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Typical intracranial myiasis in Nigerian red river hogs (*Potamochoerus porcus*) caused by an unknown bot fly (Diptera: Oestridae)

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ABSTRACT

We report an unknown taxon of bot fly (Diptera: Oestridae: Oestrinae) in red river hogs (*Potamochoerus porcus* Linnaeus, 1758) in Cross River State, Nigeria. From direct observation and interviews with local hunters, we document that, remarkably, the parasite typically occurs within the intracranial supra-meningeal space – i.e., between the inner wall of the skull and the brain – but without causing visible inflammation or clinical signs. The parasite is most similar (up to 87.9%) to *Rhinoestrus usbekistanicus* based on cytochrome oxidase subunit 1 DNA sequencing but is sufficiently divergent phylogenetically to represent a new or previously un-sequenced taxon. Morphologically, the parasite shares some, but not all, features with *R. nivarleti*. Local cultural belief systems attribute aspects of red river hog behavior (e.g. intelligence, elusiveness) to the parasite, suggesting a prolonged presence in the red river hog population. The parasite's unusual anatomic location may be aberrant, or it may be a protective adaptation to life in red river hogs, which forage vigorously with their snouts.

1. Introduction

Bot flies (Diptera: Oestridae) are obligate parasites affecting animal health, livestock agriculture, and human health worldwide (Colwell, 2001; Colwell et al., 2006; Colwell and Otranto, 2021). Bot fly larvae infect the body cavities or tissues of hosts (myiasis) with generally mild effects on growth, survival, and reproduction (Colwell, 2001; Colwell et al., 2006; Colwell and Otranto, 2021). In rare cases, bot fly infection can be severe or lethal, usually resulting from aberrant migration of larvae to the brain secondary to trauma or surgery (Hall et al., 2016; Navarro and Alves, 2016; Terterov et al., 2010). Bot flies cause sufficient economic losses to animal agriculture to warrant control strategies such as applications of insecticides/parasiticides and vaccination (Colwell, 2001; Colwell et al., 2006). Some bot flies are also zoonotic (Colwell, 2001; Colwell et al., 2006). Human infection with the sheep bot fly (*Oestrus ovis* Linnaeus, 1758), for example, is geographically widespread although uncommon, mainly affecting the oropharynx and eye (Abosdera and Morsy, 2013; Beristain et al., 2001; Bertone et al., 2020; Fathy et al., 2006; Hemmersbach-Miller et al., 2007; Masoodi and Hosseini, 2003).

Despite a long history of bot flies in animals and humans, the diversity and natural history of these parasites remain incompletely understood, especially in wildlife (Colwell, 2001; Colwell and Otranto, 2021). This knowledge gap is significant because bot flies of wildlife could become established in domestic animals, leading to new health challenges (Colwell et al., 2006). A better understanding of bot fly diversity in nature would also inform the field of parasitology, particularly with regard to evolution and taxonomy (Cardoso et al., 2021; Li et al., 2020b; Marinho et al., 2012; Stevens, 2003).

This study reports an oestrid parasite in red river hogs (*Potamochoerus porcus* Linnaeus, 1758) in Cross River State, Nigeria. Red river hogs inhabit West African forests from Senegal to the Democratic Republic of the Congo (Reyna et al., 2016). They typically occur in rain-forest and gallery forest, but they also frequent cultivated areas (Leus and Vercammen, 2013). They forage using their snouts, tusks, and forefeet, feeding primarily on wild (and cultivated) tubers, as well as fruit, grasses, dead animal and plant remains, insects, and lizards (Melletti et al., 2017). Red river hogs are the only wild suids in Cross River State, Nigeria, where they are a preferred target for hunters, and they are used not only for consumption but also for medicinal and

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cultural purposes ((Friant et al., in press); Friant et al., 2020; Lameed et al., 2015).

The bot fly we discovered in red river hogs shows unusual clinical, morphological, and genetic features that suggest that it may represent a novel or poorly characterized taxon. Our results thus expand the known host range, clinical presentation, and evolutionary diversity of the bot flies as well as the role of red river hogs as bot fly hosts.

2. Materials and methods

2.1. Field studies

On February 2, 2019, a hunter-killed red river hog was butchered in a village in Cross River National Park, Nigeria (Fig. 1A; see Friant et al., 2015 for map and full description of study site). Larvae were clearly visible in the frontal sinus and the intracranial supra-meningeal space of the animal (Fig. 1B). The two larvae shown in Fig. 1B were removed (with permission of the hunter), placed in sterile cryogenic vials containing 2 ml of RNAlater nucleic acid preservation solution (Thermo Fisher Scientific, Waltham MA), and exported to the USA.

Interviews were conducted as part of a long-term ethnographic study of traditional hunting practices in the area, where hunting occurs at high rates within “enclave” communities deep within the forest. Specifically, dietary surveys, key informant interviews, hunter storytelling, and participant observation with field notes were used to elicit information on the cultural and health-related significance of red river hogs, among other animals (Friant et al., 2015, 2019; Friant et al., in press). As part of the approximately 400 interviews conducted, hunters were asked to free-list and describe animals with infectious diseases, tell stories passed down through generations about animals, and recount personal stories of encounters with animals. Interviews were conducted in Nigerian Pidgin English, the *lingua franca* in Nigeria, using the terms “nyama nyama”, which refers to “dirty things” including “germs,” “sickness”, “infection” and “worm.” Observations and information spontaneously offered during butchering of the red river hog and collection of the larvae were recorded via field notes.

Research activities were approved by Nigeria National Parks Service and community leaders of each study community. The Pennsylvania State University Institutional Review Board (#00011190/#00018470),

City University of New York Integrated Institutional Review Board (#2016-0352), and Nigeria Health Research Ethics Committee (#NHREC/01/01/2007-18/05/2017) also approved all research activities. Fly larvae were shipped internationally under CDC PHS permit no. 20190425-2665A.

2.2. Laboratory studies

A larva retrieved from the intracranial supra-meningeal space of the red river hog was prepared for morphological analysis and imaging as outlined previously (Young et al., 2016). Briefly, images were captured using a JVC KY-F75U digital camera attached to a Leica Z16 APO dissecting microscope with apochromatic zoom objective and motor focus drive, using a Syncrosopy Auto-Montage System and software. The larva was illuminated with an LED ring light attached to the end of the microscope column and by a gooseneck illuminator with bifurcating fiber optics. Image stacks (15 per figure) were white-balance-corrected using the system software. Montaged images were then cropped and adjusted using Adobe Illustrator to form the final figure plates. Resulting images of a left parasagittally sectioned larval habitus, left caudo-lateral image, and left caudal spiracular plate were compared with characters and descriptions for larvae of *Rhinoestrus* (Zumpt, 1965).

DNA barcoding by sequencing of the cytochrome oxidase subunit 1 (cox1) gene was conducted as previously described (Ramírez-Martínez et al., 2021). To infer the phylogenetic position of the parasite, the resulting cox1 sequence was aligned with homologous oestrine cox1 sequences available in GenBank as of November 2021 using a codon-based version of the Prank algorithm (Löytynoja, 2014), implemented in TranslatorX (Abascal et al., 2010). A maximum likelihood phylogenetic tree was then inferred using PhyML 3.3 (Guindon et al., 2010) with smart model selection (Lefort et al., 2017) and 1000 bootstrap replicates to assess statistical confidence in clades, and the tree was displayed using FigTree v1.4.4. Based on results of these analyses (see below), a second region of cox1 was amplified using published primers UEA7 and UEA10 (Otranto et al., 2003) and the HotStarTaq system (Qiagen, Hilden, Germany) and sequenced as previously described (Ramírez-Martínez et al., 2021).

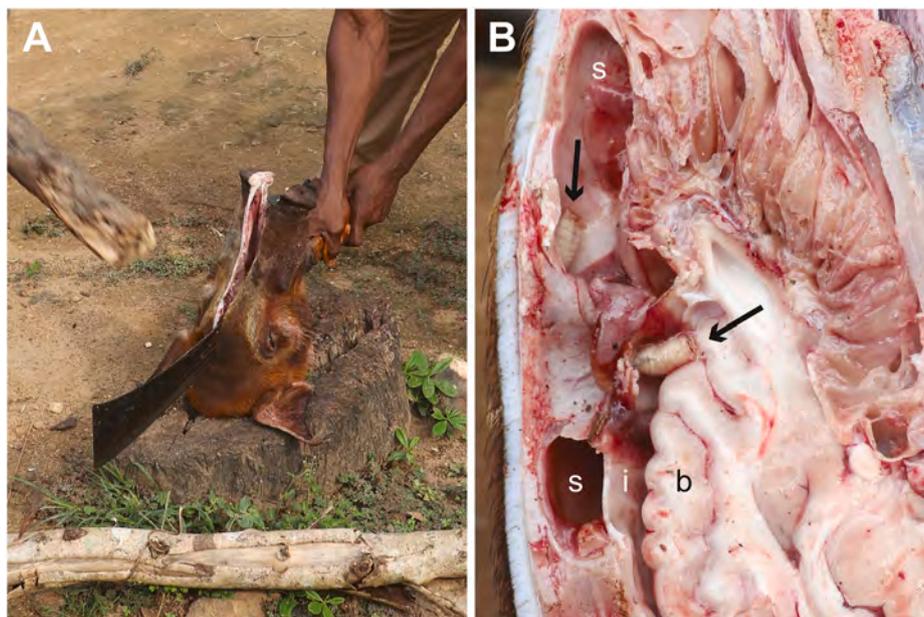


Fig. 1. A) hunter in Cross River National Park splitting the head of a recently killed red river hog with a machete. B) bot fly larvae (arrows) visible in the frontal sinus (s) and the intracranial supra-meningeal space (i) next to the brain (B) of the red river hog. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Field studies

We observed a hunter return from the forest to a village with a red river hog killed that same day. The animal was butchered in the village and the meat was shared within the community. It is tradition among Ejagham hunters to share the head of the red river hog among the hunter's "age grade" (a group of men from the same age cohort in the village). The snout may also be shared with special visitors. As part of this tradition, the head of the red river hog is typically bisected and then cut into pieces. It was during this process that larvae were observed in the frontal sinus and the intracranial supra-meningeal space (Fig. 1).

During key informant interviews, respondents described the presence of "maggots" in the "brain" of red river hogs. Respondents reported that all hunted red river hogs have "brain maggots" and that the older the animal is, the more parasites it is likely to have. Although only one hunter responded about these parasites unprompted, three additional hunters, a female butcherer, and one additional community member confirmed the typical presence of larvae in the intracranial space and the near-universal prevalence of the parasites within hunted red river hogs. Respondents also noted a lack of overt clinical disease associated with the parasites. Hunter stories further revealed that the parasites are believed to confer intelligence and elusiveness to red river hogs, making the animals challenging to hunt. One hunter described the mechanism by which larvae contribute to the intelligence of red river hogs. Specifically, the story (passed down from prior generations) is that when the animal is resting in the forest, the larvae emerge from its nose to act as sentinels of danger. When the larvae perceive a threat, they then retreat back into the hog's brain and communicate danger to the hog, which then runs away before the hunter arrives.

3.2. Laboratory studies

The parasite recovered from the intracranial supra-meningeal space of the red river hog was an approximately 1.0×0.5 cm 3rd instar larva with the characteristic morphology of a member of the family Oestridae (Colwell et al., 2006) (Fig. 2). Compared to published descriptions of third instar larvae of known *Rhinoestrus* species (Zumpt, 1965), detailed images of anatomical structures (Fig. 3A–C) showed that the ventral armature clearly possesses spinule bands ("pointed spines" *sensu* Zumpt) arranged in characteristic rows or partial rows (Fig. 3A–B: vsb). Dorsal spinule bands (Fig. 3A: dsb) are present on "at least a few anterior segments" (Zumpt, p. 160: couplet 8). Likewise, the "latero-ventral bulges" of some segments possess spinule bands (Fig. 3A: lsb) at the posterior margin (Zumpt: couplet 10), thus eliminating *Rhinoestrus hippopotami* Grünberg from consideration. Segment 11 (abdominal segment 8) does not appear to bear spinules, thus terminating in Zumpt's key at *R. nivarleti*.

However, comparing the specimen with the description of the third instar *R. nivarleti* (Zumpt: p. 167), there are several inconsistencies with respect to numbers of spinule bands (rows *sensu* Zumpt). Examining the caudal spiracular plate, referred to as the "posterior peritreme" by Zumpt (Fig. 3A–C: spl), there are structural similarities to the illustration provided by Zumpt (p. 168: Figure 227), but the actual peritreme in our specimen (Fig. 3C: ptr) forms a distinctly sclerotized rim around the periphery of the spiracular plate, becoming less defined into the plate emargination near the site of the ecdysial scar (Fig. 3C: es). The respiratory units (*sensu* Zayed et al., 2008) or spiracular papillae (Fig. 3C: sp; *sensu* Keilin, 1944) are numerous, reniform, and arranged in a stellate or radiate manner from the center of the spiracular plate to the peritreme.

Cox1 DNA barcoding (GenBank accession OK148469) showed the larva to be a member of the subfamily Oestrinae, most similar to *Rhinoestrus usbekistanicus* Gan (GenBank accession NC_045882) but with a percent nucleotide sequence similarity of only 87.9%. Phylogenetic

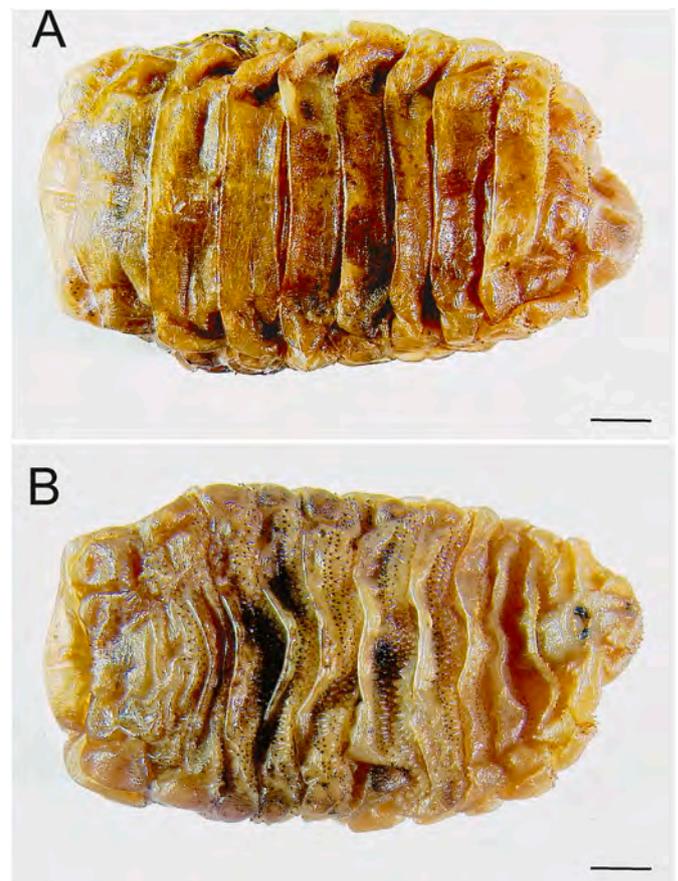


Fig. 2. Image of the third instar larva of the oestrid bot fly pictured in Fig. 1 from the intracranial supra-meningeal space of the Nigerian red river hog. A) dorsal; B) ventral. Scale bars = 1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

analysis (Fig. 4) showed the parasite to be sister taxon to *R. usbekistanicus* and *Rhinoestrus purpureus* Brauer within a clade containing the genera *Oestrus* and *Rhinoestrus*. However, the phylogenetic distance between the parasite and its closest relatives is greater than that between the genera *Pharyngomyia* and *Cephenemyia* and much greater than that among recognized species within the genus *Cephenemyia* (Fig. 4). Analysis of a second, 620 bp segment of *cox1* (GenBank accession OK148469) showed the parasite to be 85.1% similar to *R. usbekistanicus* (GenBank accession NC_045882) and 84.2% similar to *Rhinoestrus phacochoeri* Rodhain and Bequaert (GenBank accession NC_045882), the only other member of the genus *Rhinoestrus* from an African suid (a warthog, *Phacochoerus africanus* Gmelin 1788, from Senegal) with a sequence available in GenBank (Otranto et al., 2003).

4. Discussion

The diversity of Oestridae is still underexplored, and new species and even genera are still being described (Li et al., 2020a; Pape et al., 2017). Bot flies are well studied in bovids, cervids, equids, and camelids, but less is known about bot flies of suids (Colwell, 2001; Colwell et al., 2006). DNA barcoding of the bot fly in this study identified it to be a member of the Subfamily Oestrinae ("nasal bots;" Angulo-Valadez et al., 2010), most closely related genetically to *R. usbekistanus* but sufficiently divergent to represent a separate species or genus, based on divergences among other oestrine genera (Fig. 4). *Rhinoestrus nivarleti* and *R. phacochoeri* have previously been reported from red river hogs in Central Africa (Colwell, 2001) and warthogs in Senegal (Otranto et al.,

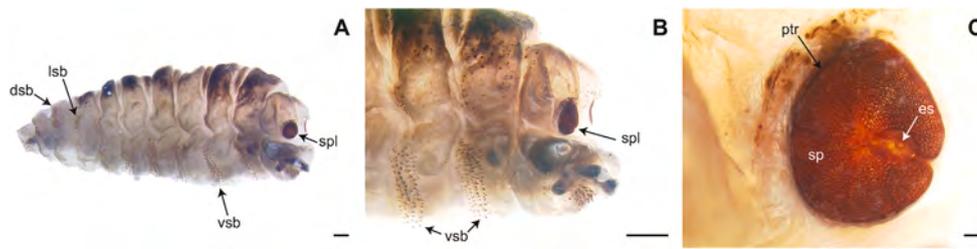


Fig. 3. Images of a third instar larva of the oestrid bot fly pictured in Figs. 1–2 from the intracranial supra-meningeal space of the Nigerian red river hog. A) left lateral habitus; B) left caudo-lateral view; C) left caudal spiracular plate. Dorsal spinule bands (dsb), latero-ventral spinule bands (lsb), ventral spinule bands (vsb), caudal spiracular plate (spl), peritreme (ptr), spiracular papillae (sp), ecdysial scar (es). Scale bars = 1 mm (A, B); 0.1 mm (C). (For interpretation of the references to colour in this figure

legend, the reader is referred to the Web version of this article.)

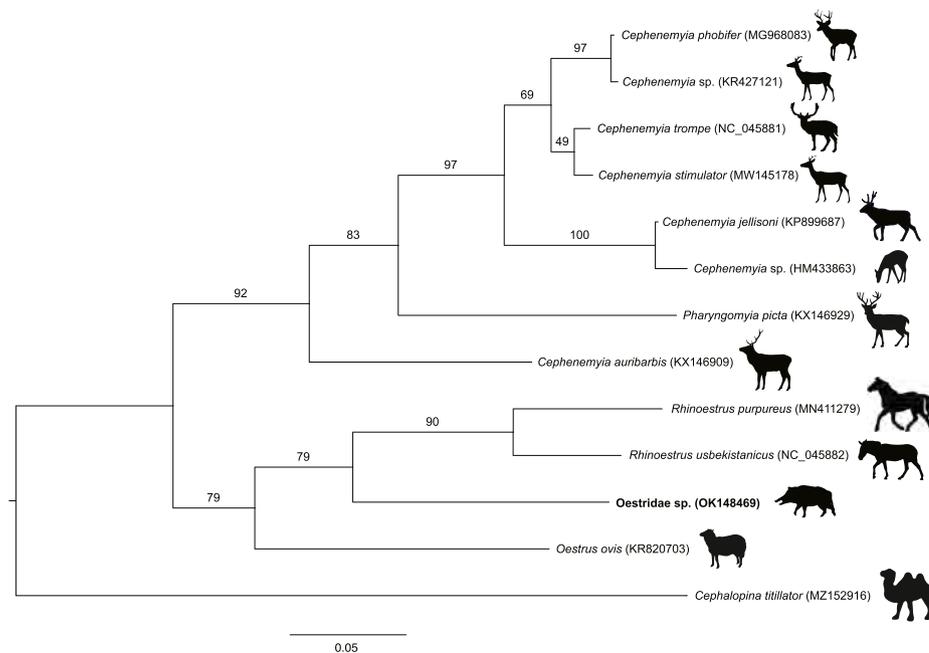


Fig. 4. Maximum likelihood phylogenetic tree of bot flies within the subfamily Oestrinae, based on a 684-position alignment of mitochondrial cytochrome oxidase subunit 1 DNA sequences. Taxon names are followed by GenBank accession numbers in parentheses; the parasite identified in the present study is shown in bold type. Silhouettes represent the typical host for each taxon. Numbers above branches are bootstrap values based on 1000 replicates, and the scale bar indicates nucleotide substitutions per site.

2003), respectively. The bot fly in this study is not a representative of *R. phacochoeri*, given its genetic difference of 15.8% in *cox1* (Otranto et al., 2003), but it could be a representative of *R. nivarleti*, for which DNA sequences are not presently available in GenBank.

Morphologically, the bot fly resembled *R. nivarleti* as described by (Zumpt, 1965) in some respects (dorsal, latero-ventral, and ventral spinule band configurations) but differed in others (number of spinule band rows on specific segments, peritreme structure, array of spiracular papillae). Complicating morphological inferences about whether the bot fly is a described or novel taxon, the morphological features considered as differential by Zumpt (1965) (the shape of the posterior spiracular plates and the distribution of spines on the third larval segment) would not allow differentiation of the two species of *Rhinoestrus* affecting horses in Italy (*R. purpureus* and *R. usbekistanicus*; Otranto et al., 2005). Thus, definitive assessment of the bot fly's taxonomy must await morphological analyses of suitably preserved larval and adult specimens, a larger sample size of infected red river hogs, and, ideally, DNA barcoding of vouchered specimens of *R. nivarleti*.

Bot flies have not, to our knowledge, previously been reported in the typical intracranial location described here. This is not likely to represent a post-mortem change, given the lack of precedent for such an unusual anatomic location and the rapidity with which red river hogs are butchered after being killed. Moreover, interviews with hunters in and near Cross River National Park revealed that intracranial infection of red river hogs with these bot fly larvae is common (nearly universal), and that an intergenerational belief system exists about their

contribution to the intelligence and elusiveness of red river hogs. Regardless of the accuracy of this knowledge, these results suggest a long-term presence of the parasite in the local red river hog population and its common intracranial presence. Notably, no hunters reported gross evidence of pathology or clinical disease in infected red river hogs, even when prompted. It is possible that such effects occur but are not readily visible. Indeed, parasite infection can cause subtle clinical effects that increase susceptibility of wildlife to predation, including hunting by humans, even when such increased susceptibility does not facilitate completion of the parasite's lifecycle (Gehman and Byers, 2017; Hudson et al., 1992; Rau and Caron, 2011). We also note that we did not record any accounts of intentional consumption of these or other parasitic larvae, as has been described elsewhere (Overstreet, 2003).

Cerebral myiasis is a lethal condition caused by infection of the brain with dipteran larvae, characteristically occurring secondary to trauma or surgery (Hall et al., 2016; Navarro and Alves, 2016; Terterov et al., 2010). Cerebral myiasis is rare and considered aberrant, in that it is not part of the parasite's typical lifecycle. By contrast, the larvae described here seem regularly to infect the intracranial space but not the brain itself, causing no clearly visible inflammation or clinical disease. Histopathologic examination and detailed analysis of red river hog behavior would likely reveal that these parasites do, in fact, cause at least some inflammation and clinical effects, respectively (it is difficult to imagine otherwise), but such effects are clearly not as dramatic as those caused by cerebral myiasis. For example, *Oestrus ovis* caused fatal cerebral myiasis in a male Sirohi goat (*Capra aegagrus hircus* Linnaeus,

1758) in India in 2011, and the associated respiratory and neurologic signs were severe and unmistakable (Shivasharanappa et al., 2011).

The mechanism by which some of these bot fly larvae migrate from the frontal sinus extra-calvarial space to the intracranial supra-meningeal space remains unknown. The frontal sinus walls are thin (Fig. 1), suggesting that the larvae may burrow directly through the cortical bone. Oestrid larvae have enzymatic adaptations for digesting host tissues that make such a mechanism plausible (Atelge et al., 2021; Boulard et al., 1996). Similarly, the reasons for the typical intracranial location of these parasites remain unknown. Infection of the intracranial supra-meningeal space could be aberrant, similar to ocular and cerebral infections of humans (Hall et al., 2016; Navarro and Alves, 2016; Shivasharanappa et al., 2011; Terterov et al., 2010). Alternatively, infection of the intracranial supra-meningeal space may be a protective adaptation. Red river hogs forage by aggressively rooting with their snouts (Leus and Vercammen, 2013), presumably leading to high air flow and particulate matter in the oropharynx and sinuses. This aspect of red river hog behavior might select for larvae capable of seeking protected anatomic sites. Such evolved protective countermeasures to host behavior have been recorded for other parasites of African wildlife, notably nasal ticks of chimpanzees (Hamer et al., 2013). The remainder of the bot fly's life cycle remains unknown but likely involves egress from the nares, deposition on the ground (perhaps aided by sneezing), pupation, hatching, mating, and laying of first instar larvae in the nares of the host, as is typical for other nasal bots (Colwell et al., 2006).

Nigeria currently has the largest national pig herd in Africa (Brown et al., 2018). Domestic pigs (*Sus scrofa domestica*) are related to wild pigs such as red river hogs and interact directly with them at wildlife-livestock interfaces (Payne et al., 2018). Unlike for other wild African suids, there is even suspected hybridization between *Potamochoerus* spp. and domestic pigs in Africa (Leus and Vercammen, 2013). Experimental infections would be necessary to determine if this parasite is capable of infecting domestic pigs, to provide additional information on its life cycle and pathogenesis, and to determine whether it could impact the prospects for an economically sustainable pig-farming industry in Nigeria.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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