Proof Delivery Form

Parasitology

Date of delivery:

Journal and vol/article ref: par 141_0/1300148

Number of pages (not including this page): 8

This proof is sent to you on behalf of Cambridge University Press.

Authors are strongly advised to read these proofs thoroughly because any errors missed may appear in the final published paper. This will be your ONLY chance to correct your proof. Once published, either online or in print, no further changes can be made.

****THESE PROOFS SHOULD BE RETURNED WITHIN 2 WORKING DAYS****

HOW TO RETURN YOUR PROOFS You can mark up proofs either on screen using the enabled electronic editing tools, or by hand on the hardcopy.

Marking up electronically All proofs are enabled to allow electronic annotation in the freely available Adobe Reader software. Using your cursor select the text for correction and use the most appropriate single tool (i.e. 'Replace', 'Cross out', 'Insert' or 'Add note to text'). Please return the file as an attachment via email to:

Lesley Bennun **<lesley.bennun@btinternet.com>**

Alternatively, please print the PDF file, mark any amendments on the proofs and list the corrections on a separate page, scan, and email them to the address above.

Changes should be limited to the correction of editing and typographical errors and the answering of any author queries. Any corrections that contradict journal style will not be transferred.

Copyright: if you have not already done so, please download a copyright form from:

http://journals.cambridge.org/images/fileUpload/documents/PAR_ctf.pdf

Please sign the form **by hand** and return by mail to the address shown on the form. Failure to send this form will delay the publication of your article.

Thank you for publishing in *Parasitology*. You will automatically receive a link to the PDF version of your article once it has been published online.

CAMBRIDGE JOURNALS

Proof Delivery Form

Parasitology

Please note:

• The proof is sent to you for correction of typographical errors only. Revision of the substance of the text is not permitted, unless discussed with the editor of the journal. Only **one** set of corrections are permitted.

• Please answer carefully any author queries.

• Corrections which do NOT follow journal style will not be accepted.

• A new copy of a figure must be provided if correction of anything other than a typographical error introduced by the typesetter is required.

• If you have problems with the file please contact **parproduction@cambridge.org**

Please note that this pdf is for proof checking purposes only. It should not be distributed to third parties and may not represent the final published version.

Important: you must return any forms included with your proof**.**

Please do not reply to this email

NOTE - for further information about **Journals Production** please consult our **FAQs** at http://journals.cambridge.org/production_faqs

Author queries:

Q1 Please provide the figure captions for figures 1–5.

Q2 The distinction between surnames can be ambiguous, therefore to ensure accurate tagging for indexing purposes online (eg for PubMed entries), please check that the highlighted surnames have been correctly identified, that all names are in the correct order and spelt correctly.

Offprint order form

CAMBRIDGE UNIVERSITY PRESS

PLEASE COMPLETE AND RETURN THIS FORM. WE WILL BE UNABLE TO SEND OFFPRINTS UNLESS A RETURN ADDRESS AND ARTICLE DETAILS ARE PROVIDED.

VAT REG NO. GB 823 8476 09

Parasitology (PAR)

Volume: ______ *no:* ______

Offprints

Offprints may be purchased at the price shown below. The total order will be sent by surface mail within one month of publication to the address given below.

Number of offprints required: ______

Email: ____________________

Offprints to be sent to (print in BLOCK CAPITALS): ____________________

____

____

____

____________________ *Post/Zip Code:* ____________________

Telephone: ____________________ *Date (dd/mm/yy):* ______ / ______ / ______

Author(s): ____________________

____

Article Title: ____________________

____

All enquiries about offprints should be addressed to ***the publisher****: Journals Production Department, Cambridge University Press, Shaftesbury Road, Cambridge CB2 8RU, UK.*

Charges for offprints (excluding VAT) *Please circle the appropriate charge:*

Number of copies	25	50	100	150	200	per 50 extra
1-4 pages	£68	£109	£174	£239	£309	£68
5-8 pages	£109	£163	£239	£321	£399	£109
9-16 pages	£120	£181	£285	£381	£494	£120
17-24 pages	£131	£201	£331	£451	£599	£131
Each Additional 1-8 pages	£20	£31	£50	£70	£104	£20

Methods of payment

If you live in Belgium, France, Germany, Ireland, Italy, Portugal, Spain or Sweden and are not registered for VAT we are required to charge VAT at the rate applicable in your country of residence. If you live in any other country in the EU and are not registered for VAT you will be charged VAT at the UK rate. If registered, please quote your VAT number, or the VAT number of any agency paying on your behalf if it is registered. *VAT Number:* ____________________

Payment **must** be included with your order, please tick which method you are using:

- ☐ Cheques should be made out to Cambridge University Press.
- ☐ Payment by someone else. Please enclose the official order when returning this form and ensure that when the order is sent it mentions the name of the journal and the article title.
- ☐ Payment may be made by any credit card bearing the Interbank Symbol.

Card Number: ______

Expiry Date (mm/yy): ______ / ______ *Card Verification Number:* ______

The card verification number is a 3 digit number printed on the **back** of your **Visa** or **Master card**, it appears after and to the right of your card number. For **American Express** the verification number is 4 digits, and printed on the **front** of your card, after and to the right of your card number.

Signature of card holder: ____________________ *Amount (Including VAT if appropriate):* £ ______

Please advise if address registered with card company is different from above

Trichodina heterodentata (Ciliophora) infestation on *Prochilodus lineatus* larvae: a host–parasite relationship study

GUSTAVO MORAES RAMOS VALLADÃO[1], SÍLVIA UMEDA GALLANI[1], SANTIAGO BENITES DE PÁDUA[1], MAURÍCIO LATERÇA MARTINS[2] *and* FABIANA PILARSKI[1]*

[1] *LAPOA – Laboratory of Aquatic Organisms Pathology, Aquaculture Center of São Paulo State University (CAUNESP), Jaboticabal, Brazil*
[2] *AQUOS-Aquatic Organism Health Laboratory, Aquaculture Department, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil*

(*Received 30 March 2013; revised 16 June, 17 July and 22 July 2013; accepted 27 July 2013*)

SUMMARY

Prochilodus lineatus is a freshwater fish species found in South America. It is common in aquaculture, but few studies regarding diseases of this fish have been performed. This study presents data of the occurrence of *Trichodina heterodentata* (Duncan, 1977), as well as the pathological alterations detected by light and scanning electron microscopy (SEM). Twenty 20-day-old larvae were harvested from an earth pond and examined. Larvae showed erratic swimming on the pond edges and some had a whitish tegument. Larval smears were either impregnated with silver nitrate or stained with Giemsa stain to observe the taxonomic features of the ciliates. Five larvae were fixed in formalin solution for histopathological analysis, and another five specimens were fixed in glutaraldehyde for SEM. All larvae were diagnosed with a severe infestation by trichodinid *T. heterodentata*. Histological sections showed discrete hyperplasia of the gill filaments with subepithelial oedema of the secondary lamellae. In the SEM, suction areas were observed on the skin, gills and eye; corrosion and ulceration of the fins were associated with the bacterial presence of cocci on the lesions. This is the first report of *T. heterodentata* in *P. lineatus* that is responsible for an acute disease that culminates in larval mortality.

Key words: fish diseases, ectoparasite, trichodinidae, pathogen, histopathology and scanning electron microscopy.

INTRODUCTION

Ciliated protozoans of the family Trichodinidae are one of the primary causes of disease in cultured fish worldwide, which leads to economic losses (Martins *et al*. 2010). Trichodinidae are a diversified group of ciliates with complex structures on their adhesive discs (Basson and Van As, 2006). These structures are utilized as taxonomic characteristics that can be used for evaluation in silver nitrate-impregnated specimens using the technique described by Klein (1958), whereas the nuclear apparatus is identified by panchromatic staining as proposed by Lom (1958) and Van As and Basson (1989). Hundreds of trichodinid species are recognized; however, few are as widely distributed as *Trichodina heterodentata* (Duncan, 1977), *Trichodina nigra* Lom, 1961, *Trichodina mutabilis* Kazubski and Migala, 1968, *Trichodina acuta* Lom, 1961 and *Trichodinella epizootica* Raabe, 1950, which are commonly found in several countries (Basson and Van As, 2006). In Brazil, recent studies of the trichodinid fauna of native fish have identified *Tripartiella pseudoplatystomae* Pinto, Garcia, Figueiredo, Rodrigues and Martins, 2009 in spotted catfish (Pinto *et al*. 2009), *Trichodina colisae* Asmat and Sultana, 2005 in pacu and its hybrid intergeneric patinga (Jerônimo *et al*. 2012) and *T. heterodentata* in pacu (Pádua *et al*. 2012). *Trichodina fariai* Cunha and Pinto, 1928 is not clearly identified because its description was not based on the silver nitrate impregnation method (Lom and Haldar, 1976).

There are few studies of the relationship between the host and parasite in trichodinid-diseased fish. Among the deleterious effects caused by trichodinids is reduced growth, as observed in *Heterobranchus longifilis* parasitized by *Trichodina maritinkae* Basson and Van As, 1991, (Ekanem and Obiekezie, 1996) whereas *Trichodina* sp. rendered *Ictalurus punctatus* more susceptible to *Streptococcus* spp. infection (Evans *et al*. 2007). Inflammation and epithelial desquamation of *Carassius auratus* gills were caused by *T. heterodentata* (Tang and Zhao, 2007). Parasitism by *T. heterodentata* also caused a decrease in the immune response of Nile tilapia vaccinated against *Streptococcus iniae* (Martins *et al*. 2011). Few studies have evaluated the effects of trichodinid

* Corresponding author: LAPOA – Laboratory of Aquatic Organisms Pathology, Aquaculture Center of São Paulo State University (CAUNESP), Via Prof. Paulo Donato Castellane, s/n, CEP: 14884-900 Jaboticabal, SP, Brazil. E-mail: fabianap@caunesp.unesp.br

Parasitology (2013), **141**, 1–8.
doi:10.1017/S0031182013001480

infestation on fish larvae; however, Basson and Van As (2006) consider the larval stage the most susceptible to trichodinid infestation.

Streaked prochilod *Prochilodus lineatus* Valenciennes, 1836, is a rheophilic freshwater fish commonly found in South American fish farms. Artificial reproduction of this fish is easy and is performed using specimens maintained in earth ponds (Hainfellner *et al*. 2012). Streaked prochilod larvae are also used as a live food for South American carnivorous fish, as dourado *Salminus brasiliensis* (Characidae) and catfish of the genus *Pseudoplatystoma* (Pimelodidae), commonly known as surubim. Parasitic fauna that affect streaked prochilod in both the wild and captivity are poorly known. Although the occurrence of *Trichodina* sp. has been reported (Eiras *et al*. 2012), no trichodinid species has been reported. This study shows the occurrence of *T. heterodentata* that parasitizes the *P. lineatus*. The histopathological and scanning electron microscopy (SEM) analysis of the fish larvae were also performed and reveals important information about parasite action on the host.

MATERIALS AND METHODS

Study area and fish

Streaked prochilod larvae within 20 days of hatching were utilized for parasitological analysis after observation for larval mortality and abnormal clinical signs. These larvae were utilized as live food for the carnivorous larvae of the cachara catfish *Pseudoplatystoma fasciatum* that were cultured in earthen ponds (15 × 10 m) in Jaboticabal city, São Paulo State, Southeast Brazil (22.14′22.7″S; 48°17′33.4″W).

Gross pathology and diagnosis of parasitic infestation

Ten larvae with abnormal behaviour and clinical signs were euthanized by cerebral commotion and examined under a light microscope. When trichodinids were present, the slides were air-dried under dim light, and several slides were fixed with methanol and then stained with Giemsa stain (one drop per 1 mL distilled water) for 120 to 180 min to show the nuclear apparatus. The other slides were impregnated with 2% silver nitrate using Klein's method (Klein, 1958) to observe the adhesive disc as described by Lom (1958). Measurements were obtained from 102 specimens impregnated with 2% silver nitrate and 38 specimens stained with Giemsa to observe the nuclear apparatus and obtain a description of the position of the micronucleus (Lom, 1958). Specimens are deposited in the National Institute of Amazonian Research (INPA), Manaus, AM, Brazil under the number 008 and 009.

The denticle span was measured from the extremity of the blade to the extremity of the ray as described by Arthur and Lom (1984). All measurements were made in micrometres and followed the recommendations of Lom (1958). Measurements were made on photomicrographs obtained with a Nikon E200® photomicroscope equipped with the Moticam 2300® image capture system. The parasite measurements were performed using the ImagePro Plus® 4.1 software. Minimum and maximum values are provided, followed in parentheses by arithmetic mean, standard deviation and number of specimens measured. Schematic drawings of the denticles, as proposed by Van As and Basson (1989), were produced by means of vectorization using CorelDraw® X5 software.

Histopathological analysis

Five streaked prochilod larvae severely infested with trichodinids were fixed in 10% buffered formalin solution, processed according to usual histopathological techniques, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin–eosin. The slides were analysed, and photomicrographs were obtained using a Nikon E200® photomicroscope equipped with the Moticam 2300® image capture system.

Scanning electron microscope (SEM)

Five streaked prochilod larvae were fixed in 2·5% glutaraldehyde and post-fixed in 1% osmium tetroxide, both in 0·1 M cacodylate buffer (pH 7·4). Next, the larvae were dehydrated with serial concentrations of ethanol and critical-point dried. The biological material was mounted on aluminium stubs, sputter-coated with gold, and examined using a JEOL JSM-5410 scanning electron microscope at an accelerating voltage of 15 kV.

RESULTS

Parasitic diagnosis

All larvae were severely parasitized by trichodinids (100% prevalence), and no preference of the infestation site was noted. The parasites were observed on the body surface, fins, gills and mouth of the fish. No mixed infestation with other parasite taxa was observed.

Trichodinid description

Trichodinids on streaked prochilod *P. lineatus* larvae showed a disc-shaped body (Fig. 1a and b) diameter of 48·4–65·9 (56·9 ± 3·6; 102), striated border membrane 2·8–5·7 (4·5 ± 0·4; 102) wide, adhesive disc diameter of 39·4–55·3 (47·7 ± 3·6; 102), denticulate ring diameter of 23·0–37·6 (29·4 ± 2·6; 102), and 20·0–26·0 (23·0 ± 0·9; 102) denticles. The denticle

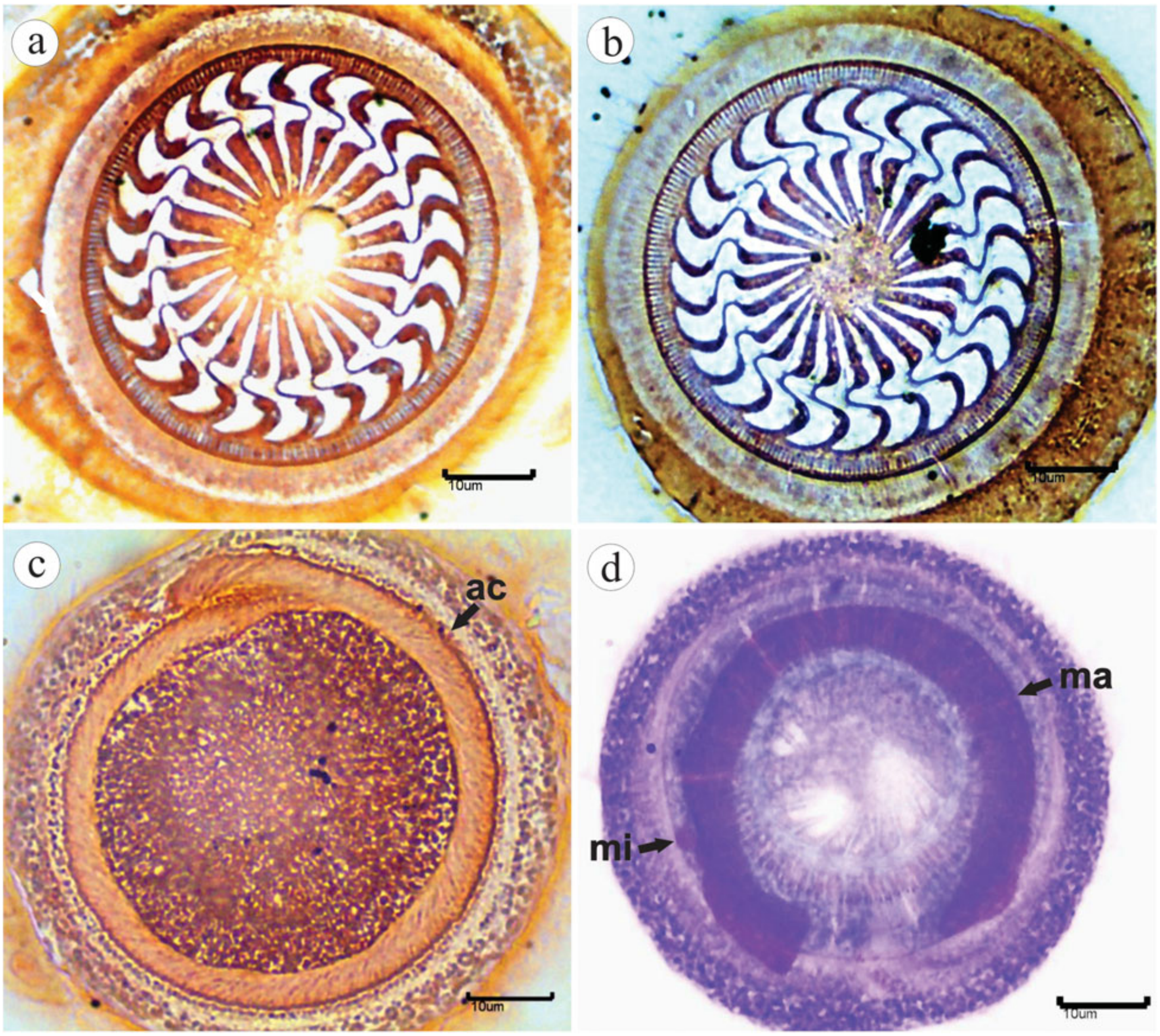

Fig. 1 - Colour online, B/W in print

Fig. 1. xxx.

Q1

length was 5·8–9·3 (7·8 ± 0·7; 102), blade length was 3·8–5·7 (4·6 ± 0·4; 102), central part width was 2·1–4·2 (3·0 ± 0·5; 102), ray length was 6·0–9·9 (7·7 ± 0·8; 102), denticle span was 13·0–17·6 (15·4 ± 1·0; 102) and 6–12 (9·8 ± 1·2; 93) pins per denticle was observed. The adoral ciliatura (Fig. 1c) in spiral was 405–430° (418·2 ± 6·7; 34). The horse-shaped macronucleus (Fig. 1d) was 39·8–59·0 (45·8 ± 4·7; 38) in diameter, 10·9–16·1 (8·5 ± 1·4; 38) in thickness and there was a distance between the macronucleus extremities of 23·3–27·5 (16·6 ± 3·9; 38) in length. Occasionally, a rounded micronucleus (Fig. 1d) situated at the $+y$ position was observed; value of $+y$ distance 13·3–25·9 (19·9 ± 4·3; 6); micronucleus length 3·8–4·6 (4·1 ± 0·3; 6) and width 1·8–3·9 (2·9 ± 0·8; 6).

Denticle description. The blade is broad and sickle-shaped and fills the space between the axes y and $y+1$, in which the apex touches the axis $y+1$, but in some specimens, crosses the axis $y+1$. Apophysis of the blade is well-developed and prominent but reduced in a few specimens. The central part of the blade is robust, triangular, with no posterior projection and fills the space between the axes $y+1$ and $y-1$. The rays are long, straight and can be projected parallel, either anteriorly or posteriorly in relation to the y axis. It is primarily sharp-pointed and occasionally tapers to a round shape with a short apophysis observed in some specimens (Fig. 2).

All morphological characteristics of the parasite on *P. lineatus* larvae are similar to *T. heterodentata*.

Clinical signs and gross pathology

The larvae showed lethargy, erratic swimming on the water surface and pond edges, and some larvae presented with irregular whitish lesions on the body surface and head. It was not possible to analyse the cachara *P. fasciatum* larvae from the same pond as the streaked prochilod *P. lineatus* larvae because of

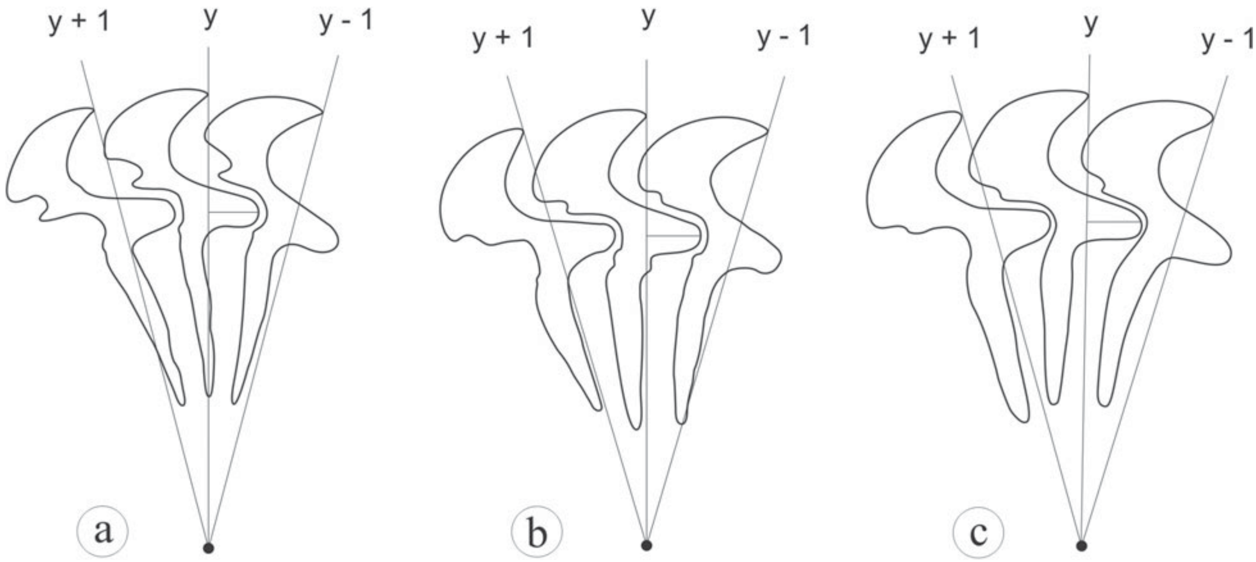

Fig. 2. xxx.

Q1

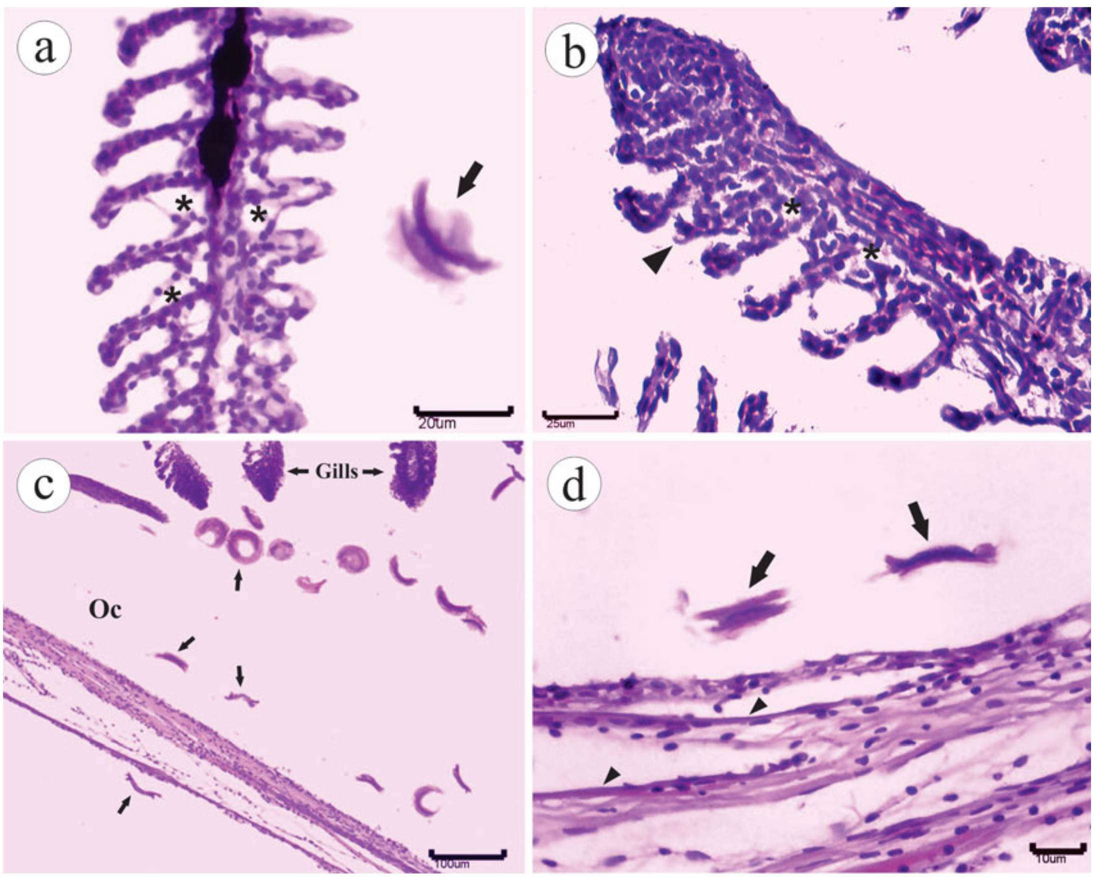

Fig. 3 - Colour online, B/W in print

Fig. 3. xxx.

Q1

the small number of animals. It was also not possible to observe the daily mortality of the streaked prochilod *P. lineatus* larvae because of their small size, although the cachara larvae did not survive, and approximately 50% of the streaked prochilod larvae stocked in the pond reached the fingerling stage.

Histopathological analysis

Major histological changes were observed on the gills and were characterized by sub-epithelial oedema with epithelial displacement of the secondary lamellae (Fig. 3a and b). There was also discrete epithelial

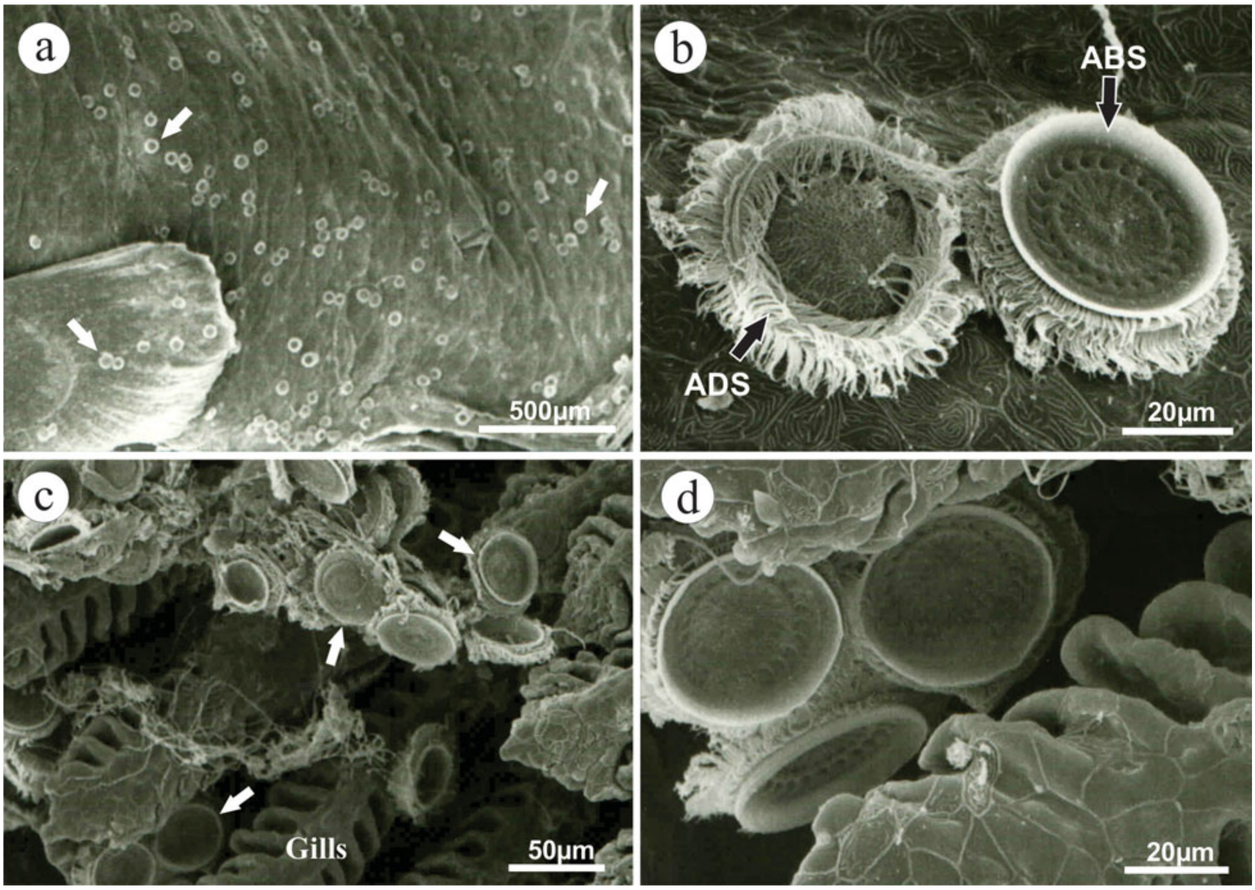

Fig. 4. xxx.

Q1

hyperplasia with focal areas of lamellar fusion and a slight mononuclear inflammatory infiltrate (Fig. 3a and b). Histological changes in the skin of the larvae could not be found. A large number of *T. heterodentata* was observed on the gills (Fig. 3c), skin (Fig. 3d), inside the mouth and occasionally in the intestine, which presented with rupture signs.

SEM observations

We observed a large number of *T. heterodentata* on the skin and fins (Fig. 4a), gills (Fig. 4c), cornea and oral cavity of the larvae by SEM. *T. heterodentata* ciliature and adhesive disc details were observed (Fig. 4b and d). Suction areas in the gill, eye (Fig. 5a) and skin near the nostril (Fig. 5b) caused by attachment of the adhesive disc were observed. Tissue injuries were epithelial desquamation with the formation of ulcers on the skin and fins. On these locations, high numbers of bacteria in the form of cocci associated with the injuries (Fig. 5c). These bacteria were also found among the cilia and on the surface of trichodinids (Fig. 5d).

DISCUSSION

Trichodina heterodentata is widely distributed in several countries and has significant morphometric variations. The measurements obtained in this study are similar to the descriptions of Basson *et al.* (1983), Albaladejo and Arthur (1989), Al-Rasheid *et al.* (2000) and Asmat (2004). The specimens with the largest-sized dimensions in the majority of taxonomic characteristics were reported in population B of the studies of Duncan (1977) and Martins *et al.* (2010), whereas Pádua *et al.* (2012) reported a Brazilian population with a smaller adhesive disc and denticulate ring diameters, denticle span, central part width and adoral ciliature than the present specimens. The variation observed in the schematic drawing of the *T. heterodentata* isolated from the streaked prochilod are consistent with the description of Van As and Basson (1989) in which well-developed and robust rays differ from the slender ray described in *T. heterodentata* isolated from *Rhinella pombali* tadpoles (Dias *et al.* 2009). Ecological and environmental factors could favour the occurrence of a varied shape of this ciliate group (Kazubski, 1971).

The behavioural alterations and gross pathology observed in the streaked prochilod larvae parasitized by *T. heterodentata* were not specific, and other Brazilian farmed fish parasitized by *Chilodonella hexasticha* showed similar changes (Pádua *et al.* 2013). In this study, no severe histological alterations were observed that were similar to those observed in

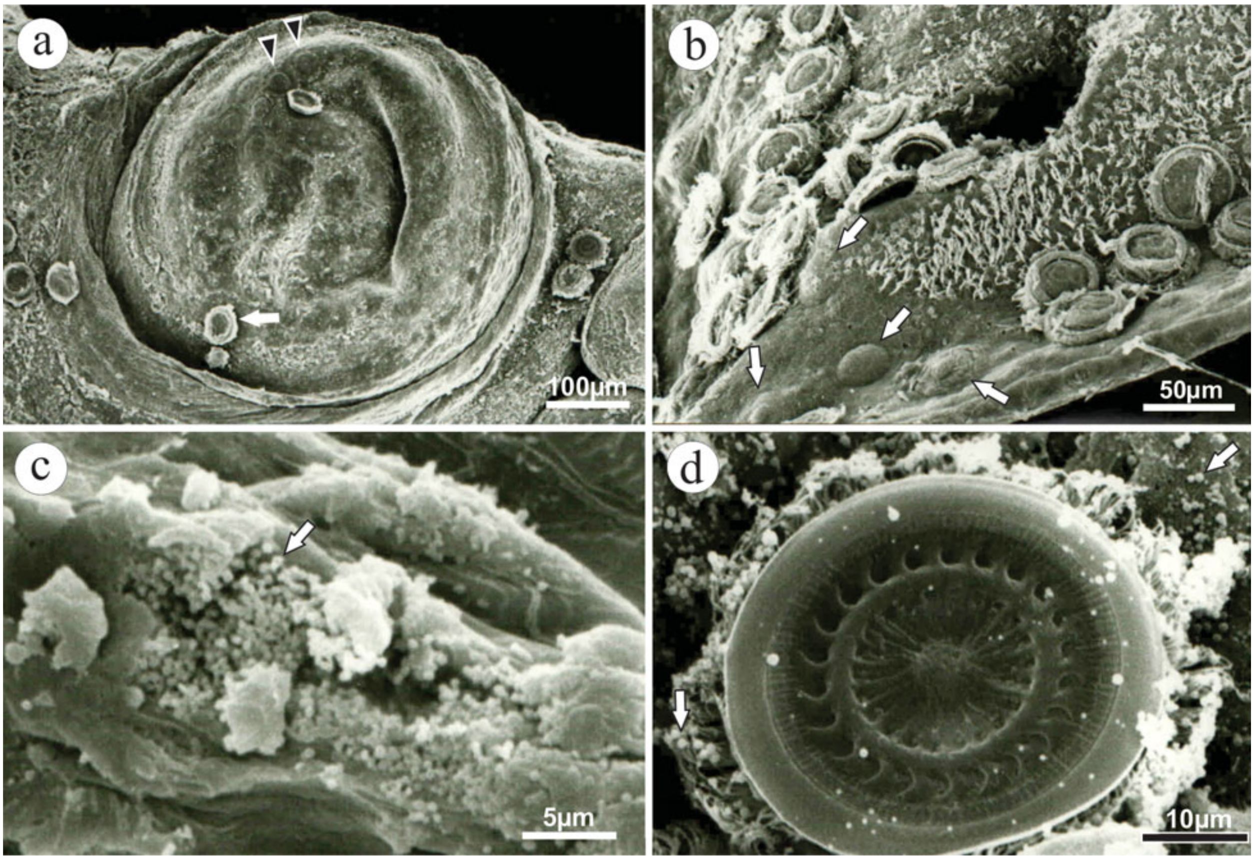

Fig. 5. xxx. **Q1**

Tetraodon fahaka parasitized by *Trichodina fahaka* Al-Rasheid, Ali, Sakran, Baki and Ghaffar, 2000 (Abdel-Baki *et al*. 2011) or *Solea aegyptiaca* parasitized by *Trichodina gobii* Raabe, 1959 (Yemmen *et al*. 2011*a*). In these cases, there were significant proliferative gill disturbances and lamellar fusion, whereas during the inflammatory reaction of *Trichodina puytoraci* Lom, 1962 in *Mugil cephalus*, degeneration and gill necrosis were related (Yemmen *et al*. 2011*b*). *Trichodina heterodentata* in streaked prochilod larvae also caused proliferative alterations; however, these alterations were discrete. Tang and Zhao (2007) reported the occurrence of inflammation and structural disorders with epithelial desquamation in the gills of *C. auratus* infested by *T. heterodentata*. The immune competent cells of the fish immune system are formed during the first days post-fertilization (Zapata *et al*. 2006), therefore, streaked prochilodontid larvae may not have a well-developed immune system necessary for a good inflammatory response.

The pathological alterations observed in this study were acute with the formation of lesions that may result in mortality. Severe proliferative lesions with complete fusion of the gill lamellae during trichodinid parasitism (Abdel-Baki *et al*. 2011; Yemmen *et al*. 2011*a*; Valladão *et al*. 2013) are chronic alterations that develop because of prolonged parasite contact. Consistent with these observations, dysfunctions in the respiratory and osmoregulatory capacities cause metabolic disturbances that are lethal to the host. By contrast, the severe infestation of fish larvae observed in this study may cause abrasion that culminates in acute epithelial lesions followed by oedema and displacement of the gill tissue.

From the SEM analysis, suction areas on the cornea, skin and gills were similar to those found on the urinary bladder of *Xenopus laevis* parasitized by *Trichodina xenopodos* Fantham, 1924 (Kruger *et al*. 1991). These authors argued that the parasite strategy of attachment to the host prevents the excretion of trichodinids from the bladder, but if trichodinids detach the suction areas disappear with very little if any damage caused. Based on our observations, we agree that this may also be an attachment strategy of *T. heterodentata* to the external surface of the fish. Particularly, these changes in the cornea may contribute to abnormal clinical signs, as erratic swimming, which may be due to obscured vision and the presence of ciliates on the eyes.

Bacteria associated with trichodinids were also identified by SEM (Khan *et al*. 1974), but no information on host lesions was reported. Trichodinid infestation can induce host susceptibility to bacterial infection as shown by Evans *et al*. (2007) and as demonstrated during infestation by other parasite ciliates (Xu *et al*. 2012*a, b*). Our study showed a large number of bacteria associated with the lesions on the host caused by the massive presence of *T. heterodentata*. The results of this study support the hypothesis that parasitism increases tissue injury that facilitates a secondary bacterial infection, which may favour disease outbreaks.

CONCLUSIONS

This is the first record of *T. heterodentata* in *P. lineatus*, a South American freshwater fish. The parasitism caused acute disease associated with an opportunistic bacterial infection, ocular lesions, ulceration on the body surface and fins, as well as compromised respiratory function.

ACKNOWLEDGEMENTS

The authors thank Dr Fernando José Zara for the critical read of the manuscript and some suggestions during the graduation course on Electron Microscopy Applied to Zoology. The SEM procedure was carried on the Electronic Microscopy Laboratory of FCAV (UNESP, Jaboticabal). We thank Claudia Aparecida Rodrigues by the SEM procedure. Thank are also due to Dra. Maria Cecília Rui Luvizotto, Dr Klaus Casaro Saturnino and Camila do Nascimento for histological sections, CNPq (National Council for Scientific and Technological Development) for a grant to Dr M. L. Martins (CNPq 302493/2010-7), FAPESP for scholarships to S. B. Pádua (2010/14490-1) and G. M. R. Valladão (2012/19414-7).

REFERENCES

Abdel-Baki, A. S., Sakran, T., Fayed, H. and Zayed, E. (2011). *Trichodina fahaka* (Ciliophora: Peritrichia) in *Tetradon fahaka* from Nile River, Egypt: seasonality and histopathology. *Scientific Research and Essays* **6**, 1583–1587. doi: 10.5897/SRE10.1046.

Albaladejo, J. D. and Arthur, J. R. (1989). Some trichodinids (Protozoa: Ciliophora: Peritrichida) from freshwater fishes imported into the Philippines. *Asian Fisheries Science* **3**, 1–25.

Al-Rasheid, K. A. S., Ali, M. A., Saakran, T., Baki, A. A. A. and Ghaffar, F. A. A. (2000). Trichodinid ectoparasites (Ciliophora: Peritrichida) of some River Nile fish, Egypt. *Parasitology International* **49**, 131–137. doi: 10.1016/S1383-5769(00)00042-8.

Arthur, J. R. and Lom, J. (1984). Trichodinid protozoa (Ciliophora: Peritrichida) from freshwater fishes of Rybinsk Reservoir, USSR1. *Journal of Eukaryotic Microbiology* **31**, 82–91. doi: 10.1111/j.1550-7408.1984.tb04294.x.

Asmat, G. S. M. (2004). First record of *Trichodina diaptomi* (Dogiel, 1940) Basson and Van As, 1991, *T. heterodentata* Duncan, 1977 and *T. oligocotti* (Lom, 1970) (Ciliophora: Trichodinidae) from Indian fishes. *Pakistan Journal of Biological Sciences* **7**, 2066–2071. doi: 10.3923/pjbs.2004.2066.2071.

Basson, L. and Van As, J. G. (2006). Trichodinidae and other ciliophorans (Phylum Ciliophora). In *Fish Diseases and Disorders*, 2nd Edn, Vol. I, *Protozoan and Metazoan Infections* (ed. Woo,, P. T. K.), pp. 154–182. CABI, Wallingford, UK.

Basson, L., Van As, J. G. and Paperna, I. (1983). Trichodinid ectoparasites of cichlid and cyprinid fishes in South Africa and Israel. *Systematic Parasitology* **5**, 245–257. doi: 10.1007/BF00009159.

Dias, R. J. P., Fernandes, N. M., Sartini, B., Silva-Neto, I. D. and D'Agosto, M. (2009). Occurrence of *Trichodina heterodentata* (Ciliophora: Trichodinidae) infesting tadpoles of *Rhinella pombali* (Anura: Bufonidae) in the Neotropical area. *Parasitology International* **58**, 471–474. doi: 10.1016/j.parint.2009.06.009.

Duncan, B. L. (1977). Urceolariid ciliates, including three new species, from cultured Philippines fishes. *Transactions of the American Microscopical Society* **96**, 76–81.

Eiras, J. C., Takemoto, R. M., Pavanelli, G. C. and Luque, J. L. F. (2012). Checklist of protozoan parasites of fishes from Brazil. *Zootaxa* **3221**, 1–25.

Ekanem, D. A. and Obiekezie, A. I. (1996). Growth reduction in African catfish fry infected with *Trichodina maritinkae* Basson and Van As, 1991 (Ciliophora: Peritrichida). *Journal of Aquaculture in the Tropics* **11**, 91–96.

Evans, J. J., Klesius, P. H., Pasnik, D. J. and Shoemaker, C. A. (2007). Influence of natural *Trichodina* sp. parasitism on experimental *Streptococcus iniae* or *Streptococcus agalactiae* infection and survival of young channel catfish *Ictalurus punctatus* (Rafinesque). *Aquaculture Research* **38**, 664–667. doi: 10.1111/j.1365-2109.2007.01710.x.

Hainfellner, P., Souza, T. G., Moreira, R. G., Nakaghi, L. S. O. and Batlouni, S. R. (2012). Gonadal steroids levels and vitellogenesis in the formation of oocytes in *Prochilodus lineatus* (Valenciennes) (Teleostei: Characiformes). *Neotropical Ichthyology* **10**, 601–612. doi: 10.1590/S1679-62252012005000021.

Jerônimo, G. T., Marchiori, N. D. C., Pádua, S. B. D., Dias Neto, J., Pilarski, F., Ishikawa, M. M. and Martins, M. L. (2012). *Trichodina colisae* (Ciliophora: Trichodinidae): new parasite records for two freshwater fish species farmed in Brazil. *Revista Brasileira de Parasitologia Veterinária* **21**, 366–371. doi: 10.1590/S1984-29612012005000008.

Kazubski, S. L. (1971). Morphological variability of *Semitrichodina sphaeronuclea* (Lom, 1956). *Acta Protozoologica* **8**, 251–259.

Khan, R. A., Barber, V. C. and McCann, S. (1974). A scanning electron microscopical study of the surface topography of a trichodinid ciliate. *Transactions of the American Microscopical Society* **93**, 131–134.

Klein, B. M. (1958). The dry silver method and its proper use. *Journal of Protozoology* **5**, 99–103. doi: 10.1111/j.1550-7408.1958.tb02535.x.

Kruger, J., Basson, L. and Van As, J. G. (1991). Redescription of *Trichodina xenopodos* Fantham, 1924 (Ciliophora: Peritrichida), a urinary bladder parasite of *Xenopus laevis laevis* Daudin, 1802, with notes on transmission. *Systematic Parasitology* **19**, 43–50. doi: 10.1007/BF00010301.

Lom, J. (1958). A contribution to the systematics and morphology of endoparasitic trichodinids from amphibians, with a proposal of uniform specific characteristics. *Journal of Protozoology* **5**, 251–263. doi: 10.1111/j.1550-7408.1958.tb02563.x.

Lom, J. and Haldar, D. P. (1976). Observations on trichodinids endocommensal in fishes. *Transactions of the American Microscopical Society* **95**, 527–541. doi: 10.2307/3225373.

Martins, M. L., Marchiori, N. C., Nunes, G. and Rodrigues, M. P. (2010). First record of *Trichodina heterodentata* (Ciliophora: Trichodinidae) from channel catfish, *Ictalurus punctatus* cultivated in Brazil. *Brazilian Journal of Biology* **70**, 637–644. doi: 10.1590/S1519-69842010000300022.

Martins, M. L., Shoemaker, C. A., Xu, D. H. and Klesius, P. H. (2011). Effect of parasitism on vaccine efficacy against *Streptococcus iniae* in Nile tilapia. *Aquaculture* **314**, 18–23. doi: 10.1016/j.aquaculture.2011.01.022.

Pádua, S. B., Martins, M. L., Carraschi, S. P., Cruz, C. and Ishikawa, M. M. (2012). *Trichodina heterodentata* (Ciliophora: Trichodinidae): a new parasite for *Piaractus mesopotamicus* (Pisces: Characidae). *Zootaxa* **3422**, 62–68.

Pádua, S. B., Martins, M. L., Carrijo-Mauad, J. R., Ishikawa, M. M., Jerônimo, G. T., Dias-Neto, J. and Pilarski, F. (2013). First record of *Chilodonella hexasticha* (Ciliophora: Chilodonellidae) in Brazilian cultured fish: a morphological and pathological assessment. *Veterinary Parasitology* **191**, 154–160. doi: 10.1016/j.vetpar.2012.07.030.

Pinto, E., Garcia, A. M., Figueiredo, H. C. P., Rodrigues, M. P. and Martins, M. L. (2009). Primeiro relato de *Tripartiella* sp. (Ciliophora: Peritrichia) em *Pseudoplatystoma corruscans* (Osteichthyes: Pimelodidae) cultivado no Estado de Mato Grosso do Sul, Brasil, com descrição de nova espécie. *Boletim do Instituto de Pesca* **35**, 91–97.

Tang, F. H. and Zhao, Y. J. (2007). Taxonomic study on three species of *Trichodina* Ehrenberg, 1838 with pathologic research on gill tissue of *Carassius auratus* caused by *Trichodina heterodentata* Duncan, 1977. A study on trichodinids from freshwater fishes in Chongqing II. *Journal of Chongqing Normal University* **24**, 8–12.

Valladão, G. M. R., Pádua, S. B., Gallani, S. U., Menezes-Filho, R. N., Dias-Neto, J., Martins, M. L. and Pilarski, F. (2013). *Paratrichodina africana* (Ciliophora): a pathogenic gill parasite in farmed Nile tilapia. *Veterinary Parasitology*, in press. doi: 10.1016/j.vetpar.2013.04.043.

Van As, J. G. and Basson, L. (1989). A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. *Systematic Parasitology* **14**, 157–179. doi: 10.1007/BF02187051.

Xu, D-H., Pridgeon, J. W., Klesius, P. H. and Shoemaker, C. A. (2012*a*). Parasitism by protozoan *Ichthyophthirius multifiliis* enhanced invasion of *Aeromonas hydrophila* in tissues of channel catfish. *Veterinary Parasitology* **184**, 101–107. doi: 10.1016/j.vetpar.2011.09.020.

Xu, D-H., Shoemaker, C. A., Martins, M. L., Pridgeon, J. W. and Klesius, P. H. (2012*b*). Enhanced susceptibility of channel catfish to the bacterium *Edwardsiella ictaluri* after parasitism by *Ichthyophthirius multifiliis*. *Veterinary Microbiology* **158**, 216–219. doi: 10.1016/j.vetmic.2012.02.007.

Yemmen, C., Quilichini, Y., Ktari, M. H., Marchand, B. and Bahri, S. (2011*a*). Morphological, ecological and histopathological studies of *Trichodina gobii* Raabe, 1959 (Ciliophora: Peritrichida) infecting the gills of *Solea aegyptiaca*. *Protistology* **6**, 258–263.

Yemmen, C., Ktari, M. H. and Bahri, S. (2011*b*). Seasonality and histopathology of *Trichodina puytoraci* Lom, 1962, a parasite of flathead mullet (*Mugil cephalus*) from Tunisia. *Acta Adriatica* **52**, 15–20.

Zapata, A., Diez, B., Cejalvo, T., Gutiérrez-de Frías, C. and Cortés, A. (2006). Ontogeny of the immune system of fish. *Fish and Shellfish Immunology* **20**, 126–136. doi: 10.1016/j.fsi.2004.09.005.