Descriptions of strigea cercariae from the Gauteng and North West Provinces, South Africa

Freshwater snails are known to serve as first intermediate hosts for various parasitic diseases such as schistosomosis, amphistomosis and fasciolosis. Two freshwater snail species, *Lymnaea natalensis*, Krauss 1848 and *Bulinus tropicus*, Krauss 1848 were sampled from five localities in Gauteng and one locality in the North West Province from 2007 to 2010. These snails were collected in order to study their cercarial sheddings. They were found to be infected with three different types of strigea cercariae, of which the morphology was studied using standard light and scanning electron microscopy techniques.

Introduction

Strigea cercariae are characterised by a tail with long furcae (longifurcate), a pharynx (pharyngeal) and the presence of both oral and ventral suckers (distome). These cercariae are known to encyst in snails, tadpoles, reptiles and fish. Adults, as intestinal parasites of birds and mammals, are usually classified in either the family Strigeidae or Diplostomatidae (Frandsen & Christensen 1984).


Surveys on cercarial sheddings in the Tshwane area, Gauteng Province and one locality in the North West Province revealed two freshwater snail species to shed three different types of strigea cercariae. Lifecycle studies on these parasites frequently failed, mainly because of their extremely high host specificity with regard to their first, second and definitive hosts. The main aim of this project was to gather morphological information on these cercarial types, which, in the future, may assist in establishing possible linkages with strigea metacercarial stages in local fish populations from these areas.

Materials and methods

The freshwater snails, *Lymnaea natalensis*, Krauss 1848 and *B. tropicus* were collected over a period of four years (2007–2010) from four localities, namely, Supersand Dam (25°35′02.42″ S – 28°10′38.98″ E, altitude 1198 m a.s.l.) situated ± 2 km north of the Bon Accord Dam in the Onderstepoort area, Gauteng; the Boekenhoutskloof farm Dam (25°32′45.18″ S – 28°26′12.47″ E, altitude 1214 m a.s.l.) approximately 15 km north of the Roodeplaat Dam Nature Reserve, Gauteng; Mesi-pepa (26°11′52.63″ S – 27°09′51.92″ E, altitude 1473 m a.s.l.) along the N14 Krugersdorp–Votersdorp main road and ± 35 km from Vetersdorp, North West Province; and Northern Farm (25°55′49.18″ S – 27°58′19.11″ E, altitude 1375 m a.s.l.) situated north of Johannesburg, Gauteng, bordering the Diepsloot informal settlement. The snails were collected using metal scoops, as described by Van Eeden (1960), or collected manually from the ventral surface of water lily leaves. In the laboratory they were kept in plastic containers and fed fish flakes. They were exposed to indirect daylight to stimulate the natural shedding of cercariae.

The cercariae were stained with Nile blue sulphate or neutral red vital stains and studied alive using a Nikon compound microscope (Nikon, Tokyo, Japan). One or two stained cercariae were placed in a drop of water on a microscope slide, covered with a glass cover slip and moved once or twice over a Bunsen burner to slow down cercarial movement. Drawings were made by means of a drawing tube. In all cases, 20 specimens were measured, with all measurements given in...
micrometres (µm) and with minimum and maximum values indicated along with the mean value and standard deviation in parenthesis. Owing to the low infections in the snails, no other intra-molluscan stages were studied.

For scanning electron microscopy (SEM), specimens were fixed overnight in 2.5% glutaraldehyde, washed in Millonig’s phosphate buffer (pH = 7.2) and dehydrated through a graded ethanol series for 30 s – 60 s in each concentration. Thereafter, they were critically point dried (Polaron, Watford, UK), mounted on aluminium stubs, sputter coated with gold (Emscope; Quorum Technologies, Ashford, UK) and examined using a Leica Stereoscan 420 SEM (Leica Electron Optics, UK) at the Electron Microscope Unit of the University of Limpopo (Medunsas campus).

Ethical considerations

This research project met the requirements of the Research Ethics Committee (BP 05/2005) and the Animal Ethics Committee (AEC 02/05) of the University of Limpopo. Snails were kept under controlled laboratory conditions with sufficient light, air and food according to acceptable standards. They were individually placed in containers and cercariae collected after natural shedding.

Results

The freshwater snails, L. natalensis and B. tropicus were found to host three types of strigea cercariae. Table 1 accounts for the total collection of these two snail species from the four localities over a period of four years from 2007 to 2010. The prevalence of each infection is also indicated.

Strigea cercaria A

Snail host: L. natalensis.
Locality: Supersand Dam, Gauteng Province.

The cercaria (Figure 1a; Figures 2a and 2b) consists of an elongated and cylindrical body, measuring 164 µm – 185 µm (180 µm ± 7 µm) long × 49 µm – 60 µm (52 µm ± 4 µm) wide and a tail stem ending in two caudal rami (Figure 1b). The tail stem is shorter than the length of the body, measuring 198 µm – 206 µm (201 µm ± 3 µm) × 24 µm – 29 µm (26 µm ± 2 µm), with the caudal rami shorter than the tail stem, at 169 µm – 174 µm (172 µm ± 2 µm) × 16 µm – 18 µm (17 µm ± 1 µm).

A pear-shaped oral sucker, 52 µm – 69 µm (59 µm ± 4 µm) × 18 µm – 31 µm (27 µm ± 4 µm) is situated at the anterior end of the body. Sensory receptors with short cilia were observed alongside the oral sucker (Figure 2c). A pre-branch, 4 µm – 7 µm (6.5 µm ± 1.0 µm) × 3 µm – 4 µm (3.5 µm ± 1.0 µm) connects the oral sucker to the pharynx, 12 µm – 19 µm (14 µm ± 2 µm) × 10 µm – 21 µm (13 µm ± 3 µm), followed by an oesophagus, 7 µm – 12 µm (8 µm ± 1 µm) × 4 µm – 6 µm (5 µm ± 1 µm) that extends to just anterior of the acetabulum. The oesophagus divides into two intestinal caeca, 82 µm – 85 µm (83 µm ± 1 µm) × 5 µm – 6 µm (5.5 µm ± 1.0 µm) that extend posteriorly and terminates close to the posterior end of the body.

The acetabulum (Figure 2d), 35 µm – 39 µm (36 µm ± 1 µm) × 33 µm – 37 µm (35 µm ± 2 µm) is aspinose and located 28 µm – 38 µm (33 µm ± 4 µm) from the oral sucker. It is notably smaller than the oral sucker. Four penetration glands posterior to the acetabulum (Figure 1a; Figure 2b) are arranged as follows: two penetration glands, 7 µm – 15 µm (10 µm ± 2 µm) × 9 µm – 16 µm (13 µm ± 2 µm) flanking each other, followed by two large unpaired penetration glands lying tandem and posterior to the first pair. The first unpaired penetration glands measure 6 µm – 10 µm (8 µm ± 2 µm) × 16 µm – 24 µm (19 µm ± 2 µm) and the second pair, 6 µm – 10 µm (8 µm ± 1 µm) × 15 µm – 27 µm (19 µm ± 3 µm).

A small bipartite excretory bladder, 7 µm – 10 µm (8 µm ± 1 µm) × 7 µm – 11 µm (9 µm ± 3 µm) is situated at the posterior end of the body (Figure 1a). Two main collecting tubes arise from the bladder, extend anteriorly and divide into anterior and posterior collecting tubules anterior to the acetabulum. Fourteen pairs of flame cells were found in the body, of which six pairs are located in the anterior half and the remaining eight pairs are located in the posterior half of the body. The flame cell formula is: 2(2 + 2 + 2 + 1 + 2) = 28. The excretory pores open sub-terminally on either side of the caudal rami (Figure 1b).

Posteriorly directed minute spines that are denser in the anterior part of the organism cover the body tegument (Figure 2c), whereas fewer and more widely separated spines cover the tail stem (Figure 2e), with sparse sensory receptors with long cilia on its surface (Figure 2f). Six hair-like structures (Figure 1b), three anteriorly and three posteriorly, were observed on each side of the tail stem. A series of caudal bodies were also observed in the tail stem (Figure 1b). The caudal rami are covered with spines that are visually fewer when compared to the spines found on the tail stem.

Strigea cercaria B

Snail host: B. tropicus.
Locality: Northern farm Dam, Gauteng Province.

Strigea cercaria B (Figures 1c and 1d; Figures 3a and 3b) comprises a cylindrical body, 88 µm – 155 µm (109 µm ± 21 µm) × 45 µm – 60 µm (50 µm ± 4 µm) and a fork tail. The tail stem, 75 µm – 127 µm (105 µm ± 16 µm) × 28 µm – 36 µm (33 µm ± 3 µm) is shorter than the body length, with the caudal rami measuring 123 µm – 161 µm (149 µm ± 11 µm) × 10 µm – 21 µm (16 µm ± 3 µm). The oral sucker (Figure 3c), 16 µm – 40 µm (30 µm ± 7 µm) × 25 µm – 33 µm (30 µm ± 3 µm) is covered by fairly large and sharp backwardly pointing spines (Figures 3c and 3d). The oral sucker opens directly into a pharynx, 7 µm – 10 µm (9 µm ± 1 µm) × 7 µm – 12 µm (10 µm ± 2 µm), followed by a short oesophagus, 6 µm – 30 µm (13 µm ± 7 µm) × 3 µm – 6 µm (4 µm ± 1 µm) (Figure 1d).

The oesophagus bifurcates into two intestinal caeca, 31 µm – 49 µm (42 µm ± 5 µm) × 4 µm – 9 µm (6 µm ± 1 µm), encircling
TABLE 1: Prevalence of infections of two freshwater snail species collected from four different localities across the Gauteng and North West Provinces, South Africa.

<table>
<thead>
<tr>
<th>Year of collection</th>
<th>Lymnaea natalensis</th>
<th>Strigea cercaria A</th>
<th>Strigea cercaria C</th>
<th>Bulinus tropicus</th>
<th>Strigea cercaria B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of snails</td>
<td>Prevalence (%)</td>
<td>Number of snails</td>
<td>Prevalence (%)</td>
<td>Number of snails</td>
</tr>
<tr>
<td></td>
<td>collected from</td>
<td></td>
<td>collected from</td>
<td></td>
<td>collected from</td>
</tr>
<tr>
<td></td>
<td>Supersand dam</td>
<td></td>
<td>Metsi-Pepa</td>
<td></td>
<td>Boeken houtskloof</td>
</tr>
<tr>
<td>2007</td>
<td>39</td>
<td>3.0</td>
<td>18</td>
<td>7.7</td>
<td>13</td>
</tr>
<tr>
<td>2008</td>
<td>38</td>
<td>2.7</td>
<td>21</td>
<td>7.3</td>
<td>17</td>
</tr>
<tr>
<td>2009</td>
<td>5</td>
<td>2.0</td>
<td>57</td>
<td>7.9</td>
<td>27</td>
</tr>
<tr>
<td>2010</td>
<td>123</td>
<td>4.0</td>
<td>107</td>
<td>8.0</td>
<td>0</td>
</tr>
</tbody>
</table>

FIGURE 1: Light microscope projection drawings of three types of strigea cercariae shed by Lymnaea natalensis and Bulinus tropicus, (a) head and (b) forked tail of strigea cercaria A, (c) head and (d) forked tail of strigea cercaria B and (e) head and (f) forked tail of strigea cercaria C.

the acetabulum and terminate posteriorly to the acetabulum. The acetabulum (Figure 3e), 18 µm – 30 µm (25 µm ± 4 µm) × 18 µm – 25 µm (22 µm ± 2 µm) is smaller than the oral sucker and situated 43 µm – 90 µm (58 µm ± 15 µm) mid-ventrally from the anterior end of the body. Two non-pigmented eyespots are situated 46 µm – 48 µm (47 µm ± 1 µm) from the anterior end of the body and measure 7 µm – 10 µm (9 µm ± 1 µm) × 4 µm – 9 µm (6 µm ± 2 µm) (Figure 1d).

Four small penetration glands (Figure 1d), 4 µm – 7 µm (6 µm ± 1 µm) in diameter, are situated at the terminal end of each intestinal caecum. A small bipartite excretory bladder, 3 µm – 9 µm (7 µm ± 2 µm) × 7 µm – 15 µm (10 µm ± 3 µm), is located at the posterior end of the body. Two main collecting tubes arise from the bladder, extend anteriorly and divide into anterior and posterior collecting tubules anterior to the acetabulum giving rise to capillaries that terminate in flame cells (Figure 1d). The flame cell formula is: 2[(2 + 1) + [2 + 2]] = 14. The excretory pore opens sub-terminally on the lateral side of each caudal ramus (Figure 1c).

The body surface is covered with many posteriorly facing spines that are concentrated more at the anterior end of the body. These spines are large and concentrated around the oral sucker (Figure 3c), and become smaller towards the posterior. A row of large spines surrounds the acetabulum wall (Figure 3e). Four hair-like structures (Figure 1d) were observed: three on the anterior half and one posterior on both sides of the body. The tail stem (Figure 1d) was covered with only a few short spines that are randomly positioned. The caudal rami are covered with fewer posteriorly directed spines.

Both the tail stem and the caudal rami have hair-like structures, eight were found on both sides of the tail stem and two on the postero-lateral side of each caudal ramus (Figure 1c).
Strigea cercaria C

Snail host: *L. natalensis.*
Locality: Metsi-pepa, North West Province.

Strigea cercaria C is elongated with a large oval-shaped body, 151 µm – 184 µm (171 µm ± 10 µm) x 45 µm – 52 µm (50 µm ± 3 µm), and a fork tail that is almost twice as long as the body (Figures 1e and 1f; Figures 4a and 4b). The tail stem measures 413 µm – 508 µm (479 µm ± 31 µm) x 66 µm – 90 µm (72 µm ± 9 µm). The caudal rami, at 407 µm – 497 µm (477 µm ± 29 µm) x 30 µm – 60 µm (38 µm ± 8 µm), are as long as the tail stem and taper posteriorly into sharp-ending points (Figure 1f).

The body (Figure 1e; Figure 4b) displays a pear-shaped oral sucker, 48 µm – 64 µm (57 µm ± 5 µm) x 22 µm – 33 µm (28 µm ± 4 µm), that is longer than wide and very protrusible. Sensory receptors with short cilia dominate the area around the mouth opening (Figures 4d and 4e). Posteriorly directed spines (Figures 4d and 4e) are concentrated at the anterior end of the body, especially around the oral sucker, but are fewer in number at the posterior end of the body. Sensory receptors with long cilia were observed dorsally on the body surface (Figure 4f).

A small oral opening (Figure 4d) situated in the middle of the oral sucker opens into a pre-pharynx, 9 µm – 15 µm
(12 \mu m \pm 2 \mu m) \times 4 \mu m - 7 \mu m (6 \mu m \pm 1 \mu m). It continues into a muscular pharynx, 10 \mu m - 15 \mu m (11 \mu m \pm 2 \mu m) \times 9 \mu m - 15 \mu m (11 \mu m \pm 2 \mu m), leading to a short oesophagus, 7 \mu m - 12 \mu m (9 \mu m \pm 2 \mu m) \times 3 \mu m - 6 \mu m (4 \mu m \pm 1 \mu m). The oesophagus divides at the level of the acetabulum into two very large intestinal caeca, 67 \mu m - 97 \mu m (80 \mu m \pm 8 \mu m) \times 10 \mu m - 16 \mu m (13 \mu m \pm 2 \mu m) that terminate just above the excretory bladder at the posterior end of the body.

An acetabulum, 12 \mu m - 39 \mu m (33 \mu m \pm 8 \mu m) \times 30 \mu m - 39 \mu m (34 \mu m \pm 3 \mu m), with spines on the luminal wall is situated 37 \mu m - 40 \mu m (39 \mu m \pm 1 \mu m) posterior to the elongated oral sucker. Three finely granular penetration glands occur in tandem on either side of the body, just posterior to the acetabulum. The first pair measures, 9 \mu m - 15 \mu m (13 \mu m \pm 2 \mu m) \times 10 \mu m - 13 \mu m (11 \mu m \pm 1 \mu m), the second pair, 7 \mu m - 15 \mu m (13 \mu m \pm 2 \mu m) \times 10 \mu m - 13 \mu m (11 \mu m \pm 1 \mu m) and the third pair, 10 \mu m - 16 \mu m (14 \mu m \pm 2 \mu m) \times 10 \mu m - 13 \mu m (12 \mu m \pm 1 \mu m).

The excretory system consists of an oval-shaped bladder, 7 \mu m - 10 \mu m (8 \mu m \pm 1 \mu m) \times 6 \mu m - 10 \mu m (8.0 \mu m \pm 1.9 \mu m) from where the main excretory ducts on each side of the bladder run anteriorly towards the middle of the body. These ducts then divide at the level of the acetabulum into anterior and posterior collecting tubules which branch into...
smaller capillaries bearing flame cells. The flame cell formula is as follows: \(2 (3 + 3) = 12\). Two hair-like structures were observed bilaterally on the anterior end and seven pairs on the posterior half of the tail stem (Figure 1f). Caudal bodies in the tail stem are irregular in size and shape, and vary in number (Figure 1f; Figure 4c).

**Trustworthiness**

**Reliability**

Repeated measurement \((n = 20)\) of morphological characteristics by both line drawings and comparative information from SEM micrographs, corroborate our findings and thus improves reliability.

**Validity**

All measurements included are of internationally accepted and well-recognised taxonomic descriptors in this discipline.

**Discussion**

All three of the cercariae were identified as strigea cercariae according to the identification keys provided by Frandsen and Christensen (1984), classifying them as longifurcate-pharyngeal distome cercariae of the superfamily Strigeoidea.

Porter (1938) described three fork-tailed cercariae shed by *L. natalensis*, namely *Cercaria scheerpoortia* and *Cercaria magaliesia* both from Hartebeestpoort Dam (North West

---

**FIGURE 4:** Strigea cercaria C, depicted using light micrographs of (a) whole mount, (b) head of cercaria and (c) caudal bodies (Cb) in the tail stem, as well as scanning electron micrographs of, (d) oral sucker (Os), (e) sensory receptors with short cilia (Scr) surrounding the oral sucker and (f) sensory receptors with long cilia (Lcr) anteroventrally on the body.
Province) and *Cercaria maritzburgensis* from Pietermaritzburg (KwaZulu-Natal Province). Fain (1953) described nine *strigea* cercariae, which included one secreted by *L. natalensis*. Furthermore, Vercammen-Grandjean (1960) described six *strigea* cercariae which included five secreted by *Biomphalaria* species and one by *L. natalensis* from Lake Kivu. More recently, King and Van As (2001) described a fork-tailed cercaria secreted by *B. tropicus* from the Free State Province, whilst Jansen van Rensburg (2001) described a fork-tailed cercaria from *L. natalensis* from the Okavango Delta, Botswana and Nadasan and Appleton (2003) described a pharyngeal longifurcate distome cercaria shed by *B. tropicus* from Durban, KwaZulu-Natal. The present study adds to the descriptions of strigeid cercariae by the abovementioned authors from various regions in Africa.

With its unique grouping of penetration glands, *strigea* cercaria A is closely related to the strigea cercaria described by Fain (1953) but has penetration glands that are located at the post-acetabular region, whereas the one previously described has penetration glands situated at the pre-acetabular region. Other differences were size and shape of the body, as well as the flame cell formula.

With its cluster penetration glands, *strigea* cercaria B is similar to the *strigea* cercaria described by King and Van As (2001) secreted by *B. tropicus* in the Free State Province. The present cercaria is, however, smaller and has fewer penetration glands and flame cells. *strigea* cercaria B has seven pairs of flame cells, three pairs anteriorly and four pairs posteriorly to the acetabulum; whereas, the one described by King and Van As (2001) has two pairs anteriorly and two pairs posterior to the acetabulum. Although the internal morphology of these two cercariae is similar, SEM revealed external differences; for example, the surface spines and features of the acetabulum. The cercariae described by Porter (1938) and Jansen van Rensburg (2001) were all shed by *L. natalensis*. All of the abovementioned cercariae differ from the description of the present cercaria based on the position and shape of the penetration glands, body size and caudal bodies in the tail. Interestingly, *strigea* cercaria B was collected only once from *L. natalensis* from Boekenhoutskloof farm Dam. It is too soon to comment whether or not this was an accidental infection or whether the parasite is adapting to a new first intermediate host.

With regard to the position and size of the penetration glands, *strigea* cercaria C is clearly different in most morphological aspects to that described by King and Van As (2001) and Jansen van Rensburg (2001). This cercaria, however, resembles most of the characteristic features of *C. scheerpoortia*, as described by Porter (1938), especially the three pairs of penetration glands on each side of the body posterior to the acetabulum. *strigea* cercaria C has three pairs of flame cells anteriorly and three pairs posteriorly, unlike *C. scheerpoortia*, which has three pairs of flame cells occurring anteriorly, two pairs occurring posteriorly and one pair occurring in the tail stem. The cercaria described here also has hair-like structures found on either side of the body and the tail, which differs from the *strigea* cercaria described by Porter (1938). Other differences are the size of the intestinal caeca, tail stem morphology and pharynx size and shape.

Four types of metacercariae were obtained from local freshwater fish from the same localities. Two types of *strigea* metacercariae were found to encyst in the muscle tissue of *Tilapia sparrmanii*, Smith 1840 and *Pseudocrenilabrus philander*, Weber 1897, one was found in the cranial cavity of *Clarias gariepinus*, Burchell 1822 and a fourth in the vitreous chamber of *T. sparrmanii*.

According to Olsen (1974) members of the superfamily Strigeoidae belong to the families Diplostomatidae and Strigeidae, which both include intestinal parasites of fish-eating and frog-eating birds and mammals. Gibson, Jones and Bray (2001) mentioned that members of the Strigeidae are mainly specific to birds but are also found in reptiles. Only one genus, *Duboisella* Baer, 1938 occurs in mammals. The lifecycles of these *strigea* cercariae are presently still unknown, but they may develop into adults in either of the families mentioned above. With these parasites infecting a wide spectrum of freshwater fish, it is important to gather much more information on their larval stages first in order to attempt lifecycle studies in the future.

**Conclusion**

In this study, *L. natalensis* was found to host two different *strigea* cercariae. The first is characterised by two penetration glands flanking each other, followed by two unpaired penetration glands lying tandem and posterior to the first pair. The second cercaria is characterised by three pairs of granular penetration glands found in tandem posterior to the acetabulum. *Bulinus tropicus* secreted one *strigea* cercaria characterised by two non-pigmented eyespots and a cluster of four penetration glands situated at the terminal end of each intestinal caecum.

The three *Strigea* cercariae described in the present study were unrelated to any previous investigations described in the text. This investigation thus adds important findings with regard to morphological characterisation of these parasites. Our preliminary studies have shown that many freshwater fish species are infected with various *strigea* metacercariae which were collected from the same waterbodies that are presently utilised by the neighbouring communities. It is therefore imperative to trace the origin of these cysts in local fish species that may serve as the main source of protein.

**Acknowledgements**

Scanning electron microscopy equipment utilised during this investigation was supported by the Department of Science and Technology in partnership with the National Research Foundation of South Africa.
Competing interests
The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Authors’ contributions
E.B.E.M. (University of Limpopo) was the principal investigator, as this study formed part of her doctoral thesis. P.H.K. (University of Limpopo) was the postgraduate supervisor of this study and C.B. (University of Limpopo) was a co-worker involved with all aspects of micrography and technical finishing of photographic plates.

References
Fain, A., 1953, Contribution à l’étude des formes larvaires des trematodes au Congo Belge et spécialité de la larva de Schistosoma mansoni [Contribution to the study of the larval forms of the trematodes in Belgian Congo and especially of the larvae of Schistosoma mansoni], Mémoires Institut Royal Colonial Belge Section de Sciences Naturelles et Médicales 22, 1–312.