Syst Parasitol (2014) 89:59–72
DOI 10.1007/s11230-014-9511-6

A revision of the genus *Neogrubea* Dillon & Hargis, 1968 (Monogenea: Mazocraeidae): new morphological and molecular data from off the Patagonian coast of Argentina

Jesús Servando Hernández-Orts · Gema Alama-Bermejo · Enrique Alberto Crespo · Juan Antonio Raga · Francisco Esteban Montero

Received: 12 May 2014 / Accepted: 17 July 2014

Abstract The genus *Neogrubea* Dillon & Hargis, 1968 (syn. *Asymmetria* Suriano, 1975) is revised based on examination of type- and voucher material, and from new specimens collected from the gills of *Seriolella porosa* Guichenot and *Stromateus brasiliensis* Fowler from off Patagonia, Argentina. Morphological comparisons based on light and scanning electron microscopy and molecular data (partial SSU and LSU rDNA sequences) of the monogeneans from off Patagonia suggest that *N. seriolellae* Dillon & Hargis, 1968 (syns *N. stromateae* Gibson, 1976, *A. asymmetria* Suriano, 1975 and *A. platensis* Rey & Meneses, 1985) is currently the only species of the genus. *Neogrubea soni* Evdokimova, 1969 is considered a *species inquirenda*. An emended diagnosis of *Neogrubea* is presented, and new host and locality records for *N. seriolellae* are given in detail. Morphological characters of the members of the mazocraeid subfamily Grubeinae Price, 1961 are also discussed.

Introduction

Neogrubea Dillon & Hargis, 1968 (Monogenea: Mazocraeidae) was proposed by Dillon & Hargis (1968) to accommodate *Neogrubea seriolellae* Dillon & Hargis, 1968 from the gills of *Seriolella porosa* Guichenot and *S. brama* (Günther) off New Zealand. A second species, *N. soni* Evdokimova, 1969 was described by Evdokimova (1969) from *Stromateus brasiliensis* Fowler [= *S. maculatus* (non Cuvier)] from off the Patagonian shelf in the South West Atlantic. The third species described,

J. S. Hernández-Orts (✉) · G. Alama-Bermejo · J. A. Raga · F. E. Montero
Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, C/ Catedrático José Beltrán 2, 46980 Paterna, Valencia, Spain
e-mail: jesus.s.hernandez@uv.es

J. S. Hernández-Orts · G. Alama-Bermejo
Institute of Parasitology, Biology Centre, ASCR, v.v.i., Branišovská 31, 370 05 České Budějovice, Czech Republic

J. S. Hernández-Orts
Department of Zoology, Biology Institute, National Autonomous University of Mexico, P.O. Box 70-153, 04510 Mexico City, Mexico

G. Alama-Bermejo
Department of Microbiology, Oregon State University, 220 Nash Hall, Corvallis, OR 97331-3804, USA

E. A. Crespo
Marine Mammal Laboratory, National Patagonic Center, CONICET and University of Patagonia, Boulevard Brown 2915 (9120), Puerto Madryn, Chubut, Argentina

N. stromateae Gibson, 1976, was also collected from the gills of *S. brasiliensis* but from off Falkland Islands by Gibson (1976). Since the erection of *Neogrubea*, specimens of this genus have been reported from other hosts and localities. Butt (1974) described specimens of *Neogrubea* sp. from the gills of *Stromateus stellatus* Cuvier from off the coast of Corral, Chile. Mamaev (1982) described specimens of *N. seriolellae* from *S. brama*, *S. puncata* (Forster), *S. tinro* Gavrilov and *Seriolella* sp. from off the Australian and New Zealand coasts, and from *Scorpis violacea* (Hutton) and *Scorpis* sp. from north of the Hawaiian Islands. More recently, the microhabitat selection by *N. seriolellae* was described based on material from *S. porosa* caught in San Matías Gulf in Argentina (Schwerdt et al., 2010).

The genus *Neogrubea* was revised by Mamaev (1982), who based on his new material, considered *N. stromateae* a junior synonym of *N. seriolellae* and accepted *N. soni* as a valid species, although he recognised that there were inaccuracies in the species description by Evdokimova (1969). A second revision was made by Gibson & Meneses (1990), who considered *Asymmetria* Suriano 1975 a junior synonym of *Neogrubea*, and *A. asymmetria* Suriano, 1975 and *A. platensis* Rey & Meneses, 1985 junior synonyms of *N. soni* and *N. seriolellae*, respectively. These authors considered that *N. stromateae* may be a junior synonym of *N. seriolellae* although the specimens of *N. stromateae* from *S. brasiliensis* are consistently smaller than those of *N. seriolellae* from *Seriolellae* spp.

In the present study, the genus *Neogrubea* is revised based on re-examination of type- and voucher material of *Neogrubea* spp., and on examination of new material from *S. brasiliensis* and *S. porosa* collected in the South West Atlantic. We present metrical data for *N. seriolellae* from different hosts and localities, and provide a molecular comparison between the new specimens from off the Patagonian coast of Argentina. An emended diagnosis of *Neogrubea* is proposed herein after the re-examination of the material.

Materials and methods

Sample collection

A total of 78 specimens of *S. brasiliensis* [total length 13.7–36.4 cm (mean ± standard deviation 27.5 ± 3.8 cm)] and 38 *S. porosa* [total length 22.7–42.7 cm (33.0 ± 5.4 cm)] were collected between 2006 and 2008 from two zones along the Argentinean coast: off north (42°45′–42°59′S, 61°09′–62°58′W) and central Patagonia (47°00′–47°19′S, 61°59′–64°25′W). The fish were sampled on board of Argentine hake trawlers, and were kept fresh on ice (*S. brasiliensis*, n = 5; *S. porosa*, n = 4) or frozen in plastic bags at –20°C for subsequent examination in the laboratory. Gills were excised, placed in seawater and examined using a stereomicroscope (×40). A total of 346 monogeneans was collected from the gills of *S. brasiliensis*, fixed in 70% ethanol (n = 339) or in 100% ethanol (n = 7); whereas 54 specimens were collected from the gills of *S. porosa*, fixed in 70% ethanol (n = 49) or 100% ethanol (n = 5). All worms fixed in 100% ethanol were collected from freshly caught fish specimens.

Morphological description

Twenty-seven monogeneans collected from nine *S. brasiliensis* and five specimens from three *S. porosa*, fixed in 70% ethanol (all specimens from thawed fish), were stained with iron acetocarmine, dehydrated in an ethanol series, cleared in dimethyl phthalate, and mounted in Canada balsam. Additionally, the anterior extension of the viteline fields was examined in 30 monogeneans from the gills of ten *S. brasiliensis*. The anterior half of each worm was stained with iron acetocarmine (n = 15) or alum carmine (n = 15), and processed as described above; the posterior half of the body was stored for further molecular analysis. All specimens were examined using a compound Leica DMR microscope equipped with bright field and differential interference contrast optics. Morphometric measurements were taken from drawings made with the aid of a drawing tube attached to a light microscope, Nikon Optiphot-2 (Nikon Corporation). The terminology of clamp sclerites follows Mamaev (1981).

Infection parameters were estimated following Bush et al. (1997) and Rózsa et al. (2000). The prevalence, mean abundance and mean intensity are provided with the 95% confidence intervals (CI) in parentheses. Sterne's exact 95% CI was calculated for prevalence (Reiczigel, 2003). Mean abundance and mean intensity 95% CIs were estimated with 20,000 bootstrap replications with the statistical software Quantitative Parasitology v. 3.0 (Rózsa et al., 2000). The distribution lists follow FAO Major Fishing Areas (http://www.fao.org/fishery/area/search/en) and are expressed in numerical form.

Specimens collected in the present study were compared with type- and voucher specimens of the following *Neogrubea* spp., deposited in the United States National Parasite Collection, Beltsville, Maryland, United States (USNPC) and the Natural History Museum, London, United Kingdom (BMNH): *N. seriolellae*, two paratypes (USNPC 071192.00) and one voucher (BMNH 1996.2.9.37); *N. stromateae*, two paratypes (BMNH 1975.3.17.1–12) and one voucher (BMNH 1989.5.18.23-24); *Neogrubea* sp., one voucher (USNPC 073449.00 labeled as *N. manteri nomen nudum*). Additionally, the holotype of *A. asymmetria* (MLP 6432) from the invertebrate collection of Museo de La Plata (MLP), La Plata, Buenos Aires, Argentina, and five vouchers (No. 6/73, 1–5) from the private collection of Dr. D. L. Suriano, deposited in the Centro de Estudios Parasitólogicos y de Vectores (CEPAVE), La Plata, Buenos Aires, Argentina, were studied.

Voucher specimens from the present study were deposited in the following collections: BMNH, London, United Kingdom; USNPC, Beltsville, Maryland, United States; IPCAS, Helminthological Collection of the Institute of Parasitology, Biology Centre ASCR, České Budějovice, Czech Republic and ZMUC, Zoology Museum of the University of Concepción, Concepción, Chile.

Molecular data

For DNA extraction, two specimens of *N. seriolellae* from *S. brasiliensis* and two from *S. porosa* fixed in 100% ethanol were dissolved in 300 µl of TNES urea (10 mM Tris-HCl (pH 8), 125 mM NaCl, 10 mM ethylenediaminetetraacetic acid, 0.5% sodium dodecyl sulphate, 4 M urea). The samples were digested with 100 µg/ml proteinase K overnight at 55°C. DNA was extracted using a conventional phenol-chloroform protocol following Alama-Bermejo et al. (2011). Extracted DNA was resuspended in 30 µl of RNAse/DNAse free water and left to dissolve overnight in the fridge. Polymerase chain reactions (PCR) were performed with a programmable thermal cycler (Techne, TC-512, GMI) in a final volume of 30 µL containing *c.*0.5 U of Thermoprime Plus DNA polymerase and 3 µL of the related 10× buffer with 1.5 mM $MgCl_2$ (ABgene), 0.2 mM of each dNTP, 0.5 µM of each primer, and approximately 100 ng of template DNA.

Polymerase chain reactions (PCR) were performed to amplify partial sequences of the SSU and LSU regions of the genomic ribosomal RNA (rRNA) gene. The primers used in the PCR amplifications were WormA (forward; 5′-GCG AAT GGC TCA TTA AAT CAG-3′) and WormB (reverse; 5′-CTT GTT ACG ACT TTT ACT TCC-3′) (Littlewood & Olson, 2001) for SSU rRNA gene; and LSU5 (forward; 5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) and ECD2 (reverse; 5′-CTT GGT CCG TGT TTC AAG ACG GG-3′) (Olson et al. 2003) for LSU rRNA gene. The PCR protocol was conducted as follows: denaturation (95°C for 3 min) followed by 40 cycles of amplification (95°C for 30 s; 55°C for 30 s; and 72°C for 2 min) plus a final extension step at 72°C for 7 min.

PCR products were purified for sequencing using the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare UK Ltd.). The PCR primers and additional primer Lin3 (5′-GCG GTA ATT CCA GCT CCA-3′) (Lin et al., 1999) were used for sequencing of the SSU rDNA fragment. Cycle sequencing was conducted in a 48 capillary ABI 3730 sequencer (Applied Biosystems) using the BIG Dye Terminator v 3.1 Ready Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. The sequences were submitted to the Basic Local Alignment Search Tool (BLAST) on GenBankTM to identify the closest relatives published to date.

Family Mazocraeidae Price, 1936
Subfamily Grubeinae Price, 1961

Genus *Neogrubea* Dillon & Hargis, 1968

Syn. *Asymmetria* Suriano, 1975

Emended diagnosis

Based on Dillon & Hargis (1968). Body elongated. Haptor armed with four pairs of clamps and three pairs of larval hooks. Each clamp composed of seven sclerites. Arrangement of clamps asymmetrical with clamps on one side of haptor larger than on the other; larger clapms with central sclerites parallel to dextro-sinistral axis; smaller clamps on small peduncles, with central sclerites parallel to anteroposterior axis and at right angles to larger clamps. Three pairs of larval hooks, dissimilar in size: one pair of external large hamuli, one pair of medial small marginal hooks and one pair of internal medium-sized marginal hooks. Anterior body end with single pair of muscular buccal suckers. Muscular pharynx sub-ellipsoidal, posterior

to muscular suckers. Oesophagus long, with lateral branches, bifurcates into two caeca posterior to genital atrium. Genital atrium armed with 10–14 small inner spines and two large external spines. Testes numerous, pre-, para- and post-ovarian, extending into the haptor. Ovary elongated, folded, with mature end oriented anteriorly. Vagina absent. Vitelline field usually reaching to level of oesophageal bifurcation anteriorly. Eggs with filaments on both poles. Parasites on the gills of Centrolophidae, Kyphosidae (?) and Stromateidae. *Type-species*: *N. seriolellae* Dillon & Hargis, 1968.

Review of the species

Neogrubea seriolellae Dillon & Hargis, 1968

Syn. *Asymmetria asymmetria* Suriano (new synonymy); 1975; *Neogrubea stromateae* Gibson, 1976; *Asymmetria platensis* Rey & Meneses, 1989

Material studied

Type-material: Ex *Seriolella porosa* Guichenot (Perciformes: Centrolophidae); gills; off Cape Campbell, Marlborough Province, and Akaroa, Canterbury Province, South Island, New Zealand; paratypes USNPC 071192.00 (1 slide with 2 specimens).

Comparative material: Ex *Stromateus brasiliensis* Fowler (Perciformes: Stromateidae); gills; off Falkland Islands, U.K.; BMNH 1975.3.17.1–12 (2 specimens); ex *S. brasiliensis*; gills; South West Atlantic, off Argentinean coast; MLP 6432 (1 specimen), CEPAVE No. 6/73, 1–5 (5 specimens); ex *S. brasiliensis*; gills; Argentine/Uruguay common fishing zone, South West Atlantic; BMNH 1989.5.18.23-24 (1 specimen); ex *Stromateus stellatus* Cuvier (Perciformes: Stromateidae); gills; off Corral, Chile; USNPC 073449.00 (1 specimen); ex *S. stellatus*; gills; off Chile. BMNH 1996.2.9.37 (1 specimen).

New material: Ex *S. porosa*; gills; off Patagonian coast of Argentina; 5 specimens; voucher material BMNH 2012.7.24.7; USNPC 105892.00; IPCAS M–533/2; ZMUC No. 37160; ex *S. brasiliensis*; gills; off Patagonian coast of Argentina; 27 specimens; voucher material BMNH 2012.7.24.8, USNPC 105891.00, IPCAS M–533/1, ZMUC No. 37108.

Representative sequences: *N. seriolellae* ex *S. porosa* [GenBank KM190942 (SSU rDNA); KM190943 (LSU rDNA)]; *N. seriolellae* ex *S. brasiliensis* [GenBank KM190944 (SSU rDNA); KM190945 (LSU rDNA)].

Prevalence: Ex *S. porosa*: 55.3% (CI 39.4–70.4%); ex *S. brasiliensis*: 61.5% (CI 50.0–71.9%).

Mean abundance: Ex *S. porosa*: 1.4 (CI 0.9–2.4); ex *S. brasiliensis*: 4.4 (2.8–9.3).

Mean intensity: Ex *S. porosa*: 2.6 (CI 1.9–4.3), 1–12 monogeneans per fish; ex *S. brasiliensis*: 7.21 (CI 4.6–14.2), 1–96 monogeneans per fish.

Records

References: 1. Dillon & Hargis (1968); 2. Butt (1974) (as *Neogrubea* sp.); 3. Gibson (1976) (as *N. stromateae*); 4. Suriano (1975 (as *Asymmetria asymmetria*); 5. Rohde et al. (1980); 6. Mamaev (1982); 7. Rey & Meneses (1985) (as *A. platensis*); 8. Schwerdt et al. (2010); 9. Present study.

Descriptions: 1; 2; 3; 4; 6; 7; 9.

Hosts: *Seriolella porosa* (1, 8, 9); *S. brama* (1, 5, 6); *Stromateus stellatus* (2); *S. brasiliensis* (3, 4, 9); *S. punctata* (6, 7); *S. tinro* (6); *Seriolella* sp. (6); *Scorpis violacea* (6); *Scorpis* sp. (6).

Distribution: Area 81 (South West Pacific) (1, 5, 6); Area 87 (South East Pacific), subarea 3.3 (2); Area 41 (South West Atlantic), subarea 3.2 (3); Area 41 (4); Area 41, subarea 2.3 (7); Area 41, subarea 3.1 (8, 9); Australia (6); Area 77 (Central East Pacific) (6).

Description (Figs. 1–3)

[Based on type- and comparative material. Metrical data are given in Table 1.] Body elongated, with smooth tegument (Figs. 1A, 3A). Haptor with 4 pairs of clamps (Figs. 1A, 3A), composed of 7 sclerite pieces (Fig. 1A–C): scleritum arcuatum anterius (saa), scleritum arcuatum posterius (sap), scleritum medio-supplementarium (sms), scleritum medio-basale (smb), scleritum postero-supplementarium (sps), scleritum antero-supplementarium internum (sasi), and scleritum antero-supplementarium externum (sase). Arrangement of clamps asymmetrical with small clamps on one side of haptor (Figs. 1B, 3B) on short peduncles, with central sclerite parallel to anteroposterior axis (Fig. 1A); clamps on opposite side larger (Figs. 1C, 3C), with central sclerite parallel to dextrosinistral axis (Fig. 1A). Posterior end of haptor with 1 pair of external large hamuli (Figs. 2A–C, 3D), 1 pair of small medial marginal hooks

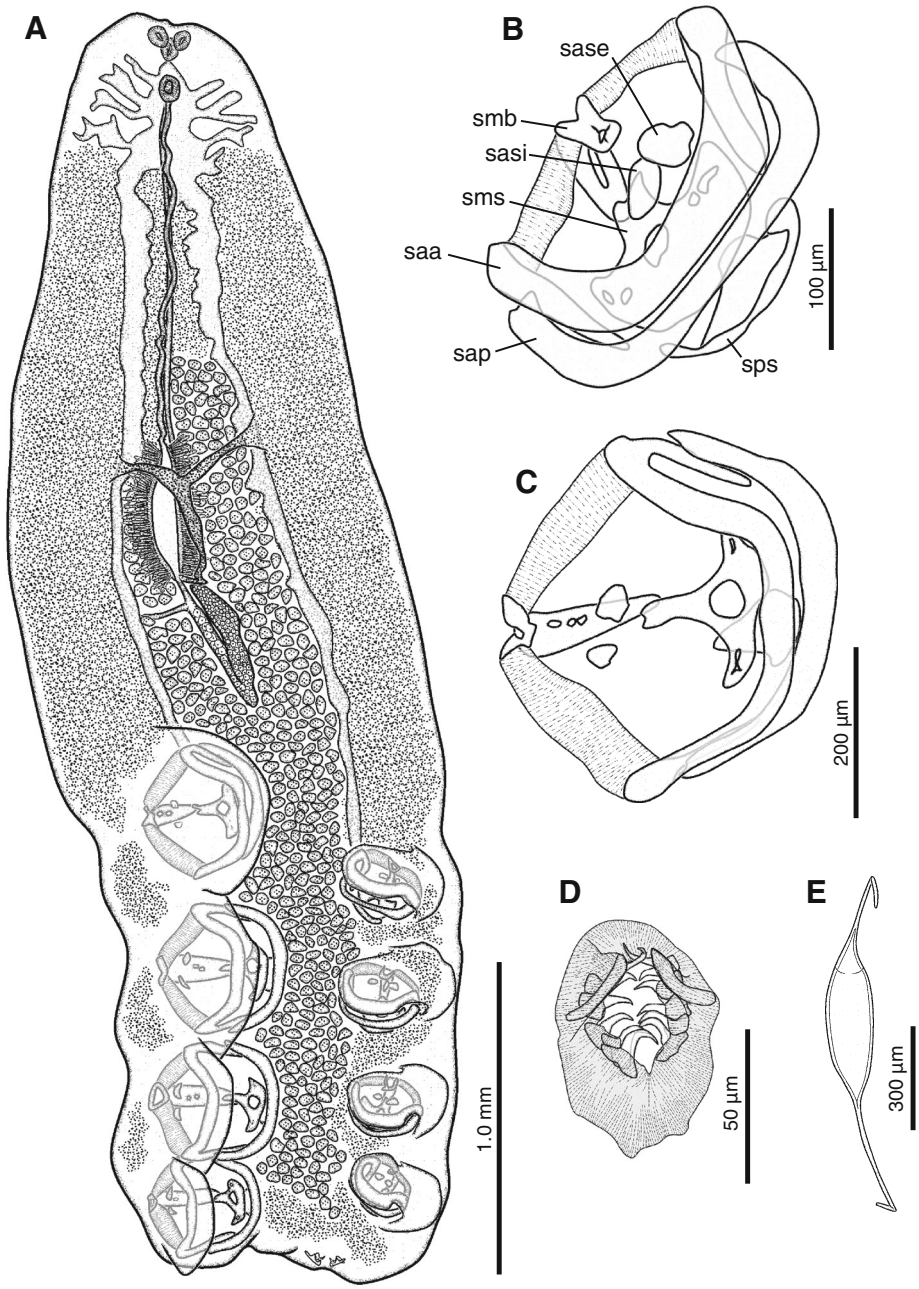

Fig. 1 *Neogrubea seriolellae* ex *Stromateus stellatus* off the Chilean coasts, South East Pacific. A, Whole worm, ventral view, voucher (USNPC 073449.00); B, Small clamp, ventral view, voucher (BMNH 1996.2.9.37); C, Large clamp, ventral view, voucher (USNPC 073449.00); D, Genital atrium, ventral view, voucher (USNPC 073449.00); E, Egg, voucher. *Abbreviations*: saa, scleritum arcuatum anterius; sap, scleritum arcuatum posterius; sase, scleritum antero-supplementarium externum; sasi, scleritum antero-supplementarium internum; smb, scleritum medio-basale; sms, scleritum medio-supplementarium; sps, scleritum postero-supplementarium

Table 1 Metrical data of specimens of *Neogrubea seriolellae* from different localities and fish hosts

Source	Dillon & Hargis (1968); Present study (USNPC 071192.00)	Present study	Present study	Butt (1974); Present study (USNPC 073449.00)	Gibson (1976); Present study (BMNH 1975.3.17.1–12)
Identified as	*Neogrubea seriolellae*	*Neogrubea seriolellae*	*Neogrubea seriolellae*	*Neogrubea* sp.	*Neogrubea stromateae*
Host	*Seriolella brama* (Günther); *Seriolella porosa* Guichenot	*Stromateus brasiliensis* Fowler	*S. porosa*	*Stromateus stellatus* Cuvier	*S. brasiliensis*
Locality	Off New Zealand	Off Patagonian shelf (Argentina)	Off Patagonian shelf (Argentina)	Off Corral (Chile)	Off Falkland Islands
Body length (mm)	6.8–11.0 (7.2–9.2)[a]	3.2–6.5	8.6–10.8	3.5–4.7 (4.2)[a]	5.2–6.7 (6.5–6.6)
Genital atrium	88–107 × 82–91 (87–102 × 87–102)[a]	58–93 × 43–77	73–98 × 62–80	72–130 × 59–85 (91 × 74)[a]	95–110 × 65–80 (95–96 × 67–78)[a]
No. of dorsal spines in genital atrium	10–14 (10)[a]	10	10	9–10 (10)[a]	10 (10)[a]
No. of ventral spines in genital atrium	2 (2)[a]	2	2	2 (2)[a]	2 (2)[a]
Large clamp width	501–976 (507–814)[a]	150–407	427–655	262–436 (341–463)[a]	540–740 (370–522)[a]
Small clamp width	324–350 (459–706)[a]	119–285	327–427	153–480 (230–281)[a]	280–360 (270–312)[a]
No. of testes	– (317–340)[a]	83–167	199–280	– (342)[a]	– (61–170)[a]
No. of haptoral hooks	4 (6)[a]	6	6	6 (6)[a]	4 (6)[a]
Hamuli	53–73 (72–94)[a]	88–96	84–100	37–93 (57–68)[a]	80–85 (83–89)[a]
Large marginal hooks	48–62 (45–61)[a]	48–52	40–72	27–63 (61)[a]	62 (43–64)[a]
Small marginal hooks	– (10–11)[a]	9–15	10–16	10–17 (10)[a]	– (10–22)[a]
Filaments on the egg	On both poles	On both poles	–	–	Not observed
Anterior limit of vitelline field	At oesophageal bifurcation	At oesophageal bifurcation	At oesophageal bifurcation	At anterior level of genital atrium (at oesophageal bifurcation)[a]	At oesophageal bifurcation

Table 1 continued

Source	Mamaev (1982)	Suriano (1975); Present study (MLP 6432; No. 6/73, 1–5)	Rey & Meneses (1989)	Present study (BMNH 1996.2.9.37)	Present study (BMNH 1989.5.18.23-24)
Identified as	*Neogrubea seriollae*	*Asymmetria asymmetria*	*Asymmetria platensis*	*Neogrubea seriolellae*	*Neogrubea stromateae*
Host	*S. brama* (Günther), *Seriolella punctata* (Forster), *Scorpis violacea* (Hutton), *Scorpis* sp.	*S. brasiliensis* Fowler	*S. punctata*	*S. stellatus* Cuvier	*S. brasiliensis*
Locality	Off New Zealand, Australia, Hawaii	Off Argentinean coast	Argentine-Uruguayan Common Fishing Zone	Off Chilean coast	Argentine-Uruguayan Common Fishing Zone
Body length (mm)	8.8–13.3	5.0–12.8 (6.5–9.3)[a]	13.0–15.0	7.7	7.7
Genital atrium	–	91 × 86[b] (70–86 × 75–96)[a]	140 × 90–130	73 × 59	96 × 67
No. of dorsal spines in genital atrium	9–13	10 (10)[a]	10[b]	10	10
No. of ventral spines in genital atrium	2	2 (2) [a]	2[b]	2	2
Large clamp width	530–1,000	300–962 (435–863)[a]	870–890	387–520	370–503
Small clamp width	400–650	208–377 (265–358)[a]	411–430	293–344	263–300
No. of testes	–	– (94–143)[a]	132	250	148
No. of haptoral hooks	6	6	4?	6	6
Hamuli	82–110	152[b] (82–94)[a]	50–70	94–100	69–70
Large marginal hooks	62–78	93 (62–63)[a]	30–60	41	24–38
Small marginal hooks	30–32	50[b] (11–23)[a]	–	11–13	8–9
Filaments on the egg	–	–	On both poles	–	–
Anterior limit of vitelline field	Posterior to genital atrium	At level of pharynx (at oesophageal bifurcation)[a]	At oesophageal bifurcation[b]	At oesophageal bifurcation	At oesophageal bifurcation

[a] Metrical data and morphological observations obtained from re-examination of type- or voucher material; [b] Obtained from the published figure

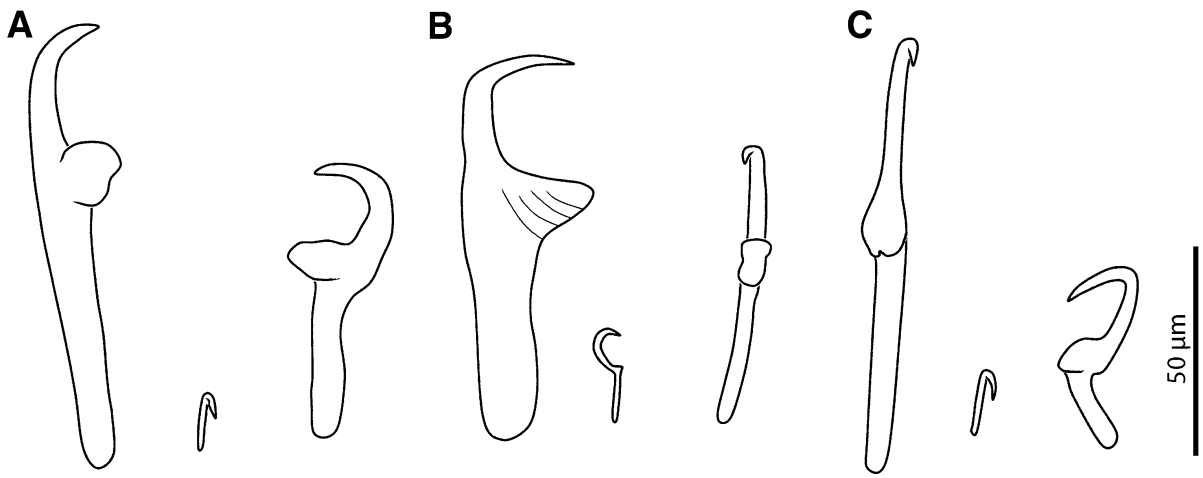

Fig. 2 Line drawings of the three pairs of larval hooks of *Neogrubea seriolellae* from different fish hosts. A, Paratype (USNPC 071192.00) ex *Seriolella porosa* off New Zealand; B, Voucher (BMNH 1975.3.17.1–12) ex *Stromateus brasiliensis* off Falkland Islands; C, Voucher (USNPC 073449.00) ex *Stromateus stellatus* off Chile

(Fig. 2A–C) and 1 pair of internal medium-sized marginal hooks (Figs. 2A–C, 3E).

Anterior part of body with 1 pair of buccal suckers. Mouth opening subterminal; pharynx muscular, subellipsoidal; oesophagus long, with lateral branches, bifurcates posterior to genital atrium, at anterior level of viteline field (Fig. 1A). Genital atrium armed with 5–7 pairs of dorsal spines with wide bases and 1 pair of large ventral spines (Fig. 1D).

Testes numerous (see Table 1), pre-, para- and post-ovarian (Fig. 1A). Vas deferens arises from region of junction of transverse vitelline ducts, extends anteriorly along midline to genital atrium (Fig. 1A). Ovary elongate, folded, with germinal and terminal portions facing anteriorly; germinal end joins oviduct anteriorly (Fig. 1A). Oviduct extends anteriorly, receives genitointestinal canal and enters oötype. Genitointestinal canal reaches right caecum at ovary level. Oötype large, dorsal to transverse vitelline ducts, surrounded by Mehlis' gland. Uterus ventral to vas deferens (Fig. 1A), extends anteriorly along midline to genital atrium.

Vitelline field extends from oesophageal bifurcation to posterior end of haptor, never reaching to level of pharynx anteriorly (Fig. 1A). Transverse vitelline ducts join at anterior level of oötype to form Y- to T-shaped medial common vitelline reservoir. Vitelline duct opens posteriorly to vitelline reservoir, joins oviduct near posterior end of oötype. Vagina not observed. Eggs fusiform, with filaments on both poles (Fig. 1E). Excretory system not observed.

Molecular characterisation

The partial SSU rDNA (1,675 bp) and LSU rDNA (748 bp) sequences for *Neogrubea seriolellae* obtained from *S. porosa* and *S. brasiliensis* were almost identical (99.9% and 99.8% sequence similarity for SSU and LSU, respectively). Submission to the BLAST server showed that the closest sequence matches for the SSU rDNA sequences were *Kuhnia scombri* (Kuhn, 1829) [GenBank AJ228783 (Littlewood et al., 1999); query coverage 100%; maximum identity 94%] and *Gotocotyla secunda* (Tripathi, 1954) [GenBank AJ276425 (Littlewood & Olson, 2001); query coverage 100%; maximum identity 92%]. The closest sequence matches for the LSU rDNA sequences were *K. scombri* [GenBank AF382044 (Olson & Littlewood, 2002); query coverage 100%; maximum identity 86%] and *Discocotyle sagittata* (Leuckart, 1842) [GenBank AF382036 (Olson & Littlewood, 2002); query coverage 100%; maximum identity 81%].

Over an LSU rDNA alignment (278 bp) of the new sequences with the two available sequences for a species of the subfamily Grubeinae Price, 1961, *Grubea cochlear* Diesing, 1858 [AF311710, 556 bp (Jovelin & Justine, 2001) and AF131730, 341 bp (Mollaret et al., 2000)], the similarities were 43.3% and 95.6% respectively.

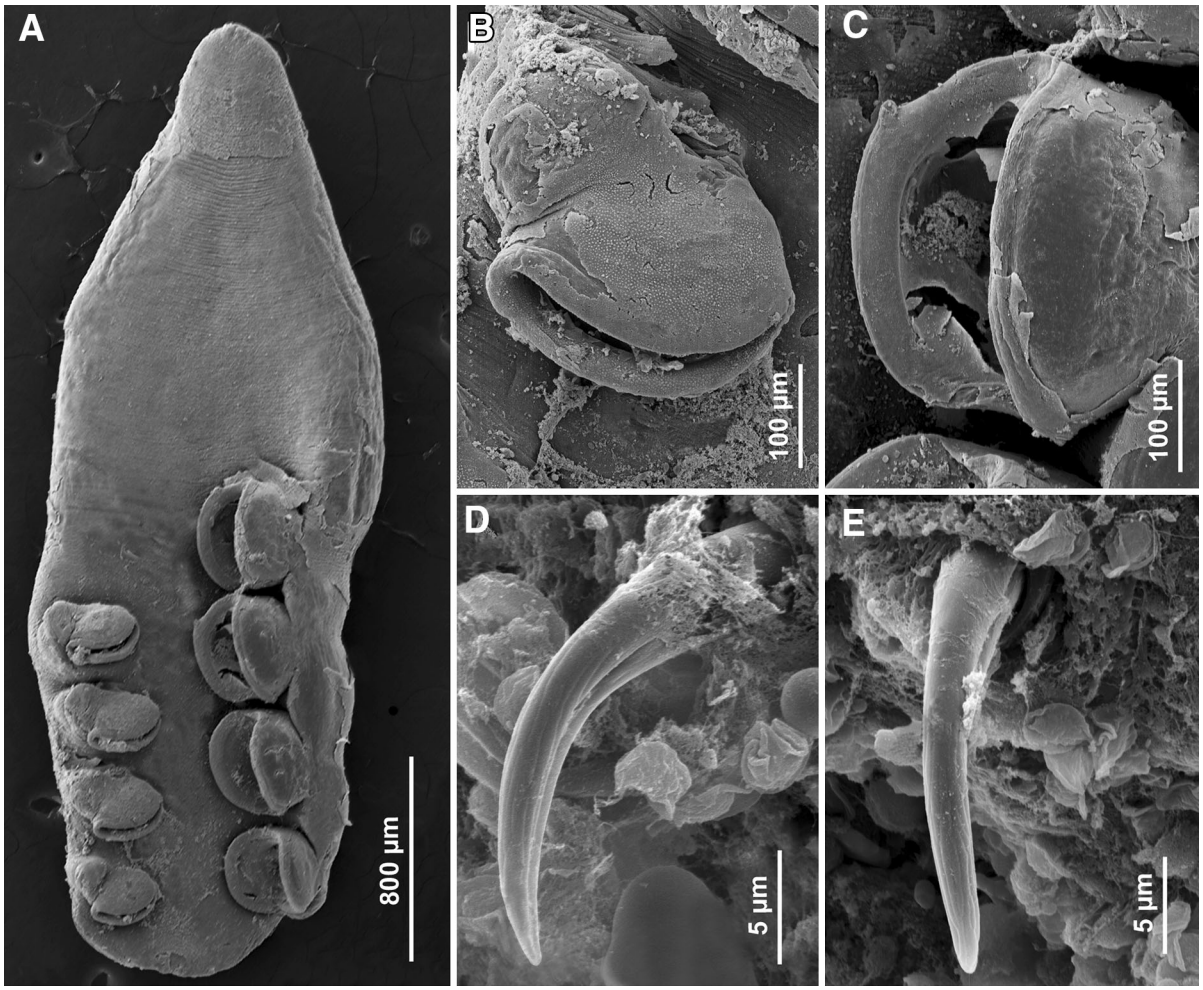

Fig. 3 Scanning electron micrographs of *Neogrubea seriolellae* ex *Stromateus brasiliensis* off Patagonian coast in Argentina. A, Whole worm, ventral view; B, Small clamp over a peduncle, ventral view; C, Large clamp, ventral view; D, External hamulus, lateral view; E, Internal large marginal hooks, dorsal view

Remarks

Neogrubea seriolellae has been described and illustrated in detail by Dillon & Hargis (1968). These authors described two pairs of larval hooks on the haptor of *N. seriolellae* (see figure 6 in Dillon & Hargis, 1968). However, our re-examination of the type-material revealed differences from the original description of *N. seriolellae* in having three pairs of larval hooks (Fig. 2A). Moreover, the re-examination of the paratypes provides additional metrical data on the number of testes and the length of the small medial marginal hooks. Finally, we also observed that in the type-material, the width of the genital atrium, the width of the small clamps and the length of the large hamuli vary above the upper ranges given in the original description of the species (Table 1).

Butt (1974) provided a detailed description of *Neogrubea* sp. from *S. stellatus* off the coast of Chile. Based on the examination on his material, we only observed one inconsistence with his description i.e. the anterior extent of the vitelline field is in fact at the level of oesophageal bifurcation (Fig. 1). The specimens described by Butt (1974) are fairly similar to specimens of *N. seriolellae* collected from *S. brasiliensis*, although one specimen collected from *S.*

stellatus exhibits a higher number of testes (Table 1). We did not find any morphological features that can be used to distinguish these specimens from other species of *Neogrubea.* We, therefore, identified his material as *N. seriolellae.*

Our re-examination of the material upon which Gibson (1976) described *N. stromateae* also revealed differences from the original description in the number of pairs of larval hooks on the haptor. This author reported two pairs of larval hooks in the specimens from *S. brasiliensis* (see figure 3D in Gibson, 1976). However, we observed three pairs of larval hooks during the re-examination of the type-material (Fig. 2B). From this material, we also obtained new data on the number of testes and the length of the small medial marginal hooks. As noted above, Mamaev (1982) considered *N. stromateae* a junior synonym of *N. seriolellae*, although it must be said that this author never examined the type-material or new material of *N. stromateae* from *S. brasiliensis*. We agree with Gibson & Meneses (1990) that material from *S. brasiliensis* is consistently smaller than material from *Seriolella* spp. (Table 1). However, based on the absence of significant differences between the two forms, and on the molecular data obtained from the present study, we agree in considering *N. stromateae* as a junior synonym of *N. seriolellae*.

Gibson & Meneses (1990) considered *Asymmetria* a junior synonym of *Neogrubea*, and *A. asymmetria* a junior synonym of *N. soni* (see below) based on the number of larval hooks (three pairs) and the anterior extension of the vitelline field (at level of pharynx). After the re-examination of the holotype and voucher specimens of *A. asymmetria* (the type-species of *Asymmetria*), we agree with Gibson & Meneses (1990) in considering *Asymmetria* a junior synonym of *Neogrubea*, since both genera are morphologically indistinguishable (Table 1). However, the re-examination of Suriano's (1975) material in the present study revealed differences from her description of *A. asymmetria* since in all the specimens examined, the anterior extent of the vitelline field is at the level of oesophageal bifurcation (Table 1). In fact, *A. asymmetria* is similar to *N. seriolellae* (syn. *N. stromateae*), since both possess three pairs of larval hooks, the vitelline field reaches anteriorly to level of oesophageal bifurcation and were described from the same host (*S. brasiliensis*) (see Table 1). Our detailed re-examination of the metrical data and morphological features of these worms, clearly suggest that *A. asymmetria* should be considered conspecific with *N. seriolellae*, and not a junior synonym of *N. sony.*

Asymmetria platensis was described from two specimens ex *S. punctata* from off the coast of Argentina (see Rey & Meneses, 1985). These authors described the species as having two pairs of larval hooks on the haptor (see figure 2G in Rey & Meneses, 1985) and anterior extent of the vitelline field at the level of pharynx. Although we were unable to examine their material, it seems possible that the small marginal hooks have also been missed by these authors. Moreover, we observed an inconsistence of this morphological character in their description, since in their figure 1 the anterior extent of the vitelline field is posterior to the oesophageal branches. Based on the metrical data (Table 1) and the molecular evidence from the newly-collected material off the Patagonian coast, we agree with Gibson & Meneses (1990) in considering *A. platensis* a junior synonym of *N. seriolellae*.

The metrical data for the newly-collected material provides the lower limits of the range for body length, genital atrium length and small and large clamps width for *N. seriolellae*. Voucher material studied from off the Chilean coast (BMNH 1996.2.9.37) and the Argentine-Uruguayan Common Fishing Zone off the coast of Argentina (BMNH 1989.5.18.23-24) exhibits intermediate values for all data (Table 1). In the absence of morphological and metrical differences, we therefore consider that the latter vouchers belong to *N. seriolellae*.

In the Southern Hemisphere, the main hosts of *N. seriolellae* are marine fishes of the families Centrolophidae and Stromateidae (both families belonging to the suborder Stromateoidei). Mamaev (1982) reported *N. seriolellae* in the gills of kyphosids (*Scorpis violacea* and *Scorpis* sp.) from the Northern Hemisphere (north of Hawaiian Islands). Gibson & Meneses (1990) questioned the presence of *N. seriolellae* in kyphosids due to the strict specificity of *Neogrubea* to stromateids. Mamaev (1982) did not provide metrical data or illustrations of the worms collected from *S. violacea* and *Scorpis* sp., and therefore, we could not compare the morphology of his specimens with that of specimens of *N. seriolellae* from *Seriolella* sp. and *Stromateus* spp. Mamaev's material was not available for examination, so the specific identification of these specimens still needs to be confirmed. Further

examination of Mamaev's (1982) material is necessary in order to clarify the status of kyphosids as hosts of *Neogrubea* spp.

Neogrubea soni Evdokimova, 1969 sp. inq.

Records
Reference: Evdokimova (1969).
Definitive host: *S. brasiliensis*.
Distribution: Area 41 (South West Atlantic).

Remarks

This species was described by Evdokimova (1969) from specimens collected from the gills of *S. brasiliensis* caught in the South West Atlantic along the Patagonian shelf (no specific localities were provided). According to her, *N. soni* differs from *N. seriolellae* in the number of pairs of larval hooks on the haptor (3 *vs* 2, respectively), the larger size of the hamuli, the smaller size of the clamps and the different host species. Latter, Gibson (1976) and Gibson & Meneses (1990) differentiated *N. seriolellae* from *N. soni* based on the anterior extension of the vitelline filed, which in *N. soni* reaches posterior to pharynx (see figure 1 in Evdokimova, 1969). Based on the morphological observations of the newly-collected and museum material examined in this study (i.e. the number of pairs of larval hooks on the haptor, the anterior extent of the vitelline field and host species; see Table 1), the taxonomic characters that differentiate *N. soni* from *N. seriolellae* are invalid. We, therefore, suggest that *N. soni* should be considered a junior synonym of *N. seriolellae*. The type-material of *N. soni* is housed in the Helminthological collection of the Zoological Institute of the Russian Academy of Sciences; however these specimens were unavailable for re-examination. Further studies of the type-material of *N. soni* are needed to establish the taxonomic status of this species.

Discussion

The ranges for measurements of some specimens of *N. seriolellae* collected from *Stromateus* spp. are generally lower than those from specimens from *Seriolellae* spp. (Table 1). Nevertheless, no clear morphological differences could be observed between *Neogrubea* spp. collected from the hosts of these two genera. Body size has been used to differentiate species of *Neogrubea* (or *Asymmetria*) (see Gibson, 1976; Rey & Meneses, 1985). However, the maximum body length reported in some specimens collected from *Stromateus* spp. (i.e. Suriano, 1975; present study) overlaps with that of specimens from *Seriolella* spp. In addition, the number of testes appears to be higher in specimens collected from the gills of *Seriolella* spp. (Table 1); it is worth noting that these soft structures are difficult to count in some specimens due to their overlapping or the difficulties to define testes limits. Therefore, the use of this morphological character to differentiate species appears questionable. Additionally, the use of soft-body features is generally not recommended in monogenean taxonomy as they can be affected during the collection or fixation of the specimens (Chisholm & Whittington, 2005).

The number of pairs of larval hooks on the haptor was one of the most important features used to differentiate species of *Neogrubea* (see Evdokimova, 1969; Butt, 1974; Rey & Meneses, 1985; Gibson & Meneses, 1990). However, our study revealed that both newly-collected and museum specimens possess three pairs of larval hooks. The length of larval hooks did not differ considerably between specimens from different host species (Table 1). The size of the hooks should be considered with caution to differentiate future species of *Neogrubea* since in a closely related species, *K. scombri*, the size of the hamuli differed from fish caught in different localities (Rohde, 1991), and to a lesser extent, as an effect of host size (Perera, 1992)

The anterior extent of the vitelline field was also used as a morphological character to differentiate species of *Neogrubea* (see Gibson, 1976; Mamaev, 1982; Gibson & Meneses, 1990). Vitelline fields reach to the level of oesophageal bifurcation in *N. seriolellae* (see Dillon & Hargis, 1968); this extent is similar to that reported in specimens of *N. seriolellae* from *Seriolellae* spp. caught off New Zealand (see figure 3 in Rohde et al., 1980), and from off the Patagonian coast of Argentina in the present study. In specimens of *N. seriolellae* from *Stromateus* spp. the vitelline field also reaches to the level of oesophageal bifurcation (Butt, 1974; Gibson, 1976). This extent was also observed in all specimens (n = 57) collected from *S.*

brasiliensis examined in the present study. Consequently, we do not consider the anterior extent of the vitelline field as a useful morphological feature to distinguish species of *Neogrubea*.

We did not observe differences in the shape and number of the spines in the genital atrium (Table 1). These are often difficult to count due to their small size and overlapping. However, all the specimens examined in this study had ten small dorsal spines and two large ventral spines. A similar number of spines in the genital atrium were also counted in specimens of *N. seriolellae* from *S. porosa* and *S. brasiliensis* enzymatically digested with Proteinase K (Hernández-Orts et al., unpublished data). Dillon & Hargis (1968) reported 10–14 dorsal spines (see their figure 2) whereas Mamaev (1982) reported 9–13 dorsal spines in the genital atrium of *N. seriolellae*; therefore it seems that the number of dorsal spines in some specimens could be higher than five pairs.

The size of the small and large clamps was also similar between the specimens examined in the present study, and varied considerably depending on their position on the haptor (e.g. clamps near the posterior body end are usually smaller) and on the developmental stage of the worm. The clamps of all specimens studied here were composed of seven sclerites of similar shape. However we observed that scleritum antero-supplementarium internum and scleritum antero-supplementarium externum were poorly sclerotised in the anterior clamps in some specimens, and for this reason difficult to observe.

In view of the morphological and molecular evidence presented in this study, it seems that most of the specimens of *Neogrubea* spp. from this work and from the published descriptions belong to a single species i.e. *N. seriolellae*. As previously stated, the validity of the only other species of the genus, *N. soni*, must be confirmed using molecular and morphological methods, mainly because this species was described based on morphological traits which are not helpful to differentiate species of *Neogrubea*.

The mazocraeid subfamily Grubeinae Price 1961 comprises three genera, *Grubea* Diesing, 1858, *Paragrubea* Mamaev, 1982 and *Neogrubea.* Members of this subfamily can be distinguished from those in other mazocraeid subfamilies by the following characters: long body; haptor with an asymmetrical arrangement of clamps with four larger clamps of mazocraeid type on one side, and one to four smaller clamps on the other; shape of sclerite pieces of clamps; genital atrium armed with a circle of small inner spines and a pair of large external spines; and vagina lacking (see Price, 1961; Mamaev, 1982).

The genus *Neogrubea* markedly differs from *Grubea* in the number of clamps on the haptor (four pairs of clamps on the haptor *vs* four clamps on one side of the haptor and one on the other side), and by the number of larval hooks (3 *vs* 2, respectively) (Dillon & Hargis, 1968; Mamaev, 1982, Yuan et al., 2013). It also differs from *Grubea* in the absence of muscular adhesion fold on the haptor (Rohde, 1986) and in the extension of the testes (pre-, para-, and post-ovarian in *Neogrubea vs* only postovarian in *Grubea*) (see Price, 1961; Dillon & Hargis, 1968; Mamaev, 1982; Rohde, 1986). Additionally, species of *Neogrubea* infect fishes of the families Centrolophidae, Stromateidae and Kyphosidae (Gibson & Meneses, 1990) while species of *Grubea* infects strictly fishes of the family Scombridae (see Rohde, 1986).

Neogrubea most closely resembles the genus *Paragrubea* in the asymmetrical arrangement of the clamps on the haptor (both have four small clamps on one side of the haptor and four large clamps on the opposite side) (see Mamaev, 1982). However, species of both genera differ from each other in the number of larval hooks (3 *vs* 2), in the distribution of the testes (intercaecal in *Neogrubea vs* inter- and extracaecal in *Paragrubea*) and in the absence of filaments on the poles on the egg in *Paragrubea* (see Mamaev, 1982). Currently *Paragrubea* only includes one species, *P. ariommae* Mamaev, 1982 from the gills of the stromateid *Ariomma luridum* Jordan & Snyder in the Pacific Ocean near Japan (Mamaev, 1982). However, our study suggests that in *Neogrubea*, the small pair of medial marginal hooks may not be observed due their small size. Also, the filaments on the eggs were not observed in some specimens of *Neogrubea* (see Gibson, 1976). Finally, species of *Neogrubea* exhibit high specificity for stromateids (Gibson & Meneses, 1990). It appears that there are few morphological and ecological characters to differentiate species of *Paragrubea* and *Neogrubea*. We were unable to study the type-material of *P. ariommae* because of the current legislation on loans of type-materials of Russian institutions. Further studies based on the type-material of *P. ariommae* together with morphological and molecular analyses of new specimens must be performed to explore the validity of *Paragrubea*.

The newly-generated sequences for *N. seriolellae* reveal a closest relationship to representative sequences for *K. scombri* rather than to sequences for *G. cochlear*, the only other member of the subfamily Grubeinae available. The LSU rDNA sequence for *G. cochlear* (AF311710) was considerably different to *N. seriolellae* whereas that for *G. cochlear* (AF131730) was much more similar. These differences suggest that one of the sequences for isolates of *G. cochlear* (AF311710) may be based on misidentified material. Obtaining complete sequences of LSU rRNA gene and other regions of the ribosomal gene as well as further exploration of the phylogenetic relationships of members of all subfamilies of the Mazocraeidae, are required to advance our knowledge on the relationships between members of this family of polypisthocotyleans.

Acknowledgements The authors thank Néstor García, Teodora Mitu, Margarita del Dedo, Barbara Berón-Vera, Marina Aversa and Soledad Leonardi for their technical assistance. Thanks are due to Prefectura Naval Argentina and ALPESCA S.A. for allowing us to collect the material on the hake trawlers. We are grateful to Pavel Gerasev for his assistance. We also thank Pat Pilitt (USNPC), Eileen Harris (BMNH) and Paula Marcotegui (CEPAVE) for specimen loans. We are indebted to the División Zoología Invertebrados, of Museo de La Plata for allowing J.S.H.O. to study type-material of *Asymmetria* housed in the helminthological collection. This study was supported by the following projects: BBVA project n. BIOCON 04, AGL2010-20892 from the Spanish Government; PROMETEO 2011-040 and ISIC 2012-003 of the Valencian Government; and the Czech Science Foundation (ECIP, No. P505/12/G112). J.S.H.O. benefited a PhD student grant from the National Council on Science and Technology (CONACYT) of the Mexican Government (scholarship number 197266). Institutional support was given by the Centro Nacional Patagónico (CONICET, Argentina) and the Institute of Parasitology (RVO: 60077344).

References

Alama-Bermejo, G., Montero, F. E., Raga, J. A., & Holzer, A. S. (2011). *Skoulekia meningialis* n. gen., n. sp. (Digenea: Aporocotylidae Odhner, 1912) a parasite surrounding the brain of the Mediterranean common two-banded seabream *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) (Teleostei-Sparidae): description, molecular phylogeny, habitat and pathology. *Parasitology International, 60*, 34–44.

Bush, A. D., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis, revised. *Journal of Parasitology, 84*, 575–583.

Butt, A. J. (1974). Contributions to the biology of the monogenetic trematode family Mazocraeidae with the description of *Neogrubea* sp. Master Thesis, Florida: University of West Florida, 75 pp.

Chisholm, L. A., & Whittington, I. D. (2005). *Empruthotrema stenophallus* n. sp. (Monogenea: Monocotylidae) from the nasal tissue of *Dasyatis kuhlii* (Dasyatidae) from Sabah, Borneo, Malaysia. *Journal of Parasitology, 91*, 522–526.

Dillon, W. A., & Hargis, W. J., Jr. (1968). Monogenetic trematodes from the southern Pacific Ocean. Part IV. Polyopisthocotyleids from New Zealand fishes: the families Mazocraeidae, Diclidophorida and Hexabothriidae. *Proceedings of the Biological Society of Washington, 81*, 351–366.

Evdokimova, E. B. (1969). New species of monogeneans from bony fishes of the Patagon Shelf. *Parazitologiya, 3*, 415–419 (In Russian).

Gibson, D. I. (1976). Monogenea and digenea from fishes. *Discovery Reports, 36*, 179–266.

Gibson, D. I., & Meneses, M. I. (1990). Some comments on the taxonomy of the species of the genus *Neogrubea* Dillon & Hargis, 1968 (Monogenea: Mazocraeidae) and their synonyms. *Systematic Parasitology, 15*, 219–221.

Jovelin, R., & Justine, J. L. (2001). Phylogenetic relationships within the polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. *International Journal for Parasitology, 31*, 393–401.

Lin, D., Hanson, L. A., & Pote, L. M. (1999). Small subunit ribosomal RNA sequence of *Henneguya exilis* (Class Myxosporea) identifies the actinosporean stage from an oligochaete host. *Journal of Eukaryotic Microbiology, 46*, 66–68.

Littlewood, D. T. J., & Olson, P. D. (2001). Small subunit rDNA and the phylum Plathyhelminthes: signal, noise, conflict and compromise. In: Littlewood, D. T. J. & Bray, R. A. (Eds) *Interrelationships of the Platyhelminthes,* London: Taylor & Francis, pp. 262–278.

Littlewood, D. T. J., Rohde, R., & Clough, K. A. (1999). The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biological Journal of the Linnean Society, 66*, 75–114.

Mamaev, Yu. L. (1981). Some new monogenean species and genera of the family Mazocraeidae. *Helminthologia, 18*, 169–187.

Mamaev, Yu. L. (1982). Monogeneans of the subfamily Grubeinae Price, 1961 (Family Mazocraeidae). *Parazitologiya, 16*, 457–462 (In Russian).

Mollaret, I., Jamieson, B. G. M., & Justine, J. L. (2000). Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *International Journal for Parasitology, 30*, 171–185.

Olson, P. D., Cribb, T. H., Tkach, W., Bray, R. A., & Littlewood, D. T. (2003). Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology, 33*, 733–755.

Olson, P. D., & Littlewood, D. T. J. (2002). Phylogenetics of the Monogenea - evidence from a medley of molecules. *International Journal for Parasitology, 32*, 233–244.

Perera, K. M. L. (1992). The effect of host size on large hamuli length of *Kuhnia scombri* (Monogenea: Polyopisthocotylea) from Eden, New South Wales, Australia. *International Journal for Parasitology, 22*, 123–124.

Price, E. W. (1961). North American monogenetic trematodes IX. The families Mazocraeidae and Plectanocotylidae. *Proceedings of the Biological Society of Washington, 74*, 127–156.

Reiczigel, J. (2003). Confidence intervals for the bionomial parameter: some new considerations. *Statistics in Medicine, 22*, 611–621.

Rey, M., & Meneses, M. I. (1985). Una nueva especie de monogenea parasito del las branquias del "saborin" *Seriolella punctata* Haedrich 1967. *Contribuciones. (Departamento de Oceanografía, Facultad de Humanidades y Ciencias). Montevideo, 2*, 107–114.

Rohde, K. (1986). *Grubea australis* n. sp. (Monogenea, Polyopisthocotylea) from *Scomber australasicus* in southeastern Australia, and *Grubea cochlear* Diesing, 1858 from *S. scombrus* and *S. japonicus* in the Mediterranean and western Atlantic. *Systematic Parasitology, 9*, 29–38.

Rohde, K. (1991). Size differences in hamuli of *Kuhnia scombri* (Monogenea: Polyopisthocotylea) from different geographical areas not due to differences in host size. *International Journal for Parasitology, 21*, 113–114.

Rohde, K., Roubal, F., & Hewitt, G. C. (1980). Ectoparasitic Monogenea, Digenea, and Copepoda from the gills of some marine fishes of New Caledonia and New Zealand. *New Zealand Journal of Marine & Freshwater Research, 14*, 1–13.

Rózsa, L., Reiczigel, J., & Majoros, G. (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology, 86*, 228–232.

Schwerdt, C. B., Guagliardo, S. E., Galeano, N. A., & Tanzola, R. D. (2010). Estructura de las infracomunidades de monogenos parásitos de *Seriolella porosa* Guichenot, 1848 (Pisces: Centrolophidae) en el Golfo San Matías, Argentina. *Revista Ibero-Latinoamericana de Parasitología, 69*, 186–193.

Suriano, D. M. (1975). Nuevo parásito branquial del pez *Stromateus brasiliensis* Fowler (Monogenea-Polyopistocotylea). *Netropica, 21*, 75–81.

Yuan, K., Xiao, Z., Ding, X., & Liu, L. (2013). Two new species of mazocraeid monogeneans from *Clupanodon punctatus* (Temminck & Schlegel) (Clupeiformes: Clupeidae) found in Daya Bay, South China Sea, with the proposal of *Chelimazocraes* n. g. *Systematic Parasitology, 86*, 271–283.