First record of *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) (Monogenea) from South Africa

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Received 8 May 2007. Accepted 30 June 2007

***Pseudodactylogyrus anguillae* is a monogenean parasite of anguillid eels that is thought to have Pacific origins. Recent identification of *Pseudodactylogyrus* in Europe and America has been attributed to human-mediated introductions. Here we report the first confirmed incidence of *P. anguillae* in South Africa and discuss whether it is a natural parasite of South African eels or an introduced exotic.**

Key words: ***Anguilla mossambica,* eel parasite, rRNA genes, ITS2, aquaculture.**

INTRODUCTION

The first recorded mention of *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) was provided by Kikuchi (1929), who described *Dactylogyrus bini* Kikuchi, 1929 from *Anguilla japonica* Temminck & Schlegel, 1847 in Japan. In this report Kikuchi also made reference to another similar monogenean that possessed larger hamuli, but provided no description for the latter specimen. Yin & Sproston (1948) recovered two similar monogenean species from *A. japonica* in China, and reassigned *D. bini* as *Neodactylogyrus bini* (Kikuchi, 1929), publishing information on the previously undescribed species as *Neodactylogyrus anguillae* Yin & Sproston, 1948. Based on studies of gill monogeneans from the eel *Anguilla reinhardti* Steindachner, 1867 caught in Australia, Gussev (1965) erected the genus *Pseudodactylogyrus* and transferred both *N. bini* and *N. anguillae* to this genus.

Anguilla japonica is generally regarded as the natural host for *P. anguillae* as it has a Pacific distribution that directly coincides with the generally accepted distribution range for *P. anguillae* (Buchmann *et al.* 1987, Hayward *et al.* 2001a). *Pseudodactylogyrus anguillae* infections of *Anguilla anguilla* have also been recorded from Japan, China and Taiwan (Kikuchi 1929; Yin & Sproston 1948; Ogawa & Egusa 1976; Chan & Wu 1984; Chung *et al.* 1984) as well as from localities throughout Europe (Buchmann 1997). Various records of *P. anguillae* infecting *A. japonica* also exist from localities outside its accepted natural distribution range within Europe. These infections are generally explained by the active introduction of *P. anguillae* via the movement of both eel species for culture purposes into Europe from the East and *vice versa*. Although some authors maintain that Europe cannot be excluded from the natural distribution range of *P. anguillae* (Nie & Kennedy 1991; Marcogliese & Cone 1993), it is generally accepted that this parasite is exotic to Europe and not a natural parasite of *A. anguillae.* Other records of this parasite from other localities and anguillid hosts suggest that although *P. anguillae* is specific to anguillid eels, it does not exhibit strict species specificity. Marcogliese & Cone (1993) reported *P. anguillae* from *Anguilla rostrata* (Le Sueur, 1817) in North America and proposed that as their findings were rare and fragmented, *P. anguillae* was possibly an indigenous species to this region. However, according to Buchmann (1997) and Hayward *et al.* (2001a), these occurrences can be explained by the accidental introduction to North America from either Europe or the Pacific.

A recent discovery of a *Pseudodactylogyrus* sp. from *Anguilla mossambica* (Peters, 1852) in South Africa represents the first record of a pseudodactylogyrid from South Africa and the second from the African continent. The first African record was that of El Naggar *et al.* (1993). Including the record of *P. anguillae* from *A. bicolor* McClelland, 1844 in Indonesia (Buchmann 1997), this discovery also represents the second record of this species in the southern hemisphere. The discovery of *P. anguillae* on *A. mossambica* in the absence of any data regarding introductions of this parasite or its potential hosts to South Africa raises questions regarding the origin and natural distribution of this parasite and whether this new distribution record is a result of a recent introduction or whether it in fact is a natural distribution of the parasite.

Pseudodactylogyrus anguillae is presumed to be

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specific to the genus *Anguilla*, a genus of freshwater eel comprising 15 species and three subspecies (Aoyama *et al.* 2001). Five species have been reported from South African waters, although one of these records (*A. obscura* Günther, 1871) is based on a single observation (Froese & Pauly 2003). Two of the species (*A. mossambica* and *A. bengalensis labiata* Peters, 1852) are found predominantly in the Indian Ocean from east Africa to Mauritius, while the other two (*A. marmorata* Quoy & Gaimard, 1824 and *A. bicolor bicolor* McClelland, 1844) have a wider distribution throughout the Indo-Pacific (Froese & Pauly 2003).

In this paper we use classical morphological comparisons and genetic data to determine the taxonomic affinities of the *Pseudodactylogyrus* species found on *A. mossambicus* and provide discussion as to its potential origin.

MATERIALS & METHODS

Sampling

Anguilla mossambica glass eels were collected for culture purposes from various water bodies between the Fish and Buffalo Rivers in the Eastern Cape, South Africa and later transported to an experimental culture facility in Stellenbosch, South Africa. Subsequent to the arrival of the eels at the culture facility, periodic mortalities were experienced and five moribund eels were euthanased and examined for parasites. These samples all revealed excessively high numbers of gill flukes later identified as *P. anguillae.* Parasites were fixed *in situ* on the gills in 96% ethanol and were subsequently removed for both molecular and morphological analysis.

Morphology

Individual parasites were removed from the gill tissue and mounted in ammonium picrate-glycerine and were used for observation, drawing and measurement of sclerotized structures. Haptoral sclerites were drawn using a LEICA DM LS compound microscope fitted with a drawing tube and measured using LIDA image analysis software for LEICA. The point-to-point morphometric measurements on the haptoral sclerites were made from images grabbed using a JVC TK 1280E colour digital video camera mounted on the compound microscope. The measurements were done according to Iwashita *et al.* (2002) and are expressed in micrometres and given in Table 1 as the mean ± standard deviation followed by the range in brackets. Only the measurements of the sclerotized structures of the current population were compared to that of other populations of *P. anguillae* as reported by other authors (Table 1), as non- sclerotized structures have been shown to distort due to preparation method and coverslip pressure (Buchmann *et al.* 1987).

DNA extraction, PCR and sequence analysis

An individual *Pseudodactylogyrus* specimen was placed in 300 μl STE with SDS 2% and Protease K (0.3 mg/ml) and incubated at 55°C for 16 hours. Genomic DNA was extracted using standard phenol-chloroform and ethanol precipitation methods (Sambrook *et al.* 2000). The ITS regions, the 5.8S and part of the 18S rRNA gene were amplified with 0.5 μM rRNA ITS primers PD-ITS-450F and PD-ITS-R (Hayward *et al.* 2001a). Amplification was catalysed with Promega Taq polymerase and 3 mM MgCl2. The amplification conditions were as follows: 94°C, 5 minutes; (94°C, 1 min, 50°C, 45 sec; 72°C, 1 min) for 30 cycles; 72°C, 10 min. Amplified products were purified using GFX® PCR Purification Kit and sequenced with the amplification primers. Sequences were blasted against the database (http://www.ncbi.nlm.nih.gov/blast) and aligned to sequences from the NCBI database using Bioedit. Partial ITS2 sequences were compared by eye to published sequences of *P. anguillae* and *P. bini* (Hayward *et al.* 2001a).

RESULTS

Pseudodactylogyrus anguillae (Yin & Sproston 1984)

Host. Anguilla mossambica wild caught as glass eels from Eastern Cape, South Africa and kept in experimental eel farm ponds, Stellenbosch, South Africa.

Site of infection. Gills

Material examined. Detailed morphometric measurements and drawings from 10 specimens mounted in ammonium picrate-glycerine (Table 1, Fig. 1).

Remarks. Pseudodactylogyrus anguillae is reported from South Africa for the first time. General morphology and measurements of the respective sclerites (Fig. 1) of the South African population did not differ significantly from those published for populations of this species in Asia and the United States confirming their identification as *P. anguillae* (Table 1).

DNA sequence. An alignment of 340 bp of ITS1

Table 1. Morphological measurements (in micrometres) of *Pseudodactylogyrus anguillae* from *Anguilla mossambica* from South Africa compared to other populations.

Measurement	*P. anguillae* Mean ± S.D. (range) (*n* = 10)	*P. anguillae* Iwashita *et al.* (2002)	*P. anguillae*[a] Ogawa & Egusa (1976)	*P. anguillae* Hayward *et al.* (2001a)
Penis				
Length	130.4 ± 5.9 (126–135)	130 (110–160)	–	–
Width	1.4 ± 0.2 (1–2)	1 (1)	–	–
Accessory piece				
Length	39.6 ± 0.6 (39–40)	35 (31–40)	28–42	–
Vagina				
Length	20.9 ± 2.4 (19–23)	24 (19–27)	–	–
Hamulus				
Total length	113.5 ± 3.4 (110–119)	99 (94–105)	100–125	99–116
Length without foldable part	95.9 ± 3.4 (92–100)	87 (81–91)	86–105	88–100
Shaft length	77.3 ± 3.7 (73–81)	73 (69–77)	60–84	71–81
Outer root	9.9 ± 1.6 (8–12)	6 (5–9)	7–14	6–10
Length of foldable part of inner root	47.1 ± 3.8 (42–51)	35 (30–41)	35–49	38–42
Inner root	69.1 ± 3.4 (65–74)	51 (43–58)	54–77	55–65
Tip length	30.4 ± 2.5 (27–33)	29 (26–31)	28–34	31–35
Width of hamulus	72.9 ± 2.6 (70–76)	68 (64–72)	–	–
Hamulus ratio	1.30 ± 0.02 (1.28–1.33)	1.29 (1.25–1.32)	–	–
Dorsal bar				
Length	55.6 ± 4.3 (51–60)	50 (44–53)	40–64	–
Width	12.3 ± 2.6 (9–15)	9 (8–12)	–	–
Marginal hooklet				
Length	16.5 ± 0.6 (16–17)	16 (15–17)	14–17	–

[a]Combined data of *P. anguillae* and *P. microrchis* according to Iwashita *et al.* 2002.

from our sample and two *Pseudodactylogyrus* sequences from the database demonstrated that our sequence was 100% identical to the *P. anguillae* sequence (AJ490163), but differed from *P. bini* sequence (AJ490163) by 10 bp. A 370 bp fragment of the ITS2 region of our sequence was identical to *P. anguillae* sequences YA11, USPA2 and USPA3, but differed from *P. anguillae* USPA1/USPA4 by

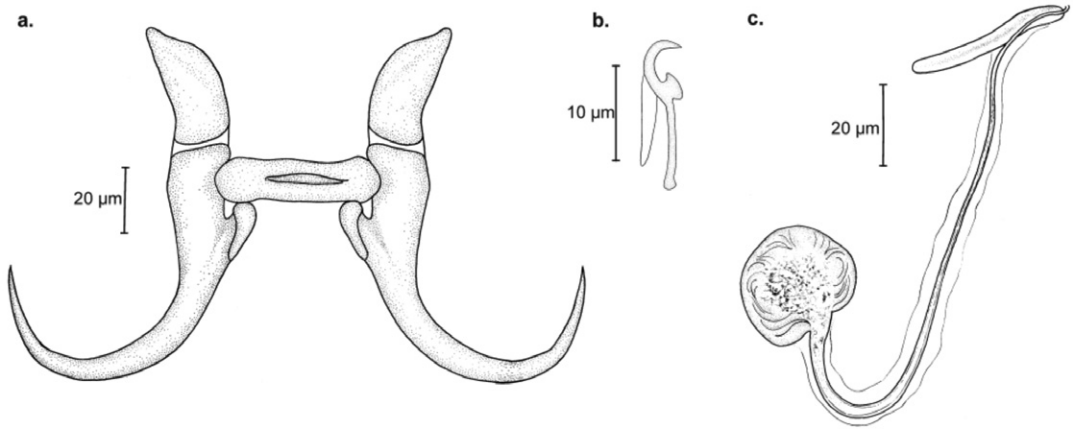

Fig. 1. Sclerotized structures of *Pseudodactylogyrus anquillae* from *Anguilla mossambica* from South Africa. **a**, Hamuli and dorsal bar; **b**, marginal hooklet; **c**, male copulatory organ (cirrus and accessory piece).

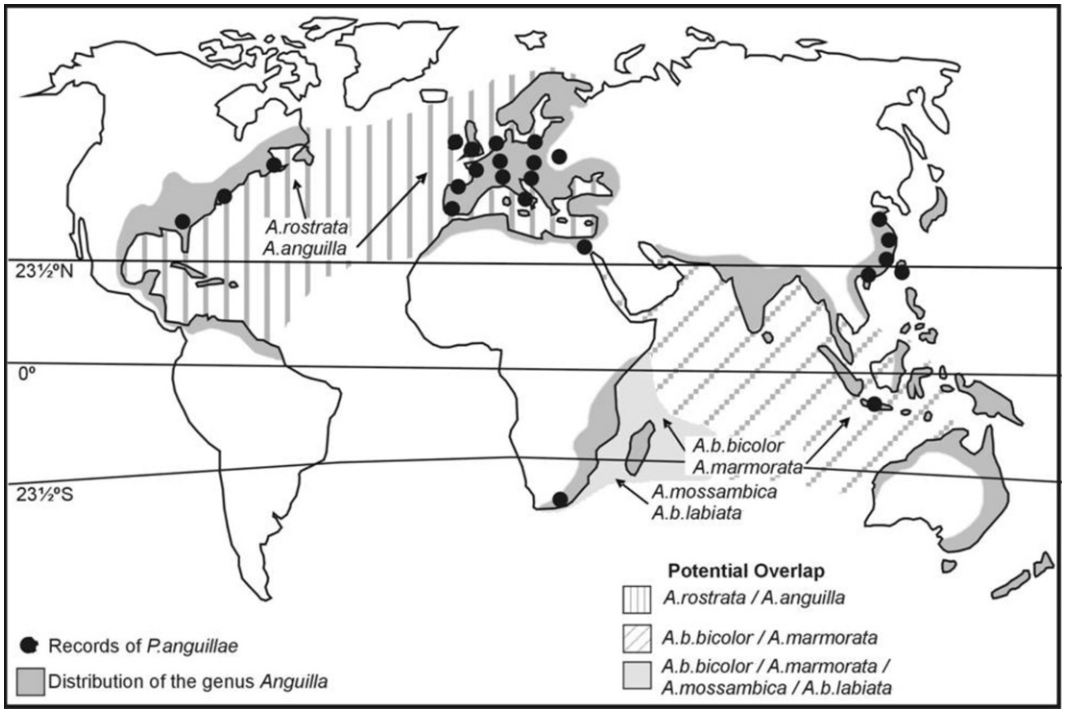

Fig. 2. Map indicating the location of all confirmed *Pseudodactylogyrus anguillae* records and the potential overlap of the *Anguilla* spp. for which spawning site data are known.

one base pair and differed from *P. bini* sequences by 5–10 bases (Hayward *et al.* 2001a). A 870 bp fragment of the ITS, 5.8S and 18S rRNA genes of the South African *Pseudodactylogyrus anguillae* was submitted to Genbank (Acc. No: AY844961).

Distribution. Figure 2 is a map indicating the location of all confirmed *P. anguillae* records and the potential overlap of the *Anguilla* spp. for which spawning site data are known.

DISCUSSION

Both classical and genetic evidence demonstrate that the parasite found on *A. mossambica* is *Pseudodactylogyrus anguillae* and is closely related, if not identical, to *P. anguillae* found elsewhere in the world. This is therefore the second confirmed record of *P. anguillae* in the Southern Hemisphere. Buchmann (1997) recorded the only other report of *P. anguillae* from *Anguilla bicolor* in Indonesia and although Gussev (1965) reported this species from Australia, Hayward *et al.* (2001a) questioned the validity of this diagnosis and as such the Australian population remains in question.

New records of *P. anguillae* elsewhere in the world have been attributed to recent introductions via the eel trade and possibly by transport in ballast waters (Hayward *et al.* 2001a; Buchmann 1997). Although recent introduction of *P. anguillae* to fresh or marine ecosystems may account for its presence in the Eastern Cape, it is possible that South Africa falls within the natural distribution range of this species. Here we examine the evidence supporting each of these scenarios.

Recent introduction

Freshwater

The easiest way for *P. anguillae* to move between continents would be for their adult hosts to be introduced from one continent to another into the freshwater system, by human intervention. It is generally accepted that *P. anguillae* was introduced into new habitats by transporting infected fish between different ports of the world for aquaculture purposes (Buchmann 1997); however, there is no documented evidence of exotic eels being introduced to South Africa via aquaculture or the ornamental fish trade, but unregulated introductions may have occurred. Anguillid eels are often difficult to distinguish and exotic species may have gone undetected in South African river systems. If exotic eels were introduced to South Africa carrying *P. anguillae,* it is expected that they

would be host to *P. bini* as well. There have been no records of *P. bini* in South Africa. Neither species has been reported from wild eels and hence no comment can be made regarding the presence or absence of these species from any South African river systems. Secondly, on the North American continent *P. anguillae* was discovered nine years before *P. bini,* firm conclusions cannot be drawn from their apparent absence.

Marine

A number of foreign introductions have been attributed to the movement of ballast water. Cone *et al.* (1994) reported on the introduction of *Dactylogyrus amphibothrium* to North America by the discharge of infected European ruffe (*Gymnocephalus cernuus*) in ship ballast waters. The possibility of the mediated transport of *Gyrodactylus anguillae* from Asia to Australia has also been acknowledged by Haward *et. al.* 2001b. Buchmann (1997) suggested a similar explanation could be offered for the presence of at least *Pseudodactylogyrus anguillae* in North America, as infected elvers could easily be transported in ship ballast from Europe or Japan. Support is added to this transmission hypothesis by the report of *Anguilla anguilla* present in ship ballast (Gollasch 2002) and consequently infected exotic anguillids may have been transported to South Africa via this route. There is a single record of *Anguilla obscura* from a tributary of the Buffalo River, near King William's Town in South Africa (Froese & Pauly 2003). This species typically has a Pacific distribution and it is not known whether this record is the result of a natural invasion or one mediated by ballast water or other means. If foreign eels were introduced to South African waters, but did not survive, there is still a strong possibility that their parasites could be transmitted to local anguillid species.

Natural distribution

Although *P. anguillae* has only just been discovered in South Africa, its presence here may be ancient and may have been previously overlooked. The lack of variation of morphology and ITS DNA sequences between individuals collected in South Africa, America and Japan can be used as evidence to support both recent translocation and natural distribution, provided that there are no barriers to gene flow within a circumglobal population of *P. anguillae.* The phylogeography of the genus *Anguilla* is highly debated. Lin *et al.* (2001) proposed that *Anguilla* first radiated about 20 myr ago and moved from the Indo-Pacific through the Central American Isthmus, a route that closed 5 myr ago. Other data (Bastrop *et al.* 2000; Aoyama *et al.* 2001) suggest an earlier radiation of representatives of the genus *Anguilla* via the Tethys Seaway. All data support a separation of Atlantic species from Pacific species at least 10 myr ago. If the host eels and their pseudodactylogyrid parasites were constrained by same genetic barriers we would expect to see sequence divergence in *Pseudodactylogyrus anguillae* parasitizing different anguillid species. The observed rate of divergence of the ITS2 gene in monogeneans is calculated to be 0.3–0.7% per million years (Zietara *et al.* 2002). If genetic isolation of their hosts occurred even as recently as 10 myr ago we would expect 3–7% sequence divergence between the ITS2 genes of *P. anguillae* parasitizing *A. mossambica, A. japonica* and *A. rostrata.* This is not the case, as ITS2 genes of *P. anguillae* from these three geographically distinct eels are identical. This suggests that *P. anguillae* is either not constrained by the same genetic barriers as its host, or that its global distribution is due to recent introductions.

Although *P. anguillae* was first described from *A. japonica,* it is not a specific parasite of this species. Natural populations of *Anguilla bicolor* from Indonesia in 1993 have also been reported to host *P. anguillae* (Buchmann 1997). This eel species is widespread in the western Pacific and tropical Indian Ocean as far west as South Africa (Skelton 1993). *Anguilla bicolor bicolor* from South Africa and *A. bicolor* from Indonesia both use the Indian Ocean basin currents to breed and to distribute their leptocephali, hence providing an overlap in their distribution allowing potential opportunities for exchange of parasites.

Figure 2 shows the overlap of breeding areas between members of the genus *Anguilla*. The specific breeding locations of all *Anguilla* species are not known, but genetically distinct species such as *A. rostrata* and *A. anguilla* are known to have sympatric distributions of leptocephali in the Sargasso Sea (Bastrop *et al.* 2000; Aoyama *et al.* 2003) and as many as five species of *Anguilla* leptocephali have been found off the coast of Sulawesi in Indonesia. If *P. anguillae* can infect leptocephali then these overlapping breeding areas provide a natural vehicle for movement of the parasite from one species of *Anguilla* to another. It has been demonstrated that leptocephali and glass eels occur sympatrically (Aoyama *et al.* 2003) and that glass eels can become infected with

P. anguillae (Buchmann 1997), suggesting that adults from one population may infect juveniles from another population with *P. anguillae* in the marine environment. *Pseudodactylogyrus anguillae* are known to be halotolerant and both adults and eggs may survive in seawater (Buchmann *et al.* 1987; Buchmann *et al.* 1992). Although *P. anguillae* is halotolerant, it is essentially a freshwater parasite. In addition, the short survivability of the eggs in seawater, 2–5 days at 20°C–30°C (Buchmann *et al.* 1987), would suggest that this proposed transmission of this parasite at sea would have to be extremely rapid. However, the gradual coastal migration of this parasite alongshore and its transmission between sympatrically occurring *Anguilla* species may be a more plausible transmission hypothesis as eels move from one estuary to the next. A similar hypothesis was proposed for *Gyrodactylus anguillae* (Hayward *et al.* 2001b). Consequently, as with *G. anguillae* (Hayward *et al.* 2001b), *P. anguillae* probably occurs throughout most of the range of its anguillid hosts. According to this hypothesis, *P. anguillae* may naturally infect eels throughout the West Pacific and Indian Ocean. The larval stages of *A. mossambica* and *A. bengalensis labiata* are unlikely to be sympatric with Pacific species, such as *A. japonica*, but may become infected by *P. anguillae* carried from Pacific breeding grounds by widely distributed species such as *A. marmorata* and *A. bicolor*. However, no records of *P. anguillae* have been recorded from *A. marmorata* and *A. bicolor* in South Africa. This hypothesis does not hold for the Atlantic species, as these species have been genetically and geographically isolated for at least 5 myr. Natural movement of *P. anguillae* between *A. anguilla* and *A. rostrata* is possible, but a human mediated translocation of *P. anguillae* from the Indian Ocean or western Pacific must have occurred in recent times.

The paucity of data regarding the distribution, intensity and prevalence of *P. anguillae* infections in wild *A. mossambica* and other anguillid populations in South African combined with the general lack of other parasite data renders it difficult to make firm conclusions as to the origins and status of *P. anguillae* locally. Using similar hypotheses as discussed here, Hayward *et al.* (2001a,b) concluded that the maintenance of genetic and morphological integrity of *Pseudodactylogyrus bini* and *Gyrodactylus anguillae* respectively on genetically distinct *Anguilla* species from three continents due to natural gene flow is unsound. This is likely to be the case for *P. anguillae* in South Africa; however, confirmation of the presence or absence of other anguillid parasites like *Pseudodactylogyrus bini*, *Gyrodactylus anguillae* and *Anguilicola crassus* from *A. mosssambica* and other South African anguillids would provide more certainty.

ACKNOWLEDGEMENTS

K.W.C. and G.C.B. were supported by the NRF/Royal Society Initiative. The authors would like to thank G. Vosloo (SA EELS) and R. Kneusel (Aquaculture Division, University of Stellenbosch) for providing the specimens.

REFERENCES

AOYAMA, J., NISHIDA, M. & TSUKAMOTO, K. 2001. Molecular phylogeny and evolution of the freshwater eel, genus *Anguilla*. *Molecular Phylogenetics and Evolution* **20**(3): 450–459.

AOYAMA, J., WOUTHUYZEN, S., MILLER, M.J., INAGAKI, T. & TSUKAMOTO, K. 2003 Short-distance spawning migration of tropical freshwater eels. *The Biological Bulletin* **204**: 104–108.

BASTROP, R., STREHLOW, B., JÜRSS, K. & STURMBAUER, C. 2000. A new phylogenetic hypothesis for the evolution of freshwater eels. *Molecular Phylogenetics and Evolution* **14**(2): 250–258.

BUCHMANN, K. 1997. Infection biology of gill parasitic monogeneans with special reference to the congeners *Pseudodactylogyrus bini* and *P. anguillae* (Monogenea: Platyhelminthes) from European eel. Ph.D. dissertation, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

BUCHMANN, K., FELSING, A. & SLOTVED, H.C. 1992. Effects of metrifonate, sodium chloride and bithional on an European population of the gill parasitic monogeneans *Pseudodactylogyrus* spp. and the host *Anguilla anguilla*. *Bulletin of the European Association of Fish Pathologists* **12**(2): 57–60.

BUCHMANN, K., MELLERGAARD, S. & KØIE, M. 1987. *Pseudodactylogyrus* infections in eel: a review. *Diseases of Aquatic Organisms* **3**: 51–57.

CHAN, B. & WU, B. 1984. Studies on the pathogenicity, biology and treatment of *Pseudodactylogyrus* in fish farms. *Acta Zoologica Sinensia* **30**: 173–180.

CHUNG, H., LIN, I. & KOU, G. 1984. Study of the parasites on the gills of cultured eel in Taiwan. *COA Fisheries Series*, No. 10. *Fish Disease Research* **6**: 24–33.

CONE, D.K., EURELL, T. & BEASLEY, V. 1994. A report of *Dactylogyrus amphibothrium* (Monogenea) on the gills of European ruffe in western Lake Superior. *Journal of Parasitology* **80**: 476–478.

EL NAGGAR, M.M., REDA, E.S. & ARAFA, S.Z. 1993. Observations on the anatomy and surface sensilla *Pseudodactylogyrus anguillae* (Yin and Sproston, 1948) Gusev, 1965, a monogenean gill parasite of *Anguilla anguilla* in Egypt. *Journal of the Egyptian-Germanic Society of Zoolology* **11**: 147–175.

FROESE, R. & PAULY, D. (eds). 2003. FishBase. http://www.fishbase.org [accessed 13 August 2003].

GOLLASCH, S. 2002. The importance of ship hull fouling as a vector of species introductions into the North Sea. *Biofouling* **18**(2): 105–121.

GUSSEV, A.V. 1965. A new genus of monogenetic trematodes from the eel, genus *Anguilla*. *Trudy Zoologicheskogo Instituta Leningrad* **35**: 119–125.

HAYWARD, C.J., IWASHITA, M., CRANE, J.S. & OGAWA, K. 2001a. First report of the invasive eel pest *Pseudodactylogyrus bini* in northern America and in wild American eels. *Diseases of Aquatic Organisms* **44**: 53–60.

HAYWARD, C.J., IWASHITA, M., OGAWA, K. & ERNST, I. 2001b. Global spread of the eel parasite *Gyrodactylus anguillae* (Monogenea). *Biological Invasions* **3**: 417–424.

IWASHITA, M., HIRATA, J. & OGAWA, K. 2002. *Pseudodactylogyrus kamegaii* sp. n. (Monogenea: Pseudodactylogyridae) from wild Japanese eel, *Anguilla japonica*. *Parasitology International* **51**: 337–342.

KIKUCHI, H. 1929. Two new species of Japanese trematodes belonging to Gyrodactylidae. *Annotations Zoologicae Japonensis* **12**: 175–186.

LIN, Y., POH, Y. & TZENG, C. 2001. A phylogeny of freshwater eels inferred from mitochondrial genes. *Molecular Phylogenetics and Evolution* **20**(2): 252–261.

MARCOGLIESE, D.J. & CONE, D.K. 1993. What metazoan parasites tell us about the evolution of American and European eels. *Evolution* **47**: 1632–1635.

NIE, P. & KENNEDY, C.R. 1991. Occurrence and seasonal dynamics of *Pseudodactylogyrus anguillae* (Yin & Sproston) (Monogenea) in eel, *Anguilla anguilla* (L.) in England. *Journal of Fish Biology* **39**: 897–900.

OGAWA, K. & EGUSA, S. 1976. Studies on eel pseudodactylogyrosis – I. Morphology and classification of three eel dactylogyrids with a proposal of a new species, *Pseudodactylogyrus microrchis*. *Bulletin of the Japanese Society of Scientific Fisheries* **42** (4): 395–404.

SAMBROOK, J., MACCALLUM, P. & RUSSEL, D. 2000. *Molecular Cloning: A Laboratory Manual* (3rd edn). Cold Spring Harbor Laboratory Press, U.S.A.

SKELTON, P.H. 1993. *A Complete Guide to the Freshwater Fishes of Southern Africa*. Southern Book Publishers, Halfway House, South Africa.

YIN, W. & SPROSTON, N.G. 1948. Studies on the monogenetic trematodes of China, Parts 1–5. *Sinensia* **19**: 57–85.

ZIETARA, M.S., HUYSE, T., LUMME, J. & VOLCKAERT, F.A. 2002. Deep divergence among subgenera of *Gyrodactylus* inferred from rDNA ITS region. *Parasitology* **124**: 39–52.

Responsible Editor: K. Prochazka