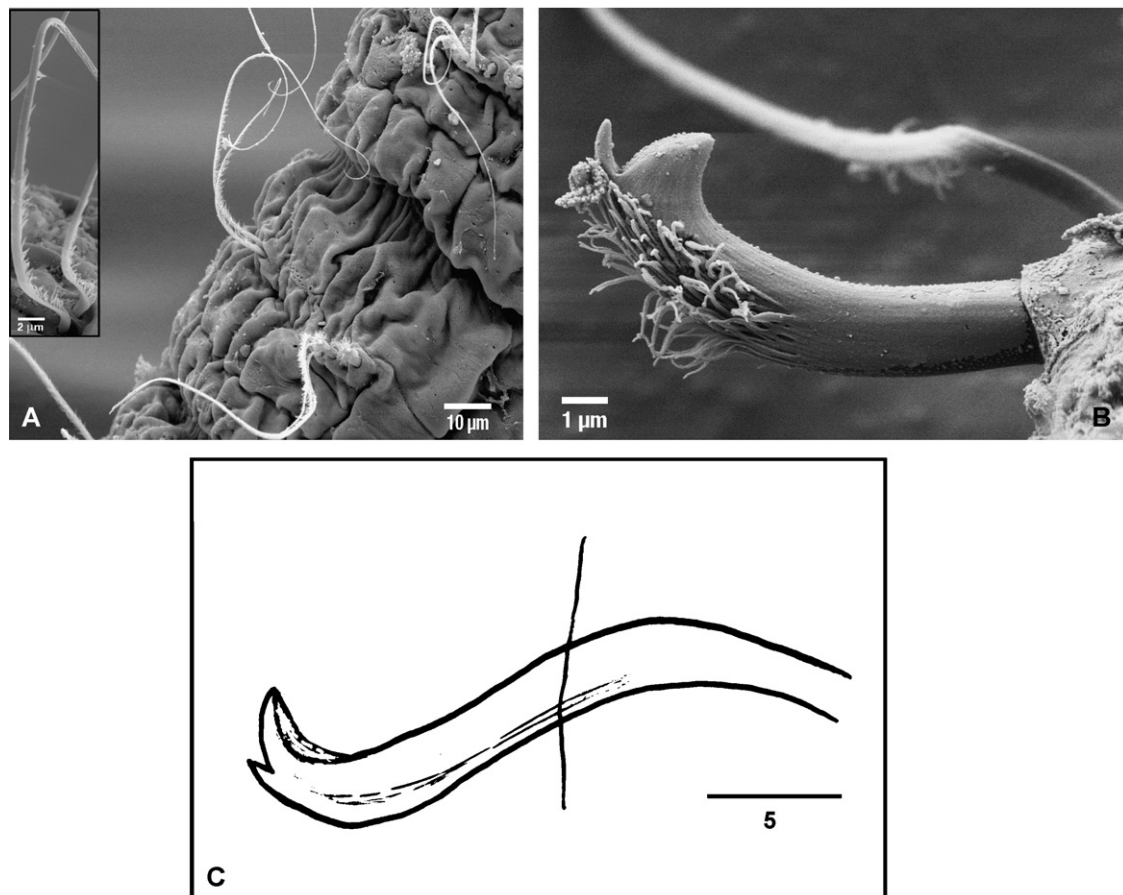


Fig. 3. *Levinsonia canariensis* comb. n.; A–B: SEM micrographs, C: camera lucida drawing. (A) Capillary or hair chaetae, showing typical frayed margin. (B), (C) Crotchets; note frill after SEM preparation in B. (C) Detail of complete female L2 IC-5 from Lanzarote, 14th chaetiger; LM preparation.

mounted on 2 slides, from type locality, 1 female with egg, 1 specimen immature; ZMUH P-23287, female on slide, and ZMUH P-23288, immature specimen in alcohol. Both from Ponta do Sul, Ilhéu de Fora, Selvagens Islands, 29°07.27'N, 16°02.36'W, 5 m, sandy bottom, May 1999; and ZMUH P-24776 and P-24777, 2 females (1 without hind end), from Playa Quemada, Lanzarote, 28°53.15'N, 13°47.57'W (Station L2 1C-5), December 1993, stained in Paracarmine, mounted in Canada Balsam on 2 slides; ZMUH P-24778, 1 submature specimen (no hind end) from Playa Quemada, Lanzarote, 28°53.15'N, 13°47.57'W, (Station L2 1C-5), December 1993, stained in Paracarmine, mounted in Canada Balsam on slide.; ZMUH P-24786 and P-24787, 2 specimens without hind ends, SEM preparations, mounted on 2 stubs, from Playa Quemada, Lanzarote, 28°53.15'N, 13°47.57'W (Station L2 1C-5).

Redescription of non-type material collected and identified by Núñez and Brito

Body size and segments. Length about 2–3 mm (coiled), width about 0.1–0.2 mm; 30–41 segments.

External morphology. Prostomium and peristomium fused, forming conical head; no eyes, terminal sense organ and nuchal organs not observed. Segments bi- to triannulate, in anterior body wider than long, in posterior body about as long as wide. Pygidium rounded, with three anal cirri.

Chaetation. Notochaetae all capillary (Fig. 3A), smooth, slender, about four per fascicle, very fine and hairlike in posterior segments, arising directly from body wall. Neurochaetae consisting of capillaries and crochets, the latter from segment 7 (chaetiger 6).

Chaetal structure. Capillary chaetae smooth, tapering to very fine tip; after preparation for SEM frayed along one side (Fig. 3A, inset); crochets bidentate, distal tooth slender, slightly bent; subdistal tooth elongate (Fig. 3B), with a thin connection from its tip to the shaft along the concave side (Fig. 3C) that is not well reproducible in SEM micrographs because of cover with homogeneous sputter material (Fig. 3B); solid shaft of crotchet S-shaped, only upper half emerging.

Internal morphology. Large eggs in segments 22–31.

Remarks

Scrutiny of the crochets with premium-class light microscopy and interference contrast revealed a depressed area or a slight groove on the concave side of the subdistal tooth, but in the present material it is not discernible whether this can be considered as a small ligament or a solid, but thin extension of the chaeta. The presence of a dorsal fold, reported by Brito and Núñez (2002) to occupy a position varying from 11–12 to 11–15 in mature males, remains questionable, since a dorsal

fold could not be found in our SEM inspections of the single male specimen of *P. canariensis* available. The lack of a pertinent figure in their study makes a structural comparison with the characteristic dorsal fold in *Questa* impossible (for comments on the absence of the dorsal fold in two specimens reported by Delgado and Núñez (1993–1994), see Giere and Erséus (1998). The ‘spermatic masses’ found by Brito and Núñez in segments 23–30 of one male pose another functional problem: In all species of *Questa* described so far, the sperm sacs become constricted to form paired globular sperm reservoirs adjacent to the dorsal fold, in which the sperm are densely coiled. From these reservoirs the sperm are then led to the fold by short, tortuous ducts. In the available material of *P. canariensis*, corresponding structures have not been observed. The dorsal anus, which *L. canariensis* has in common with *L. hawaiiensis* sp. n. is typical for many Paraonidae (compare *Aricidea*, fig. 4D, in Laubier and Ramos 1973).

Distribution and habitat

Canary Islands. Shallow sublittoral fine sand and seagrass meadows.

Levinsonia hawaiiensis sp. n.

(Fig. 4A–F)

Etymology

Named for the type locality. The species epithet is adjectival for the purposes of nomenclature.

Material examined

Holotype. ZMUH P-24772, subadult female, mounted in Canada Balsam on microscopic slide, stained in Paracarmine; SI 1997 Z R2, 21°16.53'N, 157°54.21'W, 75.6 m, 20 August 1997.

Paratypes. ZMUH P-24773, Mamala Regional St 27 (21°17.38'N, 157°55.32'W), 20.1 m, 10 August 2001; ZMUH P-24774, subadult female (3 parts), mounted in Canada Balsam on microscopic slide, stained in Paracarmine, SI 1997 B4 R5, 21°17.01'N, 157°54.24'W, 58.5 m, 20 August 1997; ZMUH P-24782, male, SEM preparation on stub, SI 1997 Z R2, 21°16.53'N, 157°54.21'W, 75.6 m, 20 August 1997; ZMUH P-24783, male, SEM preparation on stub, SI 1997 B4 R2, 21°17.01'N, 157°54.24'W, 58.5 m, 20 August 1997.

Other material. ZMUH P-24784, 1 male and ZMUH P-24785, 1 submature specimen, SEM preparations on 2 stubs; male BP86 STB R3, 21°17.12'N, 158°01.32'W, 62 m, 16 September 1986; submature BP90 HB4 R3, 21°16.58'N, 158°01.49'W, 58.5 m, 14 February 1990, SEM preparation on stub; ZMUH P-24775, 1 incomplete submature specimen, longitudinal Sections (1 µm, toluidine blue) and mounted on 1 slide, SI B4 R2, 21°17.01'N, 157°54.24'W, 58.5 m, 20 August 1997.

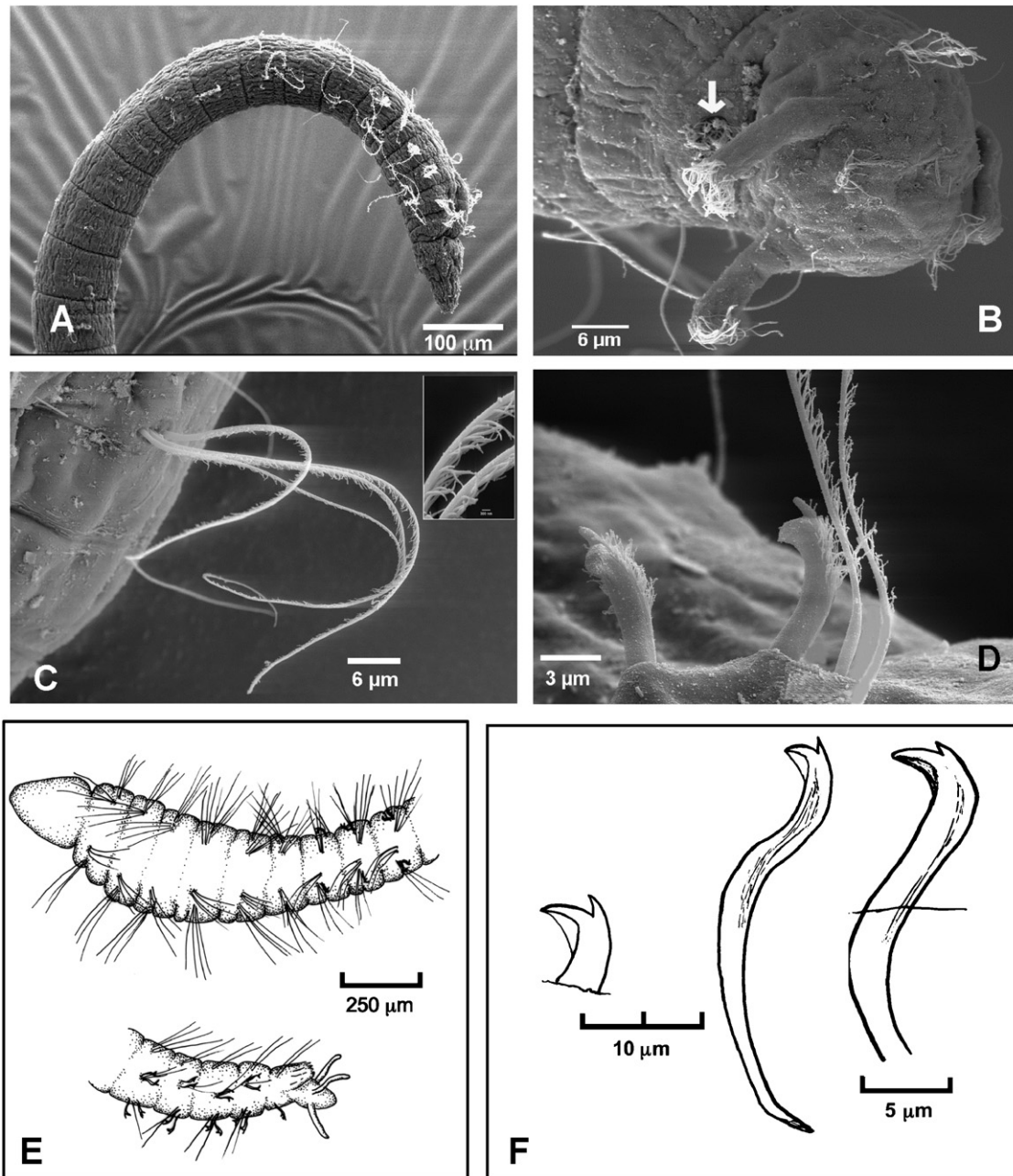


Fig. 4. *Levisenia hawaiiensis* sp. n.; A–D: SEM micrographs, E–G: camera lucida drawings, LM preparations. (A) Anterior end of adult male P-24785 (BP 86 STB R3; September 16, 1986), dorsolateral view; note absence of ‘dorsal fold’. (B) Posterior end of paratype ZMUH P-24783 (SI 1997 B4R2; August 20, 1997), dorsal view; note ciliated anal opening (arrow). (C) Capillary chaetae; inset: frills after SEM treatment. (D) Crotchets beside capillary chaetae, both frilled. (E) Top: anterior end in dorsolateral view; bottom: posterior end in ventrolateral view, note dorsal anus opening; holotype, subadult female (SI 1997 Z R2; August 20, 1997). (F) Bifid crotchets; left: tip of crotchet of 9th chaetiger; centre: complete crotchet of 44th chaetiger; right: crotchet of 14th chaetiger of *L. canariensis*.

Differential diagnosis

The only other species of *Levisenia* described to be abbranchiate is *Levisenia uncinata* (Hartman, 1965) from the Atlantic and Pacific deep sea. It differs from *L. hawaiiensis* sp. n. most prominently in the shape and distribution of crotchets along the body and in the presence of postsetal lobes in the notopodia. In *L.*

uncinata, the crotchets have a very distinct, broad membrane but no subterminal tooth, and the lobes are very much reduced (completely absent in *L. hawaiiensis*). Typically in paraonids, the neuropodial crotchets start in postbranchial setigers; in *L. uncinata* they occur first in chaetigers 13–18. In contrast, both in *L. canariensis* and in *L. hawaiiensis* sp. n. these crotchets are already

present from chaetiger 6 or 7, which underlines the close relationship between the latter two species. The crotchets also differ in shape from most *Levinsonia* species, which typically have a broad membrane on the convex side, ending more or less attached to the shaft of the crotchet and thus leaving the impression of a small subdistal tooth in some cases. In *L. canariensis* and especially in *L. hawaiiensis* sp. n., this subterminal tooth is prominently developed. In *L. hawaiiensis* sp. n. there is a clearly visible transparent membrane connecting the tip of the tooth with the shaft. The tip of the crotchets in *L. canariensis* appears slightly different: Judging from the available specimens, it is unclear whether there really is a membranous ligament or just a groove in the corresponding area, the lower part between dent and shaft. Bifid crotchets with a ligament on the concave side would be similar to crotchets in *Aricidea cerruti* Laubier 1967 (see figs. 1D, E in Laubier 1967).

Description of type material

Body size and segments. Length about 3 mm, width about 0.3 mm; 48–52 segments.

External morphology. Prostomium and peristomium fused (Fig. 4E), in juveniles relatively well separated, in adults as typical for the genus; terminal sense organ not observed. Segments about as long as wide, weakly bi- or triannulate (Fig. 4A). Branchiae absent. Pygidium with 2 dorsal and 1 ventral slender anal cirri, each with ciliary tuft. Anus dorsal (Fig. 4B, E)

Chaetation. Dorsal fascicles with capillary chaetae only, in anterior 3 chaetigers broad and more numerous than in subsequent chaetigers, then gradually becoming slender, in posterior chaetigers very fine (Fig. 4C). Ventral fascicles with few fine capillary chaetae and, beginning on chaetiger 6 or 7, with usually three relatively long, slender bifid crotchets, subterminal tooth much smaller than terminal tooth. In line between dorsal and ventral fascicle laterally an area with sensory(?) pores.

Chaetal structure. Capillary chaetae smooth, crotchets bifid, with prominent subdistal tooth, connected by thin ligament with shaft (Fig. 4D, F). Processing for SEM causes artefacts as in *L. canariensis*: transparent ligament appears solid, convex side of crotchets and one side of capillaries appear with frills formed by free ends of chitinous tubes (Figs. 3B, 4C, D). This artificial structural change after chemical treatment is not known from questids.

Internal morphology. Foregut without caeca; intestine sometimes filled with sediment.

Distribution and habitat

Hawai'i. Subtidal sediments, 5–75.6 m depth.

Phylogenetic analysis

The cladistic analysis of 15 morphological characters (described above and scored in Table 1) yielded a single

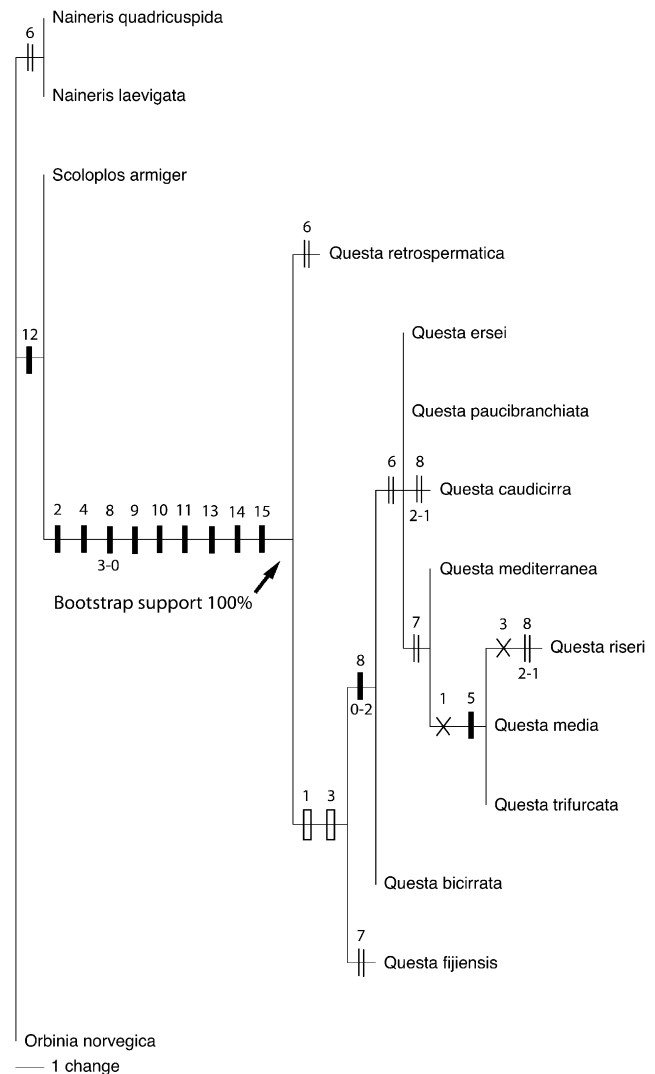


Fig. 5. Most parsimonious phylogenetic tree of *Questa*, based on morphological data matrix in Table 1. Character transformations optimized on the tree and shown by the following symbols: solid rectangle = unambiguous autapomorphy; open rectangle = autapomorphy, followed by reversal later in the tree; X = reversal; two parallel lines = convergent transformation. Character number (as defined in text) given above symbol; transformations of multistate character 8 explained under symbol (e.g. “3–0” means ‘going from state 3 to state 0’).

most parsimonious tree 23 steps long, with a consistency index of 0.74 and a retention index of 0.87 (Fig. 5). The ingroup (all *Questa* species) was strongly supported (bootstrap value 100%) due to nine unambiguous characters optimized as autapomorphies for *Questa*. None of the other nodes gained support values higher than about 50%. The tree suggests that *Q. retrospermatice* sp. n. is the sister to all other species of the genus in this most parsimonious topology, due to its lack of forks (character 1) and secondary annuli (char. 3), but the species shares this feature, as well as the four

pygidial cirri (char. 6), with many of its congeners. Further, according to our phylogenetic hypothesis, *Q. riseri*, *Q. trifurcata* and *Q. media* comprise a terminal group characterized by the lack (loss?) of branchial cilia (char. 5), but this is based on the optimization of the non-applicable state of this character for *Q. trifurcata* (see Table 1). By and large, the morphological characters within the genus are highly homoplasious.

Discussion

Taxonomy of the new species of *Questa*

Questa retrospermatICA sp. n. from Hawai'i and southern China differ from all other *Questa* species mainly by the unique position of two pairs of laterodorsal sperm receptacles in the direct vicinity of the large eggs in 12 and 13 to 14. Since the ovipore seems to be ventral, the successful transport of sperm from the receptacles to the expelled eggs is as difficult to imagine here as for other questid species with sperm receptacles in 5 or 6. Again, the laterodorsal openings of the sperm receptacles as well as of the male pores suggest a copulation of the worms 'back to back', as suggested earlier for other questid species (Jamieson and Webb 1984).

The apomorphy of sperm receptacles being shifted back into egg-bearing segments, together with other structural details, is shared by the specimens of *Questa* cf. *retrospermatICA* from New Caledonia. The large size of these worms, especially compared to those from Hawai'i, can be considered as falling within the natural variation of the species. However, the presence of loose sperm aggregations in anterior segments represents a functional and taxonomic problem that needs to be resolved by histological scrutiny; i.e., it cannot be excluded that the New Caledonian form has sperm receptacles in the 'normal' anterior location, too. On the basis of the available material, we tentatively include the specimens from New Caledonia in *Q. retrospermatICA*, but cautiously treated them separately above, to facilitate a potential revision.

Although based on only few specimens, two features of *Q. fijiensis* justify its description as a new species separate from *Q. bicirrata*: the presence of a second pair of sperm receptacles, and the capillary chaetae with only up to 10 dents each in the galleries as opposed to 12–14 in *Q. bicirrata*. However, additional material may be valuable to validate this distinction.

Systematic position of *Questa* and status of Questidae

Due to its many unique characters, *Questa* was regarded as a separate family early on (Hartman 1966;

Hobson 1970, Fauchald 1974), but it has also been suggested repeatedly that this family may have a systematic position associated with protodrilid and dinophilid 'archiannelids' (e.g. Jamieson and Webb 1984). A structural vicinity to the families Paraonidae or Orbiniidae has been suggested several times (Laubier 1967; Laubier and Ramos 1973; Hobson 1976; Blake 1996, 2000; Fauchald and Rouse 1997; Rouse and Fauchald 1997; Hausam and Bartolomaeus 2001).

In Orbiniidae as well as in *Questa* we find dorsal pairs of ciliated branchiae, one or two pairs of anal cirri, vestigial parapodia with chaetae emerging directly from the body wall rather than conspicuous podial lobes, up to three types of chaetae including crenulated capillaries, furcates and bifid crotchets (for figures see Laubier 1967; Laubier and Ramos 1973; Hartmann-Schröder 1971; Hobson 1976; Blake 1996; Hausam and Bartolomaeus 2001). The strongest support for *Questa* being nested within Orbiniidae is provided by recent studies of DNA data (Bleidorn 2005; Rousset et al. 2007).

On the other hand, the derived position of *Questa* is documented first of all by the conspicuous glandular dorsal fold in mature males, and by the sperm storage in well confined sperm receptacles in mature females. On the basis of characters such as the antecerebral loop and the sperm ultrastructure, Jamieson and Webb (1984) even claimed a unique position of questids among annelids.

Many features typical for Paraonidae are not shared by the representatives of *Questa*: The majority of paraonid species have three pygidial cirri in a characteristic two-dorsal-and-one-ventral arrangement (Laubier 1967), the dorsally turned anus with terminally rounded hind end (Fig. 4B), the smooth, unserrated hair chaetae, bifid crotchets without ligament, and the foregut developed as a simple, thin-walled pouch without deep constrictions.

The cladistic study by Garraffoni and Amorim (2003) "aims to verify the hypotheses of monophyly in Questidae and of a taxon composed of Questidae + Clitellata, and tries to determine the position of these taxa among the Annelida" (our translation). As for the Questidae, the authors used a selection of morphological features from the literature, without any inspection of material or critical discussion of homology problems (e.g. concerning structure of genital organs in questids vs. oligochaetes, histology of glandular girdle in female questids vs. clitellum in oligochaetes, hermaphroditic vs. gonochoristic condition) for compilation of a subjective character matrix. However, they omitted major differences in the buccal pad, the crenulate vs. smooth hair chaetae, and they postulated larval development in questids that is unproven. They found Questidae monophyletic, but concluded that it represents the sister group of "Clitellata", defining the two groups together as a new taxon, Apoclitellata. Apart from the refutation

of this hypothesis by several contemporary DNA studies (see introduction above), it seems difficult to perceive a more general bearing of these statements on annelid taxonomy.

Phylogeny within *Questa*

The phylogenetic analysis of *Questa* (Fig. 5) shows overwhelming support for monophyly of the genus. Six character states are unambiguous synapomorphies for all species: crotchets have ligaments (character 9); dorsal crotchets (char. 10) and sperm receptacles (char. 14) are present; males have dorsal folds (char. 11); oocytes are concentrated (char. 13); and the ventral buccal pad is complete (char. 15). A seventh feature (char. 4), the restricted (posterior) branchial distribution, is shared by all species except *Q. trifurcata*, which lacks branchia altogether. Moreover, in all species, the males have their caeca located in segments 6, 7 or 8 (char. 8, states 0–2), never farther posterior as in the other (outgroup) orbiniids (char. 8, state 3). Lastly, wherever present in *Questa* (in four spp.), forks have only one or two median dents (char. 2, state 1); in other orbiniids, there are several such dents (char. 2, state 0).

However, with regard to the detailed phylogenetic relationships among the various species of *Questa* (the topology shown in Fig. 5), the result is not as strongly supported. Although there is only one most parsimonious tree, most ingroup characters are in some conflict with each other: forks are hypothesized to have disappeared first in a main lineage, and then to have reoccurred in a more terminal part of this lineage (character 1); similarly, secondary annuli have become numerous early on, but then fewer again farther up the tree (in *Q. riseri*; char. 3); the pygidial cirri have become four twice (char. 6); the dents in the crenulation of hair chaetae have been reduced to fewer than ten, also twice (char. 7); and for the intestinal caeca, the shift to a position in segment 7 has occurred twice (char. 8, state 1). One terminal group (*Q. riseri*, *Q. media*, *Q. trifurcata*) is ‘unambiguously’ supported (as an effect of character optimization) by the presence of branchial cilia, although this feature is not applicable to *Q. trifurcata*, which lacks branchia. However, in a corresponding analysis presented by Giere and Erséus (1998, figs. 4–6), based on fewer characters and fewer taxa (with only a generalized ‘Orbiniidae’ as outgroup), there was even less resolution, with virtually no consensus between five equally most parsimonious trees. Thus, the tree of the present study can be regarded as an improvement in the sense that it gives a more resolved hypothesis open to future testing with additional data. Regarding the limited number of morphological characters available, it seems reasonable to predict that no phylogenetic hypothesis considering this genus will be

fully resolved and supported until a comprehensive DNA sequence dataset is available.

Systematic position and status of *Periquesta*

Periquesta canariensis from the Canary Islands is similar in relevant external and internal characters to the paraonid species described as *Levinsenia hawaiiensis* sp. n. in this paper: bifid crotchets only ventrally and from segment 6 backwards, ligament of crotchets absent in material at disposal, deep furrow at septum of segments 6/7.

Both species have features in common that suggest a position within Paraonidae:

- anus ciliated, opens medio-dorsally (fixed specimens) in pygidium (Fig. 4B, E);
- hind end of pygidium terminally rounded, not bilobed, without vertical cleft (Fig. 4B);
- arrangement of anal (pygidial) cirri: two dorsal, one ventral (Fig. 4B, E);
- hair chaetae smooth, lacking crenulation (galleries of dents);
- intestinal caeca absent;
- eggs of varying size and diffuse shape in segments 18–30.

The frilled appearance of hair and crotchet chaetae after processing for SEM is an additional common feature of these two species (Figs. 3A, B; 4C, D).

On the other hand, *P. canariensis* and *L. hawaiiensis* share features that deviate from those of Questidae:

- dorsal fold in segments 13–14 of mature males absent;
- dorsal branchial appendages on posterior segments absent (in most specimens);
- position and arrangement of genital organs;
- eversible ventral buccal pad (pharyngeal bulb) with characteristic musculature absent;
- antecerebral loop of blood vessel absent;
- tortuous, deeply constricted and thin-walled intestine absent;
- intestinal caeca absent.

With this in mind (see also the remarks on *Lewinsenia canariensis* above), we conclude that *Periquesta* is not closely related to *Questa*, but instead a representative of Paraonidae. Following the generic diagnoses by Strelzov (1973) and Blake (1996), the absence of a median antenna and the presence of bent hooks in the neuropodia place *P. canariensis* in the genus *Levinsenia*, together with the closely related species *L. hawaiiensis* sp. n.

Zoogeographical aspects

Questa retrospermatica sp. n. (sensu lato) with its occurrence in shallow waters of Hawai'i, southern China and New Caledonia seems to be widespread in the Pacific Ocean. The available material of *Q. fijiensis* does not yet allow any conclusions about its distributional range outside the Fiji Islands. *Q. ersei* has been found in western Australia, Queensland, and off New South Wales (Australia); the findings in New Caledonia now extend its occurrence eastward beyond the Coral Sea. The wide geographic distance between the findings of closely related taxa, however, is not unusual for meiobenthic animals (Giere 1993; Schmidt and Westheide 2000).

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