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VERSITA

Gyrodactylus derjavinoides sp. nov. (Monogenea, Platyhelminthes) on *Salmo trutta trutta* L. and *G. derjavini* Mikailov, 1975 on *S. t. caspius* Kessler, two different species of *Gyrodactylus* – combined morphological and molecular investigations

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Abstract

The paper deals with two morphologically similar but molecularly clearly different species of *Gyrodactylus*: *G. derjavinoides* sp. nov. on *Salmo trutta trutta* L. in Western Europe and *G. derjavini* Mikailov, 1975 collected on *Salmo trutta caspius* Kessler in Iran. The new species is described and its opisthaptoral hard parts compared to those of *G. derjavini*. Our molecular analysis of *G. derjavinoides* and *G. derjavini* confirmed that the morphological differences between them are species differences and not intraspecific variations. Phylogenetic analysis using the ITS rDNA region placed both species within the subgenus *G.* (*Limnonephrotus*) and within the *G. wageneri*-group, quite in accordance with morphological results. The two species, however, did not cluster as sister taxa. The correspondence between molecular-based clades within *G.* (*Limnonephrotus*) and the morphological shapes of marginal hooks within these clades are discussed. The importance of combined molecular and morphological analyses when describing or redescribing *Gyrodactylus* species is stressed.

Key words

Platyhelminthes, Monogenea, Gyrodactylidae, *Gyrodactylus* sp. nov., fish, *Salmo trutta*, Western Europe, Iran

Introduction

In 1975, the disastrous problems with *Gyrodactylus salaris* Malmberg, 1957 in salmon farms and natural waterways in Norway were first revealed. Fear of spread of the parasite prompted investigations to increase knowledge of gyrodactylids infecting salmonids not only in Norway but also in other areas with salmonid populations and salmonid farming. The *Gyrodactylus* fauna on salmonids was sparsely known and the limited knowledge of differences defining *Gyrodactylus* species caused problems with species discriminations, e.g. *Gyrodactylus* species on *Salmo trutta trutta*. In Western Europe the species here described as *G. derjavinoides* was called, i.a. *G. derjavini*[1].

The first account of *G. derjavinoides* was in 1958, from *S. t. trutta* in a fish farm in Western Sweden (Malmberg 1973, 1993; fig. 7). Its opisthaptoral hard parts were documented according to Malmberg (1970) and its protonephridial system mapped. The species was found to be a typical member of the subgenus *G.* (*Limnonephrotus*), species group *G. wageneri*. In 1972, the species was also found on *S. t. trutta* in natural water and on farmed *Oncorhynchus mykiss* (Walbaum), *Salvelinus alpinus* (L.) and *S. fontinalis* (Mitchill). Since then specimens of the species have been documented from many different

[1]For previous recording of *G. derjavinoides* as *G. derjavini* see footnote in the species description section.

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Table I. *Gyrodactylus derjavinoides* sp. nov. from *Salmo trutta trutta*, *Salmo salar*, *Oncorhynchus mykiss*, *Salvelinus alpinus* and *Salvelinus fontinalis*, specimens analysed

Locality and coordinates	Date of collection	Preparation	Number studied	Number drawn	Number digitalised	Number measured
Host: *Salmo trutta trutta* L.						
River Dalälven, at Älvkarleby, Sweden	23.11.2001	DNA-96% ethanol-am.picr.glycerine	+10	5	5	5
[1](N6717002, E1589360)						
(60°33´632″N, 17°26´057″E)	01.10.1990	am.picr.glycerine	5	2	5	3
River Mörrumsån at Mörrum fish farm, Sweden	22.11.2001	96% ethanol-DNA-am.picr.glycerine	+10	3	2	2
[1](N6229967, E1434389)	06.08.1989	am.picr.glycerine	7	2	6	3
(56°11´603″N, 14°44´902″E)	16.06.1990	am.picr.glycerine	+10	0	0	0
Mörrum fish farm, trugs	31.08.1989	am.picr.glycerine	2	2	0	0
	17.06.1990	am.picr.glycerine	+10	0	0	0
Gadie Burn, River Don System, Scotland, UK	28.07.1993	96% ethanol-DNA	10	1	1	1
[2](NJ6541826084)						
(57°19´26″N, 2°34´33″W)						
Salmon fish farm, Forsmo at River Ängermanälven, Sweden	29.10.1989	am.picr.glycerine	11	1	0	0
River Emån, at Em, Sweden	01.09.1989	am.picr.glycerine	13	0	5	0
[1](N6333980, E1541967)						
(57°7´765″N, 16°29´911″E)						
River Morupsån, Falkenberg, Sweden	11.07.1991	am.picr.glycerine	8	0	1	0
[1](N6322692, E1290628)						
(56°58´991″N, 12°21´645″E)						
Källefall fish farm, Hökensåsen, Tidaholm, Sweden	03–05.12.1958	am.picr.glycerine	3	0	0	0
[1](N6428643, E1397845)	12–14.12.1958	am.picr.glycerine	17	6	0	0
(57°58´214″N, 14°4´738″E)	06–08.06.1972	am.picr.glycerine	7	1	0	0
Small creek beside Källefall fish farm	06–15.06.1972	am.picr.glycerine	5	1	0	0
River Rödån at Källefall fish farm	06–07.06.1972	am.picr.glycerine	6	1	0	0
River at Zealand, Denmark: slides from Marianne Køie, Helsingør	03.09.1990	am.picr.glycerine	+10	2	0	0
River Sandvikselva, Norway: specimens from Kjetil Tanum, Oslo	26.05.1981	am.picr.glycerine	+2	+1	0	0
Loch Airthrey, Stirling, Scotland, UK	04.05.1992	am.picr.glycerine	+7	1	0	0
Temporary host: *Salmo salar* L.						
River Dalälven, at Älvkarleby, Sweden	01.10.1990	am.picr.glycerine	9	0	0	0
[1](N6717002, E1589360)						
(60°33´632″N, 17°26´057″E)						
River Mörrumsån at Mörrum fish farm, Sweden	09.08.1989	am.picr.glycerine	1	1	0	0
[1](N6229967, E1434389)						
(56°11´603″N, 14°44´902″E)						
River Emån, at Em, Sweden	01.09.1989	am.picr.glycerine	4	0	0	0
[1](N6333980, E1541967)						
(57°7´765″N, 16°29´911″E)						
River Säveån, at Gothenburg, Sweden	29.08.1989	am.picr.glycerine	1	1	0	0
[1](N6408432, E1283737)	23.11.1998	am.picr.glycerine	3	0	1	0
(57°44´903″N, 12°10´342″E)						
River Högvadsån, at Ullared, Sweden	29–30.04.1993	am.picr.glycerine	1	0	1	0
[1](N6338441, E1313250)						
(57°08´045″N, 12°43´234″E)						

Table I continued. *Gyrodactylus derjavinoides* sp. nov. from *Salmo trutta trutta*, *Salmo salar*, *Oncorhynchus mykiss*, *Salvelinus alpinus* and *Salvelinus fontinalis*, specimens analysed

Locality and coordinates	Date of collection	Preparation	Number studied	Number drawn	Number digitalised	Number measured
River Ätran, at Vessige Bro, Sweden [1](N6320767, E1308147) (56°58′411″N, 12°38′994″E)	17.11.1994 19.12.1995	am.picr.glycerine am.picr.glycerine	6 1	1 0	0 1	0 0
Temporary host: *Oncorhynchus mykiss* (Walbaum) Källefall fish farm, Hökensåsen, Tidaholm, Sweden [1](N6428643, E1397845) (57°58′214″N, 14°4′738″E)	06.07.1972	am.picr.glycerine	3	1	0	0
Brøns dammbrug (fish farm), Denmark	16.05.1972 15.12.1972	am.picr.glycerine am.picr.glycerine	12 12	1 0	5 1	0 0
Fole dammbrug (fish farm), Denmark	16.05.1972	am.picr.glycerine	3	1	0	0
Kolding dammbrug (fish farm), Denmark	15.05.1972	am.picr.glycerine	6	1	0	0
Temporary host: *Salvelinus alpinus* (L.) Källefall fish farm, Hökensåsen, Tidaholm, Sweden [1](N6428643, E1397845) (57°58′214″N, 14°4′738″E)	06.06.1972	am.picr.glycerine	3	1	0	0
Temporary host: *Salvelinus fontinalis* Mitchill Källefall fish farm, Hökensåsen, Tidaholm, Sweden [1](N6428643, E1397845) (57°58′214″N, 14°4′738″E)	29–30.05.1972	am.picr.glycerine	7	1	0	0

[1]Swedish coordinates are from the National Land Survey of Sweden, homepage: www.gis.lst.se/lanskartor; [2]Scottish coordinates are from the Ordenance Survey National Landmapping Agency of Great Britain, homepage: www.ordnancesurvey.co.uk

localities and areas (Table I; Malmberg and Malmberg 1991, 1993), and in Denmark, the species became an important experimental organism (see e.g., Buchmann *et al*. 1995; Buchmann 1997, 2004; Buchmann and Uldal 1997).

The opisthaptoral hard parts of *G. derjavinoides*, especially its marginal hook sickles, are very similar to the opisthaptoral hard parts of *G. derjavini*. The differences could indicate intraspecific variations, but also to be species differences. As there was little knowledge about these differences until recently, *G. derjavini* sensu Malmberg et Malmberg, 1987 was used for *G. derjavinoides*. Drawings in Ergens (1983) and Malmberg (1993) illustrate the differences. For discriminating very similar species of *Gyrodactylus*, Cunningham *et al*. (1995a, b) successfully introduced molecular analysis. For the present paper combined morphological and molecular analyses are used.

Materials and methods

The *G. derjavinoides* material from *S. t. trutta* and the *G. derjavini* material from *S. t. caspius* used for DNA and morphological analyses are presented in Tables I and II. The Swedish material of *G. derjavinoides* was obtained from the River Dalälven (Middlesweden) and the River Mörrumsån (south-eastern Sweden). Live specimens of *S. t. trutta* were caught and directly transferred into bottles with 96% ethanol on 22.11.2001 and 23.11.2001. Ammonium picrate-glycerine slides of *Gyrodactylus* specimens from these fish were prepared for morphological analyses and molecular examinations carried out on additional specimens. Ammonium picrate-glycerine slides of material from the same rivers from October 1, 1990 and August 6, 1989, respectively were included for comparative morphological studies.

The Scottish material of *G. derjavinoides* is from the River Don, Aberdeenshire, and was collected on July 28, 1993. Ten ethanol-preserved specimens, secondarily mounted in ammonium picrate-glycerine from this material were studied in detail morphologically. Regarding other material (ammonium picrate-glycerine slides) for comparative studies of *G. derjavinoides* from other localities, and from temporary hosts, see Table I.

The material of *G. derjavini* was collected in Iran on two different occasions (Table II). Specimens from June 1990 were mounted live in ammonium picrate-glycerine. The specimens from May 2002 were collected in 96% ethanol and used for ammonium picrate-glycerine slides or DNA investigations. A number of ethanol specimens were used for both mo-

lecular and morphological analyses. The body of such a specimen was dissected from the haptor and used for molecular analysis. The haptor in turn was subjected to partial digestion to allow better visualisation of various species characters. It was placed in a drop of lysis buffer (proteinase K 60 µg per ml, IGEPAL 0.45%, Tween 20 0.45% in TrisHCl 10 mM, EDTA 1 mM, pH 8.0; see Cunningham *et al*. 2001) on a microscope slide, covered with a cover slip, and observed under × 400 magnification until the tissue had partially digested, so the marginal hooks were clearly seen. Then the digestion was stopped by the addition of ammonium picrate-glycerine. The method is similar to that described in Mo and Appleby (1990) and in Harris *et al*. (1999), but the digestion presented here is performed with the haptor in the final position between the microscope slide and the cover slip.

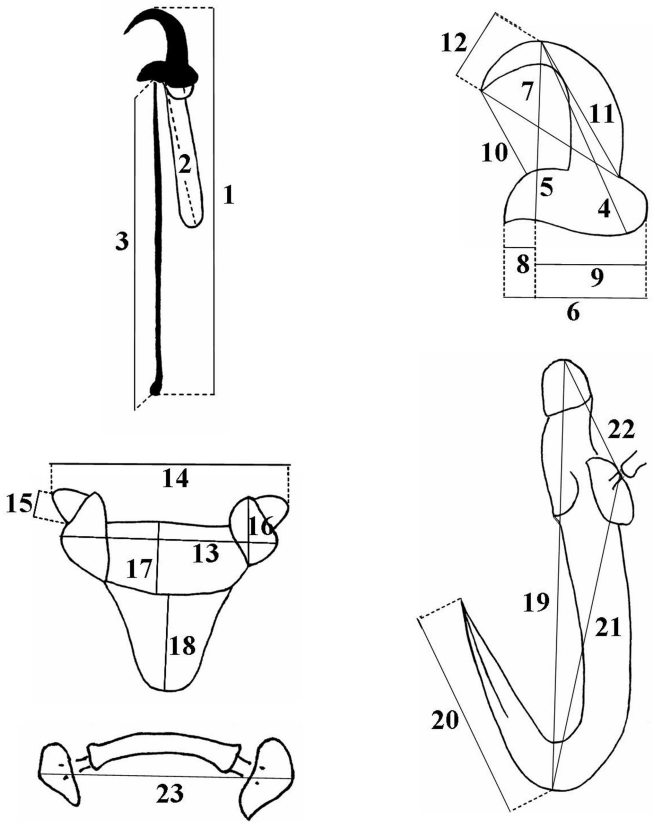

Fig. 1. Method of measuring the opisthaptoral hard parts of *Gyrodactylus derjavinoides* sp. nov. and *G. derjavini* Mikailov, 1975 from Iran. **Marginal hook:** 1. Total length of marginal hook. 2. Length of marginal hook filament loop. 3. Length of marginal hook handle. **Marginal hook sickle:** 4. Length of sickle. 5. Length of marginal hook sickle to shaft attachment. 6. Proximal width of sickle. 7. Distal width of sickle. 8. Toe length of sickle. 9. Heel length of sickle. 10. Aperture distance of sickle. 11. Shaft length of sickle. 12. Point length of sickle. (Area and perimeter of sickle measured by detection at image analysis). **Ventral bar:** 13. Length of ventral bar. 14. Distance between tips of ventral bar processes. 15. Length of ventral bar processes. 16. Basal width of ventral bar. 17. Median width of ventral bar. 18. Ventral bar membrane, length. **Anchor:** 19. Total length of anchor. 20. Length of anchor point. 21. Length of anchor shaft. 22. Length of anchor root. **Dorsal bar:** 23. Length of dorsal bar

The morphological analyses were carried out using oil immersion (× 90 objective), phase contrast and a Leitz drawing equipment (see Malmberg 1970), a Leica DC 300 digital camera and archiving system. Photographic images of the haptoral hard parts of all specimens and of embryos in the uterus, when present, were stored and printed (LazerPrint system; Reality Imaging System, Munich, Germany) for further analysis. The results were compared to drawings made by means of the drawing equipment (above). Measurements of marginal hook sickles were performed using image analysis (Leica Q-500/W with a Hamamatsu 3 CCD camera, C5810), the sickle area and perimeter by detection, and the other measurements by interactive measuring on a computer screen. The features of opisthaptoral hard parts are shown in Figure 1. The classification of species based on morphological characteristics follows Malmberg (1970).

The internal transcribed spacer (ITS) region of the ribosomal RNA gene array was isolated and sequenced from individual *Gyrodactylus* specimens as described by Cunningham (1997). Sequences were analysed using Sequencher software (Intelligenetics Corp). Alignment of ITS sequence from specimens of *G. derjavinoides* and *G. derjavini* was carried out using the CLUSTAL W multiple sequence alignment program (Thompson *et al*. 1994).

To confirm the subgeneric position of the newly sequenced *G. derjavini*, its ITS sequence was included in phylogenetic analysis with sequences of species from the following four subgenera: *G*. (*Mesonephrotus*), *G*. (*Metanephrotus*), *G*. (*Paranephrotus*) and *G*. (*Limnonephrotus*) (see Table III). *Gyrodactyloides bychowskii* (AJ566379) was used as an outgroup for the analysis.

To examine relationships between *G. derjavinoides*, *G. derjavini* and other members of the *G. wageneri*-group, ITS sequences were obtained from the GenBank database for additional *Gyrodactylus* species of the *G. wageneri*-group as given by Ziętara and Lumme (2004). Only species for which both ITS1 and ITS2 sequences are available were utilised. These species and their accession numbers are listed in Table III. *G. gracilihamatus* was used as an outgroup for the analysis.

Sequences were aligned and manually edited using the BioEdit program (Hall 1999). All gaps and ambiguous regions were deleted from the alignment. Maximum likelihood (ML) and neighbor joining (NJ) methods were used to infer phylogenetic relationships. The program PAUP, vers 4.0b10 PPC (Swofford 1999) was used to apply the methods. The ML analysis was applied using the best-fit model and parameters generated by ModelTest 3.01 (Posada and Crandall 1998). The model selected, based on Akaike Information Criterion (AIC) was a General Time-reversible model (TVM + I + G) of DNA evolution with computed gamma distribution rate parameters (0.42 and 0.82 respectively for genus and species level analyses). The likelihood settings generated were also used for calculating the distance matrix in the NJ analysis. A heuristic search was used and nodal support was obtained by

running a bootstrap of 1000 replicates. Bootstrap values of 50% or greater were recorded.

Results

Morphological analysis

Size differences were found between the marginal hook sickles of *G. derjavinoides* and *G. derjavini* (Fig. 2). The anchors of *G. derjavinoides* are not as robust as those of *G. derjavini*. Comparative drawings of ventral bars and anchors of the two species are presented in Figures 3 and 4. Measurements of the haptoral hard parts of the two species are presented in Table IV. The protonephridial system of *G. derjavinoides* is typical for the subgenus *G.* (*Limnonephrotus*), see Malmberg (1970, 1998).

Molecular analysis

The genetic divergence between *G. derjavinoides* and *G. derjavini* was represented by a genetic distance of 3.4% (uncorrected pairwise genetic distance) over the entire ITS region once gaps and ambiguous nucleotide positions had been removed (863 nucleotides remaining in alignment). Comparison with genetic distances obtained for other species of the *G. wageneri*-group indicates that the level of divergence seen between *G. derjavinoides* and *G. derjavini* is similar to that seen between previously well defined species of the group (Table V).

Phylogenetic analysis using the ITS rDNA placed *G. derjavini* within the subgenus *G.* (*Limnonephrotus*) and within the *G. wageneri*-group. Further phylogenetic analysis using species restricted to the "wageneri group" (see Ziętara and Lumme 2004), with *G. gracilihamatus* as an outgroup, grouped *G. derjavinoides* with *G. lucii* as previously found by Ziętara and Lumme (2002, 2004), but the grouping was not well supported. The phylogenetic analysis did not resolve relationships of *G. derjavini* with other members of the *G. wageneri*-group. ML and NJ analyses provided low nodal support and different topologies, as did other outgroup species. Tree topologies for all analyses were similar with respect to those grouping with nodal support greater than 50% and were as found previously by Ziętara and Lumme (2002, 2004).

Gyrodactylidae Cobbold, 1864
Gyrodactylus Nordmann, 1832
G. (*Limnonephrotus*) Malmberg, 1964
G. wageneri-group Malmberg, 1970

Amended species group diagnosis: Species with marginal hook sickles of a type similar to that in *G. pungitii* Malmberg, 1964; fig. 15e, and *G. aphyae* Malmberg, 1957 (see also Malmberg 1970; fig. 35). Ventral bars with short, antero-laterally pointing processes and a triangular ventral bar membrane. Small cirrus spines in a single arched row.

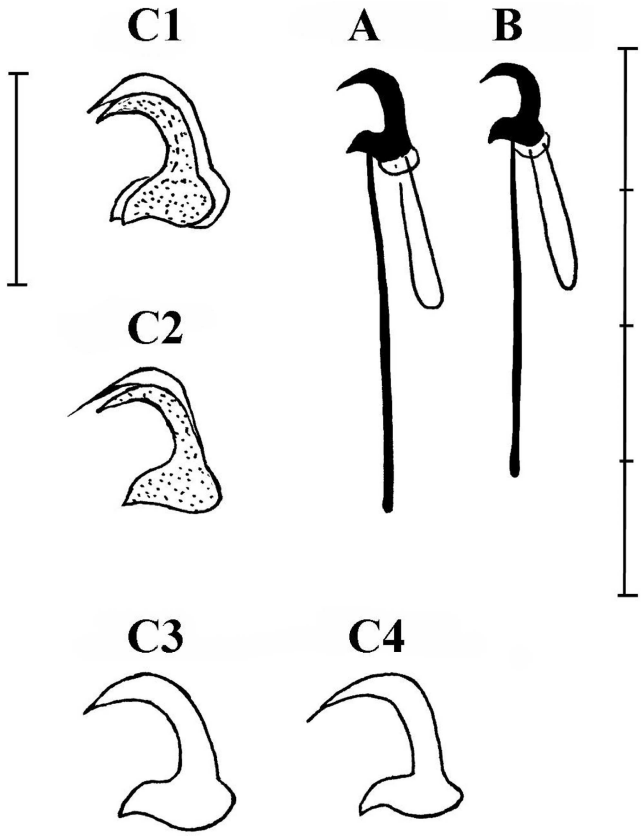

Fig. 2. Comparative drawings of marginal hooks of (**A**) *G. derjavini* Mikailov, **B** – *G. derjavinoides* sp. nov., **C4** – *G. truttae* Gläser. **C1-C4**: **C1** – for comparison, the sickle of *G. derjavinoides* is shown dotted against the sickle of *G. derjavini*, **C2** – the sickle of *G. derjavinoides* is shown dotted against the sickle of *G. truttae*, **C3** and **C4** – the sickle of *G. derjavini* and *G. truttae*, respectively. Note the longer marginal hook sickle point of *G. truttae*. Scale bars = 40 μm (A, B), 10 μm (Cl-C4)

***Gyrodactylus derjavinoides* sp. nov.**[2] (Figs 2–6; Tables I and V)

Type-host and locality: *Salmo trutta trutta* L., 1758; River Dalälven, at Älvkarleby, Sweden (N6717002, E1589360; 60°33′632″N, 17°26′057″E).

Site on host: Body and fins.

Date of collection: 23.11. 2001.

[2]*G. derjavinoides* is previously recorded as *G. derjavini* in Buchmann (2004); Lautraite *et al*. (1999); Malmberg (1987a, b, 1989, 1993); Malmberg and Malmberg (1987, 1991, 1993); Matějusová *et al*. (2001); Mo (1993, 1997); Platten *et al*. (1994); Shinn *et al*. (1995, 1996); Ziętara and Lumme (2002, 2004); as *G. truttae* in Mo (1983) and as *G*. sp. in Tanum (1983).

The following sequences in the public databases, classified as *G. derjavini* from sites in western Europe at the time of deposition in the database, are identical to the *G. derjavinoides* sequence DQ357215: AF484530, AJ001840, AJ132259. Based on the current paper, these sequences should be reclassified as being from *G. derjavinoides*.

Table II. *Gyrodactylus derjavini* Mikailov, 1975 from *Salmo trutta caspius*, specimens analysed

Locality and coordinates	Date of collection	Preparation	Number studied	Number drawn	Number digitalised	Number measured
Host: *Salmo trutta caspius* Kessler						
Fish farm, Iran: specimens from B. Jalali, Teheran	03.05.2002	DNA-96% ethanol-am.picr.glycerine	3	3	3	3
Fish farm, Iran: slides from Maryam Berzegar	03.05.2002	am.picr.glycerine	8	1	5	0
	03.05.2002	96% ethanol-am.picr.glycerine	+10	1	1	1
Fish farm, Iran: slides from B. Jalali, Teheran	01.06.1990	am.picr.glycerine	5	2	1	2

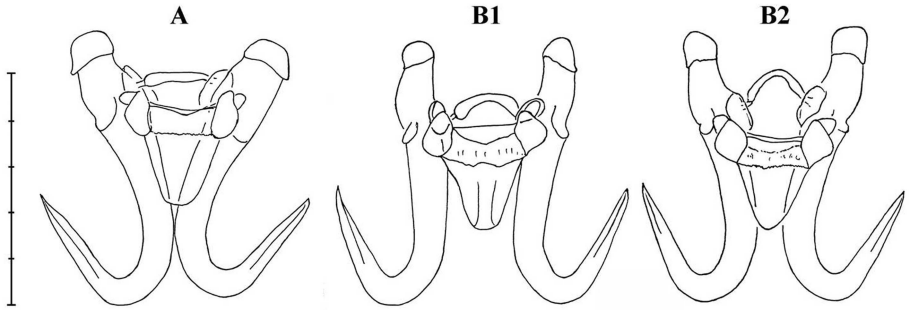

Fig. 3. Anchors, dorsal and ventral bars of ammonium picrate-glycerine specimens of *G. derjavini* Mikailov, 1975, Iran from *Salmo trutta caspius* and *G. derjavinoides* sp. nov. from *Salmo trutta trutta*: **A** – *G. derjavini*, Iran, 01.06.1990, same specimen as in Fig. 4A2 and Fig. 5A2. **B1** and **B2** – *G. derjavinoides*: **B1** – from River Dalälven, Sweden, 1.10.1990, the same specimen as in Fig. 4B2 and Fig. 5B2. **B2** – from River Mörrumsån, Sweden, 06.08.1989, the same specimen as in Fig. 4B4 and Fig. 5B4. The anchors of *G. derjavini* from Iran (A) are more robust than those of *G. derjavinoides* (B). Scale bar = 50 μm

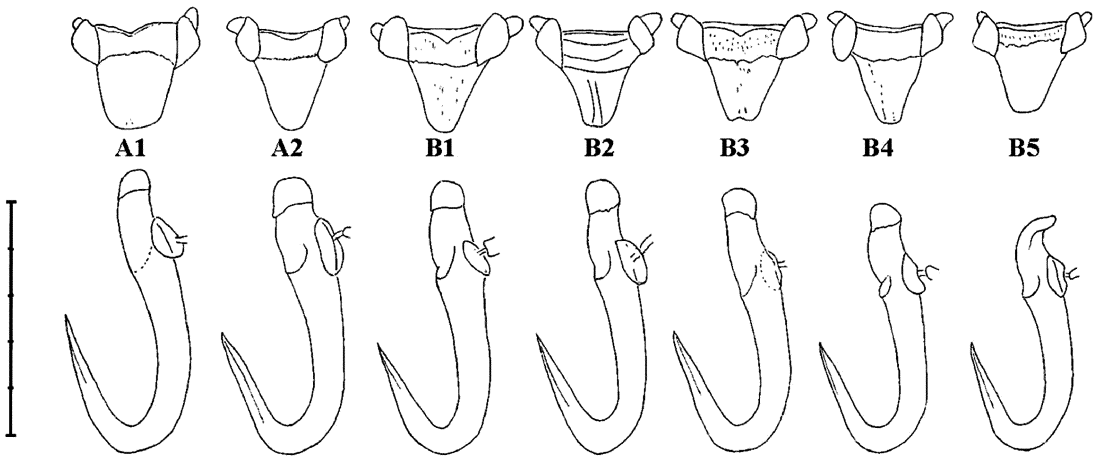

Fig. 4. Comparative drawings of ventral bars and anchors, ethanol and ammonium picrate-glycerine specimens of *G. derjavini* Mikailov, 1975 from Iran and *G. derjavinoides* sp. nov. **A1** and **A2** – *G. derjavini*: **A1** – Iran, 03.05.2002, ethanol specimen; the same as in Fig. 5A1. **A2** – Iran, 01.06.1990, am.picr.glyc. specimen, the same as in Figs 3A and 5A2. **B1-B5** – *G. derjavinoides*: **B1** – from River Dalälven, Sweden, 23.11. 2001, ethanol specimen. **B2** – from River Dalälven, Sweden, 01.10.1990, am.picr.glyc. specimen; the same specimen as in Figs 3B1 and 5B2. **B3** – from River Mörrumsån, Sweden, 22.11.2001, ethanol specimen; the same specimen as in Fig. 5B3. **B4** – from River Mörrumsån, Sweden, 06.08.1989, am.picr.glyc., the same specimen as in Fig. 5B4. **B5** – River Don, Scotland, 28.07.1993, ethanol specimen, the same specimen as in Fig. 5B6. The anchors of *G. derjavini*, Iran are more robust than those of *G. derjavinoides*. Ethanol = ethanol fixed specimen mounted in ammonium picrate-glycerine. Scale bar = 50 μm

Table III. *Gyrodactylus* species used in phylogenetic analysis to determine the position of *G. derjavini* Mikailov, 1975 from Iran to subgenus and species group level

Genus	Subgenus	Species	Accession No.	Author (Accession No.)
Gyrodactyloides		*Gyrodactyloides bychowskii*	AJ249348	Bruno *et al*. (2001)
Gyrodactylus	G. (*Metanephrotus*)	*Gyrodactylus rarus*	AY338445	Huyse *et al*. (2003)
	G. (*Metanephrotus*)	*Gyrodactylus branchicus*	AF156669	Ziętara *et al*. (2000)
	G. (*Mesonephrotus*)	*Gyrodactylus nipponensis*	AB063295	Hayward *et al*. (2001)
	G. (*Mesonephrotus*)	*Gyrodactylus arcuatus*	AY338442	Huyse *et al*. (2003)
	G. (*Paranephrotus*)	*Gyrodactylus rugiensis*	AF328870	Ziętara *et al*. (2002)
	G. (*Paranephrotus*)	*Gyrodactylus micropsi*	AF328868	Ziętara *et al.* (2002)
	G. (*Limnonephrotus*)	*Gyrodactylus macronychus*	AY061980	Ziętara & Lumme (2003)
		Gyrodactylus gracilihamatus	AF484531	Ziętara & Lumme (2002)
		Gyrodactylus kobayashii	AJ132985	Cable *et al*. (1999)
		Gyrodactylus pungitii	AF328869	Ziętara *et al*. (2002)
		Gyrodactylus luciopercae type1	AF484540	Ziętara & Lumme (2002)
		Gyrodactylus longiradix	AF484538	Ziętara & Lumme (2002)
		Gyrodactylus cernuae	AF484529	Ziętara & Lumme (2002)
		Gyrodactylus truttae	AJ132260	Cunningham (1997)
		Gyrodactylus aphyae type1	AF484527	Ziętara & Lumme (2002)
		Gyrodactylus aphyae type 2	AF484528	Ziętara & Lumme (2002)
		Gyrodactylus leucisci type1	AF484536	Ziętara & Lumme (2002)
		Gyrodactylus salaris	Z72477	Cunningham (1997)
		Gyrodactylus lavareti	AF484535	Ziętara & Lumme (2002)
		Gyrodactylus pannonicus	AF484542	Ziętara & Lumme (2002)
		Gyrodactylus teuchis	AJ249350	Cunningham *et al*. (2001)
		Gyrodactylus lucii	AF484539	Ziętara & Lumme (2002)
		Gyrodactylus gasterostei	AF328867	Ziętara *et al*. (2002)
		Gyrodactylus gobiensis	AY278041	Ziętara & Lumme (2004)
		G. sp. (*Rutilus rutilus*) type 1	AF484545	Ziętara & Lumme (2002)
		G. sp. (*Rutilus rutilus*) type 2	AF484546	Ziętara & Lumme (2002)
		G. sp. (*Alburnus alburnus*)	AF484547	Ziętara & Lumme (2002)
		Gyrodactylus derjavinoides	DQ357215	present paper
		Gyrodactylus derjavini	DQ355975	present paper

Other host: *S. salar* L., temporarily *Oncorhynchus mykiss* (Walbaum), in fish farms (see also Table I).

Other localities: River Mörrumsån, at Mörrum fish farm, Sweden (N6229967, E1434389; 56°11´603˝N, 14°44´902˝E); River Don, Scotland (O.S. NJ6541826084; 57°19´26˝N, 2°34´33˝W). For further localities, see also Table I.

Record of specimens, morphological analysis: The holotype specimen and 4 other specimens from the River Dalälven, Älvkarleby, Sweden were collected on 23.11. 2001, transferred *in vivo* to 96% ethanol, then prepared for slides in ammonium picrate-glycerine. The opisthaptoral hard parts were drawn, digitally photographed and measured. Five other specimens from the same locality, collected on 01.10.1990, were directly prepared *in vivo* on slides in ammonium picrate-glycerine, all 5 were digitally photographed, 3 were measured and 2 were drawn (see Table I). Specimens studied from the River Mörrumsån, Sweden: More than 10 specimens were collected on 22.11. 2001 in ethanol, then prepared for slides in ammonium picrate-glycerine (Table I). Seven other specimens from the same locality, collected on 06.08.1989, were directly prepared *in vivo* on slides in ammonium picrate-glycerine (Table I). The number of specimens photographed, measured and drawn from these slide preparations is given in Table I. Ten specimens from the River Don, Scotland, collected on 28.07.1993 in ethanol were prepared for slides in ammonium picrate-glycerine (Table I). Regarding data of specimens of *G. derjavinoides* sp. nov. from other localities in Sweden, Denmark, Norway and Scotland, and from temporary hosts, see Table I. The holotype specimen (Acc. No. 6183) and paratypes (Acc. Nos. River Dalälven 6184–6193; River Mörrumsån 6194–6207; River Don 6208–6217; from other localities 6218–6241) are deposited at the Swedish Museum of Natural History (SMNH).

Records of specimens, molecular analysis: The ITS ribosomal spacer region was amplified by PCR and sequenced from 7 specimens from the River Dalälven, Älvkarleby, Sweden, collected on 23.11.2001, and from 6 specimens from the River Mörrumsån, Sweden, collected on 22.11.2001. These specimens were identical in their ITS sequence. The ITS sequence was submitted to the GenBank database under Accession No. DQ357215. The ITS PCR amplification product for *G. derjavinoides* was approximately 1280 nucleotides in

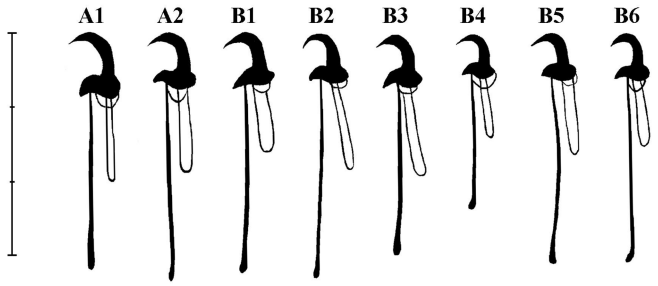

Fig. 5. Comparative drawings of marginal hooks, ethanol and ammonium picrate-glycerine specimens of *G. derjavini* Mikailov, 1975, Iran and *G. derjavinoides* sp. nov. **A1** and **A2** – *G. derjavini* from Iran: **A1** – Iran, 03.05.2002, ethanol specimen, the same specimen as in Fig. 4A1; compare Figs 6A1a and 6A1b. **A2** – Iran, 01.06.1990, am.picr.glyc. specimen, the same specimen as in Figs 3A and 4A2; compare Fig. 6A2. **B1-B5** – *G. derjavinoides*: **B1** – from River Dalälven, Sweden, 23.11.2001, ethanol, marginal hook of a large embryo; the marginal hook sickle is bigger than that of the adult in B3. **B2** – from River Dalälven, Sweden, 01.10.1990, am.picr.glyc. specimens, the same specimen as in Figs 3B1 and 3B2; compare Fig. 6C1-C5. **B3** – from River Mörrumsån, Sweden, 22.11.2001, ethanol specimen, the same specimen as in Fig. 4B3; compare Fig. 6C1-C2. **B4** – from River Mörrumsån, Sweden, 06.08.1989, am.picr.glyc. specimen, the same as in Figs 3B2 and 4B4; compare Fig. 6C3-C5; the handle in B4 of an exceptional short length. **B5** – from River Mörrumsån, Sweden, 06.08.1989, am.pic.glyc. specimen, handle of normal length. **B6** – from River Don, Scotland, ethanol specimen, the same specimen as in Fig. 4B5; compare Fig. 6D2. Note: The Swedish specimens in B1-B5 indicate seasonal variations – the marginal hook sickles of the specimens in B1 and B3 – "winter form" – have a larger area/perimeter than that of the marginal hook sickles in B2, B4 and B5 – "summer form", respectively. Ethanol = ethanol fixed specimen mounted in ammonium picrate-glycerine. Scale bar = 30 μm

length, consisting of an ITS region of 1195 nucleotides flanked by partial 18S and 28S rDNA sequence. The ITS1 region was 610 nucleotides, the 5.8S region was 157 nucleotides, and the ITS2 region was 428 nucleotides in length.

Morphological diagnosis: (Figs 2–6; Table IV. For measurements of coverslip-flattened specimens in ammonium picrate-glycerine, see Table IV). Haptor delimited from body. Pharynx with long processes. Cirrus situated posteriorly to pharynx, with a single large spine and small spines in a single arched row. Protonephridial system lacking bladders and lateral flames in the main canals. Marginal hook sickle of a similar shape to that of *G. derjavini* described here (Figs 2 and 5) and *G. derjavini* Mikailov, 1975 (his fig. 25) but smaller and not as pointed as in *G. truttae* Gläser, 1974 (Abb./fig. 6a not 6b; this paper Fig. 2); marginal hook point as in *G. derjavini* only slightly extending beyond the toe. Ventral bar similar but usually slightly smaller than in *G. derjavini* with small anterolaterally pointing processes and a triangular ventral bar membrane (Figs 3 and 4). Anchors of a similar shape as in *G. derjavini* but more gracile; anchor roots slightly curved to the median line of the body (Figs 3 and 4). Dorsal bar lacking a median notch (Fig. 3).

Etymology: The specific name *derjavinoides* means similar to *derjavini*.

***Gyrodactylus derjavini* Mikailov, 1975**[3] (Figs 2–6; Tables II and IV)

Host and locality: *Salmo trutta caspius* Kessler; Kelardasht fish farm south of New-sahr (Behiar Jalali), with water supply from River Sardab-rud, emptying into the Caspian Sea, Northern Iran.

Site on host: Fins.

Date of collection and water temperature: 01.06.1990 and 03.05.2002, 10°C and 8°C, respectively.

Record of specimens, morphological analysis: The opisthaptoral hard parts of 2 specimens, collected on 03.05.2002 in 96% ethanol and used for DNA analysis were mounted in ammonium picrate-glycerine and drawn, digitally photographed and measured. Nine other specimens from the collection of 03.05.2002 were mounted directly *in vivo* in Iran in ammonium picrate-glycerine and were studied morphologically in Stockholm as described previously. Ten more ethanol preserved specimens from that collection were mounted in ammonium picrate-glycerine and studied morphologically in Stockholm. Iranian specimens collected from the same region on 01.06.1990 were likewise prepared and studied in Stockholm (Table II). The specimens of *G. derjavini* (Acc. Nos. Iran 84485–84510) are deposited at the Swedish Museum of Natural History (SMNH).

Record of specimens, molecular analysis: The ITS spacer region of the ribosomal gene array was amplified by PCR and sequenced from 9 specimens collected on 03.05.2002. Two of the specimens sequenced were also used for morphological analysis. All 9 specimens analysed were identical in their ITS sequence. The sequence has been submitted to the EMBL database under Accession No. DQ355975. A PCR product of approximately 1300 nucleotides was obtained following PCR amplification of the ITS region of *G. derjavini* from *S. t. caspius*. Sequencing of the product revealed an ITS region 1211 nucleotides in length flanked by partial 18S and 28S rDNA sequence. The ITS1 region was 631 nucleotides, the 5.8S region was 157 nucleotides, and the ITS2 region was 428 nucleotides in length.

Phylogenetic analysis of the sequenced Iranian *G. derjavini* placed it within the subgenus *G.* (*Limnonephrotus*) (ML/NJ; 92/74%) and within the *G. wageneri* species group sensu Ziętara and Lumme (2002) (ML/NJ; 90/84%).

Morphological diagnosis (Figs 2–6; Tables II and IV): Haptor delimited from body. Pharynx with long processes. Cirrus situated posteriorly to pharynx, with a single large

[3]The ITS sequence for *G. derjavini* presented here is identical to that of the ITS sequence DQ323402 from *Gyrodactylus* specimens, presented as *G. derjavini*, infecting *O. mykiss* in Iran.

Table IV. *Gyrodactylus derjavinoides* sp. nov. from Sweden, River Dalälven or River Mörrumsån and from Scotland, River Don and *G. derjavini* Mikailov, 1975 from Iran

***Gyrodactylus* species**	***G. derjavinoides***			***G. derjavinoides***			***G. derjavinoides***			***G. derjavinoides***		
Host species	***Salmo trutta trutta***			***Salmo trutta trutta***			***Salmo trutta trutta***			***Salmo trutta trutta***		
Origin	**Dalälven 23.11.2001**			**Dalälven 01.10.1990**			**Mörrumsån 22.11.2001**			**Mörrumsån 06.08.1989**		
Fixative	**ethanol-am.picr.glycerine**			**am. picr. glycerine**			**ethanol-am.picr.glycerine**			**am. picr. glycerine**		
	no.	**range**	**average**	**no.**	**range**	**average**	**no.**	**range**	**average**	**no.**	**range**	**average**
Marginal hook												
Total length of marginal hook	3	29.46–33.26	31.93	3	31.80–34.14	33.07	1	32.78		2	25.65–31.95	28.8
Length of marginal hook filament loop	4	12.92–14.27	13.09	3	14.39–17.15	15.4	1	15.28		2	12.88–16.09	14.49
Length of marginal hook handle	3	23.90–27.43	25.84	3	25.98–28.25	27.2	1	26.1		2	20.47–26.39	23.43
Length of marginal hook sickle	5	6.30–7.47	6.83	3	6.38–6.89	6.66	5	6.78–7.72	7.08	3	5.95–6.76	6.42
Length of marginal hook sickle to shaft attachment	5	5.56–6.89	6.01	3	5.82–5.91	5.87	5	5.93–6.68	6.2	3	5.18–5.73	5.49
Proximal width of marginal hook sickle	5	4.48–5.73	4.91	3	4.54–5.38	4.84	5	4.61–5.42	5.04	3	4.29–4.97	4.67
Distal width of marginal hook sickle	5	5.52–6.33	5.93	3	5.24–5.85	5.59	5	5.39–5.90	5.68	3	5.13–5.24	5.19
Marginal hook toe length	5	1.21–2.00	1.64	3	1.48–2.00	1.68	5	1.48–1.91	1.72	3	1.31–1.76	1.52
Marginal hook heel length	5	2.64–3.90	3.31	3	2.86–3.91	3.26	5	2.91–3.77	3.4	3	2.91–3.99	3.16
Marginal hook sickle aperture distance	5	3.45–4.18	3.8	3	2.96–3.58	3.27	5	3.42–3.76	3.59	3	3.18–3.45	3.28
Marginal hook sickle shaft length	5	3.35–3.77	3.6	3	3.31–3.61	3.49	5	2.55–3.38	3.14	3	2.73–2.88	2.83
Marginal hook sickle point length	5	4.58–5.68	4.99	3	4.86–5.22	5.04	5	4.56–5.56	5.06	3	4.27–4.79	4.47
Area of marginal hook	5	16.07–22.68	19.26	3	17.83–21.21	19.53	5	17.23–23.02	19.66	3	14.70–18.14	16.86
Perimeter of marginal hook sickle	5	25.95–30.21	28.11	3	26.48–27.95	27	5	25.95–29.60	27.15	3	24.39–26.39	25.64
Ventral bar												
Length of ventral bar	3	27.35–29.56	28.27	3	23.76–25.69	24.91	2	26.38–28.59	27.49	2	24.03–24.86	24.45
Distance between tip of processes	3	32.19–34.26	33.06	2	28.73–29.42	29.08	2	30.80–31.77	31.29	2	26.24–27.08	26.66
Outermost distance between processes												
Length of ventral bar processes	6	3.31–4.97	4.24	5	3.59–5.25	4.56	4	3.18–5.53	4.22	4	2.90–5.53	3.87
Basal width of ventral bar	6	10.08–12.85	11.58	6	7.60–10.65	9.51	4	9.53–10.91	10.29	4	8.71–9.26	9.23
Median width of ventral bar	3	7.46–8.84	8.06	3	6.22–9.39	7.74	2	6.08–7.87	6.98	2	6.63–6.63	6.63
Length of ventral bar membrane	3	14.23–15.75	14.92	3	12.85–13.67	13.35	2	12.71–14.23	13.47	2	14.09–14.65	14.37
Anchors												
Total length of anchor	3	59.39–60.91	60.13	3	57.32–58.70	58.01	2	58.43–59.26	58.85	2	54.42–54.97	54.7
Length of anchor point	3	28.18–30.11	29.1	3	27.07–29.42	28.18	2	28.04–29.56	28.8	2	28.59–28.60	28.6
Length of anchor shaft	3	43.92–44.48	44.15	3	40.33–43.79	42.13	2	40.33–43.37	41.85	2	38.95–40.33	39.64
Length of anchor root	3	18.78–19.48	19.06	3	18.09–20.17	18.97	2	16.71–20.72	18.72	2	16.71–16.85	16.78
Dorsal bar												
Length of dorsal bar	1	23.2		1	26.5					2	26.66–28.18	27.42

Table IV continued. *Gyrodactylus derjavinoides* sp. nov. from Sweden, River Dalälven or River Mörrumsån and from Scotland, River Don and *G. derjavini* Mikailov, 1975 from Iran

***Gyrodactylus* species**	***G. derjavinoides***			***G. derjavini***			***G. derjavini***		
Host species	***Salmo trutta trutta***			***Salmo trutta caspius***			***Salmo trutta caspius***		
Origin	**Scotland 28.07.1993**			**Iran 03.05.2002**			**Iran 01.06.1990**		
Fixative	**ethanol-am.picr.glycerine**			**ethanol-am.picr.glycerine**			**am. picr. glycerine**		
	no.	**range**	**average**	**no.**	**range**	**average**	**no.**	**range**	**average**
Marginal hook									
Total length of marginal hook	1	32.04		4	33.64–35.75	34.97	2	35.88–37.04	36.46
Length of marginal hook filament loop	1	12.59		4	13.11–15.19	13.97	1		11.37
Length of marginal hook handle	1	26.4		4	25.62–28.03	27	2	28.58–30.36	29.47
Length of marginal hook sickle	1	6.34		4	8.28–9.06	8.84	2	7.83–8.06	7.95
Length of marginal hook sickle to shaft attachment	1	5.64		4	7.52–8.16	7.97	2	6.68–7.30	7.00
Proximal width of marginal hook sickle	1	4.67		4	5.54–6.18	5.99	2	5.58–5.61	5.60
Distal width of marginal hook sickle	1	3.2		4	6.89–7.42	7.14	2	6.60–6.70	6.65
Marginal hook toe length	1	1.71		4	2.27–2.49	2.35	2	1.92–2.00	1.96
Marginal hook heel length	1	2.7		4	3.79–4.21	3.97	2	3.83–3.90	3.87
Marginal hook sickle aperture distance	1	3.3		4	4.41–4.88	4.61	2	3.84–4.41	4.13
Marginal hook sickle shaft length	1	2.77		4	3.89–4.32	4.15	2	3.57–3.64	3.61
Marginal hook sickle point length	1	4.81		4	5.99–6.53	6.3	2	5.72–6.10	5.91
Area of marginal hook	1	14.83		4	22.92–30.51	26.84	2	26.37–33.14	29.76
Perimeter of marginal hook sickle	1	25.17		4	30.30–34.12	32.3	2	33.94–34.81	34.38
Ventral bar									
Length of ventral bar	1	24.73		4	26.93–28.18	27.52	2	24.17–24.86	24.52
Distance between tip of processes	1	24.17		4	25.69–30.53	28.35	2	25.00–26.52	25.76
Outermost distance between processes	1	25.14							
Length of ventral bar processes	2	2.64–3.59	3.12	8	2.21–4.97	3.97	4	2.63–3.59	3.28
Basal width of ventral bar	2	8.98–9.67	9.33	8	9.39–11.74	10.9	4	9.67–10.78	10.14
Median width of ventral bar	1	4.83		4	4.97–6.64	5.94	2	5.80–6.36	6.08
Length of ventral bar membrane	1	14.51		4	16.16–16.99	16.58	2	14.23–15.47	14.85
Anchors									
Total length of anchor	1	51.11		4	60.91–61.88	61.53	2	58.98–60.08	59.53
Length of anchor point	1	25.56		4	30.25–32.05	31.15	2	29.01–29.70	29.36
Length of anchor shaft	1	38.95		4	45.58–49.45	47.1	2	46.27–46.69	46.48
Length of anchor root	1	13.54		4	16.85–19.20	18.13	2	17.13–17.54	17.36
Dorsal bar									
Length of dorsal bar	1	29.01		4	22.52–25.28	24.28	2	25.00–25.69	25.35

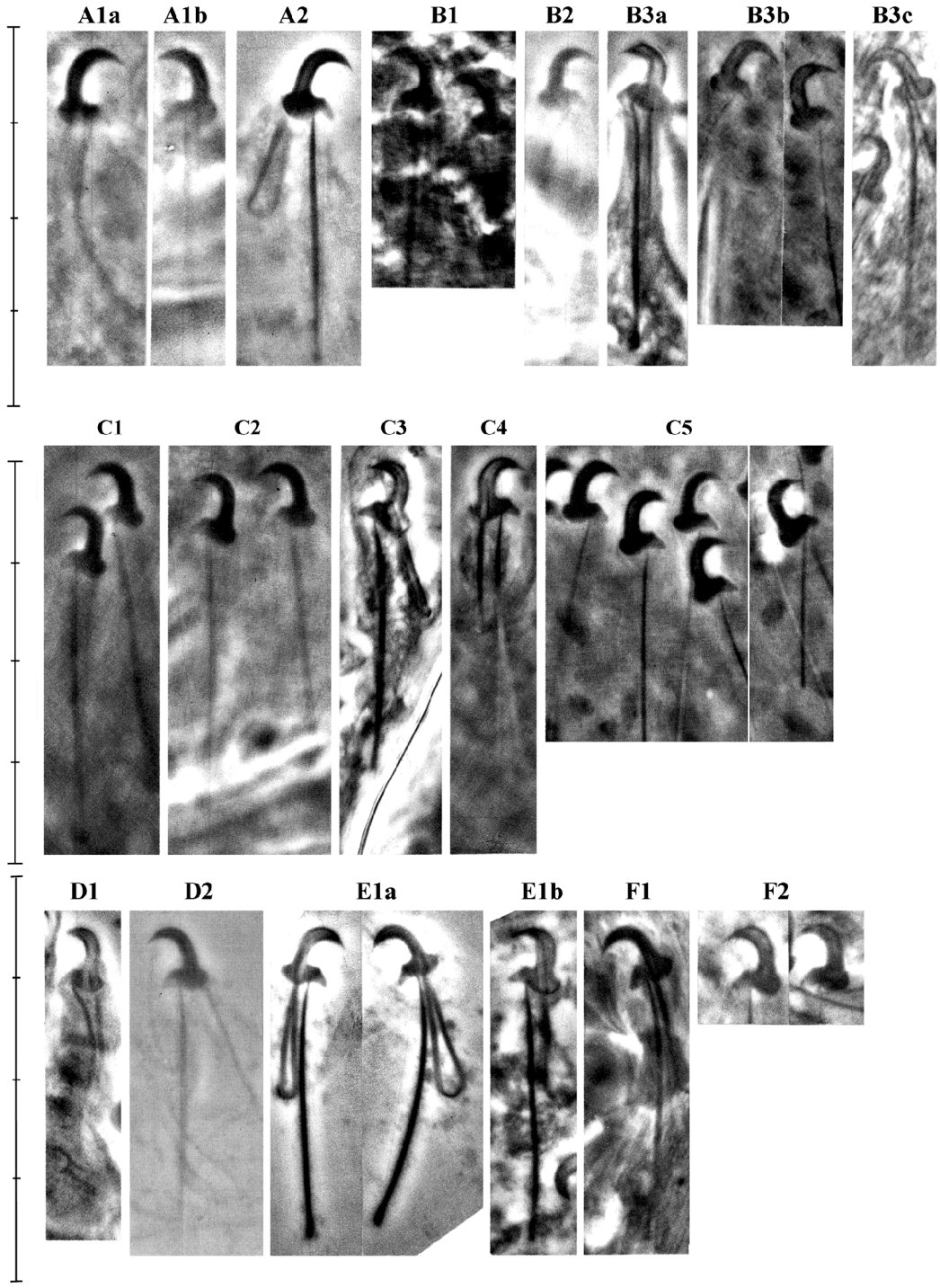

Fig. 6. Comparative micrographs (phase contrast) of marginal hooks, ethanol and ammonium picrate-glycerine specimens of *G. derjavini* Mikailov, 1975, Iran and *G. derjavinoides* sp. nov. The shape of marginal hook sickles of adults and of embryos are presented. **A1-A2** – *G. derjavini* from Iran: **A1** – Iran, 03.05.2002, ethanol – **A1a** from an adult, **A1b** – from an embryo. **A2** – Iran, 01.06.1990, am.picr.glyc., adult; compare Fig. 5A1-A2. **B-F2** – *G. derjavinoides*: **D2**, **F1** and **F2** from *Oncorhynchus mykiss*, otherwise from *Salmo trutta trutta*. **B1-B3** – from River Dalälven, Sweden. **B1-B2** – 23.11.2001, ethanol, embryo. **B3** – 01.10.1990, am.picr.glyc. **B3a** – adult, **B3b** and **B3c** – from the smallest and the largest embryo in uterus. **C** – from River Mörrumsån, Sweden. **C1** and **C2** – 22.11.2001, ethanol, embryo, two different specimens. **C3-C5** – 06.08.1989, am.picr.glyc. **C3** and **C4** – from adults of two different specimens. **C5** – from an embryo of a third specimen. Regarding **B1**-**C5** compare Fig. 5B1-B5. **D** – from River Don, Scotland, 28.07.1993, ethanol, adult; compare Fig. 5B6. **D2** – from *Oncorhynchus mykiss*, from Argyll, Scotland, 19.10.1982, am.picr.glyc., adult. **E** – River Emån, Sweden, 01.09.1989, am.picr.glyc. **E1a** – from an adult. **E1b** – from an embryo of E1a. **F1** and **F2** – from *Oncorhynchus mykiss*, Brøns fish farm, Denmark, 16.05.1972, am.picr.glyc., marginal hook in F1 from an adult, the two in F2 from an embryo of another specimen. Ethanol = ethanol fixed specimen mounted in ammonium picrate-glycerine. Scale bars = 40 μm

Table V. Uncorrected pairwise genetic distances between *G. derjavinoides* sp. nov. and *G. derjavini* Mikailov, 1975 from Iran and other members of the *G. wageneri*-group, subgenus *G.* (*Limnonephrotus*) as defined by Zietara and Lumme (2002, 2004). *G. gracilihamatus* belongs to an older clade (Zietara and Lumme 2002, see also present discussion) within the subgenus *G.* (*Limnonephrotus*)

***Gyrodactylus* spp.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
1. *Gyrodactylus gracilihamatus*	–																					
2. *Gyrodactylus derjavinoides*	0.095	–																				
3. *Gyrodactylus derjavini*	0.086	0.034	–																			
4. *Gyrodactylus teuchis*	0.089	0.037	0.028	–																		
5. *Gyrodactylus lucii*	0.092	0.027	0.027	0.039	–																	
6. *Gyrodactylus gobiensis*	0.097	0.037	0.048	0.048	0.041	–																
7. *Gyrodactylus gasterostei*	0.100	0.044	0.046	0.046	0.042	0.056	–															
8. *Gyrodactylus aphyae* type1	0.096	0.043	0.045	0.045	0.041	0.054	0.006	–														
9. *Gyrodactylus aphyae* type2	0.095	0.042	0.044	0.044	0.039	0.053	0.006	0.003	–													
10. *Gyrodactylus lavareti*	0.086	0.027	0.019	0.025	0.022	0.031	0.043	0.042	0.041	–												
11. *G* . sp. (*Rutilus rutilus* type1)	0.097	0.037	0.036	0.041	0.039	0.049	0.053	0.052	0.051	0.029	–											
12. *G* . sp. (*Rutilus rutilus* type1)	0.092	0.034	0.027	0.036	0.028	0.044	0.048	0.046	0.045	0.017	0.037	–										
13. *Gyrodactylus pannonicus*	0.095	0.038	0.034	0.032	0.038	0.042	0.044	0.043	0.042	0.027	0.043	0.037	–									
14. *Gyrodactylus salaris*	0.088	0.034	0.028	0.029	0.030	0.034	0.043	0.042	0.041	0.019	0.034	0.029	0.031	–								
15. *G* . sp. (*Alburnus alburnus*)	0.092	0.034	0.032	0.037	0.034	0.043	0.048	0.046	0.048	0.025	0.013	0.035	0.042	0.030	–							
16. *Gyrodactylus leuciści* type1	0.095	0.034	0.031	0.036	0.037	0.044	0.052	0.051	0.050	0.027	0.010	0.034	0.038	0.031	0.014	–						
17. *Gyrodactylus cernuae*	0.090	0.035	0.030	0.025	0.036	0.043	0.045	0.044	0.043	0.029	0.037	0.034	0.028	0.030	0.031	0.032	–					
18. *Gyrodactylus luciopercae* type1	0.088	0.038	0.029	0.027	0.037	0.051	0.048	0.046	0.045	0.028	0.038	0.032	0.029	0.034	0.032	0.034	0.010	–				
19. *Gyrodactylus truttae*	0.092	0.045	0.035	0.037	0.042	0.059	0.053	0.050	0.049	0.037	0.043	0.037	0.041	0.042	0.041	0.039	0.024	0.023	–			
20. *Gyrodactylus rogatensis*	0.096	0.039	0.041	0.038	0.044	0.054	0.052	0.051	0.050	0.037	0.046	0.042	0.039	0.042	0.041	0.045	0.021	0.020	0.031	–		
21. *Gyrodactylus longiradix*	0.087	0.035	0.025	0.023	0.034	0.048	0.044	0.043	0.042	0.024	0.035	0.029	0.025	0.030	0.029	0.030	0.007	0.003	0.020	0.016	–	
22. *Gyrodactylus pungitii*	0.090	0.038	0.029	0.027	0.035	0.051	0.045	0.044	0.043	0.028	0.038	0.032	0.029	0.034	0.032	0.034	0.010	0.007	0.023	0.020	0.003	–

spine and small spines in a single arched row. Marginal hook sickle of a similar shape as that in *G. derjavinoides* but larger as in *G. derjavini* Mikailov, 1975, and not so pointed as in *G. truttae* Gläser, 1974 (fig. 6a, not 6b; this paper Figs 2 and 5); marginal hook point, as in *G. derjavinoides* only slightly extending beyond the toe. Ventral bar similar but mostly bigger than in *G. derjavinoides* with small antero-laterally pointing processes and a triangular ventral bar membrane (Figs 3 and 4). Anchors of a similar shape as in *G. derjavinoides* but more robust; anchor roots slightly curved to the median line of the body (Figs 3 and 4). Dorsal bar lacking a median notch (Fig. 3).

Discussion

Since 1958, *G. derjavinoides*, based on its opisthaptoral hard parts, especially its marginal hooks has been known as one of the less varying *Gyrodactylus* species in Sweden, Norway and Denmark. Our morphological and molecular data show that *G. derjavinoides* is a distinct species from *G. derjavini* Mikailov and *G. derjavini* sensu Ergens. The two species are as different from each other morphologically as other similar species of the *G. wageneri*-group. Our Iranian *G. derjavini* specimens agree not only with Ergens' (1983) redescription of *G. derjavini* Mikailov, 1975 but also with Mikailov's (1975) own drawings (including the marginal hook sickles) in the original description. Thus from a morphological point of view our Iranian *Gyrodactylus* material represents specimens of *G. derjavini*. Type material of the species, however, was not present in the *Gyrodactylus* collection in the Zoological Institute, St. Petersburg, Russia.

Mikailov did not define the type locality of *G. derjavini* but stated that his material was collected in Azerbaijan, the River Kura area, south-western Caspian Sea area. Our material of *G. derjavini* is from the southern (Iranian) Caspian Sea area. Mikailov stated that his species was found on i.a., *Salmo trutta caspius*, *Salmo gairdneri* (i.e., *Oncorhynchus mykiss*), *Chondrostoma cyri* and *Cyprinus carpio*. The two latter hosts are cyprinids and most likely "temporary" hosts. *Oncorhynchus mykiss* was introduced as eggs to Europe and is most likely a secondary host, with a huge capacity to harbour several European *Gyrodactylus* species, e.g. the here described *G. derjavinoides*. Most likely Ergens (1983, fig. 4; 1985, fig. 478; 1992, fig. 2c) statement that *S. t. caspius* is the true host of *G. derjavini* is correct.

Gyrodactylus derjavinoides on specimens of *S. t. trutta* has been reported from different parts of Western Europe. The parasite seems also to be present on *S. t. fario* in Poland (Prost 1991 – and judging from morphological examination of specimens from M. Prost in GM's collection). It is also present on *S. t. lacustris* in the Czech Republic (Ergens 1983, 1985, 1992) and on *S. t. fontinalis* (see Matějusová *et al*. 2001). The northern limit for the species on *S. t. trutta* seems to be from south-eastern Norway (Tanum 1983, Mo 1997), eastwards to the River Dalälven area in Sweden (Malmberg and Malmberg

1991). The species is not reported from Finland (Koski and Malmberg 1995), which may imply that the River Dalälven area also represents its north-eastern European distribution. *G. derjavini*, however, is described from eastern Europe and may have a more eastern distribution than *G. derjavinoides*.

The morphological classification of *G. derjavinoides* and *G. derjavini* to subgenus and species group agrees with molecular analyses of the species. Previous analyses of the ITS ribosomal RNA region from different *Gyrodactylus* subgenera (Cable *et al*. 1999, Ziętara and Lumme 2002) placed *G. derjavinoides* (therein referred to as *G. derjavini*) in the subgenus *G*. (*Limnonephrotus*) and in the "*wageneri* group" (see Ziętara and Lumme 2002, 2004). This classification also applies to *G. derjavini*, the ITS sequence of which is presented here.

On the basis of molecular analysis, Ziętara and Lumme (2002, fig. 1) placed both *G. derjavinoides* and *G. derjavini* in a clade, the "wageneri group" which embraces several other members of the *G.* (*Limnonephrotus*), e.g. *G. pungitii* Malmberg, 1964, *G. cernuae* Malmberg, 1957, *G. truttae* Gläser, 1974, *G. teuchis* Lautraite *et al*., 1999, *G. salaris* Malmberg, 1957, *G. derjavini* (i.e., *G. derjavinoides*) and *G. lucii* Kulakovskaya, 1952. Another clade includes i.a. the species *G. gracilihamatu*s Malmberg, 1964, *G. jussi* Ziętara and Lumme 2003, *G. kobayashii* Hukudu, 1940 and *G. macronychus* Malmberg, 1957. The results of Ziętara and Lumme (2002, 2004) support Malmberg's (1970, p. 113) opinion, based on the aberrant shape of the marginal hook sickles of, e.g., *G. macronychus* and *G. gracilihamatus*, that *G.* (*Limnonephrotus*) should be divided into further species groups.

From a morphological point of view, members of each clade have a common basic type of marginal hook sickles. In the clade (including i.a. *G. derjavinoides* and *G. derjavini*) the marginal hooks have a broader proximal part (the foot with a distinct toe and heel), broader than that in species of the other clade. In the other clade, in turn the distal part (the point) of the marginal hook sickle is more protruding, see e.g., *G. macronychus* and *G. gracilihamatus*. Within the basic type of both clades, the marginal hook sickles exhibit a species specific shape, discriminating it from the marginal hook sickles of other species of the clade.

Within each clade, in turn, there appears to be little correlation between the DNA (based on ITS sequences) and the morphological species differences or similarities. This applies to the present analyses as well as to previous analyses (Cable *et al*. 1999, Ziętara and Lumme 2002). Thus, based on the present results of genetic distances, *G. derjavinoides* and *G. derjavini* though morphologically similar, did not group together. *G. derjavinoides* grouped with *G. lucii* (see also Ziętara and Lumme 2002, 2004).

Since the first molecular studies of *Gyrodactylus* specimens (see Cunningham *et al*. 1995a, b) the existence of several morphologically similar *Gyrodactylus* species have been confirmed (e.g., Cunningham *et al*. 2001, Ziętara and Lumme 2003, Huyse *et al*. 2004). Our combined morphological and molecular investigations of *G. derjavinoides* from Western Europe and *G. derjavini* from the Caspian Sea area likewise revealed the existence of two morphologically very similar species. The methods presented can be used for live specimens as well as for a specimen in ethanol, using the haptor for the morphological and the body for the molecular analyses. Such a combined molecular and morphological approach for analyses of *Gyrodactylus* specimens, as also recommended by other authors (Harris *et al*. 1999), would help ensure a correct identification of species so that information, incorporating hosts, site, locality, distribution, pathogenicity and other factors, can be accurately discussed and better insights obtained.

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