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VERSITA

Gyrodactylids (Gyrodactylidae, Monogenea) infecting *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae): A pan-global survey

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Abstract

Gyrodactylus infections in intensively-reared populations of Nile tilapia, *Oreochromis niloticus niloticus*, have been associated world-wide with high mortalities of juvenile fish. In this study, 26 populations of *Gyrodactylus* parasitising either *O. n. niloticus* or Mozambique tilapia, *Oreochromis mossambicus*, were sampled from fourteen countries and compared with type material of *Gyrodactylus cichlidarum* Paperna, 1968, *Gyrodactylus niloticus* (syn. of *G. cichlidarum*) and *Gyrodactylus shariffi* Cone, Arthur et Bondad-Reantaso, 1995. Representative specimens from each population were bisected, each half being used for morphological and molecular analyses. Principal component analyses (PCA) identified five distinct clusters: (1) a cluster representing *G. cichlidarum* collected from *O. n. niloticus* from 13 countries; (2) the *G. shariffi* paratype; (3) three specimens with pronounced ventral bar processes collected from two populations of Mexican *O. n. niloticus* (*Gyrodactylus* sp. 1); (4) four specimens collected from an Ethiopian population nominally identified as *O. n. niloticus* (*Gyrodactylus* sp. 2); (5) nine gyrodactylids from South African *O. mossambicus* (*Gyrodactylus* sp. 3). Molecular analyses comparing the sequence of the ribosomal transcribed spacer regions (ITS 1 and 2) and the 5.8S gene from the non-hook bearing half of worms representative for each population and for each cluster of parasites, confirmed the presence of *G. cichlidarum* in most samples analysed. Molecular data also confirmed that the DNA sequence of *Gyrodactylus* sp. 2 and *Gyrodactylus* sp. 3 (the morphologically-cryptic group of South African specimens from *O. mossambicus*) differed from that of *G. cichlidarum* and therefore represent new species; no sequences were obtained from *Gyrodactylus* sp. 1. The current study demonstrates that *G. cichlidarum* is the dominant species infecting *O. n. niloticus*, being found in 13 of the 15 countries sampled.

Keywords

Monogenea, *Gyrodactylus*, tilapia, *Oreochromis niloticus niloticus*, *O. mossambicus*, cichlid, aquaculture

Introduction

Tilapia production has increased significantly in the last two decades, with the global production of farmed Nile tilapia, *Oreochromis niloticus niloticus* (L.), now exceeding 2 million tonnes (FAO 2006, Fitzsimmons 2006). *O. n. niloticus* and *O. mossambicus* (Peters) (Mozambique tilapia) are the most commonly reared but at least eight other species of tilapia are also cultured in a variety of production systems including net pens (hapas), open and closed water cage culture, and in recirculation systems. The intensification of systems to produce greater tonnage has placed increasing stress upon livestock, through density-dependent effects and degradation of the culture environment, which can exacerbate infectious disease problems. If not appropriately managed, regularly monitored and assessed, the health status and environmental conditions can deteriorate rapidly, resulting in large-scale fish mortalities. For many small-scale pond-based systems, a common

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practice is to stock fish seed derived either directly from wild populations or from a non-biosecure hatchery (Suresh 2003). Subsequent stock performance can be unpredictable and may be characterised by poor feeding appetence, reduced growth and the establishment of a non-indigenous parasite species which, in certain situations, has had catastrophic impacts on indigenous fish populations.

The introduction of the capsalid *Nitzschia sturionis* Abildgaard, 1794 a parasite of *Acipenser stellatus* (Pallas) in the Caspian Sea, for example, caused mass mortality of *Acipenser nudiventris* (Lovetsky) contributing to its near extinction when introduced to the Aral Sea (Rohde 1984, Bauer *et al*. 2002). Similarly, fish mortalities have resulted from the global dissemination of, for example, capsalids via exhibits

Table I. Summary details of the fish populations that were sampled for the current study, the *Gyrodactylus* material that was analysed by morphology (n = 276) and number of specimens that were sequenced (n = 79). [a]*G. cichlidarum* Paperna, 1968 (holotype), [b]*G. niloticus* (syn. of *G. cichlidarum*) Cone, Arthur et Bondad-Reantaso, 1995 (holotype and 2 paratypes), [c]*G. shariffi* Cone, Arthur et Bondad-Reantaso, 1995 (paratype)

Biogeographical regions	Longitude/latitude	Host	Microhabitat	No. of worms measured/sequenced
Afrotropic				
Gambela, Ethiopía	8°36′N/40°14′E	*O. n. niloticus* (L.)	fin	1/1
		unidentified cichlid	fins	3/1
Accra Plaíns, Ghana	6°13′N/0°05′E[a]	*Sarotherodon galilaeus galilaeus* (L.)†	skin	1/0
Private aquarium, Stellenbosch, South Africa	33°55′S/18°51′E	*O. mossambicus* (Peters)‡	skin & fins	36/7
Indo-Malaya				
China*	13°16′N/113°11′E	*O. n. niloticus* (L.)	skin & fins	9/3
Nueva Ecija Province,	15°42′N/120°54′E[b]	*O. n. niloticus*†	skin & fins	3/0
Philippines	15°42′N/120°54′E	*O. n. niloticus*	skin & fins	12/5
Iloilo Province, Philippines	10°10′N/123°36′E[c]	*O. n. niloticus*†	fin	1/0
Ban Sang district, Thailand	14°04′N/101°22′E	*O. n. niloticus*	skin & fins	14/2
Kasetsart, Thailand	13°46′N/100°28′E	*O. n. niloticus* (chitralada strain)		14/2
Private aquarium, Bac Ninh, Vietnam	21°05′N/105°05′E	*O. n. niloticus*	skin & fins	21/7
Neotropic				
Villavicencio, Colombia (V)	3°52′N/73°46′W	*O. n. niloticus* (chitralada strain)	skin & fins	9/5
Neiva, Colombia (N)	2°45′N/75°24′W	*O. n. niloticus*		15/6
Guayaquil, Ecuador	2°11′S/79°54′W	*O. n. niloticus*	skin & fins	13/4
San Pedro Sula, Honduras	15°30′N/88°00′W	*O. n. niloticus*	skin & fins	14/4
Private aquarium, Veracruz, México	23°18′N/106°28′W	*Oreochromis* hybrid (UN**)	skin & fins	10/1***
Mazatlán, México	20°12′N/96°46′W	*O. n. niloticus*‡		4/3
Mérida, México	21°01′N/89°37′W	*O. n. niloticus*‡		12/4
Private aquarium, Tabasco, México	17°56′N/92°33′W	*O. n. niloticus*‡		3/0
Private aquarium, Veracruz, México	23°18′N/106°28′W	*O. mossambicus* (MO)		9/4***
		O. n. niloticus (NG)		10/3***
		(NR)		10/3***
Palearctic				
Dakahlia Province, Egypt	31°30′N/32°16′E	*O. n. niloticus*	skin & fins	11/3
Helmond, Holland	51°28′N/5°39′E	*O. n. niloticus*	skin & fins	12/3
Beit She' an Valley, Jordan River, Israel	32°30′N/35°29′E	*O. n. niloticus*	skin & fins	11/3
Private aquarium, Stirling, UK	56°08′N/3°54′W	*O. n. niloticus*	skin & fins	18/5

†Includes type material accessed from museum archives; ‡samples collected on our behalf and only detached gyrodactylids were sent. *Site details are withheld at the request of commercial producers providing specimens. **_Oreochromis_ Pargo-UNAM is a variety of 25% rocky mountain tilapia (hybrid of *O. aureus* Steindacher × *O. n. niloticus*) and 25% red variant *O. n. niloticus* and 50% Florida red tilapia (hybrid of *O. mossambicus* × *O. urolepis hornorum* Trewavas). ***These represent samples taken at different time points throughout 2006–2007.

Table II. Univariate statistics for 25 variables measured (in µm) on the 276 gyrodactylid specimens collected from the 29 populations of fish. Figures shown in a bold font represent measured variables with a coefficient of variation greater than 10% which were subsequently rejected prior to analysis using principal components analysis

Variables	Mean (µm)	Range (µm)		Var.	CV (%)
		min.	max.		
Hamulus					
HAD	22.7	15.4	28.0	3.2	7.9
HPSW	7.6	6.1	9.6	0.4	8.3
HPL	26.2	21.4	30.1	2.8	6.4
HDSW	3.9	2.2	6.3	0.3	**13.2**
HSL	35.4	29.2	39.7	3.2	5.0
HICL	2.0	0.7	5.2	0.4	**33.2**
HAA	0.7	0.5	0.9	0.01	6.1
HPCA	6.3	1.7	15.5	3.8	**30.7**
HIAA	0.7	0.5	0.8	0.01	6.8
HRL	21.4	12.7	27.8	5.0	**10.5**
HTL	56.9	46.4	66.3	11.3	5.9
Ventral bar					
VBTW	23.1	16.7	31.3	3.8	8.5
VBTL	22.9	19.1	33.0	3.2	7.8
VBPML	2.6	1.0	13.3	1.7	**49.1**
VBML	7.1	5.1	9.5	0.6	**11.2**
VBPL	2.3	1.1	11.8	1.4	**51.1**
VBMemL	13.5	7.7	23.3	2.3	**11.2**
Marginal hook					
MHTL	28.5	19.4	34.0	3.1	6.1
MSHL	21.4	15.1	26.7	2.0	6.6
MHSiL	7.4	4.3	9.3	0.3	7.3
MHSiPW	4.3	2.9	5.3	0.1	7.0
MHSiDW	4.6	2.7	5.9	0.2	9.1
MHToeL	1.4	1.0	2.0	0.02	**11.3**
MHA	7.0	3.5	8.3	0.3	7.5
MHI/AH	0.3	0.0	0.6	0.01	**26.9**

The coefficient of variation (CV), given here as a percentage, is the square root of the variance divided by the mean. HAA – hamulus aperture angle; HAD – hamulus aperture distance; HDSW – hamulus distal shaft width; HIAA – hamulus inner aperture angle; HICL – hamulus inner curve length; HPCA – hamulus point curve angle; HPL – hamulus point length; HPSW – hamulus proximal shaft width; HSL – hamulus shaft length; HRL – hamulus root length; HTL – hamulus total length; MHA – marginal hook aperture; MHI/AH – marginal hook instep/arch height; MHSL – marginal hook shaft length; MHSiL – marginal hook sickle length; MHSiDW – marginal hook sickle distal width; MHSiPW – marginal hook sickle proximal width; MHTL – marginal hook total length; MHToeL – marginal hook toe length; VBML – ventral bar median length; VBMemL – ventral bar membrane length; VBPL – ventral bar process length; VBPML – ventral bar process-to-mid length; VBTL – ventral bar total length; VBTW – ventral bar total width.

introduced into public aquaria (Thoney and Hargis 1991, Whittington 2004) and the importation of *Gyrodactylus salaris* Malmberg, 1957 into Norway via Swedish-reared stocks of Atlantic salmon, *Salmo salar* L. and rainbow trout, *Oncorhynchus mykiss* (Johnsen and Jensen 1991, Mo 1994, Hansen *et al*. 2003).

Prior to the current study, four species of *Gyrodactylus* Nordmann, 1832 were known to parasitise tilapiine fish. The first of these, *G. cichlidarum* Paperna, 1968 is known from its type host the mango tilapia, *Sarotherodon galilaeus galilaeus* (L.), and also from Ghanian populations of red belly tilapia, *Tilapia zillii* (Gervais) (syn. *Chromis zilii*), banded jewelfish, *Hemichromis fasciatus* (Peters), and the jewelfish, *H. bimaculatus* (Gill). *Gyrodactylus nyanzae* Paperna, 1973, the second species to be documented is described from a Ugandan population of Victoria tilapia, *O. variabilis* (Boulenger) (Paperna 1973, 1979). The third species, *G. shariffi* Cone, Arthur et Bondad-Reantaso, 1995 is known from a farmed population of *O. n. niloticus* reared in the Philippines. *Gyrodactylus aegypticus* El-Naggar et El-Tantawy, 2003 is recorded from the gills of an Egyptian population of *T. zillii* in the River Nile (El-Naggar and El-Tantawy 2003). A full description of this latter species, however, is lacking and it is regarded as a *nomen nudum* (Harris *et al*. 2004). The records of *G. niloticus* Cone, Arthur et Bondad-Reantaso, 1995, reported from Philippines *O. n. niloticus*, is according to García-Vásquez *et*

al. (2007), a junior synonym of *G. cichlidarum*. The study of García-Vásquez *et al*. (2007) documented the occurrence of *G. cichlidarum* on an aquarium-reared stock of *O. n. niloticus* in the UK and also in a commercial farm at Gania de Pucté, Municipalidad de Chablé, Tabasco, México demonstrating that *G. cichlidarum* now has a wide distribution. During the completion of this study, a fifth species, *Gyrodactylus ergensi* Přikrylová, Matějusová, Musilová et Gelnar, 2009 (Přikrylová *et al*. 2009) was described from Senegalese populations of *O. n. niloticus* and *Sarotherodon g. galilaeus*. These authors also reported the occurrence of *G. cichlidarum* on *H. fasciatus* from Senegal.

The current study investigates specimens of *Gyrodactylus* collected from 29 cultured and natural populations of *O. n. niloticus* and *O. mossambicus* and looks at the extent of morphological and molecular variation between parasite specimens and populations.

Materials and methods

Hosts and parasites

Material for the current study was collected from cultured (n = 23) and wild (n = 3) stocks of *O. n. niloticus* and *O. mossambicus*. Yolk-sac fry (2 weeks post hatch) and fry (length 2–3 cm) were collected from 15 biogeographical regions representing 26 populations (29 populations including the type material borrowed from museum collections; Table I), during the period June 2004-September 2007. The sample also included gyrodactylids collected in Veracruz, México, from Pargo-UNAM a fertile *Oreochromis* hybrid resulting from the cross-breeding of 25% rocky mountain tilapia (a hybrid of *O. aureus* Steindachner × *O. n. niloticus*), 25% red variant *O. n. niloticus* and 50% Florida red tilapia (hybrid of *O. mossambicus* × *O. urolepis hornorum* Trewavas) (see Muñoz-Córdova and Garduño-Lugo 2003). Each sample of fish was fixed and stored in 95% ethanol until examination. Gyrodactylids were separated from their host tissue using triangular, surgical, mounted needles (size 16, Barber of Sheffield, U.K.) and were prepared for morphological and molecular evaluation. Type material deposited in national collections was also included within this study, namely: the holotype of *G. cichlidarum* (acc. no. 35584) from *S. g. galilaeus* from Ghana deposited in the Musée Royal de l'Afrique Centrale (MRAC), Tervuren, Belgium; the holotype (acc. no. 084007) and two paratypes (acc. no. 084008) of *G. niloticus* (*sic G. cichlidarum*) and a paratype (acc. no. 084010) of *G. shariffi*, from the USDA U.S. National Parasite Collection, Maryland, USA. Both *G. niloticus* and *G. shariffi* originate from *O. n. niloticus* from the Philippines. Type material for *G. nyanzae* and *G. ergensi*, which are morphologically similar, were not included in the current study. Both latter species possess hamuli which measure over 85 µm in total length; the largest hamuli found in the current study measured 66 µm.

Morphological studies

The haptor of each specimen was removed using a scalpel and air-dried on a glass slide; the corresponding body was transferred to a labelled Eppendorf tube containing 95% ethanol and stored at –20°C until required for molecular evaluation. Air-dried haptors were then subjected to digestion using a pro-

Table III. The component loadings and the percentage of the variance explained by each variable (n = 15) for each successive round of principal components analysis. PCA 1 (n = 276; 29 sites), PCA 2 (n = 268; 27 sites) and PCA 3 (n = 233; 8 regional groups).Values above ± 0.70 are shown in a bold font

Variable	PCA1			PCA2			PCA3		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Cos HAA	0.02	**–0.89**	–0.40	–0.38	**0.89**	0.04	–0.44	**0.85**	–0.04
Cos HIHC	0.05	**–0.86**	–0.44	–0.34	**0.90**	0.07	–0.38	**0.88**	–0.01
HAD	–0.59	0.64	0.38	–0.26	**–0.90**	–0.04	–0.33	**–0.87**	0.03
HPSW	–0.32	–0.57	0.45	–0.62	–0.01	–0.09	–0.62	0.01	–0.13
HPL	**–0.77**	–0.41	–0.00	**–0.85**	0.09	–0.04	**–0.87**	0.04	–0.01
HSL	**–0.73**	–0.39	0.16	**–0.82**	–0.04	–0.08	**–0.83**	0.00	–0.10
HTL	**–0.85**	–0.29	0.07	**-0.88**	–0.01	–0.05	**–0.88**	–0.03	–0.05
VBTL	–0.42	–0.28	0.44	–0.47	–0.16	0.08	–0.47	–0.12	0.11
VBTW	–0.35	–0.36	0.43	–0.67	–0.12	–0.00	–0.67	–0.10	0.03
MHTL	**–0.81**	0.10	–0.12	–0.67	–0.16	0.61	–0.67	–0.05	0.64
MHSL	**–0.72**	–0.03	–0.03	–0.61	–0.13	**0.71**	–0.62	–0.00	**0.72**
MHSiL	**–0.76**	0.37	–0.33	–0.69	–0.01	–0.29	**–0.76**	–0.16	–0.22
MHSiPW	–0.68	0.01	–0.24	–0.57	0.06	–0.23	–0.56	0.05	–0.20
MHSiDW	**–0.71**	0.23	–0.20	–0.57	–0.09	–0.32	–0.55	–0.19	–0.34
HAD	–0.69	0.47	–0.36	–0.54	–0.09	–0.41	–0.56	–0.10	–0.41
% Tot. var.	38.89	22.16	9.75	39.01	16.95	8.79	41.01	15.91	9.11
Cum. %	38.89	61.05	70.80	39.01	55.96	64.75	41.01	56.92	66.03

For abbreviations see Table II.

teinase K-based method. Each haptor was digested using 2.5 µl of a 100 mg/ml proteinase K solution, 10 mM EDTA, 5% SDS and 75 mM Tris-HCl. The digestion of each specimen was monitored on an Olympus SZ40 dissecting microscope using the ×4 objective (total magnification of ×40). Once all the tissues enclosing the haptoral hooks had been removed, the digestion was stopped by the addition of 3.5 µl formalin: glycerine (50:50) solution. Each specimen was then mounted with a square 18 × 18 mm "0" thickness (VWR International®) coverslip and sealed with nail varnish. The haptoral armature was studied using a ×100 magnification using an immersion oil objective on an Olympus BH2 compound microscope to which a JVC KY-F30B 3CCD camera with an interfacing ×2.5 top lens was fitted. Measurements were made on the attachment hooks using the Point-R software (version 1.0 © University of Stirling, 2003) employing the Zeiss KS300 iC/Windows release version 3.0 (1997) (Carl Zeiss Vision GmbH, München, Germany) application platform. From each specimen, a total of 25 point-to-point measurements were made following the protocol detailed in Shinn *et al*. (2004). Digital images of the attachment hooks of each type species and from representative specimens within clusters of interest highlighted by the subsequent morphological, statistical and molecular studies were captured using the same equipment previously described.

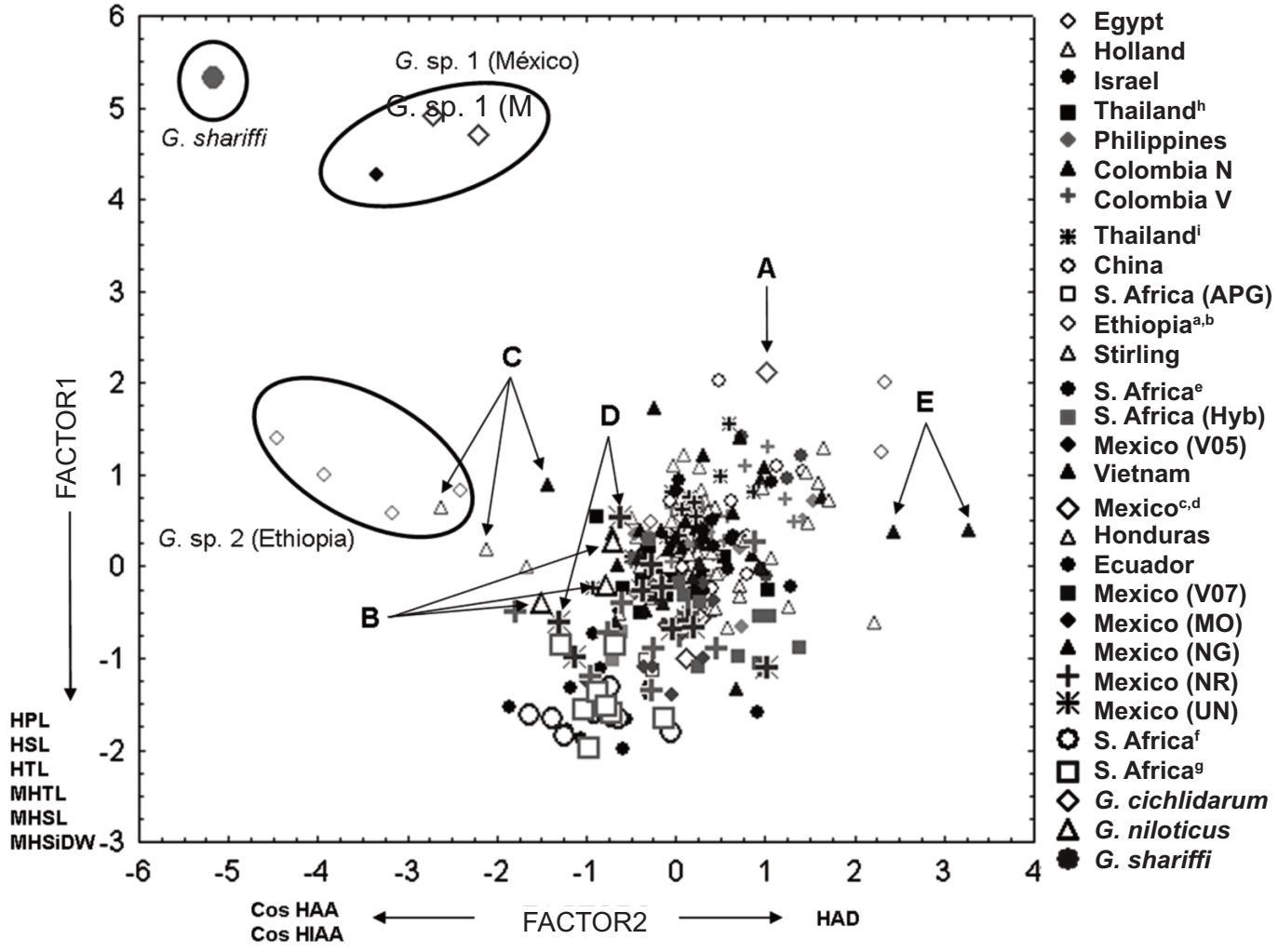

Fig. 1. Plot of the 276 specimens of *Gyrodactylus* von Nordmann, 1832 shown in the first plane of the PCA Factor 1 *vs* Factor 2 based on 15 log-transformed morphometric variables with a coefficient of variation <10%. Four discrete clusters are identified: *G. shariffi* Cone, Arthur et Bondad-Reantaso, 1995 from *O. n. niloticus* (L.) from the Philippines; *G*. sp. 1 from Mexican *O. n. niloticus*, the ventral bars of which bear large processes; and, *G*. sp. 2 from *O. n. niloticus* and an unidentified cichlid host from Ethiopia. The variables making a major contribution to the separation of specimens and the direction in which they act are given on each factor. The free-hand ellipses highlight specimens of interest. **Abbreviations:** A – holotype of *G. cichlidarum* Paperna, 1968; B – holotype and two paratypes of *G. niloticus* (syn. of *G. cichlidarum*); C – two specimens of *Gyrodactylus* sp. from aquarium-held *O. n. niloticus* UK (Stirling) and one from *O. n. niloticus* from Vietnam; D – *Gyrodactylus* sp. from the *Oreochromis* hybrid Pargo-UNAM from Veracruz, México; E – *Gyrodactylus* sp. from *O. n. niloticus* from Neiva, Colombia; HAA – hamulus aperture angle; HAD – hamulus aperture distance; HIAA – inner hamulus angle; HPL – hamulus point length; HSL – hamulus shaft length; HTL – hamulus total length; MHSiDW – marginal hook sickle distal width; MHSL – marginal hook shaft length; MHTL – marginal hook total length

Molecular analysis

The DNA from the bodies of approximately 5 individuals from each population were extracted using the QIAamp DNA Mini Kit (Qiagen). The fragment between the 3' end of the 18S subunit, the ITS1, ITS2 and 5.8S gene and the 5' end of the 28S subunit was amplified (PCR) using the primers ITS1A (5'-GTAACAAGGTTTCCGTAGGTG-3') and ITS2 (5'-TCCTCCGCTTAGTGATA-3') (Matějusová *et al*. 2001). If the first amplification failed, an attempt to amplify only ITS2 was tried with the primers ITS4.5 and ITS2 (Matějusová *et al*. 2001). The PCR reaction contained 3 µl of DNA template, 1 µl of each primer (10 pmol), 20 µl Milli-Q water added to PuReTaq Ready-To-Go PCR beads (GE Healthcare) in a 0.2 ml tubes in a final volume of 25 µl. The reaction was performed in a GeneAmp PCR system 9700 (Applied Biosystems) using the following protocol: 4 mins at 95°C followed by 35 cycles of 1 min at 95°C, 1 min at 55°C and 2 mins at 72°C. To purify the PCR products, the NucleoSpin® Purification Kit (Macherey-Nagel) was used following the manufacturer's protocol. Sequencing was performed with the PCR primers and different combinations of internal primers; ITS2F (5'-TGGTGGATCACTCGGC TCA-3') with ITS1R (5'-ATTTGCGTTCGAGAGACCG-3') (Ziętara and Lumme 2003) or ITSR3A (5'-GAGCC-GAGTGATCCACC-3') with ITS2R (5'-GGTAATCACGC

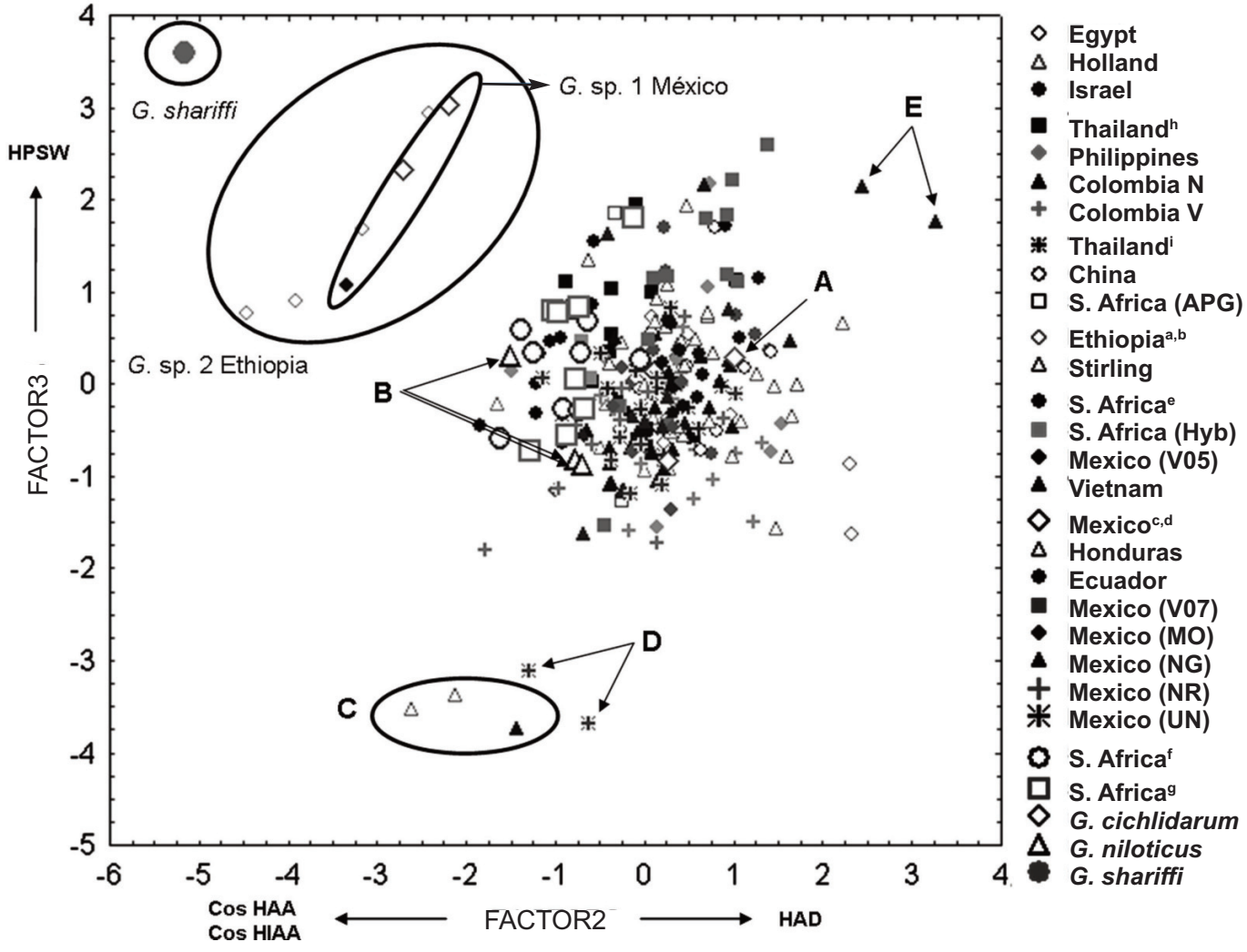

Fig. 2. PCA 1 plot of Factor 2 *vs* Factor 3 for 276 specimens of *Gyrodactylus* von Nordmann, 1832. The distribution highlights a number of discrete groups for either removal based on morphological differences or for further characterisation using molecular based approaches. The variables making a major contribution to the separation of specimens and the direction in which they act are given on each factor. The free-hand ellipses highlight specimens of interest. **Abbreviations:** A – holotype of *G. cichlidarum* Paperna, 1968; B – holotype and two paratypes of *G. niloticus* (syn. of *G. cichlidarum*); C – two specimens of *Gyrodactylus* sp. from aquarium-held *O. n. niloticus* UK (Stirling) and one from *O. n. niloticus* from Vietnam; D – *Gyrodactylus* sp. from the *Oreochromis* hybrid Pargo-UNAM from Veracruz, México; E – *Gyrodactylus* sp. from *O. n. niloticus* from Neiva, Colombia; HAA – hamulus aperture angle; HAD – hamulus aperture distance; HIAA – inner hamulus angle; HPSW – hamulus proximal shaft width

TTGAATC-3') (Matějusová *et al*. 2001, Ziętara and Lumme 2003). Sequencing was carried out on a MegaBace 1000 analysis system (GE Healthcare) using DYEnamic ET dye terminators. For a few specimens a rough molecular identification was obtained by sequencing the ITS1 fragment with the ITS1R primer only and then comparing them to the full length sequences in this study. Sequences were proofread and assembled using Vector NTI 10 (Invitrogen). Mega 4 (Tamura *et al*. 2007) was used to align sequences and to calculate genetic distances. The sequences were submitted to a BLASTN search (Zhang *et al*. 2000) with default parameter settings to establish possible identity with other species.

Statistical analysis

Principal Component Analysis (PCA) using the statistical package Statistica 6.0 (StatSoft, Inc., 1997) was used to identify clusters in the dataset and individual specimens for further detailed PCA and molecular characterisation. A total of 25 point-to-point measurements were made on each of the 276 specimens. Variables with coefficient of variation (CV) values greater than an arbitrarily set level of 10% were removed prior to PCA analysis (Table II). The remaining 15 variables were log (ln)-transformed to correct for increasing variance with increasing mean size of the measured variables (Shinn *et al*. 1996). The two angular-based variables (HIHC – hamulus

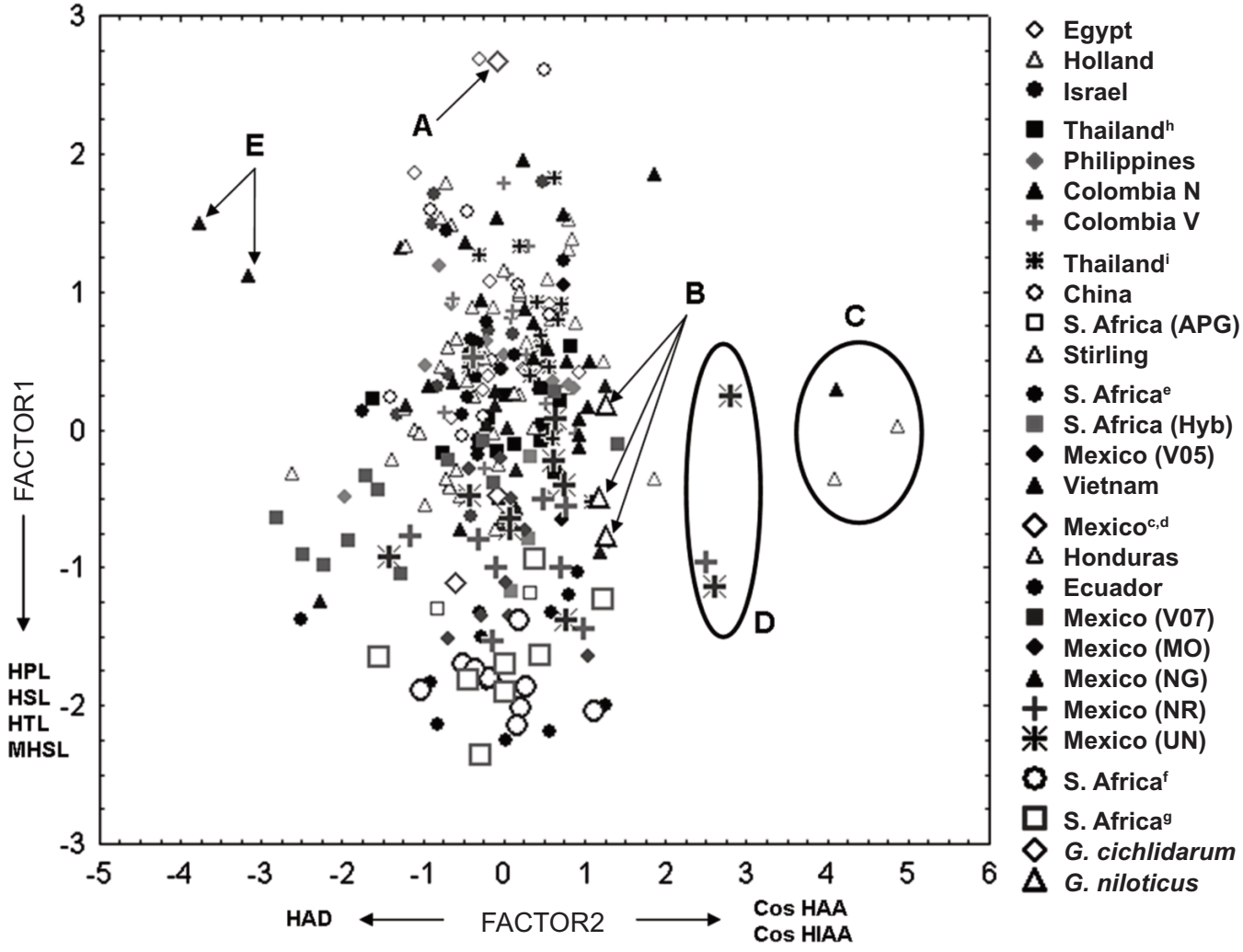

Fig. 3. PCA 2 plot of the first two principal factors for 268 specimens of *Gyrodactylus* von Nordmann, 1832. The plot highlights two further clusters of specimens, C and D, for removal based on their differing morphology from *G. cichlidarum* Paperna, 1968. The free-hand ellipses highlight specimens of interest. **Abbreviations:** A – holotype of *G. cichlidarum*; B – holotype and two paratypes of *G. niloticus* (syn. of *G. cichlidarum*); C – two specimens of *Gyrodactylus* sp. from aquarium-held *O. n. niloticus* (L.) UK (Stirling) and one from *O. n. niloticus* from Vietnam; D – *Gyrodactylus* sp. from the *Oreochromis* hybrid Pargo-UNAM from Veracruz, México and one specimen from *O. niloticus* (red variant); E – *Gyrodactylus* sp. from *O. n. niloticus* from Neiva, Colombia; HAA – hamulus aperture angle; HAD – hamulus aperture distance; HIAA – inner hamulus angle; HPL – hamulus point length; HSL – hamulus shaft length; HTL – hamulus total length; MHSiL – marginal hook sickle length

inner angle; HAA – hamulus aperture angle) were transformed to a linear measurement by taking their cosine. Several rounds of PCA were performed, removing discrete, clearly separated clusters at each stage.

Results

Principal components analysis of the morphometric data (n = 276 gyrodactylids) revealed that the specimens formed a number of discrete clusters: the paratype of *G. shariffi*; 3 specimens collected from two populations of Mexican *O. n. niloticus* (*G.* sp. 1); 4 specimens collected from two Ethiopian fish, one *O. n. niloticus*, the other nominally identified as *O. n. niloticus* (*G.* sp. 2); a major cluster of gyrodactylids parasitising a population of South African *O. mossambicus* (*G.* sp. 3); and the remaining cluster representing *G. cichlidarum* collected from *O. n. niloticus* from 13 countries. Representative specimens from each of these PCA clusters were then subjected to molecular characterisation (i.e. sequencing of the ITS1, 2 and 5.8S). Additional specimens from each population were selected at random and sequenced. The findings of the molecular study confirmed that each morphologically different type of *Gyrodactylus* had specific sequences, different from each other, and with little or no intra-individual variation.

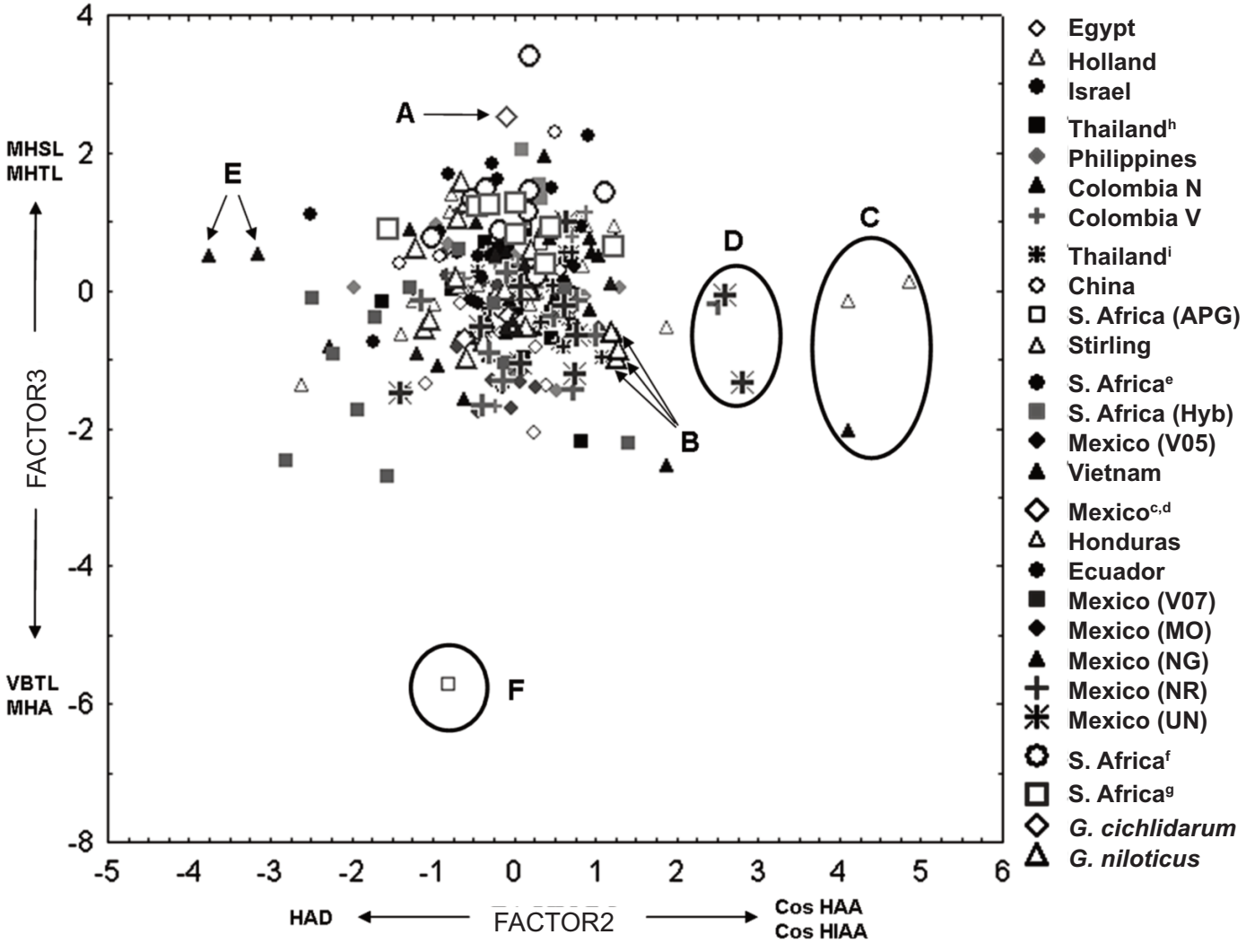

Fig. 4. PCA 2 plot of Factor 2 *vs* Factor 3 highlights a further specimen, F, for removal. The free-hand ellipses highlight specimens of interest. **Abbreviations:** A – holotype of *G. cichlidarum* Paperna, 1968; B – holotype and two paratypes of *G. niloticus* (syn. of *G. cichlidarum*); C – two specimens of *Gyrodactylus* sp. from aquarium-held *O. n. niloticus* (L.) UK (Stirling) and one from *O. n. niloticus* from Vietnam; D – *Gyrodactylus* sp. from the *Oreochromis* hybrid Pargo-UNAM from Veracruz, México and one specimen from *O. niloticus* (red variant); E – *Gyrodactylus* sp. from *O. n. niloticus* from Neiva, Colombia; F – *Gyrodactylus* sp. from South Africa (APG); HAA – hamulus aperture angle; HAD – hamulus aperture distance; HIAA – inner hamulus angle; MHA – marginal hook aperture distance; MHSiL – marginal hook sickle length; MHSL – marginal hook shaft length; VBTL – ventral bar total length

Table IV. Details of the gyrodactylid specimens removed after each round of principal components analysis based on the 15 log-transformed measurements taken on each specimen

PCA	No. of specimens	No. removed	Host	Site	Specimen features/justification for removal
1	276	1	*O. n. niloticus* (L.)	Mérida, México	HTL<50 µm; MHTL<20 µm; *G.* sp. 1
		2	*O n. niloticus*	Tabasco, México	HTL<50 µm; MHTL<20 µm; *G.* sp. 1
		3	unidentified cichlid	Ethiopia	HAD<21 µm; HAA<36 µm; HIHC<42 µm; *G.* sp. 2
		1	*O. n. niloticus*	Ethiopia	HAD<21 µm; HAA<36 µm; HIHC<42 µm; *G.* sp. 2
		1	*O. n. niloticus*	Philippines	HTL<50 µm; MHTL<20 µm; *G. shariffi* paratype (USDA no. 084008)**
2	268	2	*Oreochromis* hybrid	México (UN)	HAD<21 µm; HAA<35 µm; HIHC<40 µm; Group D
		1	*O. n. niloticus*	México (NR)	HAD<21 µm; HAA< 35µm; HIHC<40 µm; Group D
		2	*O. n. niloticus*	UK (Stirling)	HAD<17 µm; HAA<33 µm; HIHC<39 µm; Group C = *G.* sp. variant in García-Vásquez *et al.* (2007)
		1	*O. n. niloticus*	Vietnam	HAD<17 µm; HAA<33 µm; HIHC<39 µm; Group C = *G.* sp.*
		1	*O. mossambicus* (Peters)	South Africa (APG)	VBTL>27 µm; MHTL<25 µm; Group F = *G.* sp. 3**
		16	*O. n. niloticus*	UK (Stirling)	Aquarium held stock – original source unknown
		12	*O. n. niloticus*	Holland	Aquarium held stock – original source unknown

Abbreviations: HAA – hamulus aperture angle; HAD – hamulus aperture distance; HIAA – hamulus inner angle; HTL – hamulus total length; MHTL – marginal hook total length; NR – red *O. n. niloticus* from Veracruz, México; UN – *Oreochromis* hybrid Pargo-UNAM from Veracruz, México; VBTL – ventral bar total length. *Removed on the basis that they were morphologically similar to *Gyrodactylus* sp. variant described in García-Vásquez *et al.* (2007); **specimens were fixed and sent already mounted and could not be sequenced.

Multivariate analyses of morphometric data

Three PCAs were performed on the ln-transformed data of 276 specimens from 29 separate populations including the type material of *G. cichlidarum*, *G. niloticus* (syn. of *G. cichlidarum*) and *G. shariffi*, to identify outlying specimens. The component loadings for the 15 variables used in each round of PCA are presented in Table III; these indicate which variables had a major effect (i.e., a value of ± 0.7) on the separation of specimens through each principal axis in each PCA.

Identification of discrete clusters

PCA1. The PCA plot of the first two factors (Factor 1 *vs* Factor 2; Fig. 1) accounted for 38.89% and 61.06% of the total variance, respectively, and revealed four discrete clusters. Using the factor loadings (Table III), the hamulus point length (HPL), shaft length (HSL), total length (HTL) and the marginal hook total length (MHTL), shaft length (MHSL) and the sickle distal width (MHSiDW) had the greatest effect on the separation of specimens acting through Factor 1. For Factor 2, the two angle-based variables (HAA and HIAA) acting in a negative direction and the hamulus aperture distance (HAD) acting in a positive direction, were key to the separation of specimens along Factor 2. Eight specimens formed three discrete clusters away from the majority of specimens (see Table IV). The paratype of *G. shariffi* (approx. co-ords +5.5 Factor 1, –5.0 Factor 2; Fig. 1) possesses, among other features, the smallest hamulus point length of all the measured specimens (Fig. 1; Table V). The second cluster (approx. co-ords +4.6 Factor 1, –2.75 Factor 2; Fig. 1) consisted of three specimens from Mexican *O. n. niloticus*, all of which possessed small hamulus point lengths. The ventral bars of these three specimens were distinct. Based on these morphological differences, these three specimens were classified as *Gyrodactylus* sp. 1.

A third cluster, labelled "*G.* sp. 2 Ethiopia" (Figs 1 and 2), comprised four specimens including one gyrodactylid removed from *O. n. niloticus* and three other prepared from an unidentified cichlid nominally identified as *O. n. niloticus* caught in Lake Baro, Gambela, Ethiopia. These four specimens could be separated from the main cluster of specimens along Factor 2 on the basis of having large cosine values for the two angle based variables, HAA and HIAA. This corresponds to comparatively small hamulus aperture angles (HAA = 34.6 ± 4.3° *vs* 43.6 ± 2.9° for all 276 specimens; HIAA = 40.5 ± 5.7° *vs* 48.8 ± 1.7° for all 276 specimens).

The fourth cluster consisted of the remaining specimens. Within this large cluster, however, some additional specimens labelled C-E, were also of interest and most evident in the PCA plot of Factor 2 *vs* Factor 3 (Fig. 2). Those labelled as "C" consisted of two specimens from *O. n. niloticus* stock held in a Scottish aquarium and one specimen from Vietnam while those labelled "D" consisted of two specimens from the *Oreochromis* hybrid from Veracruz, México (UN). Specimens be-

Table V. Morphological measurements (mean ± st. dev. followed by the range in parentheses) of the *Gyrodactylus* from each cluster after morphological and molecular analysis. *Gyrodactylus* sp. 1 from Tabasco and Mérida, México (*O. n. niloticus* L.), *Gyrodactylus* sp. 2 from Ethiopia (*O. n. niloticus*), *Gyrodactylus* sp. 3 from South Africa (*O. mossambicus* Peters), *Gyrodactylus* sp. 4 the variant from Stirling, UK and Vietnam (*O. n. niloticus*)

Measurement	*G. cichlidarum* holotype n = 1	*G. cichlidarum* (syn. *G. niloticus*) n = 3	*G. shariffi* paratype n = 1	*G.* sp. 1 n = 4	*G.* sp. 2 n = 4	*G.* sp. 3 n = 9	*G.* sp. 4 n = 3	*G. cichlidarum* n = 251
HAD	22.2	21.4 ± 0.4 (21–22)	15.5	16.8 ± 1.1 (15–18)	18.7 ± 1.8 (17–21)	23.3 ± 1.2 (22–26)	15.8 ± 0.5 (15–16)	22.9 ± 1.3 (19–28)
HPSW	7.7	8.3 ± 0.7 (7–9)	8.7	7.9 ± 0.5 (7–8)	9.1 ± 0.5 (8–10)	8.0 ± 0.5 (7–9)	7.2 ± 0.5 (6–8)	7.5 ± 0.6 (6–9)
HPL	24.3	27.1 ± 0.2 (26–27)	21.9	22.7 ± 0.5 (22–23)	28.4 ± 0.9 (27–30)	28.5 ± 1.0 (27–30)	27.3 ± 0.7 (26–28)	26.1 ± 1.6 (21–30)
HSL	29.2	35.8 ± 0.9 (35–36)	31.2	32.2 ± 0.7 (31–33)	37.6 ± 0.5 (37–38)	37.6 ± 1.4 (35–40)	33.3 ± 0.5 (33–34)	35.3 ± 1.6 (29–39)
HAA	45.2	39.6 ± 0.3 (39–40)	30.3	37.9 ± 2.4 (34–40)	34.6 ± 4.3 (31–40)	41.1 ± 2.5 (36–45)	31.1 ± 2.2 (28–33)	43.5 ± 2.9 (34–57)
HIAA	51.5	44.7 ± 0.6 (44–45)	33.5	43.3 ± 3.3 (39–46)	40.5 ± 5.7 (34–47)	46.7 ± 3.0 (41–50)	37.2 ± 2.0 (35–38)	48.7 ± 3.0 (38–58)
HTL	54.3	58.4 ± 0.9 (57–59)	47.5	48.4 ± 1.1 (47–49)	57.5 ± 1.0 (57–59)	61.9 ± 2.1 (59–65)	55.0 ± 1.3 (53–56)	56.8 ± 3.2 (46–66)
VBTW	19.6	22 ± 2.0 (20–24)	21.6	20.4 ± 0.6[a] (20–21)	27.6 ± 2.6 (25–31)	24.7 ± 2.0 (22–29)	22.4 ± 0.2 (22–23)	23.0 ± 1.9 (17–31)
VBTL	19.2	23.8 ± 1.6 (22–25)	32.8	24.7 ± 1.3[a] (23–26)	21.2 ± 1.6 (19–23)	24.4 ± 1.3 (22–26)	23.0 ± 0.3 (22–23)	22.8 ± 1.6 (19–33)
MHTL	27.9	27.5 ± 0.7 (26–28)	19.4	22.3 ± 1.0 (22–24)	27.7 ± 0.5 (27–28)	31.3 ± 0.7 (30–32)	27.4 ± 1.1 (26–28)	28.5 ± 1.5 (21–34)
MHSL	21.6	20.8 ± 0.6 (20–22)	15.1	18.0 ± 1.4 (17–20)	22.4 ± 0.6 (22–23)	23.7 ± 0.7 (22–25)	20.4 ± 1.5 (19–22)	21.4 ± 1.3 (16–27)
MHSiL	6.5	7.4 ± 0.2 (7–8)	4.3	4.5 ± 0.2 (4–5)	5.5 ± 0.1 (5–6)	7.7 ± 0.1 (7–8)	8.0 ± 1.1 (7–9)	7.4 ± 0.3 (6–8)
MHSiPW	2.9	4.4 ± 0.2 (4–5)	3.3	3.3 ± 0.2 (3–4)	4.0 ± 0.1 (4–5)	4.4 ± 0.2 (4–5)	4.4 ± 0.2 (4–5)	4.3 ± 0.3 (3–5)
MHSiDW	3.9	4.6 ± 0.2 (4–5)	2.7	3.2 ± 0.1 (3–4)	3.6 ± 0.3 (3–4)	4.7 ± 0.3 (4–6)	5.1 ± 0.6 (4–6)	4.6 ± 0.3 (3–6)
MHA	6.8	7.3 ± 0.06 (7–8)	3.5	4.2 ± 0.3 (4–5)	5.0 ± 0.2 (5–6)	7.1 ± 0.2 (6–8)	7.0 ± 0.2 (6–8)	7.1 ± 0.3 (6–8)

For abbreviations see Table II.

longing to C and D were separated from the main aggregation of specimens principally on the basis that these specimens possessed small hamulus shaft proximal width values. The two specimens labelled "E" (Figs 1 and 2) represent gyrodactylids from Neiva, Colombia (Colombia N) which possessed slightly larger hamulus aperture distances (HAD) than those of the main cluster i.e., 27.53 ± 0.66 (Neiva) *vs* 23.0 ± 1.3 (all specimens). All the specimens belonging to groups C-E were retained for the second round of analysis.

PCA2. The *G. shariffi* paratype (n = 1), three *G.* sp. 1 (México) and four *G.* sp. 2 (Ethiopia) specimens were removed prior to the second PCA (n = 268, Table V). The second PCA analysis and plot of Factor 1 *vs* 2 (Fig. 3) revealed further clusters within the remaining specimens. Factor loadings presented in Table III highlight which variables (i.e., ± 0.7 and shown in a bold font) have a major effect in the separation of specimens. For Factor 1, the total, shaft and point lengths of the hamuli (HTL, HSL, HPL) are important features in separating specimens. For Factor 2 it is the hamulus aperture angle (Cos HAA) and the inner hamulus angle (Cos HIAA) acting in one (positive) direction and the hamulus aperture distance (HAD) acting in the other (negative) direction along Factor 2 that are important. In this analysis, the specimens detected in the first PCA as C and E were separated from the main cluster for having small Cos HAA and Cos HIAA values (Figs 3 and 4). The second cluster included two specimens labelled "D" from the *Oreochromis* hybrid from Veracruz, México which possessed smaller HPL, HSL and HTLs than those of the main cluster of specimens (Figs 3 and 4). The PCA shows another cluster including the holotype of *G. cichlidarum* and a specimen from Egypt and another from China. The plot of Factor 2 against Factor 3, shows the same three groups of C-E specimens with the addition of a further specimen from South Africa (F) which appears to have a large VBTL (27.1 *vs* all specimens 22.9 ± 1.7) and MHA (7.7 *vs* all specimens 7.08 ± 0.30) values (Fig. 4). The specimens belonging to C, D and F were subsequently removed. In addition, the 28 gyrodactylids collected from the

O. n. niloticus stock held in Holland and Stirling were removed prior to the third round of PCA because the origin of this stock was unknown.

PCA3. The third PCA analysis investigated whether specimens coded by the biogeographical region from which they were collected, grouped together in the PCA plots. The *G. cichlidarum* (including "*G. niloticus*") type specimens were retained as were the gyrodactylids labelled "E" and all the gyrodactylids sampled from both *O. n. niloticus* and *O. mossambicus* from México. Following the removal of the 35 specimens identified from the second PCA (see Table IV), the organisation of the remaining specimens (n = 233) was examined and classified by biogeographical region. The PCA plot of Factor 1 *vs* Factor 2 as presented in Figure 5 revealed three identifiable clusters. The first cluster consists of three specimens comprising the holotype of *G. cichlidarum* (arrowed "A" in Fig. 5) bordered by one specimen from Egypt and one from China. The second cluster consists of two specimens collected from *O. n. niloticus* from Neiva, Colombia and are arrowed as "E" in Figure 5. The third cluster comprises most of the specimens originating from Sub-Saharan Africa *O. mossambicus*; these are identified by a free-hand ellipse enclosing them in Figure 5. When the specimens in PCA3 were labelled by host, Figure 6 shows that there is good separation between the gyrodactylids on *O. n. niloticus* and those on South African *O. mossambicus* but poor separation between those on Mexican *O. mossambicus* and *O. n. niloticus*.

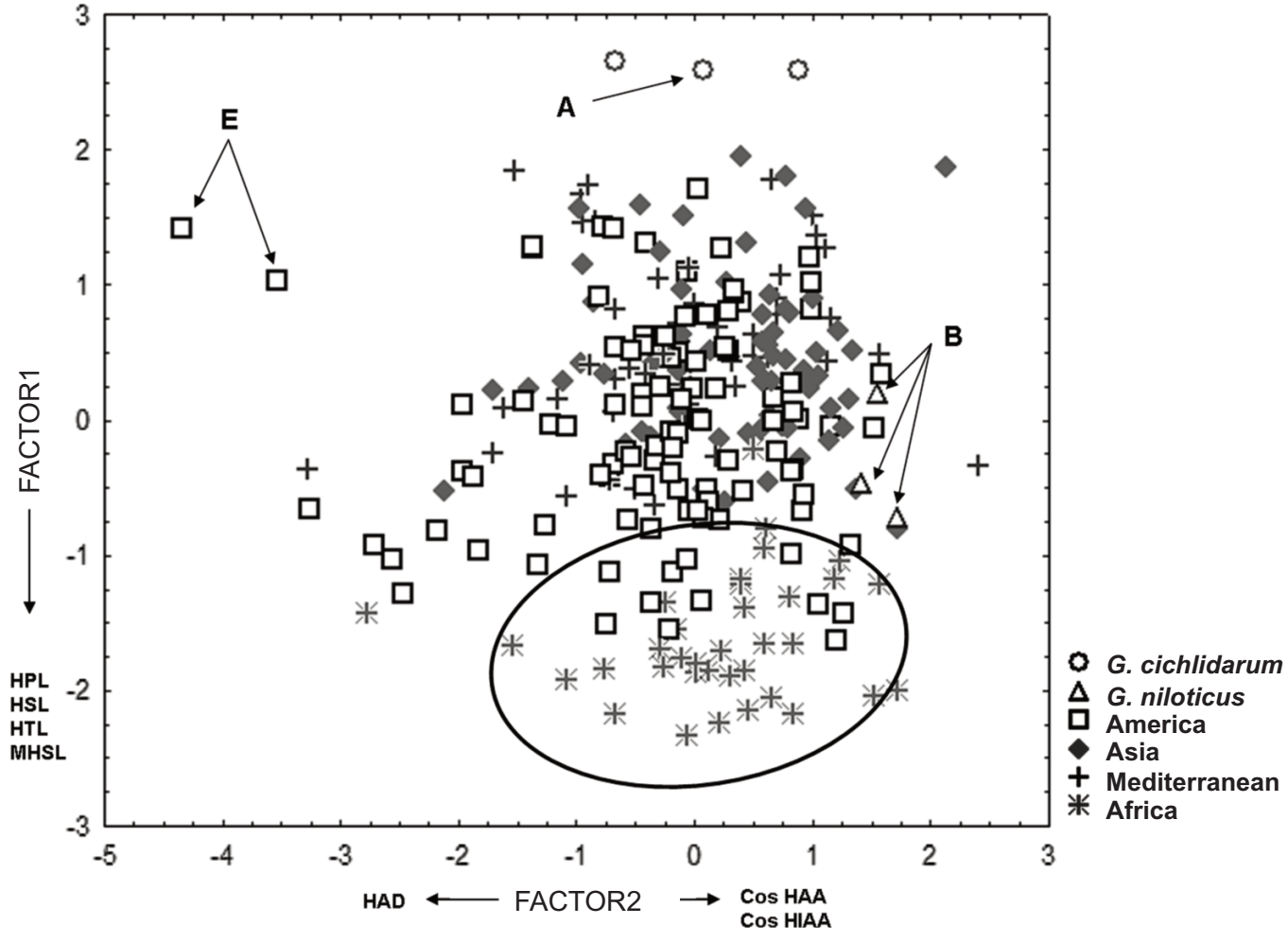

Fig. 5. PCA 3 plot of Factor 1 *vs* Factor 2 for the remaining 233 specimens of *Gyrodactylus* identified by the biogeographical region from which the fish hosts were collected. Afrotropic: South Africa (✳); Indo-Malaya: China, Philippines, Thailand and Vietnam (◆); Neotropic: Villavicencio Colombia, Ecuador and Honduras (□); Neiva Colombia (▲) and México (✹); Palearctic: Egypt and Israel (+). Type material of *G. cichlidarum* (◇) and *G. niloticus* (syn. of *G. cichlidarum*) (△) are highlighted. **Abbreviations:** A – holotype of *G. cichlidarum* Paperna, 1968; B – holotype and two paratypes of *G. niloticus* (syn. of *G. cichlidarum*); E – *Gyrodactylus* sp. from *O. n. niloticus* (L.) from Neiva, Colombia; HAA – hamulus aperture angle; HAD – hamulus aperture distance; HIAA – inner hamulus angle; HPL – hamulus point length; HSL – hamulus shaft length; HTL – hamulus total length; MHSiL – marginal hook sickle length

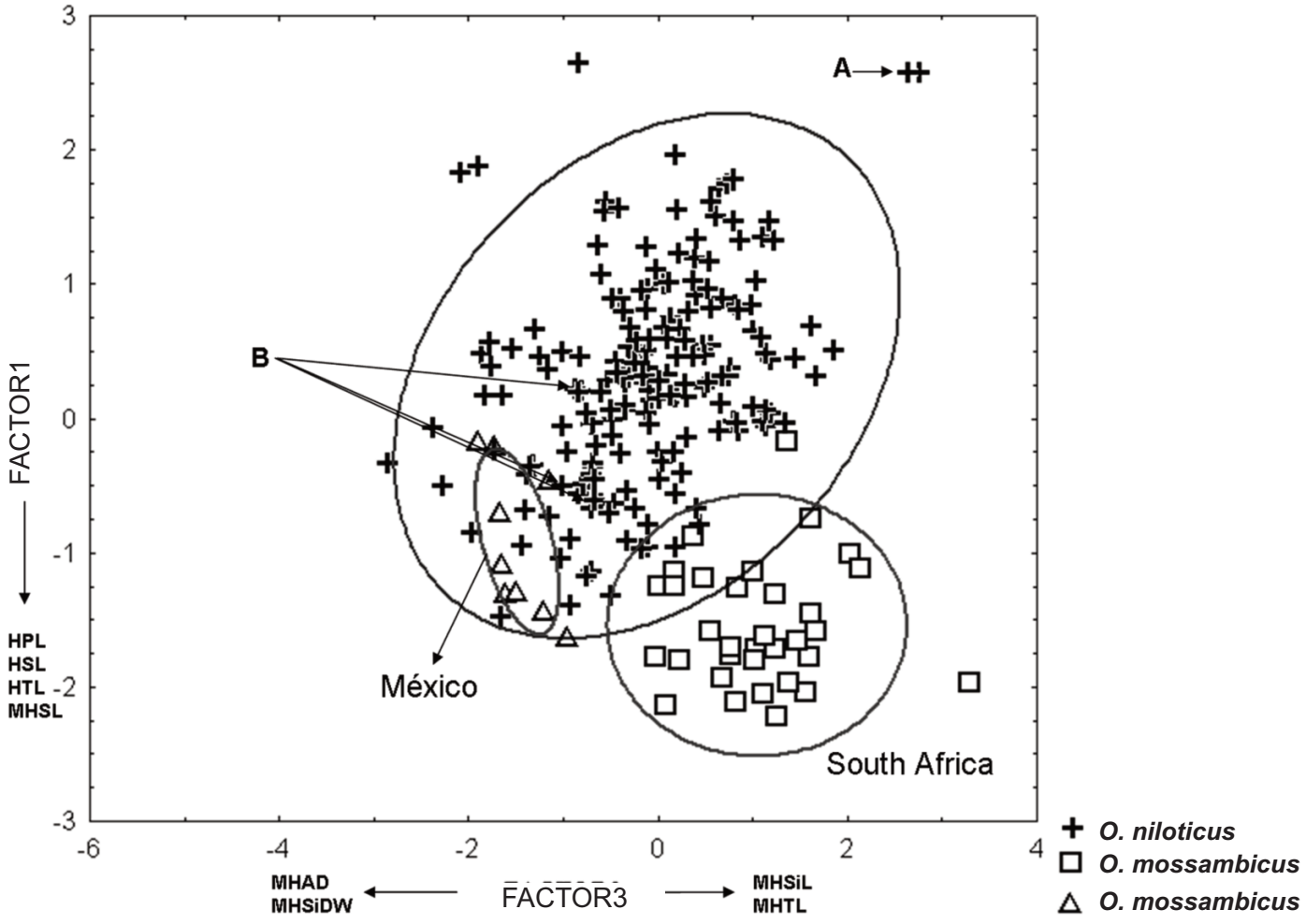

Fig. 6. PCA 4 plot Factor 1 *vs* Factor 3 for the remaining 233 specimens of *Gyrodactylus* identified by the host they parasitised: *O. n. niloticus* (L.) (✚) and *O. mossambicus* (Peters) from México (△) and South Africa (□). **Abbreviations:** A – holotype of *G. cichlidarum* Paperna, 1968; B – holotype and two paratypes of *G. niloticus* (syn. of *G. cichlidarum*); HAA – hamulus aperture angle; HAD – hamulus aperture distance; HIAA – inner hamulus angle; HPL – hamulus point length; HSL – hamulus shaft length; HTL – hamulus total length; MHSiL – marginal hook sickle length

Molecular characterisation

Following morphological analysis, the full fragment consisting of the ribosomal transcribed spacer regions (ITS1 and ITS2) and the 5.8S gene were sequenced and compared for 79 specimens (see Table I for details). No usable sequences were obtained from the four specimens of *G.* sp. 1 from México; the use of formalin during the collection procedure is suspected. Molecular analysis and a subsequent BLASTN search of the sequences in GenBank (July 2008), confirmed that the three specimens of *G.* sp. 2 (accession number FJ231869) were different from *G. cichlidarum* (accession number DQ124228) and that they represent a new *Gyrodactylus* species. In addition, the molecular data revealed that the nine gyrodactylids sampled from the South African population of *O. mossambicus* (accession number FJ231870), which were morphologically similar to *G. cichlidarum* (Table V) were found to differ significantly in their sequence (García-Vásquez *et al*., submitted). Although the 5.8S sequence of this latter species, identified as *G.* sp. 3, was identical to *G. cichlidarum*, there were 42 nucleotide substitutions (0.049 uncorrected "p" distance) in the two ITS regions. These substitutions can be attributed to 24 differences in ITS1 (12 transitions and 12 transversions) and 18 substitutions in ITS2 (9 transitions and 9 transversions), in addition to a single indel. The 5 gyrodactylids that were sequenced from the Philippines were identical to those of *G. cichlidarum*. The findings of the molecular study support those of the PCA in that each morphologically different type of *Gyrodactylus* had specific sequences with little or no intra-individual variation.

Table VI. A summary of the current study's findings with regard to the global distribution of each *Gyrodactylus* species infecting tilapiine species, including previously published records

***Gyrodactylus* species**	**Host**	**Country**
G. cichlidarum Paperna, 1968	*Hemichromis bimaculatus* Gill	Ghana[a]
	Hemichromis fasciatus Peters	Ghana[a]
	Hemichromis flaviijosephi Lortert	Israel[b], Jordan[b]
	Oreochromis aureus Steindachner	Israel[b], Jordan[b], Puerto Rico[c], UK (private aquarium)[d], México[e]
	Oreochromis Pargo-UNAM hybrid	México*[e]
	Oreochromis karongae Trewavas	UK (private aquarium)**
	Oreochromis mossambicus Peters	México*[e]
	Oreochromis niloticus niloticus L.	China*, Colombia*, Egypt*, Ecuador*, Holland*, Honduras*, Israel*, México*[f], Philippines*, South Africa*, Thailand*, UK (private aquarium)*, Vietnam*
	Sarotherodon galilaeus galilaeus L.	Ghana[a]
	Sarotherodon melanotheron heudelotii	Dumöril Israel[b], Jordan[b]
	Tilapia guineensis Günther	Ghana[b]
	Tilapia zillii Steindachner	Israel[b], Jordan[b]
	Tristamella simonis simonis Günther	Israel[b], Jordan[b]
G. shariffi Cone, Arthur et Bondad-Reantaso, 1995	*O. n. niloticus*	Philippines*
Gyrodactylus sp. 1	*O. n. niloticus*	México*
Gyrodactylus sp. 2	*O. n. niloticus*	Ethiopia*
Gyrodactylus sp. 3	*O. mossambicus*	South Africa*
Gyrodactylus sp.[g]	*O. n. niloticus*	UK[g], Vietnam[g]

*Current study; **experimental challenge with *G. cichlidarum* where infections were maintained for two weeks (García-Vásquez, unpublished data); [a]*G. cichlidarum* type material; [b]additional hosts and locations (Paperna 1979); [c]Bunkley-Williams and Williams (1994); [d]identity based on morphology only (Shinn unpublished data); [e]Salgado-Maldonado *et al*. 2005; [f]Lopez-Jimenez (2001); [g]*Gyrodactylus* specimens found to have different morphology (García-Vásquez *et al*. 2007) but same molecular profile as *G. cichlidarum*.

Discussion

The current study demonstrates that *G. cichlidarum* is the dominant species infecting *O. n. niloticus*, being found in 13 of the 15 countries sampled (Table VI). From this study, the distribution of *G. cichlidarum* extends, at least, from Scotland and Holland (52°–56°N) to the north and South Africa (33°55´S) to the south, and to the Philippines (120°54´E) to the east and to México (106°28´W) to the west.

The morphometric analysis identified four discrete clusters suggesting that the type specimen of *G. shariffi* parasitising *O. n. niloticus* in the Philippines is different to *G. cichlidarum* and the gyrodactylids forming discrete clusters. In addition, the PCA identified a discrete cluster of 3 gyrodactylids from Mexican *O. n. niloticus* (*G.* sp. 1) and 4 gyrodactylids from Ethiopian *O. n. niloticus* (*G.* sp. 2). A fourth cluster, representing 9 gyrodactylids parasitising a population of *O. mossambicus* from South Africa (*G.* sp. 3), was suggested by PCA. Sequencing the ITS regions from representative samples from each of the clusters (except *G. shariffi* where no material was available) confirmed each morphologically different type of *Gyrodactylus* had specific sequences with little or no intra-individual variation. Specimens belonging to *G.* sp. 3, however, were morphologically cryptic; their discrimination from *G. cichlidarum* using PCA proved to be difficult and they were only effectively identified through sequencing.

Gyrodactylus sp. 1 from México differs markedly from the other known species of *Gyrodactylus* and from the other specimens examined here in that they possess large ventral bar processes and a square ventral bar membrane (Table V) (García-Vásquez *et al*., submitted).

Only the morphometric features with a coefficient of variation smaller than 10% were included within the PCA analysis. The variables with higher coefficients of variation were mainly those associated with the ventral bar and the hamuli. The variables which were on average less than 5 µm in size were found to contribute little to the separation and determination of species. Despite the rejection of 10 morphometric variables prior to PCA analysis, the 15 remaining variables were sufficient to separate and discriminate the three new species (*G.* sp. 1–3). Clear differences were noted in the morphology of the marginal hooks and the hamuli between the species found in México (Tabasco and Mazatlán) (*G.* sp. 1) and Ethiopia (*G.* sp. 2), consistent with the results of the molecular analyses (only for the Ethiopian species). This was not, however, the case for the species found on South African *O. mossambicus* (*G.* sp. 3), which presented morphological features similar to those of *G. cichlidarum* while displaying clearly different molecular profiles.

The specimens labelled "C" in PCA 1 and 2 (Figs 1–4) represent one specimen from Vietnam and two specimens collected from a captive population of *O. n. niloticus* from Stir-

ling, UK. The latter specimens were identified as a *G. cichlidarum* variant in the study of García-Vásquez *et al*. (2007). Although the morphology of the hamuli and marginal hooks of the three specimens labelled "C" were subtlely different from *G. cichlidarum*, the specimens had exactly the same molecular profile as *G. cichlidarum*.

The type locality for "*G. niloticus*" in the Philippines (Bureau of Fisheries and Aquatic Resources National Freshwater Fish Hatchery, Muñoz, Nueva Ecija Province) sampled by Cone *et al*. (1995) was resampled for the current study. The collected gyrodactylids were confirmed by morphological and molecular analyses to be identical to the descriptions of *G. cichlidarum*. Although this supports the synonymisation of *G. cichlidarum* and *G. niloticus*, it does not rule out the possibility that the topotypes re-sampled from the farm are not representative of the original infection.

The morphology of *G.* sp. 1 specimens described from Mexican *O. n. niloticus* (García-Vásquez *et al*., submitted) is similar to *G. shariffi* in that both species have large ventral bar processes. *G. shariffi* was originally described from *O. n. niloticus* from the Philippines but this species was not encountered within the samples taken from the Philippines in the current study. As no molecular sequences were obtained from the four specimens of *G.* sp. 1 and since no sequence data exists for *G. shariffi*, a final conclusion has to wait.

This study demonstrates that *G. cichlidarum* has a widespread distribution (Table VI) facilitated through the global movement of *Oreochromis* species, notably *O. n. niloticus*. With this in mind, it is most likely that *G. cichlidarum* is responsible for the majority of reported gyrodactylosis outbreaks on *O. n. niloticus*. What is currently unknown, however, is how many tilapiines *G. cichlidarum* infects, whether *O. n. niloticus* is the true host and whether it has acquired *G. cichlidarum* following its movement and exposure to other tilapiines one of which might be the true host of *G. cichlidarum*. If *G. cichlidarum* has switched hosts, this may explain why this gyrodactylid represents a serious health threat to *O. n. niloticus*, but other factors including stress, environmental and culture conditions are equally likely.

Paperna (1968) described *G. cichlidarum* from *S. g. galilaeus* (syn. *Tilapia galilaea*) from the Accra plains and Akuse lagoon, lower Volta Ghana, *Tilapia zillii* (Gervais) (syn. *C. zillii*) and two hemichromids, *H. fasciatus* and *H. bimaculatus*, from locations around the Volta lake. Given this latter report of *G. cichlidarum* occurring on different tilapiine genera and that the current study has demonstrated that cryptic species are resident on African populations of *Oreochromis* spp., a reinvestigation of the gyrodactylid fauna on the two hemichromids is warranted. While the latter study, if conducted, would provide a clear picture of the host range of *G. cichlidarum* given the translocation of tilapiine stocks, it is unlikely to establish whether *O. n. niloticus* represents the original or a recently acquired host. Experimental infections with other tilapiine species such as *O. karongae* (Trewavas) (García-Vásquez, unpublished data) and *O. aureus* (an infection observed on aquarium held-stock in the UK; Shinn, unpublished data), suggests that *G. cichlidarum* can infect a range of *Oreochromis* species and that infections of these hosts in the wild are possible. The finding of *G. cichlidarum* on *Oreochromis* Pargo-UNAM, a genetically complex hybrid, lends support to the low host specificity of *G. cichlidarum*. Further studies, however, are now required to investigate the host specificity of each of the *Gyrodactylus* species considered here. Given the commercial and ecological importance of tilapiine fish, there is much that remains to be established if we are to fully appreciate the potential risks of translocating parasites into new environments through the movement of fish stocks. Such investigations should include an assessment of the pathogenic potential of the host's parasite fauna on the indigenous fish communities into which they are introduced. While it may be too late for species like *O. n. niloticus*, *O. aureus* and *O. mossambicus* which already have a pan-global distribution, the introduction of new species into new environments, especially open water systems which are already occupied by other tilapiine species, must be undertaken with extreme caution.

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References

Bauer O.N., Pugachev O.N., Voronin V.N. 2002. Study of parasites and diseases of sturgeons in Russia: a review. *Journal of Applied Ichthyology*, 18, 420–429. DOI: 0175-8659/2002/1804-06-0420.

Bunkley-Williams L., Williams E.H.R., Jr. 1994. Parasites of Puerto Rican freshwater sport fishes. Puerto Rico. Department of Natural and Environmental Resources, San Juan, Puerto Rico and Department of Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico, 168 pp.

Cone D.K., Arthur J.R., Bondad-Reantaso M.G. 1995. Description of two new species of *Gyrodactylus* von Nordmann, 1832

(Monogenea) from cultured Nile tilapia, *Tilapia nilotica* (Cichlidae), in the Philippines. *Journal of the Helminthological Society of Washington*, 62, 6–9.

El-Naggar A.A., El-Tantawy S.A. 2003. The dynamics of gill monogenean communities on cichlid fish hosts inhabiting Damietta Branch of the River Nile: long-term changes in species richness and community structure. *Journal of the Egyptian-German Society of Zoology*, 41D, 187–220 [Arabic summary].

FAO. 2006. The state of aquaculture fisheries and aquaculture. *FAO Fisheries and Aquaculture Department Food Agriculture Organization of the United Nations Rome*, 2007.

Fitzsimmons K. 2006. Prospect and potential for global production. In: (Eds. C.E. Lim and B.L. Webster) *Tilapia Biology, Culture and Nutrition*. Chapter 2. Second edn., Food Products Press®, an imprint of The Haworth Press, Inc. London, UK, 51–72.

García-Vásquez A., Hansen H., Shinn A.P. 2007. A revised description of *Gyrodactylus cichlidarum* Paperna, 1968 (Gyrodactylidae) from the Nile tilapia, *Oreochromis niloticus niloticus* (Cichlidae) and its synonymy with *G. niloticus* Cone, Arthur et Bondad-Reantaso, 1995. *Folia Parasitologica*, 54, 129–140.

García-Vásquez A., Hansen H., Bron J.E., Shinn A.P. 2010. Description of three new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) described from *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae), (submitted).

Hansen H., Bachmann L., Bakke T.A. 2003. Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling and rainbow trout in Norway and Sweden. *International Journal for Parasitology*, 33, 1471–1478. DOI: 10.1016/S0020-7519(03)00200-5.

Harris P.H., Shinn A.P., Cable J., Bakke T.A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology*, 59, 1–27. DOI: 10.1023/B:SYPA.0000038447.52015.e4.

Johnsen B.O., Jensen A.J. 1991. The *Gyrodactylus* story in Norway. *Aquaculture*, 98, 289–302. DOI: 10.1016/0044-8486(91)90270-H.

López-Jímenez S. 2001. Estudio parasitológico de los peces de aguas dulces del Estado de Tabasco. *Gaceta Sigolfo Sistema de Investigación del Golfo de México*, 8–10.

Matějusová I., Gelnar M., McBeath A.J.A., Collins C.M., Cunningham C.O. 2001. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). *International Journal for Parasitology*, 31, 738–745. DOI: 10.1016/S0020-7519(01)00176-X.

Mo T.A. 1994. Status of *Gyrodactylus salaris* problems and research in Norway. In: (Eds. A.W. Pike and J.W. Lewis) *Parasitic Diseases of Fish*. Samara Publishing Limited, Dyfed, UK, 43–58.

Muñoz-Córdova G., Garduño-Lugo M. 2003. Mejoramiento genético en tilapia: sistemas de cruzamiento y mecanismos genéticos en la determinación de color. Veracruz: Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México. Sistema de Investigación del Golfo de México del Consejo Nacional de Ciencia y Tecnología, 84 pp.

Paperna I. 1968. Monogenetic trematodes collected from fresh water fish in Ghana second report. *Bamidgeh*, 20, 88–90.

Paperna I. 1973. New species of Monogenea (Vermes) from African freshwater fish. A preliminary report. *Revue de Zoologie et de Botanique Africaines*, 87, 505–518.

Paperna I. 1979. Monogenea of inland water fishes in Africa. Museé Royal de l'Afrique Centrale, Tervuren, Ser. 8, 226, 1–127.

Přikrylová I., Matějusová I., Musilová I., Gelnar M. 2009. *Gyrodactylus* species (Monogenea: Gyrodactylidae) on the cichlid fishes of Senegal, with the description of *Gyrodactylus ergensi* n. sp. from Mango tilapia, *Sarotherodon galilaeus* L. (Teleostei: Cichlidae). *Parasitology Research*, 106, 1–6. DOI: 10.1007/s00436-009-1600-0.

Rohde K. 1984. Ecology of marine parasites. *Helgoländer Meeresuntersuchungen*, 37, 5–33.

Salgado-Maldonado G., Pineda-López R., García-Magaña L., López-Jiménez S., Vidal-Martínez V.M., Aguirre-Macedo M.L. 2005. Helmintos parásitos de peces dulceacuícolas. In: (Eds. J. Bueno, F. Álvarez and S. Santiago) *Biodiversidad del Estado de Tabasco*. Instituto de Biología, Universidad Nacional Autónoma de México. Mexico, D.F., 145–166.

Shinn A.P., des Clers S., Gibson D.I., Sommerville C. 1996. Multivariate analyses of morphometrical features from *Gyrodactylus* spp. (Monogenea) parasitising British salmonids: light microscope based studies. *Systematic Parasitology*, 33, 115–125. DOI: 10.1007/BF00009427.

Shinn A.P., Hansen H., Olstad K., Bachmann L., Bakke T.A. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitologica*, 51, 239–252.

Suresh V. 2003. Tilapias. In: (Eds. J.S. Lucas and P.C. Southgate) *Aquaculture*, *Farming Aquatic Animals and Plants*. Blackwell Publishing Company, Oxford, 321–345.

Tamura K., Dudley J., Nei M., Suhdhir K. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599. DOI: 10.1093/molbev/msm092.

Thoney D.A., Hargis W.J., Jr. 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases*, 1, 133–153. DOI: 10.1016/0959-8030(91)90027-H.

Whittington I. 2004. The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note on species complexes. *Folia Parasitologica*, 51, 109–122.

Zhang Z., Schwartz S., Wagner L., Miller W. 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, 7, 203–214. DOI: 10.1089/10665270050081478.

Ziętara M., Lumme J. 2003. The crossroads of molecular typological and biological species concepts: two new species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). *Systematic Parasitology*, 55, 39–52. DOI: 10.1023/A:1023938415148.

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