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Gyrodactylus pictae n. sp. (Monogenea: Gyrodactylidae) from the Trinidadian swamp guppy *Poecilia picta* Regan, with a discussion on species of *Gyrodactylus* von Nordmann, 1832 and their poeciliid hosts

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Abstract

Gyrodactylus pictae n. sp. is recorded from *Poecilia picta* in heterospecific shoals with the guppy *P. reticulata* in Northern Trinidad. *G. pictae* is morphologically similar to *G. turnbulli* Harris, 1986, but the hamuli and marginal hooks are slightly smaller and more gracile. The toe and the point of the marginal hook have a distinctly different shape, providing the best morphological characters for distinguishing the two species. The rDNA ITS1 and ITS2 sequences differ from those of *G. turnbulli* (the closest relative) by >5%, suggesting that these two taxa are not sibling species. The origin of the two species on poeciliids of the subgenus *Micropoecilia* is discussed, and it is suggested that this may represent a case of host–parasite co-evolution.

Introduction

A range of gyrodactylid monogeneans have been collected from poeciliid fishes from tropical Central America. The best known are *G. bullatarudis* Turnbull, 1956 and *G. turnbulli* Harris, 1986 from the guppy *Poecilia reticulata* Peters, but other species have been recorded from *Xiphophorus* spp., *P. sphenops* Valenciennes, *P. caucana* (Steindachner) and *Gambusia affinis* (Baird & Girard) (see Rogers & Wellborn, 1965; Kritsky & Fritts, 1970; Lucky, 1973; Harris, 1986; Harris & Cable, 2000; Richards et al., 2000). These studies indicate that poeciliid fishes have a complex gyrodactylid fauna dominated by *G. bullatarudis*-like species, including *G. bullatarudis*, *G. gambusiae* Rogers & Wellborn, 1965, *G. poeciliae* Harris & Cable, 2002, *G. rasini* Lucky, 1973 and *G. costaricensis* Kritsky & Fritts, 1970, and by *G. turnbulli*-like species, including *G. turnbulli* and possibly *G. milleri* Harris & Cable, 2000. In the current work, samples of gyrodactylids were collected from the swamp guppy *Poecilia picta* Reagan. This poeciliid occurs in sympatry with *P. reticulata* in some rivers in Trinidad and Tobago, forming mixed-species shoals. Specimens of *G. pictae* were first recognised as different because of their reluctance to infect *P. reticulata*. Further studies revealed morphological and molecular differences. On the basis of this material, *G. pictae* n. sp. is described.

Materials and methods

Shoals of poeciliids were collected using a siene net from streams in the Northern Mountain Range of Trinidad during June and July, 2003. Shoals were sorted according to species, either *P. picta* or *P. reticulata,* and fish of the two species were either individually fixed in 95% ethanol or examined for gyrodactylids using a stereo-microscope. Specimens of *Gyrodactylus* von Nordmann, 1832 found on *P. picta* were removed individually and transferred to fresh 95% ethanol for storage

and return to the UK. Samples were also collected of *G. turnbulli* (from the Aripo River) and *G. bullatarudis* (lowland site of the Lopinot (Arouca) River) from *P. reticulata* in the Northern Mountain Range of Trinidad.

Morphological analysis

Parasites removed from ethanol-fixed hosts were mounted in 10% sodium dodecyl sulphate (SDS) to allow flattening of hooks and measured at ×1,000 magnification using an Olympus OM-10 microscope. Measurements of the hamuli, marginal hooks and dorsal and ventral bars were as described by Harris (1986). The holotype of *G. pictae* was washed free of SDS with distilled water, stained with Borax Carmine, dehydrated in ethanol and mounted in Canada Balsam for museum deposition. Paratypes for deposition were prepared by allowing distilled water to seep under the coverslip to remove SDS. A drop of ammonium picrate–glycerine (Malmberg, 1970) was allowed to seep under the coverslip. The slide was then sealed with several layers of nail varnish. Three specimens of *G. pictae* treated with Proteinase K were prepared for Scanning Electron Microscopy (SEM) and DNA extracted as described by Harris et al. (1999).

Molecular analysis

Polymerase Chain Reaction (PCR) amplification of ribosomal DNA was undertaken using gyrodactylid specific primers (P3b 5′ TAGGTGAACCTGCAGAAGGATCA 3′; R1 5′ ACTCCATG TGGTGGATC 3′; F3 5′TTGCTGCACTCTTCATC 3′; and P4 5′ GTCCGGATCCTCCGCTTATTGATATTGC 3′) spanning the internal transcribed spacers (ITS) and the 5.8S RNA (Cable et al., 1999). PCR products were purified using a GeneClean III kit (Anachem) and sequenced using an ABI PRISM TM Dye Terminator Cycle Sequencing Ready Reaction kit following the manufacturer's guidelines, and run on an Applied Biosystems 3100 automatic sequencer. Four individuals of *G. pictae* (the three specimens morphologically identified by SEM, plus one other specimen in which the haptor was examined by light microscopy) were sequenced at least twice with each primer combination. Sequences were aligned using Sequencher V3.1.2 (Gene Codes Corporation Inc.) and CLUSTALX 1.81 (Thompson et al., 1997). Positions of the 5.8S and 28S genes were determined with BLAST searches and subsequent alignment with other gyrodactylid sequences deposited within GenBank. In view of the increasingly large sequencing database available for gyrodactylid monogeneans, positions of the 18S, 5.8S and 28S genes were reviewed for other poeciliid gyrodactylids. In the light of anomalies in the original *G. bullatarudis* ITS sequence AJ011410 (Cable et al., 1999), DNA from three new specimens of this species collected (01/07/03) from *P. reticulata* from the Lower Lopinot River (E681850.0, N1174487.5) was sequenced and deposited in GenBank.

Consensus sequence of *G. pictae* rDNA was aligned with that of *G. turnbulli*, *G. bullatarudis*, *G. poecilae* and *G. arcuatus* Bychowsky, 1933 (outgroup) using CLUSTALX 1.81 (Thompson et al., 1997). PHYLIP (Felsenstein, 1993) was used to create phylogenetic trees by parsimony (DNAPARS) and maximum likelihood (DNAML). Consensus trees generated from 1,000 bootstrapped replicates were drawn using TREEVIEW (Page, 1996).

***Gyrodactylus pictae* n. sp.**

Type-host and site of infection: *Poecilia picta* Regan, skin and fins. Sample host specimens are deposited in The Natural History Museum, London.
Type-locality: Lower Marianne River, Northern drainage of the Northern Mountain Range, Trinidad (E685837.5, N1193375.0), collected 04/vii/03.
Type-material: Holotype 2004.7.28.1 and paratypes 2004.7.28.2-5 are deposited in The Natural History Museum, London.
Etymology: Named after the host, *Poecilia picta*.

Description (Figure 1)

Medium-sized gyrodactylid; body length up to 0.7 mm. Body, anterior to haptor, fusiform, with two anterior processes bearing spike sensilla. Pharynx with long processes. Cirrus, when present, spherical, armed with single large hook and single row of small spines. Hamuli of holotype 53 (range of paratypes, 49–55) μm in total length, very slender, with maximum diameter of shafts <5 μm. Hamulus shafts slightly curved, 38.5 (36–41) μm

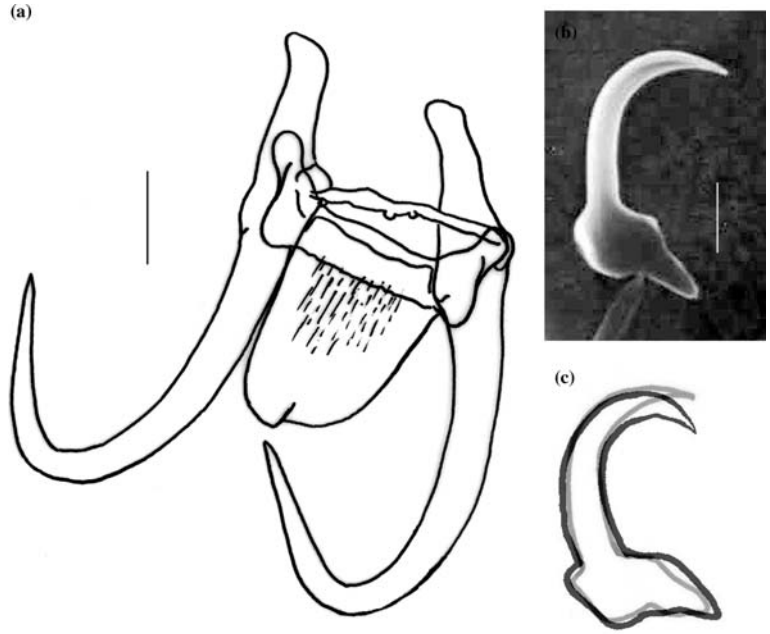

Figure 1. Hooks and bars of *Gyrodactylus pictae* n. sp. (a) Hamuli and bars, composite image based on light and scanning electron microscopy. (b) Scanning electron micrograph of marginal hook sickle. (c) Superimposition of *G. pictae* marginal hook (grey outline) on *G. turnbulli* Harris, 1986 marginal hook (black outline). Difference between the species can be resolved to the point and toe of the hook sickle. *Scale-bars*: (a) 10 μm; (b) 2 μm.

long. Hamulus roots 16 (12–17) μm long, giving shaft/root ratio of 3. Ventral bar similar to that of *G. turnbulli*, with broad, blunt membrane frequently divided posteriorly to depth of up to 5 μm (Figure 1a). Membrane and posterior edge of bar proper bear deep longitudinal striations visible with light microscope, but these are not conspicuous with SEM. Ventral bar has two distinctive large processes, which are *ca.* 6.5 μm long and angled anterolaterally; these processes appear relatively easily distorted even following light microscope preparation and account for large variance in ventral bar width between these processes (Table 1). Dorsal bar curved, of uniform diameter throughout and lacks notch proper, although it bears two lugs on either side of mid-point (Figure 1a). Marginal hooks 28.5 (26–29) μm long, with shafts 21.5 (19–22) μm long and sickles 6 (6–7) μm long. Prominent filaments extend from base of shafts, back into body of haptor. Point open, not curved to toe. Toe of marginal hook sickle bears distinct ledge (Figure 1b, c) and tapers to narrow point which lies below point of insertion of marginal hook shaft.

Comments

Gyrodactylus pictae n. sp. is similar to *G. turnbulli*. We are currently reviewing morphometric variation in *G. turnbulli* (unpublished), but there is overlap between smaller individuals of *G. turnbulli* and larger *G. pictae*. *G. pictae* is however more gracile overall, with relatively slender hamulus shafts (5 μm in diameter as opposed to 8 μm in *G. turnbulli*) and roots. This impression is heightened by the proportionally longer hamulus roots of *G. pictae* (shaft/root ratio 3, as opposed to 2.4 in *G. turnbulli*). The most characteristic difference between the species is in marginal hook sickle shape. The sickle blade of the two species is almost identical, but the toe of the marginal hook sickle of *G. pictae* is longer and angled away from the tip of the sickle (Figure 1). The toe of *G. pictae* also has a more definite shelf on its upper surface. The other species with a close resemblance to *G. turnbulli* collected from poeciliid hosts is *G. milleri* from Venezuela (see Harris & Cable, 2000). However, although the hamuli and bars of *G. milleri* are similar, the marginal hooks are very distinct, with

Table 1. Comparison of attachment hook dimensions (micrometres) of *Gyrodactylus pictae* n. sp. with *G. turnbulli* Harris, 1986 (mean ± variance).

Species	*G. pictae* n. sp.	*G. turnbulli*
Host	*Poecilia picta*	*Poecilia reticulata*
Source	Marianne river, Trinidad, July 2003	Aripo River, Trinidad, July 2003
n	21	23
Hamulus		
Total length	52.1 ± 3.2	58.3 ± 1.9
Shaft length	38.6 ± 1.1	41.6 ± 1.9
Root length	15.6 ± 1.4	17.4 ± 0.6
Point length	21.3 ± 1.0	25.0 ± 1.6
Marginal Hook		
Total length	27.6 ± 1.3	33.1 ± 1.7
Shaft length	21.0 ± 1.1	25.4 ± 1.1
Sickle length	6.5 ± 0.2	7.6 ± 0.1
Ventral Bar		
Total length	29.6 ± 3.0	28.3 ± 3.8
Total width	24.3 ± 1.3	28.8 ± 4.7
Width between processes	29.1 ± 6.9	32.0 ± 2.5
Bar length	5.4 ± 0.4	
Membrane length	16.9 ± 1.3	15.4 ± 1.3
Process length	6.7 ± 0.5	5.7 ± 0.5
Dorsal Bar		
Total length	20.7 ± 3.0	

two sharply angled bends in the blade, and therefore this species is unlikely to be confused with either *G. turnbulli* or *G. pictae*. All other species of *Gyrodactylus* from poeciliid fishes resemble more closely *G. bullatarudis* having ventral bars with distinct, bulbous, forward-pointing processes and ventral bar membrane that lack striae. The marginal hooks also have a completely different shape, with a more attenuated point. These characters make confusion with *G. pictae* unlikely.

Molecular analysis

A fragment of 999 bp was amplified and sequenced, and found to include the ITS-1 (464 bp), 5.8S rDNA gene (157 bp) and ITS-2 (378 bp). The sequence, deposited with GenBank (accession number AY692023), differs from *G. turnbulli*, the most similar sequence currently within the database, by 5.5%. The similarity to *G. bullatarudis* was, however, much weaker, with a difference of 27.5% between *G. pictae* and wild-type *G. bullatarudis* (accession number AY692024).

Both parsimony and maximum likelihood methods grouped *G. pictae* with *G. turnbulli* with a high degree of confidence (Figure 2), whereas *G. bullatarudis* was genetically more similar to *G. poeciliae*.

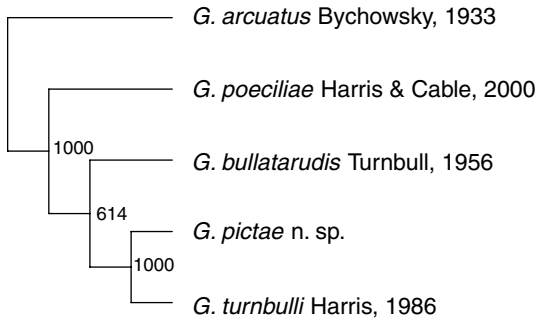

Figure 2. Parsimony analysis of rDNA data for *Gyrodactylus pictae* n. sp. and related species from poeciliids (*G. arcuatus* included as outgroup because of morphological resemblance to *G. bullatarudis*/*G. poeciliae*). Based on consensus of 1,000 bootstrapped trees. With Maximum Likelihood analysis, *G. poeciliae* and *G. bullatarudis* (with 94.5% bootstrap support), as well as *G. pictae* and *G. turnbulli* (100%), formed a distinct clade.

Discussion

G. pictae n. sp. represents the third *G. turnbulli*-like species to be described from poeciliid fishes. It is more similar to *G. turnbulli* than *G. milleri*. It can be distinguished from the latter by its overall smaller size, by the distinctive shape of the marginal hook sickles and by marked differences in host preference (Cable et al., unpublished). Although the host of *G. pictae, Poecilia picta,* has a preference for more brackish water than *P. reticulata*, mixed-species host shoals are observed in the lower regions of the Marianne River in Trinidad and the Kendall River in Tobago. This indicates that, at least occasionally, *G. pictae* and *G. turnbulli* do occur in sympatry. Despite the close morphological resemblance, however, ITS DNA sequence divergence between *G. pictae* and *G. turnbulli* is >5%, suggesting that the two species have been reproductively isolated for some considerable time. Sibling species of gyrodactylids, such as *G. rarus* Wegener, 1910 and *G. branchicus* Malmberg, 1964, differ by as little as 1% in the ITS sequence (Ziętara & Lumme, 2002), while *G. thymalli* Ziętnan, 1960 and *G. salaris* Malmberg, 1957 are incipient species having identical ITS sequences (Cunningham, 1997; Ziętara & Lumme, 2002; Hansen et al., 2003).

The available molecular (ITS) phylogeny groups *G. pictae* with *G. turnbulli* and *G. bullatarudis* with *G. poeciliae* (current study; Harris & Cable, 2000). *G. bullatarudis*-like species appear to be widespread on poeciliids. Within *Poecilia,* they have been recorded from *P. reticulata* (see Turnbull, 1956), *P. caucana* (see Harris & Cable, 2000) and *P. sphenops* (see Kritsky & Fritts, 1970), although these hosts are not closely related (Breden et al., 1999). Indeed *G. bullatarudis* and *G. rasini* Lucky, 1973 have been collected from *Xiphophorus* spp. (see Harris, 1986; Dove & Ernst, 1998); although Richards et al. (2000) suggested that *G. rasini* and *G. bullatarudis* (*sensu* Harris, 1986) should be synonymised. The remaining *G. bullatarudis*-like species, *G. gambusiae*, infects *Gambusia affinis* (see Rogers & Wellborn, 1965). *Xiphophorus* and *Gambusia* are placed within the Poecilinae, but these fishes are even more distantly related to *Poecilia*, emphasising that the *G. bullatarudis*-like species are widespread on poeciliine fishes. *G. turnbulli* and *G. pictae* are, however, much more restricted, being collected only from fishes of the subgenus *Micropoecilia*. Although *G. milleri*, from *P. caucana*, is most similar to *G. turnbulli*-like species (Harris & Cable, 2000), morphologically this form is very distinct from both *G. pictae* or *G. turnbulli*, and is likely to be only distantly related.

Breden et al. (1999) recorded distances of 15% between *P. picta* and *P. reticulata* using a mitochondrial ND2 marker; conventionally a figure of *ca.* 7–8% divergence per million years is assumed for this mitochondrial locus in fish (Fajen & Breden, 1992; Hrbek & Larson, 1999; Hrbek & Meyer, 2003), suggesting that these taxa diverged some two million years bp. Within Trinidad, specimens of *P. reticulata* show strong differentiation between the Caroni (western) and Oropuche (eastern) watersheds (Carvalho et al., 1991; Fajen & Breden, 1992), which Fajen & Breden (1992) date to 100–200,000 years bp. The short (3 month) generation time of micropoeciliids may result in more rapid divergence at mitochondrial loci compared to other teloests. Even allowing for this, the divergence time is likely to be considerable, suggesting that populations of *Poecilia* on Trinidad were structured before the island was fully separated from mainland South America some 10,000 years ago (Carvalho et al., 1991). Endemic poeciliids do occur on Trinidad (Poeser, 2003), but these too may reflect ancient speciation events in the highland watersheds which currently remain in the northern mountains of Trinidad and previously were continuous with the Venezuelan mainland.

The considerable (5%) sequence differentiation between *G. pictae* and *G. turnbulli* suggests that these two represent an ancient divergence, rather than the outcome of a recent host-switching event. Huyse et al. (2003) recorded similar distances between heterospecific gyrodactylids of gobies in the North Sea and related this to glaciation patterns 10,000–100,000 years bp. This suggests that separation of *G. pictae* and *G. turnbulli* was complete before the isolation of Trinidad, and that they are likely to be found infecting their respective hosts on the mainland also. This is certainly true of *G. turnbulli*, which occurs in Venezuela (Harris & Cable, unpublished observations). Further work on the systematics of gyrodactylids from poeciliids of the subgenus *Micropoecilia* might provide important clues about the evolution of this group of parasites and their hosts.

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