Folia Parasitologica 2015, 62: 046
doi: 10.14411/fp.2015.046

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

Three new species of *Pseudodactylogyrus* (Monogenea: Pseudodactylogyridae) from Australian eels

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Abstract: Three species of *Pseudodactylogyrus* Gusev, 1965 (Monogenea: Pseudodactylogyridae) were collected from the gills of *Anguilla reinhardtii* Steindachner and *A. australis* Richardson from several localities in Australia and eels imported to Japan from Australia. *Pseudodactylogyrus gusevi* sp. n. from *A. reinhardtii* (type host) and *A. australis* in Queensland, Australia is most similar to *P. bini* (Kikuchi, 1929), but can be differentiated by the shorter male copulatory tube, heavy sclerotisation of the vaginal tube and the presence of a small projection of the supplementary piece of the hamulus. *Pseudodactylogyrus rohdei* sp. n. from *A. australis* (type host) in Queensland, Australia is most similar to *P. anguillae* (Yin et Sproston, 1948), but differs in the possession of a longer cement gland and the presence of a small projection on the supplementary piece of the hamulus. *Pseudodactylogyrus bini* sensu Gusev, 1965 and *P. anguillae* sensu Gusev, 1965 are synonymised with *P. gusevi* sp. n. and *P. rohdei* sp. n., respectively. *Pseudodactylogyrus mundayi* sp. n. from *A. australis*, originating in Tasmania, Australia and sent alive to Japan, is most similar to *P. kamegaii* Iwashita, Hirata et Ogawa, 2002, from which it can be discriminated by the shorter male copulatory tube and the shorter vaginal tube. *Dactylogyrus bialatus* Wu, Wang et Jian, 1988 from *Synechogobius ommaturus* (Richardson) (Gobiidae) is transferred to *Pseudodactylogyrus* as *P. bialatus* comb. n. A phylogenetic tree based on the ITS2 region of six species of *Pseudodactylogyrus* including *P. gusevi* and *P. mundayi* shows that *P. haze* from a goby diverged first, and that species from eels are monophyletic, forming three lineages differing by their zoogeographical distribution. With the three new species and one new combination proposed in this paper, *Pseudodactylogyrus* is now comprised of eight species infecting anguillid and gobiid fish, and a key to species is presented.

Keywords: taxonomy, morphology, phylogeny, ITS2 rDNA, evolution, *Anguilla*

Monogeneans of the genus *Pseudodactylogyrus* Gusev, 1965 are gill parasites of fresh and brackish water fishes. Four species have been described: (i) *Pseudodactylogyrus bini* (Kikuchi, 1929) [syns. *Dactylogyrus bini* Kikuchi, 1929 and *Neodactylogyrus bini* (Yin et Sproston, 1948)] from the Japanese eel, *Anguilla japonica* Temminck et Schlegel, European eel, *A. anguilla* (Linnaeus), American eel, *A. rostrata* (Lesueur), speckled longfin eel, *A. reinhardtii* Steindachner, giant mottled eel, *A. marmorata* Quoy et Gaimard, and African longfin eel, *A. mossambica* (Peters); (ii) *Pseudodactylogyrus anguillae* (Yin et Sproston, 1948) (syns. *N. anguillae* Yin et Sproston, 1948 and *P. microrchis* Ogawa et Egusa, 1976) from *A. japonica*, *A. anguilla*, *A. reinhardtii*, *A. marmorata* and *A. mossambica*; (iii) *Pseudodactylogyrus kamegaii* Iwashita, Hirata et Ogawa, 2002 from *A. japonica*; and (iv) *Pseudodactylogyrus haze* Ogawa, 1984 from the yellowfin goby, *Acanthogobius flavimanus* (Temminck et Schlegel) (Kikushi 1929, Gusev 1965, Ogawa and Egusa 1976, Golovin 1977, Ogawa 1984, Cone and Marcogliese 1995, Hayward et al. 2001, Iwashita et al. 2002, Sasal et al. 2008). In addition, *Pseudodactylogyrus pseudobagrus* Ling, 1973 was reported on the gills of the yellow catfish *Pseudobagrus fulvidraco* (Richardson) [= *Tachysurus fulvidraco* (Richardson)] from China; this species was transferred to *Sinidactylogyrus* Zhang, 1981 by Zhang (1981).

We had the opportunity to examine parasites of wild Australian eels, *A. reinhardtii* and *A. australis* Richardson. We collected specimens comprising three species that all belonged to *Pseudodactylogyrus* and differed from the known species. Here, we describe these specimens as three new species, and amend the generic diagnosis of *Pseudodactylogyrus* accordingly. In addition, a new combination is proposed to add another species to the genus.

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ZooBank number for article: urn:lsid:zoobank.org:pub:E3342927-FA7F-41BF-BF28-7B7BB0548CBE

MATERIALS AND METHODS

Wild *Anguilla reinhardtii* from the River Burnett at Bundaberg, Noosa and Redland Bay, Queensland, Australia and *A. australis* from Noosa, Queensland, Australia were caught commercially and sent to the Zoology Department at the University of New England, Armidale. Wild *A. australis*, caught commercially in Tasmania and sent to Japan, were also examined. The eels were anesthetised by MS222 or FA100 and the gills were removed. Monogeneans were collected under a stereomicroscope, subsequently flattened and fixed in AFA and stained with Heidenhein's iron haematoxylin, Delafield's haematoxylin or alum carmine for observations of general characters. Specimens fixed in glycerin-ammonium picrate (GAP), dehydrated and mounted in Canada balsam were used for observations and measurements of sclerotised parts of the parasite. Some of the specimens were compared with the GAP-fixed voucher specimens of *P. bini* collected from *A. japonica* in Japan and deposited at the Meguro Parasitological Museum, Tokyo (MPM – Collection No. 20997).

Measurements and terminology of sclerotised parts are similar to those of Iwashita et al. (2002), except for the foldable part of the root of the hamulus, which is termed here as the supplementary piece as in Ogawa (1986) (Fig. 1). All figures were drawn using a drawing tube. Measurements, using a calibrated ocular micrometer or digital photo equipment (DS-Fi1 and DS-L2, Nikon), were given in micrometres as the range and mean and the number of specimens in parentheses. Since the male copulatory tube and sclerotised vaginal tube were irregularly curved, these parts were measured on a computer using the ImageJ program (image processing program available at: http://rsb.info.nih.gov/ij/) as described previously (Ogawa et al. 2012). Fish names follow Froese and Pauly (2015).

To elucidate phylogenetic relationships between two new species and known congeners, the Internal Transcribed Spacer 2 (ITS2) region of five species of *Pseudodactylogyrus* was sequenced (Table 1); *Pseudodactylogyroides apogonis* (Yamaguti, 1940) from *Apogon semilineatus* (Temminck et Schlegel) (Apogonidae) was used as the outgroup. Prior to fixation in 70% ethanol, worms were identified microscopically by morphological characters of hamuli and/or genital organs. Genomic DNA was extracted from fixed worms using a QIAquick DNA extract kit (QIAGEN, Inc., Valencia, California, USA) following the manufacturer's instructions. The small subunit ribosomal DNA including the ITS regions was amplified using the oligonucleotide primers PD-F (5'-AACCTGGTTGATCCTGCCAG-3') and PD-ITS-R (5'-TAATGCTTAAATTCAGCGGGT-3').

PCR was carried out in 20 µl volume containing 0.1 µl of Takara EX Taq DNA polymerase (Takara Bio, Inc., Otsu, Japan), 2.0 µl of PCR buffer (Takara Bio, Inc.), 1.6 µl of dNTP mixture (Takara Bio, Inc.), 1.0 µl of 10 µM each primers, 2 µl of extracted DNA and 12.3 µl of distilled water. PCR conditions were as follows: first denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 2 min, and the final extension step at 72 °C for 2 min. PCR products were checked by running on 1% agarose gel containing ethidium bromide and purified using a Qiagen DNA purification kit (QIAGEN, Inc.). The cycle sequencing was carried out using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, USA) with the PD-ITS-R primer and PD-ITS-450F (5'-CGATGAAGAGTGCAGCAAAC-3') as the sequence primer. Sequencing products were purified by Centi-cep columns. Subsequently, products were electrophoresed by an ABI 377 DNA Sequencer (Applied Biosystems, Inc.).

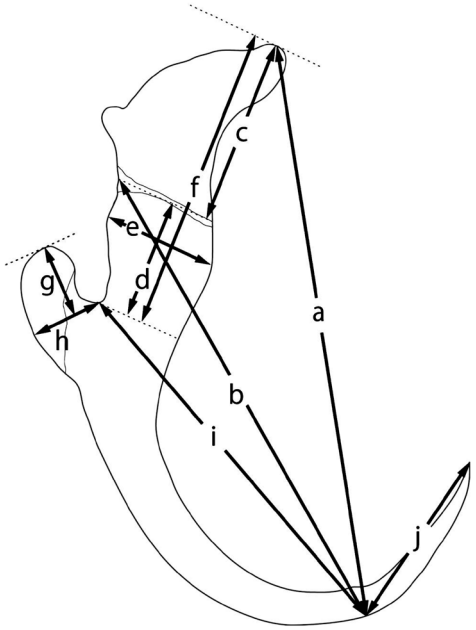

Fig. 1. Measurements and terminology of the sclerotised parts of the hamulus of *Pseudodactylogyrus* spp. a – length of the hamulus with the supplementary piece; b – length of the hamulus without the supplementary piece; c – length of the supplementary piece; d – length of the internal process; e – width of the internal process; f – length of the internal process with supplementary piece; g – length of the external process; h – width of the external process; i – length of the hamulus base; j – length of the point.

The obtained sequence data were edited by the Genetix Mac 9.0 sequence editor (Software Development Co., Ltd., Tokyo, Japan). Determination of the position of the ITS2 region was carried out by a comparison with the sequence of the Genbank record of the sequence of *P. bini* (Accession No. GQ478293). The multiple alignments of the obtained sequence data were performed using MAFFT v7.215 (Katoh and Standley 2013) with the option L-INS-i. To remove unreliably aligned regions within the datasets, we used Gblocks v0.91b (Castresana 2000) to identify the conserved regions with the following parameter settings: minimum number of sequence for a conserved position: 21; minimum number of sequence for a flank position: 34; maximum number of contiguous nonconserved positions: 8; minimum length of a block: 10; allowed gap positions: all. As a result, 438 of 483 positions were used for following phylogenic analysis.

The phylogenic trees were constructed with MEGA6 (Tamura et al. 2013) for maximum likelihood method (ML) and maximum parsimony method (MP). The best-fitting model of evolution selected was Kimura 2-parameter model with invariant sites (K2 + I) by MEGA6. Gaps or missing data were treated using complete deletion option in MEGA6. Bootstrap values were estimated from 1 000 replicates and a heuristic search was performed implementing the estimated model parameters using nearest-neighbor-interchange (NNI) branch swapping for ML and Subtree-Pruning-Regrafting (SPR) branch swapping for MP. Evolutionary distance between *P. haze* and *Pseudodactylogyrus* spp. of eels and mean

Table 1. List of specimens of *Pseudodactylogyrus* spp. used for DNA analysis.

Species	Host	Locality	No.	Accession No.
Pseudodactylogyrus anguillae (Yin et Sproston, 1948)	*Anguilla japonica* Temminck et Schlegel	Yoshida, Shizuoka Pref., Japan	5	LC041237–LC041241
P. bini (Kikuchi, 1929)		Yoshida, Shizuoka Pref., Japan	5	LC041242–LC041246
P. gusevi sp. n.	*A. reinhardtii* Steindachner	Burnett River, Queensland, Australia	13	LC041208–LC041220[1]
		Noosa, Queensland, Australia	8	LC041221–LC041228
		Redland Bay, Queensland, Australia	4	LC041229–LC041232
		South Brisbane, Queensland, Australia	1	LC041233
P. haze Ogawa, 1984	*Acanthogobius flavimanus* (Temminck et Schlegel)	Miyakoda River, Hamamatsu, Shizuoka Pref., Japan	1	LC041207
P. kamegaii Iwashita, Hirata et Ogawa, 2002	*Anguilla japonica*	Minato River, Futtsu, Chiba Pref., Japan	1	LC041236
P. mundayi sp. n.	*A. australis* Richardson	Hamamatsu, Shizuoka Pref., Japan*	2	LC041234, LC041235
Pseudodactylogyroides apogonis (Yamaguti, 1940)**	*Apogon semilineatus* (Temminck et Schlegel)	Sagami Bay, Misaki, Kanagawa Pref.	1	LC041206

No. – no. of specimens; * caught in Tasmania, Australia and sent live to Hamamatsu, Shizuoka Pref., Japan; ** outgroup; [1] MPM Coll. No. 20990 (Clade A), 20991A–20991F (Clade B).

distance within *Pseudodactylogyrus* spp. of eels were calculated with the K2 model using MEGA6.

Some differences in ITS2 sequences were found among individuals initially identified as one of the new species (see Table 1). To determine whether these differences corresponded with any differences in morphology, seven additional specimens from the Burnett River were fixed in 100% ethanol and the body was sectioned into two parts under a stereomicroscope. The anterior part was used for molecular analysis and the posterior (mainly the haptor) was remounted in GAP for examination of haptoral sclerites.

RESULTS

Family **Pseudodactylogyridae** Ogawa, 1986

Subfamily **Pseudodactylogyrinae** Ogawa, 1986

Genus ***Pseudodactylogyrus*** Gusev, 1965

Pseudodactylogyrus gusevi sp. n. Fig. 2

ZooBank number for species:
urn:lsid:zoobank.org:act:50DC807D-4E5E-4717-B3B3-744A699C8A0F

Synonym: *Pseudodactylogyrus bini* sensu Gusev, 1965 ex *Anguilla reinhardtii*

Description (based on 9 stained and 19 GAP specimens): Body elongate, slightly tapering at both ends, with widest part at level of testis or post-testicular region, 940–1 580 (1 340; n = 9) long, 220–310 (270; n = 9) wide. Haptor transversely wide, 89–123 (106; n = 9) long, 128–173 (150; n = 9) wide. Pair of hamuli (anchors), stout and robust, situated medially in haptor. Supplementary piece united with root of internal process of hamulus, folded ventrally or unfolded, its junction thin and flexible. Hamulus with or without supplementary piece 74–84 (80; n = 17) and 67–75 (71; n = 19) long, respectively. Supplementary piece curved inward, tapering distally, 21–27 (24; n = 19) long, with small projection near base of outer ridge. Internal process almost straight and square or slightly curved outward from middle, 16–19 (17; n = 19) long, 16–19 (17; n = 18) wide. When supplementary piece unfolded, combined length of supplementary piece and internal process 36–43 (40; n = 17) long. External process short, width much narrower than internal process, 8–14 (10; n = 19) long, 8–11 (10; n = 19) wide. Base of hamulus curved inward and slightly tapered, 50–62 (57; n = 19) long, leading to point, 27–32 (29; n = 19). Transverse bar connecting hamuli straight, situated ventrally to hamuli, swollen at both ends, 44–54 (49; n = 19), 10–17 (12; n = 19) wide. Marginal hooks of larval type, in 7 pairs, 16–19 (17; n = 19) long: 6 pairs located along margin of the haptor; 1 pair in centre of haptor, just beside hamuli. Cement gland well developed, 203–306 (246; n = 8) long, i.e. 15.5–23.0% of body length, situated posterior to vitellarium in body proper, opening on ventral surface of haptor.

Anterior end of body somewhat truncated. Three pairs of head organs opening subterminally on both sides of anterior region. Two pairs of eye spots located near anterior end of pharynx. Secretory cells of head organ in 2 pairs, located on each side just posterior to head organ and posterior to pharynx. Mouth opening at same level as eye spots. Pharynx barrel-shaped, 56–81 (68; n = 9) long, 42–74 (57; n = 9) wide. Oesophagus short. Postpharyngeal gland cells of 2 types, opening to each side of oesophagus, one a mass of small cells closely attached to oesophagus, the other consists of 2 or 3 large, granular cells located outside small cells. Intestine bifurcated, run on both sides of body, uniting in front of cement gland.

Testis ellipsoidal or elongate, medial, 100–245 (190; n = 9) long, 109–144 (120; n = 9) wide. Vas deferens emerging from front edge of testis, looping around sinistral intestinal limb, narrowing in width and ascending in intercaecal area, forming vesicula seminalis before turning backward. Vesicula seminalis directed anteriorly and then posteriorly, sausage-shaped, 35–50 (41; n = 9) long in straight line, 8–15 (11; n = 9) wide, leading into base of copulatory tube. Prostatic reservoir spherical, surrounded by thick muscle bundles, 21–37 (30; n = 9) in diameter. Prostatic cells distributed around male copulatory complex in intercaecal space. Male copulatory complex consisting

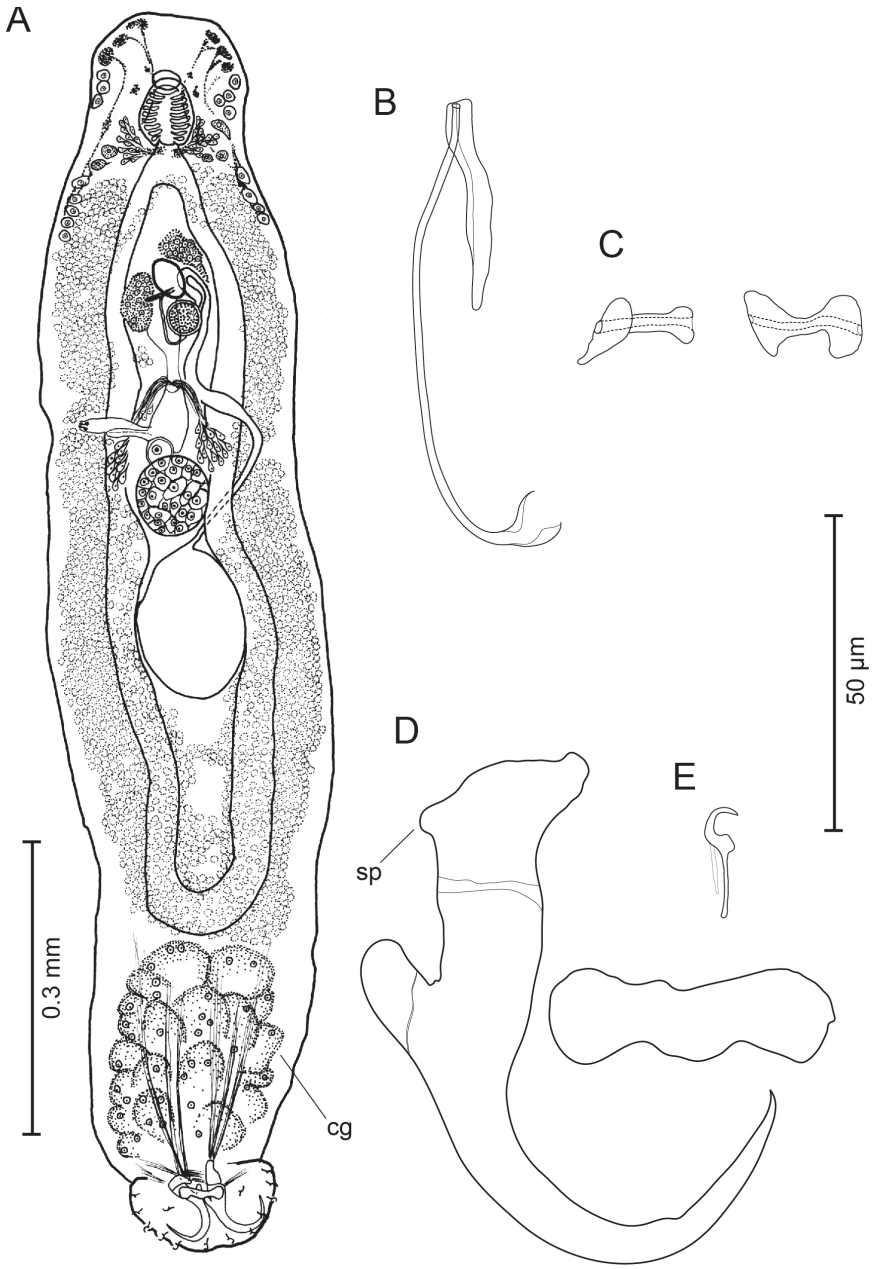

Fig. 2. *Pseudodactylogyrus gusevi* sp. n. from *Anguilla reinhardtii*. **A** – holotype (MPM 20983), ventral view; **B–E** – sclerotised parts of paratypes; **B** – male copulatory complex (MPM 20988); **C** – vagina (left: weakly sclerotised vagina from young adult, right: well sclerotised vagina from fully developed adult) (MPM 20987); **D** – hamulus and bar (MPM 20988); **E** – marginal hook (MPM 20988). *Abbreviation*: cg – cement gland; sp – small projection.

of arch-form tube and accessory piece. Tube ensheathed by muscles along whole length, curved inward almost at right angle toward its base, rather straight toward its tip, 78–96 (85; n = 19) long along its curved line, having constant width, 1–2 (2; n = 19) wide, except at funnel-shaped base, 5–8 (6; n = 19) wide. Accessory piece a straight, grooved rod, 34–41 (38; n = 19) long, 2–5 (3; n = 19) wide. Tube reaching genital pore, located anterior to prostatic reservoir.

Ovary spherical, situated immediately anterior to testis and smaller than testis, sometimes overlapping with anterior part of testis, 46–87 (75; n = 9) long, 56–84 (71; n = 9) wide. Oviduct starting from anterior edge of ovary to form fertilisation chamber, having small outgrowth on right side. Vagina almost straight, opening 17–37 (26; n = 8) from dextral lateral margin. Distal end a sclerotised, dumbbell-shaped tube, with both ends thickened, 15–21 (17; n = 18) in a straight line, 16–22 (19; n = 18) long along its curve, 2 (n = 17) wide. Fertilisation chamber leading to ootype. Mehlis' glands opening at posterior end of ootype, with gland cells on both sides of ovary. Uterus leads to genital pore together with male copulatory complex. No egg observed in uterus. Vitellarium coexistent with intestine.

Type host: *Anguilla reinhardtii* Steindachner (Anguillidae).

Other host: *Anguilla australis* Richardson (Anguillidae).

Type locality: Burnett River at Bundaberg, Queensland, Australia (24°52'S; 152°21'E), 9 and 10 January 1998.

doi: 10.14411/fp.2015.046

Table 2. Comparison of measurements of *Pseudodactylogyrus* spp. reported from *Anguilla* spp. Measurements are given in micrometres (μm), range followed by mean in parenthesis.

Species	*Dactylogyrus bini* Kikuchi, 1929	*D. bini* (another form)	*Neodactylogyrus bini* (Yin et Sproston, 1948)	*Pseudodactylogyrus bini* (Kikuchi, 1929)	*P. bini*	*N. anguillae* Yin et Sproston, 1948	*P. microrchis* Ogawa et Egusa, 1976[a] and *P. anguillae*[b] (Yin et Sproston, 1948)	*P. anguillae*	*P. anguillae*	*P. kamegaii* Iwashita, Hirata et Ogawa, 2002	***P. gusevi*** sp. n.	***P. rohdei*** sp. n.	***P. mundayi*** sp. n.
Locality	Japan	Japan	Shanghai, China	Chiba, Shizuoka and Aichi Prefs., Japan	Australia	Shanghai, China	Chiba, Shizuoka and Aichi Prefs., Japan	Shizuoka Pref., Japan	Australia	Chiba Pref., Japan	Queensland, Australia	Queensland, Australia	Tasmania, Australia
Host	*Anguilla japonica* Temminck et Schlegel	*A. japonica*	*A. japonica*	*A. anguilla* (Linnaeus)	*A. reinhardtii* Steindachner	*A. japonica*	*A. anguilla*	*A. anguilla*	*A. reinhardtii*	*A. japonica*	*A. reinhardtii, A. australis* Richardson	*A. australis*	*A. australis*
Reference/ Structure	Kikuchi (1929)	Kikuchi (1929)	Yin and Sproston (1948)	Ogawa and Egusa (1976)	Gusev (1965)	Yin and Sproston (1948)	Ogawa and Egusa (1976)	Iwashita et al. (2002)	Gusev (1965)	Iwashita et al. (2002)	present study	present study	present study
Body length	1100–1400	900–1000	563–1210	539–1626 (896)	1400	482–854	374–1259	-	-	808–1414 (1041)	940–1580 (1340)	1470	640–960 (830)
Body width	200	180–190	135–207	120–311 (209)	260	167–213	138–282	-	-	182–303 (233)	220–310 (270)	360	91–177 (230)
Haptor length	70–80	90–100	68–90	65–107 (85)	80	112–136	91–170	-	-	95–185 (122)	89–123 (106)	150	83–177 (137)
Haptor width	140	120–150	104–130	85–172 (117)	150	117–131	94–190	-	-	100–245 (173)	128–173 (150)	221	128–173 (126)
Cement gland length	-	140–180	200–250	-	-	66–100	-	-	-	-	203–306 (246)	168	51–86 (68)
Cement gland length/ body length	-	-	-	-	-	-	-	-	-	-	15.5–23.2%	11.4%	5.7–11.0%
Pharynx length	-	-	-	40–91 (62)	-	-	35-77	-	-	53–75 (64)	56–81 (68)	91	44–72 (61)
Pharynx width	-	-	50–67	34–80 (52)	65–75	45–57	32-67	-	-	43–73 (56)	42–74 (57)	75	44–61 (51)
Male copulatory tube length	100	-	-	-	100	-	-	110–160 (130)	136	120–150 (140)	78–96 (85)	124–125	80–94 (88)
Male copulatory tube width	-	-	-	-	2–3; 6 (base)	-	-	1 (1)	2	2 (2)	1–2; 5–8 (base)	1; 4–5 (base)	2; 3–6 (base)
Accessory piece length	30–46	-	36–39	29–50 (41)	35	36	28–42	31–40 (35)	47	32–39 (35)	34–41 (38)	42–48	25–33 (29)
Prostatic resorvoir diameter	30	-	25–29	13–37 (22)	30	36–47	12–45	-	-	28–40 (32)	21–37 (30)	19	20–35 (30)
Ovary length	-	-	39–77	302–114 (64)	-	43–77	36–143	-	-	38–100 (75)	46–87 (75)	140	61–155 (80)
Ovary width	-	-	50–91	32–108 (67)	70–90	55–104	35–86	-	-	63–120 (90)	56–84 (71)	106	51–92 (69)
Testis length	-	-	73–191	67–265 (156)	200	91–154	22–225	-	-	88–270 (202)	100–245 (190)	266	110–230 (169)
Testis width	-	-	86–163	52–184 (114)	150	96–132	17–112	-	-	90–200 (139)	109–144 (120)	140	99–143 (108)
Vaginal tube length	-	-	-	-	20	-	-	-	30	21–32 (26)	15–21 (17)	22–28	9–16 (12)
Vaginal tube width	-	-	-	-	3	-	-	-	4	3–7 (4)	2	3	3–7 (5)
Hamulus[c] (a)	44–53	80–90	40–63	63–76 (69)	75–81	104–120	100–125	94–105 (99)	90–102	86–100 (95)	74–84 (80)	97–105	85–100 (93)
Hamulus[c] (b)	-	-	-	53–63 (58)	-	-	86–105	81–91 (87)	-	78–85 (82)	67–75 (71)	85–89	81–88 (85)
Hamulus[c] (c)	-	-	-	-	-	-	35–49	69–77 (73)	-	28–37 (33)	21–27 (24)	31–32	23–32 (26)
Hamulus[c] (d)	-	-	-	-	-	-	-	-	-	-	16–19 (17)	21–22	18–23 (20)
Hamulus[c] (e)	-	-	-	-	-	-	-	-	-	-	16–19 (17)	18–19	15–17 (16)
Hamulus[c] (f)	-	-	30–41	-	25–39	29–32	54–77	26–31 (29)	60	40–54 (47)	36–43 (40)	53	38–47 (44)
Hamulus[c] (g)	-	-	-	-	8–10	-	7–14	-	12–15	5–9 (6)	8–14 (10)	13–16	3–7 (5)
Hamulus[c] (h)	-	-	-	-	-	-	-	-	-	-	8–11 (10)	11–13	4–6 (6)
Hamulus[c] (i)	-	-	-	-	49–58	-	60–84	5–9 (6)	72–73	63–71 (68)	50–62 (57)	72–73	70–74 (72)
Hamulus[c] (j)	-	-	-	-	25–27	-	28–34	30–41 (35)	31	28–32 (30)	27–32 (29)	34–35	32–38 (35)
Connecting bar length	32–40	30–40	37–43	35–46 (40)	41–47	51–58	40–64	44–53 (50)	57	45–53 (49)	44–54 (49)	55–61	41–53 (48)
Connecting bar width	-	-	-	-	8–12	-	-	8–12 (9)	15	5–11 (9)	10–17 (12)	12–17	8–13 (10)
Marginal hook length	10–14	same as typical *D. bini*	25	15–18 (17)	16–18	10	14–17	15–17 (16)	14	13–14 (14)	16–19 (17)	18–19	15–16 (16)

[a] syn. of *P. anguillae;* [b] combined data of *P. anguillae* and *P. microrchis*; [c] measurements of the components of the hamulus shown in Fig. 1. .

Other localities: Noosa, Queensland, Australia (26°29'S; 153°01'E), 8 and 9 January 1999; South Brisbane, Queensland, Australia (27°28'S; 153°02'E), 6 February 1998; Redland Bay, Queensland, Australia (27°36'S; 153°18'E), 11 January 1999.

Site: Gills.

Type material: Holotype: Meguro Parasitological Museum, Tokyo (MPM 20983) and 27 paratypes (MPM 20984–20989), Queensland Museum, Brisbane (QM G234715, G234716) and the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice (IPCAS M-584).

Etymology: The species is named after the late Alexander V. Gusev, who erected the genus *Pseudodactylogyrus* in 1965, based on specimens collected from *Anguilla reinhardtii* by the late John C. Pearson.

Remarks. Gusev (1965) identified specimens from *Anguilla reinhardtii* as *P. bini*, but they differed from those of *P. bini* as originally described by Kikuchi (1929) in the shape of the male copulatory tube: arch-form in Gusev (1965) *vs* curved in a circle in Kikuchi (1929). *P. bini* sensu Gusev, 1965 were very similar to *P. gusevi* sp. n., but unfortunately, his specimens were lost (P.I. Gerasev, Zool. Inst., St. Petersburg, Russia – pers. comm.) and thus could not be compared with the present specimens (see Table 2). The general structure and measurements of his *P. bini* are almost completely identical with the present measurements, except for some parts of the female reproductive organs and the hamuli. The vaginal tube is heavily sclerotised at both ends in *P. gusevi*, whereas it was described as sclerotised at only one end in Gusev's *P. bini*. There is a small outgrowth on the right side of the fertilisation chamber and a small projection on the outer side of the supplementary piece of hamulus in *P. gusevi*, while neither of such structures was described in Gusev (1965). Gusev's description was based on specimens collected by J.C. Pearson. No mention was made on how the specimens were fixed, but most probably they were stained specimens, in which some minute structures like the sclerotised part of the vagina and the supplementary piece of the hamulus are often difficult to observe. The present description of such sclerotised parts were based on specimens fixed in GAP and the small outgrowth of the fertilisation chamber was observed only in specimens fixed in AFA and stained with Heidenhain's iron hematoxylin. Thus, it is thought that the observed morphological differences between our specimens and those of Gusev (1965) are insufficient to consider them as two separate species. We thus conclude that *P. bini* sensu Gusev, 1965 is identical with *P. gusevi* sp. n.

Pseudodactylogyrus gusevi can be differentiated from congeners by the morphological characters of the sclerotised parts such as hamuli, male copulatory complex and vagina, and from the most similar species, *P. bini*, by the combination of the following morphological characters: considerably shorter male copulatory tube (78–96 µm in *P. gusevi vs* 167–212 µm in *P. bini*; n = 8; vaginal tube short (16–22 µm) with heavily sclerotised ends in *P. gusevi*, compared with a simple, longer (25–32 µm; n = 8) and lightly sclerotised tube in *P. bini*; and the presence of a small projection on the supplementary piece of the hamulus in *P. gusevi vs* the absence of such structure in *P. bini*.

Pseudodactylogyrus rohdei sp. n. Fig. 3

ZooBank number for species:
urn:lsid:zoobank.org:act:AA2CEF0B-58EB-4F78-8179-E8F48C11DB5E

Synonym: *Pseudodactylogyrus anguillae* sensu Gusev, 1965 ex *Anguilla reinhardtii*.

Description (based on 1 stained and 2 GAP specimens): Body elongate, slightly tapering at both ends, widest part at level of post-testicular region, 1470 (n = 1) long, 360 (n = 1) wide. Haptor transversely wide, 150 (n = 1) long, 221 (n = 1) wide. Pair of hamuli large and slender. Hamulus with or without supplementary piece 97–105 (n = 2) and 85–89 (n = 2) long, respectively. Supplementary piece curved inward, tapering distally, 31–32 (n = 2) long, with small projection near base of outer ridge as in *P. gusevi*. Internal process almost straight, slightly wider distally, 21–22 (n = 2) long, 18–19 (n = 2) wide. When supplementary piece unfolded, combined length of supplementary piece and internal process 53 (n = 2) long. External process short, straight or slightly curved inward, width about half that of internal process, 13–16 (n = 2) long, 11–13 (n = 2) wide. Base of hamulus curved inward and tapered, 72–73 (n = 2) long. Point 34–35 (n = 2) long. Transverse bar straight, swollen at both ends, 55–61 (n = 2) long, 12–17 (n = 2) wide. Marginal hooks 18–19 (n = 2) long. Cement gland well developed, 168 (n = 1) long or 11.4% of body length.

Head organs not clearly observed. Two pairs of eye spots located near anterior end of pharynx. Secretory cells of head organ in 2 pairs, as in *P. gusevi*. Mouth opens at same level as eye spots. Pharynx barrel-shaped, 91 (n = 1) long, 75 (n = 1) wide. Oesophagus short. Postpharyngeal gland cells of 2 types, opening to each side of oesophagus. Intestine bifurcated and running on both sides of body, uniting in front of cement gland.

Testis elongate, medial, 266 (n = 1) long, 140 (n = 1) wide. Vas deferens looping around sinistral intestinal limb, narrowing in width and ascending in intercaecal area, turning backward, leading into base of copulatory tube. Vesicula seminalis not clearly formed. Prostatic reservoir 19 (n = 1) in diameter. Prostatic cells distributed around prostatic reservoir in space between intestinal limbs. Male copulatory complex a sclerotised tube, 124–125 (n = 2) long along its curved line, 1 (n = 2) wide with funnel-shaped base 4–5 (n = 2) wide, accompanied by accessory piece, straight and grooved, 42–48 (n = 2) long, 4–5 (n = 2) wide.

Ovary spherical, overlapping with anterior part of testis, 140 (n = 1) long, 106 (n = 1) wide. Oviduct forming fertilisation chamber anterior to ovary. No outgrowth of fertilisation chamber observed. Vagina dextral, 47 (n = 1) from lateral margin. Distal end of vagina a sclerotised tube, straight or bent medially, 22–28 (n = 2) long, 3 (n = 2) wide. No egg observed in uterus. Vitellarium co-existent with intestine.

Type host: *Anguilla australis* Richardson (Anguillidae).

Type locality: Noosa, Queensland, Australia (26°29'S; 153°01'E), 9 January 1999.

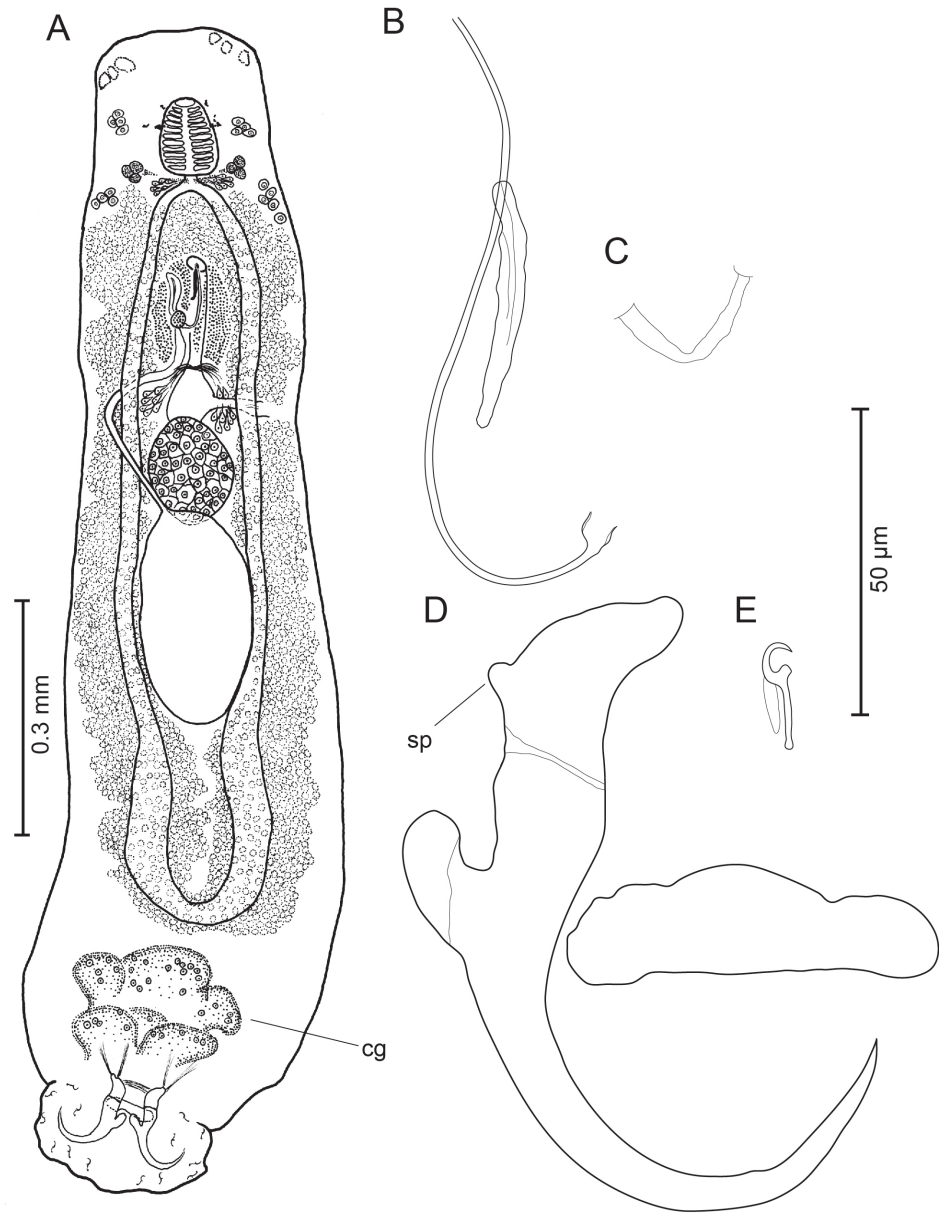

Fig. 3. *Pseudodactylogyrus rohdei* sp. n. from *Anguilla australis* Richardson. **A** – holotype (MPM 20992), dorsal view; **B**–**E** – sclerotised parts of paratypes; **B** – male copulatory complex (QM G234717); **C** – vagina (QM G234717); **D** – hamulus and bar (MPM 20993); **E** – marginal hook (MPM 20993). Abbreviation: cg – cement gland; sp – small projection.

Site: Gills.

Type material: Holotype (MPM 20992) and 2 paratypes (MPM 20993 and QM G234717).

Etymology: The species is named after Klaus Rohde, a distinguished fish parasitologist, Emeritus Professor of the University of New England, Australia.

Remarks. We compare *P. rohdei* sp. n. with specimens from *A. reinhardtii* described as *P. anguillae* by Gusev (1965). His description was simple and no measurements were given of the body, haptor, pharynx, prostatic reservoir, testis or ovary. His measurements of the sclerotised parts correspond very well with ours, except for the length of marginal hooks: 14 according to Gusev, compared with 18–19 in GAP specimens of the present study. A small projection on the outer side of the supplementary piece of the hamulus was also not described in Gusev (1965). However, considering that these differences are small, we concluded that *P. anguillae* sensu Gusev, 1965 collected from *A. reinhardtii* is conspecific with *P. rohdei* sp. n. described in this paper. His specimens were collected from *A. reinhardtii*, whereas ours were collected from *A. australis*.

P. rohdei sp. n. is most similar to *P. anguillae*, but can be easily discriminated by the length of the cement gland (much shorter in *P. anguillae*) and the shape and size of the hamulus (small projection of the supplementary piece absent in *P. anguillae*; external process shorter in *P. anguillae*) (Yin and Sproston 1948, Ogawa and Egusa 1976). *P. rohdei* sp. n. is also similar to *P. gusevi*, but can be distinguished by the size of the male copulatory tube (124–125 µm in *P. rohdei vs* 78–96 µm in *P. gusevi*) and the size of hamuli (without the supplementary piece) (85–89 µm *vs* 67–75 µm).

Pseudodactylogyrus mundayi sp. n. Fig. 4

ZooBank number for species:
urn:lsid:zoobank.org:act:C73E73FD-F48F-4268-B88E-5FEE1C17D176

Description (based on 8 stained and 16 GAP specimens): Body elongate, slightly tapering at both ends, widest part at level of testis or post-testicular region, 640–960 (830; n = 8) long, 91–177 (230; n = 8) wide. Haptor transversely wide, 83–177 (137; n = 6) long, 128–173 (126; n = 8) wide. Pair of hamuli slender. Hamulus with and without supplementary piece 85–100 (93; n = 12) and 81–88 (85; n = 16) long, respectively. Supplementary piece curved inward and tapering distally, 23–32 (26; n = 14) long. No small projection formed along outer ridge of supplementary piece. Internal process almost square and slightly curved outward, 18–23 (20; n = 16) long, 15–17 (16; n = 16) wide. When supplementary piece unfolded, combined length of supplementary piece and internal process being 38–47 (44; n = 13) long. External process short, width being much narrower than that of internal process, 3–7 (5; n = 16) long, 4–6 (6; n = 16) wide. Base of hamulus curved inward, slightly tapered, 70–74 (72; n = 16) long, leading to point, 32–38 (35; n = 16). Transverse bar straight, swollen at both ends, 41–53 (48; n = 16) long, 8–13 (10; n = 16) wide. Marginal hooks of larval type, in 7 pairs, 15–16 (16; n = 14) long: 6 pairs located along margin of haptor; 1 pair in centre of haptor, just beside hamuli. Cement gland short, 51–86 (68; n = 7) long or 5.7–11.0% of body length.

Three pairs of head organs opening subterminally on both sides of anterior region. Two pairs of eye spots located near anterior end of pharynx. Secretory cells of head organ in 2 pairs, located on each side just posterior to head organ and pharynx. Mouth opening at same level as eye spots. Pharynx barrel-shaped, 44–72 (61; n = 8) long, 44–61 (51; n = 8) wide. Oesophagus short. Postpharyngeal gland cells of 2 types open on each side of oesophagus. Intestine bifurcated and running on both sides of body, uniting in front of cement gland.

Testis ellipsoidal or elongate, situated in middle of body, 110–230 (169; n = 8) long, 99–143 (108; n = 8) wide. Vas deferens looping around left intestinal limb, narrowing in width and ascending in intercaecal area, forming vesicula seminalis before turning backward. Vesicula seminalis directed anteriorly and then posteriorly, sausage-shaped, 29–38 (33; n = 7) long in straight line, 56–70 (62; n = 7) long along a curved line, 8–17 (14; n = 8) wide, leading into base of copulatory tube. Prostatic reservoir spherical, surrounded by thick muscle bundles, 20–35 (30; n = 8) in diameter. Prostatic cells distributed around male copulatory organ in intercaecal space. Male copulatory tube short, 80–94 (88; n = 16) long along its curved line, of constant width, 2 (n = 16) wide and its base, funnel-shaped, 3–6 (4; n = 16) wide. Accessory piece a straight, grooved rod, 25–33 (29; n = 16) long, 3–5 (4; n = 16) wide. Genital pore located anterior to prostatic reservoir.

Ovary spherical, situated just in front of testis, smaller than testis, sometimes overlapping with anterior of testis, 61–155 (80; n = 8) long, 51–92 (69; n = 8) wide. Oviduct with small outgrowth on right side in front of ovary, leads to ootype and uterus. Vagina opening dextrally, 6–29 (17; n = 8) from right lateral margin, almost straight tube. Distal end of vagina a poorly sclerotised tube, sometimes unrecognisable, 9–16 (12; n = 11) long in a straight line, 9–17 (13; n = 9) long along its curve, 3–7 (5; n = 13) wide. Genital pore opening at level of vesicula seminalis. Vitellarium co-existent with intestine.

Type host: *Anguilla australis* Richardson (Anguillidae).

Type locality: Tasmania, Australia (precise locality not specified). The host eels were transferred alive to Shizuoka Prefecture, Japan (34°42'N; 137°38'E) and sampled on 23 March 2010 for parasitological examination.

Site: Gills.

Type material: Holotype: (MPM 20994) and 23 paratypes (MPM 20994–20996; QM G234718, G234719; IPCAS M-585).

Etymology: The species is named after the late Barry L. Munday, an outstanding Tasmanian authority in fish pathology and parasitology.

Remarks. *P. mundayi* sp. n. is most similar to *P. kamegaii* in general morphology, but can be discriminated from the latter in that the male copulatory tube (80–94 µm long) is considerably shorter than that of *P. kamegaii* (120–150 µm long), and the vaginal tube (9–16 µm long) is shorter than that of *P. kamegaii* (21–32 µm long) (Iwashita et al. 2002). *Pseudodactylogyrus mundayi* differs from *P. kamegaii* not only in morphology, but also in host species (*A. australis* compared with *A. japonica*) and habitat (fresh water *vs* sea water) (Iwashita et al. 2002, Katahira et al. 2012).

Molecular analysis

The phylogenetic tree of six *Pseudodactylogyrus* spp. including two of the three newly described species (*P. gusevi* and *P. mundayi*) is shown in Fig. 5. All *Pseudodactylogyrus* spp. analysed in this study formed a monophyletic group. Among them, *P. haze* is located in the most basal position. Its ITS2 sequence is very different from that of the other species; the evolutionary distance between *P. haze* and *Pseudodactylogyrus* spp. of eels is 0.5447, whereas mean distance among *Pseudodactylogyrus* spp. of eels is 0.0370. In contrast, *Pseudodactylogyrus* spp. from Australian and Japanese eels are closely related. They are divided into three lineages by their distribution, i.e. from Japanese waters (*P. anguillae*, *P. bini* and *P. kamegaii*), Australian mainland waters (*P. gusevi* sp. n.) and Tasmanian waters (*P. mundayi* sp. n.). Specimens of *P. gusevi* are divided into two groups (Clade A and B). Clade A consists of specimens collected only from the Burnett River (n = 6), whereas clade B consists of specimens collected from all localities, namely Burnett River (n = 7), Noosa (n = 8), Redland Bay (n = 4) and South Brisbane (n = 1). Among the seven additional specimens of *P. gusevi* from the Burnett River examined for both morphology (haptoral sclerites) and the ITS2 sequence, one (MPM 20990) belonged to Clade A, whereas the remaining six (MPM 20991A–20991F) belonged to Clade B. No morphological differences were de-

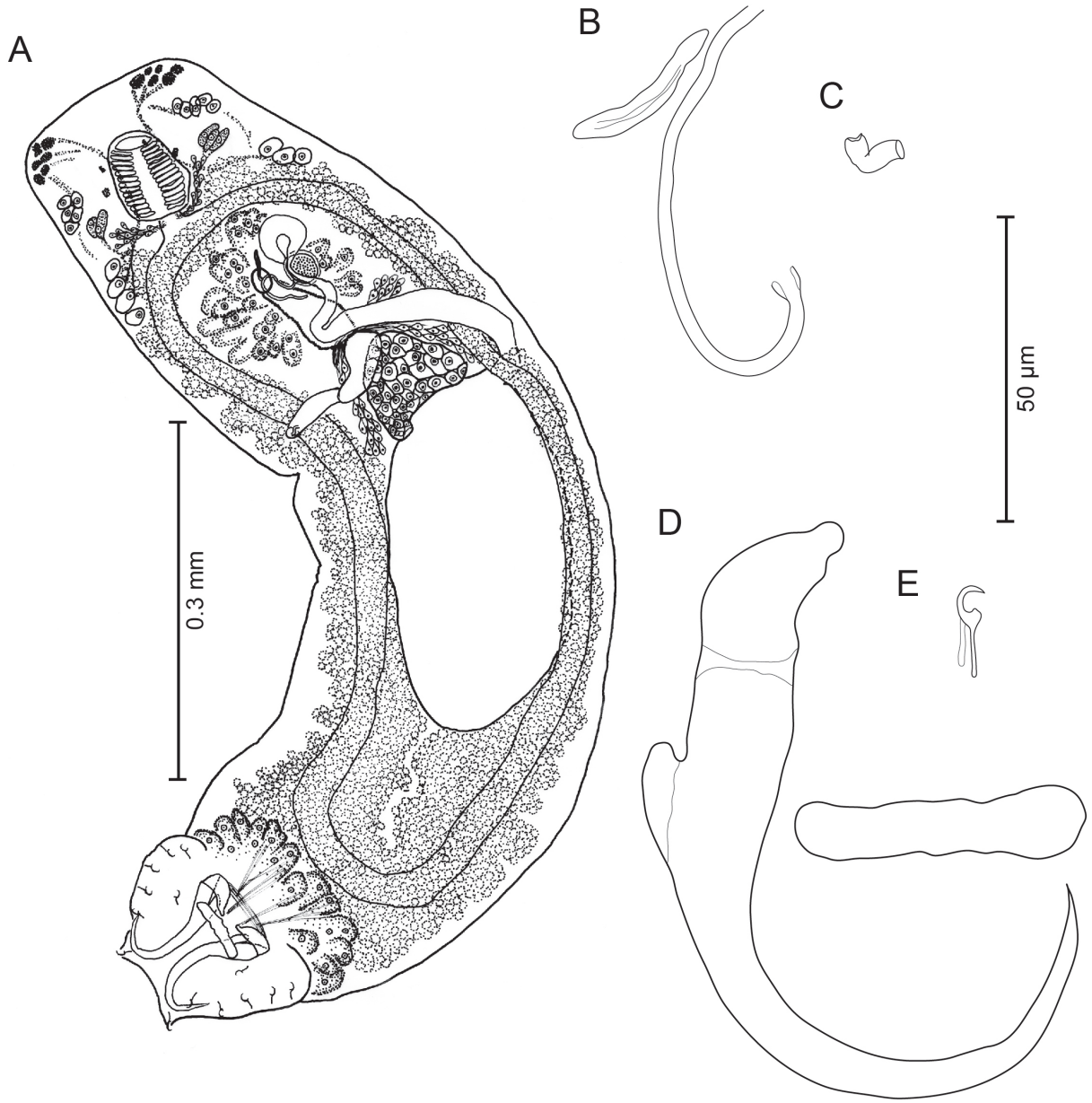

Fig. 4. *Pseudodactylogyrus mundayi* sp. n. from *Anguilla australis* Richardson. **A** – holotype (MPM 20994), ventral view; **B**–**E** – sclerotised parts of paratypes; **B** – male copulatory complex (MPM 20996); **C** – vagina (MPM 20996); **D** – hamulus and bar (MPM 20996); **E** – marginal hook (MPM 20996).

tected in the hamulus and transverse bar among the seven specimens.

DISCUSSION

Pseudodactylogyrus bini and *P. anguillae* were originally described from the gills of Japanese eel, *Anguilla japonica* in Japan and in China, respectively (Kikuchi 1929, Yin and Sproston 1948). Since then, these monogeneans have been reported from different species of ells (*Anguilla* spp.) in different geographical regions. There is evidence that infection of *Anguilla* spp., other than the type host *A. japonica*, are results of human activities, by which infected *A. japonica* was introduced to localities outside of its natural distribution, or other *Anguilla* spp. were introduced to localities where *A. japonica* was naturally distributed. Once the *Pseudodactylogyrus* infection became established in some parts of Europe, commercial movements of infected *A. anguilla* became another source of parasite invasion within other parts of Europe and elsewhere. There are also some unconfirmed sources of origin like *P. anguillae* infection of *A. mossambica* in South Africa (Christison and Baker 2007), *P. bini* and *P. anguillae* infection of *A. mossambica* and *A. marmorata* in the Island of Reunion (Sasal et al. 2008), and *P. anguillae* infection of *A. bicolor* McClelland in Indonesia (K. Buchmann, Univ. of Copenhagen, Frederiksberg C, Denmark – pers. comm.).

Live *A. australis* is imported to Japan from Australia and kept in fresh water at an eel dealer's facilities until consumed for food. *Pseudodactylogyrus mundayi* was collected from such eels purchased from the dealer. *Anguilla japonica* and *A. marmorata* are eel species native to Japan, from which three species of *Pseudodactylogyrus*, i.e.

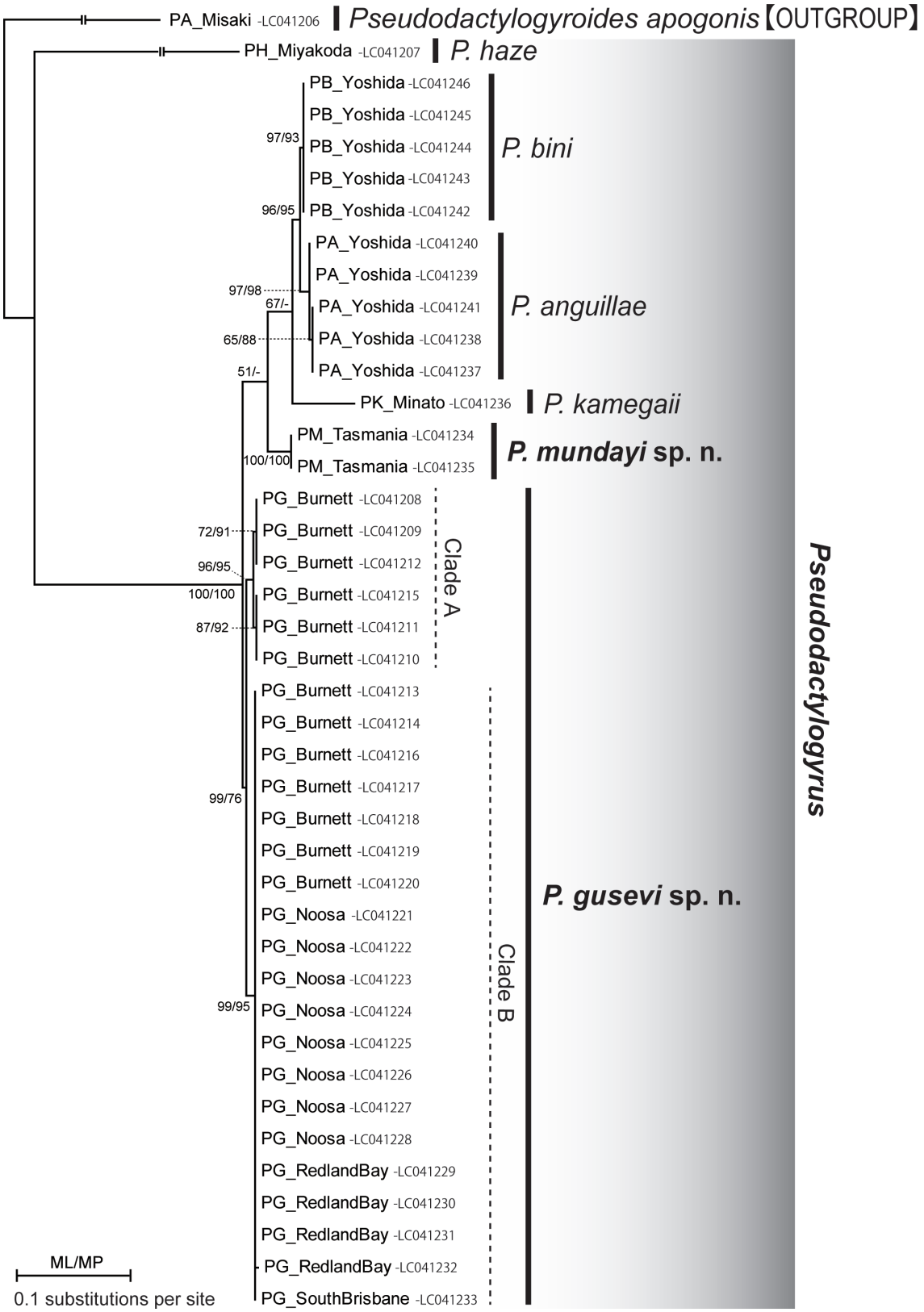

Fig. 5. Phylogenetic tree of species of *Pseudodactylogyrus* Gusev, 1965 constructed based on ITS2 sequences.

P. bini, *P. anguillae* and *P. kamegaii* from the former species of eel [note: *P. anguillae* was first reported in Japan by Kikuchi (1929) as another form of *Dactylogyrus bini*; Table 2] and *P. bini* and *P. anguillae* from the latter have been recorded (Kikuchi 1929, Iwashita et al. 2002; Katahira and Nagasawa 2014). No infection of *P. mundayi* has been detected in eels in Japan so far, but there is a risk of introduction of this new species infecting native eels in Japan. Japanese eel, *A. japonica*, is a highly valuable fish species and extensively cultured in Japan. This eel species

has been included in the red list of endangered species by the International Union for Conservation of Nature and Natural Resources after a long history of overfishing of young elvers used for aquaculture (Tatsukawa 2003). Preventive measures should urgently be taken against possible infection of Japanese eelswith *P. mundayi*.

Kennedy (1998) reported *P. bini* and *P. anguillae* infection of *Anguilla reinhardtii* in several localities of Queensland, Australia. It is possible that his *Pseudodactylogyrus* spp. correspond to the present *Pseudodactylogyrus*, especially *P. gusevi* and *P. rohdei*. As three new species are proposed in this paper and Kennedy (1998) did not describe morphological characteristics of the two species he collected, confirmation of the identification of his specimens is required. His specimens had been deposited at International Institute of Parasitology, UK, but they have been lost after the closure of the institute in 1998 (D.I. Gibson, Nat. Hist. Mus., London, UK – pers. comm.). This made it impossible to compare his specimens with the present ones.

Wu et al. (1988) proposed a new species *Dactylogyrus bialatus* Wu, Wang et Jian, 1988 from the gills of *Synechogobius ommaturus* (Richardson) (Gobiidae) in Zhejian Province, China. They described the sclerotised parts only, but this species apparently belongs to the genus *Pseudodactylogyrus*, as it has the characteristic supplementary piece at the root of the hamulus and seven pairs of marginal hooks are of larval type. Thus a new combination, ***Pseudodactylogyrus bialatus*** (Wu, Wang et Jian, 1988) comb. n. ZooBank number for species: urn:lsid:zoobank.org:act:36152620-64DB-4A1D-B59D-DE76CD94BB8F, is proposed here, which can be discriminated from the most similar *P. haze* by having a larger hamulus (78–89 µm long *vs* 60–71 µm long) and a larger accessory piece of the male copulatory complex (45–48 µm long *vs* 25–32 µm long). With the three new species and one new combination proposed in this paper, the genus *Pseudodactylogyrus* is emended and is now comprised of eight species. A key to species of *Pseudodactylogyrus* is given below.

Amended diagnosis of *Pseudodactylogyrus*

Pseudodactylogyridae. Body elongated with three pairs of head organs. Head truncated. Haptor with one pair of hamuli, one connecting bar and 14 marginal hooks. One pair of hamuli directing ventrally, with connecting bar and supplementary pieces, which are separated from or united with roots of hamuli. Marginal hooks of larval type, one pair central and six pairs peripheral. Cement gland well developed. Two pairs of eye spots present. Pharynx well developed. Postpharyngeal gland present. Intestinal limbs without diverticula, united posteriorly. Testis rounded or elongated, median, equatorial. Vas deferens looping around left intestinal limb. Vesicula seminalis formed by dilatation of vas deferens. Male copulatory complex consisting of a simple tube and accessory piece. Prostatic reservoir single, directly attached to copulatory tube. Genital pore postbifurcal. Ovary rounded or elongated, median, immediately pretesticular. Vagina opening dextrally, armed or unarmed. Receptaculum seminis present. Vitellarium coexistent with intestine. Parasites of freshwater and marine teleosts.

Type species: *Pseudodactylogyrus bini* (Kikuchi, 1929).

Key to species of *Pseudodactylogyrus* Gusev, 1965

1 Accessory piece of male copulatory complex rod-shaped, much shorter than copulatory tube 2
– Accessory piece of male copulatory complex hook-shaped terminally, almost same length as copulatory tube 6

2 Copulatory tube longer than 100 µm; 2 µm wide .... *P. kamegaii* Iwashita, Hirata et Ogawa, 2002
– Copulatory tube longer than 100 µm; 1 µm wide 3
– Copulatory tube shorter than 100 µm 5

3 Supplementary piece of hamulus with small projection near base of outer ridge ..... *P. rohdei* sp. n.
– Supplementary piece of hamulus without such projection 4

4 Hamulus stout, less than 80 µm long including supplementary piece ..... *P. bini* (Kikuchi, 1929)
– Hamulus slender, nearly 100 µm or longer including supplementary piece .... *P. anguillae* (Yin et Sproston, 1948)

5 Vagina poorly sclerotised, curved ..... *P. mundayi* sp. n.
– Vagina well sclerotised, thickened on both ends, almost straight ..... *P. gusevi* sp. n.

6 Accessory piece of male copulatory complex longer than 60 µm; transverse bar longer than 60 µm .... *P. bialatus* comb. n.
– Accessory piece of male copulatory complex shorter than 50 µm; transverse bar shorter than 50 µm .... *P. haze* Ogawa, 1984

Ling (1973) described *Pseudodactylogyrus pseudobagrus* from the gills of *Pseudobagrus fulvidraco* (= *Tachysurus fulvidraco*) in China. The parasite was characterised by a pair of hamuli, a pair of identical, long connecting bars, seven pairs of marginal hooks of larval type, the male copulatory complex consisting of a simple tube and an accessory piece with a succate base and bifurcate tip. This species is different from the members of *Pseudodactylogyrus* in the presence of two connecting bars and absence of a supplementary piece of the hamulus. Ling (1973) may have had no knowledge of *Pseudodactylogyrus*. His genus was later transferred to *Sinidactylogyrus* by Zhang (1981), but the systematic position of *S. pseudobagrus* (Ling, 1973) is not clear.

In the present phylogenetic tree, *P. gusevi* collected from *A. reinhardtii* in the Burnett River was divided into two groups (Clades A and B). Specimens collected from the other localities, namely Noosa, South Brisbane and Redland Bay, all belonged to Clade B. Although no morphological differences were observed among *P. gusevi*

specimens from the Burnett River, a possibility remains that *P. gusevi* is comprised of two cryptic species.

Morphologically, *P. gusevi* is most similar to *P. bini*, while molecularly, *P. gusevi* and *P. bini* were located in a distant position among *Pseudodactylogyrus* spp. of eels. The apparent morphological similarity between *P. gusevi* and *P. bini* is probably a result of convergence. Among the three new species of *Pseudodactylogyrus*, *P. gusevi* and *P. rohdei* are closely related morphologically; both have very similar anatomy (testis, ovary, cement gland, etc.), male copulatory complex and hamulus, which has a small projection on the outer ridge of its supplementary piece. This indicates that the two monogeneans may have evolved from a common ancestral species. When the present data and those of Gusev (1965) are combined, both *A. reinhardtii* and *A. australis* are hosts of *P. rohdei* in the Australian mainland. Phylogenetic analysis of the host eels indicates that *A. reinhardtii*, together with *A. japonica*, belongs to the Indo-Pacific group, whereas *A. australis* belongs to the Oceanian group (Minegishi et al. 2005). Although the geographical distribution of the two Australian eels overlaps, they are rather distant from each other phylogenetically. It is possible that *P. rohdei* has evolved either on the Indo-Pacific or on the Oceanian group host in the Australian mainland and later expanded its host range to the other group host.

The present phylogenetic tree suggests that among the eel pseudodactylogyrids, *P. gusevi* diverged first, followed by *P. mundayi*, which forms a sister clade with the three *Pseudodactylogyrus* spp. of *A. japonica*, although we could not obtain high statistical supports. The latter species of *Pseudodactylogyrus* may have evolved into three independent species as ancestral *A. japonica* dispersed to the North Pacific region. Similarly, *P. mundayi* and *P. gusevi* may have evolved as ancestral *A. australis* and *A. reinhardtii* dispersed to the Oceanic region, respectively.

The natural host of *P. bini* and *P. anguillae* is *A. japonica* and records of these pseudodactylogyrids from other *Anguilla* spp. like *A. anguilla* and *A. rostrata* were the results of international translocations of *A. japonica* to areas outside its natural distribution area. In contrast, the record of *P. bini* and *P. anguillae* from wild *A. marmorata* in Japan (Katahira and Nagasawa 2014) shows the two pseudodactylogyrids have two natural hosts, *A. japonica* and *A. marmorata*. Similarly, *A. reinhardtii* and *A. australis* are considered as natural hosts of *P. rohdei*. Whether or not *P. mundayi* infects *A. reinhardtii* in Tasmania remains to be clarified.

Members of *Pseudodactylogyrus* comprise those infecting anguillid eels and gobiid fishes. The present molecular data indicate that *Pseudodactylogyrus* spp. infecting *Anguilla* spp. (*P. bini*, *P. anguillae*, *P. kamegaii*, *P. gusevi* and *P. mundayi*) are monophyletic, suggesting their speciation occurred parallel to the host speciation. It is probable that a similar process of speciation has occurred in *P. haze* and *P. bilalatus* comb. n. on the gobiid hosts.

Species of *Pseudodactylogyroides* Ogawa, 1986 retain one pair of vestigial dorsal hamuli, implying that they are an ancestral group of the members of *Pseudodacylogyrus* – see Ogawa (1986). *Pseudodactylogyroides* spp. have been recorded on fishes of the families Apogonidae, Gobiidae and Eleotridae (the latter two belonging to the suborder Gobioidei) (Ogawa 1986, Lim 1995, Li 2004). It is hypothesised that ancestral species of *Pseudodactylogyrus* were parasites of unspecified marine fishes including gobioids and apogonids, and that they switched hosts to ancestral anguillids to have evolved to the present eel pseudodactylogyrids.

Three hypotheses can be proposed about the origin of the present *Pseudodactylogyrus* of eels. A host switch might have occurred twice independently: one among ancestral eels of the Oceanic group including *A. australis*, and the other among the Indo-Pacific group including *A. japonica* and *A. reinhardtii* (first hypothesis). Alternatively, the present species originated from a host switch that occurred before the separation of the Atlantic group (*A. anguilla* and *A. rostrata*) and Oceanic group from the Indo-Pacific group (second hypothesis), or from a host switch within the Indo-Pacific group after their separation from those of the Atlantic and Oceanic group (third hypothesis) (see Minegishi et al. 2005, Aoyama 2009 for information regarding separation of the anguillid groups).

There has been no record of natural *Pseudodactylogyrus* infections among the Atlantic group, except for infections caused by anthropogenic activities. Besides, Hine (1978) did not find even a single pseudodactylogyrid from 459 New Zealand longfin eel, *Anguilla dieffenbachii* Gray and 839 *A. australis* (both belonging to the Oceanic group) collected from different localities of New Zealand. This implies that no host switch had occurred within the Oceanic and Atlantic group. If this is the case, infection of *A. australis* with *P. rohdei* on the Australian mainland and *P. mundayi* in Tasmania, may have been the result of a host shift of these monogeneans from *A. reinhardtii* to *A. australis*, both eels showing overlapping distributions. Indeed, such shifts have occurred frequently, like that of *P. bini* and *P. anguillae* from *A. japonica* to *A. anguilla*, *A. rostrata* and *A. marmorata*. From these, the third hypothesis may seem most plausible. Based on this hypothesis, ancestral pseudodactylogyrids may have switched to ancestral Indo-Pacific eels, which later dispersed to the North Pacific and evolving to *A. japonica*, and to the tropical Pacific and evolving to *A. reinhardtii* (see Aoyama et al. 2001). *Anguilla reinhardtii* and *A. japonica* diverged at an early time within the Indo-Pacific group (Aoyama et al. 2001), and consequently, undiscovered *Pseudodactylogyrus* species might infect other eels of the Indo-Pacific group and be distributed through tropical regions, where no information about the parasite fauna of anguillid eels has yet been reported. Further studies of other eel species in different localities are required to better understand the co-evolution of anguillid eels and pseudodactylogyrids.

Acknowledgements. We thank David I. Gibson, Natural History Museum, London, Clive R. Kennedy, University of Exeter, UK and Pavel Gerasev, Zoological Institute, St. Petersburg, Russia for their efforts to search for the *Pseudodactylogyrus* specimens used in Kennedy (1998) and Gusev (1965). Thanks are also due to Tingbao Yang, Sun Yat-sen University, Guangzhou, China for sending us copies of Chinese references unavailable in Japan.

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Received 6 April 2015 Accepted 5 June 2015 Published online 28 August 2015

Cite this article as: Ogawa K., Iwashita M., Hayward C.J., Kurashima A. 2015: Three new species of *Pseudodactylogyrus* (Monogenea: Pseudodactylogyridae) from Australian eels. Folia Parasitol. 62: 046.